From PCL/HA powder to bioresorbable scaffold via laser powder bed fusion

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SUMMARY

Bone bioresorbable scaffold substitutes bone physiological functionalities during its life span. In addition to biological and mechanical performances, bioresorbable scaffolds have to show structure material homogeneity, non-toxic degradation products and degradation rate proportional to new bone growth. In this study, the compositional and structural characteristics of bioresorbable polycaprolactone (PCL)/hydroxyapatite (HA) scaffolds, produced *via* laser powder bed fusion in lattice forms (with diamond and rhombic dodecahedron elementary unit cells, respectively), were analyzed in terms of comparison between starting powder and manufactured scaffolds. Scaffolds showed the achievement of: i) isotropic structure, necessary to obtain good mechanical properties; ii) chemical composition enhancing cells adhesion to the scaffold surface; and iii) crystallinity that preserves structure integrity in physiological environment.

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Introduction

Bone bioresorbable scaffold provides the performances of bone segment to be substituted during its lifetime, gradually until its total degradation. In particular, mechanical behavior must be guaranteed in addition to biological ability of stem cell adhesion, proliferation and differentiation (Oryan *et al*., 2014). Once defined scaffold material, all these features are modulable through scaffold geometry and surface properties to bone tissue interface. Generally, the goal to achieve is scaffold design that mimic trabecular bone. Lattice-based scaffold could be a response to this aim (Ali *et al*., 2020). The choice of material is due to the kind of bone replacement and to the scaffold degradation time needed for new bone tissue growth. Furthermore, degradation products must be non-toxic in physiological environment and removable *via* metabolic pathways of organism. Hydroxyapatite (HA) was widely employed as artificial bone in orthopedics, due to its chemical composition similar to natural bone (Shin *et al*., 2014), excellent biocompatibility both *in vitro* and *in vivo* and osteoconductivity. Moreover, Kim *et al*. (2017) demonstrated that the increase of phosphate ion concentration during calcium phosphate scaffold degradation plays a key role inducing the osteogenic differentiation of stem cells. Unfortunately, HA exhibits low crack resistance (Abedalwafa *et al*., 2013) and, for this reason, it is often mixed in percentage as high as 30 wt.% with polymers such as polycaprolactone (PCL) (Causa *et al*., 2005). PCL is a cheap Food and Drug Administration (FDA)-approved polymer for load-bearing application with high thermal stability. However, pure PCL scaffolds show limitations as low biocompatibility and slow degradation, due to its semi-crystalline nature and hydrophobicity (Abedalwafa *et al*., 2013; Siddiqui *et al*., 208). Thus, the combination of PCL and HA promotes scaffolds properties connected to geometry, such as biological and mechanical answers, and scaffold surface performances for bone tissue regeneration and restoration of physiological functions (Hajiali *et al*., 2018). The last step in the production process of bioresorbable bone scaffold is focused on the production technology. Additive manufacturing is getting more and more attention thanks to high accuracy and low cost for customized production (Wubneh *et al*., 2018). In particular, powder bed fusion is a high-resolution technique, which assure smooth scaffold surface for cells attachment and mechanical performances suitable to clinical applications.

In this study, bioresorbable lattice scaffolds were produced by the laser powder bed fusion (LPBF) technology, by starting from PCL/HA (30 wt.%) powder mixture. Scaffolds were built by repeating diamond (DO) and rhombic dodecahedron (RD) elementary unit cell, to allow scaffold suitability to load bearing of daily stresses and cells colonization. The problem of this approach is that LPBF process could change the properties of PCL/HA starting material and damage bioresorbable scaffolds performances, due to material-laser interaction. Therefore, in this study a double check, pre- and post-production, of PCL/HA main prerequisites was carried out in order to investigate:

i) Material homogeneity to assure scaffold isotropic properties during implant life span;
ii) Non-toxic degradation products;
iii) Low degradation rate for assuring new bone formation (Wiria *et al*., 2007).

