Systematic characterization of 3D-printed PCL/β-TCP scaffolds for biomedical devices and bone tissue engineering: Influence of composition and porosity

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This work aims at providing guidance through systematic experimental characterization for the design of 3D-printed scaffolds for potential orthopedic applications, focusing on fused deposition modeling with a composite of clinically available polycaprolactone (PCL) and β-tricalcium phosphate (β-TCP). First, we studied the effect of the chemical composition (0–60% β-TCP/PCL) on the scaffold’s properties. We showed that surface roughness and contact angle were, respectively, proportional and inversely proportional to the amount of β-TCP and that degradation rate increased with the amount of ceramic. Biologically, the addition of β-TCP enhanced proliferation and osteogenic differentiation of C3H10. Second, we systematically investigated the effect of the composition and the porosity on the 3D-printed scaffold mechanical properties. Both an increasing amount of β-TCP and a decreasing porosity augmented the apparent Young’s modulus of the 3D-printed scaffolds. Third, as a proof of concept, a novel multimaterial biomimetic implant was designed and fabricated for potential disc replacement.

I. INTRODUCTION

Design and manufacturing of synthetic scaffolds to stimulate bone repair has been extensively studied. It is reported that a construct should ideally present the following properties: biocompatibility, high porosity with interconnected pores to allow cell ingrowth, sufficient mechanical strength, promotion of cell adhesion (osteocductive) and activity (osteoinductive), appropriate degradation, and custom-fit geometry. The design of a construct is therefore complex, and different manufacturing processes have been explored in this regard, such as casting, molding, or electrospinning. Recently, additive manufacturing (AM) has received increasing attention in medical devices and tissue engineering because it allows the manufacturing of constructs with a controllable and accurate material layout, leading to a high potential for complex geometries and better control over the porosity and pore layout in the construct. In particular, fused deposition modeling (FDM) is of interest for bone tissue engineering because of its ease of use, the variety of biocompatible polymers and composites that can be exploited, and because porous structures can be accurately generated. Numerous studies have been published regarding this topic, and a large number of geometries as well as materials have been explored.

Regarding material composition, medical grade poly (ε-caprolactone) (PCL) and beta tricalcium phosphate (β-TCP) are two widely used materials for orthopedic applications. PCL is a biocompatible synthetic polymer that is used for biodegradable implants due to its biocompatibility, long-term biodegradability, FDA approval, and relatively low cost. In addition, PCL can easily be manufactured and manipulated, thanks to its low melting point, its ease of mixture with other materials (polymers and ceramics), and its compatibility with most AM methods, in particular FDM. β-TCP is a calcium phosphate derivative, similar to the calcium phosphate material that comprises between 60 and 70% of natural...
bone, thus presenting an inherent biomimetic potential. It is biodegradable and has been demonstrated to have osteo-
conductive properties in encouraging new bone growth, thus reducing patient recovery time due to the generation of
natural bone tissue.\textsuperscript{15} The combination of these two
materials offers unique properties and presents great interest
for regenerative medicine, and many studies have been
conducted on this particular composite for medical devices,
implants, and constructs for orthopedic applications.\textsuperscript{16–18}
During the design of the construct, its porosity and its
composition must be carefully defined since both parameters
can be related to the scaffold’s properties (chemical, physical, and biological) known to be of influence in tissue
engineering, such as surface composition, degradation, stiffness, surface morphology and hydrophilicity, cell prol-
liferation, and differentiation. Several studies have linked
some of these properties to the porosity and/or the composition of the construct. For instance, Huang et al.\textsuperscript{14}
showed that the addition of β-TCP in PCL improved the
3D-printed scaffold’s mechanical performance, and Yeo
et al. studied in detail the degradation of PCL/β-TCP
scaffolds (80:20) both in vitro and in vivo.\textsuperscript{16} Hollister
et al.\textsuperscript{19,20} focused on the effect of the
composition. They correspond to a plain surface. For mechanical testing, the specimens
to deposit struts of width ranging from 350 to 400
10 mm, thickness 600
mm, and strut distance 0.4 mm (0%
porosity) were manufactured, resulting in a scaffold with
100/0, 80/20, 60/40, and 40/60.

