IntechOpen

# Biomaterials

## Physics and Chemistry - New Edition

*Edited by Rosario Pignatello and Teresa Musumeci*

# BIOMATERIALS - PHYSICS AND CHEMISTRY - NEW EDITION

Edited by **Rosario Pignatello** and **Teresa Musumeci**

**Biomaterials - Physics and Chemistry - New Edition**
http://dx.doi.org/10.5772/intechopen.69128
Edited by Rosario Pignatello and Teresa Musumeci

**Contributors**

Ana Colette Maurício, Daniel Santos, José Domingos, Vitor Sencadas, Pedro Gomes, Maria Helena Fernandes, C. LaShan Simpson, Jenna Mosier, Nancy Nguyen, Kadie Parker, Jonathan Lakey, Sarah Lee, Mayilone Sathialingam, Michael Alexander, Eurico Felix Pieretti, Olandir Correa, Marina Pillis, Maurício David Martins Neves, Edson Cavalcanti, Rosario Pignatello, Teresa Musumeci



**Notice**



First published in London, United Kingdom, 2018 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Biomaterials - Physics and Chemistry - New Edition
Edited by Rosario Pignatello and Teresa Musumeci

p. cm.

Print ISBN 978-1-78923-064-2

Online ISBN 978-1-78923-065-9

eBook (PDF) ISBN 978-1-83881-376-5

# Meet the editors

Prof. Rosario Pignatello is a Full Professor of Pharmaceutical Technology and Legislation at the Department of Drug Sciences, University of Catania (Italy). He is a scientific coordinator of NANO-*i* (Research Centre on Ocular Nanotechnology) at the University of Catania. He is the Coauthor of about 140 papers in peer-reviewed journals and about 150 oral and poster communications to international symposia. His research activity in the area of innovative pharmaceutical technology focuses on: polymer micro- and nanoparticles for the controlled drug delivery and drug targeting, synthesis and technological investigation on chemically engineered (bio)polymers for drug delivery, liposomes, lipid-based nanocarriers, and micelles, advanced ocular drug delivery systems, prodrugs and conjugates for drug lipidization, drug-membrane interaction studies using calorimetry on biomembrane models.

Teresa Musumeci is an Assistant Professor at the Department of Drug Sciences of the University of Catania. She received her PhD degree in Pharmaceutical Technology from the University of Palermo in 2007. She is the co-author of 32 papers in peer-reviewed journals and 2 book chapters. She teaches “Laboratory of Pharmaceutical Technology” and “Quality Assurance System.” Her scientific activity focuses on design and characterization of nanocarriers for controlled and targeted drug delivery.

# Contents

# Preface

Scientists who dedicate their research activity to biomaterials pass through the typical dichotomy that often characterizes the basic research: on one hand is the wish of exploring new frontiers of chemistry, physics, biology, medicine, pharmaceutics, food science, and all the fields to which biomaterials can be applied. The constantly advancing scientific knowledge would prompt the researchers to explore new strategies for producing materials with improved and tailored features. On the other hand, the attempts to give an "official" definition for biomaterials impose strong limitations to the potentiality of basic research. Just as examples, biomaterials are defined as "a nonviable material used in a medical device, intended to interact with biological systems" (Consensus Conference of the European Society for Biomaterials, 1986) or "any substance or combination of substances, other than drugs, synthetic or natural in origin, which can be used for any period of time, which augments or replaces partially or totally any tissue, organ, or function of the body, in order to maintain or improve the quality of life of the individual" (American National Institute of Health) or even "a systematically and pharmacologically inert substance designed for implantation within or incorporation with living systems" (Clemson University Advisory Board for Biomaterials).

Essentially, the only common property is that a biomaterial is different from a biological material that is produced by a biological system. Clearly, none of the proposed definitions can succeed to cover the whole setting of properties and applications of this plethora of natural or synthetic compound, but they can only fit a particular aspect of their function.

These considerations have been one of the bases of the present editorial task that reached its second edition and involves volumes focused on the recent developments and applications of biomaterials. This contribution book collects review articles and experimental reports from eminent experts who are actively involved in this scientific area, striving to cover the interdisciplinary aspects necessary for an effective development and the use of biomaterials. Contributors were asked to give their personal and recent experience on biomaterials, regardless of any specific limitation to fit into one definition or the other. In our opinion, this book will give readers a wider idea on the new and ongoing potentialities of different synthetic and engineered macromolecular materials.

In the meantime, an editorial choice was not to limit the selection of papers concerning market or clinical applications of biomaterials, but results coming from very fundamental studies are welcomed in these textbooks. This preference will also allow to gain a more general view of how the various biomaterials can be applied, along with the methodologies necessary to design, develop, and characterize them, without the restrictions necessarily imposed by industrial or profit concerns.

This volume contains six chapters related to the recent studies and researches on new materials, with a particular attention to their physical, mechanical, and chemical characterizations.

I hope that readers will be driven toward an improved understanding of the range of disciplines and characterization methodologies used to develop biomaterials having the physicochemical and biological properties needed for any specific clinical application.

**Rosario Pignatello**
Section of Pharmaceutical Technology
Department of Drug Sciences
University of Catania
Catania, Italy

**Teresa Musumeci**
Section of Pharmaceutical Technology
Department of Drug Sciences
University of Catania
Catania, Italy

Chapter 1

# Introductory Chapter: Reduce the Gap from Bench to Bedside for Nanomedicines Increasing the Stability to Long-Term Storage

Teresa Musumeci and Rosario Pignatello

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76541

## 1. The issue of physical stability in nanosized drug delivery systems

Stability to storage of a new pharmaceutical product is a key step for medical and commercial success. As with other pharmaceutical products, nanomaterials and nanotechnological drugs (nanomedicines) must be rigorously designed and tested to demonstrate the favorable benefit/risk ratio needed by health authorities to issue marketing authorizations.

Innovative pharmaceutical products, such as micro and nanocarriers, have to face two important aspects for regulatory approval: firstly, the demonstration of efficacy and safety profile is necessary, and secondly suitable storage conditions must be identified for the final product. In the case of colloidal (nanosized) carriers, it is important to obtain nearly unchanged physico-chemical properties during storage [1, 2].

The issue of stability to long-term storage of nanosized drug carriers will be briefly commented in this chapter, since this aspect can become, and often actually is, crucial for the translation of basic researches to clinically valid products. Liposomes, that would often be promising colloidal systems for drug delivery and targeting, are a sound example of how the question of stability strongly influences the translation from lab scale to therapeutics. Apart some few products which were able to reach a clinical significance, a huge number of projects, although promising at the preliminary phases, are unable to undergo industrial and commercial scaling-up, in most cases because of their very limited physico-chemical stability and difficulty to preserve the chemical integrity of liposomal formulations.

Another important aspect is related to the requirement of sterilization, mandatory for products that must come in contact with blood, eye or other damaged or sensitive tissues. Gamma

or UV radiation and autoclaving or chemical (ethylene oxide) sterilization techniques are usually applied to sterilize polymer-based products or devices. These processes expose the polymeric materials to a certain degree of physico-chemical stress and thus fundamental to ensure that the sterilized biomaterial retains its characteristics and features.

Of course, for some applications of biomaterials, a robust chemical stability is paradoxically unwanted. For resorbable materials, for instance, such as materials for sutures or fixing screws for bone fractures, a relatively rapid degradation in the body after the healing process is required.

Considering colloidal systems, we should pay attention to three principal properties, such as size and size distribution (homogeneity), surface charge, and shape (the rule of 4S), that should not significantly change during storage and that influence physico-chemical stability (**Figure 1**) [3].

Looking into this matter, we can highlight some important properties that separately influence physico-chemical stability. It is widely known that a mean particle size below 100 nm, correlated with a narrow size distribution (PdI < 0.2), allows increasing the stability of a nanosized system. These experimental evidences are necessary but not sufficient conditions, for instance also zeta potential values (i.e., a surface charge not less than ±35 mV) stabilize the nanoformulation. When this situation is not verified, several instability conditions have been reported.

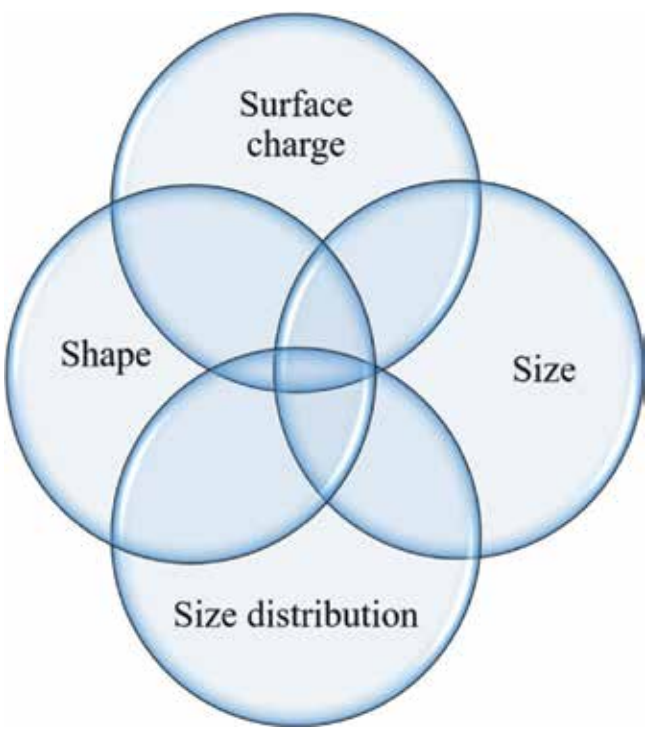



**Figure 1.** Venn diagram on properties that influence the physico-chemical stability of colloidal suspensions.

## 2. Freeze-drying (lyophilization) as a strategy to increase nanocarrier stability

To improve the stability of colloidal delivery systems upon storage, the removal of solvents, if required during the preparation method, is necessary, to obtain a final aqueous suspension. Storage of aqueous suspensions could determine some changes in the product such as increase of size, due to coalescence or aggregation phenomena, or even a reduction of it, due to degradation of the polymeric matrix, undesirable drug leakage, changes in the surface charge, and so on.

To remove water, freeze-drying (lyophilization) is one of the most efficient techniques used in the pharmaceutical industry and a strategy to reduce and control the instability phenomena [4, 5]. However, several physico-chemical phenomena, such as air adsorption and modification of nanoparticle surface during the various steps of the process may lead difficulty in redispersion of colloidal carriers in aqueous media for the subsequent administration [6, 7]. The addition of inert additives, defined as lyoprotectants or cryoprotectants) at relatively high concentrations (10–30%, but in some instances also up to 50% by weight), could protect the colloidal suspension against stress induced by the freeze-drying process [8–10].

During the last three decades, different studies were performed to select the additives to use during the freeze-dried process to prevent lyophilization stress. Several authors described the protective effect due to the formation of an amorphous vitreous layer on the surface of particles. Carbohydrates and polyalcohols are the most investigated ones [11].

**Figure 2** shows how this field is a current topic of interest for scientists, as highlighted by the increased number of papers published in the last two decades (Pubmed source).

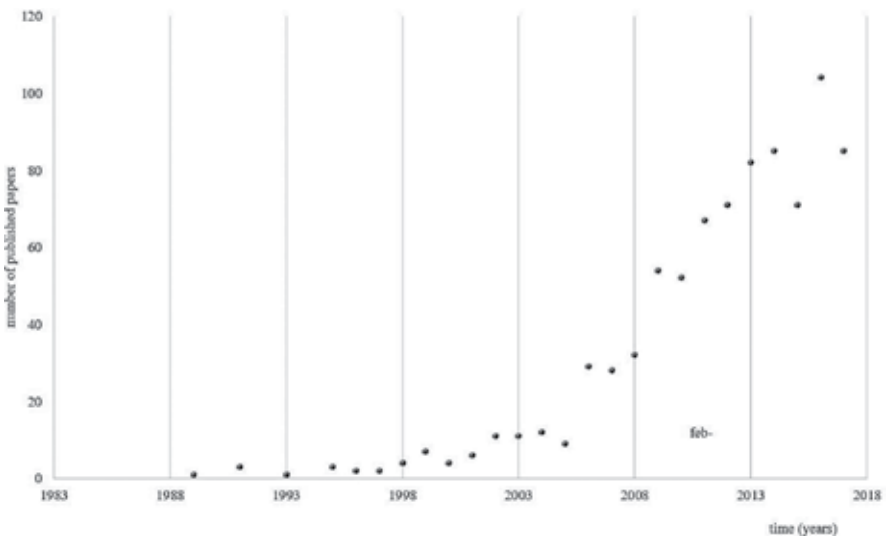



**Figure 2.** Trend of article publication in the field 'freeze-dried and nanoparticles' (Pubmed database; update: February 2018).

The protective action of cryoprotectants is principally a surface phenomenon, for which the proper amount of this component should also be evaluated. The concentration should be established as a function of total superficial area correlated to the size and the presence or not of the adsorbed drug onto the surface that could change the disposition of cryoprotectant at a molecular level on the surface. In the case of sugars, it was showed a reduction in the suitable amount to be used going from monosaccharides and disaccharides to oligosaccharides, therefore as a function of the type [9].

To better understand what happens during the freeze-drying process, the involved steps are recalled below. Scientists do not often distinguish between cooling and freezing steps and they consider as a single process. Conversely, cooling is related to the decrease of temperature of the chamber and the product inside it, while freezing corresponds to the modification of the physical state of the formulation from liquid (water) to solid (ice). During this step, water starts to nucleate and ice crystals are formed. Supercooling occurs if the formulation is cooled below its freezing point.

Lyophilization of nanosystems would however deserve a peculiar discussion. The first question is whether it is better a slow or fast freezing speed to obtain redispersible nanoformulations? The answer should consider some aspects: first of all, the state of excipient (e.g., the cryoprotectant) during freezing. For instance, mannitol crystallizes during slow freezing, while it is made more amorphous upon a fast freezing that makes mannitol to be able to protect nanoparticles. Another aspect is the concentration; several authors reported an enhanced protective action with increasing the amount of cryoprotectant. In summary, fast freezing rate and high concentration of cryoprotectant should produce a dry nanoformulation that preserve its better initial physico-chemical properties upon redispersion with an aqueous medium. However, a critical concentration for each cryoprotector seems to exist, above which an additional amount is prejudicial in many cases [12].

During primary drying, it is appropriate to obtain a non-collapsed cake, whose consequence would be a long time necessary for the redispersion of the freeze-dried nanoparticles. If primary drying occurs below the Tc of the nanoformulation, it is possible to avoid this phenomenon and the product will preserve the initial macroscopic structure.

Also the secondary drying is very important to be considered. Several authors sometimes underestimate the evaluation and standardization of operative conditions during this step. Currently, the drying time and shelf temperature can be optimized using simulation softwares, thus reducing experimental errors [13].

Raw materials used for the preparation of the colloidal carriers should be considered in the design and in the selection of the cryoprotectant. Liposomes are more sensitive to the freezing step, while polymers used to realize micro and nanocarriers are resistant to the low temperatures, but could suffer in the dehydration process, with a difficulty to restore the initial particle morphology and size (e.g., because of aggregation).

## 3. Regulatory aspects

Polymer- and lipid-based nanomedicines are checked during stability assays for physico-chemical, chemical, and microbiological aspects, such as macroscopic and microscopic physical

appearance, particle mean size and size homogeneity, zeta potential (surface charge), morphology and surface chemistry, drug loading/content and release profile, and *in vitro* drug stability/degradation. ICH guidelines (e.g., ICH Q1A and Q1C [14, 15]) can be helpful to project a suitable and efficacious stability plan, in terms of temperature/humidity conditions and duration.

For formulations that contain or encapsulate biotechnological products, like monoclonal antibodies, peptides, proteins, and gene material, the procedures described in ICH guideline Q5C for stability testing of these compounds must be considered [16].

Moreover, for medical devices and especially for sterile devices, apart an extrapolation from the above ICH guidelines, the ISO 13485:2016 rules are useful to manage the quality and stability requirements of these products, with the aim of demonstrating their ability to meet customer needs and regulatory requisites.

In April 2014 in London, during the "SME workshop for micro-, small- and medium-sized enterprises: focus on quality for medicines containing chemical entities", organized by EMA, Dolores Hernán Pérez de la Ossa presented a report entitled "Quality aspects of nano-based medicines" [17].

The report identifies the challenges that a nanomedicine product should overcome to pass the regulatory process and safely reach the market. Such document fixes not only the physico-chemical aspects of the proposed nanomedicine, but also any complex supramolecular structure, for example the presence of ligands on the surface. These molecules determine the interactions with biological substrates and influence the physical stability of the so-called third generation nanoformulations. The presence of these substances often limits the effects of others additives added to stabilize the colloidal carriers. Cryoprotectant agents could not cover the surface and not preserve from aggregation during a freeze-dried process. The ligands could significantly modify the zeta potential value, a predictable parameter of colloidal carrier stability.

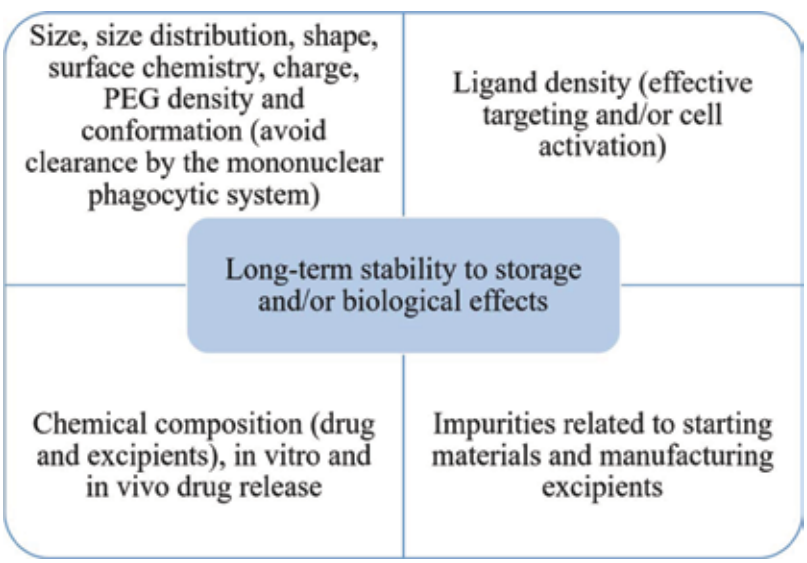



**Figure 3.** The main properties of nanomedicines that influence both long-term storage stability and biological interactions.

Another important aspect worthy of note is that only a small number of scientists, during the planning of an experimental work, taken into account the regulatory aspects, which instead must be considered before the development of a project. The biggest threshold is the scalable, controlled, and reproducible manufacturing of nanomedicines under good manufacturing practice (GMP) and, in many cases, under sterile conditions.

Why it is important to control physico-chemical properties of nanocarriers? Safety and efficacy of nanomedicines upon biological substrates are strictly correlated with these properties (**Figure 3**).

For this reason, there is a growing interest in quality-by-design approach (QbD): it is in fact very important to establish which critical quality attributes (CQA) govern the quality, safety, and efficacy of nanomedicines, also considering their specific route of administration or application. Applying this or a similar approach in preclinical studies helps to obtain reproducible drug batches [18–20].

## Author details

Teresa Musumeci and Rosario Pignatello*

*Address all correspondence to: r.pignatello@unict.it

Section of Pharmaceutical Technology, Department of Drug Sciences, University of Catania, Catania, Italy

## References

[1] Muthu MS, Feng S-S. Pharmaceutical stability aspects of nanomedicines. Nanomedicine. 2009;**4**(8):857-860

[2] Vauthier C, Ponchel G. Polymer Nanoparticles for Nanomedicines: A Guide for their Design, Preparation and Development. Cham (CH): Springer; 2017. ISBN: 978-3-319-41419-5

[3] Carbone C, Manno D, Serra A, Musumeci T, Pepe V, Tisserand C, Puglisi G. Innovative hybrid vs polymeric nanocapsules: The influence of the cationic lipid coating on the "4S". Colloids and Surfaces. B, Biointerfaces. 2016;**141**:450-457

[4] Barley J. Basic Principles of Freeze Drying. Available at: https://www.spscientific.com/freeze-drying-lyophilization-basics/ [Accessed: March 2018]

[5] Tang X, Pikal MJ. Design of freeze-drying processes for pharmaceuticals: Practical advice. Pharmaceutical Research. 2004;**21**(2):191-200

[6] Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: Formulation, process and storage considerations. Advanced Drug Delivery Reviews. 2006;**58**:1688-1713

[7] Beirowski J, Gieseler H. Stabilisation of nanoparticles during freeze drying: The difference to proteins. European Pharmaceutical Review. 2011;**4**. Available from: https://www.europeanpharmaceuticalreview.com/article/8883/stabilisation-of-nanoparticles-during-freeze-drying-the-difference-to-proteins/

[8] Bonaccorso A, Musumeci T, Carbone C, Vicari L, Lauro MR, Puglisi G. Revisiting the role of sucrose in PLGA-PEG nanocarrier for potential intranasal delivery. Pharmaceutical Development and Technology. 2018;**23**(3):265-274

[9] Musumeci T, Vicari L, Ventura CA, Gulisano M, Pignatello R, Puglisi G. Lyoprotected nanosphere formulations for paclitaxel controlled delivery. Journal of Nanoscience and Nanotechnology. 2006;**6**(9-10):3118-3125

[10] Picco AS, Ferreira LF, Liberato MS, Mondo GB, Cardoso MB. Freeze-drying of silica nanoparticles: Redispersibility toward nanomedicine applications. Nanomedicine (London, England). 2018;**13**(2):179-190

[11] Fonte P, Reis S, Sarmento B. Facts and evidences on the lyophilization of polymeric nanoparticles for drug delivery. Journal of Controlled Release. 2016;**225**:75-86

[12] Abdelwahed W. A pilot study of freeze drying of poly(epsilon caprolactone) nanocapsules stabilized by poly(vinyl alcohol): Formulation and process optimization. International Journal of Pharmaceutics. 2006;**309**:178-188

[13] Kodama T, Takeuchi M, Wakiyama N, Terada N. Optimization of secondary drying condition for desired residual water content in a lyophilized product using a novel simulation program for pharmaceutical lyophilization. International Journal of Pharmaceutics. 2014;**469**:59-66

[14] ICH Q1A (R2). Stability Testing Guidelines: Stability Testing of New Drug Substances and Products. Step 5; February 2003

[15] ICH Q1C. Stability Testing for New Dosage Forms. Step 5; November 1996

[16] ICH Q5C. Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products. Step 5; November 1995

[17] de la Ossa DHP. Quality Aspects of Nano-Based Medicines. 2014. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Presentation/2014/04/WC500165444.pdf

[18] Havel HA. Where are the nanodrugs? An industry perspective on development of drug products containing nanomaterials. The AAPS Journal. 2016;**18**(6):1351-1353

[19] Mühlebach S, Borchard G, Yildiz S. Regulatory challenges and approaches to characterize nanomedicines and their follow-on similars. Nanomedicine (London, England). 2015;**10**(4):659-674

[20] Tyner KM, Zou P, Yang X, Zhang H, Cruz CN, Lee SL. Product quality for nanomaterials: Current U.S. experience and perspective. WIREs Nanomedicine and Nanobiotechnology. 2015;**7**(5):640-654. DOI: 10.1002/wnan.1338

Chapter 2

# Spray Drying: An Overview

Daniel Santos, Ana Colette Maurício,
Vitor Sencadas, José Domingos Santos,
Maria H. Fernandes and Pedro S. Gomes

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72247

**Abstract**

Spray drying is a well-known method of particle production which comprises the transformation of a fluid material into dried particles, taking advantage of a gaseous hot drying medium, with clear advantages for the fabrication of medical devices. In fact, it is quite common the production of microspheres and microcapsules designed for drug delivery systems. This review describes the different stages of the mechanism of the spray-drying process: atomization, droplet-to-particle conversion and particle collection. In particular, this work addresses the diversity of available atomizers, the drying kinetics and the importance of the configuration of the drying chamber, and the efficiency of the collection devices. The final properties of the dried products are influenced by a variety of factors, namely the spray dryer design, the feed characteristics and the processing parameters. The impact of those variables in optimizing both the spray-drying process and the synthesis of dried particles with desirable characteristics is discussed. The scalability of this manufacturing process in obtaining dried particles in submicron-to-micron scale favors a variety of applications within the food, chemical, polymeric, pharmaceutical, biotechnology and medical industries.

**Keywords:** spray drying, dry particles, atomizer, drying chamber, collector

## 1. Introduction

Spray drying is a well-known method of particle production which consists on the transformation of a fluid material into dried particles, taking advantage of a gaseous hot drying medium [1]. Its first observation is dated 1860 and a primitive spray dryer device was patented by Samuel Percy in United States in 1872 [1–3].

Ever since it was first discovered, the spray-drying technique has been improved concerning its operational design and applications. In fact, the primordial spray dryer devices lacked process efficiency and safety. After overcoming these issues, spray drying became an attractive method for food industry purposes, ending up to be used in milk powder production in the 1920s, remaining one of the most important applications until the current days. Spray-drying evolution was directly influenced by World War II, where there was an imperative need to reduce the weight and volume of food and other materials to be carried [3, 4]. As a result, spray drying has become an industry benchmark, namely in the dairy products' fabrication. In the post-war period, the spray-drying method continued progressing, reaching the pharmaceutical, chemical, ceramic and polymer industries [3, 5].

Even after more than a century of research, spray drying is still a target of study and innovation due to the increasing demand for complex particles with specific characteristics. This is considered a powerful technological process since it brings feasibility to the production of free-flowing particles with well-defined particle size. Besides, bearing in mind the ability to use different feedstocks as well as its high productivity and broad applications, makes this technique more and more attractive to the scientific community [2, 3].

Spray-drying mechanism is based on moisture elimination using for that a heated atmosphere to which the feed product is subjected. The process may be described by three major phases (atomization, droplet-to-particle conversion and particle collection), although some authors use four or five minor steps to describe it in more detail [2, 4, 6]. As shown in **Figure 1**, a solution is pumped to an atomizer, breaking up the liquid feed into a

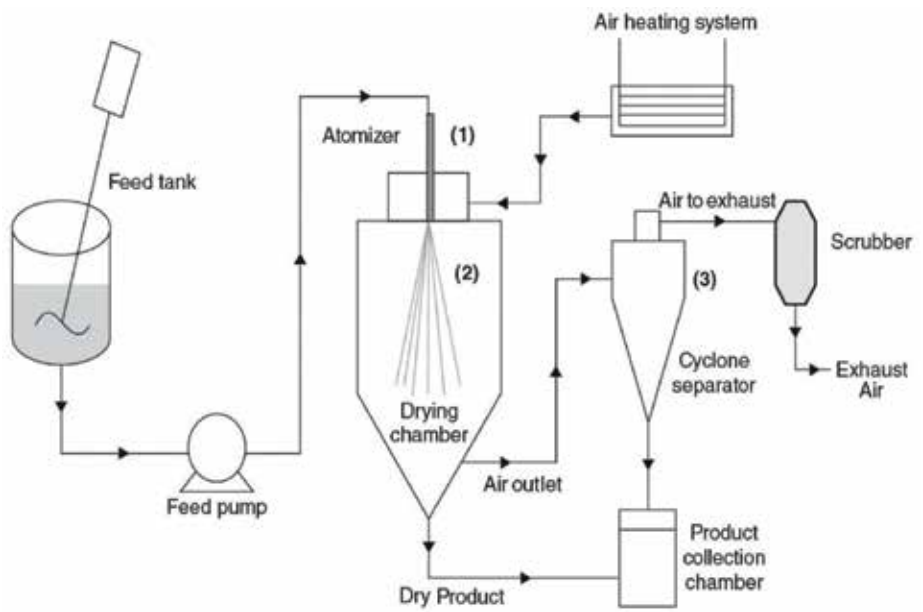



**Figure 1.** Schematic representation of spray-drying mechanism. (1) Atomization. (2) Droplet-to-particle conversion. (3) Particle collection. Adapted from [2, 7].

spray of fine droplets. Then, the droplets are ejected into a drying gas chamber where the moisture vaporization occurs, resulting in the formation of dry particles. Finally, using an appropriate device, the dried particles are separated from the drying medium, being then collected in a tank.

All of these stages, conjointly with the conditions, in which they are processed, play a crucial impact in the yield of spray-drying mechanism as well as in the final particle properties [1, 3]. Thus, every step of this mechanism will be meticulously described below.

## 2. Design and mechanism stages

### 2.1. Atomization

The spray-drying process is initiated with the feed solution atomization in small droplets due to a decrease of surface tension. This is considered a crucial step for the subsequent phases, namely during the drying chamber exposition. In fact, breaking up the initial solution into many droplets increases their surface area, optimizing therefore the heat and mass transfers between the heated drying gas and the liquid particles. In other words, this gathers the ideal conditions for evaporation process, which will be preponderant for the formation of dried particles [1, 6, 8].

The physical principle behind the atomization transformation process is based on the liquid disintegration phenomenon. Several authors have addressed different interpretations and analytical models to explain such event [2].

In 1873, Joseph Plateau was a pioneer in this issue, realizing that a liquid jet of constant radius, falling due to gravity, experiences a progressive increase of its length [2]. As soon as a critical value is reached, the cylindrical shape of the jet is disintegrated into small spherical droplets, which essentially takes place due to a decrease in surface tension (**Figure 2A**). Later, Lord Rayleigh (1878) [2] validated Plateau's work and postulated the "Liquid jet theory". In broad terms, he described the existence of perturbations waves in a simple laminar jet. For certain wavelengths, the optimum wavelength ($\lambda_{opt}$ = 4.51d, where d is the initial jet diameter), such perturbations grow larger in time, causing the droplet formation (**Figure 2B**) [2].

Weber (1931) [2] and Ohnesorge (1936) [2] built a more complete model to describe the liquid instability. Besides the surface tension and inertial forces underlying the previous works, they

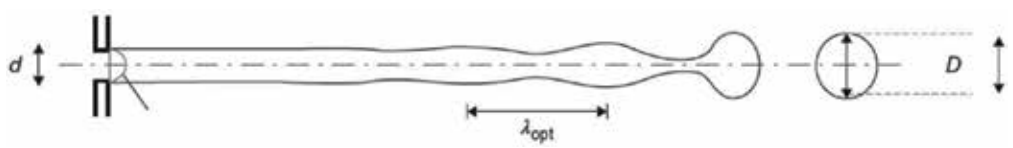



**Figure 2.** Schematic representation of droplet formation mechanism showing the optimum wavelength introduced by Rayleigh [2].

described the impact of other factors in the system, namely feed viscosity, the surrounding air and the atomization gas. Weber proved that the frictional forces of the surrounding air cause a decrease in the $\lambda_{opt}$ for drop formation. Moreover, the increase of the relative velocity between the liquid jet and the surrounding air also provokes a decrease in the $\lambda_{opt}$, and consequently a reduction in the final droplet size. Regarding Ohnesorge's contribution, he reported the propensity of a liquid jet to breakup into droplets through a relationship (Eq. (1)) between its viscosity, density, surface tension and jet size [2].

$$Oh = \frac{\sqrt{We}}{Re} = \frac{\mu}{\sqrt{\rho \cdot \gamma \cdot L}} = \frac{Viscous\ forces}{\sqrt{(inertia \times surface\ tension)}} \quad (1)$$

*Oh* is the Ohnesorge number (dimensionless) that expresses a ratio between the Weber number (*We*) and the Reynolds number (*Re*). $\mu$, $\rho$ and $\gamma$ are the viscosity, density and surface tension of the feed solution, respectively. *L* is the volume per unit area of the feed droplet.

The atomization process into droplet form may be accomplished by pressure, centrifugal, electrostatic or ultrasonic energy, using specific devices called atomizers [6, 8]. There are different atomizers (**Figure 3**), which are used according the desired product characteristics (shape, structure and size) as well as depending on the nature of the feed solution. The most common devices used in the majority of atomization processes are explained below and summarized in **Table 1** [1, 2]. In fact, there is a mathematical equation (Eq. (2)) which expresses the relation between the droplet diameter ($D_d$), the atomizer type and feed solution properties (surface tension ($\gamma$), viscosity ($\mu$) and density ($\rho$)) [6].

$$D_d = K_f \cdot Q^n \left[\rho^a \cdot \gamma^b \cdot \mu^c\right] \quad (2)$$

$K_f$, $Q$ and $n$ are the equipment constant, feed solution volumetric flow rate, and the power constant of volumetric flow rate, respectively. The power constants of solution properties are represented by *a*, *b* and *c*.

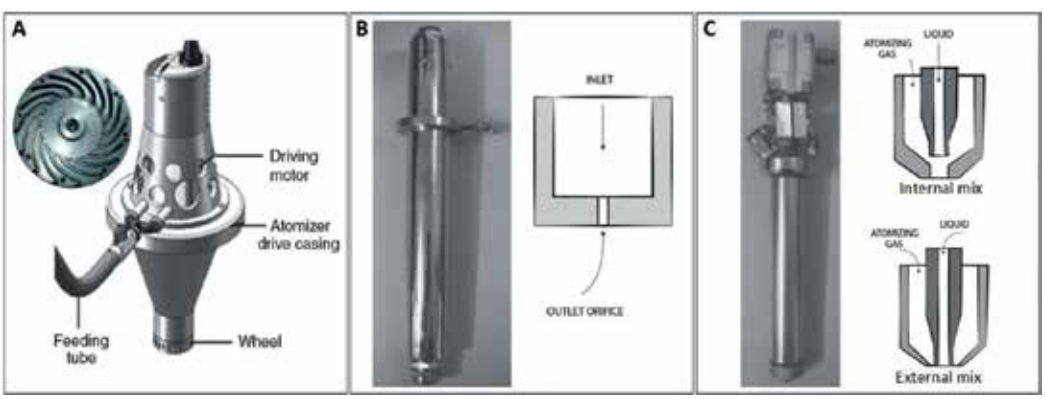



**Figure 3.** Schematic representation of conventional atomizers used on spray drying. (A) Rotary atomizer with respective disc atomizer in detail. (B) Hydraulic nozzle atomizer, (C) pneumatic nozzle atomizer—internal and external two-fluid nozzles. Adapted from [1, 2, 7, 9, 10].

| | Rotary atomizer | Hydraulic nozzle atomizer | Pneumatic nozzle atomizer |
|---|---|---|---|
| Atomization energy | Centrifugal | Pressure | Kinetic |
| Atomization parameters | Disc speed 10,000–30,000 rotations per minute (rpm) | Nozzle pressure 250–10,000 PSI | Nozzle pressure 250–10,000 PSI |
| Type of spray | Fine, medium, coarse | Coarse, less homogeneous | Medium coarseness, poor homogeneity |
| Mean droplet size | 30–120 μm [2] | 120–250 μm [2] | 30–150 μm [2] |
| | 10–200 μm [11] | 30–350 μm [11] | 5–100 μm [11] |
| | | 20–600 μm [4] | 10–200 μm [4] |
| Relation between $D_d$ and the feed solution properties | $D_d \alpha Q$ | $D_d \alpha Q$ | $D_d \alpha Q$ |
| | $D_d \alpha \mu$ | $D_d \alpha \mu$ | $D_d \alpha \mu$ |
| Relation between $D_d$ and atomization | $D_d$ is inversely proportional to disc speed and diameter | $D_d$ is inversely proportional to atomization pressure | $D_d$ is inversely proportional to atomization pressure |
| Advantages | Handle high feed rates without clogging; formation of uniform size particles; low pressure operation; high efficiency | Low price; formation of particles with little occluded air (i.e. particle production of higher density); enables the use of narrow drying chambers | Better control over particle size than in the hydraulic nozzle; useful for feeds of high viscosity; ideal to laboratory scale since it requires small drying chamber; good efficiency |
| Drawbacks | High price; not suitable to viscous feed; Inability to use a horizontal and small spray dryer chambers | Not suitable to high viscous feed; high feed rates cause coarse and less homogeneous sprays (i.e. broad particle size distribution) | High operation costs due to the need of high amounts of compressed gas for atomization; production of particles with high occluded air; Downstream turbulence |

Adapted from [1–3, 7, 11].

