Chapter 6

Perspective of Additive Manufacturing Selective Laser Melting in Co-Cr-Mo Alloy in the Consolidation of Dental Prosthesis

Marcello Vertamatti Mergulhão,
Carlos Eduardo Podestá and
Maurício David Martins das Neves

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Abstract

This chapter seeks to compare the properties of samples manufactured by additive manufacturing (AM) by the selective laser melting (SLM) technology and compare with the precision casting (PC) processes using the Co-Cr-Mo (ASTM F75) alloy to manufacture of dental prosthesis. This AM process can be manufactured three-dimensional models by means of a laser beam that completely melts particles of powder deposited layer by layer. However, it is still relevant to know the properties of: performance, dimensional, mechanical and microstructural of this laser melting process and compare with a convencional process. The results of mechanical evaluation showed that the SLM technique provides superior mechanical properties compared to those obtained by the PC technique. It is possible to verify that the consolidation by SLM technique results in lower presence of porosity than PC technique. In addition, PC samples presented a gross dendritic microstructure of casting process. Microstructural analysis of SLM samples results in a characteristic morphology of layer manufacturing with ultrafine grains and a high chemical homogeneity. In this way, the development of the present study evidenced to improve the manufacture of customized components (copings) using the SLM technology.

Keywords: Co-Cr-Mo alloy, biomaterial, additive manufacturing, selective laser melting, precision casting

1. Introduction

Metal powders of cobalt-chromium (Co-Cr) alloy are widely used in various sectors of the automotive, aeronautics, and aerospace industry, because of its high wear resistance and adequate corrosion resistance also being used in surface coating to increase performance components [1, 2]. In addition, the biocompatibility properties are suitable and are being used in the manufacture of medical and dental prosthetics [3–7]. The use of Co-Cr alloys is widely discussed to manufacture medical and dental implants or prostheses [7–9] presenting positive aspects in relation to biocompatibility analysis. The necessity for characterization and biological evaluations, physical-chemical, and mechanical are basic requirements for the development of new biomaterials applied in medical devices. In general, biomaterials need to present a final clinical characteristic (bio-functionality) and biocompatibility [10, 11].

Since 1930, Cobalt-Chromium-Molybdenum (Co-Cr-Mo) alloys processed by casting were used as dental alloys and later adapted for use in orthopedic implants [12, 13]. According to Jabbari et al., Co-Cr alloys are used almost exclusively in the manufacture of metal structures prostheses and recently is replacing Ni-Cr alloy or alternatively for the production of restorations in porcelain fused to metal (PFM), because Co-Cr alloy is Ni-free and does not have allergic responses or toxic effects related to Nickel [14].

The coefficient of thermal expansion (CTE) is a thermal property of the alloy, is of great interest in cases of applications in dental components, that requires ceramic coating, such as the dental crown. In this case, it is shown by Refs. [6, 15, 16] that Co-Cr alloys should have a CTE value in the range of 14.0–14.6 × 10^{-6} °C^{-1} at temperatures from 500 to 600°C to the correct ceramic firing process, as coating of the metal component. The CTE of ceramic materials for coating applications in metallic materials should be close, providing a good adjustment due to contraction and expansion during heating, thus avoiding the possibility of voids or cracks occurring during the firing process [15, 17].

Currently, the lost wax casting method is the most widely used, but has faced competition from other manufacturing processes [17]. Several authors [18–20] describe the development of AM technologies providing the creation of final customized implants. Techniques such as stereolithography were implemented to manufacture resin models for posterior manufacture of dental prostheses (crowns and bridges) by conventional process of lost wax castings. Mechanical, chemical, and microstructural properties are evaluated in comparison to new AM technologies, for example, the selective laser melting (SLM) in relation to conventional techniques as lost wax casting [21–23]. In this way, the preparation of medical and dental components provides customized final components with high mechanical properties, compared to conventional techniques (see **Figure 1**) [24].

Notably in health area, this technology is competitive over other traditional manufacturing processes by advances occurred in the area of processes using powder metallurgy techniques [25, 26]. Selective laser melting technology is one of the innovative technologies in additive manufacturing development in the middle of the 1980s after the creation of selective laser sintering (SLS) process. SLM is a process based on the 3D construction in which it is possible by laser beam to completely melt the metallic powder particles on a previous layer [27–31].

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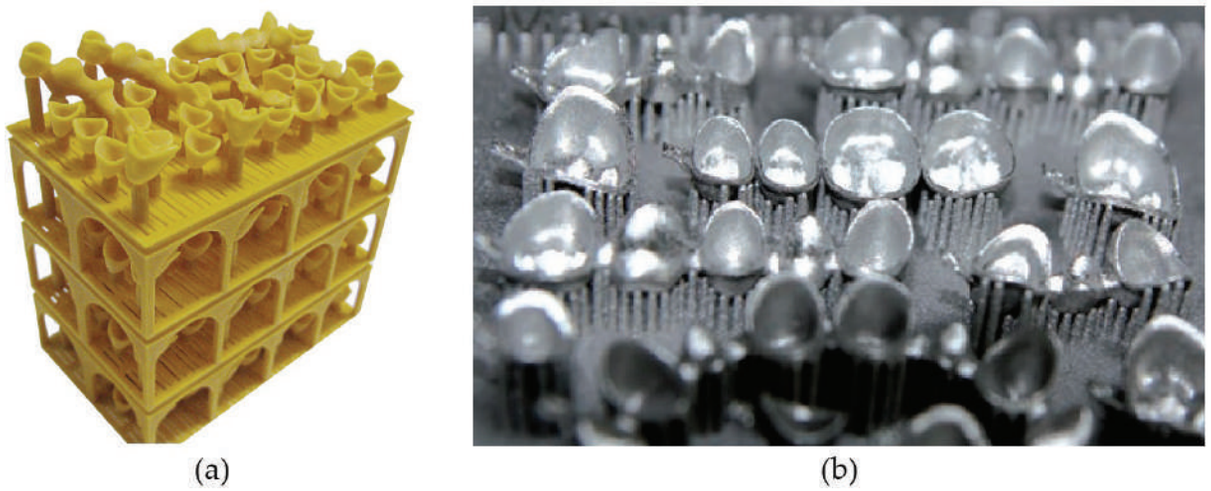

Figure 1. Models of dental components manufactured by AM techniques in (a) resin model by stereolithography for posterior precision casting process and (b) copings manufactured by SLM technique [17].

In SLM technique, the raw material is in metallic powder form and the thermal energy required for the complete melting of the powder layer comes from a laser beam, usually is used a source of Ytterbium (Yb) fiber [29]. The maximum laser power on SLM machines is approximately 400 W and the laser focus may have a diameter of approximately 100 μm [30]. In turn, the laser beam is commanded by an interface that transmits it to the optical assembly, which selectively directs (X–Y plane) the laser beam, causing the powders to melt [28–33]. The metal powder is stored in a container, which may or may not be the distributor of powder (deposited by gravity), which in turn uniformizes the powder layer (between 50 and 100 μm) on an object consolidation platform. At each consolidated layer, the platform moves on the Z–axis, according to the next layer until the component is completely consolidated. The process of component consolidation occurs in a consolidation chamber (internal environment of the SLM equipment) that is under inert atmosphere protection (argon gas) [28–33]. The basic scheme of the components present in the consolidation process by SLM can be observed in **Figure 2**, and other machine parameters and working conditions are presented in **Table 1**.

Details of the main parameters of the SLM technique are shown schematically in **Figure 3**.

The SLM technique has several process parameters and can be grouped in five families, being these related to laser, scanning, material (powder), temperature, and consolidation chamber [28, 29, 31].

- Laser: power "P", beam diameter, pulse duration, pulse frequency;
- Scanning: speed "v", track distance, strategy;
- Powder (material): material properties, particle size, distribution, bed density of powder, layer thickness;
- Temperature: consolidation layer, powder feeder, uniformity;
- Compounding chamber: composition of protective atmosphere.

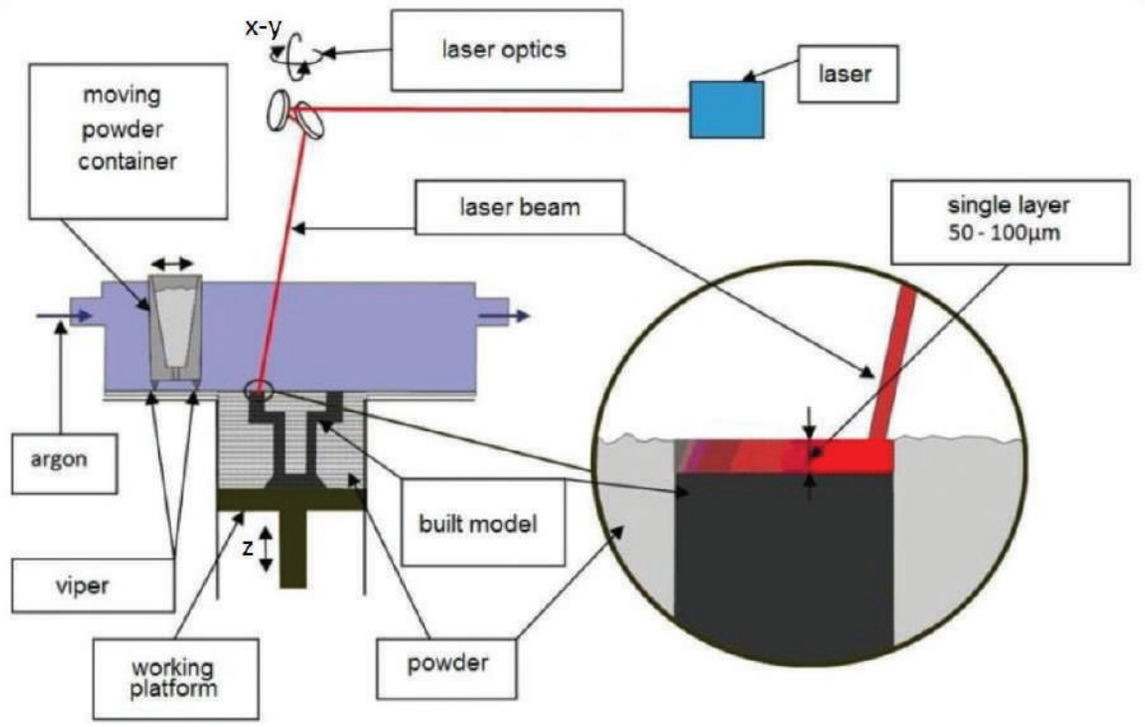

Figure 2. Basic schematic of components present in a SLM machine [33].

The most common parameters to be adjusted in the SLM process to optimize the manufacture of components are: laser power (P), scan speed (v), track (hatch) distance, and layer thickness. According to Refs. [27, 34, 35], the volumetric energy density of the laser (ψ, given in J.mm^{-3}) relates the main parameters of consolidation, in relation to the laser as shown in Eq. (1), being: laser power (P), track distance (t), scan speed (v), and layer thickness (L).

$$\psi = \frac{P}{t \cdot v \cdot L} \tag{1}$$

System parameters	
Laser power	400 W Yb-Fiber-laser
Build speed	20 ccm/h
Pract. layer thickness	20–75 µm min
Scan line/wall thickness	150 µm
Operational beam focus	80–120 µm
Scan speed	15 m/s
Inert gas consumption in operation	Ar/N_2, 2.5–3.0 l/min
Inert gas consumption venting	Ar/N_2, 1700 l @ 100 l/min
Compressed air requirement	18 l/min @ 1.5 bar

Table 1. Typical technical parameters of the SLM®280^{HL} machine.

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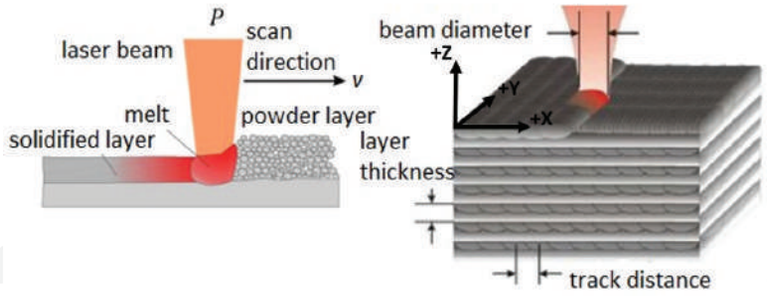

Figure 3. Schematic of parameters involved in SLM consolidation [31].

Several authors in Refs. [27, 34, 35] report that these parameters affect the volumetric energy density, determinant in the powder melting and that in turn influences the mechanical properties and roughness of the surface of the consolidated parts. The combination of these variables can generate excess (or insufficient) energy during the consolidation process, which can lead to the balling phenomenon at consolidated specimen, which corresponds to the dissimilar or noncontinuous scan tracks [30, 36, 37]. Additionally, the balling phenomenon can generate uniform deposition of next powder layer, can cause uncontrollable porosity and delamination by the absence of inter-fusion between layers [37, 38].

As observed, the parameters of the SLM process involve a certain complexity, in order to obtain the fabrication of components of complete density. In order to optimize the mechanical and physical-chemical properties of the final components manufactured the consolidation strategies are the subject of discussion and study [39]. The consolidation strategies refer to the consolidation parameters already presented, as well as to the direction and orientation of laser beam scanning, angle of rotation between the layers, and the number passes of laser beam (per layer), as seen in **Figure 4a–h** [40–42]. Also, the physical properties of the components may be associated with the manufacturing anisotropy of the samples, see **Figure 4i** [43–45].

An interesting point to consider for AM processes is the feedstock (or raw material–metal powder). These new technologies demanded a characteristic powder size distribution, format and physical properties (flowability and packing) [36]. In this case, to produce spherical metal powder the most common process is gas atomization [29, 30]. However, it is remarkable that the use of gas-atomized powders in the SLM process by the better physical properties is compared to water-atomized powders. The characteristic format of powders (gas and water atomized) is possible to observe in **Figure 5**.

In addition, the capability to reusability of feedstock material in AM processes is a significant issue to promote economic and environmental manufacture processes [28, 46]. However, the effects and influence of the powder reuse on manufactured parts are the subject of much discussion [47–50]. The conclusions of Tang et al. [48] study, about reuse of Ti-6Al-4V powders of electron beam melting (EBM) process, appoint the increased oxygen content and particles became less spherical. Although the reuse powder improved the flowability (by little presence of satellite particles), increased the yield strength and the ultimate tensile on the AM process of Ti-6Al-4V [48].

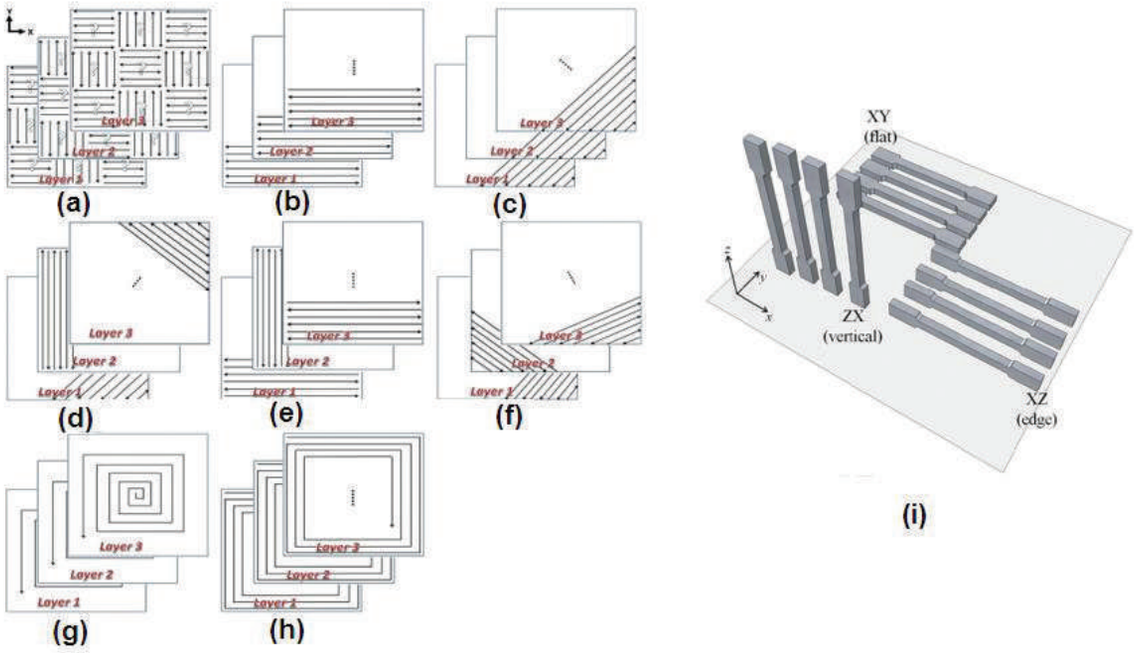

Figure 4. Representation of laser scanning strategies for sample consolidation via SLM, scanning in: (a) island, (b) line, (c) line at 45°, (d) line and rotation line at 45°, (e) line and rotation line at 90°, (f) line rotate at 67°, (g) internal spiral, (h) external spiral [42], and (i) building orientation of specimens [45].

Considering this important field in expansion, this chapter is part of this scenario with a focus on the dental sector, more specifically on the evaluation of mechanical properties and microstructural analysis of Co-Cr-Mo alloy to manufacture dental prostheses (copings). The aim of this chapter is to evaluate the mechanical properties and microstructures of standardized specimens made by powder metallurgy techniques using SLM from powdered gas powder of the Co-Cr-Mo alloy. The results obtained by SLM will be compared with the results of samples manufactured by precision casting.