In this paper, we report on a systematic approach to this
problem, by manufacturing and assessing the properties of
3D-printed PCL/β-TCP scaffolds with different compositions
and porosities. We first focused on the effect of the
composition. For that purpose, we synthesized PCL/β-TCP
composites with different proportions of ceramic (0, 20, 40,
60% β-TCP) and 3D-printed nonporous scaffolds. For each
composition, we examined the surface morphology, evalu-
ated the hydrophilicity, quantified the degradation speed,
determined the mechanical properties of the material.
We then performed in vitro biological experiments to assess
the composition effect on cell proliferation and osteogenic
differentiation. Next, we systematically assessed the effect
of both the composition and the porosity on mechanical
properties. We produced 20 groups of scaffolds with
different porosities and composition, measured their vol-
ume, and characterized their mechanical performances.
In the last part of the paper, as a proof of concept, a 3D-printed
implant composed of multiple materials and porosities was
designed and fabricated for the application of disc re-
placement. The choice of the design parameters was
detailed and a prototype was manufactured.

II. EXPERIMENTAL

A. Synthesis and composition analysis of PCL/
β-TCP materials

PCL (Sigma-Aldrich, St. Louis, Missouri) and β-TCP
powder with particle size averaging 100 nm (Berkeley
Advanced Materials Inc., Berkeley, California) were
weighed with respect to the required PCL/β-TCP ratio.
10% (wt/v) PCL solution and 5% (wt/v) β-TCP solution
in dimethylformamide were prepared at 80 °C and stirred
for 3 h, before being mixed together and thoroughly
stirred for another 1 h. This solution was then precipitated
in a large volume of water at room temperature to remove
the solvent. The material was then dried for 24 h at room
temperature and manually cut into pellets of approxi-
ately 5 mm in diameter. This synthesis process was
repeated for each ratio of PCL to β-TCP explored in this
study: 100/0, 80/20, 60/40, and 40/60.

Similar to the test performed by Lepoittevin et al.,\textsuperscript{22} the
composition of the synthesized composites was validated
by thermal gravimetric analysis (TGA), using a TA in-
strument Q500 TGA (TA Instruments, New Castle,
Delaware). Briefly, the samples were heated up to
550 °C with a constant increase of 20 °C per minute,
while monitoring their mass over time. Because of the
thermal properties of both materials, the remaining mass at
the end of the test was considered to be pure β-TCP.

B. Filament fabrication and scaffold 3D printing

Using an in-house built screw extruder (see Fig. S1),
the solid pellets of PCL/β-TCP were melted at 90 °C
and extruded into a filament of constant diameter for FDM
3D printing. Average filament diameter for each group
was measured and the values were used for each group,
respectively, during the 3D printing process. Scaffolds
were manufactured using a Lulzbot Mini (Aleph Objects
Inc., Loveland, Colorado) with a nozzle of 500 µm. The
printing temperature was set to 160 °C so that each ratio
could be printed smoothly. The layer thickness was set to
200 µm, each layer being constituted of parallel struts
with an orientation of 90° relative to the previous layer.
The printing speed was set to 5 mm/s and was calibrated
to deposit struts of width ranging from 350 to 400 µm.
Two types of scaffolds were manufactured. For surface
characterization and biological studies, discs of diameter
10 mm, thickness 600 µm, and strut distance 0.4 mm (0%
porosity) were manufactured, resulting in a scaffold with
a plain surface. For mechanical testing, the specimens
were cylinders of diameter 10 mm and height 5 mm. Five
different porosities were explored for each material
composition. They correspond to five different strut
distances: 0.4, 0.5, 0.71, 1.25, and 2.5 mm. 0.4 mm is
the theoretical distance for a porosity of 0%, and 2.5 mm
has been empirically defined as the maximum value that
still ensures scaffold integrity.
C. Surface characterization

Hydrophobicity was evaluated using a contact angle goniometer, Ramé-Hart 290 (Ramé-Hart Instrument Co., Succasuna, New Jersey). Briefly, a droplet (4 µL) was deposited at the center of the disc scaffold, and the contact angle was measured 1 min after deposition using image processing. Five samples were tested for each group. Because the contact angle is linked to surface chemistry as well as morphology and because 3D printing affects the surface morphology, two assays were carried out. The first assay aimed at studying the impact of the composition only. To homogenize the surfaces after 3D printing, post-processing was performed. The scaffolds were placed in an oven at 80 °C onto a glass substrate for 10 min to melt the samples and obtain similar surfaces for all the compositions. The second assay was performed on the scaffolds directly after printing to observe the influence of both the material composition and the manufacturing process.