**Table 1.** Conventional atomizers used on spray drying.

### *2.1.1. Rotary atomizers*

The rotary atomizers have a horizontal wheel or disc, and the feedstock solution is driven to its center. There, a centrifugal force is applied which accelerates the feed solution to the periphery, forming a spray of droplets. It is common to find grooves in the atomizer disc, since these structures maximize the control over the solution dispersion caused by the rotary high velocities [1, 2].

### *2.1.2. Hydraulic nozzle atomizers*

Also known as one-fluid nozzles, the operation principle of the hydraulic nozzles consists on the conduction of the feed solution, under pressure, through a pipe with gradually decreasing diameter. The fluid emerges from a small nozzle orifice (usually ranging from 0.4 to 4 mm in

diameter) at high velocity with a simultaneous loss in its pressure, undergoing atomization, and thus it is disintegrated in the form of droplets [1, 2].

*2.1.3. Pneumatic nozzle atomizers*

Pneumatic nozzles are also called multi-fluid nozzles. The most common configuration of these devices is based on a two-fluid nozzle atomizer, where two phases are fed into the nozzle, namely the feedstock solution and the compressed gaseous atomizing medium. The gas flows separately from the feed solution, meeting it whether within or outside the nozzle. Due to the high frictional forces over the liquid surface caused by the high gas velocity, atomization takes place and the feedstock solution is broken down into a cloud of droplets, as stated by Weber. Similarly to the previous atomizers, atomization is influenced by feed properties. Herein, gas velocity and density, as well as its direction and the ratio liquid/gas, also have an important effect in the atomization process [1–3].

**2.2. Droplet-to-particle conversion**

After atomization, the spray-drying mechanism proceeds with the particle formation phase, a crucial stage marked by two events: spray-air contact and droplet drying step, resulting, as a whole, in the removal of the droplets' solvent content and consequently on their transformation into dried particles [4, 6].

*2.2.1. Droplet-air-contact*

Atomized droplets are exposed to a hot gas within the drying chamber, resulting on first rapid moisture evaporation. Usually, this drying gas is heated (and filtered) atmospheric air, although in some cases there is a need to use other inert gases to avoid eventual instabilities between the gas and the droplets [2]. In what concerns drying chamber's size and shape, it should be consistent with the spray dryer setup, that is, it should be chosen according the used atomizer and the pretended particle properties. Thus, there are different sized drying chambers (smaller or taller), though they should be big enough to guarantee that particles have the necessary time to dry before reaching the chamber's walls (otherwise they would deposit in the chamber walls, which is undesirable). The majority of vertical chambers are cylindrical, ending with an inverted cone on its base, as depicted in **Figure 4** [1].

There are different drying chamber configurations, in which the flow pattern between the hot gas and the spray of droplets is distinct: co-current flow, counter-current flow and mixed flow [1].

In the co-current flow (**Figure 4A**), both the atomized spray and the heated gas enter at the top of the drying chamber, flowing through it in the same direction. The dried particles are dropped at the bottom of the chamber, where they are released together with the gas. In such configuration, there is no time for the drying gas to exchange some of its heat with the surroundings, and thus the atomized droplets meet the highest temperatures inside the drying chamber. However, this implies an instantaneous high rate of solvent evaporation (thermal energy of hot air is utilized for evaporation, cooling it), enabling the dried particles to contact with moderate temperatures which avoid undesirable thermal degradation [1, 2, 8].

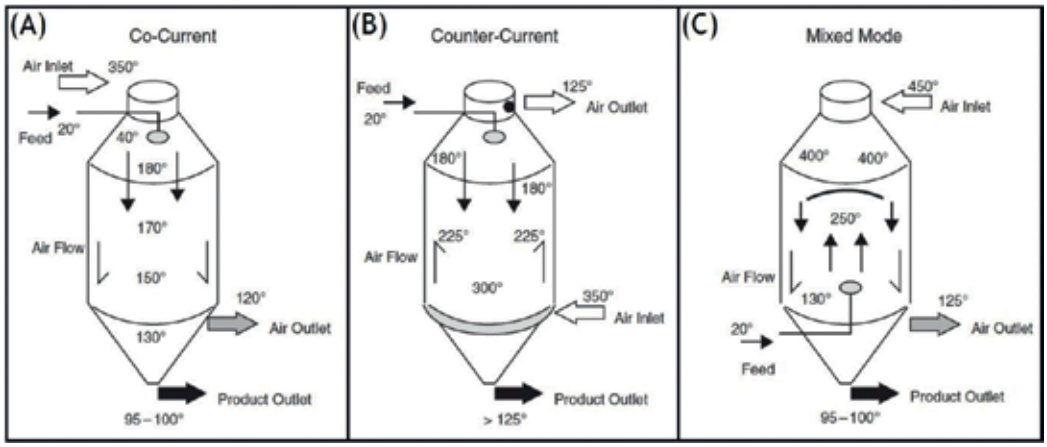



**Figure 4.** Air-droplet flow patterns within the drying chamber. An exemplificative temperature profile is also represented. (A) Co-current flow. (B) Counter-current flow. (C) Mixed flow [7].

In the counter-current design (**Figure 4B**), spray and hot gas are introduced at opposite ends of the chamber, being the atomizer positioned at its top and the air supplied from the bottom. This flow pattern is considered the most efficient use of the chamber's heat since the upward streamline of the hot gas will reduce the downward flow velocity of the atomized spray, subjecting it to a longer period of time inside the chamber [3]. However, in contrast with what happens in the co-current flow, during the upward flow of the hot gas some of its heat is released to the surroundings. Thus, atomized droplets with high amounts of solvent will hit the gas at lower temperatures, whereas the driest particles will contact with the highest temperatures of the drying medium at the bottom of the chamber, limiting the application of this process to heat resistant materials. The dried particles are released at the bottom of the chamber while the gas leaves it through its upper part [1].

Mixed flow dryer construction (**Figure 4C**) combines both co-current flow and counter-current flow, that is, atomized droplets are fed from the bottom of the chamber in counter-current relative to the downward streamline of the drying gas which is supplied from the top. The dried particles as well as the drying gas are then released at the bottom of the chamber. First, the atomized spray experiences an upward flow, and due to the impact of the downward flow of the drying medium, there is a reversion on its path, ultimately falling in the bottom of the chamber. Hence, the residence time of spray inside the chamber is maximized, being this method preferential to dry coarse droplets, even in small chambers [3]. This arrangement is also appropriated only for heat resistant materials.

*2.2.2. Droplet drying: moisture evaporation*

As introduced above, as soon as the aerosol droplets contact with the drying medium within the chamber, they undergo evaporation and solute condensation, resulting in solvent removal. This phenomenon reflects a heat and mass balance problem driven by the difference between the solvent's vapor pressure and its partial pressure toward a gas phase. Thus, the hot gas

temperature triggers a heat exchange from it to the droplets, whereas the vapor pressure difference causes a moisture transfer in the opposite direction. As a result, dried particles are obtained [5, 8].

Drying kinetics of the spray-drying process comprise several steps with different durations and specific events, as shown in **Figure 5** [2, 12].

Immediately upon gas-droplet contact, heat transfer from the gas to the droplet causes an increase of droplet's temperature, from its initial temperature ($T_i$) to a constant value, the equilibrium evaporation temperature ($T_{eq}$) (**Figure 5AB**). The drying process proceeds at a constant evaporation rate, that is, a fast water diffusion from the droplet core to its surface allows a constant moisture removal. Thus, the droplet surface remains sufficiently cool and saturated with moisture, keeping its temperature constant at the wet-bulb temperature (**Figure 5BC**). Wet-bulb temperature is the name given to the temperature of the gas when it gets saturated with vapor from the liquid. This is the lowest temperature that the drying gas can reach due to the evaporative cooling phenomenon, that is, the gas is cooled as it spends latent heat of vaporization. This stage of intense moisture evaporation is also marked by droplet shrinkage, as represented in **Figure 6**—step 1 [2, 12].

During the constant drying rate period, the evaporation of a liquid droplet with a given diameter $d$, is proportional to its surface area. This is reflected in the "$d^2$ law" (Eq. (3)), a mathematical model that expresses how the drying process is mainly controlled by the Peclet number ($Pe$) [2, 13].

$$\frac{\partial C}{\partial r} = Pe \cdot C \tag{3}$$

$C$ and $r$ are the mass concentration of solid fraction and droplet radius, respectively. Peclet number is defined by (Eq. (4)) [2, 13].

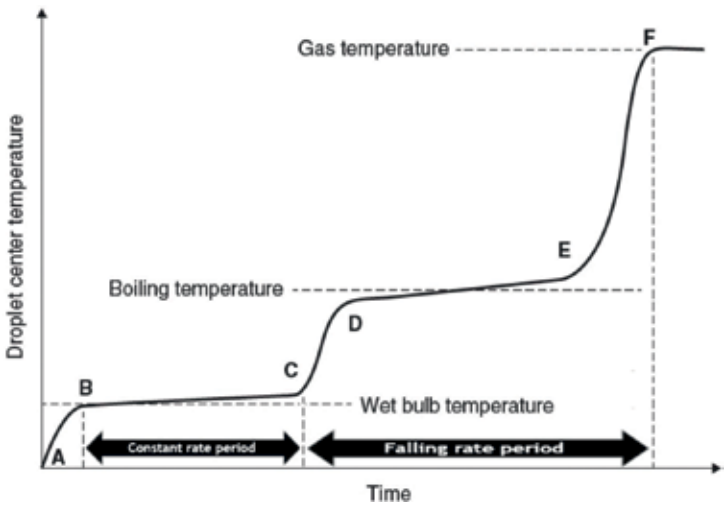



**Figure 5.** Temperature evolution during drying kinetics of a sprayed liquid droplet. Adapted from [12].

$$Pe = \frac{K}{D} \approx \frac{evaporation\ rate}{diffusion\ rate} \tag{4}$$

Moisture evaporation occurs at a constant rate until a critical value of the droplet water content is reached. In other words, when the solute dissolved in the liquid reaches almost its saturation, a thin shell (also known as crust) is formed at the droplet surface (**Figure 6**—step 2), and, as a result, evaporation slows down and becomes dependent on the water diffusion rate through such surface shell [2, 3]. This marks the beginning of the drying kinetics' falling rate period, being immediately noticeable an increasing particle temperature (**Figure 5CD**). When droplet temperature reaches the moisture boiling point, vaporization takes places, a transition which requires a large amount of energy (**Figure 5DE**). This means there is no longer a sensible heating of the particles and thus, the drying process proceeds driven by external heat transfer from the air to the particle. Once again, there is an increase of particle temperature until it becomes equal to that of the surrounding gas, marking the end of the drying process (**Figure 5EF**) [12]. Along the falling rate period, when the partial pressure of moisture vapor at the droplet core overcomes the ambient pressure, bubble formation inside the droplets may occur (**Figure 6**—step 3) [2].

In order to achieve a successful droplet-to-particle conversion, an optimization of the process conditions is required. Regarding the drying mechanism, two major aspects have a huge impact in the final products: the minimum temperature ($T_G$) which allows a completely solvent removal and the minimum residence time ($t$) of the particles inside the chamber that ensures a sufficient drying time. $T_G$ can be predicted from (Eq. (5)), an Antoine equation [6].

$$T_{wb} = K_1 \cdot \left(\frac{T_b}{K_2}\right)^m log(T_G) + K_3 \tag{5}$$

$T_{wb}$ and $T_b$ are the wet-bulb temperature and the boiling temperature, respectively. $K_1$, $K_2$, $K_3$ and $m$ are Antoine constants.

Regarding $t$ calculation, a mathematical mass balance can be defined through (Eq. (6)) [6].

$$C_m = C_0 \left(1 - \frac{t}{\tau_D}\right)^{-\frac{3}{2}} \tag{6}$$

$C_m$, $C_0$ and $\tau_D$ are the desired final concentration of the main component of the dried particle after spray-drying process, the initial concentration and the maximum droplet drying time,

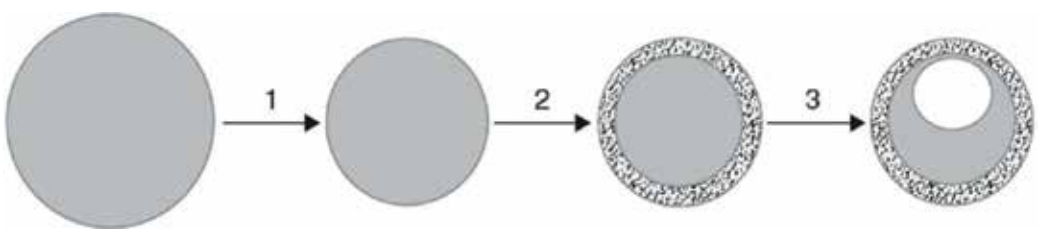



**Figure 6.** Schematic representation of droplet morphological changes during spray drying. Adapted from [2].

respectively. $\tau_D$ can be extrapolated from (Eq. (7)) [6], where $D_d$ is the initial droplet diameter and $K$ is the evaporation rate, as stated above in (Eq. (4)).

$$\tau_D = \frac{D_d^2}{K} \tag{7}$$

### 2.3. Particle collection

Once the droplet-to-particle conversion is concluded, it is necessary to collect the dried particles. This implies a separation procedure, in which the dried particles are disassociated from the drying gas. Such separation occurs in general in two phases, called primary and second separation. In the primary separation, the most dense particles are recovered at the conical bottom of the drying chamber, as they settle on it [2, 8]. On the second separation, the finest or smallest particles are transferred to external devices, where they are separated from the humid air. The most common dry collectors include the cyclone separator, the bag filter and the electrostatic precipitator (**Figure 7**); equipment with different efficiencies and which are

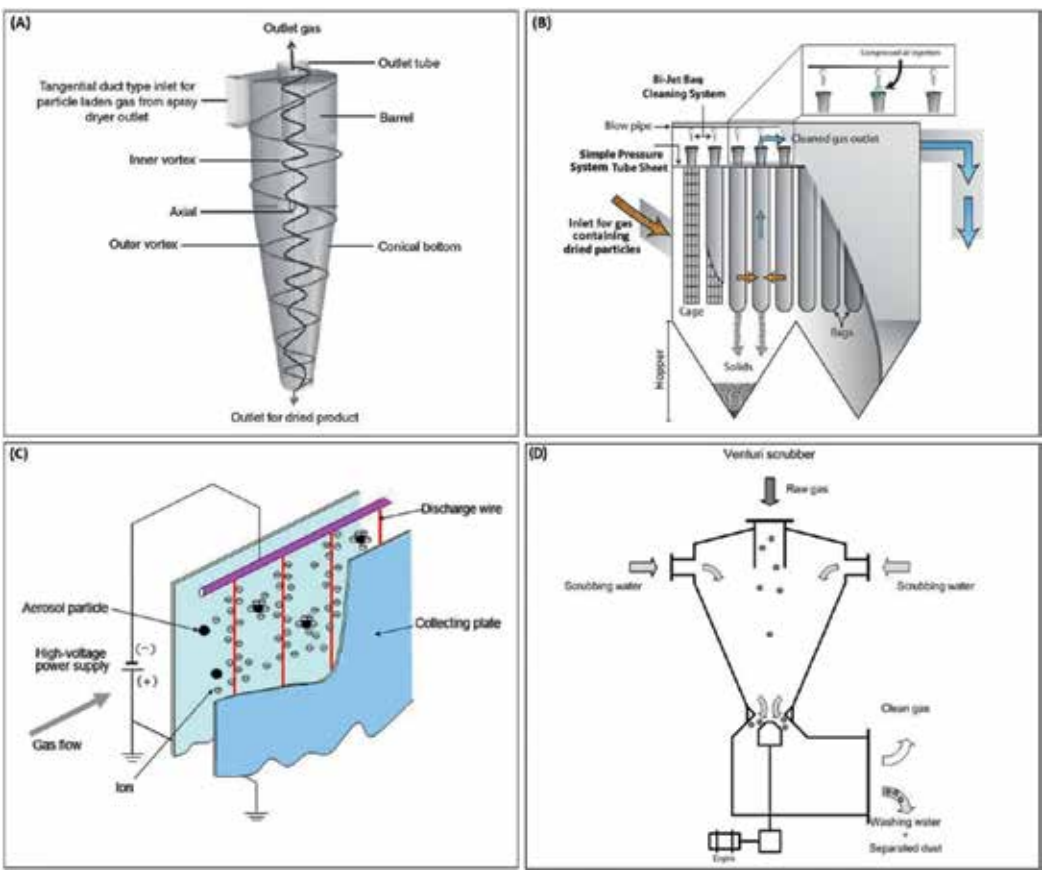



**Figure 7.** Typical collectors used on spray drying. (A) Cyclone separator. (B) Bag filter. (C) Electrostatic precipitator. (D) Venturi wet scrubber. Adapted from [14–17].

used according to the size of particles exhausted with the humid gas and the desired characteristics of the final product [6]. Moreover, it is also common to use wet scrubbers after the dry collectors in order to perform an extra gas cleaning step, as it will be explained below [2].

#### *2.3.1. Cyclone separator*

The separation mechanism of the cyclone separator is based on centrifugal force. This device presents a cylindrical upper part, the barrel, and a conical part on its bottom, the cone (**Figure 7A**). The streamline of air containing the dried particles coming from the drying chamber is supplied into the cyclone at its top, namely tangentially to the barrel. Then, this streamline follows a downward flow, creating an outer vortex. The high velocities of the outer vortex create a centrifugal force on the particles, allowing the particles-gas stream separation. As soon as the gas reaches the cone, an inner vortex is created in the opposite direction. Thus, the gas is expelled from the cyclone at its top, whereas the particles settle into a collection chamber placed on its bottom [2].

#### *2.3.2. Bag filter*

Filtration based on bags is extensively used in the spray-drying process. Herein, the air streamline containing the dry particles enters the bag filter under pressure or suction by its hopper and is passed through a fabric, which halts the particles path (**Figure 7B**). This means that the dry particles are retained on the bag surface while the clean air passes through it, being expelled from the device. The accumulated particles on the bag surface are then collected due to pulses of compressed air injected in counter-current flow inside the bags [2, 18]. Bag filters present high operation efficiency, especially when they are arranged in filtering units with decreasing fabric pores diameter [1].

#### *2.3.3. Electrostatic precipitator*

Electrostatic precipitation is a method of particle collection whose principle is based on electrostatic forces. A high voltage is applied to discharged wires, forming an electric field between them and the collecting plates that constitute the precipitator (**Figure 7C**). As a result, the gas around these wires is ionized, being capable of charging the particle content of the drying air flowing around this area. Due to Columbic forces, the charged particles converge to the collecting plates and thus the air becomes devoid of dust [2]. Electrostatic precipitators are also very efficient but seldom used taking into account the high equipment costs [1].

#### *2.3.4. Wet scrubbers*

In a spray-drying process, it is usual to find some particles escaping in the air stream after the dry collection. Owing to this, it is quite common to install an additional collecting system after the dry collectors, the wet scrubbers. These devices are economical and effective alternatives which perform a final gas cleaning step, being thus capable of minimizing the particle content or even some odor intensity before releasing the gas streamline to the atmosphere [2, 18].

Venturi wet scrubbers (**Figure 7D**) are well studied devices of easy cleaning or maintenance, being therefore one of the most used equipment in the spray-drying process. They present a converging section, a throat (narrowest part) and a divergent section (diffuser). The inlet air carrying fine particles enters the scrubber and is mixed up with the scrubbing liquid (usually water). This mixture flows throughout the converging section, reaching the throat at high velocity. As a result, a spray of droplets is formed with the dust particles entrapped inside them (scrubbing liquid could also be injected at the throat level). Lastly, the fluid content is separated from the clean gas, being the former discharged and the latter released into the atmosphere [16, 18].

## 3. Process parameters

As it could be noted above, the final properties of the dried products are directly influenced by a set of equipment parameters, such as the atomization devices, the drying chamber configuration and the collector type choice. Additionally, a variety of feedstock specificities and process parameters also play a crucial role in the final particle characteristics, conferring different morphologies, sizes or residual moisture amounts. It is thus fundamental to realize how these variables influence the spray-drying mechanism in order to achieve an optimized operation [2].

### 3.1. Atomization pressure

Atomization stage is carried under pressure, namely when nozzle atomizers are used. The pressure involved during this process has an impact on droplet size. For a given atomizer device and feed solution, droplet size decreases with increasing pressure, as expressed in the following mathematical correlation (Eq. (8)) [1, 2]:

$$\frac{D_f}{D_i} = \left(\frac{P_f}{P_i}\right)^{-0.3} \tag{8}$$

$D_i$ and $D_f$ are the initial and final droplet sizes when the atomization pressure changed from $P_i$ to $P_f$, respectively.

In the particular case of rotary atomizers, droplet size exhibits an inverse relationship with wheel rotation speed and wheel diameter [2].

### 3.2. Feed flow rate

Feedstock solution is pumped into the atomizer at a controllable rate. Keeping the atomization pressure constant, there is an increase in the droplet size with increasing feed flow rates. This is easily understandable bearing in mind that the nozzle would have the same energy amount to spend in the atomization process of higher feeding volumes. Thus, the droplet fissions are minimized, provoking a small reduction of its size [2].

### 3.3. Feed viscosity

When feed viscosity is increased, a great percentage of atomization energy supplied to the nozzle is used to overcome the large viscous forces of the solution. Hence, a small amount of energy is left for the droplet fission, resulting in larger droplet sizes. This mechanism follows (Eq. (9)).

$$\frac{D_f}{D_i} = \left(\frac{\mu_f}{\mu_i}\right)^{0.2} \quad (9)$$

$D_i$ and $D_f$ are the initial and final droplet sizes when the solution viscosity changed from $\mu_i$ to $\mu_f$, respectively. Feed density also follows this principle [2, 7].

### 3.4. Feed surface tension

As stated above, atomization occurs due to the disruption of the feed surface tension. This means that a feedstock solution with higher surface tension hinders the atomization process. In that sense, before starting the spray-drying process, feedstocks are usually emulsified and homogenized in order to reduce their surface tension [2].

### 3.5. Inlet temperature

The inlet temperature refers to the heated drying gas temperature, measured right before its entry into the drying chamber. The thermal charge of inlet drying gas reflects its capacity to dry the humid atomized droplets and, thereby, higher inlet temperatures enable higher solvent evaporation rates. Nevertheless, the inlet temperature should not just be increased to achieve better drying performances because it also has an impact in the wet-bulb temperature of the surrounding air. In fact, lower inlet temperatures lead to lower surrounding air wet-bulb temperature, preventing therefore thermal degradation of the final product. Hence, a wise choice of inlet temperature, balanced on these factors, should be done according the feedstock properties [1, 2].

### 3.6. Drying gas flow rate

Drying gas flow rate is the volume of drying gas which is supplied to the drying chamber per unit time. High gas flow rates will increase particle movements inside the chamber, minimizing air-droplet interaction time. Besides, it is also reported that the higher the drying gas flow rate, the greater efficiency will be obtained during cyclone separation. This means that the drying gas flow rate should be low enough to ensure a complete particle moisture removal, but on the other hand, it should be suitable for the subsequent separation procedure [1].

### 3.7. Outlet temperature

Outlet temperature is the temperature of the air containing the dried particles just before such content to be piped into the collection devices. Theoretically, the outlet temperature is the highest temperature to which the dried powder can be heated, although in the counter-current dryers the final product may present a higher temperature than the outlet air (**Figure 4B**) [1, 2].

Outlet temperature results from all heat and mass exchanges inside the drying chamber, and thus is not directly regulated by the operator. However, this is a function of parameters like the inlet temperature, the drying gas flow rate, as well as the feed properties (solvent evaporation enthalpy and droplet solid concentration) [1].

### 3.8. Residence time inside drying chamber

Residence time refers to the exposition period of the atomized droplets inside the drying chamber, being another important factor with a direct influence on the final product quality. Residence time should be long enough to guarantee that the main goal of the drying stage is accomplished, that is, to obtain dried particles. On the other hand, it is fundamental to keep the product characteristics and when the dried particles are subjected to longer residence times, thermal degradation may occur, especially upon heat-sensitive materials. It is hard to experimentally predict the minimum residence time to be used, although it could be approximately calculated using the (Eq. (6)), defined above. Notwithstanding, it should be remarked that the residence time is usually in the order of a few seconds (e.g. in general, fine particles should not stay more than 10–15 s inside the drying chamber) [2, 19].

### 3.9. Glass transition temperature ($T_g$)

Glass transition temperature is an important thermophysical property of amorphous polymers. Above $T_g$, the material changes from a rigid glassy state to a more rubbery state. Hence, this could be related somehow with the material stickiness on the drying chamber, being therefore an obstacle to the spray-drying process. Product agglomeration problems are, for example, one of the major undesirable issues. The $T_g$ of a feed solution is dependent on its solute constituents. (Eq. (10)), the Gordon-Taylor equation, expresses the $T_g$ of a given feed solution consisting of more than one solute [2, 20].

$$T_g = \frac{w_1 \cdot T_{g1} + k \cdot w_2 \cdot T_{g2}}{w_1 + k \cdot w_2} \tag{10}$$

$w_1$ and $T_{g1}$ are the weight fraction and the glass transition temperature, respectively, of the blend component with the lower $T_g$. $w_2$ and $T_{g2}$ are the weight fraction and the glass transition temperature, respectively, of the blend component with the higher $T_g$. $k$ is the ratio of specific heat change of component 1 to component 2 at the glass transition temperature.

Summing up, the importance of processing parameters on the spray-drying efficiency is clear. Therefore, the advantages and drawbacks of each parameter should be weighed in order to produce products with desirable characteristics. The trade-off between some of the spray-drying parameters is summarized in **Table 2**.

Throughout this review, the importance of the spray-drying technique is evidenced. It enables the production of particles with high yield and made up of several raw materials on an industrial scale, thus proving to be a cost-effective process. Spray drying is preferred over conventional particle production approaches, such as emulsion/solvent evaporation method, due to its unique properties: rapid, continuous and single-step method that displays great versatility and reproducibility [22]. Additionally, spray drying does not require a final drying step, as is the case of the majority of conventional methods, and allows to deal with heat-sensitive materials. Considering these ideas, the use of the spray drying in the production of several drug particles or polymeric carriers with well-defined particle size and good flowability (in opposition to the conventional methods) comes as no surprise. Some examples are detailed below [22].

However, spray drying presents some challenges as it was being explained in the previous sections. It is worth highlighting issues like product loss associated to particle deposition

| Increasing these variables | | Outlet temperature | Particle size | Final product moisture | Efficiency |
|---|---|---|---|---|---|
| | Drying air flow rate | Lower heat losses of the inlet energy ↑ | --------- | Lower partial pressure of evaporated water ↓ | Better separation in cyclone ↑ |
| | Air humidity | More energy contained in moisture ↑ | --------- | Higher partial pressure of drying air ↑ | More moisture may lead to adherence of the product to chamber walls ↓ |
| | Inlet Temperature | Direct proportion ↑↑ | --------- | Lower relative humidity of air ↓ | Eventually dryer product prevent adhering ↑ |
| | Atomizing air flow | Higher amount of cold air to be heated ↓ | Higher amount of available energy for atomization ↓↓ | --------- | --------- |
| | Feed rate | More solvent to be evaporated ↓↓ | More liquid to disperse ↑ | Higher amount of water leading to its higher partial pressure ↑ | Depends on application ↑↓ |
| | Solid concentration in feed | Less water to be evaporated ↑ | More solid available for particle formation ↑↑ | Less water evaporation, lower partial pressure ↓ | Bigger particles are easier to separate in cyclone ↑ |
| ..... | Organic Solvent (instead of water) | Less energy required for evaporation ↑↑ | Lower surface tension. More available energy to spend on particle fission ↓ | Lack of water in feed leading to very dry product ↓↓ | Lack of hygroscopicity results in easier drying ↑↑ |

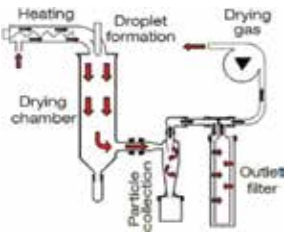
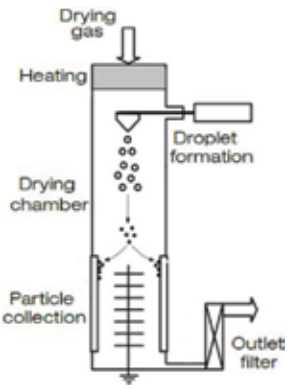
↑ Minor increase ↑↑ Moderate increase ↑↑↑ High increase ↓ Minor decrease ↓↓ Moderate decrease ↓↓↓ High decrease

**Table 2.** Relationships between spray- drying parameters. Adapted from [1, 21].

inside the walls of the drying chamber, as well as due to the inability of the separation devices to collect the smallest particles. As a result the process yield tend to decrease, while in optimal conditions would be close to 100%. Moreover, it is important to note that it is very challenging to obtain very small particles (nanometer scale) by spray drying, not only due to the inefficiency of the collecting devices but also due to the inherent difficulty of disintegrating the feedstock solution (atomization step) into submicron droplets [22].

## 4. Spray dryers

Spray-drying mechanism can be carried out in a pilot-spray dryer developed on a laboratory scale or performed in commercially available instruments. The best spray dryer configuration depends on the purpose for which the instrument is used, that is, the equipment must be

| | **Mini Spray Dryer B-290** | **Nano Spray Dryer B-90 HP** |
|---|---|---|
| Schematic representation of the equipment |  |  |
| Needed sample amount | 30–1000 mL | 1–200 mL |
| Sample viscosity limit | Up to 10 cps | Up to 10 cps |
| Droplet formation (atomization device) | Two-fluid nozzle | Ultrasonic nebulizer |
| Atomization principle | Fine droplets are formed using compressed gas. Nozzle tip diameter: 0.7, 1.4 or 2.0 mm | Piezoelectric actuator with a thin stainless steel membrane which vibrates at ultrasonic frequencies. Membrane micro sized holes: 4.0, 5.5 or 7.0 μm |
| Droplet size distribution | Broader | Narrow |
| Drying chamber temperature (inlet temperature) | Up to 220°C | Up to 120°C |
| Mean residence time | 1.0–1.5 s | 1–4 s |
| Particle collection | Cyclone technology | Electrostatic particle collector |
| Process speed | High | Low |
| Particle diameter range | 1–25 μm | 200 nm–5 μm |
| Yield | Up to 70% | Up to 90% |
| Applications | Pharmaceutical industry, life and material sciences | |
| Special remarks | Already used in more than 700 publications and 400 patents | Newest generation of laboratory scale spray-dryers provided by Büchi |

Adapted from [22, 28–31].

**Table 3.** Major properties of Mini Spray Dryer B-290 and Nano Spray Dryer B-90 HP commercialized by Büchi.

compatible with the feedstock solution and meet the processing conditions, which lead to the desirable particle specificities.

Büchi (Switzerland) have been developing reliable and versatile spray dryers, which have been used for various intents [22–27]. Mini Spray Dryer B-290 and Nano Spray Dryer B-90 HP are two of those instruments and thus, their major characteristics and benefits are organized in **Table 3** [22].

## 5. Spray-drying applications in the biomedical area

Spray drying medical applications are mainly focused on the production of microparticles designed for encapsulation purposes and drug delivery systems, which can be then administered orally, pulmonary, ophthalmologically, parenterally, nasally or even vaginally [22]. The fact that this technological process enables to dry heat-sensitive components, like enzymes or proteins, without compromising their biological activity makes the production of such systems possible [32, 33]. As a result, within the biomedical field, spray drying is primarily used to produce dry powder aerosols and to tune active pharmaceutical compounds, making them useful and suitable for drug delivery [11, 32]. In that sense, different strategies have been used to tailor the sprayed products according to the desired goals.

Regarding the spray-drying approach aiming drug encapsulation and delivery systems, it usually takes advantage of a complex initial system containing the active drug substance and an aqueous/organic phase (solution, emulsion or suspension) to produce either microspheres or microcapsules [11, 32].

The fabrication of biodegradable microspheres filled with an active drug is one of the most common strategies of spray drying. Polyesters gather important requirements, such as good biocompatibility, biodegradability and easiness to process, and thus they are usually chosen for the manufacturing of such products. As a result, it is possible to control the drug release over time as the polymer fraction is gradually degraded toward the physiological environment [32, 34]. There are several studies which have reported the production of a wide variety of polymeric microspheres using spray drying. Exemplificative findings involve the use of co-poly (D,L-lactic/glycolic acid) (PLGA) encapsulating a drug designed to fight solid tumors [34], polylactide (PLA) and poly(lactide-co-glycolide) (PLG) entrapping antigens [35], and a mix of PLGA and poly(ε-caprolactone) (PCL) incorporating a specific chemotherapy agents used in the treatment of ovarian and breast cancer [36].

The incorporation of hydrophilic domains in the spray-dried particles is an alternative way of controlling the drug release behavior. In fact, the hydrophilicity of the polymers used to encapsulate the drug has a direct impact on the behavior it, as a more hydrophilic polymer enables fast gelation, consequently slowing the drug release rate. On the other hand, a low concentration of hydrophilic polymeric coating can enhance the drug release rate of poorly water soluble drugs, since it will improve the wettability of the surrounding fluids. Derivative cellulose polymers such as sodium carboxymethyl cellulose, hydroxypropylmethyl cellulose and methylcellulose are just three examples of hydrophilic polymers which have been purposed for these endings [11, 32, 37].