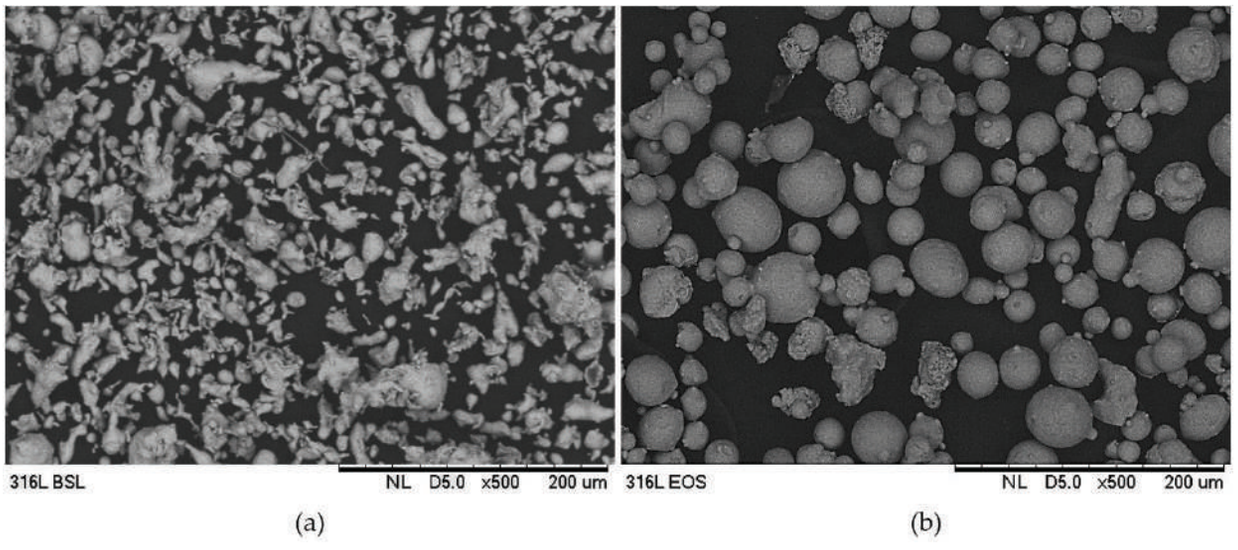

Figure 5. Characteristic format of powders (316L alloy) produced by water atomization (a) and gas atomization (b). Magnitude ×500.

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2. Experimental procedure

2.1. Powder characterization

Co-Cr-Mo alloy gas atomized (H.C Starck®, Lübeck, Germany) was provided by the HighBond® (Indaiatuba, Brazil) in the particle size (granulometric range) of 15–45 μm. The confirmation of the chemical composition was performed by energy dispersive X-ray (Shimadzu EDX-720 equipment and by LECO). This study was based on alloy/powder with certification of ANVISA (Brazilian agency) for use in health care segment.

Several physical properties of gas-atomized powder were obtained such as flow time (ASTM B212 [51]), apparent density (ASTM B213 [52]), and tap density (ASTM B527 [53]). The particle size distribution was performed using a particle analyzer by laser scattering (Cilas–Model 1064). The particle format and microstructural characterization of powders were performed via optical and scanning electron microscopy (OM–Olympus BX51M and SEM-EDS Philips XL30).

To evaluate the internal porosity of powders sample were measured by the pycnometer density in comparison to theoretical density. The density by Helium pycnometry considered only the internal porosity (excluding the open porosity) and was performed using the Micromeritics equipment (Model Accu PYC 1330 Pycnometer).

Differential scanning calorimetry (DSC) analysis was performed using a sample of gas-atomized Co-Cr-Mo powder. Three runs of heating curves at rates of 10, 20, and 30°C/min were performed and under static atmosphere constituted in argon (99.999%) for minimizing the oxidation of the samples. In all experiments, both the crucible (sample holder and the reference–empty during all tests) were composed of alumina (Al_2O_3) with a volume of approximately 100 μL. The equipment used was Setsys 16/18, from Setaram with a thermocouple rod of Pt/Pt Rh 10%.

2.2. Manufacturing specimens using precision casting and selective laser melting

Precision casting and selective laser melting techniques performed the manufacture of gas-atomized Co-Cr-Mo powders. The tensile and three-point bending specimens were manufactured in standard dimensions according to ISO 22674-06 [54] and ASTM B528-12 [55]. **Figure 6** shows specimens manufactured.

The precision casting (PC) samples were performed according to ASTM F75-12 [13] by HighBond® (Indaiatuba, Brazil). The PC fabrication process method satisfied the following steps: machining of wax disks in the standard dimensions of tensile and flexural test specimens, assembly and shell building, dewaxing and pouring the Co-Cr-Mo alloy was by an induction furnace at a temperature of 1489°C.

The consolidation of SLM samples was carried out by SLM Solutions™ using a selective laser melting machine SLM®280HL with a single Ytterbium laser beam (maximum power 400 W). The building consolidation of specimens was parallel to laser beam and performed using parameters such as: layer thickness of 30 μm and diameter of laser beam of 76 μm.

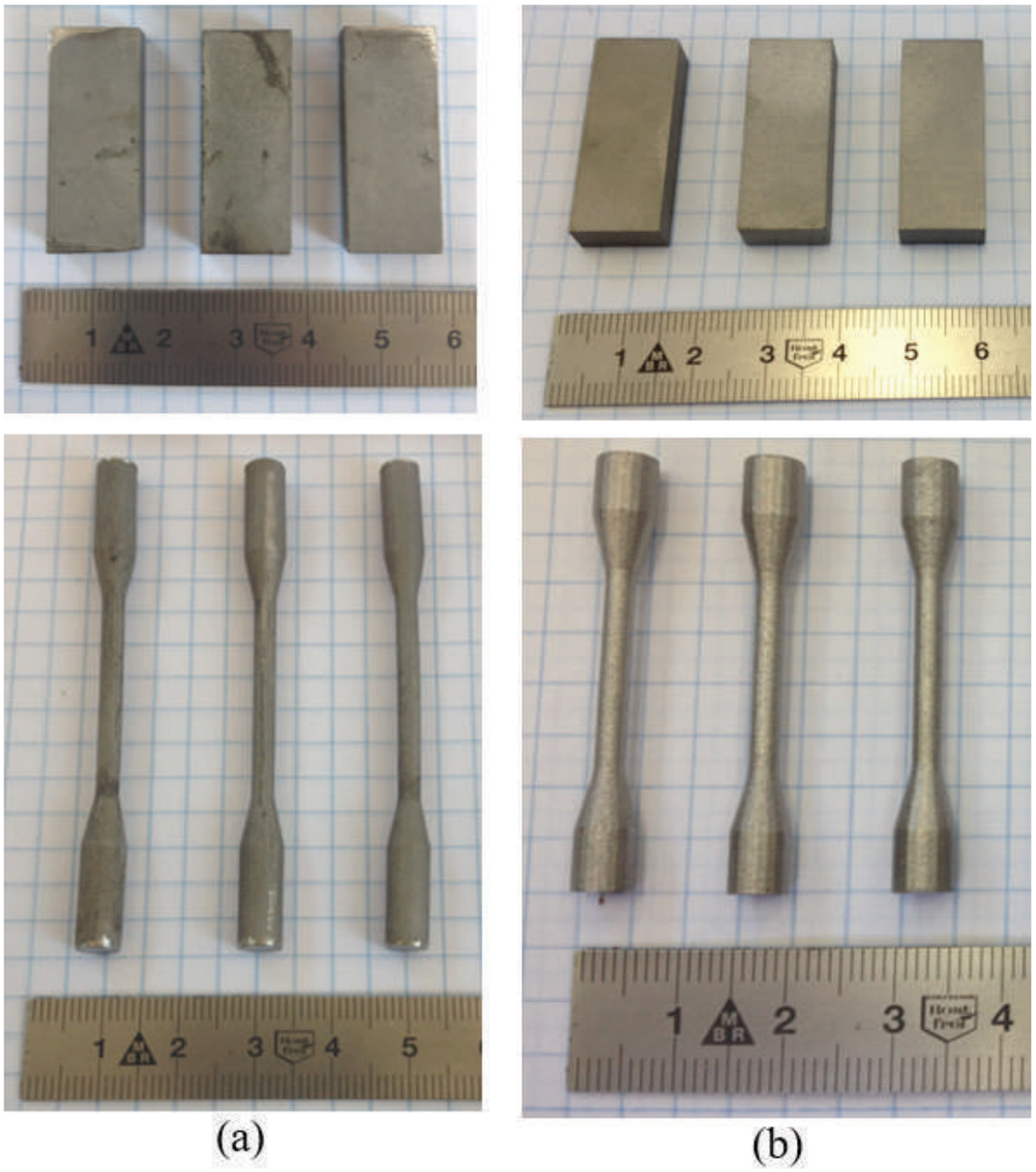

Figure 6. Specimens manufactured of Co-Cr-Mo alloy: (a) specimens made by FP technique and (b) specimens made by SLM technique.

2.3. Characterization of samples manufactured

To evaluate the susceptibility to cell growth in the Co-Cr-Mo alloy after the consolidation processes (PC and SLM) was performed by the cytotoxicity analysis, according to ISO 10993-5 [56]. The determination of the cytotoxicity was obtained by the quantitative evaluation method, which is carried out by the measurement of cell death, cell proliferation or formation of cellular colonies.

To evaluate the internal porosity of cast, and SLM samples were measured by the pycnometry density in comparison to the theoretical density. The density by Helium pycnometry, considered only the internal porosity (excluding the open porosity) was measured using the Micromeritics equipment–Model Accu PYC 1330 Pycnometer.

The thermomechanical analysis (TMA) was performed on samples consolidated by SLM and PC of the Co-Cr-Mo alloy. The purpose of the technique was to obtain the coefficient of thermal expansion (CTE). In addition, the SLM samples were analyzed in the parallel and transversal building direction (SLM 1—parallel direction and SLM 2—transversal direction). The routine of the TMA remained the heating rate was from 10°C/min until the temperature of 1300°C. The equipment used was a Setaram–Setsys 16/18, using a thermocouple rod of Pt/Pt Rh 10% under a static atmosphere (argon—99.999%) to exclude the sample oxidation.

2.4. Mechanical characterization

Mechanical characterization of consolidated samples by PC and SLM techniques was held in five samples of each test (tensile and three-point bending), respectively, according to ISO 22674-06 [54] and ASTM B528 [55]. The three-point bending test determined the transversal rupture strength (TRS) of specimens. The TRS relates to the applied load (P) and the distance between the supports (L), over the cross area of the sample (thickness "*t*" and width "*w*"), as show in Eq. (2). Mechanical tests were performed using a universal testing machine (Instron 3366) under a crosshead speed of 0.2 mm/min at room temperature.

$$TRS = \frac{3 \cdot P \cdot L}{2 \cdot t2 \cdot w} \tag{2}$$

2.5. Microstructural evaluation

The microstructural characterization of consolidated Co-Cr-Mo and the fracture analysis were evaluated after tensile test. Metallography preparation consisted of mechanical grinding in SiC paper #1200 and final chemical polishing with OP-S 0.02 μm with addition of 10% HCl. The specimens were etching in solution: 100 ml HCl and 2 ml H_2O_2 (1–2 min at room temperature). The microstructural characterization was performed in both building directions using an optical microscope (OM) Olympus–BX51M and scanning electron microscope (SEM) with energy dispersive X-ray (EDS) Philips XL30 and JEOL–JSM6701F.

3. Results and discussion

3.1. Powder characterization

The confirmation of chemical composition was performed in Co-Cr-Mo powder alloy, as also in the samples manufactured (PC and SLM). The chemical composition is presented in **Table 2** comparing with the standard ASTM F75-12 [13].

Elements (%)	Powder	PC	SLM	ASTM F75
Co	63.93 ± 0.16	66.38 ± 0.15	65.38 ± 0.32	Balanço
Cr	28.83 ± 0.19	26.76 ± 0.21	27.68 ± 0.13	27.00 – 30.00 ± 0.30
Mo	7.07 ± 0.31	6.68 ± 0.03	6.61 ± 0.16	5.00 – 7.00 ± 0.15
Fe	0.17 ± 0.01	0.18 ± 0.08	0.33 ± 0.06	0.75 ± 0.03
C	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.350 ± 0.020
S	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.010 ± 0.003
N_2	0.0820 ± 0.0011	0.0416 ± 0.0015	0.1330 ± 0.0015	0.250 ± 0.020
O_2	0.0940 ± 0.0015	0.0187 ± 0.0016	0.0240 ± 0.0010	–

Table 2. Chemical composition (weight %) of Co-Cr-Mo samples (powder, PC and SLM) in accordance with standard ASTM F75.

The characteristic format of the powder process fabrication by gas atomization is observed in **Figure 7**. The analysis in SEM shows that the powders are spherical and presented satellites (appointed by arrows—**Figure 7a**,**b**). The satellites can be formed in the surface particles during the cooling process of the spherical powder particles during gas atomization. It is noteworthy that the shape of the particle influences on packing properties, flow hate, and compressibility, as well as reports on the powder metallurgy process [2, 57, 58]. The cross-sectioned powder (**Figure 7d,e**.) shows the dendritic morphology with the primarily arms and ramifications, characterizing the rapid solidification of gas atomization process.

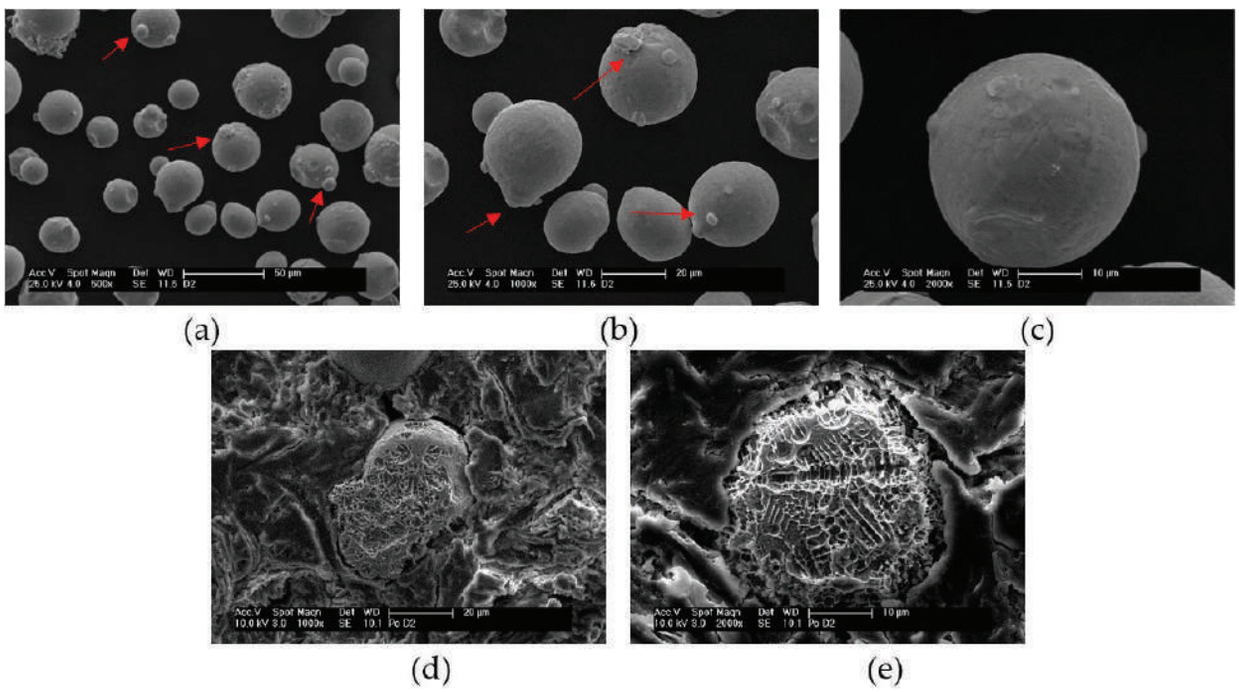

Figure 7. SEM images of Co-Cr-Mo powder: (a) magnitude ×500, (b) magnitude ×1000, (c) magnitude ×2000, (d) and (e) cross-section powder after chemical etch (etch solution: HCl, H_2SO_4, and HNO_3 for 60–240 s at 45°C, respectively, magnitude ×1000 and ×2000).

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Physical properties	Co-Cr-Mo powder			
Granulometric distribution (μm)	D10	D50	D90	D mean
	20.88	31.11	46.10	32.36
Flow rate (s/50g)	15.85 ± 0.11			
Apparent density (g/cm^3)	4.51 ± 0.01			
Tap density (g/cm^3)	5.26 ± 0.05			
Theoretical density (g/cm^3)	8.38			
Helium pycnometry (g/cm^3)	8.30 ± 0.001			

Table 3. Results of physical properties for Co-Cr-Mo powder in the SLM range.

The powders to manufacture samples via SLM technique have a mean diameter less than 50 μm to improve the physical properties such as flow time, apparent density, and tap density [33]. The results of physical powder properties are summarized in **Table 3**.

According to Haan et al. [59], Co-Cr-Mo powders with diameter D90 equals to 39 μm, the flowability was 18.60 s/50 g. The results were similar to those obtained for the present study, such as 15.86 s/50 g for D90 equals to 46.10 μm.

The result of tap density tends to be higher than the result of the apparent density, because of the particle's accommodation there is a decrease in the amount of voids between the particles [2]. Also, the smaller the apparent density, the greater the percentage of increase the tap density.

It is possible to verify the presence of closed porosity that is not considered as a measure of the volume of Helium and consequently reduces the value of pycnometry density (8.30 g/cm^3). The presence of internal porosity calculated in relation to theoretical density (8.38 g/cm^3) is approximately 1.3%.

To investigate and confirm the thermal events present in the Co-Cr-Mo alloy, the heating curves (different rates: 10, 20, and 30°C/min) of the DSC analysis obtained using the Co-Cr-Mo powder are shown in **Figure 8**. The presence of three events occurring in the heating curves of DSC is observed, being the first exothermic, the second endothermic, and the final event corresponding to the fusion of the Co-Cr-Mo alloy. It is possible to verify the temperature variation of the events between the different temperature rates.

In relation to the first event (exothermic), occurring around 582.81°C, it is related to the phase transformation of the alloy (precisely from Co), from the cubic face (αCo) phase to the compact hexagonal phase (εCo). In a similar analysis, Santos [16] obtained a slight peak at 600°C in the thermal analysis (DTA), however the author does not approach the occurrence. Facchini [60] describes this occurrence, the event occurs at approximately 650°C, but is described by an endothermic peak, diverging from the present analysis, in which the curve of 20°C/min occurs at approximately 600°C and which describes an exothermic peak.