The morphology of the surface was evaluated qualitatively using second electron emission imaging. The samples were first cleaned in ethanol and cut to size using a scalpel. The samples were sputter-coated with gold (10 nm) (SPI Sputter, SPI Supplier Division of Structure Prob Inc., West Chester, Pennsylvania). A scanning electron microscope (SEM; Zeiss Sigma FESEM, Carl Zeiss Microscopy, Thornwood, New York) was then used to image the samples at three different magnifications: 150, 1000, and 10,000, with an acceleration voltage of 3 kV.

Surface roughness (arithmetic average roughness $R_a$) of the disc scaffolds was quantified using a profilometer (Dektak XT, Bruker, Massachusetts). The profiles were measured over a line of 1 mm length following a single strut, with a stylus force of 1 mg and a measuring range of 6.5 µm. Five samples were tested per group.

D. Degradation

Because PCL is a slow biodegradable polymer, accelerated degradation was performed following the protocol described by Lam et al.\textsuperscript{23} Pieces of filaments (length 20 mm) were used as specimens to quantify the degradation of the material itself and not the scaffold after printing. The specimens were immersed in a 5 M NaOH solution (15 mL) and incubated at 37 °C. At predefined time-points, they were removed, dried in an oven at 30 °C for 45 min to remove any liquid, and weighed. Degradation was then quantified through measuring mass loss, i.e., the change in mass of the bulk recovered filament sample throughout the duration of the experiment.

E. Biological study

For all biological studies, multipotent mouse C3H10T1/2 fibroblasts (ATCC, Manassas, Virginia) were used as a model to study osteogenic differentiation of mesenchymal stem cells. They were cultured in DMEM (Life Technologies, Carlsbad, California) supplemented with 10% fetal bovine serum (FBS; Life Technologies, Carlsbad, California) and 1% PS. The medium was changed every two days. The cells were incubated at 37 °C, 5% CO$_2$ in a humidified incubator.

Biological experiments were performed using flat nonporous scaffolds to assess the impact of the composition only and for practical reason of image analysis of nontransparent samples (details are provided in Supplementary Material 2). After manufacturing, disc samples were immersed in a 70% ethanol solution for 20 min, rinsed in PBS 3 times, and dried overnight. Cell seeding was performed in 24-well culture plates by depositing cells suspended in media on the surface of the disc with a concentration of $0.8 \times 10^4$ cells/cm$^2$, incubating them for 20 min before filling the well with 1 mL of media. Cell proliferation and osteogenic differentiation were both assessed at days 1, 7, and 11. Proliferation was evaluated by moving the scaffolds to new wells, detaching the cells from the scaffold using 0.05% trypsin (Life Technologies, Carlsbad, California), suspending them in media, and counting them using a Z2 particle counter (Beckman Coulter, Brea, California). For cell differentiation, ALP activity of cells was assessed through semiquantitative staining. Alkaline phosphatase kit (Sigma-Aldrich, St. Louis, Missouri) was used and staining was performed following the manufacturer’s instructions. At designated time-points, cells were fixed for 1 min in 3.7% formaldehyde and the samples were incubated for 1 h with ALP stain. After staining, the scaffolds were imaged and the ALP levels were quantified using image processing performed with Matlab R2013 (MathWorks, Natick, Massachusetts). Briefly, color features of the images of the scaffolds were extracted, quantified, and the pixel values were averaged over the entire surface of the scaffold. For each composition, the obtained values at days 7 and 11 were normalized using the average value at day 1.