A singular method focused on the encapsulation of sensitive bioactive compounds (e.g. peptides, proteins) or other drugs can be also achieved through spray-drying technology associated to sol-gel polymerization process. In other words, sol-gel process is carried under soft conditions, and when combined with spray drying can deal with the production of microspheres with sensitive molecules entrapped on them [32, 38]. Spray-dried silica gel microspheres are reported in the literature as a promising system to be administered in the form of a drug injectable [38, 39].

Some literature findings are assembled in the following subsections, which have reported the use of spray drying for different applications within the medical area.

### 5.1. Pulmonary drug delivery

Pulmonary drug delivery route is considered an interesting alternative to the oral drug delivery system, as the lung presents a high surface area covered by a rich blood supply. Thus, a targeted delivery to the lungs enables the use of reduced doses, consequently minimizing the side effects associated with systemic drug administration [24, 40]. Notwithstanding, this strategy implies the use of aerosol drugs, whose production is indeed feasible through the spray-drying technology. As reported in the literature, spray drying has revealed to be a powerful tool in fabricating an inhalable powder suitable for lung delivery, since it allows the production of dried, stable and well-defined solid particles within the "respirable size range for pulmonary delivery" [25, 26, 41, 42].

Adi et al. [43] used spray-drying method to produce dry powders for inhalation-containing doxycycline or ciprofloxacin or even a combination of these two chemicals. The cospray formulation of doxycycline and ciprofloxacin (1:1 ratio) has proved to be more efficient for lung delivery than each one of the single spray-dried antibiotic since it exhibited improved physical stability which favored the drug deposition profile.

Beck-Broichsitter et al. [24] investigated the release profile of phosphodiesterase V (PDE-5) (a drug prescribed in the treatment of severe pulmonary hypertension) encapsulated within spray-dried polymeric particles. In that sense, they produced particles from organic PLGA solutions and also composite particles obtained from aqueous PLGA nanosuspensions. They concluded that both spray-dried products were aerodynamically suitable for deep lung deposition but the particles produced from the organic solution are preferred over the composite ones to work as pulmonary drug delivery vehicles as they exhibited an extended drug releasing profile.

Grenha et al. [44] developed microencapsulated protein (insulin)-loaded chitosan/tripolyphosphate nanoparticles by spray drying. In particular, by taking advantage of the ionotropic gelation of chitosan with the tripolyphosphate, they prepared the nanoparticles and then incorporated the protein content within such particles using aerosol excipients (mannitol and lactose). As a result, suitable microspheres for lung protein delivery were produced. In fact, the microspheres presented a good protein loading capacity, being released in a matter of a few minutes toward the lung environment. Nevertheless, the authors realized that the mannitol/protein ratio impacts the microspheres morphology, namely spherical shapes are obtained in the presence of higher amounts of nanoparticles.

### 5.2. Nasal drug delivery

Similarly to the pulmonary drug delivery, nasal drug delivery present some remarkable advantages when compared to drug delivery using the central nervous system, standing out the rapid onset action. Nasal surface presents a large surface area with rich porous vascularized epithelium, favoring the drug adsorption. Dry powder-based mucoadhesive products are ideal for nasal administration and thus, spray-drying technique has been used for such purposes [26, 45, 46].

Tadwee et al. [46] prepared, by spray drying, hydoxypropyl methylcellulose microspheres loading carbamazepine, an antiepileptic drug which is used in epileptic seizures. Along this study, they found in this system a promising strategy to allow the drug absorption by the mucosal membrane. This is of extreme importance considering that carbamazepine has a poor aqueous solubility which hinders its use by oral administration.

Alhalaweh et al. [45] developed zolmitriptan-chitosan microparticles by spray drying. Zolmitriptan is a drug prescribed to fight the migraine symptoms (pain, nausea, photophobia and phonophobia). In the present days, it already exist a tablet and a nasal spray (based on zolmitriptan drug) solutions used to treat such health condition. Nevertheless, in both alternatives, the absolute bioavailability of this drug does not exceed 40% [47]. In that sense, Alhalaweh et al. intended to use chitosan in the preparation of a zolmitriptan powder, considering that the mucoadhesive property of chitosan may have an important impact on increasing the bioavailability of the drug in the nose. Some important notes can be drawn from this study, such as: spray drying allowed the production of chitosan spherical particles entrapping good amounts of zolmitriptan; zolmitriptan dispersion around the chitosan matrix is dependent on the chitosan quantity; zolmitriptan release is also affected by proportion and molecular weight of chitosan.

Recently, Zadeh et al. [48] used spray drying to produce insulin loaded microspheres for intranasal delivery application, which is indeed an alternative route of insulin administration that has been tested in the treatment of diabetic mellitus. For that purpose, Zadeh et al. used spray drying to produce microspheres of chitosan and polyvinyl alcohol (PVA) working as the carrier vehicle of insulin. As expected, the properties of the microspheres varied upon different material ratios. In what concerns the rate and drug release from the microspheres, it is important to highlight that chitosan microspheres led to an initial fast insulin release, followed by a slower release rate, but with effective insulin absorption, while the microspheres made up of chitosan and PVA allowed rapid insulin release but without absorption effectiveness.

### 5.3. Orthopedic field

Spray-drying mechanism can also be used in the materials production focused in the orthopedic field, as it will be hereafter explained. Aiming bone repair purposes, Quinlan et al. [27] proposed the development of a collagen-hydroxyapatite scaffold enhanced with spray-dried alginate particles loading vascular endothelial growth factor (VEGF). In other words, VEGF was primarily encapsulated in the alginate particles which were then incorporated within the collagen-hydroxyapatite matrix. Upon this biomaterial implantation in a bone damage site, it was registered VEGF release for 35 days. As a result, there was an effective vessel formation with consequent improved bone regeneration outcomes, which did not happen at all in the presence of a scaffold without the growth factor.

Sequeira et al. [49] pointed the use of two inorganic oxides, zirconia and alumina, as potential candidates to be used in the orthopedic implantology field. A biocompatible material for such purpose may be obtained combining these two materials, since the stiffness and long-term stability of alumina is merged to the chemical stability and mechanical strength of zirconia. Herein, different zirconia-toughened alumina and alumina-toughened zirconia composite granules were produced by spray drying. Both composites exhibited good mechanical properties as well as allowed osteoblastic cytocompatibility.

Ceramic powders are of major importance in bone tissue engineering. Innovative approaches like 3-D printing makes use of calcium phosphate ceramic powders to shape complex and precise products aiming the regeneration of bone defects. Nevertheless, conventional powder fabrication procedures are far from being ideal for the subsequent 3-D printing usage, as they lack flowability and dispersity which cause agglomeration and irregular morphology issues [50]. Following along with this idea, spray drying has proved to be a credible method to overcome those problems, and thus being suitable in the production of such powders. Ben et al. [50] used spray drying to fabricate monodispersed and spherical β-tricalcium phosphate (β-TCP) powders to be used in 3-D printing. They obtained ceramic materials with good dispersity and flowability, satisfying a high density and uniform porous architecture, meeting therefore important requirements for bone regeneration.

Cholas et al. [51] embedded spray-dried hydroxyapatite (HA) microspheres within a collagen matrix. HA microspheres presented a mesoporous structure which in combination with the collagen matrix formed a good composite scaffold for human bone tissue engineering. Moreover, the authors realized that promising outcomes may be obtained using a simple modification of this system. These HA microspheres could be loaded for drug delivery purposes or even to control the pore structure of the ceramic particle.

## 6. Spray drying patents in the biomedical area

Due to the increasing interest of spray-drying technology as well as the numerous applications that spray-dried products can hit, specially within the medical field, several patents were issued until these days. **Table 4** compiles some of those patents of the past 10 years.

| Title and number of the patent | Technical field | Brief description/main goal | Publication year | Ref. |
|---|---|---|---|---|
| Thermostable spray-dried rotavirus vaccine formulation and process thereof (US20170173145) | Vaccine development (pharmaceutical) | Provide an enhanced spray-drying process to obtain said rotavirus vaccine formulation. In particular, it was obtained a vaccine with improved heat-stability, ease-of-use, ease-of-transportation and affordability features | 2017 | [52] |

| Title and number of the patent | Technical field | Brief description/main goal | Publication year | Ref. |
|---|---|---|---|---|
| Inhalable epinephrine (US20170119699) | Inhalable drug delivery | Development of particles for delivery of epinephrine to the respiratory system. It was intended to spray-dry particles exhibiting aerodynamic characteristics that enable targeted delivery of epinephrine to the site of action | 2017 | [53] |
| Pharmaceutical composition with improved bioavailability (US20170000764) | Solubility and bioavailability enhancement | It was intended to use spray drying to get improved bioavailability (good solubility/dissolution rate), safety and tolerability of a compound for therapeutic endings | 2017 | [54] |
| Method for improving the pharmaceutical properties of microparticles comprising diketopiperazine and active agent (US20160101049) | Pharmaceutical formulations for pulmonary delivery | Spray drying was used to fabricate diketopiperazine-insulin particles, improving the aerodynamic performance, active agent stability and efficiency delivery, when compared to diketopiperazine-insulin particles obtained by lyophilization | 2016 | [55] |
| Spray drying vancomycin (US20150231197) | Injectable antibiotic (pharmaceutical) | As an alternative to lyophilization, spray-dried vancomycin (an injectable antibiotic to fight bacterial infections in the body) was proposed. Spray-dried antibiotic demonstrated favorable reconstitution times and water content | 2015 | [56] |
| Spray drying of high molecular weight hyaluronic acid (US20140155347) | Spray drying of polysaccharides | Hyaluronic acid is commonly used in several medical applications due to its single physical and biological properties. This invention allows a minimal molecular weight loss of the spray-dried polysaccharide | 2014 | [57] |
| Liposomal formulations of lipophilic compounds (US20130259922) | Liposomal preparation for drug delivery | Liposomes have been used in the pharmaceutical industry for drug delivery purposes, being usually administrated by injection. Long-term stability and preparation procedures of liposomes involve dehydration and rehydration steps, which can be accomplished by spray drying | 2013 | [58] |
| Adhesive containing microparticles (WO2012158483) | Medical devices for skin delivery | A liquid containing active agents was spray dried, resulting in microparticles which can be subsequently incorporated in an adhesive | 2012 | [59] |

| Title and number of the patent | Technical field | Brief description/main goal | Publication year | Ref. |
|---|---|---|---|---|
| Nanoparticle carriers for drug administration and process for producing same (US20110033550) | Nanoparticle carriers for oral administration | Herein, a double emulsion of water-oil-water emulsion was spray dried to form spherical nanometric particles loaded with a specific drug. It is worth mentioning that the nanoparticle carrier itself is made up of polymeric material, the drug is delivered into one of the emulsion phases and the oil-phase or the outer-water phase was doped with a carbohydrate | 2011 | [60] |
| Method of producing porous microparticles (US20100092453) | Production of porous microparticles | The production of porous particles is feasible using spray drying under specific conditions. In particular, combining desirable organic compounds with a volatile solvent system. | 2010 | [61] |
| Pulmonary delivery of polyene antifungal agents (US20090081302) | Spray-dried polyene compositions | Polyenes (e.g. amphotericin) are efficient antifungal compounds, but lack solubility, either in water or organic solvents. In that sense, the current invention deals with complex issues to get a chemical stable and dispersible powder polyene antibiotic. Nevertheless, the obtained particles are good candidates to be administered by inhalation to the lung to fight pulmonary fungal infections | 2009 | [62] |
| Inhalable powders comprising protein, phenylalanine, and other protein stabilizers (US20080089849) | Production of phenylalanine-containing powders | This invention deals with the production of spray-dried powders containing a phenylalanine fraction and other active substance to serve as an inhalative pharmaceutical composition. The resulting powders revealed good aerodynamic characteristics and stabilization of the extra active agent | 2008 | [63] |

Adapted from [52–63].

**Table 4.** Some patents about spray-drying technology in the past 10 years.

## 7. Conclusions

Spray drying is considered a powerful technological process since it brings feasibility to the production of free-flowing particles with well-defined particle size. This is indeed a cost-effective

manufacturing process capable of producing dried particles in submicron-to-micron range [2, 3]. Moreover, the ability to use different feedstocks and the high productivity and broad appli cations of this technique makes it more and more attractive to the scientific community [2, 3]. It was noticed that the spray-drying conditions form a network of mutual relationships which have a direct influence on the spray-drying efficiency. The advantages and drawbacks of each parameter should be weighed in order to produce products with desirable characteristics. Such parameters should not be analyzed separately, but rather looked as a complex model, which as a whole contributes to the success of the spray-drying process [2, 64]. The scalability and the cost-effectiveness of this manufacturing process in obtaining dried particles in submicron-to-micron scale favors an increasing variety of applications within the food, chemical, polymeric, pharmaceutical, biotechnology and medical industries.

## Author details

Daniel Santos[1], Ana Colette Maurício[2,3]*, Vitor Sencadas[4,5], José Domingos Santos[6], Maria H. Fernandes[7] and Pedro S. Gomes[7]

*Address all correspondence to: ana.colette@hotmail.com

1 Department of Metallurgical and Materials Engineering, Faculty of Engineering of the University of Porto (FEUP), Porto, Portugal

2 Animal Science and Study Centre (CECA), Institute of Sciences, Technologies and Agroenvironment of the University of Porto (ICETA), Porto, Portugal

3 Department of Veterinary Clinics, Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto, Porto, Portugal

4 School of Mechanical, Materials, Mechatronics and Biomedical Engineering, University of Wollongong, Wollongong, NSW, Australia

5 ARC Center of Excellence for Electromaterials Science, University of Wollongong, NSW, Australia

6 REQUINTE/LAQV, Department of Metallurgical and Materials Engineering, Faculty of Engineering of the University of Porto (FEUP), Porto, Portugal

7 Faculty of Dental Medicine of University of Porto (FMDUP), Porto, Portugal

## References

[1] Cal K, Sollohub K. Spray drying technique. I: Hardware and process parameters. Journal of Pharmaceutical Sciences. 2010;**99**(2):575-586

[2] Anandharamakrishnan C. Spray Drying Techniques for Food Ingredient Encapsulation. John Wiley & Sons; 2015

[3] Ortega-Rivas E, Juliano P, Yan H. Food Powders: Physical Properties, Processing, and Functionality. Springer Science & Business Media; 2006

[4] Patel R, Patel M, Suthar A. Spray drying technology: An overview. Indian Journal of Science and Technology. 2009;**2**(10):44-47

[5] Vehring R, Foss WR, Lechuga-Ballesteros D. Particle formation in spray drying. Journal of Aerosol Science. 2007;**38**(7):728-746

[6] Nandiyanto ABD, Okuyama K. Progress in developing spray-drying methods for the production of controlled morphology particles: From the nanometer to submicrometer size ranges. Advanced Powder Technology. 2011;**22**(1):1-19

[7] Anandharamakrishnan C. Handbook of Drying for Dairy Products. John Wiley & Sons; 2017

[8] Gharsallaoui A, Roudaut G, Chambin O, Voilley A, Saurel R. Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Research International. 2007;**40**(9):1107-1121

[9] GlobalSpec I. Spray Guns and Applicators Information [Internet]. 2017. Available from: http://www.globalspec.com/learnmore/manufacturing_process_equipment/surface_coating_protection/coating_paint_spray_guns. [Accessed: 2017-08-16]

[10] IndiaMART. Atomizer Disc [Internet]. 1996-2017. Available from: https://www.indiamart.com/divyaengineering-works/atomizer-disc.html. [Accessed: 2017-08-05]

[11] Jain Manu S, Lohare Ganesh B, Chavan Randhir B, Barhate Shashikant D, Shah CB. Spray drying in pharmaceutical industry: A review. Research Journal of Pharmaceutical Dosage Forms and Technology. 2012;**4**:74-79

[12] Handscomb C, Kraft M, Bayly A. A new model for the drying of droplets containing suspended solids. Chemical Engineering Science. 2009;**64**(4):628-637

[13] Huang D, editor Modeling of particle formation during spray drying. European Drying Conference, October 26-28, Palma Balearic Island, Spain; 2011

[14] NPTEL. General Design Consideration of Cyclone Separators [Internet]. 2014. Available from: http://nptel.ac.in/courses/103103027/module5/lec1/2.html. [Accessed: 2017-09-02]

[15] SpA RG. Bag Filters [Internet]. 2014-2016. Available from: http://www.redecam.com/bag-filters/. [Accessed: 2017-08-20]

[16] VITO. Venturi Scrubber [Internet]. 2015. Available from: https://emis.vito.be/en/techniekfiche/venturi-scrubber. [Accessed: 2017-08-10]

[17] © Hitachi L. Principles of Electrostatic Precipitator and Factors Affecting Performance [Internet]. 2014. Available from: http://www.hitachi-infra.com.sg/services/energy/dust-collection/principle/dustcollection.html. [Accessed: 2017-08-16]

[18] Mujumdar AS. Handbook of Industrial Drying. CRC Press; 2014

[19] Schmitz-Schug I, Foerst P, Kulozik U. Impact of the spray drying conditions and residence time distribution on lysine loss in spray dried infant formula. Dairy Science & Technology. 2013;**93**(4-5):443-462

[20] Kulshreshtha AK. Handbook of Polymer Blends and Composites. iSmithers Rapra Publishing; 2002

[21] AG BL. Training Papers Spray Drying [Internet]. 1997-2002. Available from: http://static1.buchi.com/sites/default/files/downloads/Set_3_Training_Papers_Spray_Drying_en_01.pdf?996b2db24007502bd69c913b675467cfc63880ba. [Accessed: 2017-08-16]

[22] Sosnik A, Seremeta KP. Advantages and challenges of the spray-drying technology for the production of pure drug particles and drug-loaded polymeric carriers. Advances in Colloid and Interface Science. 2015;**223**:40-54

[23] Lee SH, Heng D, Ng WK, Chan H-K, Tan RB. Nano spray drying: A novel method for preparing protein nanoparticles for protein therapy. International Journal of Pharmaceutics. 2011;**403**(1):192-200

[24] Beck-Broichsitter M, Schweiger C, Schmehl T, Gessler T, Seeger W, Kissel T.Characterization of novel spray-dried polymeric particles for controlled pulmonary drug delivery. Journal of Controlled Release. 2012;**158**(2):329-335

[25] Wu X, Hayes Jr D, Zwischenberger JB, Kuhn RJ, Mansour HM. Design and physicochemical characterization of advanced spray-dried tacrolimus multifunctional particles for inhalation. Drug Design, Development and Therapy. 2013;**7**:59

[26] B Patel B, K Patel J, Chakraborty S. Review of patents and application of spray drying in pharmaceutical, food and flavor industry. Recent Patents on Drug Delivery & Formulation. 2014;**8**(1):63-78

[27] Quinlan E, López-Noriega A, Thompson EM, Hibbitts A, Cryan SA, O'Brien FJ. Controlled release of vascular endothelial growth factor from spray-dried alginate microparticles in collagen–hydroxyapatite scaffolds for promoting vascularization and bone repair. Journal of Tissue Engineering and Regenerative Medicine. 2017;**11**(4):1097-1109

[28] Büchi. Mini Spray Dryer B-290—Technical Data Sheet [Internet]. 2017. Available from: https://www.buchi.com/sites/default/files/downloads/B-290_Data_Sheet_en_D.pdf?83b925aae302a8e76f002d1ce679fa06904d1039. [Accessed: 2017-11-02]

[29] Büchi. Nano Spray Dryer B-90 HP—Technical data sheet [Internet]. 2017. Available from: https://www.buchi.com/sites/default/files/technical-data-pdf/B-90_Data_Sheet_en.pdf?507a2adc7486d8ee2dfa592c9638749918452ed3. [Accessed: 2017-11-02]

[30] Büchi. Mini Spray Dryer B-290 [Internet]. 2017. Available from: https://www.buchi.com/en/products/spray-drying-and-encapsulation/mini-spray-dryer-b-290. [Accessed: 2017-11-02]

[31] Büchi. Nano Spray Dryer B-90 HP [Internet]. 2017. Available from: https://www.buchi.com/en/products/spray-drying-and-encapsulation/nano-spray-dryer-b-90-hp

[32] Ré M-I. Formulating drug delivery systems by spray drying. Drying Technology. 2006; **24**(4):433-446

[33] Sollohub K, Cal K. Spray drying technique: II. Current applications in pharmaceutical technology. Journal of Pharmaceutical Sciences. 2010;**99**(2):587-597

[34] Fu Y-J, Shyu S-S, Su F-H, Yu P-C. Development of biodegradable co-poly (D, L-lactic/glycolic acid) microspheres for the controlled release of 5-FU by the spray drying method. Colloids and Surfaces B: Biointerfaces. 2002;**25**(4):269-279

[35] Bt B, Benoit M-A, Poulain-Godefroy O, Schacht A-M, Capron A, Gillard J, et al. Vaccine properties of antigens entrapped in microparticles produced by spray-drying technique and using various polyester polymers. Vaccine. 2000;**18**(15):1495-1505

[36] Lopez-Gasco P, Iglesias I, Benedi J, Lozano R, Teijón J, Blanco M. Paclitaxel-loaded polyester nanoparticles prepared by spray-drying technology: In vitro bioactivity evaluation. Journal of Microencapsulation. 2011;**28**(5):417-429

[37] Wan LS, Heng PW, Chia CG. Spray drying as a process for microencapsulation and the effect of different coating polymers. Drug Development and Industrial Pharmacy. 1992;**18**(9):997-1011

[38] Kortesuo P, Ahola M, Kangas M, Jokinen M, Leino T, Vuorilehto L, et al. Effect of synthesis parameters of the sol–gel-processed spray-dried silica gel microparticles on the release rate of dexmedetomidine. Biomaterials. 2002;**23**(13):2795-2801

[39] Kortesuo P, Ahola M, Kangas M, Kangasniemi I, Yli-Urpo A, Kiesvaara J. In vitro evaluation of sol–gel processed spray dried silica gel microspheres as carrier in controlled drug delivery. International Journal of Pharmaceutics. 2000;**200**(2):223-229

[40] Sung JC, Pulliam BL, Edwards DA. Nanoparticles for drug delivery to the lungs. Trends in Biotechnology. 2007;**25**(12):563-570

[41] Muralidharan P, Malapit M, Mallory E, Hayes D, Mansour HM. Inhalable nanoparticulate powders for respiratory delivery. Nanomedicine: Nanotechnology, Biology and Medicine. 2015;**11**(5):1189-1199

[42] Alves GP, Santana MHA. Phospholipid dry powders produced by spray drying processing: Structural, thermodynamic and physical properties. Powder Technology. 2004; **145**(2):139-148

[43] Adi H, Young PM, Chan HK, Stewart P, Agus H, Traini D. Cospray dried antibiotics for dry powder lung delivery. Journal of Pharmaceutical Sciences. 2008;**97**(8):3356-3366

[44] Grenha A, Seijo B, Remunán-López C. Microencapsulated chitosan nanoparticles for lung protein delivery. European Journal of Pharmaceutical Sciences. 2005;**25**(4):427-437

[45] Alhalaweh A, Andersson S, Velaga SP. Preparation of zolmitriptan–chitosan microparticles by spray drying for nasal delivery. European Journal of Pharmaceutical Sciences. 2009;**38**(3):206-214

[46] Tadwee I, Shahi S, Thube M. Spray dried nasal mucoadhesive microspheres of carbamazepine: Preparation and invitro/ex-vivo evaluation. International Journal of Pharmaceutical Sciences and Research. 2011;**2**:23-32

[47] Rapoport AM, Bigal ME, Tepper SJ, Sheftell FD. Zolmitriptan (Zomig®). Expert Review of Neurotherapeutics. 2004;**4**(1):33-41

[48] Zadeh S, Rajabnezhad S, Zandkarimi M, Dahmardeh S, Mir L. Mucoadhesive microspheres of chitosan and polyvinyl alcohol as a carrier for intranasal delivery of insulin: In vitro and in vivo studies. MOJ Bioequivalence & Bioavailability. 2017;**3**(2):00030

[49] Sequeira S, Fernandes M, Neves N, Almeida M. Development and characterization of zirconia–alumina composites for orthopedic implants. Ceramics International. 2017; **43**(1):693-703

[50] Ben Y, Zhang L, Wei S, Zhou T, Li Z, Yang H, et al. PVB modified spherical granules of β-TCP by spray drying for 3D ceramic printing. Journal of Alloys and Compounds. 2017;**721**(Supplement C):312-319

[51] Cholas R, Padmanabhan SK, Gervaso F, Udayan G, Monaco G, Sannino A, et al. Scaffolds for bone regeneration made of hydroxyapatite microspheres in a collagen matrix. Materials Science and Engineering: C. 2016;**63**:499-505

[52] Gill D, Saigal N, Kale S, Sharma T, Shukla N, Sikriwal D, et al. Thermostable spray dried rotavirus vaccine formulation and process thereof. US20170173145; 2017

[53] Batycky RP, Caponetti G, Childs M, Ehrich E, Fu K, Hrkach JS, et al. Inhalable epinephrine. US20170119699; 2017

[54] Lomuscio S, Ma H, Matchett MA, Sandhu HK, Shah NH, Zhang Y-e. Pharmaceutical composition with improved bioavailability. US 20170000764; 2017

[55] Wilson BR, Grant M. Method for improving the pharmaceutic properties of microparticles comprising diketopiperazine and an active agent. US20160101049; 2016

[56] Fragale C, Brueck D. Spray drying vancomycin. US20150231197; 2015

[57] Toemmeraas K, Bach P. Spray drying of high molecular weight hyaluronic acid. US20140155347; 2014

[58] Haas H, Fattler U. Liposomal formulations of lipophilic compounds. US20130259922; 2013

[59] Wibaux AM, Johnson P. Adhesive containing microparticles. WO2012158483; 2012

[60] Kalombo L. Nanoparticle carriers for drug administration and process for producing same. US20110033550; 2011

[61] Healy AM, Mcdonald B, Corrigan OI, Tajber L. Method of Producing Porous Microparticles. US20100092453; 2010

[62] Weikert M, Gordon MS, Kumar S, Yang B, Sarwar R. Pulmonary delivery of polyene antifungal agents. US20090081302; 2009

[63] Schultz-fademrecht T, Garidel P, Fischer B, Bechtold-peters K. Powders for inhalation. US20080089849; 2008

[64] Sadek C, Schuck P, Fallourd Y, Pradeau N, Floch-Fouéré C, Jeantet R. Drying of a single droplet to investigate process–structure–function relationships: A review. Dairy Science & Technology. 2015;**95**(6):771-794

Chapter 3

# Calcification of Biomaterials and Diseased States

Jenna Mosier, Nancy Nguyen, Kadie Parker and
Chartrisa LaShan Simpson

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71594

**Abstract**

Calcification is one of the most common issues that arise concerning biocompatibility, known to affect many systems in the body. It is often associated with an increase in free phosphate and calcium particles in the serum that leads to mineral deposition. Calcification is problematic both in the naturally occurring state of the body, as well as when it exists as result of biomaterial implants. While calcification is prominent in many different forms, not all mechanisms and processes associated with the phenomenon are completely understood. In this chapter, materials affected by calcification, potential mechanisms of action, and potential treatments will be discussed. Both bioprosthetic and polymer heart valves and urinary implants will be evaluated for material composition, application, and failure. Current research on the assessment of these materials will be reported, with the associated chemical and biological mechanisms explained. The chapter will also detail diseased states of the arteries that induce calcification and what treatments can be used for both arterial and bioprosthetic calcification. Finally, the chapter will conclude by detailing future designs for biomaterials to prevent and treat calcification in both natural and synthetic applications.

**Keywords:** aortic valves, biomaterials, bioprosthetic, calcification, tissue engineering, urinary regeneration

## 1. Introduction

Calcification is one of the most common issues that arise concerning biocompatibility, known to affect many systems in the body. It is often associated with an increase in free phosphate and calcium particles in the serum that leads to mineral deposition [1]. Calcification is problematic both in the naturally occurring state of the body, as well as when it exists as result of biomaterial implants [2]. While recent research confirms that it is an active, cell-mediated

process rather than a passive association of age, the various mechanisms of calcification and related factors that are involved with this phenomenon are still not completely understood.

The cardiovascular system is one that is majorly affected by calcification, both naturally and via biosynthetic and bioprosthetic implants. Natural vascular calcification is associated with the stiffening of arterial walls and the deposition of free calcium and phosphate particles in the serum [3]. Vascular calcification is also seen to increase in patients on dialysis, due to serum being stripped of natural inhibitors of mineralization [4]. Valve implants, coronary stents, and balloon angioplasty are all affected by mineralization due to immune response of biomaterials used, calcium affinity, or even elastin/collagen injury post-implantation [5]. The consequence of this calcification is often associated with implant failure and stiffness of tissue. This also occurs in implants in the urinary system due to adhesion of various minerals and cells to the surface of the implant [6]. Implants such as urinary catheters and ureteral stents calcify as a result of interaction of the bacteria and the device.

While calcification is prominent in many different forms, not all mechanisms and processes associated with the phenomenon are completely understood. In this chapter, materials affected by calcification, potential mechanisms of action, and potential treatments will be discussed. Both bioprosthetic and polymer heart valves and urinary implants will be evaluated for material composition, application, and failure. Current research on the assessment of these materials will be reported, with the associated chemical and biological mechanisms explained. The chapter will also detail diseased states of the arteries that induce calcification and what treatments can be used for both arterial and bioprosthetic calcification. Finally, the chapter will conclude by detailing future designs for biomaterials to prevent and treat calcification in both natural and synthetic applications.

## 2. Heart valves

A major contributor to morbidity and mortality worldwide is valvular heart diseases (VHDs). Valvular dysfunction is related to an insufficient opening or closing of the valve caused by either stenosis, regurgitation or both [7]. Stenosis can be described as a stiffening of the leaflets, leading to improper opening and closing of the valves. Regurgitation occurs when blood flows back through the valve indicating inadequate valve closure [8]. Almost 2.5% of the U.S. population is affected by VHDs. With 300,000 surgeries completed annually, heart valve replacements come in second for the most common cardiovascular surgical procedure to treat this issue [7]. There are currently two strategies for this treatment: repair or valve replacement [7].

Valve replacements generally exist in two forms: mechanical heart valves (MHVs) or bioprosthetic (biological) heart valves (BHVs) [9]. There are five categories of biological heart valves: autograft, autologous, homografts, pericardial valves, and porcine xenografts [7]. Autograft heart valves are implanted using the Ross procedure, which replaces the problematic aortic valve with a healthy valve that is already within the patient [10]. To create an autologous heart valve, cells from a patient must be harvested and transplanted onto a scaffold using tissue engineering techniques. The resulting tissue that has formed within the scaffold is then placed back inside the same patient [11]. Homografts that used for valve replacements are typically taken from

organ donors. Grafts obtained from these donors, or sources other than the receiving individual are known as allografts. Pericardial valves are fabricated from bovine pericardium and are fixed onto a stented frame during implantation [12]. Xenografts are any valves transplanted from an animal source, including porcine and bovine pericardial valves [12].

Bioprosthetic valves can also be in one of three forms: stented, stentless, and percutaneous [13]. Mechanical valves are typically created from non-biological materials like polymers, metal, carbon, and various alternatives [9]. Of the two valve replacement types, roughly half of U.S. patients receive bioprosthetic valves. These are usually either porcine xenograft or bovine pericardial valves. Another 43% of patients undergoing heart valve surgery will receive mechanical prosthesis (**Table 1**) [7].

### 2.1. Bioprosthetic vs. polymer valves

When choosing between a bioprosthetic and a mechanical valve, there are some important factors that should be taken into consideration. These include the patient's age, preference, life expectancy, comorbidities, and indication/contraindication for warfarin therapy [13]. MHVs and BHVs not only have different compositions, but also differ in features like thrombogenicity, durability, and hemodynamic properties [15]. MHVs have superb durability but require lifelong anticoagulation therapy because of their increased risk of thromboembolism, thrombotic obstruction, and hemorrhage. In contrast, bioprosthetic valves do not require anticoagulation therapy because they are less thromboembolic; however, due to calcific

| **Bioprosthetic heart valves** | **Material** | **Purpose** | **Implantation methods** | ***In vivo* response** |
|---|---|---|---|---|
| Stented | Porcine valve leaflets and bovine pericardium fabricated into pericardial valves are both mounted onto a polymer or metallic supporting stent | Unlike mechanical valves, stented valves are not susceptible to thrombo-embolic effects | Requires open heart surgery | These biological valves do not present the patient with thrombo-embolic problems but they do lead to calcification and tissue hardening due to immune response |
| Stentless | Made from bovine pericardium or porcine aortic valves | Used to improve hemodynamics and durability of the valves | Requires open heart surgery | These present the same problems *in vivo* as stented valves but have been shown to have a 10% larger effective valve area compared to stented valves |
| Percutaneous | Biologic porcine or bovine pericardium is affixed to a supporting stent or cage | A less invasive surgery for valve replacement in patients with high operative risks | Implanted into the body by a percutaneous transfemoral method | Presents same problems as stented and stentless but is a very novel technique and needs further investigating |

**Table 1.** Table summarizing the differences between stented, stentless and percutaneous bioprosthetic heart valves [12–14].

tissue degradation, their durability is finite [13]. MHVs and BHVs last around 20–30 years and 10–15 years, respectively. Biological valves are used more often than mechanical valves because of their ease of implantation, safety, functionality, and the fact that they do not require anticoagulant therapy [7, 15].

Most BHVs used are fabricated from porcine heart valves or from bovine pericardium. While bioprosthetic valves are competent, they are still lacking in that they have significant structural deterioration due to calcification [16]. Younger age, renal insufficiency, mitral valve position, and hyperparathyroidism are all predictive factors thought to be associated with structural valve deterioration (SVD). Patient age is a major factor of SVD in bioprostheses. Implant failure ten years after application occurs in less than 10% of elderly patients, while reaching 20–30% in patients less that forty years old [13].

Other factors contributing to calcification are the pre-implantation techniques used on bioprosthetic valves. For example, prior to implantation, most bovine or porcine valves are decellularized which make them less antigenic; however, this process removes all the endothelial cells present. Therefore, adjoining tissue and/or circulating cells cannot then be reseeded after decellularization occurs [7, 17]. Along with decellularization, stabilization of the extracellular matrix (ECM) components, and masking of xenogeneic epitopes are important. For this reason, all animal pericardium must be treated with specific crosslinking agents such as glutaraldehyde prior to implantation. However, glutaraldehyde stimulates many destructive effects such as structural damage, cytotoxicity, and calcific deterioration [18]. Because of problems associated with prosthetic valves, approximately 60% of all patients receiving heart valve replacements will need to have a revision surgery [7]. Also, all studies thus far have neither confirmed nor rejected the use of pericardial valves over porcine valves or *vice versa* (**Figure 1**) [13].