The second event (endothermic) occurs around 944.52°C, may be related to allotropic transformation of element Co, by the transition of the phase of compact hexagonal structure (εCo) to the phase of cubic structure of face centered (αCo). This transformation can be confirmed

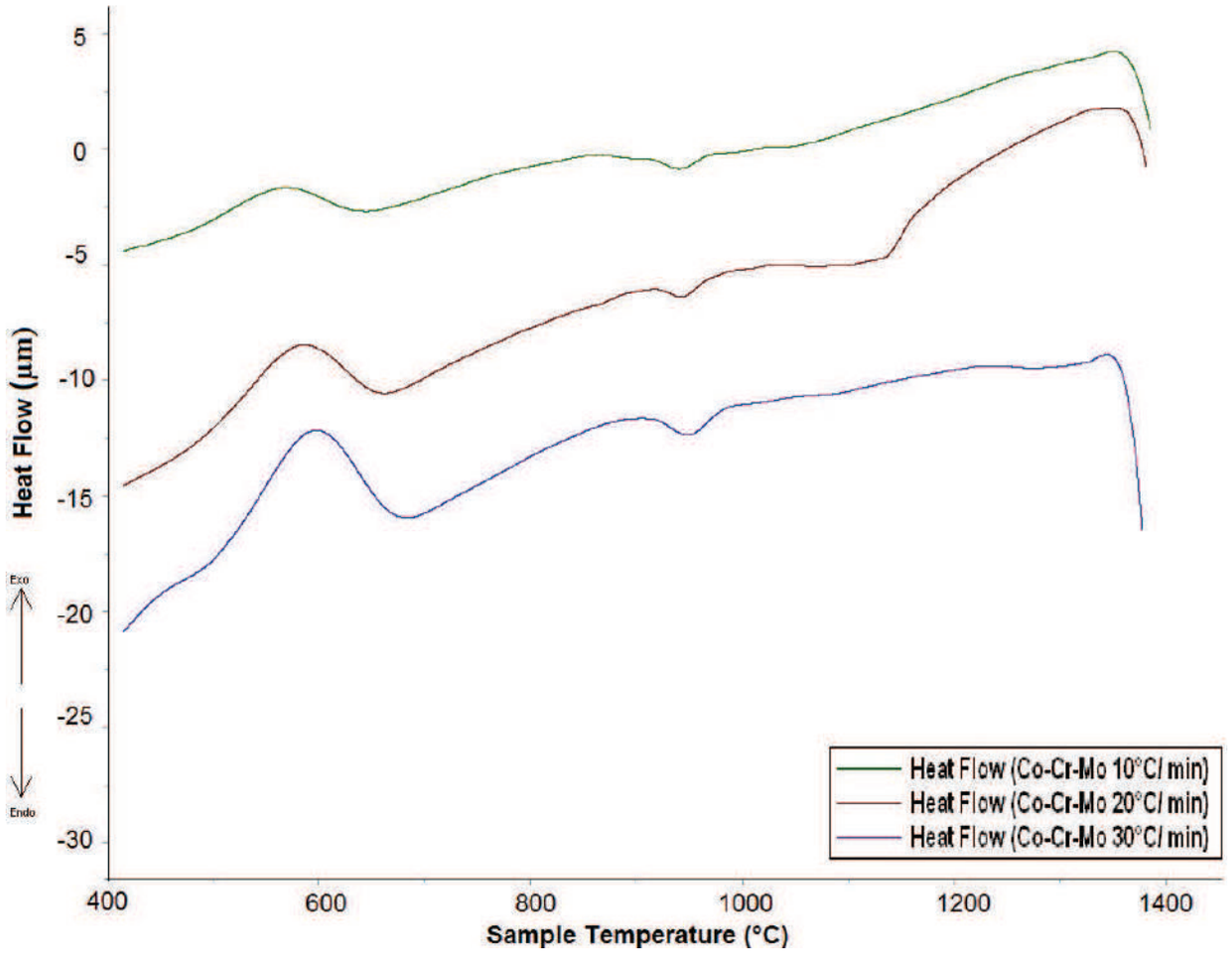

Figure 8. Heating curves of DSC analysis for Co-Cr-Mo powder in different rates (10, 20, and 30°C/min).

in the Co-Cr binary diagram occurring at about 950°C). This occurrence is similar to that described in Ref. [16, 60], which obtains endothermic peaks, respectively, at approximately 970 and 1000°C, relative to that obtained in the present study of 944.52°C. This temperature difference is associated with the chemical composition of the alloy (64Co-29Cr-7Mo of the present study), which represent alloys according to ASTM F75-12 (stoichiometry is 66Co-28Cr-6Mo) and therefore there are temperature difference of 26 and 56°C relative to the cited references. This difference can be associated to different calibrations, among the equipment used in the analysis.

In interpreting the DSC curves, it is possible to verify the melting temperature of the Co-Cr-Mo alloy. By means of the average value of the three heating rates, the melting temperature is approximately 1354.5°C, with a variation of 4°C between rates.

3.2. Evaluation of samples manufactured

The result of the cytotoxicity analysis for the processed samples is shown in **Figure 9**. According to the cytotoxicity assay with respect to the pure extract, without dilution, the samples processed by precision casting and selective laser melting showed no toxicity.

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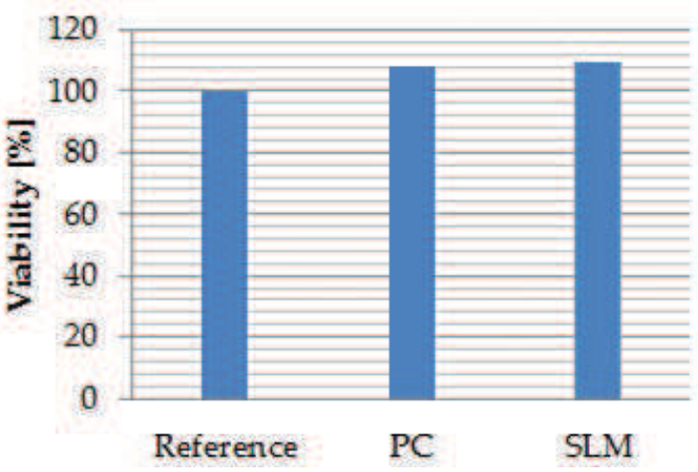

Figure 9. Result of viability of cell growth (cytotoxicity analysis) for Co-Cr-Mo specimens manufactured by PC and SLM techniques.

The results showed the expected results for the Co-Cr-Mo alloy, because as there is a need for specific mechanical properties to be reached, the use of Co-Cr alloys for the manufacture of medical and dental implants or prostheses did not show any toxicity with the medium biological [7, 8, 61].

The porosity of the samples was determined and a comparison was made with average densities: theoretical, volumetric, and by helium pycnometry. The results of the mean densities obtained for the samples consolidated by PC and SLM can be seen in **Table 4**.

Analyzing the results of volumetric density, it is possible to identify that PC samples present a lower result than the one obtained in the SLM samples. This premise is confirmed by the result obtained, evidencing that the PC process presents superior open porosity than SLM technology. When correlating with the density by Helium pycnometry, it can be verified that the open porosity results in 0.24% for PC sample and 0.12% for SLM sample.

Analyzing the Helium pycnometry, it is possible to verify that both consolidation processes have the same theoretical density (8.24 g/cm^3). Relating the Helium pycnometry density to the theoretical density is possible to check the internal porosity, that results, respectively, for the PC and SLM process of 2.14 and 1.80%. It can be concluded that the SLM consolidation process produces samples with lower internal porosity, and can obtain components with densities around 98.20% of theoretical, in contrast to the PC process that obtains components with density of 97.86% of theoretical.

The heating curves obtained from TMA in the form of PC and SLM sample are presented in **Figure 10**. To understand the events occurred at TMA, the heating curves of DSC analysis were juxtaposed. The CTE for the consolidated samples has a different behavior between the processes of the Co-Cr-Mo alloy in a similar analysis to the present study. The CTE for the consolidated samples has a distinct behavior between the processes of the Co-Cr-Mo alloy. As it is possible to verify the CTE at temperature of 500°C of samples (SLM 1, SLM 2, and PC) is, respectively, 15.0/19.5/22.0 × 10^{-6} °C^{-1}. At the temperature of 600°C, the coefficient value decreases to the values of 12.5/14.5/18.5 × 10^{-6} °C^{-1}. This difference is greater for the

Sample	Densities (g/cm³)		
	Theoretical	Helium pycnometry	Volumetric
PC	8.42	8.24 ± 0.01	8.22 ± 0.10
SLM	8.39	8.24 ± 0.01	8.23 < 0.01

Table 4. Results of densities (medium values): theoretical, Helium pycnometry and volumetric for samples PC and SLM.

alloy processed by PC, which is associated to the dendritic microstructure formed and in relation to the casting process because it characterizes a fine grain microstructure and more packaging.

As can be seen in the TMA and DSC curves are similar in the temperature ranges of events. Facchini [60] shows the DSC and TMA curves (heating rate of 20°C/min) performed in an ASTM F75-12 composition alloy and processed by electron beam fusion (EBM). EBM has an effect similar to the SLM because both processes have a concentrated heat source and is possible to relate the DSC analysis, in which, the first peak (565–900°C) is associated with the transition from the FCC (αCo) phase to the HCP (εCo) phase, and the second peak (900–1000°C) reduces the HCP phase (εCo) and reappearance of the FCC phase (αCo).

Is possible observed two events, the first event occurs in the range of 514–614°C and the second event at 923–961°C. In the case of the PC sample (TMA curve), it is possible to verify that the events occur in a higher temperature in relation to the samples processed by SLM. This occurrence is associated to the microstructure samples of analysis performed. The powder

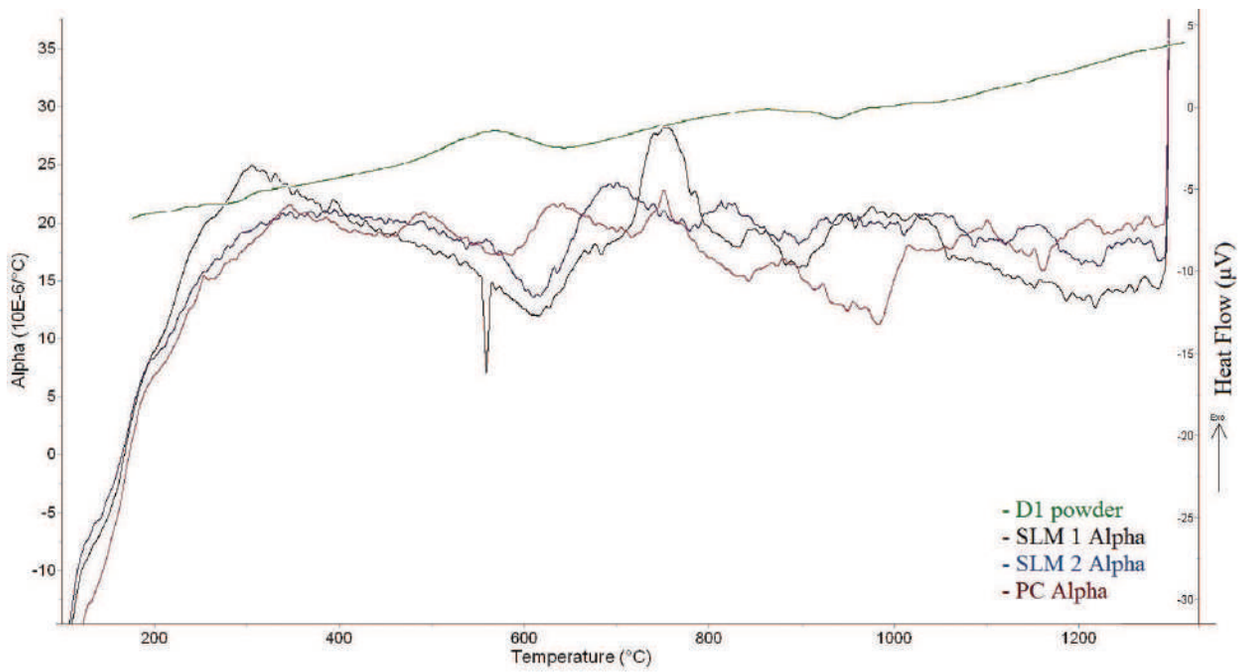

Figure 10. Heat curve of thermal analysis for Co-Cr-Mo samples: DSC curve of Co-Cr-Mo powder sample and TMA curves of consolidated samples PC and SLM (SLM 1 and SLM 2).

sample (at DSC analysis) has a dendritic microstructure, as the same of the PC sample (at TMA). TMA curves of SLM samples (SLM 1 and SLM 2) show more evident peaks, but the transition of these events set at increased temperatures. Thus, the laser fusion process has a refined and more homogeneous microstructure what hinders phase transitions and requires higher temperatures.

3.3. Mechanical behavior

The mechanical results of the tests for the PC and SLM specimens are present in **Table 5**. Analyzing the values is possible to verify that in all properties the SLM technique results in higher properties than PC technique. According to standard ISO22674:06 [54], the SLM and PC specimens satisfied the type 5 criteria in all mechanical properties.

The result of TRS samples (SLM = 2501.2 ± 9.7 MPa and PC = 1072.3 ± 4.6 MPa) was satisfactory. However, there was no rupture of PC sample (test interrupted) evidenced the ductility of the precision casting process, that was confirmed by the value of higher elongation. According to Mengucci et al. [62], the TRS result for a similar composition of Co-Cr-Mo alloy, after the shoot-peened treatment followed by heat treatment for strain relief, resulted a TRS equal to 2700 ± 25 MPa. Therefore, the present results are acceptable comparing the data obtained with the study by Mengucci et al. [62].

The heat treatment, as hot isostatic pressing (HIP), after the additive manufacturing process (by laser melting process–SLM and EBM) of parts has been used successfully by medical and aeronautic manufactures. The HIP process is effective to obtain better results of mechanical properties (ductility and fatigue resistance) and decrease the porosity [60, 63]. Although this present study evaluated the mechanical properties of SLM and PC samples without any post-process of heat treatment is possible, check the relevant mechanical properties obtained by manufacture process. According to the results present evaluation is presented in **Table 6** to compare the mechanical properties to those presented in the literature [14, 41, 44, 62, 64].

Mechanical properties	Consolidation technique		Standards	
	PC	SLM	ISO 22674 "type 5"	ASTM F75 "casting"
Yield strength (MPa)	646.7 ± 44.4	731.5 ± 40.3	500	450
Rupture strength (MPa)	742.2 ± 106.8	1127.9 ± 0.1	–	–
Ultimate tensile strength (MPa)	771.7 ± 103.3	1136.9 ± 1.0	–	655
Elongation (%)	14.20 ± 2.8	13.7 ± 5.3	2	8
Elastic modulus "E" (GPa)	223.42 ± 15.7	225.2 ± 14.4	150	–
Hardness Vickers (HV)	272.2 ± 20.5	334.8 ± 16.0	–	266–345
TRS (MPa)	1072.3 ± 4.6	2501.2 ± 9.7	–	–

Table 5. Mechanical properties of Co-Cr-Mo alloy manufactured by PC and SLM compared to the minimum properties required by standards.

References		Alloy (wt%)	σ_{YS} (MPa)	El (%)	σ_{UTS} (MPa)	TRS (MPa)	Hardness (HV)
Present work Co-Cr-Mo	PC	64Co-29Cr-7Mo	646.76 ± 44.36	14.20 ± 2.76	771.70 ± 103.32	1072.3 ± 4.6	256.7 ± 12.9
	SLM		731.50 ± 40.31	13.73 ± 5.32	1136.95 ± 0.92	2501.2 ± 9.7	358.1 ± 9.8
Takaichi et al. [64]	C	Co-28Cr-6Mo	296 ± 25	9.6 ± 2.5	912 ± 39	–	–
	SLM		516 ± 28	10.7 ± 2.9	591 ± 37	–	–
Qian et al. [41]	C	60-65Co 26-30Cr 5-7Mo	610	–	741	–	–
	SLM		873 ± 76	–	1303 ± 73	–	–
Kajima et al. [44]	C	63Co-29Cr-6Mo	571 ± 23	11.2 ± 2	775 ± 67	–	–
	SLM	60-65Co 26-30Cr 5-7Mo	877 ± 37	12.3 ± 3	1170 ± 29	–	–
Mengucci et al. [62]	SLM	63.8Co-24.7Cr-5.1Mo-5.4W	–	–	1340 ± 20	2700 ± 25	434 ± 22
Jabbari et al. [14]	C	61.6Co-30Cr-6.5Mo	–	–	–	–	320 ± 12
	SLM	Co-29Cr-5.5Mo	–	–	–	–	371 ± 10
Liverani et al. [40]	SLM	Co 27-30Cr 5-7Mo	677	–	–	–	361 ± 31

Table 6. Comparative results for mechanical properties obtained in the present study with Co-Cr alloys manufactured by selective laser melting (SLM) and casting (C) process presented in the literature.

3.4. Microstructural analysis

To understand the mechanical properties improved in the SLM specimens in relation to the casting process technique carried out the microstructural analysis by OM and SEM-EDS.

The microstructural analysis by OM of PC samples (**Figure 11a,b**) describe dendritic arms and ramifications with different solidification orientations [14]. In addition, PC sample present porous (microporous) as the SLM samples, but are uneven (a little larger but in small quantity). This occurrence is possible to form by problems of dispersing the powder in the bed layer and the presence of satellites/porous in the powder particles. SLM specimens show a characteristic morphology (weld-like structure) of laser beam melting. Is possible to check the layers formed during the manufacture process (**Figure 11c,d**)? The vertical section of SLM sample is to observe the building direction of specimen (indicated by arrow—**Figure 11d**) characterized by the overlapping of each layer and the morphology formation by the action of the laser beam such as the weld pool.