F. Porosity measurement

To assess the actual porosity of each scaffold, they were imaged using a micro-CT imaging device eXplore CT120 (TriFox Imaging, Chatsworth, California). Reconstruction was performed using MicroView software (Parallax Innovations, Ilderton, Canada). Each composition was processed independently because of their different sensitivities to X-ray imaging. For each, a threshold value was identified using the automatic tool provided by the software. Using this value, the volume of each individual sample was computed. To get the porosity $p$, the construct volume $V_c$ was compared to...
the overall volume of the cylinder $V_t$, using the following equation:

$$p = 1 - \left(\frac{V_c}{V_t}\right).$$

**G. Mechanical analysis**

First, the mechanical properties of the bulk materials were evaluated to examine the properties of the material independently of the manufacturing process. For this purpose, 5 cm pieces of filament were directly used as specimens. Tensile testing was performed using an Instron 5944 uniaxial testing system with a 2 kN load-cell (Instron Corporation, Norwood, Massachusetts). A preload of 1 N was applied, at a speed of 1% strain/s until 25% strain. The values of the Young’s modulus as well as the tensile strength at zero slope were extracted, the first one being the value of the initial slope of the stress–strain curve, the second being the ordinate of the zero slope point of the curve. Five specimens were tested per material composition.

Then, 3D-printed porous scaffolds were mechanically tested in compression using the same instrument, following guidelines adapted from Huang et al. A preload of 1 N was applied, and tests were performed at a speed of 1% strain/s until 25% strain. Five porosities were independently tested for each composition to study the influence of both the amount of β-TCP and the porosity on the mechanical properties. Five specimens were tested for each group, and for each specimen, the apparent Young’s modulus and the yield strength at 1% were measured. The first one was identified as the slope of the initial linear portion of the stress–strain curve. The second one corresponds to the ordinate of the intersection between the stress–strain curve and a line with a slope equal to the Young’s modulus starting at an offset of 1% strain.

**H. Manufacturing of a multimaterial 3D-printed implant**

To 3D print a single piece construct consisting of several materials, an algorithm was developed. Knowing the layout of the construct and the position of each material in the construct’s bulk volume, the corresponding length of the filament for each volume required was computed. The filament pieces were then manually fused in the order in which the printing would complete each separate section to form a single multimaterial construct. The recomposed filament was then used by the printer to manufacture the multimaterial construct in a single iteration, with each major section of the construct utilizing a different material. To prove the feasibility of multimaterial constructs, a novel implant for disc reconstruction was proposed. The design as well as a prototype is detailed in Sec. III.

**I. Statistical analysis**

Data are presented using mean ± standard deviation. Statistical analyses were performed using the $t$-test method when two groups were involved, and one-way analysis of variance with ad hoc Tukey’s test for three or more groups. Differences were considered significant for $P < 0.05$, as labeled in figures by the * symbol. Analyses were performed using Matlab R2013 software (MathWorks).

**III. RESULTS AND DISCUSSION**

**A. Material composition**

Using the TGA curves presented in Fig. 1(a), the ratios of β-TCP in the samples were quantified as the remaining mass at 550 °C. The values are 2.62%, 21.06%, 41.55%, and 59.31% for theoretical compositions of 0%, 20%, 40%, and 60% β-TCP, respectively, which represents relative errors of 2.62%, 1.06%, 1.55%, and 0.69%.

![FIG. 1. TGA of PCL/β-TCP composites with a theoretical ceramic composition of 0, 20, 40, and 60%. (a) Variation of mass loss according to temperature. (b) Differential TGA.](https://www.cambridge.org/core)
respectively. This disparity can either be the result of impurities in PCL and/or experimental errors. This analysis validates the material composition after synthesis, and therefore confirms that the solvent method used in this study efficiently mixes PCL and β-TCP with a ratio up to 60% ceramic. For future reference, higher amounts of β-TCP were attempted, but the synthesis failed as the amount of PCL was too low to bind such a large amount of ceramic, resulting in a collapsing composite after precipitation.

Figure 1(b) presents the differential thermogravimetric curves, which are the first derivative of the TGA curves, providing further information on material thermal degradation. The abscissa of the larger peak for each composition are 378.3 °C, 398.3 °C, 402.1 °C, and 409.2 °C, respectively for 0%, 20%, 40%, and 60% of β-TCP.
showing a slight increase in thermal stability due to the addition of β-TCP, which corroborates the results presented by Huang et al.\textsuperscript{14}

Filament diameters following extrusion were also measured, averaging 2.75 mm ± 0.10, 2.43 mm ± 0.08, 2.45 mm ± 0.03, and 2.66 mm ± 0.04, respectively, for β-TCP ratios of 0%, 20%, 40%, and 60%.