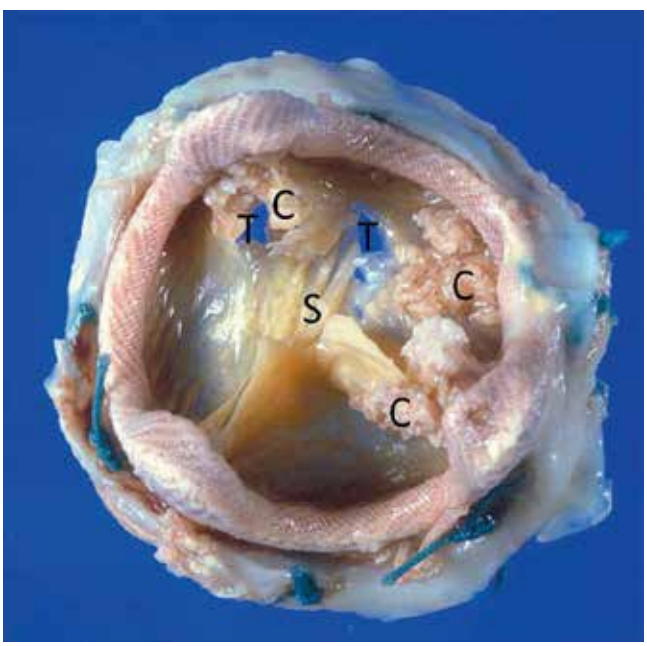



**Figure 1.** Image of a porcine bioprosthetic heart valve. (C) is showing calcification, (T) is showing cusp tears, and (S) is showing stenosis of the valve [16].

### *2.1.1. Mechanism*

#### *2.1.1.1. Modes and mechanisms of valve failure*

The specific mechanisms leading to VHD are not fully known, meaning it is unclear how important genetics, cellular characteristics, and microenvironmental characteristics are in this disease. However, in light of recent evidence, it is believed that alterations in developmental morphogenesis signaling pathways could play a role in VHD [8]. One affected pathway is that of Notch1. The Notch1 pathway is engaged in numerous cell-to-cell communication processes. With this pathway being an intercellular signaling mechanism, it is believed that the loss of Notch1 results in deformation of leaflet morphology throughout embryo development and the inability to suppress calcification during adulthood [19].

One known major cause of failure in bioprosthetic heart valves is calcification [17]. The exact mechanism of tissue degeneration leading to calcification is not fully known. However, IgM/IgG antibodies entering the valve matrix initiate the process. This then leads to deposition of macrophages on the valve surface which is followed by collagen breakdown and calcification [15]. These macrophages are critical factors in the innate immune response. Macrophages are in charge of inducing phagocytosis and killing bacteria. When these macrophages become overwhelmed, they induce an inflammatory response [20]. This inflammatory response causes an increase in inflammatory cytokines that cause calcification [13]. For this reason, the immune system is thought to be a key factor in the initiation of calcification (**Figure 2**) [15].

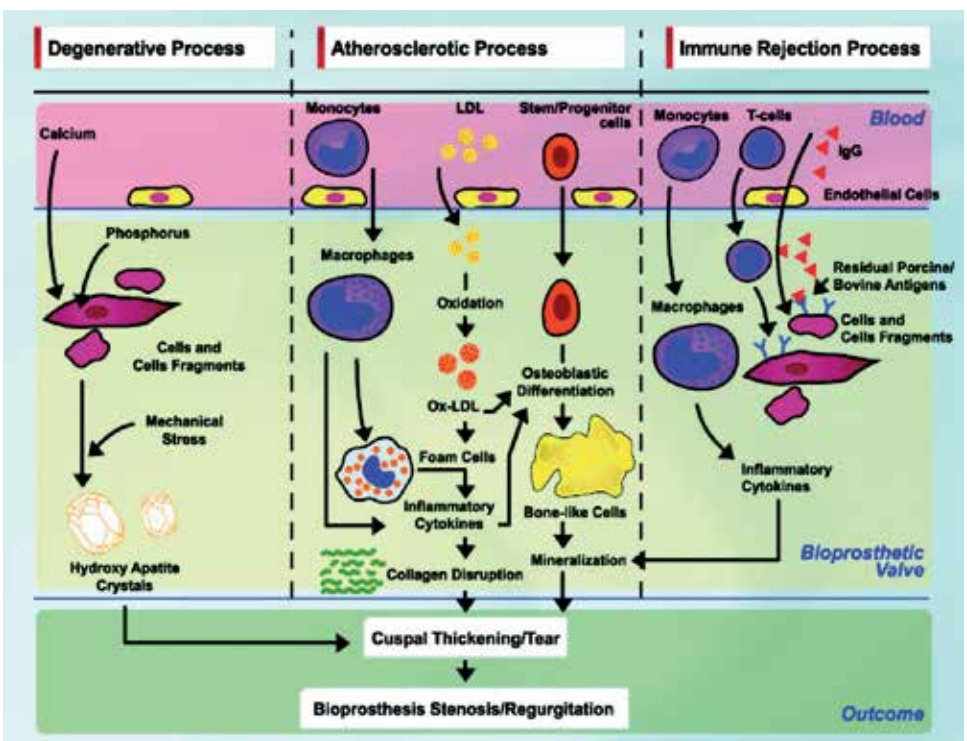



**Figure 2.** This is a theoretical model showing the degenerative, atherosclerotic, and immune rejection processes involved in the structural degradation of bioprosthetic heart valves [13].

To further determine the reason for calcification involved with BHVs, their composition must be examined. BHVs are fixed in glutaraldehyde to reduce immunogenicity and ameliorate the mechanical strength of the heart valve; however, this fixation reduces antigen presentation and chemical stabilization by concealing antigens and eventually leading to an influx in calcium [9]. Consequently, glycoproteins and other substances are lost during glutaraldehyde fixation which allows for the formation of a calcium phosphate precipitate that would not occur under normal cardiac conditions [17]. This glutaraldehyde fixation is also thought to cause chemical interactions between aldehyde groups, phospholipids and circulating calcium ions which can also cause calcification in bioprosthetic valves [15].

Surface heparin has been used as a preventative method for dealing with tissue calcification in heart valve replacements. This heparin treatment is meant to replace glutaraldehyde fixation. In one study, it was discovered that porcine aortic valves that were pretreated with surface heparin showed a decrease in the accumulation of calcium in valve tissue [17]. While the exact mechanisms of heparin are unclear, it is thought that the heparin molecules block calcium phospholipid-binding sites that glutaraldehyde fixation targets. Ingrowth and antiproliferative effects are also characteristics of heparin which may potentially influence small muscle cell growth during implantation which would indirectly inhibit calcification [17].

In addition to glutaraldehyde fixation causing calcium influx and tissue degradation deterioration, recent studies have suggested that SVD is also due to active mechanisms such as atherosclerosis and immune rejection. This immune rejection could be due to bioprosthetic valves not being "immunologically inert" [13]. This results in humoral and cellular immune responses that lead to tissue disruption and/or mineralization. This would explain why younger patients with a more vigorous immune system might experience faster SVD.

Bioprosthetic SVD might also be due to atherosclerotic processes from associated risk factors [13]. The oxidation and infiltration of low-density lipoproteins within bioprosthetic tissue might trigger an inflammatory process. This would result in osteoblastic differentiation of stem/progenitor cells caused by the oxidized low-density lipoproteins and inflammatory cytokines [13]. Another reason for bioprosthetic valve failure is calcific deposits found in tears in the commissural and basal areas of the cusp. Within 15 years of implantation, over 50% of porcine valves show some form of functional degradation, usually due to regurgitation caused by these cusp tears [15].

### *2.1.2. Prevention*

#### *2.1.2.1. Biomaterial alterations and coatings*

One of the major reasons that implants calcify is due to the biocompatibility of the material. In several studies, either altering the chemical makeup and properties of the biomaterial or coating the material with anticalcification agents have been used to reduce these effects.

Crosslinking surface material of various implants has become a topic of interest in current research, specifically because of the mechanical properties it supplies to implants. Crosslinking chemistry provides protection to various extracellular matrix components in bioprosthetic heart valves in order to retain structural strength [21]. Several crosslinking methods, specifically crosslinking with glutaraldehyde, provide strength by preventing degradation, but the

elastin in the ECM is not protected. This leads to stiffness, tears, and deformations in the surface of the material, a condition known as "permanent set" [5]. A modified form of cross-linking involving treating the surface of biomaterial implants with pentagalloyl glucose does not cause damage to collagen or surface deformations. In a study using bovine pericardium tissue, the combined cross-linking was prepared by first soaking the tissue in neomycin trisulfate and a buffer, then incubating it in a cross-linking carbodiimide solution with pentagalloyl glucose. The treated leaflets of tissue were then tested *in vivo* in rats to determine calcification after a thirty day period. Little to no calcification was found on any of the leaflets. The study showed the potential benefits of using this cross-linking method to prevent or delay calcification in implants, though further data should be collected (**Figure 3**) [5].

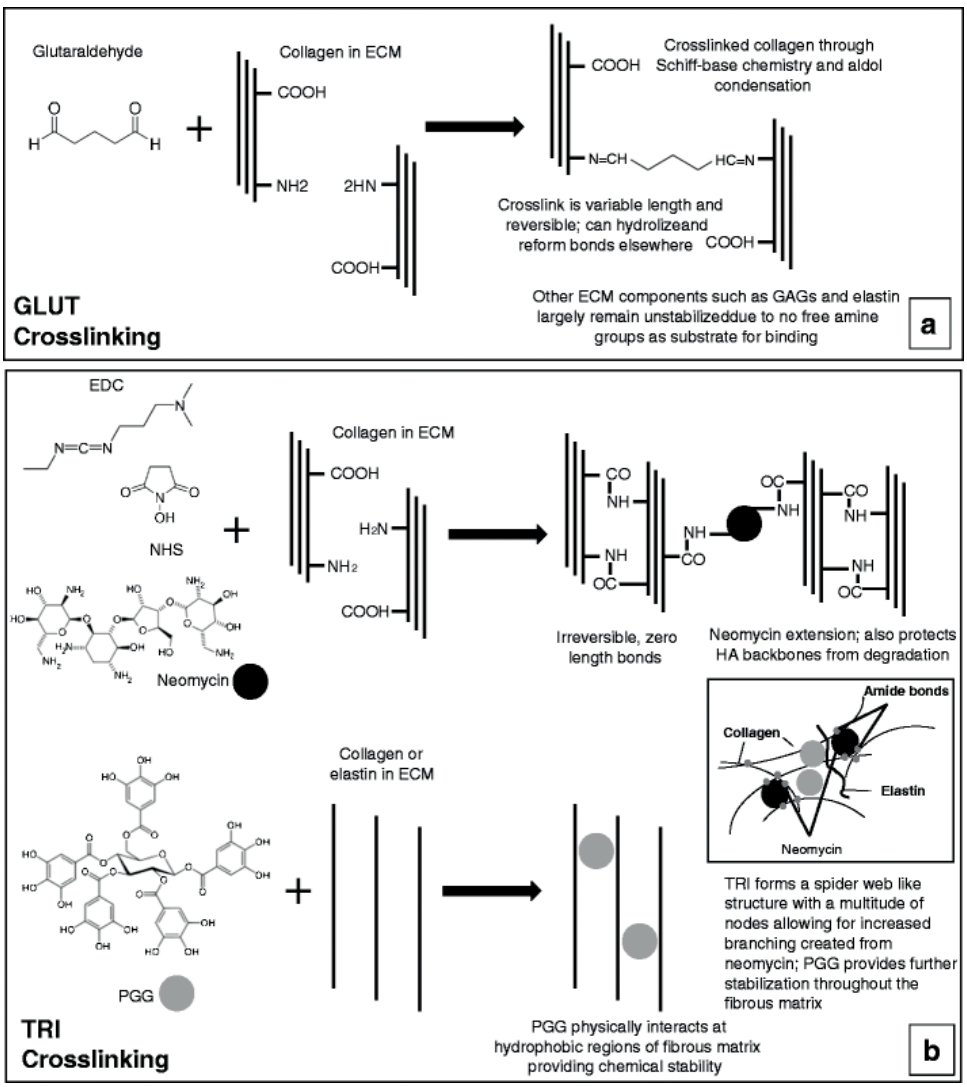



**Figure 3.** Treating the biomaterial with POSS to form POSS-PCU changes existing receptors so that free calcium ions are no longer able to bind, preventing deposition and mineralization [5].

Another method of preventing calcification is by targeting the free aldehydes present in bioprosthetic tissue. As previously mentioned, glutaraldehyde is used to set tissue to be used for implants because of the strength it provides, and the aldehydes remaining on the tissue are thought to promote calcification [22]. When bovine pericardium is treated with alternatives to cap the free aldehydes, the tissue shows significantly reduced levels of calcium content and mineralization [23]. Reducing agents such as glycine, glutamate, and sodium bisulfite can form a Schiff base and effectively neutralize the aldehydes present [23]. This process allows the tissue to maintain its mechanical strength but inhibits the formation of calcification [23].

Nanocomposites are growing in popularity for use in biomaterials due to their biocompatibility and anticalcification properties [24]. Using polyhedral oligomeric silsesquioxane (POSS) nanoparticles with poly(carbonate-urea)urethane (PCU) has proven to increase mechanical strength, and potentially work as a calcification-resistant material. **Figure 3** illustrates the treatment of POSS-PCU [24]. These composites have been shown to significantly decrease deposition compared to glutaraldehyde-fixed bovine pericardium tissue. The treated tissue shows decreased platelet adhesion to its surface compared to typical bovine pericardium, a mechanism thought to be associated with calcification resistance [25]. These nanocomposites show increased promise for use in biomaterials.

### 2.2. Tissue engineered and ion-loaded scaffolds

Because of their biocompatibility and regenerative capabilities, tissue engineered scaffolds are becoming a popular source for heart valves replacements. There are three different types of scaffolds: porous, fibrous, and hydrogels. These can be either acellular or seeded with autologous cells to promote regeneration and avoid a negative immune response [7]. A key component in the scaffold is its ability to degrade in a controlled time period [7]. The tissue can then be regenerated while the synthetic scaffold degrades or is remodeled, leaving behind growth and proliferation resembling natural tissue [26].

Tissue-engineered scaffolds seeded with vascular interstitial cells (VICs) have been shown to regenerate valvular tissue, while still retaining the alpha-smooth muscle actin ($\alpha$-SMA) marker expressed in smooth muscle cells [7]. The tissue engineered scaffolds have a lower risk of ECM damage than the decellularized tissue used in many heart valve implants; therefore, the collagen and fibers behave in a more normal manner, retaining their smooth muscle phenotype. Porosity can be controlled, compared to the unintentional porosity created in decellularized tissue, making the scaffolds more resistant to calcification [7].

These scaffolds can be manipulated before entering the body, from seeding them with autologous cells to promote new growth to loading them with ions as a form of drug delivery to mitigate negative responses post-implantation. Metal ions, including iron, aluminum, and magnesium, are gaining popularity in current research because of their ability to bind to forming hydroxyapatite crystals in the serum and prevent further deposition [27]. Though the mechanism is not fully understood, it is hypothesized that ions such as magnesium bind where calcium typically would, preventing calcium deposits [28]. They also are thought to interrupt alkaline phosphatase activity [27]. These ions often exist naturally in the body for

this process, but by loading tissue-engineered scaffolds with different metals (aluminum, magnesium, iron) they can specifically target calcium deposition at the site of the implant to prevent failure. The problem that rises with this design is that, after implantation, there is no way to reload the matrices with more ions, so it cannot act as a long term inhibitor.

## 3. Urinary

### 3.1. Urinary tract materials

In the entire urinary system, the organs most commonly and significantly affected by calcifications are the kidneys, followed by ureters, and then the urinary bladder. Urinary tract calculi are formed when the urine is supersaturated with salt and minerals such as calcium oxalate, struvite (ammonium magnesium phosphate), uric acid, and cysteine [29]. This supersaturation can be caused by a variety of genetic and dietary factors, urinary calcium excretion, and environmental triggers [30]. Urinary calculi are solid particles in the urinary system that cause pain, nausea, vomiting, hematuria, and chills/fever due to secondary infection.

Urinary tract calcification is organized in three different categories: renal calcification, ureteral calcification, and bladder calcification. These are further classified by their orientation, position, shape, size, mobility, opacity, chemical composition, and location in the kidneys, ureters, or bladder, and their relation to pathologic conditions [31]. Calcification in the urinary tract can also occur from infection of the implants. Healthcare associated infections are the fourth leading cause of disease [33]. Studies indicate that biofilm infections cause up to 80% death [33]. One of the leading causes of infection is the insertion of catheters and ureteral stents [33].

#### *3.1.1. Mechanism*

##### *3.1.1.1. Urinary infection mechanisms*

Catheters and stents used in patients are subject to biofilm formation when different particles in the urine, blood, or surrounding tissue attach to the surface of the implant. Biofilms begin to form when colonized bacteria attaches to the surface of the device, altering the surface properties [32]. Once attached, the bacteria binds with target molecules and, after an extended period of time, the attachment becomes permanent and the process is irreversible. Overtime, as the biofilm becomes more developed, it will repeat these processes to form a new biofilm formation on an unpopulated area of the implantation device [34].

One of the main reasons for encrustation of a device is infection due to bacteria that produce urease. This enzyme uses urea to create an alkaline environment from ammonia, raising the pH [35]. *E. faecalis, Proteus mirabilis, Staphylococcus aureus*, and *Candida tropicalis* are considered the strongest strains to form biofilms; however, *P. mirabilis* is hydrolyzes urea ten times faster than the rates of other strains [34]. Under these conditions, hydroxyapatite and struvite crystals form on the surface of the device, resulting in encrustation [34]. This process will continue and repeat until the flow of urine is blocked due to encrustation resulting in the complete device failure [35].

Encrustation of ureteral stents occurs for a variety of reasons. One reason is the failure of patients to return for stent removal after surgery or inadequate counsel by professional healthcare [36]. The material of the stent may also contribute to encrustation. Silicone containing stents seems to be more resistant to encrustation, followed by polyurethane, silitek, percuflex, and hydrogel coated polyurethane [36]. Stents fracturing after being *in situ* for a long period due to hardening and loss of tensile strength can also be another reason for encrustation. Other factors that contribute may include urinary composition (hypercalciuria, hyperoxaluria, hypocitraturia, homocystinuria, and hyperuricosuria), history of urolithiasis, and congenital urinary tract anomalies [37].

### *3.1.2. Prevention*

#### *3.1.2.1. Urinary implant coatings*

Coatings of the urinary implantation devices are one of approaches that prevents bacterial adherence. Surface coatings of the devices inhibit bacterial biofilm formation to prevent infection and encrustation [38]. The coating needs to have certain properties to inhibit bacterial adherence which includes biocompatible, resists biofilm formation, and antimicrobial [39].

Hydrogels are hydrophilic, cross-linked polymers capable of absorbing large amounts of liquid. They form a thin layer of water on the surface of the device, preventing biofilm formation and bacterial adherence [34]. Studies have shown hydrogel-coated catheters have less bacterial adherence compared to non-hydrogel coated catheters [40]. In addition, hydrogel-coated catheters also cause less irritation and inflammation [34].

Similar to hydrogels, antimicrobial peptides (AMPs) are hydrophilic polymers that have antibiotic resistance which inhibits bacterial adhesion [39]. In one study, AMPs were coated on titanium implants and inhibited bacterial adhesions both *in vitro,* and *in vivo* using rat models. In addition to inhibiting bacterial growth, it also has wound healing benefits. However, there are some conditions with AMPs that include potential local toxicity, pH sensitivity, susceptibility to proteolysis, and high cost of synthesis [34].

Polyvinylpyrrolidone (PVP) is also hydrophilic and has excellent lubricant properties. Therefore, the implantation device has less bacterial adhesion and encrustation *in vivo* compared to uncoated catheters [34]. Heparin is a glycosaminoglycan which is a natural inhibitor of crystallization. Naturally, heparin is considered to prevent bacterial attachment and encrustation; however, studies concluded that there is not a significant decrease in bacterial adherence despite its overall good quality [38].

On the contrary, hyaluronic acid shows promising results *in vitro.* Hyaluronic acid is a type of glycosaminoglycan that inhibits nucleation, growth, and aggregation of salts. Covalently bound hyaluronic acid catheters increase hydration, while decreasing adsorption of proteins and bacterial adhesion. Even though hyaluronic acid coating shows promising results, it has yet to be fully analyzed. Gendine is another antimicrobial coating that contains gentian violet and chlorhexidine [34]. Compared to uncoated controls, devices that are coated in gendine are resistant to the adherence of multi-drug-resistant bacteria [34].

Researchers are continuously searching for an ultimate biocompatible material that can substitute segments of the urinary tract [41]. This involves urinary system cells or other cell sources that can be seeded onto biodegradable scaffolds [42]. Experts have reported that cells isolated from urine can express smooth muscle, endothelial and interstitial cells, and markers of urothelial [42].The ideal biomaterial has to be biocompatible and biodegradable, promote vascular regeneration, nerve regeneration, and cellular differentiation; also, it should be watertight and stretchable, resist encrustation and biofilm formation, and regain its shape [41]. However, most biomaterial includes natural collagens, and natural collagens scaffolds cannot maintain their physical properties in an *in vivo* environment resulting in graft failure or formation of fibrosis [43]. There is no ideal biomaterial available yet, but they can be modified to enhance biological properties for cellular integration. Smart polymers are also optimal for use in urinary construction [41].

## 4. Diseased state

In addition to affecting different biomaterials and biosynthetic implants, calcification also occurs naturally throughout the cardiovascular and the urinary system due to various states and diseases.

Cardiovascular disease is the leading cause of death in the United States, with a high mortality rate among end stage renal disease (ESRD) patients [44]. ESRD and other forms of kidney disease are marked by elevated levels of calcium phosphate in the serum, leading to mineral deposition and calcification of the arterial wall. This occurs as vascular smooth muscle cells differentiate from their typical phenotype to osteoblast-like cells that cause bone formation in atypical regions [45]. This risk increases with patients on dialysis, due to the fact that important calcification-inhibitory molecules, such as Fetuin-A, are stripped from the body [45]. This high level of phosphate also leads to the activation of the Wnt signaling pathway. When high levels of phosphate accumulate in smooth muscle cells, $\beta$-catenin is upregulated [46]. This leads to an increased expression of bone-morphogenetic protein 2 (BMP-2) and runt-related transcription factor-2 (Runx2) in smooth muscle cells, though the two factors are typically only seen in bone cells [47].

Primary hyperparathyroidism (PHPT), often associated with cardiovascular disease, is another condition potentially linked to calcification. It has been observed that as levels of parathyroid hormone increase in PHPT patients, there is an associated increase in abdominal aortic calcification [48].

Another form of calcification associated with imbalance of minerals in the body is nephrocalcinosis. When calcium intake increases and it begins to build up in the kidneys, it leads to the deposition of minerals in the renal parenchyma and tubules [49]. These calcified regions are also formed through an osteopontin deficiency. This calcification in the urinary system can contribute to renal dysfunction and potentially lead to ESRD [49].

Calcification of both the vascular and urinary system in the body can be driven by various diseases and conditions, typically due to some sort of mineral or chemical imbalance in the

serum. Taking this into account, as well as the increased rate of calcification seen in bio-implants, it is critical for current research to move toward both prevention and treatment of this phenomenon.

### 4.1. Mechanism

#### *4.1.1. General mechanisms*

Calcification is a pathological process that occurs with an imbalance of several genetic, chemical, and physical properties. The process can depend on the levels of proteins and ions present in the serum, like metal ions that bind with hydroxyapatite or proteins that chaperone free calcium and phosphate particles. It can also be induced by physical damage to cells and tissue, whether by chemical means or foreign implants in the body. Though many individual factors are associated with the formation of mineral deposits and calcification in the body, the mechanisms inducing calcification are still being researched and understood.

Vascular calcification (VC) is a prominent issue affecting both the intimal and medial layers of the arterial wall. Intimal calcification is usually associated with plaque rupture and thickening of the endothelium layer in the vessels while medial calcification occurs as smooth muscle cells differentiate into osteoblast-like cells, which are associated with bone growth. This phenotypic switch in the medial layer is often associated with various osteogenic predecessors [50].

Osteoprotegerin (OPG) is a glycoprotein that works by inhibiting bone resorption, and an increase in OPG is often associated with an increase in calcification [51]. It participates in the OPG/RANK/RANKL pathway to act as a decoy receptor binding to RANKL, where RANK is supposed to bind. This in turn prevents RANK's intended mechanism of osteoblast differentiation into osteoclasts [51].

BMP-2 is also thought to play an important role in VC, since it is expressed in higher levels in chronic kidney disease patients, and is a part of the Wnt/β-catenin pathway [52]. Typically, it is associated with bone and tooth formation, but the high phosphate levels in uremic patients activate this pathway and causes BMP-2 expression.

Apart from the osteogenic markers and factors associated with calcification is another major protein, matrix Gla protein (MGP). In its activated form, it antagonizes BMP-2 signaling as a negative feedback regulator due to its carboxylated glutamate residues [53]. It also acts by binding to forming hydroxyapatite crystals to prevent deposition and calcification. However, it must be carboxylated by vitamin-K in order to be active, which may be why vitamin-K deficient kidney disease patients show calcified vessels [54].

In biomaterials, the surface structure of the material can determine the post-implantation calcification. Materials with a higher porosity have the increased potential for calcification, because the larger pores allow for more calcium deposition [55].

## 4.2. Prevention

### *4.2.1. Dietary changes*

Because most causes of calcification are rooted in a mineral imbalance, dietary modifications or supplementation are currently being studied for potential use in attenuating the effects of calcification in different regions in the body. Magnesium ions are known to inhibit calcium deposition, though the mechanism is not clearly understood [56]. When supplied to vascular smooth muscle cells, the rate of cell damage by apoptosis is significantly decreased as the magnesium levels increase in the media [57]. Decreasing the rate of apoptosis decreases arterial stiffness and calcification since apoptosis of smooth muscle cells often leads to the disruption and remodeling of plaque in the arteries [58]. Increased magnesium levels also decrease the expression of Runx2, inhibiting the differentiation of smooth muscle cells into osteoblast-like cells [57].

In a Framington Heart Study of 2695 participants, a dietary assessment was used to measure magnesium intake levels and determine whether adding the supplement could prevent or inhibit calcification. It was found that both coronary artery and abdominal aortic calcification decreased as magnesium intake increased, with a 22% decrease in coronary artery calcification for every 50 mg increase in daily magnesium intake, and significant decrease in abdominal aortic calcification with magnesium increase [59]. Even though the mechanism is not fully understood, the metal does correlate with an inhibition of calcification.

MGP is another inhibitor of calcification that prevents the differentiation of vascular smooth muscle cells into osteoblasts [60]. However, MGP can only inhibit calcification if it is activated via carboxylation, making it a vitamin K-dependent protein. In patients with chronic kidney disease, there is a vitamin K deficiency and MGP remains inactivated [54]. For this reason, vitamin K was investigated as a dietary supplement to activate circulating MGP and inhibit calcification.

In a randomized, controlled trial, male and female patients were given either a control or vitamin K supplement, and CT scans were used for analysis of calcification levels [61]. Blood samples were also taken and analyzed with a radioimmunoassay to determine MGP levels in the serum. While results showed that the supplement reduced the levels of calcification currently existing, it did not prevent the new formation of calcium deposits. MGP levels also showed no significant difference between the control and vitamin K group [61]. This shows that vitamin K could be used as a supplement to slow the progression of existing calcium deposits, though it has not been proven to prevent the formation of new calcification.

### *4.2.2. Protein therapy*

Several naturally occurring proteins in the body act as inhibitors of calcification. MGP, as previously mentioned, is a naturally occurring inhibitor of calcification, requiring carboxylation to prevent osteoblastic-differentiation. Fetuin-A, also known as alpha-2-Heremans-Schmid glycoprotein, is another protein that acts by binding to free calcium and phosphate particles in the serum and preventing deposition [62]. In dialysis patients, fetuin levels in the body are significantly lower than in healthy patients, correlating with an increase in vascular

calcification [63]. Because of this correlation, fetuin has been considered as a potential therapeutic protein, as treatment for vascular calcification [4].

Osteopontin (OPN) is a protein associated with bone remodeling and resorption. When phosphorylated, it can easily bind with calcium ions to prevent calcification [49]. It resists calcification in a dose-dependent manner when supplemented to smooth muscle cells to protect their phenotype, and is currently being researched to determine its therapeutic abilities [49].

#### *4.2.3. Drug-coated stents and balloon angioplasty*

There are two main classifications of stents: bare metal stents and drug-eluting stents. The latter has been used to treat calcification and prevent restenosis by incorporating anti-proliferation and anti-inflammatory agents into the material of the stent [64]. However, in regions of high calcification, it is common for the stent to improperly deploy within the vessel, leading to further plaque build-up and implant failure [64].

In order to prevent improper placement of the stent and reduce adverse effects, drug-coated angioplasty balloons are often favorable to stents. Balloon angioplasty is a common treatment for calcification in the arteries, working as an immediate clearing of vessels to allow blood flow [65]. By modifying this design and using drug-coated balloons, obstructions in the vessels can be immediately broken up while also delivering various agents to prevent the return blockage without leaving a permanent implant behind [66]. Paclitaxel-coated balloons have been used because of the drug's ability to stop cell division so that when it is delivered to regions with increased plaque buildup, further growth is inhibited. The drug is delivered uniformly to the arterial wall with immediate release and incorporation into the tissue [67].

## 5. Assessment

### 5.1. Assessment of biomaterials calcification

Many different techniques are used to investigate and examine the calcification of biomaterials. This can be done with either morphologic or chemical techniques. Morphological testing yields important qualitative information like the detection, characterization, and distribution sites of calcific deposits as detailed below, but still lacks quantitative information. While chemical techniques reveal more qualitative data such as identification of elemental composition and determination of crystalline mineral phases, they require a complete ruination of the tissue specimen [68]. Furthermore, techniques such as microcomputer tomography (micro CT) are recent technologies available for both *in vitro* and *in vivo* samples that are non-invasive and non-destructive [68].

Morphological assessment of calcification uses many different techniques, including scanning electron microscopy (SEM), radiographs (X-rays), light microscopy, transmission electron

microscopy (TEM), and microcomputer tomography (micro CT) [68, 69]. Calcific deposit dispersal can be seen from X-rays, and most calcification is studied using morphological techniques done outside of the body once the implantation is removed. As mentioned previously, calcific deposit morphology, quantification, and localization can be seen from micro CT. Both X-ray and CT techniques require gross specimen sample preparation. Light microscopy is used in conjunction with various staining techniques to identify mineral deposits with either a calcium or phosphorus-specific stains. Alizarin red is a calcium-specific stain and von Kossa is a phosphate-specific stain [68]. Hematoxylin/eosin, Mallory's trichrome and alcian blue stains are known as histological stains associated with light microscopy, both readily available and easily applied to tissue [69].

Two types of microscope techniques mentioned previously, SEM and TEM, are electron microscopes that use a highly focused electron beam contained in a vacuum to passes the specimen [68]. In one study, SEM was used to analyze bovine pericardium samples in vitro for calcification using SEM. To prepare the samples for analysis, they were first soaked in a simulated body fluid containing ionic concentrations similar to natural body plasma fluid, then placed in a controlled environment. After seven days, samples were rinsed, deionized, and frozen in liquid nitrogen. Finally, samples had to be lyophilized before SEM analysis could be performed [70]. Other methods of calcification testing include Fourier transform infrared spectroscopy, which is used to determine structure coatings and x-ray diffraction of lyophilized samples using a diffractometer with Cu-K$\alpha$ radiation [70].

## 6. Future works

There are many important factors to review when looking at heart valve replacements. Cost should be considered since valvular heart disease is prominent worldwide, especially in underdeveloped countries. Post-implantation failure is another factor, largely due to age of the patient given that children and young adults have a more competent immune system and experience a higher rate of BHV failure. In some countries lacking adequate ways of monitoring patients, mortality is an increased risk [9]. Calcification is also a major cause of deterioration in BHV replacements. The complications associated with calcification of artificial heart valves can lead to the need for revision surgery in patients. Mechanical valve replacements potentially require additional surgery due to thrombosis, thromboembolism, or spontaneous bleeding can occur; additionally, these replacements require lifelong anticoagulation therapies [9].

For these reasons, experts are trying to further understand the mechanism of biomaterial calcification and exploring more biocompatible materials. As mentioned before, there is not a specific mechanism that leads to VHD, so further understanding of the various processes involved will improve treatment strategies that include tissue engineering and drug-coated biomaterials [8]. Some studies have reported that tissue engineering scaffolds have similar uniaxial mechanical properties but need more investigation with biaxial mechanical properties that are more related to soft tissue. There are also clinical studies that combined both

synthetic and natural polymers to construct a scaffold that could be similar to the native mechanical properties of a heart valve which may improve their biocompatibility [7]. Another approach to prevent calcification is to modify the surface of the device; for example, heparin can be used to inhibit tissue calcification [69].

In addition, urinary stents and catheters need more attention to overcome the two main causes that lead to calcification: infection and encrustation. Currently, studies are focusing on innovating stent designs, biomaterials, and surface coatings [41]. Many studies have attempted to combine multiple antimicrobial agents into one coating, for example using several antibiotics. Another approach that most researchers have recently used is constructing urinary tissue from organ-specific stromal cells resulting in better biomechanical properties similar to human than non-specific stromal cells [71]. However, most biomaterials include natural collagens that are unable to maintain the same physical properties, resulting in graft failure [43]. Further investigation and clinical studies are needed to introduce the ideal biomaterials and coating [34].

In conclusion, further development will include better understanding of VHD to improve our treatment strategies. More trials and clinical studies are needed to create an "ideal" biomaterial for tissue engineering and drug-coated biomaterials. Additional experiments will be needed to test innovating stent designs, heart valves, and surface coatings to treat implantation calcification.

## Acknowledgements

Authors would like to acknowledge Mississippi State University and MAFES for funding.