Figure 12 represents the SEM images of PC samples and the semi-quantitative analysis of interesting points by EDS. It is possible to identify the cast specimen with a second phase (white area) in the matrix. The semi-quantitative analysis with the EDS and the respective spectrums (**Figure 12c,d**) show that the composition of white area (point 1) is rich in Mo element, and the matrix (point 2) is composed by Co-Cr elements, with a small percentage of Mo. The phase (point 1) shows the confirmation of carbide ($M_{23}C_6$) presence, rich in chromium and

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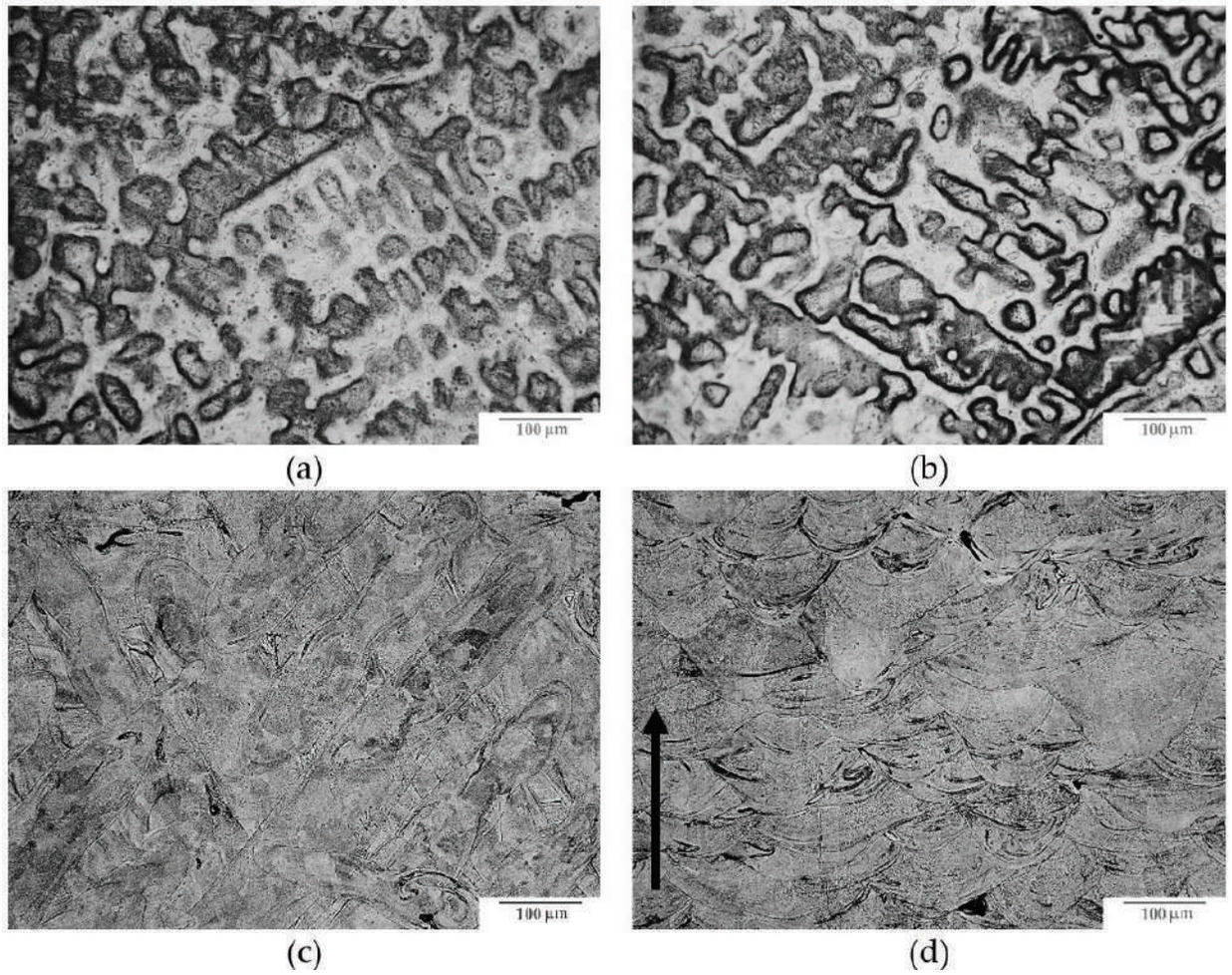

Figure 11. MO images of Co-Cr-Mo alloy consolidated: (a–b) transversal and longitudinal view of precision casting sample and (c–d) transversal and longitudinal (arrow indicates building consolidation) view of selective laser melting (Etch: 100 ml HCl and 2 ml H_2O_2. Magnitude: ×200).

molybdenum [14]. The $M_{23}C_6$ carbide results in a micro hardness of 699 ± 131 HV (1 mN/15 s) in opposition of 338 ± 14 HV (1 mN/15 s) to the micro hardness of matrix.

Figure 13 shows the microstructure of SLM specimen. It is observed that a microstructure is formed with small grains characterizing the rapid solidification during the SLM manufacturing process. The semi-quantitative analysis in the fine grains shows that it does not have different elements compositions. SLM specimen presents a homogeneous matrix with Co-Cr-Mo elements. The morphology formation of the laser melting sample was also observed after electrolytic attack [59, 61]. Also confirms that the fine grains are oriented in direction of the laser scanning. This characteristic microstructure of laser melting technique allows to achieve better mechanical properties than the cast technique.

The fractures of the tensile samples were SEM analyzed (**Figure 14**) observed the formation of dimples homogeneously distributed in the microfracture of both samples. Regions with the presence of dimples are ductless and with higher toughness. However, it is apparent that the dimple formations on the SLM sample extends completely by the fracture planes and are of

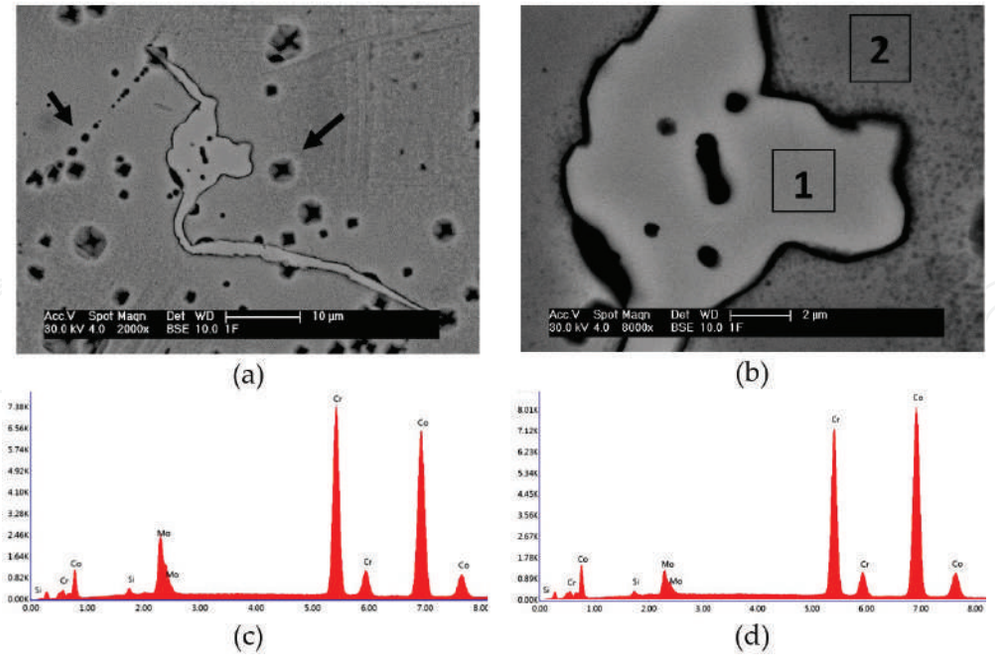

Figure 12. SEM images of PC sample: (a) magnitude ×2000, (b) magnitude ×8000, and (c–d) EDS spectrograms of analysis at point 1 and point 2.

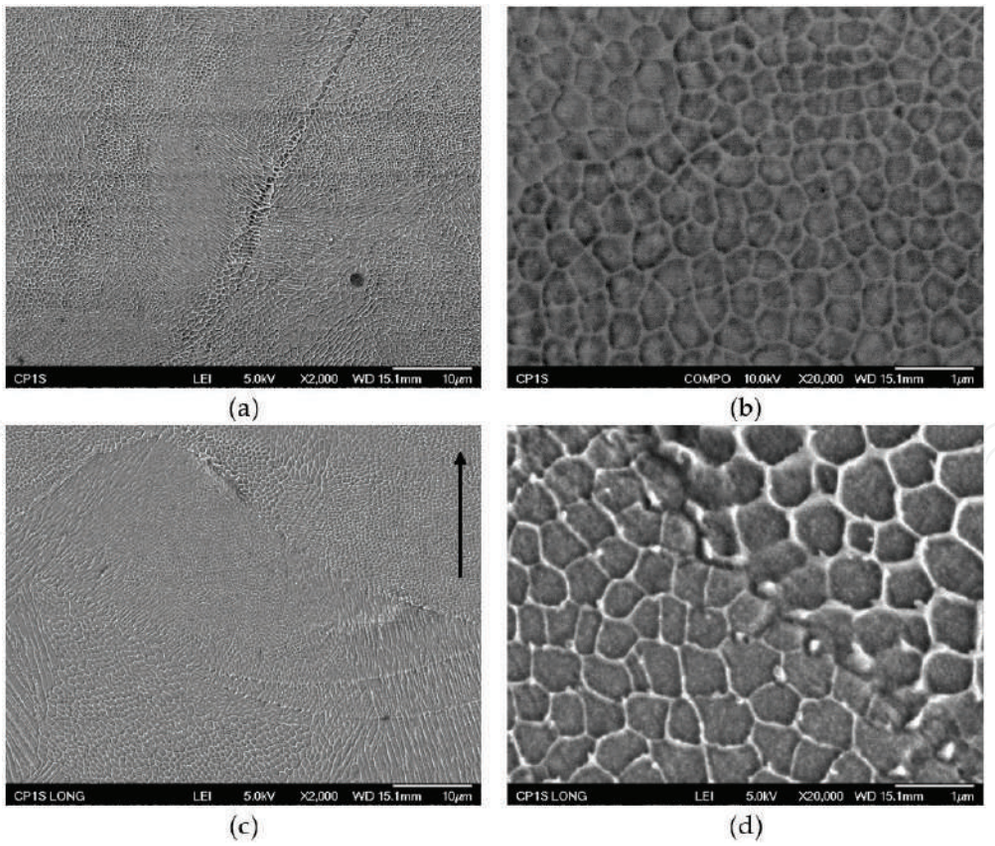

Figure 13. SEM images of SLM specimens: (a) horizontal section from backscattered electrons, and (b) from secondary electrons, (c–d) vertical section from backscattered electrons (black arrow indicates the building consolidation).

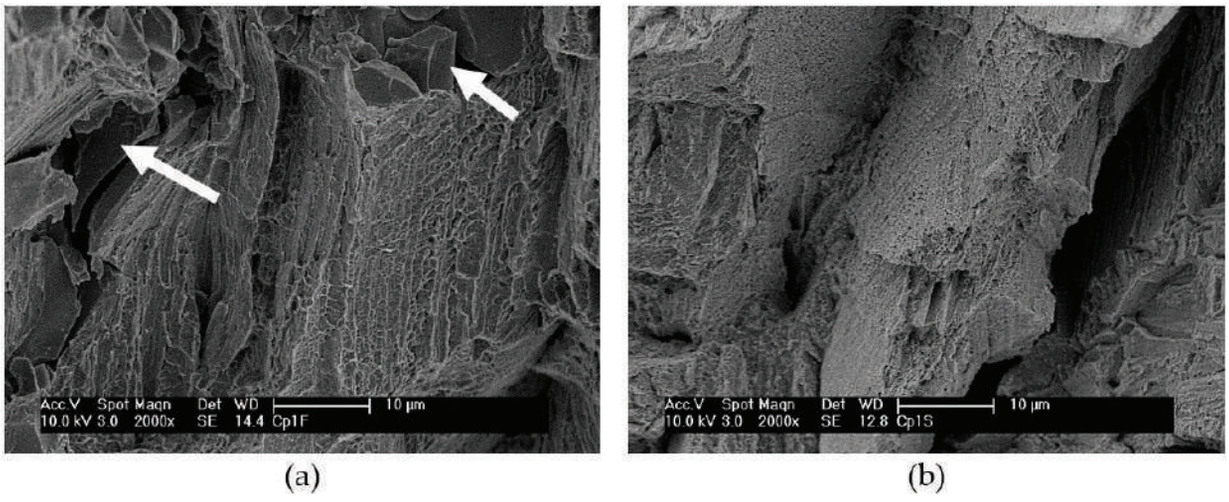

Figure 14. SEM images of tensile fracture: (a) PC sample and (b) SLM sample (magnitude: ×2000).

finer size, compared to the fracture with dimples geometrically larger of PC sample. It can be verified with SLM samples and confirmed with the mechanical results in relation to PC samples. In addition, some planar regions (indicated by arrows) show a semi-cleavage morphology. The type of fracture observed in the samples, according to Takaichi et al. [64], describes the formation of dimples along the fracture surface, as well as cracking of the wedge is appointed as a possible formation of cleavage fracture over favorable crystallographic planes.

4. Conclusions

In general, the results of powders characterization showed that the granulometric range of 20–50 μm is the one that best fits in the properties of packaging, for the consolidation by SLM.

The biocompatibility of the samples obtained a positive result for both processing techniques. In this way, the development of the present study evidenced to improve the manufacture of customized dental components (copings) using the SLM technique.

Microstructural analysis obtained for SLM samples results in a characteristic morphology of layer manufacturing with ultrafine grains and a high chemical homogeneity. The conventional technique presented a differentiated microstructure by the gross dendritic microstructure of casting process.

The mechanical evaluation showed that the SLM technique provides superior mechanical properties (as yield strength, rupture strength, ultimate tensile strength, TRS, and hardness) compared to those obtained by the precision casting technique.

The thermal analyses showed the present phase transitions of Co-Cr-Mo alloy, as well as being possible to correlate them (TMA to DSC curves). The coefficient of thermal expansion (CTE) resulted for both processes a similar value to alloys used in dental materials.

The processing using laser melting proved better mechanical and thermal properties to precision casting processing technique without post-processing (thermal treatment). SLM technique evidenced a promising use to manufacture prosthetics and dental implants. Nevertheless, it is still of great concern and promising further development of laser melting process (SLM and EBM) in relation to the parameters and variables of process, as also to the post-processing method apply to AM parts. Such characteristics should be addressed to new materials and investigate in relation to the performance and bio-functionality of specific application part.

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Author details

Marcello Vertamatti Mergulhão*, Carlos Eduardo Podestá and
Maurício David Martins das Neves

*Address all correspondence to: marcellovertamatti@gmail.com

Nuclear and Energy Research Institute (IPEN/CNEN-SP), CCTM, São Paulo, Brazil

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Chapter 7

Application of 3-D Printing for Tissue Regeneration in Oral and Maxillofacial Surgery: What is Upcoming?

Seied Omid Keyhan, Hamidreza Fallahi,
Alireza Jahangirnia,
Mohammad Taher Amirzade-Iranaq and
Mohammad Hosein Amirzade-Iranaq

Additional information is available at the end of the chapter

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Abstract

The ultimate goal of any surgical procedure is to improve perioperative form and function and to minimize operative and postoperative morbidity. In recent years, many exciting and novel technological advances have been introduced in the field of oral and maxillofacial surgery. One example of such technology that is continuing to increase in prevalence is the use of 3-dimensional (3-D) printing techniques with special properties, which seems hopeful for practitioners in the field of regenerative medicine. Tissue engineering is a critical and important area in biomedical engineering for creating biological alternatives for grafts, implants, and prostheses. One of the main triad bases for tissue engineering is scaffolds, which play a great role for determining growth directions of stem cells in a 3-dimensional aspect. Mechanical strength of these scaffolds is critical as well as interconnected channels and controlled porosity or pores distribution. However, existing 3-D scaffolds proved less than ideal for actual clinical applications. In this chapter, we review the application and advancement of rapid prototyping (RP) techniques in the design and creation of synthetic scaffolds for use in tissue engineering. Also, we survey through new and novel merging era of "bioprinting."

Keywords: 3-D printing, prototyping, tissue engineering, scaffolds, bioprinting, stem cells, regenerative medicine, oral surgery, maxillofacial surgery

1. Introduction

Three-dimensional printing—also known as rapid prototyping—was first introduced in 1980s; during past three decades, enormous changes and development have been performed by scientists through modifying this technology by uses, material, and also accuracy.

With increasing attention of scientific societies, recently, scientific literature bolded feasibility of 3-D-printed tissues and organs and its usage within laborious clinical situations. Also, this technology was used largely in accurate and highly customized devices, such as tracheobronchial splints, bionic ears, and even more. Within the field of craniofacial surgery, 3-D surgical models have been used as templates to create bone grafts, tailoring bioprosthetic implants, plate bending, cutting guides for osteotomies, and intraoperative oral splints. Using 3-D models and guides has been shown to shorten operative time and potentially reduce the complications associated with prolonged operative times.

The goal of surgical procedures for a clinician is to improve perioperative form, recovery of function, and also minimizing operative and postoperative morbidity. Many exciting and new technological advances have ushered in a new era in the field of oral and maxillofacial surgery over the last years, which within no exaggeration 3-D printing is the novelist and controversial one.

The aim of this chapter is to introduce 3-D printing method and its role in the contemporary oral and maxillofacial surgery and to review current advantages of its application in the field of regenerative medicine.

1.1. History of the technology

Three-dimensional (3-D) printing has been utilized in diverse aspects of manufacturing to produce different objects from guns, boats, and food to models of unborn babies. From over 1450 articles related to 3-D printing listed in PubMed, nearly a third of them were solely published in the last 2 years [1].

Three-dimensional (3-D) printing is a manufacturing process that objects are fabricated in a layering method during fusing or depositing different materials such as plastic, metal, ceramics, powders, liquids, or even living cells to build a 3-D matter [2, 3]. It is a process of generating physical models from digital layouts [4, 5]. This technology demonstrates a technique that a product designed through a computer-aided scheme is manufactured in a layer-by-layer system [6]. This process is also cited as rapid prototyping (RP), solid freeform technology (SFF), or additive manufacturing (AM) [7].

3-D printing techniques are not brand new and have been existed since 30 years ago [8–10]. This technology is first introduced and invented by Charles Hull in 1986, and at first, it was utilized in the engineering and automobile industry for manufacturing polyurethane frameworks for different models, pieces, and instruments [11]. Originally, Hull employed the phrase "stereolithography" in his US Patent 4,575,330, termed "Apparatus for Production of Three—Dimensional Objects by Stereolithography" published in 1986. Stereolithography technique included subjoining layers over the top of each other, by curing photopolymers with UV lasers [12, 13].

Since then, 3-D models have been used for a diversity of different objectives. Since 1986, this process has started to accelerate and has honored recognition globally and has influenced different arenas, such as medicine.

The developing agora for 3-D desktop printers encourages wide-ranging experimentations in that subject. Generally, medical indications of these printers are such as treatment planning, prosthesis, implant fabrications, medical training, and other usages [4].