**B. Composition influence**

1. Surface characterization

Contact angle tests were first performed on post-processed scaffolds to quantify the influence of the composition alone. As shown in Fig. 2(a), all values are under 90 °C (between 70° and 80°), indicating that the materials tend to be hydrophilic. Moreover, a small decrease in contact angle is shown when the amount of ceramic is increasing, with statistical significance between 0, 20, and 60%. Figure 2(b) presents the results of similar tests on scaffolds without post-processing, to assess both the influence of the composition and the manufacturing process on the contact angle. Contrary to the previous test, no significant difference is noted between the different groups although their chemical compositions are different. Similar results are demonstrated in Ref. 25, where pure PCL presents a contact angle of 75°, while the addition of β-TCP did not significantly affect the contact angle. These two tests show that the surface morphology resulting from FDM 3D printing has a non-negligible impact on surface hydrophilicity. This morphology is the result of the layer formation strut by strut and is inherent to FDM 3D printing technology.

Surface morphology was observed using SEM imaging, presented in Figs. 2(c)–2(f). Cross-sectional images are also introduced in Fig. 3. At every scale, imaging shows a relatively smooth surface for pure PCL, and an increase in surface roughness for higher β-TCP ratios. At low magnification (×150), the morphology is the result of two phenomena. First, the junction between struts of a single layer can be observed, resulting in linear ridges on the surface, as indicated in Figs. 2(c)–2(f)(I). This is the result of the strut by strut deposition of FDM 3D printing processes. Although careful calibration can attenuate it, it is related to the inherent variability of the process and therefore cannot be entirely removed. Second, the addition of ceramic nanoscopic powder is introducing bumps at the surface. At this scale, they are the results of aggregates of ceramic particles that assemble together because of the high surface tension of nanoparticles and the separation of the hydrophilic ceramic particles from the PCL matrix. Although both components of the composite were put in solution separately and thorough mixing was carried out, interaction between particles was evidently stronger. At 1000× magnification, these aggregates can be better observed [Figs. 2(c)–2(f)(II)]. Because of the extrusion process, they are usually under a thin layer of polymer, sometimes even creating holes in the surface of the material. Consequently, the higher the amount of ceramic in the composite, the rougher the surface will be. At high magnification (10,000×), the ceramic particles can be distinguished, their average size being 100 nm [Figs. 2(c)–2(f)(III)]. Some of them are apparent, but judging by the texture of the surface, not all of them seem to be exposed, and similar to aggregates, some of them are covered with a thin layer of polymer.

Surface roughness measurements $R\textsubscript{a}$ are introduced in Fig. 2(g), with values of 178.3 ± 67.6 nm, 645.7 ± 84.7 nm, 1193.6 ± 97.6 nm, and 1837.6 ± 317.6 nm, respectively, for β-TCP ratios of 0%, 20%, 40%, and 60%. A significant increase of $R\textsubscript{a}$ is observed for increasing amount of ceramic, confirming the observations of the SEM images.

2. Degradation

Significant differences in degradation rates under accelerated conditions were observed between the different materials depending on the ratio of ceramic to polymer utilized (Fig. 3). Quantifiable mass loss in the two materials with higher ceramic content (60 and 40%) commenced within 24 h, with the 60% filament experiencing over 50% mass loss within 10 h. Lower ceramic content filament (20%) showed <5% mass loss after the total 54 h period of the trial, and all samples of pure PCL filament experienced <1% mass loss over the same duration.

These results are consistent with results presented in similar studies. Indeed, polyester degradation under alkaline conditions is a well-known accelerated

![FIG. 3. Accelerated degradation of the PCL/β-TCP material under alkaline conditions for ceramic composition of 0, 20, 40, and 60%.](https://doi.org/10.1557/jmr.2018.112)
It is thought that polyester degradation under these conditions mimics typical degradation under aqueous conditions where ester–ester linkages are hydrolytically severed, breaking apart the bulk material. The presence of additional –OH ions from alkaline solution catalyzes this process, speeding up a degradation process that can require 3–4 years in vivo. Lei et al. suggested that the addition of β-TCP speeds up the degradation because β-TCP particles are only physically mixed in the composite, and submersion in the alkaline media frees the β-TCP particles to convert into its more thermodynamically favorable form of apatite in solution. The void left by dissolving β-TCP particles additionally increases the available surface area for the aforementioned hydrolytic attack on ester–ester linkages, while also opening up more regions of β-TCP to be freed. Visually, it was noted that solutions further along in the degradation process possessed a white powder-like substance that precipitated along the bottom of the vials used for degradation; these are theorized to be the aforementioned released β-TCP particles. Higher ceramic content filaments thus experienced accelerated rates of degradation due to the presence of more β-TCP particles, allowing for greater amounts of the ceramic to be released and quickening the rate at which the filaments lost structural integrity. As such, it is worth noting that the extremely high degradation rates of the higher ceramic content (40 and 60%) is a result of disassembly of the composite, i.e., loss of structural integrity, in combination with our assay method of mass measurement of the samples, but not the dissolution/disappearance of the ceramic particles themselves. However, this result does offer an approach via manipulation of the ceramic ratio to control degradation rates and further enables the creation of a bioresorbable bone implant that can ideally be designed to match the natural variations found in bone healing rates.