Materials and Methods

Bioresorbable scaffolds in PCL/HA (30 wt.%) were produced by LPBF technology. The machine system employed was an EOS P770 with power laser 1.2 W. Scaffolds lattice structures (1 cm^3) were filled with DO and RD elementary unit cells, respectively, as shown in Figure 1.

Double check on pre- and post-production process was conducted as comparison between PCL/HA starting powder and DO and RD manufactured scaffolds. Information on morphology of starting powder and its dispersion in scaffold matrix was obtained by a Tescan VEGA3 scanning electron microscope (SEM). Chemical composition analysis of scaffolds compared to starting powder was performed by energy dispersive spectroscopy (EDS) through an EDAX Element System. Results were obtained by averaging data taken from five different sample areas observed at the same magnification value (500x). Crystallographic data from powders and scaffolds were obtained by X-ray diffraction (XRD) measurements, carried out by a Bruker D8 Advance diffractometer operating at V=40 kV and I=40 mA, with Cu-Kα radiation, in the angular range 2θ=11°- 55°. Pattern was analyzed by the DIFFRAC.EVA software package including the ICDD–PDF 2 license for search/match analysis.

Results

SEM images of PCL/HA starting powder and its dispersion on a manufactured scaffold are visible in Figure 2 A and B, respectively. HA particles (brighter) are clearly homogeneously dispersed in PCL powder (darker), as evidenced in the electron backscattered SEM image of Figure 2A. PCL particles are irregularly shaped with a wide range of sizes (20-200 µm). On the other hand, HA particles are spherical (see inset in Figure 2A) with sizes from 5 to 30 µm. These particles are not subjected to melting caused by laser and remain as a solid dispersion in the PCL matrix of scaffold (Figure 2B). On the contrary, some PCL particles remain partially melted on surface layers of DO and RD scaffolds (Figure 3 A and B, respectively), by receiving sufficient energy through the laser beam to attach to the structure but not enough to completely melt.

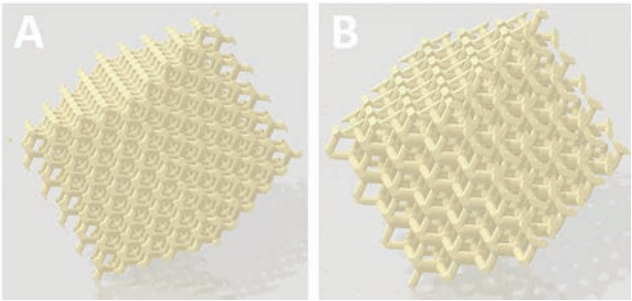

Figure 1. PCL/HA lattice scaffolds with diamond (A) and rhombic dodecahedron (B) elementary unit cells.

The chemical compositions of the starting powder and scaffolds, obtained by EDS, are listed in Table 1. DO and RD scaffolds composition slightly differs from powder. In particular, the ratio between Ca and P, the main species of HA, is about the same for powder (2.2), DO (2.0) and RD (2.3) scaffolds suggesting a comparable amount of HA in all the analyzed samples. Chemical elements belonging to PCL (O, C, H) are not reported because EDS is not able to quantify light elements. Gold is due to the conductive coating layer deposited on samples for SEM observation reasons. The presence of silicon, only in scaffolds, is certainly due to contamination in the production phase.

XRD patterns of PCL/HA powder, DO and RD scaffolds are reported in Figure 4. Peaks from PCL are indicated as full dots and all the other are the peaks from HA ($Ca_{10}(PO_4)_6(OH)_2$, according to ICDD 740565). PCL and HA in the powder (red pattern) are well-crystallized, as results from high intensity and small width of diffraction peaks. The DO scaffold (blue pattern) maintains a good crystallinity of both compounds, suggesting maintenance of semi-crystallinity property of polymer after the production process. In contrast, RD scaffold (black pattern) exhibits sensible decrease of crystallization of both PCL and HA.

Discussion

Bone bioresorbable lattice scaffolds with DO and RD elementary unit cells (Figure 1) in PCL/HA (30 wt.%) were produced by LPBF. In this study, the compositional and structural characteristics of scaffolds after the additive manufacturing process were investigated.