Having being used in military, food industry, and art, rapid prototyping is receiving a lot of attention in the field of surgery in the last 10 years [6, 14].

The pioneering usage of stereolithography in oral and maxillofacial surgery was by Brix and Lambrecht in 1985. Later this technique was used by them for treatment planning in craniofacial surgery [15].

In 1990, stereolithography was used by Mankovich et al. for treating patients having craniofacial deformities [16, 17]. They used it to simulate bony anatomy of the cranium using computed tomography with complete internal components [17, 18].

By aiding in complex craniofacial reconstructions, 3-D printing has recently earned reputation in medicine and surgical fields [19–21].

Today, maxillofacial surgery can benefit from additive manufacturing in various aspects and different clinical cases [22]. This technique can help with bending plates, manufacturing templates for bone grafts, tailoring implants, osteotomy guides, and intraoperative occlusal splints [23–27]. Rapid prototyping can shorten surgery duration and simplify pre and intraoperative decisions. It has enhanced efficacy and preciseness of surgeries [10].

2. Current 3-D printing techniques used in oral and maxillofacial surgery

From first innovation till nowadays, there are different kind of technologies introduced for 3-D printing. Binder jetting (BJ), electron beam melting (EBM), fused deposition modeling (FDM), indirect processes, laser melting (LM), laser sintering (LS), material jetting (MJ), photopolymer jetting (PJ), and stereolithography (SL) are well-known technologies of 3-D printing [14, 28, 29].

There are many different 3-D printing techniques. Benefits and disadvantages are factors to differ each technology system [14]. Among this variety of different techniques, there is a huge discussion and usage in oral and maxillofacial region for SL, FDM, and PJ [1, 28, 30].

Each technology has its own characteristics, properties, and advantages which **Table 1** summarizes some different three dimensional printing technologies.

3. Biomaterials available for 3-D printing

As researchers aim to investigate new materials for 3-D printing in last decade, it is obvious to see variety of biomaterials with different properties and also different applications. As **Table 2** summarizes all biomaterials used within studies all over the world for generating scaffolds for bone tissue engineering, it has to be noticed that from this large spectrum of biomaterials

Techniques	Advantages	Disadvantages
Light cured resin		
1. Stereolithography (SLA) Light sensitive polymer cured layer by layer by a scanning laser in a vat of liquid polymer.	Rapid fabrication. Able to create complex shapes with high feature resolution. Lower cost materials if used in bulk.	Only available with light curable liquid polymers. Support materials must be removed. Resin is messy and can cause skin sensitization and may irritate by contact and inhalation. Limited shelf life and vat life. Cannot be heat sterilized. High cost technology.
2. Photojet—light sensitive polymer is jetted onto a build platform from an inkjet type print head, and cured layer by layer on an incrementally descending platform.	Relatively fast. High resolution, high-quality finish possible. Multiple materials available various colors and physical properties including elastic materials. Lower cost technology.	Tenacious support material can be difficult to remove completely. Support material may cause skin irritation. Cannot be heat sterilized. High cost materials.
3. DLP (digital light processing) Liquid resin is cured layer by layer by a projector light source. The object is built upside down on an incrementally elevating platform.	Good accuracy, smooth surfaces, relatively fast. Lower cost technology.	Light curable liquid polymers and wax-like materials for casting. Support materials must be removed. Resin is messy and can cause skin sensitization, and may be irritant by contact Limited shelf life and vat life. Cannot be heat sterilized. Higher cost materials.
Powder binder		
Plaster or cementaceous material set by drops of (colored) water from 'inkjet' print head. Object built layer by layer in a powder bed, on an incrementally descending platform.	Lower cost materials and technology. Can print in color. Un-set material provides support Relatively fast process. Safe materials.	Low resolution. Messy powder. Low strength. Cannot be soaked or heat sterilized.
Sintered powder		
Selective laser sintering (SLS) for polymers. Object built layer by layer in powder bed. Heated build chamber raises temperature of material to just below melting point. Scanning laser then sinters powder layer by layer in a descending bed.	Range of polymeric materials including nylon, elastomers, and composites. Strong and accurate parts. Self-supported process. Polymeric materials—commonly nylon may be autoclaved. Printed object may have full mechanical functionality. Lower cost materials if used in large volume.	Significant infrastructure required, e.g., Compressed air, climate control. Messy powders. Lower cost in bulk. Inhalation risk. High cost technology. Rough surface.
Selective laser sintering (SLS)—for metals and metal alloys. Also described as selective laser melting (SLM) or direct metal laser sintering (DMLS). Scanning laser sinters metal powder layer by layer in a cold build chamber as the build platform descends. Support structure used to tether objects to build platform.	High strength objects can control porosity. Variety of materials including titanium, titanium alloys, cobalt chrome, stainless steel. Metal alloy may be recycled. Fine detail possible.	Elaborate infrastructure requirements. Extremely costly technology moderately costly materials. Dust and nanoparticle condensate may be hazardous to health. Explosive risk. Rough surface. Elaborate post-processing is required: Heat treatment to relieve internal stresses in printed objects. Hard to remove support materials. Relatively slow process.
Electron beam melting (EBM, Arcam). Heated build chamber. Powder sintered layer by layer by scanning electron beam on descending build platform.	High temperature process, so no support or heat treatment needed afterwards. High speed. Dense parts with controlled porosity.	Extremely costly technology moderately costly materials. Dust may be hazardous to health. Explosive risk. Rough surface. Less post-processing required. Lower resolution.

Techniques	Advantages	Disadvantages
Thermoplastic		
Fused deposition modeling (FDM) First 3-DP technology, most used in 'home' printers. Thermoplastic material extruded through nozzle onto build platform.	High porosity. Variable mechanical strength. Low- to mid-range cost materials and equipment. Low accuracy in low cost equipment. Some materials may be heat sterilized.	Low cost but limited materials—only thermoplastics. Limited shape complexity for biological materials. Support material must be removed.

Table 1. 3-D printing modalities and materials [14, 31].

Composed scaffolds	Synthetic scaffolds		Natural scaffolds	
Nano-hydroxyapatite/ collagen/PLLA	Ceramic	Polymeric	Inorganic	Organic
Octacalcium phosphate/ collagen	Calcium Magnesium Phosphate cement (CMPC)	PLGA	Silver	Collagen sponge
Nano-hydroxyapatite/ polyamide 6	βTCP	PLG	Coral	PRP
Nano-hydroxyapatite/ polyamide66	HA/TCP	PLLA	Silk fibroin protein	Gelatin sponge
Hydroxyapatite-coated PLGA	Flurohydroxyapatite	PGA	Premineralized silk fibroin protein	Gelatin Hydrogel
HA/PLGA	Ca deficient hydroxyapatite (CDHA)	PLA	ABB	PuraMatrix
βTCP/collagen		PLA-PEG	Deer horn	Alginate
DBM/PLA		Fibronectin-coated PLA		Partially demineralized bone matrix
Nano-hydroxyapatite/ polyamide		PEG-DA		Bio-Oss
OsteoSet		PEG-MMP		Allograft
Octacalcium phosphate precipitated (OCP) alginate		PVDC		Fibrin sealant
Demineralized bone powders/PLA		Polycaprolactone		Gelatin foam
Apatite-coated PLGA				Collagen gel
				Hyaluronic acid based hydrogel

TCP, tri-calcium phosphate; HA, hydroxyapatite; DBM, demineralized bone matrix; PLGA, poly(lactic-co-glycolic acid); PLA, poly(D,L-lactic acid); PGA, poly(glycolic acid); PLLA, poly(L-lactic acid); PVDC, polyvinylidene chloride; PEG, polyethylene glycol; DA, diacrylate; MMP, matrix metalloproteinases; ABB, anorganic bovine bone; Puramatrix, a self-assembling peptide nanomaterial.

Table 2. Types of scaffolds used in bone tissue engineering in maxillo-craniofacial region [51].

just a whole bit of them are available for application in 3-D printing. As follows, we discuss four large categories of materials for 3-D printing of scaffolds and craniofacial tissues, which researches still aim to determine these materials complete properties and advantages.

3.1. Polymers and hydrogels

Polymer hydrogels are ideal candidates for the development of printable materials for tissue engineering. Hydrogels are known for remarkable tunability of rheological also presenting great mechanical, chemical, and biological properties; high biocompatibility; and similarity to native extracellular matrix (ECM) [32]. For three-dimensional printing of polymers and hydrogels, the use of materials with controlled viscosity should been noticed. This defines the range of printability of the ink. Polymer inks, which are typically printed in the prepolymer phase, need enough viscosity allowing structural support of subsequent printed layers, also enough fluidity to prevent nozzle clogging. For avoiding these difficulties, alginate hydrogels have been cross-linked with calcium ions immediately before the ink leaves the printing head or just after extrusions [33].

In recent researches, for providing suitable ink for bioprinting applications, prepolymerized cell-laden methacrylated gelatin hydrogels have been used successfully [34, 35]. Synthetic hydrogels used for cell encapsulation may limit cell-cell interactions. These interactions are critical for efficient cell proliferation, differentiation, and finally, tissue development. This can represent one of the limitations of bioprinting cell-laden hydrogels which is not present in 3-D printed scaffolds with cells seeded onto or in bioprinting of dense cell aggregates, which will discuss as follow. Hence, the requirement for the development of ECM-derived hydrogels that have tunable physical and chemical properties, are compatible with high cell viability, and provide the adequate binding sites (RGDs) for cell attachment and matrix remodeling during their early proliferative stage [32].

Synthetic polymers are most commonly used materials for 3-D printing in biomedical applications [36, 37]. However, since high temperature is usually involved during the printing of these materials, the direct incorporation of cells or growth factors in the polymer mixture is generally avoided as the cell viability or bioactivity [37] cannot be maintained throughout the manufacturing process.

Although hydrogels provide great advantages for tissue engineering applications, such as the ability of exposing cells to highly hydrated 3-D microenvironments that is similar to the natural ECM [32]. In contrast, they generally present very low stiffness (in the kPa range) compared with the majority of load-bearing tissues in the craniofacial complex (in the GPa range). Therefore, reconstruction of tissues subjected to higher mechanical loads, such as bones and teeth, usually requires the use of ceramic materials or composite scaffolds which provide more mechanical advantages, where polymers are commonly combined with inorganic fillers to increase scaffold stiffness [38].

3.2. Ceramics

Ceramic scaffolds are usually composed of calcium and phosphate mineral phases, such as hydroxyapatite [39] or b-tricalcium phosphate [40]. The noticeable ability of these scaffolds to upregulate osteogenesis due to inherent properties of the formation of a bioactive ion-rich cellular

microenvironment, also as mentioned before their ability to mechanically provide space maintenance, makes these materials interesting choice for 3-D scaffold fabrication for craniofacial applications. In contrast, ceramic scaffolds are not compatible with cell encapsulation for bioprinting. In 3-D printed ceramic scaffolds, cells quickly populate the scaffold surface, which establishing close cell-cell interactions lead to promotion of cell proliferation and differentiation. On the other hand, ceramics with properties lead to lower rates of degradation than hydrogels, which aids in prolonged guided tissue remodeling and structural support. In contrast, ceramic scaffolds are too brittle for implantation in load-bearing defect sites. Ideal scaffolds would combine the high calcium content of calcium and phosphate ceramics with the outstanding toughness of natural bone, which perhaps can only be obtained by creating scaffolds that are biomimetically mineralized and hierarchically structured, as recent researches demonstrated that in [41].

Fused deposition of ceramics (FDC) in a direct printing mode generally consists of extruding a slurry including a high content (>50% w/v) of inorganic components [42]. The manufacturing of such scaffolds follows 3 steps:

1. Mixture phase, which involves the preparation of the slurry. The bioceramic particles are mixed in a solvent (aqueous or nonaqueous) with a low concentration of organic polymers/surfactants, called the binder, to obtain adequate flowability.
2. Green ceramic and binder burnout phase involving the deposition of filaments of slurry following a predetermined pattern prior to drying and exposure to high temperature to burn out the organic component of the mixture.
3. Sintering phase, which involves the exposure of the green form to elevated temperature (above 1000°C) to initiate the migration of atoms between adjacent ceramic particles, hence creating physical bonds called "necks."

It is critical for reproducible manufacturing of 3-D rapid prototyped bioceramics to have shape retention, a challenge that can be reached by adjusting the viscosity of the slurry and the evaporation rate of the solvent [43].

3.3. Composite materials

Printable composites, which are usually in the form of copolymers, polymer-polymer mixtures, or polymer-ceramic mixtures [44], allow ability for the combination of variety of advantageous properties of their included components, which provide a remarkable candidate as "bioink". Considering the advantages of polymer composite hydrogels, such as interpenetrating polymer networks (IPNs) or hybrid hydrogels [45], the incorporation of synthetic fillers to printable materials recently discussed in researches [33]. The addition of silicate fillers [38] and a range of nanoparticles have been used to synthesize different types of composite scaffolds [46] to promote greater control over viscosity and stiffness of polymer hydrogels. In addition, several of silica-containing hydrogels with higher expression of genes encoding morphogenetic cytokines, such as bone morphogenetic proteins (BMPs) seems promising [47]. The combination and manufacturing mixture of hydrogels with filler materials and/or natural peptides with morphogenetic capacity demonstrate great future for application in 3-D printing in aim to reach ultimate goal in regenerative craniofacial repair.

3.4. Cell aggregates and spheroids

Over recent years, many of researches aimed to evaluate and study cell aggregates and spheroids for use in tissue engineering and regenerative medicine [48]. As this method cited correctly and appropriately as "scaffold-free printing," in fact small quantities of hydrogel are used to facilitate cell aggregation. In this method for 3-D printing, or in an appropriate

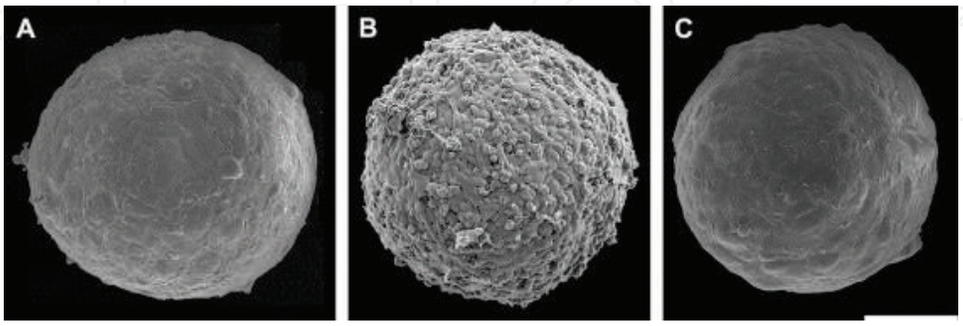

Figure 1. SEM view of multicellular spheroids of HUSMCs (A), CHO cells (B) and HFBs (C) (adapted from Norotte et al. [50]).

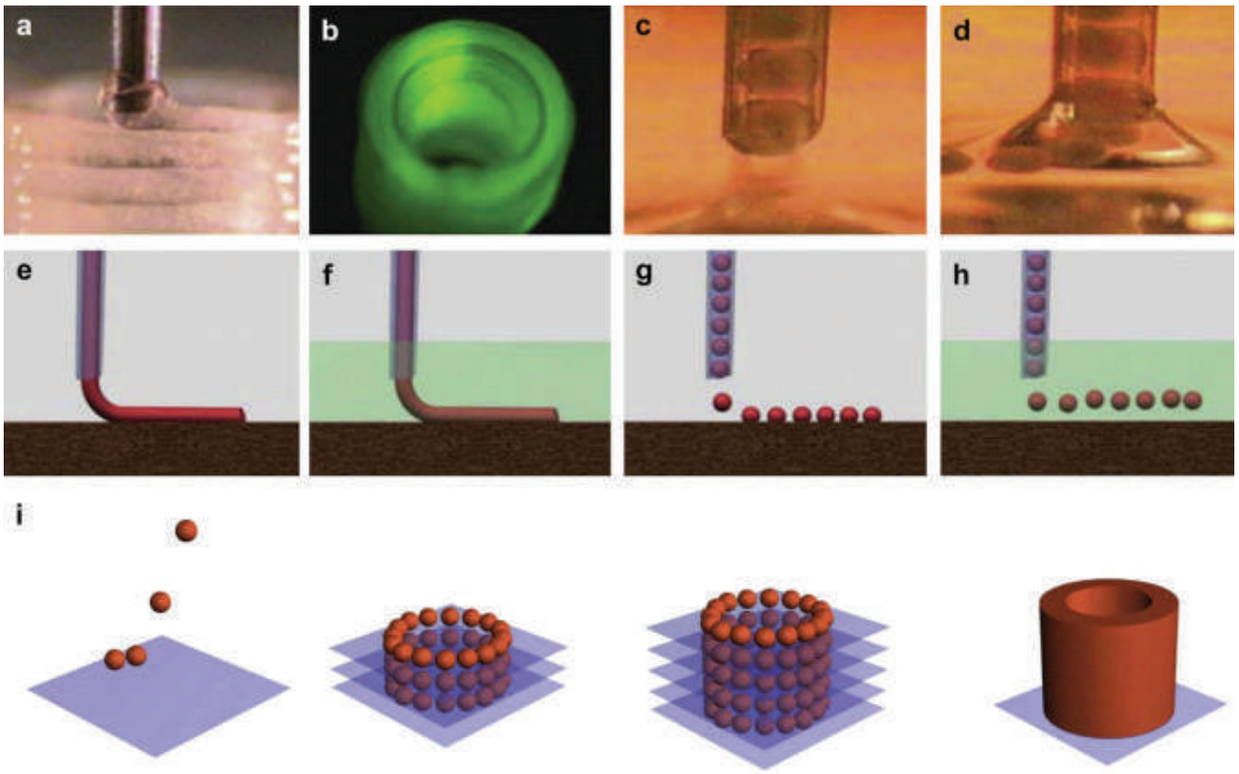

Figure 2. Principles of spheroids bioprinting technology: (a) bioprinter (general view); (b) multiple bioprinter nozzles; (c) tissue spheroids before dispensing; (d) tissue spheroids during dispensing; (e) schematic view of continuous dispensing in air; (f) schematic view of continuous dispensing in fluid; (g) schematic view of digital dispensing in air; (h) schematic view of digital dispensing in fluid; (i) schematic view of bioassembly of tubular tissue construct using bioprinting of self-assembled tissue spheroids illustrating sequential steps of layer-by-layer tissue spheroid deposition and tissue fusion process (adopted from Mironov et al. [48]).

way called "bioprinting," multicellular spheroids are deposited using extrusion printers and allowed to self-assemble into the desired 3-D structure (**Figure 1**). As it is clear, these systems allow direct fabrication of tissue constructs which in contrast to other methods have extremely high cell densities. Although in load-bearing tissues with high amount of mineral components and noticeable mechanical properties use of this methods still looks uncertain, the ability to position aggregates of heterotypic cells with microscale precision (**Figure 2**) seems promising as an excellent alternative to bioprint complex tissues consisting variety of cells [49].