## Author details

Jenna Mosier, Nancy Nguyen, Kadie Parker and Chartrisa LaShan Simpson*

*Address all correspondence to: clsimpson@abe.msstate.edu

Mississippi State University, Mississippi State, USA

## References

[1] Reynolds JL, Skepper JN, McNair R, Kasama T, Gupta K, Weissberg PL, et al. Multifunctional roles for serum protein fetuin-a in inhibition of human vascular smooth muscle cell calcification. Journal of the American Society of Nephrology [Internet]. Oct 1, 2005;**16**(10):2920-2930. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16093453 [Accessed: Sep 13, 2017]

[2] Frederick J, Schoen MD, Robert J, Levy, MD. Calcification of tissue heart valve substitutes: Progress toward understanding and prevention. Annals of Thoracic Surgery [Internet]. Mar 1, 2005;**79**(3):1072-1080. Available from: http://www.sciencedirect.com/science/article/pii/S0003497504013219 [Accessed: Sep 13, 2017]

[3] Persy V, et al. Vascular calcification and bone disease: The calcification paradox. Trends in Molecular Medicine [Internet]. Sep 1, 2009;**15**(9):405-416. Available from: http://www.sciencedirect.com/science/article/pii/S147149140900118X [Accessed: Sep 13, 2017]

[4] Westenfeld R, Schäfer C, Krüger T, Haarmann C, Schurgers LJ, Reutelingsperger C, et al. Fetuin-A protects against atherosclerotic calcification in CKD. Journal of the American Society of Nephrology [Internet]. Jun 1, 2009;**20**(6):1264-1274. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19389852 [Accessed: Sep 13, 2017]

[5] Tam H, Zhang W, Infante D, Parchment N, Sacks M, Vyavahare N. Fixation of bovine pericardium-based tissue biomaterial with irreversible chemistry improves biochemical and biomechanical properties. Journal of Cardiovascular Translational Research [Internet]. Apr 17, 2017;**10**(2):194-205. Available from: http://link.springer.com/10.1007/s12265-017-9733-5 [Accessed: Sep 13, 2017]

[6] Wu S, Wang Z, Bharadwaj S, Hodges SJ, Atala A, Zhang Y. Implantation of autologous urine derived stem cells expressing vascular endothelial growth factor for potential use in genitourinary reconstruction. Journal of Urology [Internet]. Aug 1, 2011;**186**(2):640-647. Available from: http://www.sciencedirect.com/science/article/pii/S0022534711035658 [Accessed: Sep 13, 2017]

[7] Ibrahima DM, Kakarougkas A, Allam NK. Recent advances on electrospun scaffolds as matrices for tissue-engineered heart valves. Materials Today Chemistry [Internet]. Sep 1, 2017;**5**:11-23. Available from: http://www.sciencedirect.com/science/article/pii/S2468519417300423#bib20 [Accessed: Sep 13, 2017]

[8] Cheung DY, Duan B, Butcher JT. Current progress in tissue engineering of heart valves: Multiscale problems, multiscale solutions. Expert Opinion on Biological Therapy [Internet]. 2015;**15**(8):1155-1172. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26027436 [Accessed Sep 14, 2017]

[9] Singhal P, Luk A, Butany J. Bioprosthetic heart valves: Impact of implantation on biomaterials. ISRN Biomaterials [Internet]. Mar 12, 2013;**2013**:1-14. Available from: http://www.hindawi.com/journals/isrn/2013/728791/ [Accessed: Sep 14, 2017]

[10] Vojáčeka J, El-Hamamsy I, Ondrášek J, Žáček P, Fila P, Voborník M, Špatenkad J. Current status of the ross procedure in aortic valve surgery. Cor Et Vasa [Internet]. Feb 1, 2017;**59**(1):e71-e16. Available from: http://www.sciencedirect.com/science/article/pii/S0010865016301412 [Accessed: Sep 14, 2017]

[11] Sodian R, Lueders C, Kraemer L, Kuebler W, Shakibaei M, Reichart B, Daebritz S, Hetzer R. Tissue engineering of autologous human heart valves using cryopreserved vascular umbilical cord cells. Annals of Thoracic Surgery [Internet]. Jun 1,

2006;**81**(6):2207-2216. Available from: http://www.sciencedirect.com/science/article/pii/S0003497506000403?via%3Dihub [Accessed: Sep 14, 2017]

[12] Bloomfield P. Choice of heart valve prosthesis. Heart [Internet]. Jun 2002;**87**(6):583-589. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12010950 [Accessed: Sep 14, 2017]

[13] Pibarot P, Dumesnil JG. Prosthetic heart valves. Circulation [Internet]. 2009;**119**(7):1034-1048. Available from: http://circ.ahajournals.org/content/119/7/1034.long [Accessed: Sep 14, 2017]

[14] Legg M, Mathews E, Pelzer R. The design and development of a stented tissue mitral and aortic heart valve replacement for human implantation. Cardiovascular Journal of Africa [Internet]. Apr 2012;**23**(3):126-130. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22555635 [Accessed: Sep 18, 2017]

[15] Siddiqui RF, Abraham JR, Butany J. Bioprosthetic heart valves: Modes of failure. Histopathology [Internet]. Aug 1, 2009;**55**(2):135-144. Available from: http://doi.wiley.com/10.1111/j.1365-2559.2008.03190.x [Accessed: Sep 14, 2017]

[16] Manji RA, Menkis AH, Ekser B, Cooper DK. Porcine bioprosthetic heart valves: The next generation. American Heart Journal [Internet]. Aug 1, 2012;**164**(2):177-185. Available from: http://www.sciencedirect.com/science/article/pii/S0002870312003675?via%3Dihub [Accessed: Sep 14, 2017]

[17] Yang M, Lin Y-H, Shi W-P, Shi H-C, Gu YJ, Shu Y-S. Surface heparin treatment of the decellularized porcine heart valve: Effect on tissue calcification. Journal of Biomedical Materials Research Part B: Applied Biomaterials [Internet]. Feb 1, 2017;**105**(2):400-405. Available from: http://doi.wiley.com/10.1002/jbm.b.33490 [Accessed: Sep 14, 2017]

[18] Aguiari P, Iop L, Favaretto F, Fidalgo CML, Naso F, Milan G, et al. In vitro comparative assessment of decellularized bovine pericardial patches and commercial bioprosthetic heart valves. Biomedical Materials [Internet]. Feb 3, 2017;**12**(1):15021. Available from: http://stacks.iop.org/1748-605X/12/i=1/a=015021?key=crossref.161333f57a86420980063b8a87470ff7 [Accessed: Sep 14, 2017]

[19] MacGrogan D, Luna-Zurita L, de la Pompa JL. Notch signaling in cardiac valve development and disease. Birth Defects Research Part A: Clinical and Molecular Teratology [Internet]. Jun 1, 2011;**91**(6):449-459. Available from: http://doi.wiley.com/10.1002/bdra.20815 [Accessed: Sep 14, 2017]

[20] Cole J, Aberdein J, Jubrail J, Dockrell DH. The role of macrophages in the innate immune response to Streptococcus pneumoniae and Staphylococcus aureus: Mechanisms and contrasts. Advances Microbial Physiology. 2014;**65**:125-202. DOI: 10.1016/bs.ampbs.2014.08.004. PMID: 25476766

[21] Tam H, Zhang W, Feaver KR, Parchment N, Sacks MS, Vyavahare N. A novel crosslinking method for improved tear resistance and biocompatibility of tissue based biomaterials. Biomaterials [Internet]. Oct 1, 2015;**66**:83-91. Available from: http://www.sciencedirect.com/science/article/pii/S0142961215005876 [Accessed: Sep 13, 2017]

[22] Mathapati S, Bishi DK, Guhathakurta S, Cherian KM, Venugopal JR, Ramakrishna S, Verma RS. Biomimetic acellular detoxified glutaraldehyde cross-linked bovine pericardium for tissue engineering. Materials Science and Engineering C [Internet]. Apr 1, 2013;**33**(3):1561-1572. Available from: http://www.sciencedirect.com/science/article/pii/S0928493112006315 [Accessed: Sep 13, 2017]

[23] Lim H-G, Kim SH, Choi SY, Kim YJ. Anticalcification effects of decellularization, solvent, and detoxification treatment for genipin and glutaraldehyde fixation of bovine pericardium. European Journal of Cardio-Thoracic Surgery [Internet]. Feb 1, 2012;**41**(2):383-390. Available from: https://academic.oup.com/ejcts/article-lookup/doi/10.1016/j.ejcts.2011.05.016 [Accessed: Sep 13, 2017]

[24] Vellayappan MV, Balaji A, Subramanian AP, John AA, Jaganathan SK, Murugesan S, et al. Tangible nanocomposites with diverse properties for heart valve application. Science and Technology of Advanced Materials [Internet]. Jun 20, 2015;**16**(3):33504. Available from: http://www.tandfonline.com/doi/full/10.1088/1468-6996/16/3/033504 [Accessed: Sep 13, 2017]

[25] The anti-calcification potential of a silsesquioxane nanocomposite polymer under in vitro conditions: Potential material for synthetic leaflet heart valve. Acta Biomaterialia [Internet]. Nov 1, 2010;**6**(11):4249-4260. Available from: http://www.sciencedirect.com/science/article/pii/S1742706110002813?via%3Dihub [Accessed: Sep 13, 2017]

[26] Rocco KA, Maxfield MW, Best CA, Dean EW, Breuer CK. In vivo applications of electrospun tissue-engineered vascular grafts: A review. Tissue Engineering Part B Reviews [Internet]. Dec 16, 2014;**20**(6):628-640. Available from: http://online.liebertpub.com/doi/abs/10.1089/ten.teb.2014.0123 [Accessed: Sep 13, 2017]

[27] Singh C, Wang X. Metal ion-loaded nanofibre matrices for calcification inhibition in polyurethane implants. Journal of Functional Biomaterials [Internet]. Jun 23, 2017;**8**(3):22. Available from: http://www.mdpi.com/2079-4983/8/3/22 [Accessed: Sep 13, 2017]

[28] Vasudev SC, Chandy T, Mohanty M, Up R, Sharma CP. Inhibition of bioprosthesis calcification due to synergistic effect of Fe/Mg ions to polyethylene glycol grafted bovine pericardium. Journal of Biomaterials Applications [Internet]. Oct 27, 2001;**16**(2):93-107. Available from: http://journals.sagepub.com/doi/10.1106/0JE0-M473-XATE-6EJD [Accessed: Sep 13, 2017]

[29] An imaging review on urinary tract calcifications. International Journal of Anatomy Radiology and Surgery [Internet]. 2013;**2**(1):19-28. Available from: http://www.ijars.net/articles/PDF/1957/4488_E(C)_PF1(M)_F(H)_PF1(H)_PFA(H).pdf [Accessed: Sep 17, 2017]

[30] Evan AP. Physiopathology and etiology of stone formation in the kidney and the urinary tract. Pediatric Nephrology [Internet]. May 7, 2010;**25**(5):831-841. Available from: http://link.springer.com/10.1007/s00467-009-1116-y [Accessed: Sep 13, 2017]

[31] Dyer RB, Chen MY, Zagoria RJ. Abnormal calcifications in the urinary tract. Radiographics [Internet]. 1997;**18**(6):1405-1424. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9821191

[32] Lee J, Ramasamy M, Lee J. Recent nanotechnology approaches for prevention and treatment of biofilm-associated infections on medical devices. BioMed Research International. 2016;**2016**(October):1-17

[33] Guggenbichler JP, Assadian O, Boeswald M, Kramer A. Incidence and clinical implication of nosocomial infections associated with implantable biomaterials - catheters, ventilator-associated pneumonia, urinary tract infections. GMS Krankenhhyg Interdiszip [Internet]. 2011;**6**(1):Doc18. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3252661&tool=pmcentrez&rendertype=abstract

[34] Lo J, Lange D, Chew B. Ureteral stents and foley catheters-associated urinary tract infections: The role of coatings and materials in infection prevention. Antibiotics [Internet]. 2014;**3**(1):87-97. Available from: http://www.mdpi.com/2079-6382/3/1/87/

[35] Malic S, Waters MGJ, Basil L, Stickler DJ, Williams DW. Development of an "early warning" sensor for encrustation of urinary catheters following Proteus infection. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2012;**100 B**(1):133-137

[36] Murthy KVR, Jayaram Reddy S, Prasad DV. Endourological management of forgotten encrusted ureteral stents. International Brazilian Journal of Urology. 2010;**36**(4):420-429

[37] Dakkak Y, Janane A, Ould-Ismail T, Ghadouane M, Ameur A, Abbar M. Management of encrusted ureteral stents. African Journal of Urology [Internet]. 2012;**18**(3):131-134. Available from: http://dx.doi.org/10.1016/j.afju.2012.08.013

[38] Lange D, Elwood CN, Choi K, Hendlin K, Monga M, Chew BH. Uropathogen interaction with the surface of urological stents using different surface properties. Journal of Urology [Internet]. 2009;**182**(3):1194-1200. Available from: http://dx.doi.org/10.1016/j.juro.2009.05.008

[39] Gao G, Lange D, Hilpert K, Kindrachuk J, Zou Y, Cheng JTJ, et al. The biocompatibility and biofilm resistance of implant coatings based on hydrophilic polymer brushes conjugated with antimicrobial peptides. Biomaterials [Internet]. 2011;**32**(16):3899-3909. Available from: http://dx.doi.org/10.1016/j.biomaterials.2011.02.013

[40] Desai DG, Liao KS, Cevallos ME, Trautner BW. Silver or nitrofurazone impregnation of urinary catheters has a minimal effect on uropathogen adherence. Journal of Urology [Internet]. 2010;**184**(6):2565-2571. Available from: http://dx.doi.org/10.1016/j.juro.2010.07.036

[41] Yang L, Whiteside S, Cadieux PA, Denstedt JD. Ureteral stent technology: Drug-eluting stents and stent coatings. Asian Journal of Urology [Internet]. 2015;**2**(4):194-201. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2214388215000995

[42] Zhang Y, McNeill E, Tian H, Soker S, Andersson KE, Yoo JJ, et al. Urine derived cells are a potential source for urological tissue reconstruction. Journal of Urology. 2008;**180**(5):2226-2233

[43] Bodin A, Bharadwaj S, Wu S, Gatenholm P, Atala A, Zhang Y. Tissue-engineered conduit using urine-derived stem cells seeded bacterial cellulose polymer in urinary reconstruction and diversion. Biomaterials [Internet]. 2010;**31**(34):8889-8901. Available from: http://dx.doi.org/10.1016/j.biomaterials.2010.07.108

[44] Foley RN, Parfrey PS. Cardiovascular disease and mortality in ESRD. Journal of Nephrology [Internet]. 1998;**11**(5):239-245. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9831236 [Accessed: Sep 14, 2017]

[45] Selamet U, Tighiouart H, Sarnak MJ, Beck G, Levey AS, Block G, et al. Relationship of dietary phosphate intake with risk of end-stage renal disease and mortality in chronic kidney disease stages 3-5: The modification of diet in renal disease study. Kidney International [Internet]. Jan 2016;**89**(1):176-184. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26422502 [Accessed: Sep 13, 2017];

[46] WNT/β-catenin signaling promotes VSMCs to osteogenic transdifferentiation and calcification through directly modulating Runx2 gene expression. Experimental Cell Research [Internet]. Jul 15, 2016;**345**(2):206-217. Available from: http://www.sciencedirect.com/science/article/pii/S0014482716301471?via%3Dihub [Accessed: Sep 13, 2017]

[47] Deng D, Diao Z, Han X, Liu W. Secreted frizzled-related protein 5 attenuates high phosphate-induced calcification in vascular smooth muscle cells by inhibiting the Wnt/ß-catenin pathway. Calcified Tissue International [Internet]. Jul 19, 2016;**99**(1):66-75. Available from: http://link.springer.com/10.1007/s00223-016-0117-7 [Accessed: Sep 13, 2017]

[48] Pepe J, Diacinti D, Fratini E, Nofroni I, D'Angelo A, Pilotto R, et al. High prevalence of abdominal aortic calcification in patients with primary hyperparathyroidism as evaluated by Kauppila score. European Journal of Endocrinology [Internet] Aug 1, 2016; **175**(2):95-100. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27165861 [Accessed: Sep 13, 2017]

[49] Paloian NJ, Leaf EM, Giachelli CM. Osteopontin protects against high phosphate-induced nephrocalcinosis and vascular calcification. Kidney International [Internet]. May 2016;**89**(5):1027-1036. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0085253816002854 [Accessed: Sep 13, 2017]

[50] Amann K. Media calcification and intima calcification are distinct entities in chronic kidney disease. Clinical journal of the American Society of Nephrology [Internet]. Nov 1, 2008;**3**(6): 1599-1605. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18815240 [Accessed: Sep 13, 2017]

[51] Osteoprotegerin, vascular calcification and atherosclerosis. Atherosclerosis [Internet]. Jun 1, 2009;**204**(2):321-329. Available from: http://www.sciencedirect.com/science/article/pii/S0021915008006916 [Accessed: Sep 13, 2017]

[52] Rong S, Zhao X, Jin X, Zhang Z, Chen L, Zhu Y, et al. Vascular calcification in chronic kidney disease is induced by bone morphogenetic protein-2 via a mechanism involving the Wnt/β-catenin pathway. Cellular Physiology and Biochemistry [Internet]. 2014;**34**(6): 2049-2060. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25562153 [Accessed: Sep 13, 2017]

[53] Yao Y, Bennett BJ, Wang X, Rosenfeld ME, Giachelli C, Lusis AJ, et al. Inhibition of bone morphogenetic proteins protects against atherosclerosis and vascular calcification. Circulation Research [Internet]. Aug 20, 2010;**107**(4):485-494. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20576934 [Accessed: Sep 13, 2017]

[54] Dietary vitamin K and therapeutic warfarin alter the susceptibility to vascular calcification in experimental chronic kidney disease. Kidney International [Internet]. May 1, 2013;**83**(5):835-844. Available from: http://www.sciencedirect.com/science/article/pii/S0085253815558274 [Accessed: Sep 13, 2017]

[55] Kopecek J. Hydrogels: From soft contact lenses and implants to self-assembled nanomaterials. Journal of Polymer Science Part A: Polymer Chemistry [Internet]. Nov 15, 2009;**47**(22):5929-5946. Available from: http://doi.wiley.com/10.1002/pola.23607 [Accessed: Sep 13, 2017]

[56] Louvet L, Buchel J, Steppan S, Passlick-Deetjen J, Massy ZA. Magnesium prevents phosphate-induced calcification in human aortic vascular smooth muscle cells. Nephrology Dialysis Transplantation [Internet]. Apr 1, 2013;**28**(4):869-878. Available from: https://academic.oup.com/ndt/article-lookup/doi/10.1093/ndt/gfs520 [Accessed: Sep 13, 2017]

[57] Kircelli F, Peter ME, Sevinc Ok E, Celenk FG, Yilmaz M, Steppan S, et al. Magnesium reduces calcification in bovine vascular smooth muscle cells in a dose-dependent manner. Nephrology Dialysis Transplantation [Internet]. Feb 1, 2012;**27**(2):514-521. Available from: https://academic.oup.com/ndt/article-lookup/doi/10.1093/ndt/gfr321 [Accessed: Sep 13, 2017]

[58] Clarke MCH, Littlewood TD, Figg N, Maguire JJ, Davenport AP, Goddard M, et al. Chronic apoptosis of vascular smooth muscle cells accelerates atherosclerosis and promotes calcification and medial degeneration. Circulation Research [Internet]. 2008;**102**(12). Available from: http://circres.ahajournals.org/content/102/12/1529.full [Accessed: Sep 13, 2017]

[59] Magnesium intake is inversely associated with coronary artery calcification: The Framingham heart study. JACC: Cardiovascular Imaging [Internet]. Jan 1, 2014;**7**(1):59-69. Available from: http://www.sciencedirect.com/science/article/pii/S1936878X1300778X [Accessed: Sep 13, 2017]

[60] Spronk HMH, Soute BAM, Schurgers LJ, Thijssen HHW, De Mey JGR, Vermeer C, et al. Tissue-specific utilization of menaquinone-4 results in the prevention of arterial calcification in warfarin-treated rats. Journal of Vascular Research [Internet]. Jan 29, 2004;**40**(6):531-537. Available from: http://www.karger.com/?doi=10.1159/000075344 [Accessed: Sep 13, 2017]

[61] Shea MK, O'Donnell CJ, Hoffmann U, Dallal GE, Dawson-Hughes B, Ordovas JM, et al. Vitamin K supplementation and progression of coronary artery calcium in older men and women. American Journal of Clinical Nutrition [Internet]. Jun 1, 2009;**89**(6):1799-1807. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19386744 [Accessed: Sep 13, 2017]

[62] Vasquez ES, Cunningham JL, McMahan JB, Simpson CL, Walters KB. Fetuin-A adsorption and stabilization of calcium carbonate nanoparticles in a simulated body fluid. Journal of Materials Chemistry B [Internet]. Jul 29, 2015;**3**(31):6411-6419. Available from: http://xlink.rsc.org/?DOI=C5TB00565E [Accessed: Sep 13, 2017]

[63] Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Böhm R, Metzger T, Wanner C, Jahnen-Dechent W, Floege J. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: A cross-sectional study. Lancet [Internet]. Mar 8, 2003;**361**(9360):827-833. Available from: http://www.sciencedirect.com/science/article/pii/S0140673603127109 [Accessed: Sep 13, 2017]

[64] Huisman J, van der Heijden LC, Kok MM, Danse PW, Jessurun GA, Stoel MG, can Houwelingen KG, Löwik MM, Hautvast RW, IJzerman MJ, Doggen CJ, von Birgelen C. Impact of severe lesion calcification on clinical outcome of patients with stable angina, treated with newer generation permanent polymer-coated drug-eluting stents: A patient-level pooled analysis from TWENTE and DUTCH PEERS (TWENTE II). American Heart Journal [Internet]. May 1, 2016;**175**:121-129. Available from: http://www.sciencedirect.com/science/article/pii/S0002870316000636 [Accessed: Sep 13, 2017]

[65] Zeller T, Baumgartner I, Scheinert D, Brodmann M, Bosiers M, Micari A, Peeters P, Vermassen F, Landini M, Snead DB, Kent KC, Rocha-Singh KJ; IN.PACT DEEP Trial Investigators. Drug-eluting balloon versus standard balloon angioplasty for infrapopliteal arterial revascularization in critical limb ischemia: 12-Month results from the IN.PACT DEEP randomized trial. Journal of the American College of Cardiology [Internet]. Oct 14, 2014;**64**(15):1568-1576. Available from: http://www.sciencedirect.com/science/article/pii/S0735109714057143 [Accessed: Sep 13, 2017]

[66] Kinstner CM, Lammer J, Willfort-Ehringer A, Matzek W, Gschwandtner M, Javor D, Funovics M, Schoder M, Koppensteiner R, Loewe C, Ristl R, Wolf F. Paclitaxel-eluting balloon versus standard balloon angioplasty in in-stent restenosis of the superficial femoral and proximal popliteal artery: 1-Year results of the PACUBA trial. JACC: Cardiovascular Interventions [Internet]. Jul 11, 2016;**9**(13):1386-1392. Available from: http://www.sciencedirect.com/science/article/pii/S1936879816304253 [Accessed: Sep 13, 2017]

[67] Gao L, Chen Y-D. Application of drug-coated balloon in coronary artery intervention: Challenges and opportunities. Journal of Geriatric Cardiology [Internet]. Nov 2016;**13**(11):906-913. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28133467 [Accessed: Sep 13, 2017]

[68] Grinia M. Nogueira, Andrea C.D. Rodas, Raquel F. Weska, Cassiano G. Aimoli, Olga Z. Higa, Marina Maizato, Adolfo A. Leiner, Ronaldo N.M. Pitombo, Bronislaw Polakiewicz MMB. Bovine pericardium coated with biopolymeric films as an alternative to prevent calcification: In vitro calcification and cytotoxicity results. Materials Science and Engineering C [Internet]. May 10, 2010;**30**(4):575-582. Available from: http://www.sciencedirect.com/science/article/pii/S0928493110000299 [Accessed: Sep 14, 2017]

[69] Onuki Y, Bhardwaj U, Papadimitrakopoulos F, Burgess DJ. A review of the biocompatibility of implantable devices: Current challenges to overcome foreign body response. Journal of Diabetes Science and Technology [Internet]. 2008;**2**(6):1003-1015. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2769826&tool=pmcentrez&rendertype=abstract

[70] Schoen F and Levy R. Pathological calcification of biomaterials. In: Ratner B, Hoffman A, Shoen F, Lemons J, editors. Biomaterials Science: An Introduction to Materials in Medicine. Waltham (MA): Elsevier; 2013. pp. 739-749

[71] Orabi H, Rousseau A, Laterreur V, Bolduc S. Optimization of the current self-assembled urinary bladder model: Organ-specific stroma and smooth muscle inclusion. Canadian Urological Association Journal [Internet]. 2015;**9**(9-10):E599-E607. Available from: internal-pdf://222.205.202.33/Orabi-2015-Optimization of the current self-as.pdf%5Cn http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4581925/pdf/cuaj-9-10-e599.pdf

Chapter 4

# Physical Protection of Pancreatic Islets for Transplantation

Sarah Lee, Mayilone Sathialingam,
Michael Alexander and Jonathan Lakey

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71285

**Abstract**

Type 1 diabetes is an autoimmune disorder that destroys the insulin producing cells of the pancreas. The mainstay of treatment is replacement of insulin through injectable exogenous insulin. Improvements in islet isolation techniques and immunosuppression regimens have made islet transplants a treatment options for select patients. Islet transplants have improved graft function over the years, however, graft function beyond year two is rare and notably these patients require immunosuppression to prevent rejection. Cell encapsulation has been proposed for numerous cell types but it has found increasing enthusiasm for islets. Since islet transplants have experienced a myriad of success the next step is to improve graft function and avoid systemically toxic immunosuppressive regimens. Cell encapsulation hopes to accomplish this goal. Encapsulation involves encasing cells in a semipermeable biocompatible hydrogel that allows the passage of nutrients and oxygen however blocks immune regulators from destroying the cell thus avoiding systemic drugs. Several advances in encapsulation engineering and cell viability promises to make this a revolutionary discovery. In this chapter, we will provide a review of islet encapsulation as used for the treatment of type 1 diabetes.

**Keywords:** biomaterial, islet encapsulation, type 1 diabetes, islet transplantation, immune barrier

## 1. Introduction

Islet transplantation to treat type 1 diabetes has achieved great improvements, as more recipients are able to achieve insulin independence for longer periods of time. Unfortunately, the lack of donor organs and immunosuppressive medication regimens continue to impede further progress in cell replacement therapy. Encapsulation of islets for transplantation

provides a solution to these problems. Cell encapsulation envelopes cells in a biocompatible matrix that provides a gradient which allows the diffusion of oxygen and nutrients but prevents large immune molecules from reaching the cell, avoiding host immune response. Encapsulation has been suggested since the 1930s, but noteworthy achievements have occurred over the last decade. This chapter aims to provide a review including a historical background, current research, and future applications of cell encapsulation for the treatment of type 1 diabetes.

## 2. History

Over 25 million people in the United States (US) suffer from diabetes with approximately 5% characterized as type 1, and diabetes is ranked as the 7th leading cause of death in the US [1]. Type 1 diabetes mellitus (T1DM) is an autoimmune disease that causes destruction of the β-cells of the pancreas, which results in insulin deficiency [2]. Currently, the mainstay of treatment is short-term glycemic control through injectable exogenous insulin. However, as islet transplantation has been continually improving, the scientific community has shifted views of curing T1DM toward cell replacement therapy rather than supportive care. Islet transplantation was recognized as a promising field in restoring long-term endogenous insulin production when in 1999, under the Edmonton Protocol, a total of 21 subjects out of 36 were able to achieve long-term glycemic control and insulin independence upon transplantation of islets in the portal vein and the insulin independence prolonged up to 2 years [3]. According to the Collaborative Islet Transplant Registry (CITR), there have been a total of 677 islet transplant recipients from 1999 to 2010 and the percentage of recipients that achieve insulin independence for 3 years was 44% between 2007 and 2010 compared to 27% from 1999 to 2002 [4]. Various immunosuppressive regimens have been implemented to avoid rejection and maintain graft function. However, like other organ transplants, immunosuppressive medications are implicated in adverse effects to the patient as well as toxicity to the graft [5, 6].

Additionally, the current method for islet transplantation requires invasive, difficult, and time-consuming surgeries that create stress and risk for both the patients and the islets. To circumvent these issues, cell encapsulation has been proposed as the next treatment option for islet transplants with the goal of eliminating immunosuppression. Although cell encapsulation was tested to treat other diseases such as neurodegenerative diseases and epilepsy, the greatest achievement using this method has been in the encapsulation of islets for the treatment of T1DM [7–9]. Insulin independence can successfully be achieved through the transplantation of isolated islets; to ensure that the islets can remain effective and functioning, improvements in graft viability, difficulty of procedures, and the avoidance of systemically toxic drugs can be accomplished through encapsulation [10]. In the following sections, we will discuss the more recent advances in encapsulated islet technology.

## 3. Current research

### 3.1. Animal and human trials

The first researcher to pioneer transplantation of encapsulated tissue was Biscegeli in 1933. He placed mouse tumor cells in a polymer matrix which he transplanted into the abdomen of a guinea pig and was able to maintain the host's survival without rejection [11]. This idea was replicated not until 50 years later when Lim and Sun first used encapsulated islets for transplantation in diabetic animals. They placed 2000–3000 islet equivalent (IEQ) in an alginate hydrogel for intraperitoneal transplantation of diabetic rats to achieve normoglycemia for up to 3 weeks compared to only 8 days for nonencapsulated islets [12]. Currently, there are a myriad of achievements in encapsulating islets in small and large animal studies as well as early phase clinical trials. In syngeneic transplantation (NOD mice) studies by Kobayashi et al. in 2003, the authors used a 5% agarose microcapsule encasing 1500–2000 islet equivalents (IEQ) per mouse for intraperitoneal implantation as well as omental pouch transplants, and observed prolonged euglycemia for 100 days compared to 8 days for unencapsulated islet transplants [13]. The same authors repeated the study in 2006 and observed the same period of euglycemia in the recipients; however, when they also retrieved the devices after 400 days, they observed that viable islets were recovered with a small percentage of necrotic cells [14].

In a more recent study of murine models, Nishimura et al. carried out a two-part experiment; the first experiment involved transplant of encapsulated porcine islets into the intraperitoneal cavities of streptozotocin-induced diabetic nude mice (n = 4) and observed insulin independence in all mice for 2 months. Then, to observe the long-term effects, the second part of the experiment involved the same procedure but required monitoring up to 6 month, and all mice had maintained insulin independence for the duration of the experiment although C-peptide levels were low for both experiments [14]. Encapsulation methods have improved over time, as shown in studies by Haque et al. in 2017, where non-human primate islets were encapsulated with polyethylene glycol (PEG) and confirmed functioning when transplanted into C57BL/6 and BALB/c mice. It was also observed that when compared to the naked islet transplant, the encapsulated islets had shown no graft rejection for up to 150 days [15].

Less consistent but otherwise noteworthy results were achieved in islet transplants in larger animal models. Initially, Soon-Shiong performed several encapsulated islet transplants into diabetic canine models. According to the publication from 1993, islets of 1500–20,000 IEQ/kg were encapsulated in alginate-based microcapsules and transplanted into the intraperitoneal cavity; subjects gained insulin independence for 110 days as well as the presence of C-peptide for an average of 483 days [16]. In 2010, Dufrane used porcine encapsulated islets (subcutaneous/kidney capsule transplants of alginate-based micro- and macro-encapsulated islets, 30,000 IEQ/kg) to transplant into cynomolgus primates. The authors observed euglycemia for up to 28 weeks [17]. In another study using cynomolgus monkeys as recipients by Elliott et al., neonatal pig islets were isolated (10,000 IEQ/kg) and encapsulated in alginate microcapsules resulting in a more than 40% reduction in injectable insulin dose compared to pre-implantation [18]. Based

on several noteworthy achievements in large animal studies, researchers have been granted approval for stage one and two human clinical trials. Due to the previous success by Soon-Shiong using a canine model the authors were authorized for the first human clinical trial in 1994. A 38-year-old male, with type 1 diabetes and end stage renal disease postoperative to kidney transplant and with low dose immunosuppression, became the first recipient of encapsulated islets. The patient initially received 10,000 IEQ/kg of cadaveric islets encapsulated in alginate microcapsules, followed by a repeat transplant of 5000 IEQ/kg 6 months later. The patient's insulin requirements reduced to 1–2 insulin units per day and eventually discontinued all exogenous insulin administration after 9 months [19]. In 2006, Calafiore et al. isolated islets from human cadavers (400,000–600,000 IEQ) and encapsulated them with sodium-alginate beads for intraperitoneal injection. As a result, the patients experienced improved blood glucose levels and a declined daily exogenous insulin intake; however, neither patient became insulin independent [20].

Living cell technologies has achieved the best outcomes for encapsulated islet transplants. In one of their studies, islets derived from pigs in a pathogen-free farm in New Zealand were encapsulated in alginate microcapsules for intraperitoneal xenotransplantation into human recipients. Several early phase clinical trials have been performed from this company and they have shown promising results. One of the most significant achievement has been the reduction of accidental hypoglycemic events to 40%. Several patients improved daily glucose levels and reduced exogenous insulin dosing and 2 patients became insulin independent after 4 months [21]. Despite the promising results, the lack of reproducibility threatens enthusiasm for future advances. For example, a human clinical trial by Tuch et al. involved alginate microcapsules for human cadaveric islets and observed the presence of plasma C-peptide levels for up to 2.5 years, however, there was no improvement in insulin requirements [22]. Likewise, in a follow-up publication by Elliot et al., one recipient experienced early success with a 30% reduction in insulin dose, but after 49 weeks, the subject reverted back to the original insulin dose [21]. The most recent study in 2016 by Matsumoto et al. which involved 8 human subjects, they did not observe statistically significant changes to the reduction of exogenous insulin doses; however, the group that received higher dose of islets had improved HbA1c (<7%) for a duration of 600 days, and had significantly reduced the frequency of unaware hypoglycemic events [23]. The study shows that despite the lack of insulin-secreting capabilities, as compared to exogenous injections which potentially and frequently cause accidental hypoglycemic events, the encapsulated islets are capable of a more regulated release of insulin. Although there has been a consistent observation of viability and immunoprotection of encapsulated islets, the efficacy of enabling a subject's insulin independence in-vivo is still being researched.