The first feature checked was the homogeneous dispersion of ceramic particles (HA) into the polymeric matrix (PLC) in order to avoid risk of scaffold inhomogeneous mechanical and biological properties, with further risk of implant failure. LPBF technology allows to obtain, starting from well mixed PCL/HA powder (Figure 2A), scaffolds composed of a PCL matrix with a balanced dispersion of HA particles (Figure 2B). During the additive manufacturing process, in the layering stage of the powder bed, the spherically shaped HA particles (inset in Figure 2A) act as a solid lubricant for the irregularly shaped and variously-sized PCL powder. In addition, experimental tests in literature (Eosoly *et al*., 2009; Eshraghi *et al*., 2012) showed that HA particles dispersed in solid PCL matrix, inhibits the movements of polymeric chains, improving the mechanical performances of scaffold. Thus, HA particles guarantee isotropic performances to DO and RD bioresorbable LPBF scaffolds and could potentially enhance the mechanical properties. In addition, the presence of PCL particles partially melted on scaffolds surface (Figure 3), helps to obtain roughness level allowing cells adhesion on scaffolds.

The second aspect verified in this study was the presence of unexpected elements in bioresorbable scaffolds that could potentially degrade in harmful ions. EDS analysis (Table 1) shows the presence of silicon contamination in the scaffolds. However, this is not an issue since silicon has several biological functions in the human body, improving bone health and accelerating healing (Henstock *et al*., 2014). EDS analysis also shows that the ratio

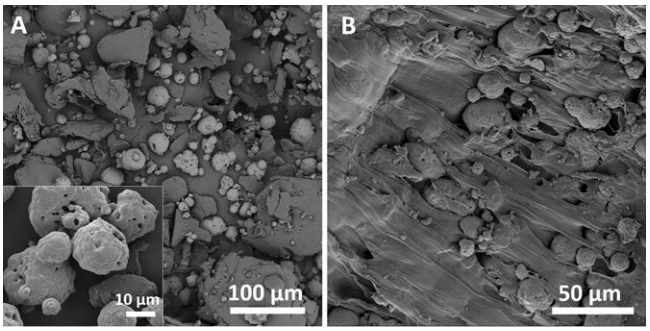

Figure 2. Backscattered SEM micrograph of (A) PCL/HA (30 wt.%) powder mixture and (B) HA particles in PCL matrix of a manufactured scaffold.

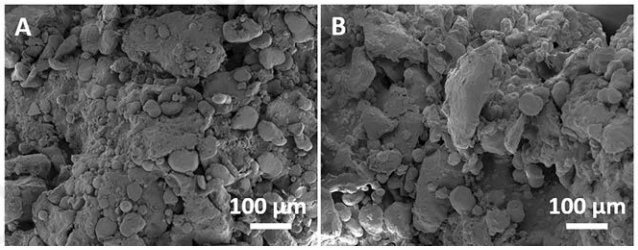

Figure 3. SEM micrographs of PCL particles partially melted on DO (A) and RD (B) scaffolds surface.

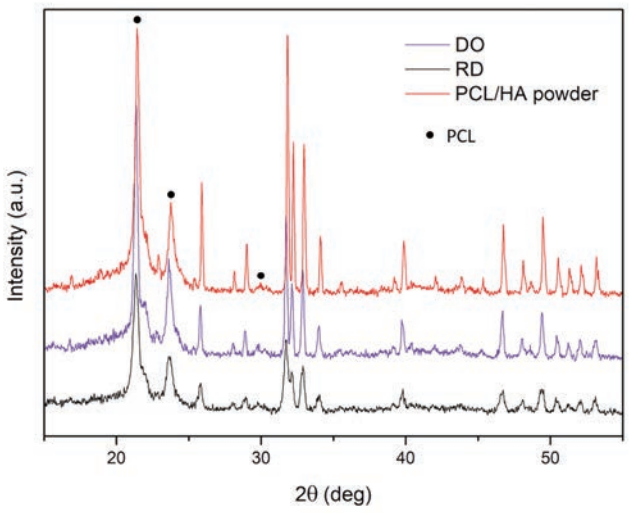

Figure 4. BXRD patterns of PCL/HA powder (red), DO (blue) and RD (black) scaffolds. PCL, full dot; HA, all the other peaks.