4. Manufacturing of scaffolds with 3-D printing technology

Researches aimed to investigate novel technologies for 3-D printing and introduced some novel methods including phase-separation, self-assembly, electrospinning, freeze drying, solvent casting/particulate leaching, gas foaming, and melt molding [52]. Using scaffolds, the architecture of native extracellular matrices can be mimicked at the nanoscale level and therefore provide the primary base for the regeneration of new tissue [53]. Originally, a "top-down" approach was used as a tissue engineering method for scaffold fabrication. In this method, cells are seeded onto a biodegradable and biocompatible scaffold and are predicted to migrate and fill the scaffold hence creating their own matrix. By using this technique, several avascular tissues such as bladder [54] and skin [55] have been engineered effectively. However, due to the limited diffusion properties of these scaffolds, this technique faces several challenges for fabrication of more complex tissues such as heart and liver [56]. Therefore, "bottom-up" methods have been developed to overcome this problem [57]. Bottom-up approaches include cell-encapsulation with microscale hydrogels, cell aggregation by self-assembly, generation of cell sheets, and direct printing of cells [58]. These complex tissue blocks can be assembled using various methods including microfluidics [59], magnetic fields [60], acoustic fields [61], and surface tension [62]. These methods are relatively easy and have provided a solid foundation for the fabrication of scaffolds. However, as mentioned previously, these conventional methods suffer from several limitations including inadequate control over scaffold properties such as pore size, pore geometry, distribution of high levels of interconnectivity, and mechanical strength. As such, it is necessary to develop technologies with sufficient control so as to design more intricate tissue-specific scaffolds. In addition, scaffolds can be coated using surface modification techniques (such as introducing functional groups) to enhance cell migration, attachment and proliferation. Three-dimensional printing allows scaffolds to become more precisely fabricated (similar to that of the computer-aided design (CAD)) with higher flexibility in the type of materials used to make such scaffolds. Three-dimensional printing uses an additive manufacturing process where a structure is fabricated using a layer-by-layer process. Materials deposited for the formation of the scaffold may be cross-linked or polymerized through heat, ultraviolet light, or binder solutions. Using this technology, 3-D printed scaffolds can be prepared for optimized tissue engineering [52].

For appropriate formation of tissue architecture, the seeding cells (often stem cells) require a 3-D environment/matrix similar to that of the ECM. The ECM acts as a medium to provide proteins and proteoglycans among other nutrients for cellular growth. The

ECM also provides structural support to allow for cellular functionality such as regulating cellular communication, growth, and assembly [63]. With this in mind, scientists and engineers originally attempted to replicate the ECM through conventional techniques, which consequently established a framework for using more advanced techniques, such as 3-D printing, to yield higher quality scaffolds. The 3-D printing technique can create defined scaffold structures with controlled pore size and interconnectivity and the ability to support cell growth and tissue formation [64–66]. The current methods for 3-D printing involve a CAD, which is then relayed to each 3-D printing system to "print" the desired scaffold structure. Through various 3-D printing technologies, discussed below, researchers are trying to fabricate biocompatible scaffolds that efficiently support tissue formation (**Table 3**).

5. Bioprinting advantages aiming for clinical use

The goal of tissue engineering is to create functional tissues and organs for regenerative therapies and ultimately organ transplantation/replacement. Trial and error was the long and tedious process mainly used to advance the field of regenerative medicine by clarifying the success of techniques.

Researchers needed to come up with a list of requirements in order to measure their successes or failures in tissue fabrication [48, 67]. This list was generated from the observations of natural human tissue.

As gold standard of fabricated tissues is to be as similar as possible to natural tissues in the human body in different parameters, then these fabricated tissues must:

1. Be able to integrate with naturally occurring tissue, and attach via microsutures, glues [68], or through cell adhesion [69–71].
2. Be capable of essential functions in vivo [48].
3. Become fully vascularized in order to sustain its functionality [68, 71].

Also, the printers used for tissue fabrication required standardization as well [67, 69].

1. The bioprinting machines required set extreme sterilization methods to eliminate unwarranted contamination with previously used materials or foreign matter from the environment.
2. The conditions for printing must be ideal for tissue fabrication, so factors such as humidity and temperature must be closely monitored.
3. Nozzle size and methods of delivery affect the viability of the materials being printed; therefore, there must be set ideals for delivery methods in relation to the various printing materials.

Printing method	Advantages	Disadvantages	Preclinical progress
Direct 3-D printing/ inkjet	• Versatile in terms of usable materials • No support is necessary for overhang or complex structures	• Potential toxicity (incompletely removed binders) • Low mechanical strength prints compared to laser sintering • Time consuming (post-processing)	• (Rat/bone) • (Rabbit/bone) • (Mouse/bone)
W/electrospinning			• (Mouse/cartilage)
Bioplotting	• Prints viable cells • Soft tissue applications	• Limitation on nozzle size* (*Must not be cytotoxic during processing) • Requires support structure for printing complex shapes	• (Rabbit/trachea) • (Rabbit/cartilage) • (Rat/cartilage) • (Mouse/cartilage) • (Mouse/tooth regeneration) • (Mouse/skin)
Fused deposition modeling	• Low cytotoxicity vs direct 3-D printing • Relatively inexpensive (printers and materials)	• Limitation on materials (often requires thermoplastics) • Materials used are nonbiodegradable • Requires support structure for overhangs and complex shapes • Post-processing may be necessary • Low Resolution	• (Swine/bone) • (Rat/bone)
Selective laser sintering	• Provides scaffolds with high mechanical strength • Powder bed provides support for complex structures • Fine resolution	• Limitation on materials (must be shrinkage and heat resistant) • Very high temp required (up to 1400°C) • Expensive and time consuming (processing and post processing)	• (Mouse/bone) • (Rat/heart) • (Rat/bone) • (Mouse/skin) • (Mouse/heart)

Printing method	Advantages	Disadvantages	Preclinical progress
Stereolithography	• Very high resolution • Speed of fabrication • Smooth surface finish	• Materials must be photopolymers • Expensive (two photon printers) • Support system is necessary for overhang and intricate objects	• (Rat/bone) • (Rabbit/trachea) • (Pig/tendon)
Electrospinning	• Speed of fabrication • Cell printing • Soft tissue engineering • Low shear stress (bioelectrospraying)	• Random orientation of fibers • Nonuniform pore sizes • High voltage (1–30 kV) requirements	• (Mouse/ biocompatibility) • (Rat/bone) • (Rabbit/vascular tissue)
Indirect 3-D printing	• Good for prototyping/preproduction • Material versatility casting once mold is obtained	• Requires proprietary waxes for biocompatibility (wax printing) • Low accuracies/resolution • Mold required for casting • Long production times (mold → cast → processing → product)	• (Rat/bone) • (Mouse/tooth regeneration)

Table 3. Preclinical researches on various 3-D printing techniques for manufacturing scaffolds for tissue engineering [52].

As a result, researchers created a few methods of printing with the goal of finding a solution to the given problems for optimal tissue biofabrication [48, 68, 69]. Thermal inkjet bioprinting with bioink and direct-write bioprinting both make use of modified inkjet printers but with varied application techniques. Organ printing with tissue spheroids is the recent achievement of researches which seems promising to fabricate tissues directly. **Table 4** review advantages and disadvantages of all three common methods "Thermal Inkjet Bioprinting," "Direct-Write Bioprinting," and "Spheroid Organ Printing." Organ printing, otherwise known as the biomedical

Type of bioprinting	Method	Tissue characteristics	Note
Thermal inkjet bioprinting	• Bottom up • Layer-by-layer	• Avascular • Aneural • Alymphatic • Thin • Only nourishable via diffusion	"Bioink," which is a water-based liquid consisting of proteins, enzymes, and cells suspended in a media or saline.
Direct-write bioprinting	• Digital control of print. • Several printing units simultaneously. • Application of variety of materials simultaneously. • Faster turnaround time for printed products.	Possibility of printing tissues with different compositions.	• The bioink of direct-write printers may consist of hydrogels of varying consistencies that are composed of agarose, alginate, collagen type I, and Pluronic F127. • This method categorized in pneumatic, mechanical, and a pneumatic-mechanical hybrid. It was concluded that the pneumatic systems work better with high viscosity materials, while mechanical systems are better suited in working with materials of low viscosity.
Spheroid organ printing	Spheroids are punched into "biopaper" which is a sprayed layer of hydrogel. Each spheroid is made of living cells, thereby creating a ball of "living materials" capable of self-assembly and self-fusion. Alternatively, the spheroids can be digitally placed, undergo self-assembly, and fuse without the use of hydrogel.	• Self-organization is defined as, "a process in which patterning at the global level of a system emerges solely from numerous interactions among the lower-level components of the system." • Self-assembly is defined to be, "the autonomous organization of components into patterns or structures without human intervention."	Researchers fabricated three types of spheroids to create a vascular tree: solid or nonlumenized spheroids, spheroids with one big lumen (mono-lumenized spheroids), and microvascularized tissue spheroids.

Table 4. 3-D bioprinting technique advantages and properties[67].

application of rapid prototyping, may be defined as additive layer-by-layer biomanufacturing of cells. Advantages of organ printing include its automated approach offering a pathway for a scalable and reproducible mass production of tissue-engineered products. This also allows the precise simultaneous 3-D positioning of several cell types, hence enabling the creation of tissue with a high level of cell density. Organ printing may be used to solve the problem of vascularization in thick tissue constructs, and moreover, this technology may be done in situ. Therefore, this emerging transforming technology has potential for surpassing traditional solid scaffold-based tissue engineering [72].

6. Current limitations

6.1. Vascularization

In order to create a complete and functional organ, the researchers must be able to create thick complex tissues with full vascularization containing lumens of various sizes, large vascular structures to microstructures, in order to sustain the surrounding organ tissue. The best way to achieve this type of vascularization is to fabricate the vascular system and tissue simultaneously, of which is easier said than done [48]. Thorough vascularization remains a common theme for current bioprinting limitations. Without a functional circulatory system, tissue constructs are limited to a means of diffusion for nutrition, which in itself is limited to just a few hundred microns [69].

Current methods of vascularization call for the infiltration of host microvessels into an implanted construct [67, 73, 74].

Yet, this strategy is lacking in control and specificity for the developing microvessels. The invading microvessels have a limited penetration depth which prevents the successful incorporation of the microvessels into larger layered constructs. Additionally, the penetration of the vascular system itself may result in a distortion of the region penetrated or in the destruction of the fabricated tissue altogether. For these reasons, it would be ideal to construct tissues with direct vessel in-growth, or vascularization created within the tissue itself, all before implantation.

6.2. Tissue components and costs

In addition to vascularization, native tissues contain unique cellular combinations and organizations. There is a need to develop techniques that mimic the complexity of native tissues in order to drive tissue recovery and replacement for medical applications [69]. With the production of organs such as kidneys, for example, at least one million glomeruli and nephrons would need to be generated. Not only would the fabrication be a massive undertaking but also the fabricated tissue would need to be scalable. Scalability of biofabricated tissues is not presently a reality. Yet, spheroids have shown promise toward being scalable with further development. Finally, another major limitation for the development of natural-like, fully functioning fabricated human tissue is economic [68]. This challenge must definitely be overcome if biofabrication technology is to allow the creation of a functional living human organ.

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7. Future aspects of 3-D printing for regenerative medicine

In this chapter, we have illustrated current guiding principles for 3-D bioprinting in tissue fabrication, as well as recent advances and technological developments. The speed at which our knowledge has advanced with additive manufacturing and automated printing systems shows a promise to expand our basic science and engineering capabilities toward addressing health care problems. One of the significant developments in 3-D bioprinting is to manufacture cell microenvironments from molecular to macroscopic scales, which are requested and suitable for tissue engineering and regenerative medicine. As novel methods and technologies introduced in recent years for 3-D printing of biomaterials, promising overview of future appears to manufacture scaffolds for tissue engineering that reach the gold standards and also better comprehensions of stem cells microenvironments and interactions. By aid of various novel technologies, such as microfluidic systems [75, 76], biopatterning [77], and layer-by-layer assembly [76, 78], researchers are now able to biomanufacture microtissue constructs within scaffolds and even also within scaffold-free environments. Considering the great and enormous improvements of biomaterial for tissue engineering, in contrast, there are still certain challenges and difficulties that need more attention. Vascularization is one of the limitations which receive most of attentions [79, 80] due to the fact that this challenge leads to hypoxia, apoptosis, and immediate cell death. For resolving this issue and providing sufficient space for vascularization, researchers attempts to fabricate porous scaffolds [81], to provide sufficient space for vascularization. However, this approach cannot overcome the vascularization challenge completely due to the diffusion of cells and other materials into these porous structures [82]. Forming interconnected, well-defined vascular structures during biomanufacturing process seems to lead to resolving this difficulty and providing better results during process. Other issues that have to be noticed are mechanical strength and stability in 3-D tissue engineering which is one of the key requirements [83]. To be clear in regeneration of hard (e.g., bone) and soft (e.g., vascular grafts) tissues, modulus of elasticity is a crucial parameter that desires improvement [84–86]. Furthermore, the development of a totally closed bioprinting system that integrates printing and post-printing processes such as in-vitro culture and maturation of tissue constructs continues to be a challenge.

With advances in near future, which help finding solutions for the challenges mentioned above, bioprinting technologies will potentially help improvements of rapid clinical solutions and advances in medical implants. Further, we envision that the integration of cells and biomaterials through bioprinting with microfluidic technologies are likely to create unique microenvironments for various applications in cancer biology, tissue engineering, and regenerative medicine [87–91]. Additionally, developments on high-throughput biomanufacturing of 3-D architectures will pave the way for further advancements of in vitro screening and diagnostic applications, potentially enabling complex organ constructs. In the meantime, it is only the effective interplay of engineering concepts in combination with the well-established fundamentals of biology that will realize the true potential of this exciting area.

Author details

Seied Omid Keyhan[1,2,3,4], Hamidreza Fallahi[5], Alireza Jahangirnia[6], Mohammad Taher Amirzade-Iranaq[7] and Mohammad Hosein Amirzade-Iranaq[8,9,10,11]*

*Address all correspondence to: h.amirzade@gmail.com

1 Department of Oral & Maxillofacial Surgery, Faculty of Dentistry, Birjand University of Medical Science, Birjand, Iran

2 Vice Presidential Organization of Technology of the Islamic Republic of Iran, Iran

3 Stem Cell & Regenerative Medicine Network, Shahid Beheshti University of Medical Sciences, Tehran, Iran

4 Cranio Maxillofacial Research Center, Tehran Dental Branch, Islamic Azad University, Tehran, Iran

5 Oral and Maxillofacial Surgery, Jundishapur University of Medical Sciences, Ahvaz, Iran

6 Oral and Maxillofacial Surgeon, Private Practice, Tehran, Iran

7 Biomaterial Medical Engineer, Department of Materials, Faculty of Materials Engineering, Islamic Azad University Najaf Abad Branch, Isfahan, Iran

8 Student Research Committee (SRC), Baqiyatallah University of Medical Sciences, Tehran, Iran

9 Universal Network of Interdisciplinary Research in Oral and Maxillofacial Surgery (UNIROMS), Universal Scientific Education and Research Network (USERN), Tehran, Iran

10 Student Research Committee, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

11 International Otorhinolaryngology Research Association (IORA), Universal Scientific Education and Research Network (USERN), Tehran, Iran

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Chapter 8

Tissue Engineering: Use of Growth Factors in Bone Regeneration

Carmen Mortellaro and Massimo Del Fabbro

Additional information is available at the end of the chapter

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Abstract

Tissue healing is a complex process involving a cascade of cellular and molecular events that are mostly shared by the different tissues of the body. Interestingly, the tissue repair process initiates immediately after a traumatic injury and is mediated and controlled by a wide range of cytokines, proteins, and growth factors released from platelets upon activation. Consequently, many growth factors have been considered as therapeutic molecules for the repair or regeneration of a wide range of tissues. Although their role has been only partially elucidated, the potential benefit of most growth factors has been demonstrated. In the last few years, the development of platelet-rich preparations has revolutionized the field of regenerative medicine, due to the repair capacities of the platelet-released growth factors that stimulate and accelerate both soft and hard tissue healing and regeneration. Today, autologous platelet concentrates (APCs) are used in a wide range of disciplines such as dentistry, oral surgery, orthopedics, sport medicine, dermatology, and plastic and reconstructive surgery. The purpose of this chapter is to describe the current evidence regarding the benefits of using autologous platelet concentrates in various oral surgery procedures, using a systematic review approach.

Keywords: platelet concentrates, growth factors, tissue regeneration, oral surgery

1. Introduction

Autologous platelet concentrates (APCs) have been widely used in many different clinical situations that require a rapid tissue healing and regeneration as it is especially the case in oral and maxillofacial surgery, orthopedics, sports medicine, ophthalmology, and in the treatment of skin ulcers. APCs are hemocomponents, obtained through centrifugation of patient's own blood, in order to collect the most active blood components: platelets, fibrin,

and in certain cases also leukocytes. The final product has a platelet concentration higher than the basal level, consequently has an increased number of platelet-derived growth factors [1]. The rationale of the clinical use of such platelet-rich preparations is based upon the concept of exploiting their contents enriched of numerous mitogenic platelet-derived growth factors (including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), endothelial growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (FGF), and hepatocyte growth factor (HGF)) as well as other key molecules in promoting tissue healing (as adhesive proteins, pro-coagulant factors, cytokines, chemokines, and anti-microbial proteins [2–5]) to stimulate many biological functions, such as chemotaxis, angiogenesis, proliferation, and differentiation (**Table 1**) in order to enhance hard and soft tissue healing.