3. Mechanical properties of the bulk material

Tensile tests were performed on a filament-shaped material of different compositions to assess the bulk mechanical properties, i.e., the properties of the material before being affected by the manufacturing process [Fig. 4(a)]. Both Young’s modulus and yield strength are introduced in Figs. 4(b) and 4(c). Young’s modulus average values were 264 MPa, 355 MPa, 495 MPa, and 1140 MPa, respectively, for 0%, 20%, 40%, and 60% of β-TCP content and yield strength average values were 14.2 MPa, 12.4 MPa, 10.74 MPa, and 10.29 MPa for the same β-TCP ratios. Young’s modulus quantifies the material stiffness and increases with the amount of ceramic in the composite, with statistical significance between all the groups. The increase seems to be linear up to 40% and displays a larger increase between 40 and 60%. The yield strength decreased compared to the amount of ceramic in the composite. It represents the maximum stress that can be applied to the material without permanent deformation. As a result, elasticity of the PCL/β-TCP composite reduces with higher quantity of ceramic.

Theoretical models have been developed to estimate the Young’s modulus of particulate-filled systems. For spherical particles added in a polymeric phase, the simplest model equation has been identified by Einstein. Under certain hypotheses (low ratio of particles, perfect adhesion between the two phases of the composite, and particles much more rigid than the matrix), a linear dependency can be highlighted between the Young’s modulus of the composite and the Young’s modulus of the polymer, according to the volume ratio of particles. For large filler concentration, a more complex model has been developed by Kerner, which under the same hypotheses predicts considerably more stiffening action of the filler compared to Einstein’s model for higher particle concentration. Both these models support the results presented in Figs. 4(b) and 4(c).
4. Cell proliferation and differentiation

Figure 5 shows the proliferation results of mouse fibroblasts (C3H10 T1/2) at days 1, 7, and 11. At day 1, no significant difference was noted between groups, highlighting the fact that scaffold composition had no influence on cell attachment. Over the three time-points, the number of cells increased for all groups. Starting at day 7, the number of cells on the sample containing β-TCP became significantly higher than the number of cells on pure PCL scaffolds (about 50% more on average at day 11). Although it may seem that the number of cells slightly increased with the ceramic ratio at day 11, no statistical difference was shown over the different β-TCP ratios. The proliferation study indicated that addition of ceramic improves cell proliferation compared to pure PCL. One possible explanation is the variation in surface morphology reported previously since it has been shown that cells proliferate better on rougher surfaces, as rougher surfaces provide larger surface area for cell growth.11

Figure 6(b) represents the relative ALP activity of the different groups for days 1, 7, and 11, and examples of scaffolds at day 11 after staining are introduced in Fig. 6(a). For composition of 0 and 20% β-TCP, no significant increase was shown over time, while 40 and 60% ratios indicated a significant increase of activity at day 11 compared to days 1 and 7, with respective increases of 5 and 10%. Moreover, the ALP activity seems to increase with the amount of β-TCP in the scaffold, with significant differences between 0% and 40%, 60%, and between 20% and 60% [see Fig. 6(b)]. These results highlight the impact of the addition of β-TCP on C3H10 osteogenic differentiation, and corresponding results can be found in the literature. Shin et al. demonstrated a higher ALP activity for human mesenchymal stem cells cultured on PCL/β-TCP compared to PCL only, pointing out a potential impact of the exposed β-TCP particles at the surface of the scaffold.21 Similar results were shown by Polini et al., where the addition of β-TCP in PCL nanofibers improved stem cell differentiation.25

The biological studies highlight the osteoconductive property of β-TCP and are in accordance with other studies on the impact of calcium phosphate-based materials (β-TCP or hydroxyapatite) for both proliferation and differentiation of stem cells.15,25 It is not clear, however, if this impact is the result of differences in chemical surface properties or of differences in physical properties of the surface (hydrophobicity, roughness, and stiffness). Although we tried to isolate the influence of the composition only, it is inherently linked to the physical properties through the manufacturing process. Post-processing techniques could be considered to modify physical properties and decrease the physical properties’ variability for more accurate assessment in the future.