Table 1. Chemical composition (at. %) of PCL/HA powder, DO and RD scaffolds by EDS analysis.

	Powder		DO		RD	
	AV	SD	AV	SD	AV	SD
Au	0.13	0.05	0.28	0.04	0.31	0.04
Si	-	-	0.32	0.04	0.19	0.05
C	66.4	1.4	63.6	1.1	62.1	0.4
O	29.4	1.2	33.2	1.3	34	1
P	1.22	0.13	0.90	0.09	1.1	0.2
C_a	2.7	0.3	1.8	0.3	2.5	0.5

AV, average value; SD, Standard deviation.

between the main constituents of HA (Ca and P) remains approximately constant in all the investigated samples. This latter evidence suggests comparable amount of HA in DO and RD scaffolds if compared to powder, confirming that the additive production process does not cause any modification of the compound's stoichiometry neither potentially inconsistent biological responses. In addition, the lattice parameters of HA obtained by XRD analysis, reveal a Ca/P ratio of 10:6, *i.e.* 1.67. Therefore, Ca/P ratios experimentally found for powder, DO and RD scaffolds result slightly higher than that nominal. This increase is a positive effect; in fact, Ca/P ratio is an essential requirement for cell growth as shown by Ergun *et al.* (2008) that by *in vitro* studies found increased osteoblast adhesion on substrates at high Ca/P ratios (up to values as high as 2.5). Thus, DO and RD bioresorbable scaffolds do not developed potentially harmful chemical elements during LPBF production process, while their chemical composition could enhance cells adhesion to the scaffold surface. Finally, bioresorbable scaffold crystallinity is an important aspect related to the degradation rate. According to XRD patterns in Figure 4, both PCL and HA compounds preserve crystallinity after scaffold production, although slightly lower than powder. As demonstrated by Guarino *et al.* (2009), the increase of crystallinity of scaffold polymeric matrix because of the HA is able to hinder the degradation of composite scaffold, by deflecting physiological fluids preferentially at the interface with PCL. In fact, polymer/ceramic interface is more susceptible to hydrolytic attack, in this way HA induces a sort of shielding effect of the polymer matrix. Therefore, the crystalline DO and RD scaffolds should preserve their properties from action of physiological fluids until new bone tissue growth.

Conclusions

Bone bioresorbable scaffold substitutes bone physiological functionalities during its life span. It is possible to tailor biological and mechanical performances of scaffolds *in vivo* by the combination of materials, geometries and production technologies.

In this study, bioresorbable lattice scaffolds were produced *via* LPBF technology, starting from PCL/HA (30 wt.%) powder mixture. Scaffolds were built by repeating DO and RD elementary unit cell, to allow scaffold suitability to load bearing applications and cells viability. However, LPBF process could change the properties of PCL/HA starting powder and damage these performances because of the material-laser interaction. For this reason, the material homogeneity, non-toxic degradation products and low degradation rate were investigated comparing the starting powder with the built scaffolds. Results have shown a homogeneous solid dispersion of HA particles in the PCL matrix suitable to guarantee isotropic performances of DO and RD bioresorbable LPBF scaffolds and potentially enhanced mechanical properties. Moreover, DO and RD bioresorbable scaffolds do not developed potentially harmful chemical elements during LPBF production process, while their chemical composition and surface morphology could enhance cells adhesion to the scaffold surface. In addition, the crystallinity of PCL/HA powder is almost conserved in DO and RD scaffolds and that should preserve their properties from action of physiological fluids until new bone tissue growth.

In conclusion, DO and RD scaffolds produced by LPBF using a PCL/HA powder own the main prerequisites of bone bioresorbable implant.

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