Category	Term	Biological activities
Adhesive proteins	VWF + propeptide, Fg, Fn, Vn, TSP-1, and laminin-8	Cell contact interactions, clotting, and extracellular matrix composition
Clotting factors and associated proteins	F V/Va, F XI, Gas6, protein S, HMWK, AT, and TFPI	Thrombin production and regulation and angiogenesis
Fibrinolytic factors and associated proteins	Pgn, PAI-I, u-PAm, OSN, α2-AP, HRGP, TAFI, and α2-M	Plasmin production and vascular modeling
Proteases and antiproteases	TIMP-4, MMP-4, inhibitor of FIX, PN-II, C1-INH, and A1AT	Angiogenesis, vascular modeling, regulation of coagulation, and regulation of cellular behavior
Growth factors, cytokines, and chemokines	PDGF, TGFβ-1 and -2, EGF, IGF-1,VEGF, bFGF, FGF-2, HGF, CCL5, IL-8, MIP-1α, CXCL5, MCP-3, ANG-1, and IL-1β, neutrophil chemotactic protein	Chemotaxis, cell proliferation and differentiation, and angiogenesis
Basic proteins and others	PF4, β-TG, PBP, CTAP III, NAP-2, and ES	Regulation of angiogenesis, vascular modeling, and cellular interactions
Antimicrobial proteins	TC	Bactericidal and fungicidal properties
Others	CS-4, AB, and Ig	Diverse
Membrane glycoproteins	αIIbβ3, αvβ3, GPIb, PECAM-1, most plasma membrane constituents, receptors for primary agonists, CD40L, TF, and P-selection	Platelet aggregation and adhesion, endocytosis of proteins, inflammation, thrombin generation, and platelet-leukocyte interactions

Extracted and readapted from Anitua et al. [2].

Table 1. Platelet α-granule contents and their functional categories.

2. Brief history of platelet concentrates

2.1. Fibrin adhesives

More than 40 years ago, these technologies were originally used as sealant-adhesive agents in the treatment of hemorrhage with the aim of blocking the blood leakage [6]. Subsequently, other molecules involved in the coagulation process were combined to such fibrin preparations to improve their adhesive properties. These preparations were referred as "platelet-fibrinogen-thrombin mixtures" and were successfully used in ophthalmology [7, 8], general surgery [9], and neurosurgery [10]. Other authors termed them as "gel foam" [11].

It is noteworthy that the application of these preparations was essentially related to their adhesive properties and the platelets were served only to reinforce the fibrin matrix architecture. A few years later, it was developed the concept that these preparations could have healing and regenerative properties. In the late 1980s, Knighton et al. [12–14] used the autologous "Platelet-Derived Wound Healing Factors (PDWHF)," which were prepared through two-step centrifugation process, in the treatment of chronic nonhealing cutaneous ulcers. In 1997, Whitman et al. used platelet concentrate referred as "platelet gel" in oral and maxillofacial surgery [15].

2.2. Platelet-rich plasma

The term "Platelet-Rich Plasma" (PRP) was, for the first time, introduced by Kingsley et al. to describe a thrombocyte concentrate [16] used for the treatment of severe thrombopenia. However, the use of PRP term really started with Marx in 1998 [1] when he published a comparative clinical study in which the PRP regenerative potential was demonstrated in a series of patients undergoing mandibular reconstruction. Afterward, the PRP product was associated with the concept of platelet growth factors and their potential contribution to the enhancement of tissue healing.

According to the PRP protocol, the blood is collected in tubes containing anticoagulants and processed by two centrifugation steps. **Figure 1** illustrates schematically the specific protocol [17]. The final PRP product can be applied to the surgical site with a syringe or be activated by thrombin and/or calcium chloride to trigger platelet activation and to stimulate the fibrin polymerization.

After blood collection into tubes with anticoagulant, the first centrifugation at low force (soft spin) allows the separation of blood into three distinct layers: red blood cells at the bottom, a cellular plasma (platelet-poor plasma (PPP)) in the upper portion, and a whitish layer called buffy coat located between them containing the highest concentration of platelets and leukocytes. For the production of Pure-PRP (P-PRP), PPP and the superficial buffy coat layer are transferred into another tube and centrifuged at high forces (hard spin), after which most of the PPP and leukocytes are discarded and the final P-PRP can be collected. For the production of PRP rich in leukocytes (L-PRP), PPP, the entire buffy coat layer and some residual red blood cells are collected and transferred in another tube to be hard spin centrifuged. To obtain

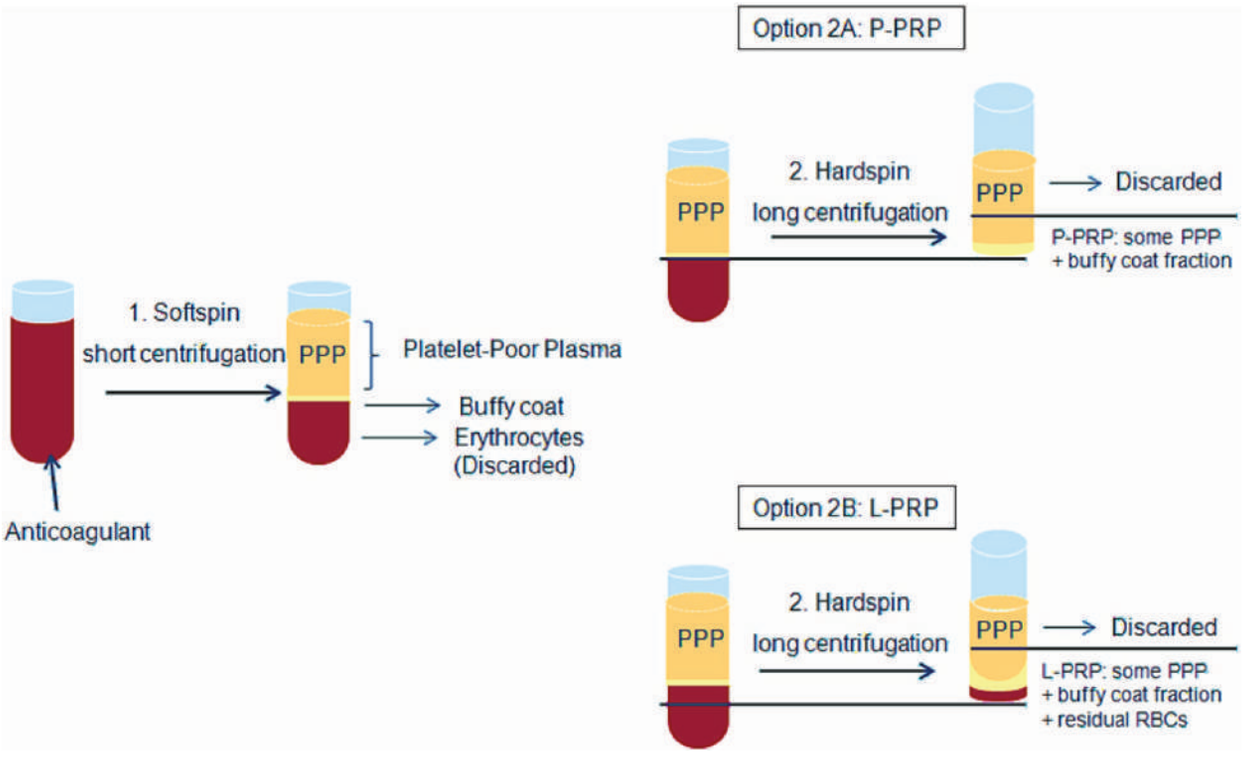

Figure 1. Protocol for PRP production.

the final L-PRP, PPP is discarded leading to an L-PRP that contains the buffy coat with most of the platelets and leukocytes, some residual red blood cells and PPP (adapted from Dohan Ehrenfest et al. [17]).

Currently, more than 20 different commercial systems for PRP preparation exist, which may lead to products with different features, especially regarding the composition and the cell concentration rate respect to baseline. On average, a 5–8× concentration is achieved though a ratio of up to 11× has been reported with PRP.

2.3. Platelet-rich fibrin

In 2001, Choukroun et al. developed a protocol for producing a hemocomponent named platelet-rich fibrin (PRF) [18]. Here, the blood is collected in tubes in the absence of anticoagulant and centrifuged with moderate forces (3000 rpm in a dedicated centrifuge) for 12 min. Afterward, three layers are formed: red blood cells and acellular plasma are located, respectively, at the bottom and at the top of the tube, and the fibrin clot, positioned between them, is PRF (**Figure 2**). Since the formation of the PRF clot naturally occurs within the tube, it has a strong fibrin matrix in which most of the platelets and leukocytes are embedded [19]. Since its introduction, PRF has undergone some development: the advanced PRF (a-PRF) was launched a few years ago, characterized by a reduced centrifugation speed and time (2700 rpm, 8–10 min), which allows a more even cell distribution within the clot [20]. Recently, the injectable PRF (i-PRF) has also been developed, which may be obtained with a further softer spin (1500 rpm, 3 min), consisting of a liquid form, very rich in white cells that can be used by infiltration into tissues and joints.

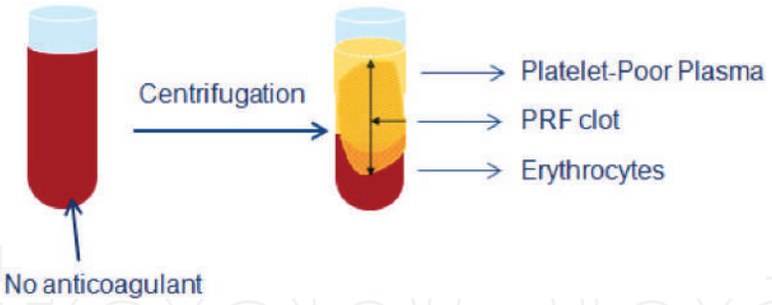

Figure 2. Protocol for PRF production. (Adapted from Dohan Ehrenfest et al. [19]).

2.4. Plasma rich in growth factors

In parallel to the introduction of PRP and PRF, Anitua in 1999 proposed another platelet concentrate protocol, denominated plasma rich in growth factors (PRGF) [21]. Briefly, blood is collected in tubes containing 0.2 ml of 3.8% trisodium citrate as anticoagulant. After a centrifugation at 580 g for 8 min, red blood cells and buffy coat layer are deposited at the bottom of the tube and the plasma component above. The latter is then manually separated into two fractions. The lower portion of about 2 ml, above the buffy coat, is the PRGF, whereas the upper portion is the plasma poor in growth factors (PPGF) (**Figure 3**). The final PRGF product may be applied as a liquid fraction to the target site or may be preactivated by adding 0.2 ml of 10% $CaCl_2$ to induce the clot formation [22].

2.5. Technical differences between PRGF, PRP, and PRF

PRGF differs from PRP for the following technical aspects:

1. Blood volume drawn is minimal (5–40 ml).
2. Requires a single centrifugation for the preparation.
3. Does not contain leukocytes.
4. Does not contain proinflammatory cytokines.
5. Platelet concentration is reduced (2–3 fold the baseline, respect to 5–8× for PRP).

In addition, PRGF also differs from PRF for these features:

1. Different products can be obtained (liquid, gel, membrane, and fibrin clot).
2. PRGF liquid can be combined with bone graft materials for bone regeneration procedure.

Differences between PRP and PRF are as follows:

1. PRP preparation requires two centrifugations.
2. Different products can be obtained (liquid and fibrin clot).
3. PRP liquid can be mixed with bone graft materials for bone regeneration procedures.

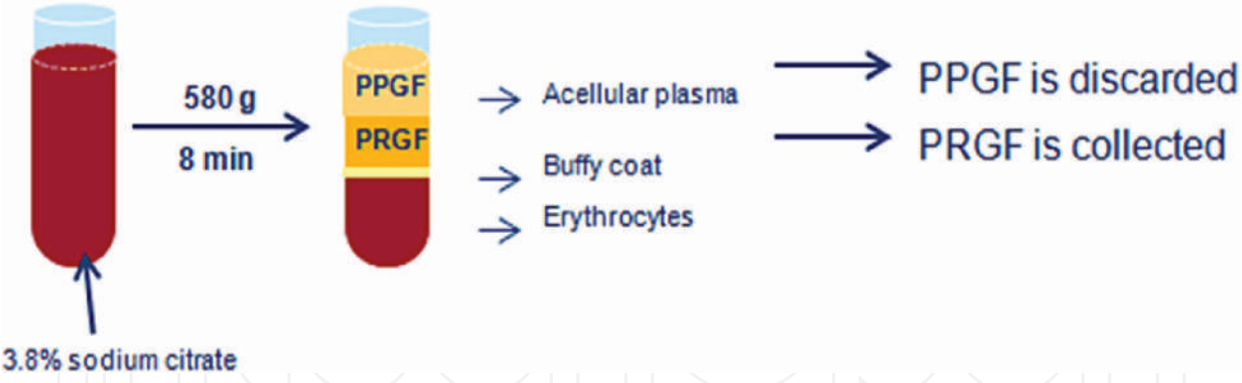

Figure 3. Process of PRGF production. Details are given in the text. (Adapted from Anitua [22]).

3. Clinical evidence of the efficacy of autologous platelet concentrates in oral regenerative surgical procedures

3.1. General aspects

In the last couple of decades, it has been observed a growing interest in the use of autologous platelet concentrates during oral regenerative surgical procedures as an adjunctive tool to enhance the hard and soft tissue healing. The following sections will summarize the recent evidence on the efficacy of autologous platelet concentrates in the dental field. The recent evidence has been obtained using a systematic review approach. The focused question was: "Does the adjunct of autologous platelet concentrate produce benefits to hard and soft tissue healing in oral surgery procedures in terms of tissue parameters, postoperative complications as well as patient's postoperative quality of life?" In order to address the aim of this chapter, electronic searches were performed on the main scientific databases (MEDLINE, Scopus, and Cochrane Central Register of Controlled Trials). Proper search terms were used, combined by Boolean operators. Only controlled clinical trials, randomized clinical trials, and existing systematic reviews and meta-analyses of the literature were included. The outcomes were complications and adverse events, treatment success, discomfort/quality of life, bone healing and remodeling assessed by histological and radiographic techniques, and soft tissue healing. The surgical procedures taken into consideration were: tooth extraction, periodontal surgery, endodontic surgery and treatment of immature necrotic teeth, maxillary sinus augmentation, and implant treatment. When possible, a quantitative analysis was undertaken by meta-analysis approach, using the software RevMan (RevMan, Version 5.3, The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark, 2014).

3.2. Alveolar postextraction healing

Several recent systematic reviews evaluated the efficacy of autologous platelet concentrates in enhancing alveolar socket healing after tooth extraction [23, 24]. Beneficial effects were generally reported in terms of better soft tissue healing, better clinical and histological epithelialization of wound margins, and a faster wound closure, although the heterogeneity of the data

could not allow sound meta-analyses. Regarding the bone formation, the qualitative synthesis of the histological analyses reported a better bone quality in biopsies retrieved from sites treated with platelet concentrate. Furthermore, the meta-analysis of the histomorphometric evaluation of the bone formation (including only few of the included studies) revealed that sites treated with platelet concentrate showed a statistically significant greater proportion of new bone than the controls, at 3 months of follow-up [23]. Even though the results of the meta-analysis suggest a beneficial effect of autologous platelet concentrates on bone formation, caution should be paid on interpreting such results, since the available evidence is scarce and of limited quality [23]. In spite of the relatively numerous randomized clinical studies assessing the value of APCs for enhancing postextraction socket healing, the main reason that prevents a wide meta-analysis is that the methods used for assessing bone regeneration and socket preservation are very different, providing different information that cannot be aggregated. In fact, different studies used different techniques like histological and immunohistological analysis, histomorphometric evaluation, scintigraphy, micro-TC, intraoral radiography, cone beam computed tomography, and clinical assessment, for the evaluation of different variables as percentage of new bone formation, osteoblasts activity, bone density, crestal bone level changes, ridge width and height, and soft tissues health status [23, 24]. All studies that evaluated postoperative symptoms like pain, swelling, trismus, and adverse events like dry socket, alveolitis, and acute inflammation concluded that APCs are effective in reducing symptoms and the incidence of adverse events, with an overall improvement of patients' quality of life [24]. The most recent systematic review on this topic concludes that APCs should be used in postextraction sites in order to improve clinical and radiographic outcomes such as bone density and soft tissue healing, as well as to reduce postoperative symptoms [24]. The actual benefit of APCs on decreasing of pain in extraction sockets, however, though consistently reported, is still not quantifiable [24]. In **Figure 4**, a brief sequence of pictures documenting a

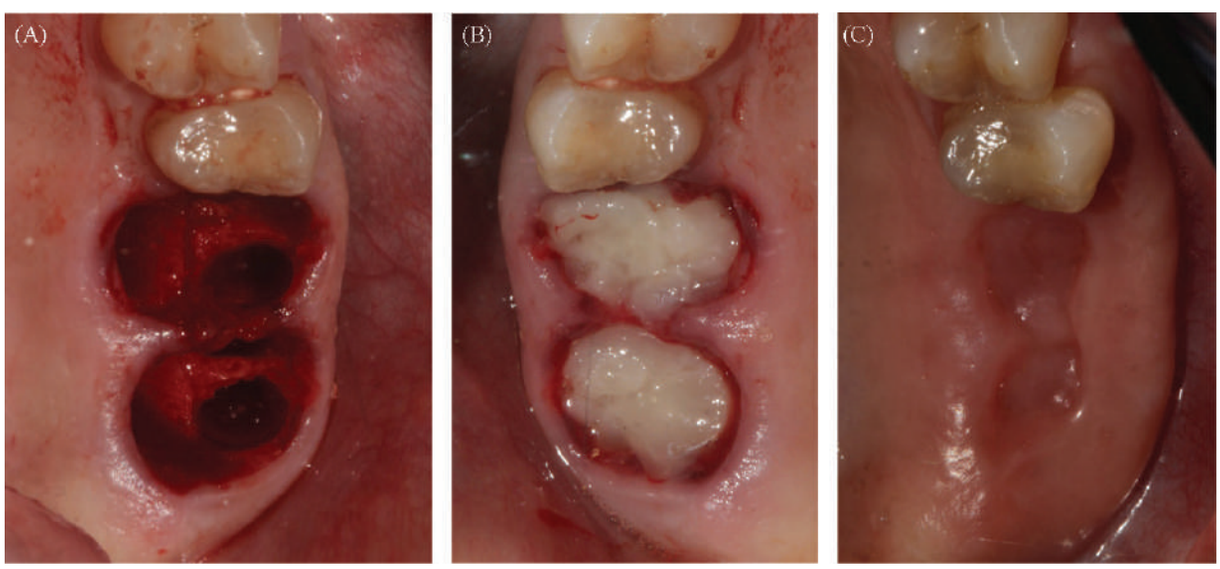

Figure 4. Double tooth extraction in the upper jaw and placement of PRGF in the extraction socket. (A) Fresh sockets after atraumatic extraction. (B) Sockets filled with PRGF clot; it can be sutured for a better stabilization. (C) 14 days after surgery: excellent healing of soft tissues is shown.

case of double extraction in the posterior upper jaw, subsequent positioning of platelet concentrate (PRGF) into extraction sockets, and the postsurgical clinical healing is shown.