Although the purpose of aforementioned early phase clinical trials is to assure safety and determine optimal dosing, it is notable that most encapsulated islet recipients do not achieve perfectly sustainable insulin independence. There is also yet to be a standardized protocol for the type of biomaterial used and the islet dose to be transplanted. However, based on novel in-vivo studies, it is evident that the type of biomaterial determines graft survival. King et al. tested several encapsulation methods for mice recipients using alginate with and without poly L-lysine (PLL), with high guluronic acid (G) or high mannuronic acid (M) and revealed

that PLL-free high M microcapsules had better results, with continuous normoglycemia for 8 weeks [24]. Likewise, Lanza et al. observed that capsule integrity and graft function could be improved by altering the concentration of alginate [25]. More recently, hyaluronic acid and collagen hydrogels (HA-COL gel), which have been steadily gaining recognition in tissue engineering, have taken a new turn and started emerging as a potential alternative to alginate for encapsulation material for islets. In 2016, Harrington et al. had proposed that HA-COL gels' biological and mechanical properties can be applied to encapsulating methods of islets as well, and in the study, they observed that the allogeneic transplantation of islets in HA-COL gels into diabetic rats had reversed diabetes and remained such for 80 weeks, all while providing immunoprotection for islets and increasing viability [26]. Discussions regarding the most optimal material for encapsulation of islet are still ongoing, and a definite consensus is yet to be reached.

### 3.2. Biomaterials in transplantation

Chang et al. were the first researchers to describe the application of semipermeable membranes for encapsulation. Chang postulated that polymer membrane-encapsulated liver enzymes and cells can be transplanted to treat a disorder [27]. Several types of encapsulation methods have been developing and currently the most widely employed method is the alginate-based microencapsulation [24, 28–30].

The capsule vehicles come in variety of structures and sizes, ranging between vascular shunts, macro-, micro-, and nanoscale devices. The original vascular device was developed as capillary fibers in culture-coated medium [31]. Maki et al. performed studies with vascular devices as arteriovenous shunts transplanted in diabetic canines. As a result, several subjects were able to achieve reduced exogenous insulin requirements [32, 33]. Ultimately, the major difficulty in utilizing the devices was the inability to provide enough islets to coat the fibers. This device type was able to achieve reduced exogenous insulin requirements [32, 33].

Devices were constructed in hopes of including more islets by elongating fibers, but complications arose due to clotting and fibrosis. To solve this issue, researches used greater amounts of islets and multiple devices in order to achieve insulin independence, but this method was eventually disused due to its high cost and relative inefficiency [34]. However, a 2017 study demonstrated the potential of human recombinant antithrombin (ATryn®), when co-administered with pancreatic islets, to reduce inflammation and intravascular coagulation in transplant recipients without posing a risk to the islets or test subjects [35]. In general, macroscale devices are not as commonly used among researchers because their increased immunogenicity and larger diffusion parameters required for oxygen and nutrients to reach islet cells lead to poor islet viability, function, and regenerative capacity [36]. However, such macroscale devices can offer several advantages, including simple implantation and retrievability using minimally invasive techniques [37]. One recent study elaborated on the ability of alginate sheets to promote vascularization and blood flow to areas of implanted sheets in mice due to a robust vascular response in the host. In response to increased blood flow and

consequently more oxygen and nutrients reaching the islets, the islets in the alginate sheets maintained high viability and function [38]. In a later attempt to improve the diffusion of nutrients, glucose, and insulin in cells in macroscale devices, a novel macroencapsulation device was developed in which islets were placed in thin, nondegradable, microwell membranes of the device. The results reveal that the device was effective in maintaining islet responsiveness and function in cell culture. However, future studies need to be conducted to test the in-vivo success of such a device [39].

Nanoencapsulation has been used to improve diffusion parameters and better islet insulin response. PEG is one of the most common materials used in nanoencapsulation devices as it can crosslink under UV or visible light exposure without threatening cell viability. Nevertheless, the shortcomings of PEG include the lack of biocompatibility with the transplant recipient and inadequate protection of islets from cytokines [40]. However, by using multi-layer PEGylation and immunosuppressive drug cocktails, islets have demonstrated increased stability and longer survival time while minimizing the immune response, as indicated by the reduction in human serum albumin, fibronectin, and immunoglobulin G [41]. In a 2017 study, majority of diabetic animals that received a transplant of PEGylated islets exhibited long-term normoglycemia [42]. Thus, despite its shortcomings, PEG encapsulation has still yielded positive results in certain studies.

Despite the success of nanoencapsulation, microencapsulation still is the most widespread method of islet encapsulation due in part to their improved surface area to volume ratio and mechanical stability. Though biocompatibility issues still exist with microencapsulation, the spherical shape and spatial characteristics of microcapsules can promote the diffusion of nutrients while limiting the host immune attacks on transplanted islets [43, 44]. One major obstacle standing in the way of sustainable and consistent clinical islet transplant is the lack of an optimal cell encapsulation approach, particularly the ideal transplantation site, encapsulation material and encapsulation device. Such standardization, as well as safety and cost effectiveness, are imperative for future widespread clinical use. Numerous tests have been conducted regarding various encapsulation materials and methods, each study showcasing the effectiveness of different encapsulation materials [43].

Both synthetic agents, from poly ethylene oxide to poly vinyl alcohol, and natural occurring hydrogels, like gelatin, chitosan, and alginate, have been utilized in encapsulation engineering and in extracellular matrixes [45–47]. Though poly glycolic and lactic acid polymers are some of the more popular synthetic agents in medical devices, they still pose the risk of increased fibrosis and loss of the encased cells. Nevertheless, synthetic biomaterials are still being frequently used, with PEG being the most widely used synthetic biomaterial for islet encapsulation, though different encapsulation strategies have varying levels of success. Such strategies include assembling a thin layered PEG-lipid structure around the surface of islets and assembling a multilayer film around islets using biotin and streptavidin [48]. It has been recently demonstrated that the simple PEGylation of islets provided modest immunoprotection in full MHC mismatched mice [42]. However, when coupled with the systematic distribution of immunosuppressive drugs, the PEGylated islets could sustain long-term normoglycemia in the mice.

Due to the complications with islet encapsulation using synthetic materials, alginate encapsulation has risen in popularity due to its improved biocompatibility and stability, easy gelation process, and relatively low cost. Alginate has typically been the most popular microencapsulation material, due to its widespread availability and ease of production, although alginate endotoxin content and purity can vary from different manufacturers [44]. The variation in alginate production and purification, in addition to the lack of research regarding the optimal transplantation site of islets and optimal donor strain and age, currently stand in the way of consistent success in transplanting alginate encapsulated islets into humans [49]. In an effort to improve capsule permeability and mechanical strength, studies have used polycations and anions in the encapsulation process, although it often results in a greater host biologic response to the transplant. To resolve this issue, one study discovered that this immune response can be minimized with the addition of another thin layer of alginate [50]. During the process of gelation, cross linking occurs via covalent, ionic, or physical bonds, which subsequently establishes the diffusion gradient for the adequate flow of nutrients and oxygen to the islet cells. Previously, problems with crosslinking arose in regard to such capsules having smaller pore size with inconsistent permselectivity [50]. However, in a recent study, alginate capsules crosslinked with $BaCl_2$ and suspended in chitosan showed similar pore size, function, and viability in vitro when compared to regular alginate capsules. When islets were encapsulated in the chitosan-coated crosslinked capsules and transplanted into mice and canines, the islets improved in graft survival and had significantly less fibrosis when compared to regular alginate capsules at 1 year post-transplantation [51].

When engineering scaffolds for islets, there are multiple considerations that need to be taken before standardizing a procedure that is both practical and safe for the islets and host. The capsules need to be non-toxic and reproducible, and their degradation should not negatively affect the islets or host, but rather following tissue growth.

Earlier experiments regarding capsule construction encountered problems with capsule fibrosis and low islet viability and function. For example, extensive capsule fibrosis was a common occurrence in these early experiments [22, 52]. Subsequent studies were able to eliminate the presence of fibrosis, but at the expense of abundant necrotic islets due to inadequate oxygen flow to the transplanted islets [53, 54]. However, multiple potential solutions have been explored since these issues were encountered. As a preliminary solution to high capsule fibrosis and lack of oxygen supply for transplanted islets, a group of scientists created a highly-vascularized bioartificial cavity using polylactide-based scaffolds for islet transplantation and implanted it under the skin and in the omentum of rats. After 4 weeks, histological analysis revealed heightened vascularization with minor fibrosis and minimal infiltration of inflammatory cells near the implant [55]. In a different experiment aimed to improve the oxygen flow to transplanted islets, a bioartificial pancreas underscored the potential of HEMOXCell® as an oxygen carrier for islets in vitro. HEMOXCell® was able to increase cell viability and decrease hypoxia indicators while restoring insulin secretion back to normal levels [56].

Other researchers have gone on to improve capsule engineering by means of co-encapsulation and stem cells to reduce instances of inflammation and fibrosis while sustaining islet function.

### 3.3. Improved capsule engineering: Co-encapsulation

Co-encapsulation methods hope to enhance the viability and function of islets by adding molecules to the capsules surrounding the cells. The essential purpose of encapsulation is to suppress the host animal's immune response to reduce inflammation and increase the survival of islets. In a novel study, the co-encapsulation of HMGB1 A box protein, an inflammation receptor antagonist, with islets provided a protective effect in islet transplants; the results showed an a 2-fold improvement in the survival rate of such co-encapsulated islets in diabetic mice. These islets were similar in bead diameter, viability, and insulin secretion function to islets encapsulated in only alginate [57]. In another study investigating co-encapsulation techniques to reduce inflammation, scientists conducted a subcutaneous screening of 16 small anti-inflammatory drugs to see each drug's corresponding effect on the formation of fibrotic cell layers, of which dexamethasone and curcumin showed to be most effective. Subsequently, pancreatic rat islets were co-encapsulated with curcumin in alginate microcapsules, resulting in an increase glycemic control and reduced instances of fibrosis in diabetic mice [58]. Clearly, co-encapsulation can be effective in reducing the recipient's immune response. However, researchers have not limited themselves to only co-encapsulation research, as recent developments in encapsulation cell technology involves the use of stem cells as a source of islets.

### 3.4. Stem cells

Due to the lack of human cadaveric donors for islet transplants, stem cells provide a promising alternative for islet transplants. In a study by Vaithilingam, mesenchymal stem cells were stimulated by a cytokine cocktail of IFN-$\gamma$ and TNF-$\alpha$ and subsequently were co-encapsulated with islets and transplanted into mice. All of the mice attained normoglycemia, as opposed to just 9.1% of the mice that received alginate encapsulated islets. In another study, mice receiving co-encapsulated stimulated MSCs also demonstrated improved viability and function with significantly less inflammatory cytokines, a significant step forward in the search to optimize encapsulated islet transplants [59]. A minimal immune response was also achieved in abdominal transplants using MSC islets coated luciferase-GFP, with reduced fibrotic formation and macrophage infiltration. Furthermore, islet endothelial cells formed chimeric blood vessel in the surrounding tissue with the transplant recipient's cells, due to the presence of MSC [60]. In another study, pancreatic islets were encapsulated using a silk-based platform including MSCs, resulting in the reduction of Th1 cytokines and improvement in blood glucose response. However, future experiments need to evaluate the viability of the islets and methods to strengthen the biocompatibility of silk for transplantation purposes [61]. Most importantly, a recent study demonstrated that an autologous stem cell transplant was able to accomplish long-term sustainable insulin independence in humans diagnosed with T1D, a phenomenon that had not occurred in previous decades [62]. Findings like these demonstrate the vast potential of stem cells in islet transplants, with more improvements bound to occur in the upcoming years.

## 4. Conclusion

Recent discoveries in the cell encapsulation of islets have made great strides in the pursuit to revolutionize the current treatment for T1DM. These studies have made significant

advancements to improve islet viability, function, and insulin response to higher blood glucose levels in transplanted islets. However, multiple obstacles still stand in the way of standardizing a method to allow widespread clinical use of this technology. Nevertheless, improvements in islet encapsulation materials and methods, as well as the growing potential of co-encapsulation and stem cell use for islet transplants provide a promising future in this field of research.

## Author details

Sarah Lee[1], Mayilone Sathialingam[1], Michael Alexander[1] and Jonathan Lakey[1,2]*

* Address all correspondence to: jlakey@uci.edu

1 Department of Surgery, University of California Irvine, Orange, CA, USA

2 Department of Biomedical Engineering, University of California Irvine, Irvine, CA, USA

## References

[1] Centers for Disease Control and Prevention. National Diabetes Statistics Report. Atlanta, GA: US Department of Health and Human Services; 2017. p. 2017

[2] American Diabetes A. Economic costs of diabetes in the U.S. in 2012. Diabetes Care. 2013;**36**:1033-1046

[3] Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, et al. International trial of the Edmonton protocol for islet transplantation. The New England Journal of Medicine. 2006;**355**:1318-1330

[4] Barton FB, Rickels MR, Alejandro R, Hering BJ, Wease S, Naziruddin B, et al. Improvement in outcomes of clinical islet transplantation: 1999-2010. Diabetes Care. 2012;**35**:1436-1445

[5] Niclauss N, Bosco D, Morel P, Giovannoni L, Berney T, Parnaud G. Rapamycin impairs proliferation of transplanted islet beta cells. Transplantation. 2011;**91**:714-722

[6] Hafiz MM, Faradji RN, Froud T, Pileggi A, Baidal DA, Cure P, et al. Immunosuppression and procedure-related complications in 26 patients with type 1 diabetes mellitus receiving allogeneic islet cell transplantation. Transplantation. 2005;**80**:1718-1728

[7] Eriksdotter-Jonhagen M, Linderoth B, Lind G, Aladellie L, Almkvist O, Andreasen N, et al. Encapsulated cell biodelivery of nerve growth factor to the basal forebrain in patients with Alzheimer's disease. Dementia and Geriatric Cognitive Disorders. 2012;**33**:18-28

[8] Jeon Y. Cell based therapy for the management of chronic pain. Korean Journal of Anesthesiology. 2011;**60**:3-7

[9] Bachoud-Levi AC, Deglon N, Nguyen JP, Bloch J, Bourdet C, Winkel L, et al. Neuroprotective gene therapy for Huntington's disease using a polymer encapsulated BHK cell line engineered to secrete human CNTF. Human Gene Therapy. 2000;**11**:1723-1729

[10] Shapiro AM, Nanji SA, Lakey JR. Clinical islet transplant: Current and future directions towards tolerance. Immunological Reviews. 2003;**196**:219-236

[11] Tomaro-Duchesneau C, Saha S, Malhotra M, Kahouli I, Prakash S. Microencapsulation for the therapeutic delivery of drugs, live mammalian and bacterial cells, and other biopharmaceutics: Current status and future directions. Journal of Pharmacology (Cairo). 2013;**2013**:103527

[12] Lim F, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. Science. 1980;**210**:908-910

[13] Kobayashi T, Aomatsu Y, Iwata H, Kin T, Kanehiro H, Hisanaga M, et al. Indefinite islet protection from autoimmune destruction in nonobese diabetic mice by agarose microencapsulation without immunosuppression. Transplantation. 2003;**75**:619-625

[14] Kobayashi T, Aomatsu Y, Iwata H, Kin T, Kanehiro H, Hisanga M, et al. Survival of microencapsulated islets at 400 days posttransplantation in the omental pouch of NOD mice. Cell Transplantation. 2006;**15**:359-365

[15] Haque MR, Kim J, Park H, Lee HS, Lee KW, Al-Hilal TA, et al. Xenotransplantation of layer-by-layer encapsulated non-human primate islets with a specified immunosuppressive drug protocol. Journal of Controlled Release. 2017;**258**:10-21

[16] Soon-Shiong P, Feldman E, Nelson R, Heintz R, Yao Q, Yao Z, et al. Long-term reversal of diabetes by the injection of immunoprotected islets. Proceedings of the National Academy of Sciences of the United States of America. 1993;**90**:5843-5847

[17] Dufrane D, Goebbels RM, Gianello P. Alginate macroencapsulation of pig islets allows correction of streptozotocin-induced diabetes in primates up to 6 months without immunosuppression. Transplantation. 2010;**90**:1054-1062

[18] Elliott RB, Escobar L, Tan PL, Garkavenko O, Calafiore R, Basta P, et al. Intraperitoneal alginate-encapsulated neonatal porcine islets in a placebo-controlled study with 16 diabetic cynomolgus primates. Transplantation Proceedings. 2005;**37**(8):3505

[19] Soon-Shiong P, Heintz RE, Merideth N, Yao QX, Yao Z, Zheng T, et al. Insulin independence in a type 1 diabetic patient after encapsulated islet transplantation. Lancet. 1994;**343**:950-951

[20] Calafiore R, Basta G, Luca G, Lemmi A, Montanucci MP, Calabrese G, et al. Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: First two cases. Diabetes Care. 2006;**29**:137-138

[21] Elliott RB, Escobar L, Tan PL, Muzina M, Zwain S, Buchanan C. Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. Xenotransplantation. 2007;**14**:157-161

[22] Tuch BE, Keogh GW, Williams LJ, Wu W, Foster JL, Vaithilingam V, et al. Safety and viability of microencapsulated human islets transplanted into diabetic humans. Diabetes Care. 2009;**32**:1887-1889

[23] Matsumoto S, Abalovich A, Wechsler C, Wynyard S, Elliott RB. Clinical benefit of islet xenotransplantation for the treatment of type 1 diabetes. eBioMedicine. 2016;**12**:255-262

[24] King A, Lau J, Nordin A, Sandler S, Andersson A. The effect of capsule composition in the reversal of hyperglycemia in diabetic mice transplanted with microencapsulated allogeneic islets. Diabetes Technology & Therapeutics. 2003;**5**:653-663

[25] Lanza RP, Jackson R, Sullivan A, Ringeling J, McGrath C, Kuhtreiber W, et al. Xenotransplantation of cells using biodegradable microcapsules. Transplantation. 1999; **67**:1105-1111

[26] Harrington S, Williams J, Rawal S, Ramachandran K, Stehno-Bittel L. Hyaluronic acid/ collagen hydrogel as an alternative to alginate for long-term Immunoprotected islet transplantation. Tissue Engineering. Part A. 2017 [Epub ahead of print]

[27] Chang TM. Semipermeable microcapsules. Science. 1964;**146**:524-525

[28] Khanna O, Larson JC, Moya ML, Opara EC, Brey EM. Generation of alginate microspheres for biomedical applications. Journal of Visualized Experiments. 2012;**66**:e3388

[29] Krishnamurthy NV, Gimi B. Encapsulated cell grafts to treat cellular deficiencies and dysfunction. Critical Reviews in Biomedical Engineering. 2011;**39**:473-491

[30] Zimmermann H, Shirley SG, Zimmermann U. Alginate-based encapsulation of cells: Past, present, and future. Current Diabetes Reports. 2007;**7**:314-320

[31] Chick WL, Like AA, Lauris V. Beta cell culture on synthetic capillaries: An artificial endocrine pancreas. Science. 1975;**187**:847-849

[32] Maki T, Otsu I, O'Neil JJ, Dunleavy K, Mullon CJ, Solomon BA, et al. Treatment of diabetes by xenogeneic islets without immunosuppression. Use of a vascularized bioartificial pancreas. Diabetes. 1996;**45**:342-347

[33] Maki T, Ubhi CS, Sanchez-Farpon H, Sullivan SJ, Borland K, Muller TE, et al. Successful treatment of diabetes with the biohybrid artificial pancreas in dogs. Transplantation. 1991;**51**:43-51

[34] Lanza RP, Borland KM, Lodge P, Carretta M, Sullivan SJ, Muller TE, et al. Treatment of severely diabetic pancreatectomized dogs using a diffusion-based hybrid pancreas. Diabetes. 1992;**41**:886-889

[35] Gmyr V, Bonner C, Moerman E, Tournoys A, Delalleau N, Quenon A, et al. Human recombinant Antithrombin (ATryn(R)) administration improves survival and prevents intravascular coagulation after Intraportal islet transplantation in a piglet model. Cell Transplantation. 2017;**26**:309-317

[36] Song S, Roy S. Progress and challenges in macroencapsulation approaches for type 1 diabetes (T1D) treatment: Cells, biomaterials, and devices. Biotechnology and Bioengineering. 2016;**113**:1381-1402

[37] Fotino N, Fotino C, Pileggi A. Re-engineering islet cell transplantation. Pharmacological Research. 2015;**98**:76-85

[38] Krishnan R, Arora RP, Alexander M, White SM, Lamb MW, Foster CE 3rd, et al. Noninvasive evaluation of the vascular response to transplantation of alginate encapsulated islets using the dorsal skin-fold model. Biomaterials. 2014;**35**:891-898

[39] Skrzypek K, Groot Nibbelink M, van Lente J, Buitinga M, Engelse MA, de Koning EJP, et al. Pancreatic islet macroencapsulation using microwell porous membranes. Scientific Reports 2017;**7**:9186

[40] Jang JY, Lee DY, Park SJ, Byun Y. Immune reactions of lymphocytes and macrophages against PEG-grafted pancreatic islets. Biomaterials. 2004;**25**:3663-3669

[41] Haque MR, Jeong JH, Byun Y. Combination strategy of multi-layered surface camouflage using hyperbranched polyethylene glycol and immunosuppressive drugs for the prevention of immune reactions against transplanted porcine islets. Biomaterials. 2016;**84**:144-156

[42] Giraldo JA, Molano RD, Rengifo HR, Fotino C, Gattas-Asfura KM, Pileggi A, et al. The impact of cell surface PEGylation and short-course immunotherapy on islet graft survival in an allogeneic murine model. Acta Biomaterialia. 2017;**49**:272-283

[43] Omami M, McGarrigle JJ, Reedy M, Isa D, Ghani S, Marchese E, et al. Islet microencapsulation: Strategies and clinical status in diabetes. Current Diabetes Reports. 2017;**17**:47

[44] Desai T, Shea LD. Advances in islet encapsulation technologies. Nature Reviews. Drug Discovery. 2017;**16**:338-350

[45] Lee KY, Mooney DJ. Alginate: Properties and biomedical applications. Progress in Polymer Science. 2012;**37**:106-126

[46] Nicodemus GD, Bryant SJ. Cell encapsulation in biodegradable hydrogels for tissue engineering applications. Tissue Engineering. Part B, Reviews. 2008;**14**:149-165

[47] Lee KY, Mooney DJ. Hydrogels for tissue engineering. Chemical Reviews. 2001;**101**: 1869-1879

[48] Foster GA, Garcia AJ. Bio-synthetic materials for immunomodulation of islet transplants. Advanced Drug Delivery Reviews. 2017;**114**:266-271

[49] Krishnan R, Ko D, Foster CE 3rd, Liu W, Smink AM, de Haan B, et al. Immunological challenges facing translation of alginate encapsulated porcine islet xenotransplantation to human clinical trials. Methods in Molecular Biology 2017;**1479**:305-333

[50] O'Sullivan ES, Johnson AS, Omer A, Hollister-Lock J, Bonner-Weir S, Colton CK, et al. Rat islet cell aggregates are superior to islets for transplantation in microcapsules. Diabetologia. 2010;**53**:937-945

[51] Yang HK, Ham DS, Park HS, Rhee M, You YH, Kim MJ, et al. Long-term efficacy and biocompatibility of encapsulated islet transplantation with chitosan-coated alginate capsules in mice and canine models of diabetes. Transplantation. 2016;**100**:334-343

[52] Suzuki K, Bonner-Weir S, Trivedi N, Yoon KH, Hollister-Lock J, Colton CK, et al. Function and survival of macroencapsulated syngeneic islets transplanted into streptozocin-diabetic mice. Transplantation. 1998;**66**:21-28

[53] Xin ZL, Ge SL, Wu XK, Jia YJ, Hu HT. Intracerebral xenotransplantation of semipermeable membrane-encapsuled pancreatic islets. World Journal of Gastroenterology. 2005;**11**:5714-5717

[54] De Vos P, Van Straaten JF, Nieuwenhuizen AG, de Groot M, Ploeg RJ, De Haan BJ, et al. Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth? Diabetes 1999;**48**:1381-1388

[55] Kasoju N, Kubies D, Fabryova E, Kriz J, Kumorek MM, Sticova E, et al. In vivo vascularization of anisotropic channeled porous polylactide-based capsules for islet transplantation: The effects of scaffold architecture and implantation site. Physiological Research. 2015;**64**(Suppl 1):S75-S84

[56] Rodriguez-Brotons A, Bietiger W, Peronet C, Langlois A, Magisson J, Mura C, et al. Comparison of Perfluorodecalin and HEMOXCell as oxygen carriers for islet oxygenation in an in vitro model of encapsulation. Tissue Engineering. Part A. 2016;**22**:1327-1336

[57] Jo EH, Hwang YH, Lee DY. Encapsulation of pancreatic islet with HMGB1 fragment for attenuating inflammation. Biomaterial Research. 2015;**19**:21

[58] Dang TT, Thai AV, Cohen J, Slosberg JE, Siniakowicz K, Doloff JC, et al. Enhanced function of immuno-isolated islets in diabetes therapy by co-encapsulation with an anti-inflammatory drug. Biomaterials. 2013;**34**:5792-5801

[59] Vaithilingam V, Evans MDM, Lewy DM, Bean PA, Bal S, Tuch BE. Co-encapsulation and co-transplantation of mesenchymal stem cells reduces pericapsular fibrosis and improves encapsulated islet survival and function when allografted. Scientific Reports. 2017;**7**:10059

[60] Fransson M, Brannstrom J, Duprez I, Essand M, Le Blanc K, Korsgren O, et al. Mesenchymal stromal cells support endothelial cell interactions in an intramuscular islet transplantation model. Regenerative Medicine Research. 2015;**3**:1-11

[61] Hamilton DC, Shih HH, Schubert RA, Michie SA, Staats PN, Kaplan DL, et al. A silk-based encapsulation platform for pancreatic islet transplantation improves islet function in vivo. Journal of Tissue Engineering and Regenerative Medicine. 2017;**11**:887-895

[62] Cantu-Rodriguez OG, Lavalle-Gonzalez F, Herrera-Rojas MA, Jaime-Perez JC, Hawing-Zarate JA, Gutierrez-Aguirre CH, et al. Long-term insulin independence in type 1 diabetes mellitus using a simplified autologous stem cell transplant. The Journal of Clinical Endocrinology and Metabolism. 2016;**101**:2141-2148

Chapter 5

# Electrochemical Characterization of an Optical Fiber Laser-Treated Biomaterial

Eurico Felix Pieretti, Olandir Vercíno Corrêa,
Marina Fuser Pillis and
Maurício David Martins das Neves

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72653

**Abstract**

The implant manufacturing process includes texturization to enhance its adhesion and marking the final products for their identification, long-term quality control and traceability. Marking is carried out after cleaning and prior to sterilization. These marks eventually can concentrate stress leading to premature failure. The marked areas are defective regions that affect the passive film formed on the metallic biomaterials used for implants favoring the onset of various corrosion types, such as pitting, crevice or fatigue. This study aims to evaluate the effect of a Yb optical fiber laser marking processes used for metallic implants on the localized corrosion resistance of the austenitic stainless steel ISO 5832-1. This is one of the most used materials for manufacturing implants. The electrochemical behavior of the marked areas obtained by this method was evaluated in a phosphate-buffered saline (PBS) solution with pH of 7.4 and the results were compared with unmarked samples. All tested surfaces were prepared according to the recommendations for the use in surgery. For localized corrosion resistance evaluation, electrochemical tests such as monitoring the open circuit potential (OCP), electrochemical impedance spectroscopy (EIS) and cyclic potentiodynamic polarization measurements were performed. The results showed that the laser marks affect the protector characteristics of the biomaterial's passive film. Lower pitting resistance was associated to the laser marked areas.

**Keywords:** biomaterials, stainless steel, electrochemistry, laser, marking

## 1. Introduction

The increase in people's life expectancy and the rising number of accidents with serious injuries have raised the number of orthopedic surgeries worldwide. In order to help or replace injured

body parts, implants or prostheses are used to perform their duties properly. These materials must have adequate chemical composition and surface condition so they are not rejected by the body.

The material selection for biomedical application should take into consideration its physical, chemical and mechanical properties. The main properties that must be taken into account are: strength, elasticity modulus, bending and torsion, fatigue resistance, corrosion resistance and hardness [1, 2]. The manufacturing process of metallic implantable medical devices is one of the most important stages of the production of implants, and this can be via casting, forging, machining, welding or by powder metallurgy. This process also involves cleaning, surface finish, marking and sterilization, according to the strictest standards of quality control [1–3].

The implants when in contact with human tissue might suffer constant wear and corrosion, and the corrosion products may cause hypersensitivity, leading to the need for implant replacement surgery. These surgeries lead to increased costs and health risks for patients. Therefore, research and development of biomaterials with improved surfaces is of great interest [1–3].

Using materials to repair or restore damaged tissues or organs in humans is not new. The first reports of biomaterial use have 4000 years of existence, describing the use of sutures for repair of wounds. The reports of use of non-organic materials are from 1550, with the use of gold thread for sutures [1]. Earliest indications are also around this period, with the medical records of Hindu, Egyptian and Greek civilizations citing bone transplants from animals to humans.

Nowadays, advances in biomedical engineering and surgery have made possible the reconstruction of various parts of the human body with biomaterials. According to Williams [2], biomaterial is any substance or combination of substances, except drugs, naturally occurring or synthetic, that can be used during any period of time, as part of a system that treat, augment or replace any tissue, organ or body functions.

Biomaterials have many applications such as orthopedic for the repair or replacement of any part of the skeletal system. Metallic biomaterials are also used for neural or neuromuscular stimulation in electronic systems in order to provide electrical stimulation to tissues with certain degree of deterioration [4, 5]. The development of biomaterials appears to be fundamentally important in the sense that it provides an improved standard of living for the people, represented by an increase in life expectancy, general health and well-being of the population.

The metallic materials used in implants or prostheses are generally passive materials and therefore are subject to localized corrosion when in contact with body fluids. According to Lyman and Villamil [1, 6], the most common types of corrosion observed in metallic implants are: galvanic, crevice, pitting and selective corrosion.

Pitting corrosion is a localized attack on a surface covered with oxide. A pit is initiated by adsorption of anion activators, particularly chloride ions. When the pitting potential is reached, the electric field strength in the thinner parts of the film is so high that chloride ions can penetrate the film, starting localized dissolution of the oxide film. Thus, once a pit has formed, it will continue to grow autocatalytic [7–10].

The mechanism of pitting corrosion occurs by nucleation and growth, which creates conditions for its propagation, which are: the chloride ions enrichment in pitting, the generation of an acidic solution inside the pit, by hydrolysis of metal ions, high conductivity of salt solution,

the limited supply of oxygen and, outside of the pit, forming a layer of hydrate, with the dilution of pits solution by diffusion and convection [11–17].

The laser marking technique is commonly used for biomaterials classification or traceability enabling posterior analysis of the implantable metallic device after its use. The laser engraving process strongly affects the biomaterials' surface properties, especially those exposed to the corrosive human body fluids, such as microstructure, composition and roughness [18–25].

Qi et al. [26] studied the effects of laser engravings on stainless steels. They have used a pulsed, Q-switched Nd: YAG laser and evaluated the influence of pulse frequency, energy and speed on the final metallic surfaces quality. They compared the depth, width and contrast generated by the laser process and observed that the pulse frequency was the parameter that most affected surface oxidation, and consequently, the contrast of the surfaces subjected to this laser beam. Similarly, Leone et al. [27] verified that the laser pulse frequency was the parameter that most interfered in the contrast obtained on the digital images of the surfaces submitted to the engravings. They have used the same type of laser and the same parameters as Qi et al. [26]. The material that underwent the markings was an AISI 304 stainless steel. They have observed that the roughness and oxidation of the etched surfaces increased as a result of increasing the pulse frequency.

Bizi-Bandoki et al. [28] studied the changes in roughness and surface tension of the AISI 316 L stainless steel and the Ti-6Al-4 V alloy. A titanium-sapphire ultra-short pulse laser was used varying only the number of pulses. The researchers have found that as the number of pulses increased, the topographic features of both materials were also altered, producing surface undulations and their changing behavior from hydrophilic to hydrophobic.

## 2. Experimental procedures

The material used in this study was the austenitic stainless steel, ISO 5832-1, which is largely used for orthopedic implants. Its chemical composition is shown in **Table 1**. The tested surfaces were marked via pulsed nanosecond ytterbium (Yb) optical fiber laser, at four different pulse frequencies, as shown in **Table 2**. For comparison reasons, unmarked surface of this biomaterial was also evaluated. The marking procedure consisted of recording the number 8 (eight) many times on the surface in order to cover the largest possible area of the samples.

| C | Si | Mn | P | S | Cr | Mo | Ni | Fe |
|---|---|---|---|---|---|---|---|---|
| 0.023 | 0.378 | 2.09 | 0.026 | 0.0003 | 18.32 | 2.59 | 14.33 | Bal. |

**Table 1.** Chemical composition of the ISO 5832-1 stainless steel (wt.%).

| Sample | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Pulse frequency (kHz) | 80 | 188 | 296 | 350 |

**Table 2.** Yb-optical fiber laser pulse frequencies.

| NaCl | KCl | $Na_2HPO_4$ | $KH_2PO_4$ |
|---|---|---|---|
| 8.0 | 0.2 | 1.15 | 0.2 |

**Table 3.** Chemical composition (g/L) of phosphate-buffered saline solution (PBS).

The aim was to evaluate the effect of the marks on the corrosion resistance of the implant. The mark is usually carried out for identification and traceability of the implant.

To evaluate the specimens' corrosion resistance, electrochemical techniques were employed such as open circuit potential *versus* time (OCP), electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization. All electrochemical tests were carried out using Biologic EC-Lab V10.33 – SP-150 potentiostat-galvanostat equipment. The electrochemical test cells were composed of a three electrode setup, with the specimen as the working electrode, a Pt counter electrode (wire with geometric area of 2.0 $cm^2$) and a saturated calomel electrode (SCE) (3 M) reference electrode. The area of the working electrode exposed to the electrolyte corresponded to 1 $cm^2$. In order to ensure reproducibility, at least four samples for each surface condition were measured. The electrolyte used was a phosphate-buffered saline (PBS) solution, pH of 7.4, with chemical composition shown in **Table 3**. The solution was prepared from high purity chemical reagents and deionized water. Potentiodynamic polarization tests were carried out at a rate of 0.167 mV/s, at (37 ± 1)°C, after monitoring the open circuit potential (OCP) for 12 h.

Surface characterization of the ISO 5832-1 stainless steel either with laser or without laser engravings was carried out by scanning electron microscopy (SEM).

## 3. Results and discussion

The corrosion resistance of the ISO 5832-1 stainless steel, greatly used for biomedical applications, was measured by electrochemical methods. **Figure 1** shows the variation of the open circuit potential as a function of immersion time for the four different types of laser marked surfaces and the blank (without laser), at 37°C, during a period of 12 h, which was reasonable time for the stabilization of corrosion potential. The tests were carried out for 10 specimens of ISO 5832-1 austenitic stainless steel, for each of the following conditions: unmarked and laser marking, changing the pulse frequencies in four levels. The analysis of **Figure 1** allows the understanding of some phenomena that have been repeated for several specimens depending on the type of surface evaluated.