3.3. Periodontal defects

Several systematic reviews and some meta-analyses evaluated the efficacy of autologous platelet concentrates in the treatment of periodontal defects, including intrabony defects, gingival recessions, and furcation defects [25–33]. Beneficial effects on clinical and radiographic outcomes in the treatment of infrabony defects were reported, although a high heterogeneity emerged among the clinical studies in terms of outcomes evaluated and bioactive agents/procedures combined with autologous platelet concentrates [25, 28, 30–32]. Furthermore, two meta-analyses [28, 30] concluded that PRP might exert positive adjunctive effects in the surgical treatment of such defects when combined with grafting materials, but no adjunctive effects were found in association with the guided tissue regeneration technique. Indeed, the latter is considered the gold standard for the treatment of periodontal intrabony defects and its use could probably mask the PRP effect. Regarding the autologous platelet concentrates' effect on gingival recessions, very few systematic reviews have been conducted, presumably due to the limited data about it. PRP or PRF did not show any clinical improvements in the treatment of gingival recessions or furcation defects [28, 33]. A Cochrane systematic review on this topic is still ongoing [34] and its results will certainly shed light on the actual evidence level regarding this topic, possibly confirming early indications of previous systematic reviews.

3.4. Endodontics and endodontic surgery

Platelet concentrates have been recently used in the clinical treatment of immature necrotic teeth, with the aim of regenerating the intracanal pulp and stimulating tooth development, as well as in the surgical treatment of teeth with apical periodontitis to enhance healing of periapical tissues. Clinical evidence on the benefits of the use of platelet concentrates in these pathologies exists but is still scarce. A recent systematic review concluded that periapical healing and apical closure were improved in those immature necrotic teeth treated with PRP compared with the control group without PRP, even though not statistically significant, and a significant better thickening of dentinal walls and root lengthening were also reported [35]. However, from the histological point of view, it seems that a true regeneration of necrotic pulp tissue of either mature or immature teeth was not achieved after using platelet concentrates. In fact, the neoformed intracanal tissues were mainly cemento-like, bone-like, and connective tissue. Root canals were repopulated with living tissue that only marginally resembled the original pulp. Despite this, the root maturation may be achieved and teeth function is not compromised [36].

Though the use of APCs in the management of periapical lesions could be considered a proper indication, very scarce studies are present regarding this topic. One randomized clinical study evaluated the postoperative quality of life in patients undergoing endodontic surgery [37]. The test group of 18 patients was treated with the adjunct of P-PRP and the control group was treated conventionally, without P-PRP. The test group showed significantly less pain and swelling, fewer analgesics taken, and improved functional activities as compared with

the control group [37]. This suggests that the adjunct of P-PRP to the endodontic surgery procedure may produce significant beneficial effect to patients' quality of life during the early postoperative period.

Another pilot clinical study on endodontic surgery compared a group of seven patients treated with the adjunct of PRF versus four control patients [38]. In addition to confirming the beneficial effects in the early postoperative period regarding significant reduction of pain and swelling, a significantly better healing of the lesion was observed after 2 and 3 months but not after 1-year follow-up [38]. This preliminary study had a very small sample size, so results should be interpreted cautiously. The latter two studies have been included in a recently published Cochrane systematic review on endodontic procedures for the retreatment of periapical defects, which concluded that there is evidence that adjunctive use of a gel of plasma rich in growth factors reduces postoperative pain compared with no grafting [39]. Regarding other possible beneficial effects of APCs in endodontic surgery, further evidence is needed.

3.5. Maxillary sinus augmentation

The use of platelet concentrates in association with grafting material during maxillary sinus augmentation procedure provided conflicting results in both preclinical and clinical studies [40, 41]. A recent meta-analysis documented that PRP combined to graft materials, in this type of surgical procedure, had no adjunctive effect on bone formation, on implant survival and implant stability as well as it did not show any statistically significant differences on marginal bone loss or alveolar bone height, compared to the bone graft alone [42]. Similar conclusions were also reported in other systematic reviews [43, 44]. However, another meta-analysis reported opposite conclusions concerning the bone formation supporting the use of PRP for sinus bone graft [45]. Furthermore, beneficial effects on soft tissue healing as well as reduction of postoperative discomfort were often reported [43]. Such variability in results could be ascribed to a number of factors. First of all, different techniques have been adopted for the preparation of platelet concentrates, leading to the products with different characteristics (final concentration of platelets and white cells, presence or absence of leukocytes, use of anticoagulants and activators, different mechanical consistence of the product, and association with different graft types) and, presumably, different biological activities. Secondly, different studies may also differ in experimental design, objectives, outcome variables, inclusion criteria, and follow-up duration. Furthermore, the sinus augmentation technique, though representing a very popular model for the assessment of bone substitutes for bone regeneration, suffers from a number of confounding factors that make standardization difficult, like patient age, residual bone quality and quantity, smoking habits, volume of graft used, porosity and general features of the graft material (e.g., intraoral or extraoral autografts, allografts, xenografts, and alloplasts), graft resorbability over time, graft healing time, and intra- and postsurgical complications like membrane perforation, infection, expertise of the clinician, techniques adopted for evaluating bone formation, including the position of the biopsy (crestal or lateral). Finally, if one aims at evaluating the effect of APCs on implant survival and success in the maxillary sinus augmentation, a number of additional factors concerning the implants and the prosthetic reconstruction must be considered, e.g., implant length

and width, shape, surface micro- and nano-geometry, type of implant-abutment connection, implant primary stability, and number and position of the implants. In fact, it is well known that implant survival in the augmented maxillary sinus is more variable than that of implants placed in the posterior maxilla.

A randomized clinical trial evaluating the effect of P-PRP adjunct on postoperative quality of life of patients undergoing maxillary sinus augmentation procedure, found a beneficial effect of P-PRP regarding pain, swelling, hematoma, and other postoperative symptoms, improving patient's acceptance of this often demanding procedure [46].

3.6. Implant dentistry

As it is claimed that platelet concentrates may promote bone regeneration, several animal studies have been conducted to assess the PRP effect on the osseointegration process, through histological and histomorphometrical evaluation, but controversial results have been reported. In fact, some studies did not demonstrate any advantages of PRP over non-PRP control groups at stimulating faster bone formation or higher bone-implant contact [47–49]. By contrast, histomorphometric analyses of the bone-implant interface in the early healing phase after implantation (6 or 8 weeks) revealed a significantly higher percentage of bone-implant contact in implants coated with liquid PRP formulation compared to those not PRP-bioactivated [50–52]. In addition to being time dependent, PRP effect is also site dependent since its effect has been reported to decrease with increasing distance from the site of application [52]. Similarly, liquid-PRP showed a tendency to increase the bone apposition to roughened titanium implants during early healing phase [53, 54].

Clinical studies reported a higher bone formation around the implants [55] and a good preservation of the alveolar crest around postextraction implants [56, 57] when APCs were used.

APCs have been also combined with several different types of grafting materials during regenerative procedures associated with implantoprosthesis rehabilitations, showing satisfying results and positive patient-related outcomes [58–61]. A long-term clinical study (10–12 years) on short implant placement in association with PRGF reported an implant survival rate of 98.9% and marginal bone loss inferior to 1 mm [62].

3.7. Systemically compromised patients

Patients affected by chronic systemic conditions like osteoporosis, cancer, diabetes type I or II, immunodeficiency, hematological/coagulation defects ,and other conditions, often present difficulty in healing even after simple surgical procedures, like the extraction of a tooth. Therefore, the use of a safe tool that may enhance the healing process in a natural way may represent a remarkable benefit for these patients.

A few studies have been published on the use of platelet concentrates in systemically compromised patients, most of them are regarding postextraction healing. Some examples will be reported.

3.8. Diabetes

In a split-mouth study in 34 patients with diabetes mellitus candidate to bilateral tooth extraction of a total of 127 teeth, alveolar sockets on the test side were treated with P-PRP and on the control side they were left to heal in a natural way [63]. The P-PRP group showed a significantly better healing (evaluated through the Healing Index) and a faster closure of the alveolus (by evaluation of the residual socket volume) at 3 and 7 days after extraction, as compared to the control group [63]. The authors concluded that platelet growth factors stimulate a faster epithelialization, protecting the alveolus in early healing steps. Hence, it is avoided occurrence of alveolitis, very common in diabetic patients following tooth extraction.

3.9. Irradiated patients

In a split-mouth study on 20 patients that underwent radiotherapy for head and neck cancer, and candidate to bilateral tooth extraction, alveolar sockets on the directly irradiated side were treated with P-PRP (test group) and on the untreated side they were left to heal in a natural way (control group) [64]. Twenty-four bilateral extractions were performed in the mandible and 33 in the maxilla for a total of 114 extractions. The P-PRP group showed a significantly better healing, in terms of Healing Index and residual socket volume at each follow-up (7, 14, and 21 days), as compared to the control group. Patients were followed up to 24 months after surgery. In the control group, two cases with bone exposure were retreated with P-PRP and subsequently healed [64].

3.10. Osteoradionecrosis (ORN)

In a series of 10 patients who developed osteoradionecrosis, debridement of necrotic bone (performed with ultrasonic instruments) was associated with the adjunct of P-PRP. All patients successfully healed, with no intraoperative or postoperative complications up to 12 months of follow-up [65]. Tissue regeneration and closure was excellent, and postoperative pain, assessed through visual analogue score (VAS) scores, was low. In spite of the absence of a control group, this study suggested that P-PRP may be beneficial as an adjunct to surgical treatment of ORN, for predictable enhancement of tissue vascularization and epithelialization in patients with a history of head and neck radiotherapy.

3.11. Coagulation defects

A case-control study was performed on 66 patients affected by severe thrombocytopenia (<50,000 platelets/μL) and in need for at least two tooth extraction each [66]. For these patients, postoperative bleeding represents an important issue. Teeth were extracted in two consecutive interventions. In one intervention, the patients received a platelet transfusion before extraction (systemic treatment) and in the other session, the postalveolar sockets were filled with P-PRP (local treatment). Patients were evaluated frequently in the first 7 days after extraction. The group treated locally with P-PRP showed a statistically significant reduction in postoperative bleeding, hematoma, and need for reintervention, as compared to the group receiving a systemic infusion of platelets [66]. Therefore, P-PRP in postalveolar sockets may strongly

reduce the risk of hemorrhage and related complications in thrombocytopenic patients, due to the ability of stimulating healing and its hemostatic properties.

3.12. Bisphosphonate-related osteonecrosis of the jaws

Recently renamed medication-related osteonecrosis of the jaw (MRONJ), it is an adverse drug reaction consisting of progressive bone destruction in the maxillofacial region of patients under current or previous treatment with a bisphosphonate or another antiresorptive or antiangiogenic drug. Since APCs demonstrated to enhance bone and soft tissue healing in oral surgery procedures, it is reasonable to believe that they might provide benefits to these patients. A recent systematic review included 18 studies, reporting on 362 patients undergoing oral surgery in combination with APCs [67]. The adjunct of APC in the surgical treatment of necrotic bone removal significantly reduced osteonecrosis recurrence as compared to control (**Figure 5**). APC was associated with a reduced, though not significant, incidence of BRONJ after tooth extraction. Heterogeneity among studies was found regarding bisphosphonate type, clinical indication, administration route, treatment duration, triggering factors, study design, follow-up duration, type of APC, and outcomes adopted to evaluate treatment success [67]. Though the results of this review must be cautiously interpreted, since they are based on low evidence level studies, with limited sample size, they are suggestive of possible beneficial effects of APC when associated with surgical procedures for treatment or prevention of BRONJ. To confirm such indication, prospective comparative studies with a large sample size are urgently needed. Another subsequent systematic review on the same topic substantially confirmed these results, highlighting the need for well-done evidence-based comparative studies [68].

3.13. Implants in patients assuming bisphosphonates

In a multicenter study on a cohort of 235 middle-aged osteoporotic women under bisphosphonates therapy, the outcome of a total of 1267 implants was evaluated after a minimum follow-up of 24 months [69]. The implants were always placed in combination with P-PRP, used as a coating over implant surface at insertion (**Figure 6**). The main outcomes (adverse

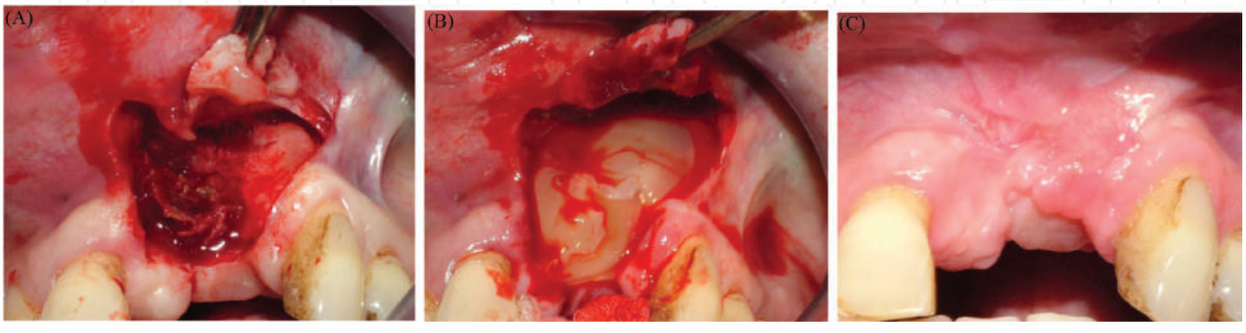

Figure 5. (A) A case of an oncologic patient under bisphosphonate affected by osteonecrosis of the upper jaw, undergoing respective surgery for removal of the necrotic bone. (B) PRGF is placed within the region involved from resection. (C) One year after surgery, the region is completely healed.

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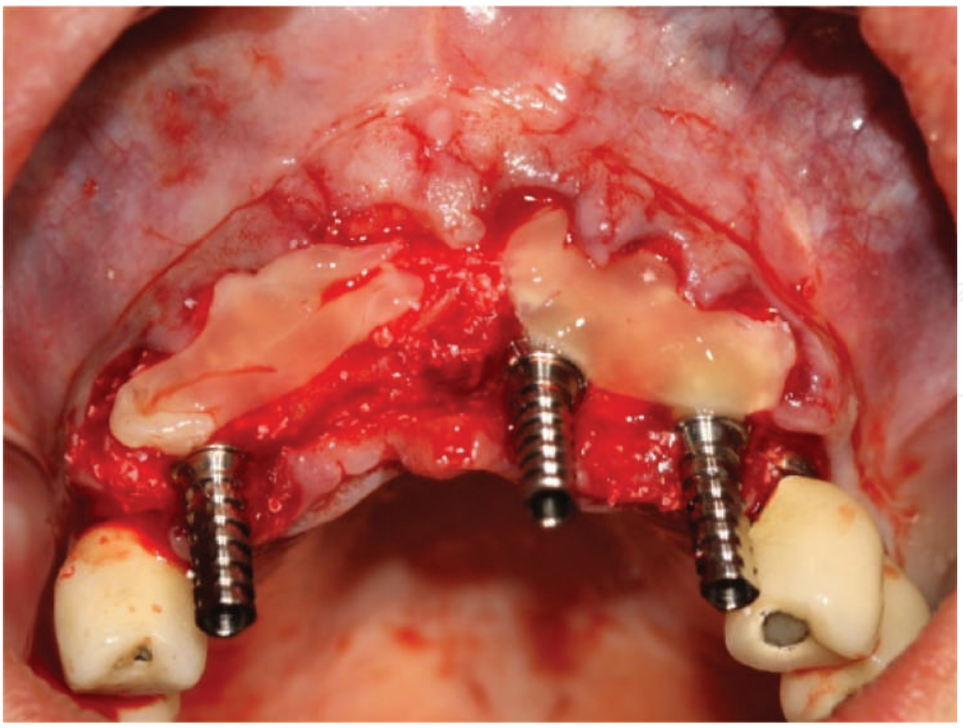

Figure 6. Multiple implant placements in an osteoporotic woman under bisphosphonates. PRGF membrane was used to cover the region involved from implant surgery.

events) were implant failure and incidence of osteonecrosis of the jaw (ONJ). Sixteen implants were lost in 16 patients up to 120 months after placement, representing a survival of 98.7% on an implant basis and 93.2% on a patient basis [69] No cases of ONJ occurred. The results are perfectly in line with those of healthy patients.

Overall, the above results suggest that the use of procedures aimed at enhancing tissue healing, such as autologous platelet concentrates, may produce relevant benefits in patients at risk due to their compromised systemic condition and should be recommended.

4. Conclusion

The use of autologous platelet concentrates generally produced beneficial effects, though the level of evidence differs among various surgical procedures. In postextraction sockets and periodontal intrabony defects, the advantage of using APCs, both alone and combined with bone substitutes, is well documented by a number of randomized clinical studies. In maxillary sinus augmentation, controversial outcomes exist, due to few published controlled studies. Also, in endodontic surgery and implant treatment, there is a paucity of evidence-based studies, even though all show beneficial effects of APC. The variability among protocols and outcomes in different studies often prevents the possibility of performing meta-analysis and is thought to be related to the controversial results sometimes observed. Better soft tissue healing, improved patients' quality of life and reduced incidence of adverse events and

complications are systematically reported when using APC. In conclusion, the use of such autologous products is recommended for improving predictability and patients' acceptance of treatment in oral surgery procedures.

Author details

Carmen Mortellaro[1]* and Massimo Del Fabbro[2,3]

*Address all correspondence to: carmen.mortellaro@med.uniupo.it

1 Department of Health Sciences, Avogadro University of Eastern Piedmont, Novara, Italy

2 Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milano, Italy

3 IRCCS Istituto Ortopedico Galeazzi, Milano, Italy

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