The curve presented in black color, referring to the steel without markings, is representative of the amount of 10 tests performed and presents a more constant open circuit potential for the period evaluated, which suggests the existence of a more stable passive film on the steel. From the first 4000 s of immersion, there is an increase in potential, which with the passage of time, exhibits more uniform behavior until reaching a value of the order of 0.18 V (SCE) in 12 h of test.

Most of the specimens marked by laser beam with parameters 2 and 4 showed a trend of increasing in potential for nobler and more stable values. Specimen 1 showed a drop in open

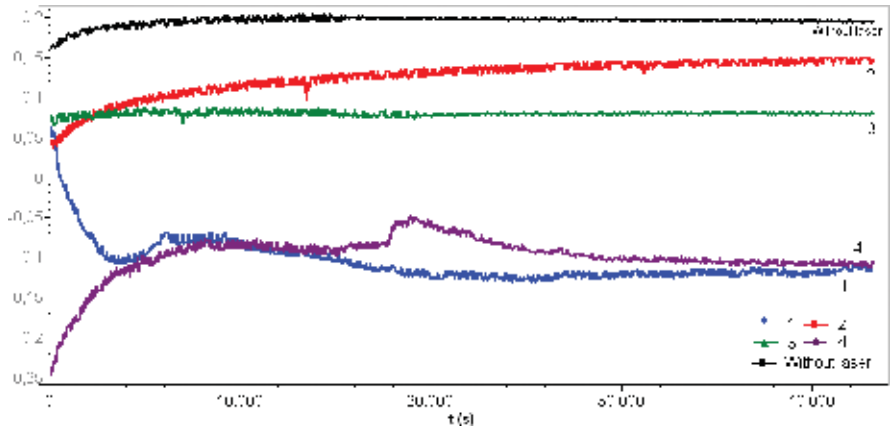



**Figure 1.** Open circuit potential variation with time of immersion for unmarked and Yb-optical fiber laser marked surface, in different frequencies, during 12 h of immersion in phosphate buffer solution (PBS).

circuit potential for the first immersion times, around 4000 s. This potential drop can be associated with the attack of more active regions of the surface due to the formation of microplates related to defects generated by the laser marking process. The attack of the more active regions can be considered as a partial "cleaning" of the surface by corrosive attack that is followed by a more homogeneous surface responsible for the recovery of potential in the final moments of the immersion. Specimen 3 presented values of more uniform potentials throughout the open circuit immersion period.

For longer immersion times in this solution, around 25,000 s, all specimens with and without laser markings, exposed in **Figure 1**, show uniform behavior of the corrosion potential, indicating the formation of a stable passive film.

The electrochemical impedance spectroscopy (EIS) diagrams for the biomaterials under the conditions studied, obtained at 37°C in PBS solution, immediately after the potential monitoring corrosion are shown in **Figure 2**.

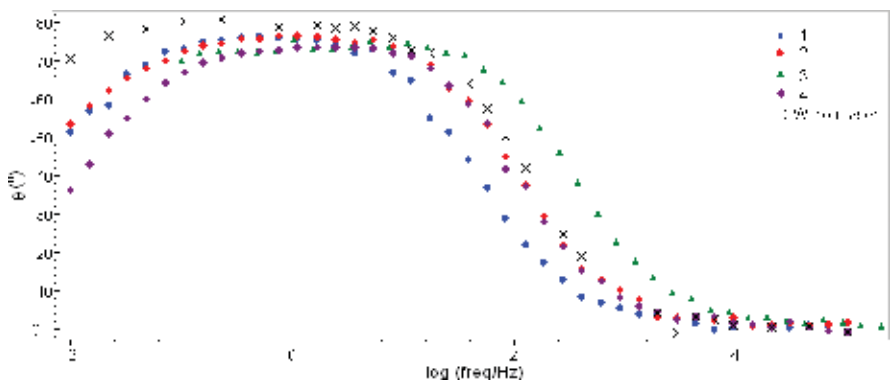



**Figure 2.** Bode diagram (phase angle) obtained after OCP for austenitic stainless steel ISO 5832-1 in PBS at 37°C.

**Figure 2** presents the Bode (phase angle) diagram for the laser marked specimens and without marks, with phase angle values between −35° and −80°, in the low-frequency region, which corresponds to that of passive metals [18, 29–32], and the samples without laser treatment had the lowest values and the highest values were obtained for the samples marked in condition 4.

The diagram shows two constants. One is in the region of high frequencies, identified with a slope between 0.01 and 0.1 Hz. The other in the region of low frequencies, characterized by a slope between 1 and 10 Hz for the surface corresponding to specimen 1 and between 10 and 100 Hz for the other conditions of marked surfaces (specimens 2, 3 and 4) and without laser. This behavior was observed for stainless steels and associated with the constant at high frequencies at the external-medium oxide interface and the constant at mean frequencies at the internal metal-oxide interface [29–31].

The Bode diagrams, shown in **Figure 3**, represent the reproducibility obtained in 10 tests for each condition and suggest that the change in the laser beam parameters used for the markings has a small influence on the impedance in the low-frequency region, the lowest values obtained for the specimens marked in conditions 1 and 4, and the highest for the material without laser treatment, in this frequency range. This fact evidenced a slight decrease in the protective capacity of the passive film for the treated specimens.

In **Figure 2**, the Bode diagrams show a narrow plateau for the laser-labeled specimens under all conditions evaluated with phase angle values around −70°, in the medium frequency range, and a wide plateau with phase angle values close to −80° over a wide frequency range, from averages to low, for specimens without laser treatment, typical of passive materials [18, 29, 30].

Higher phase angles at low frequencies for unlabeled specimens suggest passive and more protective films. The drop in the phase angles at the lowest frequencies for the labeled specimens is an indicative of the deterioration of the passive film properties. In the laser-etched samples, the peak phase angle occurs at lower frequencies compared to the untreated specimens, suggesting that the outermost layer of the oxide is less protective than the others [18, 24].

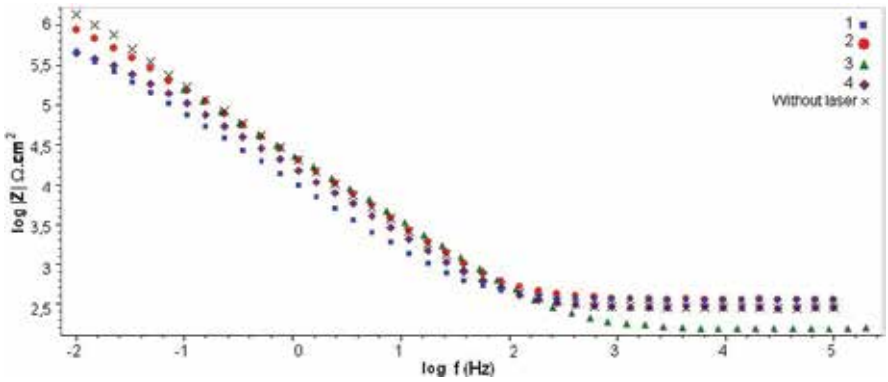



**Figure 3.** Bode diagram (Z-module) obtained after OCP for austenitic stainless steel ISO 5832-1 in PBS at 37°C.

For the same period of immersion from the open circuit corrosion potential, Nyquist impedance diagrams exhibit capacitive behavior, typical of passive materials, and showed lower-Z imaginary values for the specimens with laser treatment; when compared to the specimens without laser beam application. In **Figure 4**, for the same frequency value, the lower impedances were associated with the laser labeled specimen in condition 3, followed by the marking conditions with parameters 4, 1 and 2.

The EIS results were supported by the cyclic potentiodynamic polarization curves. The lower resistance to pitting corrosion was associated with the laser treated specimens; this is explained by the thermal effect of the process on the homogeneity of the microstructure of this analyzed biomaterial, which reduces the potential for breaking the passive film.

**Figure 5** presents the cyclic polarization curves for specimens with and without laser marks. This result represents the laser effect on the susceptibility to corrosion of this stainless steel; the curves presented values of passive film breakdown from about +0.3 V (ECS) to +0.8 V (ECS).

This variability is expected for heterogeneous surfaces such as the specimens with markings via laser beam, where the conditions of the specimens under conditions 1 and 4 showed higher resistance to localized corrosion. It is noted that the film breaking potentials, for all laser marked conditions, are below the specimens without laser marks, that is, polished to 1 μm.

The microstructure of the ISO 5832-1 austenitic stainless steel, widely used for biomaterial application, is presented in **Figure 6**.

**Figure 7** shows that pitting susceptibility is increased in the laser affected areas of the stainless steel surface.

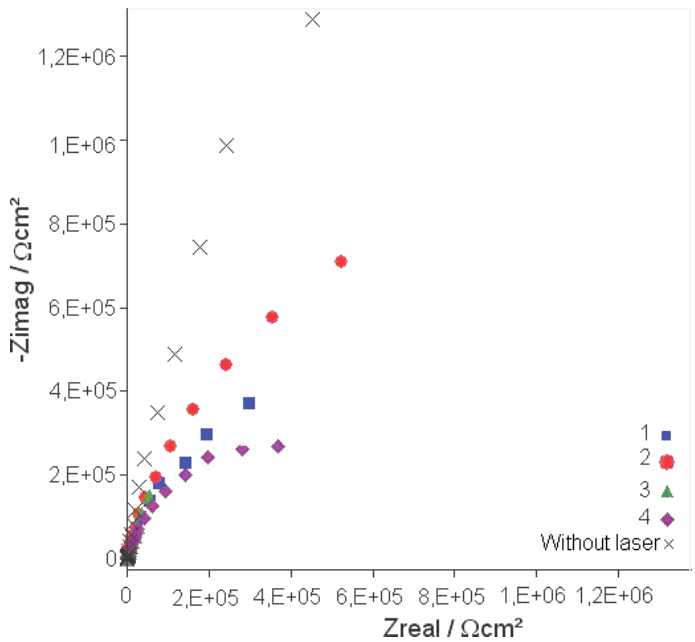



**Figure 4.** Nyquist diagram obtained after OCP for austenitic stainless steel ISO 5832-1 in PBS at 37°C for the specimens.

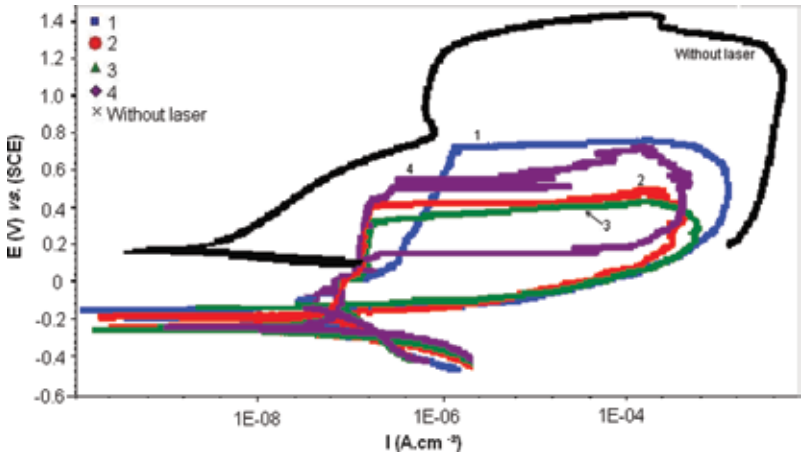



**Figure 5.** Potentiodynamic polarization curves for unmarked and laser marked ISO 5832-1 stainless steel samples obtained in phosphate-buffered saline solution (PBS).

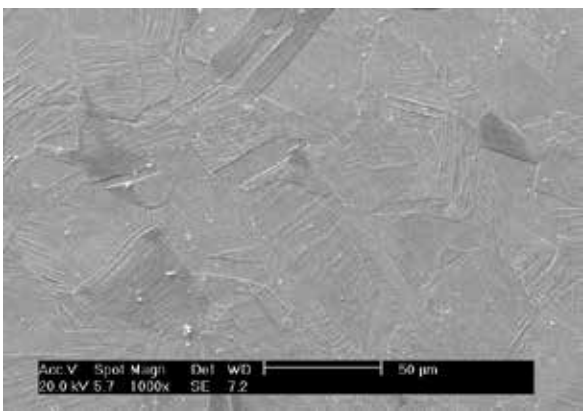



**Figure 6.** SEM microstructure of ISO 5832-1 austenitic stainless steel surface.

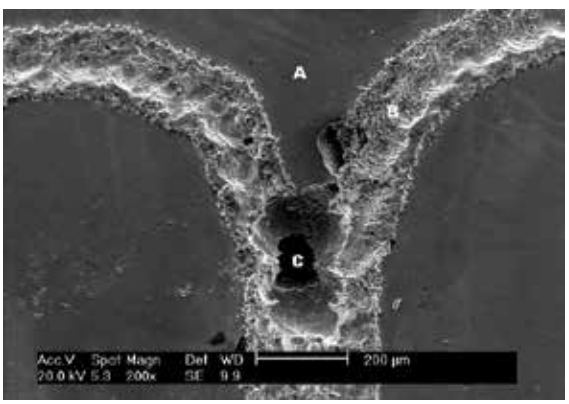



**Figure 7.** SEM image of the ISO 5832-1 stainless steel surface after polarization tests. (A) Region without laser, (B) laser marked region and (C) laser marked region showing pits associated to the melted areas.

## 4. Conclusions

The results of this study showed that susceptibility to pitting corrosion increases due to the thermal effect of the laser marking process in the surface of the stainless steel comparatively to the unmarked regions. In addition, the passive films properties are widely changed by the marking process, with the frequencies used.

The lowest pitting resistance was found for the samples with laser marks. This is explained by the thermal effect of the laser marking procedure on the stainless steel microstructure decreasing pitting resistance. The laser marking occurs by melting of the surface in order to produce the desired marks, besides that a rougher nonhomogenous surface is generated, which favors the corrosion attack.

## Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this book chapter.

## Author details

Eurico Felix Pieretti*, Olandir Vercíno Corrêa, Marina Fuser Pillis and
Maurício David Martins das Neves

*Address all correspondence to: efpieretti@usp.br

Materials Science and Technology Center, Nuclear and Energy Research Institute (CCTM-IPEN/CNEN), University City, São Paulo, Brazil

## References

[1] Lyman DJ, Seare Jr WJ. Biomedical materials in surgery. Materials Science. 1974;**4**:415-433

[2] Williams DF. Corrosion of implant materials. Materials Science. 1976;**6**:237-266

[3] Black J. Systemic effects of biomaterials. Biomaterials. 1984;**5**:11-18

[4] Gibbons DF. Biomedical materials. Biophysics and Bioengineering. 1975;**4**:367-375

[5] Anderson JM. Biological response to materials. Annual Review of Materials Research. 2001;**31**:81-110

[6] Villamil RFV, Aranha H, Afonso MLCA, Mercadante MT, Agostinho SML. Aços inoxidáveis em implantes ortopédicos: fundamentos e resistência à corrosão. Revista Brasileira de Ortopedia. 2002;**37**:471-476

[7] Tsuchiya H, Fujimoto S, Chihara O, Shibata T. Semiconductive behavior of passive films formed on pure Cr and Fe–Cr alloys in sulfuric acid solution. Electrochimica Acta. 2002;**47**:4357-4366

[8] Hakiki NE, Montemor MF, Ferreira MGS, Belo MC. Semiconducting properties of thermally grown oxide films on AISI 304 stainless steel. Corrosion Science. 2000;**42**:687-702

[9] Belo MC, Hakiki NE, Ferreira MGS. Semiconducting properties of passive films formed on nickel-base alloys type Alloy 600: influence of the alloying elements. Electrochimica Acta. 1999;**44**:2473-2481

[10] Curley-Fiorino ME, Schmid G M. The effect of the $Cl^-$ ion on the passive film on anodically polarized 304 stainless steel. Corrosion Science. 1980;**20**:313-329

[11] Hakiki NE, Boudin S, Rondot B, Belo MC. The electronic structure of passive films formed on stainless steels. Corrosion Science. 1995;**37**:1809-1822

[12] Hakiki NE, Belo, MC, Simões AMP, Ferreira MGS. Semiconducting properties of passive films formed on stainless steels. Journal of the Electrochemical Society. 1998;**145**:3821-3829

[13] Marcus P. Surface science approach of corrosion phenomena. Electrochimica Acta. 1998; **43**:109-118

[14] Taveira LV, Montemor MF, Belo MC, Ferreira MG, Dick LFP. Influence of incorporated Mo and Nb on the Mott-Schottky behaviour of anodic films formed on AISI 304L. Corrosion Science. 2010;**52**:2813-2818

[15] Schmuki P, Böhni H, Bardwell JA. In situ characterization of anodic silicon oxide films by ac impedance measurements. Journal of the Electrochemical Society. 1995;**142**:1705-1712

[16] Ningshen S, Mudali UK, Mittal VK. Semiconducting and passive film properties of nitrogen-containing type 316LN stainless steels. Corrosion Science. 2007;**49**:481-496

[17] Sunseri C, Piazza S, Quarto FD. Photocurrent spectroscopic investigation of passive films on Chromium. Journal of the Electrochemical Society. 1990;**137**:2411-2417

[18] Pieretti EF, Costa I. Surface characterisation of ASTM F139 stainless steel marked by laser and mechanical techniques. Electrochimica Acta. 2013;**114**:838-843

[19] Pieretti EF, Neves MDM. International Journal of Electrochemical Science. 2017;**12**: 9204-9211

[20] Pieretti EF, Manhabosco SM, Dick LFP, Hinder S, Costa I. Localized corrosion evaluation of the ASTM F139 stainless steel marked by laser using scanning vibrating electrode technique, X-ray photoelectron spectroscopy and Mott-Schottky techniques. Electrochimica Acta. 2014;**124**:150-155

[21] Pieretti EF, Neves MDM. Influence of laser marks on the electrochemical behaviour of the ASTM F139 stainless steel for biomedical application. International Journal of Electrochemical Science. 2016;**11**:3532-3543

[22] Pieretti EF, Costa I, Marques RA, Leivas TP, Neves MDM. Electrochemical study of a laser marked biomaterial in albumin solution. International Journal of Electrochemical Science. 2014;**9**:3828-3836

[23] Pieretti EF, Palatnic RP, Leivas TP, Costa I, Neves MDM. Evaluation of laser marked ASTM F 139 stainless steel in phosphate buffer solution with albumin. International Journal of Electrochemical Science. 2014;**9**:2435-2444

[24] Pieretti EF, Pessine EJ, Corrêa OV, Rossi W, Neves MDM. Effect of laser parameters on the corrosion resistance of the ASTM F139 stainless steel. International Journal of Electrochemical Science. 2015;**10**:1221-1232

[25] Manhabosco SM, Santos AP, Marcolin ML, Pieretti EF, Neves MDM, Dick LFP. Localized corrosion of laser marked M340 martensitic stainless steel for biomedical applications studied by the scanning vibrating electrode technique under polarization. Electrochimica Acta. 2016;**200**:189-196

[26] Qi J, Wang KL, Zhu YM. A study on the laser marking process of stainless steel. Journal of Materials Processing Technology. 2003;**139**:273-276

[27] Leone C, Genna S, Caprino G, Iorio I. AISI 304 stainless steel marking by a Q-switched diode pumped Nd:YAG laser. Journal of Materials Processing Technology. 2010;**210**:1297-1303

[28] Bizi-Bandoki P, Benayoun S, Valette S, Beaugiraud B, Audouard E. Modifications of roughness and wettability properties of metals induced by femtosecond laser treatment. Applied Surface Science. 2011;**257**:5213-5218

[29] Mansfeld F, Shih H. Detection of pitting with electrochemical impedance spectroscopy. Journal of Electrochemical Society. 1988;**1**:1171-1172

[30] Trethewey KR, Paton M. Electrochemical impedance behaviour of type 304L stainless steel under tensile loading. Materials Letters. 2004;**58**:3381-3384

[31] Krakowiak S, Darowicki K, Slepski P. Impedance investigation of passive 304 stainless steel in the pit pre-initiation state. Electrochimica Acta. 2005;**50**:2699-2704

[32] Mansfeld F. Electrochemical impedance spectroscopy (EIS) as a new tool for investigating methods of corrosion protection. Electrochimica Acta. 1990;**35**:1533-1544

Chapter 6

# Tuned Hydroxyapatite Materials for Biomedical Applications

Ewerton Gomes Vieira,
Thátila Wanessa da Silva Vieira,
Marcos Pereira da Silva,
Marcus Vinicius Beserra dos Santos,
Carla Adriana Rodrigues de Sousa Brito,
Roosevelt Delano de Sousa Bezerra,
Ana Cristina Vasconcelos Fialho,
Josy Anteveli Osajima and
Edson Cavalcanti da Silva Filho

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71622

**Abstract**

Hydroxyapatite stands out between biomaterials due to its properties of osteoconduction and osteoinduction, being adequate to be used in bone grafts. The high stability and flexibility of the structure allows for several biomedical applications, for example, the use as polysaccharide based on the scaffold formulations and the cationic substitutions occurring through the doping of the material using metals, which may enhance biological characteristics, such as improving the action of combating bacterial infections *in situ*. This study was a research of articles and patents, without and with time restriction (2007–2017), which contain information about hydroxyapatite in the tissue engineering, biomedical, doped with cerium and its properties of antibacterial activity. There were also searches of products and companies that commercialize these types of materials aimed at tissue engineering area. Scopus was used for searched of articles and were EPO, USPTO, and INPI used for patents, and to search for products and companies were used search engines. Few papers were found to associate all the keywords, but the ones found are recent works, thus showing a new area with potential to be investigated.

**Keywords:** hydroxyapatite, scaffold, doping, antibacterial activity

## 1. Introduction

Tissue bioengineering is the science that involves the applications of engineering and health sciences to assist and accelerate the regeneration of defective and damaged tissues in the human body. It aims to create and improve new therapies and to develop new biomaterials that can be used to restore, improve, or prevent worsening of compromised tissue function such as in situations with the loss of tissue integrity resulting from trauma, developmental deformities and diseases [1, 2].

In the case of loss or compromise of the bone tissue, several natural or synthetic biomaterials such as polymers, ceramics, and metals or their composites have been investigated and used as a substitution alternative in different ways. The main alternative for damaged or lost bone tissue replacement is the autogenous bone graft. This is the first alternative to be used for the regeneration of the bone tissue due to its osteogenic properties [3].

However, grafts have limited the availability due to the need for surgical procedures with possible local infections, rejection by the transplanted organism, and progressive reabsorptions of the material. As a result, scientific research is developing new biomaterials for its replacement. Synthetic grafts can be an interesting alternative, due to intrinsic characteristics such as biocompatibility and chemical similarity with the bone tissues of living beings, allied with their properties of osteoconduction and osteoinduction [4–8].

Bioceramics is the class of ceramics used for repair and replacement of diseased and damaged parts of musculoskeletal systems. They are the most widely used materials in the class of traumas such as calcium phosphates, hydroxyapatite (HAp) ($Ca_{10}(PO_4)_6(OH)_2$), octacalciumphosphate ($Ca_8H_2(PO_4)_6.5H_2O$), calcium pyrophosphate dihydrate ($Ca_2P_2O_7.2H_2O$), and β-tricalcium phosphate ($Ca_3(PO_4)_2$) [9]. Calcium phosphates are classified according to a molar ratio of calcium and phosphorus Ca/P ranging from 0.5 to 2.0. HAp is the most widely used component in biomedical applications for phosphates and is the main component of the bone and is known for its excellent cellular and tissue affinity. HAp is widely used in tissue regeneration and biomedical applications in the form of coatings on metal implants, bone and nerve tissue graft production, drug release agents, wound protection, cell culture substrates, enzymatic immobilization, bone prosthesis or graft coatings, due to their excellent biocompatibility, osteoconduction property, and similarity with the inorganic component of the natural bone [10–12].

This biomaterial has the ability to establish chemical bonds with the living tissue of the bone due to its structure and chemical composition that are similar to apatite, which is found in the human skeleton. In addition, the biocompatibility and bioactivity of HAp can promote the proliferation of osteoblasts that are new bone-forming tissue. Other studies with these materials also cover the areas of biology, chemistry, materials engineering, and so on [13–15].

Porous three-dimensional scaffolds based on HAp are the ideal materials mostly used in modeling, reconstructing, and forming new bone tissues. Scaffolds adapt indirectly to the tissue and favor tissue differentiation, migration, and proliferation or osteoblastic formation [16]. However, it is not possible to use the HAp alone as scaffolds due to mechanical defects.

The combination of biodegradable polymers and bioactive inorganic materials ultimately improves the mechanical properties, biocompatibility, and cellular affinities of individual components [17].

Biocomposites based on natural biopolymers are being studied and associated with HAp due to the biocompatible and biodegradable behavior of some of these natural polymers. This new generation of biomaterials combines with bioactive properties that resemble the natural function of bone, triggering tissue regeneration mechanisms *in vivo* [18, 19]. Biocomposites HAp-biopolymers that often closely resemble the position and structure of mineralized tissues provide excellent mechanical properties and favorable biological properties, proving to be an ideal candidate for tissue engineering as well as orthopedic and dental applications [20].

Anionic polysaccharides, such as alginate, hyaluronic acid, silk fibroin, cellulose and natural gums, and others, such as chitosan, are excellent alternatives for improving the biocompatibility of HAp when it is in association. These biocomposites are potential models for the mineralization of HAp because its anionic surface can bind the $Ca^{2+}$ ions, besides controlling the nucleation and the growth of the crystal reduce the interfacial energy between the crystal and the surface. Several materials composed of HAp can be prepared using polysaccharides in the form of scaffolds for biomedical applications and bone tissue engineering [20, 21].

Many surgical procedures involve the formation of a chemical interface of the biomaterial/bone type and, consequently, the biological fixation, which the living bone structure penetrates the free space of the biomaterial, causing the permanent fixation of the bone. However, these procedures may lead to problems of bacterial infections that are difficult to control during the postoperative period, and consequently, the excessive use of antibiotics may not provide sufficient protection, causing the loss of bone material and generating resistant strains of bacteria, which are difficult to treat [22–24]. The defense mechanisms activated by the immune system can be reinforced through the introduction of antibacterial agents that have biological interaction with the biomaterial. One of the alternatives is the substitution capacity of HAp ions by doping, and these ions have antibacterial properties: silver ($Ag^{+}$), cupper ($Cu^{2+}$), zinc ($Zn^{2+}$), selenium ($SeO_3^{2-}$), strontium ($Sr^{2+}$), lanthanides ($Ce^{3+}$, $Ga^{3+}$, $Sm^{3+}$), and so on [25–28].

The sites that have the ions that compose Hap ($Ca^{2+}$, $PO_4^{2-}$, $OH^{-}$) can be occupied by ions of similar size and charge. This ability to incorporate ions through the doping is an alternative that is based on the fact that the introduction of small amounts of some ions can cause changes that improve the biological, physical-chemical, mechanical, and antimicrobial properties of the material [26, 29–33]. Thus, this study aims to present a search for articles and patent of technological inventions, which include information about hydroxyapatite in relation to its applications in the field of tissue engineering and doping with cerium ion for biomedical applications.

This work was conducted with the help of scientific articles and technological innovation patents and products present in the market. The articles in the SCOPUS database were used, and the keywords used were as follows: *hydroxyapatite*, *scaffold, polysaccharide, doped, cerium*, and *antimicrobial activity*. These keywords were combined with each other, and quotation marks were used for searching compound words.

Keyword research related to study topics was based on the information contained in the abstract, keywords, and titles. For the search of patents for technological innovation, the research was conducted in patent database: European Patent Office (EPO), United States Patent and Trademark Office (USPTO), and Brazil's National Institute of Industrial Property (INPI).

Searches for products and companies were done by using search engines (Google, Bing, Yahoo, Bing, and Ask), and the keywords were as follows: hydroxyapatite, polysaccharides, biopolymers, scaffold, tissue engineering, odontology, osteoporosis, tooth, bone, cell growth, bone graft, and implants.

Access of both articles and patents was realized in May 2017, using the same fields of research and the same keywords for the search of articles. In the case of INPI, we used the words also in Portuguese. The researches of articles and patents were conducted in two ways: with and without time restriction from 2007 to 2017, and the search for products marketed was in September 2017 using the information provided in the catalogs and websites of the companies found in the database.

## 2. Results and discussion

### 2.1. Search for articles in the SCOPUS database

The investigation of the number of articles published revealed that the words are being combined, there is a decrease in the number of publications found, and, in some cases, there is no publication related to these words. The results were obtained using the separate and combined keywords such as *hydroxyapatite, scaffolds, polysaccharide, doped, cerium,* and *antibacterial activity* are shown in **Table 1**.

In **Table 1**, comparing the publication time of articles and analyzing the data, with and without time restriction between 2007 and 2017, it was observed that most of the publications are concentrated in this period. This shows that studies on the material have been increasing over the last decade. When using the combination of words such as *hydroxyapatite* and *scaffold* and *polysaccharide* and *doped* and *cerium* and *antibacterial activity*, which are the main keywords for this work, it is noted that no related article was found in the databases researched. The results show the specificity because there are no articles dealing with related words or the themes proposed by this manuscript.

The expression of words such as *hydroxyapatite* and *scaffold* and *polysaccharide* (**Table 1**) was found in 74 works between 1960 and 2017 and was found in 66 works between 2007 and 2017. However, only 46 articles are relatively of experimental scientific research. Thus, only the number of articles related to the abovementioned keywords (about 85.71%) was published in the last decade. In other words, this topic has been receiving more attention in the last decade from the global scientific community.

When combining the keywords such as *hydroxyapatite* and *scaffold* and *polysaccharide* and *doped*, two articles were found but only one of these is effectively experimental scientific

| Keywords | Publications (1960–2017) | Publications (2007–2017) |
|---|---|---|
| Hydroxyapatite | 49.983 | 26.970 |
| Scaffold | 98.299 | 81.265 |
| Polysaccharide | 136.782 | 60.187 |
| Doped | 333.539 | 187.096 |
| Cerium | 73.813 | 47.834 |
| Antibacterial activity | 79.671 | 48.238 |
| Hydroxyapatite and scaffold | 5.191 | 4.463 |
| Hydroxyapatite and scaffold and polysaccharide | 74 | 66 |
| Hydroxyapatite and doped | 1.219 | 1.089 |
| Hydroxyapatite and doped and cerium | 24 | 20 |
| Hydroxyapatite and doped and antibacterial activity | 71 | 70 |
| Hydroxyapatite and scaffold and polysaccharide and doped | 2 | 2 |
| Hydroxyapatite and scaffold and polysaccharide and doped and cerium | 0 | 0 |

Source: Authorship (2017).

**Table 1.** Number of publications found in the SCOPUS database.

research; the other is a review. The article entitled "Bioactivation of knitted cellulose scaffolds bystrontium" was published in the year 2008 by Brandt, Muller and Greil, researchers from the Materials Science Department of the University of Erlangen-Nuremberg, Germany. The article discusses the use of the properties of strontium ($Sr^{2+}$) in the treatment against osteoporosis, its anabolic and nonresorptive activity. The material used was in the scaffold form, which was prepared using a HAp doped with $Sr^{2+}$ plus doped cellulose composition. The study evaluated the kinetics of $Sr^{2+}$ release during static exposure to simulated body fluid to evaluate the precipitation of carbonated hydroxyapatite under conditions that simulate the inorganic part of human blood plasma.

The keywords *hydroxyapatite* and *doped* and *cerium*, which form the starting material for the scaffolds composition, according to the study of the articles found for the combinations (**Tables 1** and **2**) important information could be verified as method of synthesis, microorganisms used in antibacterial tests. In most of the articles, the goal is to develop a material with antibacterial activity and stimulate the formation of new bone tissues from the synthesis of hydroxyapatite doped with cerium. One of the articles exposes the association of cerium with strontium-doped hydroxyapatite in order to improve biological properties and antibacterial activity. **Table 2** shows some of these articles and describes a relationship between the use of the synthesized materials and their applications, and **Table 3** shows their respective objectives.

| Material | Method of synthesis | Application | Author, year of publication |
|---|---|---|---|
| Compound of HAp hydrogel based on xanthan gum | Soaking process | Bone tissue engineering | Izawa et al., 2014 |
| Scaffold based on HAp and gum Arabic | Coprecipitation and dissolution | Bone tissue engineering | Hadavi et al., 2017 |
| Scaffold nanofibrous cotton based on cellulose and nano-HAp | Electrospinning | Bone tissue engineering | Ao et al., 2017 |
| Hydroxyapatite co-substituted with strontium and cerium | Microwave irradiation | Inhibition of *Staphylococcus aureus, Escherichia coli* | Gopi et al., 2014 |
| Reinforced hydroxyapatite composite with cerium doped glass | Coprecipitation | Inhibition of *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* | Morais et al., 2015 |
| Hydroxyapatite doped with cerium (IV) | Coprecipitation | Inhibition of *Staphylococcus aureus, Escherichia coli* | Ciobanu et al., 2016 |
| Hydroxyapatite and fluorohydroxyapatite co-substituted with zirconia and cerium | Sol-gel | Inhibition of *Staphylococcus aureus, Escherichia coli* | Sanyal et al., 2016 |

Source: Authorship (2017).

**Table 2.** Materials synthesized in the articles and their applications.

It is possible to note that some of these works shown in **Table 3** employ studies of the cerium-doped hydroxyapatite as well as the combination thereof with other metals such as strontium and zirconia and assess their ability to inhibit bacterial. This doping is possible due to the chemical structure of HAp that can accommodate a variety of cationic and anionic substituents. **Figure 1** shows the projection of the unit cell (hexagonal) of the hydroxyapatite when projected down the c-axis and shows an OH group in the center of the structure and the position of the two types of calcium cations: calcium 1 (Ca(1)) and calcium 2 (Ca(2)) for a better understanding of how substitution of HAp ions occurs. Ca(1) atoms are located at the ends of a hexagonal unit cell, while Ca(2) atoms are in a more internal position around the OH group. The phosphate group (P) is the largest ion that constructs the unit cells [15].

The research in the article databases revealed that most of them develop materials for future applications of these in the field of tissue engineering, but there is still a lack of applications of these materials aimed at the field of tissue and biomedical engineering. In other words, the studied materials have the characteristics of inhibition of bacterial growth, but the search showed the nonuse of these biomaterials organized in the form of scaffolds and associated with some polysaccharides.

The four reported articles (**Table 3**) showed the main objective of doping of HAp and the improvement of its bacteriological growth inhibition properties. Majority of these articles, which aim for this purpose, present the methodologies used for the synthesis and characterization of the materials, and the biological assays are used to investigate the antibacterial properties of these materials. However, they do not elucidate how the mechanism of action of these materials with bacteria works.

| Title | Author | Objectives |
|---|---|---|
| Mineralization of hydroxyapatite upon a unique xanthan gum hydrogel by an alternate soaking process | Izawa et al., 2014 | Production of a hydrogel that will serve as an organic template for biomimetic calcium mineralization in bone tissue. |
| Novel calcified gum Arabic porous nanocomposite scaffold for bone tissue regeneration | Hadavi et al., 2017 | Study of the proportionate effects of polysaccharide/n-HAp scaffold on the mechanism of in vitro ossification |
| Fabrication and characterization of electrospun cellulose/nanohydroxyapatite nanofibers for bone tissue engineering | Ao et al., 2017 | Development of an efficient process for the manufacture of a nanocomposite based on cellulose/ nano-HAp/cotton through the electrospinning process. |
| Strontium, cerium co-substituted hydroxyapatite nanoparticles: synthesis, characterization, antibacterial activity toward prokaryotic strains and in vitro studies | Gopi et al., 2014. | Synthesis of hydroxyapatite doped with strontium and cerium to improve biomedical applications and study the antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, showing the influence of $Sr^{2+}$ and $Ce^{3+}$ concentration on size, morphology, purity, crystallinity, antibacterial activity and bone bonding ability. |
| Novel cerium-doped glass-reinforced hydroxyapatite with antibacterial and osteoconductive properties for bone tissue regeneration | Morais et al., 2015. | Development of a composite of hydroxyapatite reinforced with cerium-doped glass, and to study its physicochemical, biological, and biomechanical properties. |
| New cerium(IV)-substituted hydroxyapatite nanoparticles: preparation and characterization | Ciobanu et al., 2016. | Preparation of cerio (IV)-doped hydroxyapatite powders nano by a coprecipitation method. Influence of the effects of calcium replacement by cerium on morphology, purity, crystallinity, crystallite size, and antibacterial capacity. |
| Structural and antibacterial activity of hydroxyapatite and fluorohydroxyapatite co-substituted with zirconium-cerium ions | Sanyal et al., 2016. | The effects of co-substitution of calcium by zirconium (Zr) and cerium (Ce) ions on the structure of hydroxyapatite and fluorohydroxyapatite on crystal size, morphology, crystallinity, thermal studies, and antibacterial activity against *S. aureus and E. coli.* |

Source: Authorship (2017).

**Table 3.** Titles and objectives of the articles cited in **Table 2**.

One of the causes that leads to failure of conventional implants is the infection caused by bacteria; therefore, the articles aim to improve the biological properties of hydroxyapatite through doping with cerium. Due to this factor, ion has properties that stimulate the formation of new bone tissue and acts as an antibacterial agent. Bacteria, the main cause of infections, are classified as Gram-positive or Gram-negative depending on the difference in

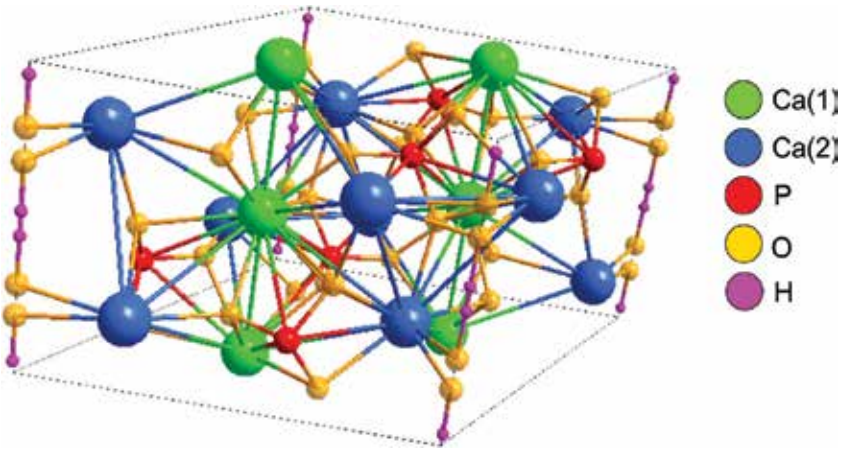



**Figure 1.** Projection of the unit cell of hydroxyapatite. Source: Authorship (2017).

cell wall architecture. The Gram-positive cell wall consists of a thick layer of peptidoglycan, while the Gram-negative cell wall shows more complex membrane structure and composition. Gram-negative has the finest peptidoglycan layer and the outer surface of the cell has a membrane composed of proteins, lipopolysaccharides, and phospholipids called the outer membrane. The space between the peptidoglycan and the outer membrane is known as the periplasmic space; this space presents in some points enzymes and proteins and performs several physiological functions. **Figure 2** shows the comparison between the compositions of the cell walls of Gram-positive and Gram-negative [34].

**Figure 2** shows that the surface of Gram-positive bacteria is mainly covered by neutral and acidic polysaccharides, a large number of different proteins, theichoic acids, whereas the outer membrane of Gram-negative has an irregular distribution of lipids on the external and internal surface, which the outer face contains all lipopolysaccharides, while the inside face contains most of the phospholipids [35]. Gram-positive bacteria are mostly studied bacteria in the articles, especially *Staphylococcus aureus. S. aureus* is an exceptionally well-adapted pathogen that can survive under different conditions, without particular nutritional or environmental requirements.

Over the years, infections caused by *S. aureus* have increased as one of the leading causes of bacterial infections in humans worldwide. In the last few decades, treatment of these infections has become more difficult, mainly because *S. aureus* develops mechanisms of resistance to the antibiotics used in the treatments [36, 37]. While for the Gram-negative, the most tested was *E. coli*, which, despite the reduced number in the cause of this type of infection, is a relevant group in clinical practice, presenting a difficulty in its treatment [38].

Gopi et al. [39] produced nanoparticles of pure hydroxyapatite (n-HAp), hydroxyapatite doped with strontium (Ca/Sr.-HA), hydroxyapatite co-substituted with strontium and cerium (Ca/Sr./Ce-HA) in different concentrations of cerium (0.05, 0.075, and 0.1 mol/L) by using the microwave irradiation method. All the synthesized materials were investigated by Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), field emission scanning

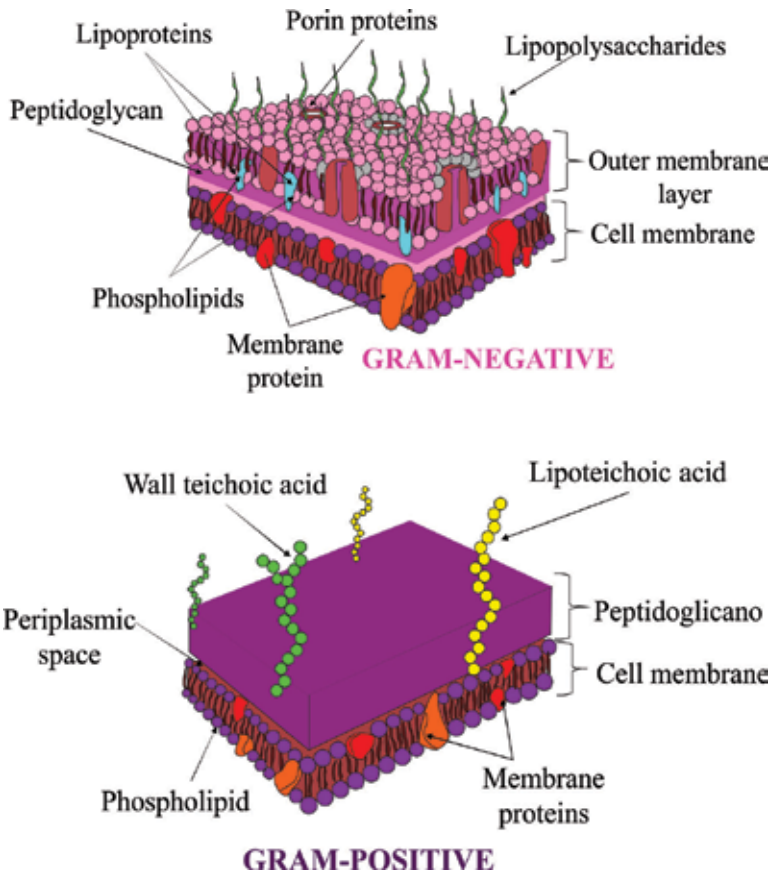



**Figure 2.** Comparison of cell wall of Gram-positive and Gram-negative bacteria. Source: Authorship (2017).

electron microscopy (SEM-FEG), energy X-ray dispersive analysis (EDX), high resolution transmission electron microscopy (HRTEM), and thermogravimetric analysis (TGA). The antibacterial activity of the nanoparticles was evaluated against two prokaryotic strains, *E. coli* and *S. aureus*, by using the disc diffusion method. The results showed that the Ca/Sr./Ce-HAp sample with the 0.1 mol/L concentration of cerium presented higher antibacterial activity in relation to the two strains tested when compared to HA and Ca/Sr./HAp results. According to Gopi et al. [39], $Ce^{3+}$ was important in increasing the antibacterial activity of the synthesized nanoparticle. In order to evaluate the bioactivity of the samples, they tested using simulated body fluid (SBF) for several days and observed that $Sr^{2+}$ and $Ce^{3+}$ ions contributed to the formation of apatite. It can be inferred that the synthesized Ca/Sr./Ce-HA nanoparticle can be a promising biomaterial for biomedical applications. In the work of Morais et al. [40], a composite of hydroxyapatite reinforced with cerium-doped glass (GR-HAp-Ce) was developed. The phases formed in the synthesized material were identified using SEM techniques coupled with energy dispersed secondary (SEM-EDS) and X-ray diffraction (XRD). In addition to the hydroxyapatite phase, the material presented the β-TCP phases, and the authors concluded that the presence of cerium in the GR-HAp-Ce composite provided an effective antibacterial

effect against bacteria *S. aureus* and *S. epidermidis*, but this effect was not observed for the bacterium *P. aeruginosa*. In addition to investigating the antibacterial activity, the osteoconductive properties of the material were also evaluated, which was performed using human osteoblastic cells and showed that the addition of cerium did not affect the cellular viability of the material and that it showed good osteoconductive capacity.

Ciobanu et al. [41] synthesized nanoparticles of pure hydroxyapatite and cerium-doped hydroxyapatite (HAp-Ce) in different concentrations in the range of 1–25% (with a 5% variation) using the coprecipitation method and studied their antibacterial property. The effects of the replacement of cerium to calcium on the morphology, purity, crystallinity, crystallite size, and antibacterial capacity of cerium HA-substituted powders were investigated using scanning electron microscopy (SEM) coupled with X-ray analysis (XRD), X-ray excited photoelectron spectroscopy (XPS), infrared spectroscopy (FTIR), and Brunauer-Emmett-Teller (BET) surface area analysis confirming the formation of hydroxyapatite and the presence of $Ce^{4+}$ and $Ce^{3+}$ ions in its crystal lattice. The doped materials obtained better results of bacterial inhibition indicating that the presence of the ion contributed to the inhibition of bacteria; however, the nanopowders of HAp-Ce were more effective against the *E. coli* bacterium than against *S. aureus*.

Sanyal and Raja [42] studied the effect of the co-substitution of zirconium (Zr) and cerium (Ce) on the structure of hydroxyapatite (HAp) and fluorohydroxyapatite (FHA) gel. The samples were confirmed by the FTIR and XRD spectra; in addition, it was observed that with the increase of the concentration of the $Zr^{4+}$ and $Ce^{3+}$ ions, the formation of the HA phase was maintained. Co-substituted materials showed better results of antibacterial activity than pure hydroxyapatite. Materials with higher cerium concentration showed better bacterial inhibition against *E. coli and S. aureus* bacteria. All the articles studied describe that the presence of cerium ion in the structure of hydroxyapatite improved the antibacterial activity and also gave the material an improvement in bioactivity and may contribute to the formation of new bone tissues.

### 2.2. Search in the main patents databases

The results of the researches at European Patent Office (EPO), USPTO, and National Institute of Industrial Property (INPI) patents using the separate and combined keywords: *hydroxyapatite, scaffolds, polysaccharide, doped, cerium* and *antibacterial activity* are shown in **Table 4**.

Using the search keywords in English and Portuguese, it was possible to find patent deposits in the main patent databases. According to the data shown in **Table 5**, INPI found 74 patents deposited with the word hydroxyapatite and 10 patents when the word scaffold was used. For the combination of *hydroxyapatite* and *scaffold*, the result of a patent filed with the INPI under number 0905514-2 has been reported. The patent PI 0905514-2 provides a process for the scaffolding of a composite hydrogel biomaterial (CNHAP) based on chitosan (CN) and hydroxyapatite (HA), with potential for application in the medical-dental area, demonstrating the biocompatibility characteristics evidenced by classical in vitro assays. These characteristics are associated to the combination of physical-chemical and biological properties of the materials that compose it. CNHAP was obtained in the form of hydrogel with good mechanical characteristics, easy handling and modeling, high porosity, leading as promising as a bone filling material. The production of the CNHAP composite hydrogel scaffold was performed

| Keywords | EPO | USPTO | INPI |
|---|---|---|---|
| Hydroxyapatite | 6.616 | 579 | 74 |
| Scaffold | >10.000 | 1870 | 10 |
| Polysaccharide | >10.000 | 3311 | 5 |
| Doped | >10.000 | 24,312 | 22 |
| Cerium | >10.000 | 2800 | 60 |
| Antibacterial activity | >10.000 | 1071 | 55 |
| Hydroxyapatite and scaffold | 192 | 10 | 1 |
| Hydroxyapatite and scaffold and polysaccharide | 2 | 0 | 0 |
| Hydroxyapatite and doped | 101 | 4 | 0 |
| Hydroxyapatite and doped and cerium | 1 | 0 | 0 |
| Hydroxyapatite and doped and antibacterial activity | 2 | 0 | 0 |
| Hydroxyapatite and scaffold and polysaccharide and doped | 0 | 0 | 0 |

Source: Authorship (2017).

**Table 4.** Number of patents found in EPO, USPTO, and INPI databases.

| Title | Classification | Country | Abstract |
|---|---|---|---|
| Polyether ether ketone/nanohydroxyapatite dental implant and manufacturing method thereof | A61L27/42; A61L27/12; A61L27/18; A61L27/54 | China | Preparation of a biomaterial for dental implant applications based on polyether ether ketone/nano-HAp doped with $Ag^{+}$ and $Zn^{2+}$ presenting antibacterial properties. |
| Method for preparing antibacterial diamond-like carbon/hydroxyapatite gradient multielement nanocoating | A61L27/30; A61L27/32; A61L27/54; C23C14/06; C23C14/35 | China | Method for the preparation of a carbon/HAp base composite having antibacterial properties to be used as coating materials in the biomedical areas. |
| Porous polysaccharide scaffold comprising nanohydroxyapatite and use for bone formation | A61L27/12; A61L27/20; A61L27/56 | France | Method for the preparation of a scaffold composed of polysaccharide and hydroxyapatite used as support for tissue mineralization. |
| Continuous gradient composite scaffold and preparation method thereof | A61L27/26; A61L27/46 | China | Scaffold composed of magnetic nanoparticles of hydroxyapatite/iron with high cellular biocompatibility and high mechanical resistance after the addition of the natural polysaccharide. |

Source: Authorship (2017).

**Table 5.** Characteristics of patents found in the EPO.

by an *in situ* mineralization procedure of the polymeric hydrogel of CN, by HA. This in situ mineralization method promoted mechanical and bioactivity characteristics to the CNHAP, which is suitable for the medical-dental application.

In addition to biocompatibility, tissue fillers should be able to promote cell adhesion, proliferation, and differentiation, essential requirements for tissue bioengineering, which have been increasingly explored within clinical practice. For the keywords such as *hydroxyapatite* and *scaffolds* and *polysaccharide* and *doped* and *cerium* and *antibacterial activity*, no deposited patents were found. Evaluating the results found, it can be understood that the results show the lack of patent filing implying that this area of research is promising.

In patent searches in the EPO database (**Table 5**), two patent records were found using the expression: *hydroxyapatite* and *doped* and *antibacterial activity*. Using the expression *hydroxyapatite* and *scaffold* and *polysaccharide*, also in the EPO, two patent records were found. **Table 6** shows the information about these patents found.

It is important to note that the polysaccharides cited in the patents (**Table 6**) were defined as a molecule composed of two or more molecules of monosaccharide units. Patents report the use of chitosan, hyaluronic acid, chondroitin sulfate, alginate, chitin, dextran, and other natural polysaccharides, which are the ideal extracellular matrix materials for the composition of scaffolds applied in the areas of tissue and biomedical engineering.

| Company | Trademarks | Characteristics | Applicability | Country |
|---|---|---|---|---|
| JHS Biomateriais | HAP-91 | Powder | Bone graft | Brazil |
| JHS Biomateriais | COL.HAP-91 | Scaffold | Bone graft | Brazil |
| Bionnovation® | HAP –Bionnovation® | Powder | Bone graft | Brazil |
| Baumer | GenPhos HA TCP | Powder | Bone graft | Brazil |
| Oral science | Remix® | Toothpaste | Dental Products | France |
| Clarion Pharmaceutical Co. | MCHC | Tablets or capsules | Osteoporosis treatment | India |
| SofSera | SHAp | Powder | Enxerto ósseo | Japan |
| SANGI CO. LTD. | Medical nano-hydroxyapatite | Toothpaste | Dental Products | Japan |
| Sewon Cellontech Co., Ltd. | OssFill | Gel | Bone graft | Korea |
| GranuLab | GranuMas® | Granules | Bone graft | Malaysia |
| Fluidinova | nanoMIX® | Powder | Biomedical/Cosmetic | Portugal |
| Berkeley Advanced Biomaterials Inc. | BABI-HAP-G2 | Granules | Bone graft/Orthopedic Surgery | USA |
| Berkeley Advanced Biomaterials Inc. | BABI-HAP-N100 | Powder | Composites, for DNA and protein purification, or as a reference material. | USA |

Source: Authorship (2017).

**Table 6.** Hydroxyapatite-based biomaterials available on the market.

The data showed that there is a small amount of number of patents related to tissue engineering; in other words, inventions associated with HAp with polysaccharides for the composition of scaffolds, which is the main theme of this work. In particular, there is a deficiency of biomaterials with antibacterial activity properties associated with HAp, polysaccharides, and scaffolds composition for bone tissue regeneration applications. Some of problems of implant may cause to the recipient organism have been addressed throughout this work, for instance, infectious problems originating from bacteria.

For better consistency to the results obtained, the product present in the market based on tuned hydroxyapatite and polysaccharide was examined. The research by companies specialized in the development and commercialization of hydroxyapatite-based biomaterials with applications in tissue engineering was conducted through search engines.

The searched keywords showed information about companies, products, and formulations. For example, the Brazilian company JHS Biomateriais develops a composite named HAP-91 constituted of porous hydroxyapatite, crystalline, biocompatible, pure, and widely tested as bone graft material and with excellent biocompatibility in living organisms. Besides, it is hydrophilic, and the powder can be used directly as a bone graft or it can be added to the patient's own blood drops.

Another biomaterial developed by JHS Biomateriais is COL.HAP-91. The COL.HAP-91 is a collagen-hydroxyapatite composite spongy (25% collagen and 75% HAP-91), with the hemostatic properties of natural collagen fiber network purified, biocompatible, easy to handle, absorbable, and osteoinductive. Both products are registered with the Ministry of Health from Brazil and have a protected trademark at INPI. Its average market price for these materials ranges from € 22.04 to 23.60 per 1000 g.

The Brazilian company Bionnovation®, in its product catalog, sells hydroxyapatite bone graft for applications in orthopedic, maxillofacial, and dental surgeries. The hydroxyapatite is synthesized from calcium hydroxide and phosphoric acid, and the product has radiopaque particles of varied sizes that support in the development of bone cells and assists the osteoconduction of bone-forming cells.

The US Company Berkeley Advanced Biomaterials Inc. develops hydroxyapatite, tricalcium phosphate, and other calcium-based products. The company's business focuses on applications in orthopedic surgeries and bone graft. The European company Fluidinova synthesizes nanohydroxyapatite and markets through the nanoMIX® product. The biomaterial company supplies companies that manufacture medical devices, cosmetics, toothpastes, and other applications.

No material was found available for commercialization when the research was carried out with the words hydroxyapatite, tissue engineering, polysaccharides, and scaffold, even with the two patents deposited in the EPO (**Table 4**). However, when it uses the keywords such as scaffold and tissue engineering, companies and products from several countries were found, for example, Atex Technologies Inc. (China), Electrospinning Company (England), Bio-Scaffold International Pte Ltd. (Singapore), GeSiM (Germany), Matricel (Germany), Silkbiomaterial (Italy), ExCel Matrix Biological Devices (P) Ltd. (USA), and Nanofiber solutions (USA).

**Table 6** shows some materials available on the market, that is, hydroxyapatite-based materials, used in the field of tissue engineering. Also present in the table are the data referring to companies, headquarters, characteristics, application and trademarks of some products on the market.

## 3. Conclusion

Analyzing the results, it can verify the use of hydroxyapatite in the areas of tissue engineering and bone regeneration. Many papers and technological innovation patents were found by searching only the keywords such as hydroxyapatite and scaffold. However, the combinations of the keywords mentioned in the experimental session showed that the number of articles and technology innovation patents was reduced. Synthesis of scaffolds is associated with natural polysaccharides or biopolymers due to their high biocompatibility, but the number of articles and patents decreases when it uses the hydroxyapatite and polysaccharides in the scaffold composition. Cerium-doped hydroxyapatite and its association with polysaccharides and biopolymers is an area that is still poorly studied and quite promising. This conclusion is supported by the small number of publications and patents. Therefore, the data presented for patent deposits and published articles show that there are no papers that contain the chosen and all combined keywords. For the use of these types of biomaterials with antibacterial properties, the research studies showed that the bacteria *Escherichia coli* and *Staphylococcus aureus* were the most investigated. This is explained considering that the bacteria are more accessible for research; in addition, *Staphylococcus aureus* is one of the most common agents present in bone infections. In the search for products, corporate brands, and companies in the areas of tissue engineering and bone regeneration, biomaterials that were found in the market used a hydroxyapatite in biomedical applications, bone graft, and composition of cosmetics. As for the association of hydroxyapatite and polysaccharides, no materials were found on the market when using the keywords such as hydroxyapatite, tissue engineering, polysaccharides, and scaffold.

## Author details

Ewerton Gomes Vieira[1], Thátila Wanessa da Silva Vieira[1], Marcos Pereira da Silva[1], Marcus Vinicius Beserra dos Santos[1], Carla Adriana Rodrigues de Sousa Brito[1], Roosevelt Delano de Sousa Bezerra[2], Ana Cristina Vasconcelos Fialho[3], Josy Anteveli Osajima[1] and Edson Cavalcanti da Silva Filho[1]*

*Address all correspondence to: edsonfilho@ufpi.edu.br

1 Interdisciplinary Laboratory for Advanced Materials – LIMAV, Federal University of Piaui, Teresina, PI, Brazil

2 Federal Institute of Education, Science and Technology of Piauí, Teresina, PI, Brazil

3 Department of Pathology and Dental Clinic, Federal University of Piaui, Teresina, PI, Brazil

## References

[1] Oliveira LSAF, Oliveira CS, Machado APL, Rosa FP. Biomateriais com aplicação na regeneração óssea – método de análise e perspectivas futuras. Revista de Ciência Médicas e Biológicas. 2010;**9**(1):37-44

[2] Tabata Y. Biomaterial technology for tissue engineering applications. Journal of the Royal Society Interface. 2009;**6**:S311-S324

[3] Kalambettu A, Dharmalingam S. Fabrication and *in vitro* evaluation of sulphonated poly-ether ether ketone/nano hydroxyapatite composites as bone graft materials. Material Chemistry and Physics. 2014;**147**:168-177

[4] Best SM, Porter AE, Thian ES, Huang J. Bioceramics: Past, present and for the future. Journal of the European Ceramic Society. 2008;**28**:1319-1327

[5] Dorozhkin SV. Bioceramics of calcium orthophosphates. Biomaterials. 2010;**31**:1465-1485

[6] Guillaume O, Geven MA, Sprecher CM, Stadelmann VA, Grijpma DW, Tang TT, Qin L, Lai Y, Alini, M, de Bruijn JD, Yuan H, Richards RG, Eglin D. Surface-enrichment with hydroxy-apatite nanoparticles in stereolithography-fabricated composite polymer scaffolds promotes bone repair. Acta Biomaterialia. 2017;**54**:386-398

[7] Kawabata K, Yamamoto T, Kitada A. Substitution mechanism of Zn ions in β-tricalcium phosphate. Physica B: Condensed Matter. 2011;**406**:890-894

[8] Ryabenkova Y, Pinnock A, Quadros PA, Goodchild RL, Möbus G, Crawford A, Hatton PV, Miller CA. The relationship between particle morphology and rheological properties in injectable nano-hydroxyapatite bone graft substitutes. Materials Science and Engineering C. 2017;**75**:1083-1090

[9] Kawachi YE, Bertran CA, dos Reis RR, Alves OL. Biocerâmicas: Tendências e Perspectivas de uma Área Interdisciplinar. Química Nova [online]. 2000;**4**(3):518-522.

[10] Lin K, Wu C, Chang J. Advances in synthesis of calcium phosphate crystals with controlled size and shape. Acta Biomaterialia. 2014;**10**:4071-4102

[11] Sadat-Shojai M, Khorasani M, Dinpanah-Khoshdargi E, Jamshidi A. Synthesis methods for nanosized hydroxyapatite with diverse structures. Acta Biomaterialia. 2013;**9**:7591-7621

[12] Supová M. Substituted hydroxyapatites for biomedical applications: A review. Ceramics International. 2015;**41**:9203-9231

[13] An S, Matsumoto T, Miyajima H, Nakahira A, Kimc K, Imazato S. Porous zirconia/hydroxyapatite scaffolds for bone reconstruction. Dental Materials. 2012;**28**(12):1221-1231

[14] Oyefusi A, Olanipekun O, Neelgund GM, Peterson D, Stone JM, Williams E, Carson L, Regisford G, Oki A. Hydroxyapatite grafted carbon nanotubes and graphene nanosheets: Promising bone implant materials. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014;**132**:410-416

[15] Gayathri B, Muthukumarasamy N, Velauthapillai D, Santhosh SB, Asokan V. Magnesium incorporated hydroxyapatite nanoparticles: Preparation, characterization, antibacterial and larvicidal activity. Arabian Journal of Chemistry (2017), In Press, http://dx.doi.org/10.1016/j.arabjc.2016.05.010

[16] Fook ACBM, Aparecida AH, Fook MVL. Desenvolvimento de biocerâmicas porosas de hidroxiapatita para utilização como scaffolds para regeneração óssea. Revista Matéria. 2010;**3**(15):392-399

[17] Ao C, Niu Y, Zhang X, He X, Zhang W, Lu C. Fabrication and characterization of electrospuncellulose/nano-hydroxyapatite nanofibers for bone tissue engineering. International Journal of Biological Macromolecules. 2017;**97**:568-573

[18] Kikuchi M, Ikoma T, Itoh S, Matsumoto HN, Koyama Y, Takakuda K, Shinomiya K, Tanaka J. Biomimetic synthesis of bone-like nanocomposites using the self-organization mechanism of hydroxyapatite and collagen. Composites Science and Technology. 2004;**64**:819-825

[19] Swetha M, Sahithi K, Moorthi A, Srinivasan N, Ramasamy K, Selvamurugan N. Biocomposites containing natural polymers and hydroxyapatite for bone tissue engineering. International Journal of Biological Macromolecules. 2010;**47**:1-4

[20] Izawa H, Nishino S, Maeda H, Morita K, Ifuku S, Morimoto M, Saimoto H, Kadokawa J. Mineralization of hydroxyapatite upon a unique xanthan gum hydrogel by an alternate soaking process. Carbohydrate Polymers. 2014;**102**:846-851

[21] Hadavi M, Hasannia S, Faghihi S, Mashayekhi F, Zadeh HH, Mostofi SB. Novel calcified gum Arabic porous nano-composite scaffold for bone tissue regeneration. Biochemical and Biophysical Research Communications. 2017;**448**:671-678

[22] Nagano S, Yokouchi M, Setoguchi T, Sasaki H, Shimada H, Kawamura I, Ishidou Y, Kamizono J, Yamamoto T, Kawamura H, Komiya S. Analysis of surgical site infection after musculoskeletal tumor surgery: Risk assessment using a new scoring system. Sarcoma. 2014;**2014**:1-9

[23] Namba RS, Inacio MC, Paxton EW. Risk factors associated with deep surgical site infections after primary total knee arthroplasty: An analysis of 56,216 knees. The Journal of Bone & Joint Surgery. 2013;**95**:775-782

[24] Radovanovic Z, Jokic B, Velijovic D, Dimitrijevic VK, Petrovic R, Janackovic D. Antimicrobial activity and biocompatibility of $Ag^{+}$- and $Cu^{2+}$-doped biphasic hydroxyapatite/$\alpha$-tricalcium phosphate obtained from hydrothermally synthesized $Ag^{+}$ and $Cu^{2+}$-doped hydroxyapatite. Applied Surface Science. 2014;**307**:513-519

[25] Ferraris S, Venturello A, Miola M, Cochis A, Rimondini L, Spriano S. Antibacterial and bioactive nanostructured titanium surfaces for bone integration. Applied Surface Science. 2014;**311**:279-291

[26] Kolmas J, Groszyk E, Kwiatkowska-Różycka D. Substituted hydroxyapatites with antibacterial properties. BioMed Research International. 2014;**2014**:1-15

[27] Morais DS, Coelho J, Ferraz MP, Gomes PS, Fernandes MH, Hussain NS, Santos JD, Lopes MA. Samarium doped glass-reinforced hydroxyapatite with enhanced osteoblastic performance and antibacterial properties for bone tissue regeneration. Journal of Materials Chemistry B. 2014;**2**:5872-5881

[28] Mishra VK, Bhattacharjee BN, Parkash O, Kumar D, Rai SB. Mg-doped hydroxyapatite nanoplates for biomedical applications: A surfactant assisted microwave synthesis and spectroscopic investigations. Journal of Alloys and Compounds. 2014;**614**:283-288

[29] Aina V, Lusvardi G, Annaz B, Gibson IR, Imrie FE, Malavasi G, Menabue L, Cerrato G, Martra G. Magnesium- and strontium-co-substituted hydroxyapatite: The effect of doped ions on the structure and chemico-physical properties. Journal of Materials Science: Materials in Medicine. 2012;**23**:2867-2879

[30] Dorozhkin SV. Calcium orthophosphates in nature, biology and medicine. Materials. 2009;**2**:399-498

[31] Farzadi A, Bakshi F, Solati-Hashjin M, Asadi-Eydivand M, Osman NAA. Magnesium incorporated hydroxyapatite: Synthesis and structural properties characterization. Ceramics International. 2014;**40**(4):6021-6029

[32] Kannan S, Goetz-Neunhoeffer F, Neubauer J, Ferreira JMF. Ionic substitutions in biphasic hydroxyapatite and $\beta$-tricalcium phosphate mixtures: Structural analysis by Rietveld refinement. Journal of the American Ceramic Society. 2008;**91**:1-12

[33] Shepherd JH, Shepherd DV, Best SM. Substituted hydroxyapatites for bone repair. Journal of Materials Science: Materials in Medicine. 2012;**23**:2335-2340

[34] Roane TM, Reynolds KA, Maier RM, Pepper IL. Chapter 2. In: Pepper IL, Gerba CP, Gentry T, Maier RM, editors. Environmental Microbiology. Academic Press, Elsevier, 2nd ed. 2009. pp. 9-36

[35] Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. Cold Spring Harbor Perspectivas em Biologia. 2010;**2**(5):1-17

[36] Baptista I, Rocha SM, Cunha A, Saraiva JA, Almeida A. Inactivation of Staphylococcus Aureus by high pressure processing: An overview. Innovative Food Science & Emerging Technologies. 2016;**36**:128-149

[37] Stefani S, Campanile F, Santagati M, Mezzatesta ML, Cafiso V, Pacini G. Insights and clinical perspectives of daptomycin resistance in Staphylococcus Aureus: A review of the available evidence. International Journal of Antimicrobial Agents. 2015;**46**(3):278-289

[38] Hsieh PH, Lee MS, Hsu KY, Chang YH, Shih HN, Ueng SW. Gram-negative prosthetic joint infections: Risck factors and outcome of treatment. Clinical Infectious Diseases. 2009;**49**:1036-1043

[39] Gopi D, Ramya S, Rajeswari D, Karthikeyan P, Kavitha L. Strontium, cerium co-substituted hydroxyapatite nanoparticles: Synthesis, characterization, antibacterial activity towards

prokaryotic strains and in vitro studies. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2014;**451**:172-180

[40] Morais DS, Fernandes S, Gomes PS, Fernandes MH, Sampaio P, Ferraz MP, Santos JD, Lopes MA, Sooraj HN. Novel cerium doped glass-reinforced hydroxyapatite with antibacterial and osteoconductive properties for bone tissue regeneration. Biomedical Materials. 2015;**10**(4):1-15

[41] Ciobanu G, Bargan AM, Luca C. New cerium (IV)-substituted hydroxyapatite nanoparticles: Preparation and characterization. Ceramics International. 2015;**41**:12192-12201

[42] Sanyal V, Raja CR. Structural and antibacterial activity of hydroxyapatite and fluorohydroxyapatite co-substituted with zirconium-cerium ions. Applied Physics A. 2016; **122**(132):2016

*Edited by Rosario Pignatello and Teresa Musumeci*

This contribution book collects five among reviews and original articles from eminent experts working in the interdisciplinary area of biomaterial synthesis and application. From their direct and recent experience, the readers can access the novel and ongoing potentialities of different synthetic and engineered biomaterials. Contributions reflect the fundamental studies, with a particular attention to the physico-chemical mechanical characterization of biomaterials, along with biocompatibility studies and potential clinical use.

After an introductory chapter on the question of storage stability for biomaterial-based devices and products and for polymeric nanomedicines, a first review deals with the use and commercial sources of hydroxyapatite in tissue engineering and other biomedical applications. A study follows on optical fiber laser marking on the properties of stainless steel in implant manufacturing. Two other reviews, respectively, focused on the approaches to prevent or treat the effects of calcification that occurs in vivo on biomaterial-based implants and on the encapsulation of pancreatic islet cells for the treatment of type I diabetes will be presented. Finally, an overview on the physical bases and application in biomaterial science of the spray-drying process will close the volume. This setting will allow to achieve a general view of how classical and novel biomaterials can be applied, along with the methodologies necessary to design, develop, and characterize them, without the restrictions necessarily imposed by industrial or profit concerns.

Readers will be apprised about the methodologies used to develop biomaterials possessing the physical and biological properties needed for specific medical and clinical applications.

Published in London, UK


IntechOpen

ISBN 978-1-83881-376-5