C. Effect of composition and porosity on mechanical properties

Characterization of the influence of porosity and composition on the mechanical properties of 3D constructs was performed in a systematic manner. 20 different groups were manufactured using FDM [Fig. 7(a)] with \( n = 5 \) for each group. The average values of porosity for each group are presented in Fig. 7(d). As expected, porosity was mostly guided by the distance between each strut in the scaffold, and very low variation is noted between the β-TCP ratios. After being tested under compression, apparent Young’s modulus and yield strength were computed for each group, as presented in Figs. 7(b) and 7(c). These values, respectively, ranged from 12 MPa (β-TCP ratio: 60%, strut...
distance: 2.5 mm) to 188 MPa (β-TCP ratio: 60%, strut distance: 0.4 mm), and 0.7 MPa (β-TCP ratio: 0%, strut distance: 2.5 mm) to 15.4 MPa (β-TCP ratio: 0%, strut distance: 0.4 mm). In comparison, Gibson tested cancellous bone from varying regions of the body and demonstrated Young’s modulus values that varied between an order of magnitude of 10 and 10² MPa, a range is similar to the Young’s modulus range demonstrated in Fig. 7(b). This study could therefore be used to design and tailor constructs specific to the type of bone considered in the application, improving its biomimicry, and hypothetically enhancing bone regeneration rates.

To assess the extent of the influence of the composition versus the porosity, each mechanical parameter was graphically related to the porosity in Figs. 7(e) and 7(f). For the apparent Young’s modulus, a linear trend can be identified, the slope being steeper with the amount of β-TCP. As a result, the composition of the construct has very little influence on its apparent Young’s modulus for high porosity. For lower porosity, an increasing amount of ceramic results in higher Young’s modulus. Comparing the values of the lowest porosity (close to 0%) to the results of the tensile tests performed on the same materials in a bulk form, a large discrepancy can be noted, especially for higher amounts of ceramic, which highlights the important influence of the FDM process and the geometry of the construct. Regarding yield strength [Fig. 7(f)], the curve of each composition overlays, underscoring the fact that β-TCP amount has very little influence and that the yield strength of a 3D construct is guided by the layout of its layers. Indeed, during compression testing, yield is the result of a collapse of the construct when loaded, which makes the geometrical layout of the construct of higher impact compared to the composition.

Interestingly, a similar apparent Young’s modulus value can be achieved by constructs with very distinct design parameters. For instance, scaffolds with 15% porosity/0% β-TCP and 45% porosity/60% β-TCP will both have an apparent Young’s modulus of about 100 MPa. This overlap results in more freedom regarding construct design and allows for researchers to take other parameters into consideration other than solely the mechanical performances since these two vastly different compositions can result in the same mechanical values. For this purpose, the results presented in this section and the rest of the paper can act as guidelines and assist in the design process.

D. Multimaterial implant for disc reconstruction: Proof of concept

Using the results previously presented, and to prove the feasibility of manufacturing multimaterial constructs...
3D printed in a single piece, a novel design for disc reconstruction is presented. Disc implants aim at replacing a collapsing disc between two vertebrae. Typically, they are composed of four assembled parts: two inner elements sliding between each other to allow relative movement between the vertebrae, placed in between two metallic endplates connecting the implant to each vertebra. An alternative design is introduced in Fig. 8(a), using the data collected in this study. For high stiffness and better osteoconduction, the two endplates consist of 45% porous material with a high amount of b-TCP. They are connected through a section of highly porous (70–75%) pure PCL designed to mimic biological cartilage stiffness and elasticity. Being 3D printed in a single piece, no assembly is required and the construct can be patient specific. A prototype is pictured in Fig. 8(b), validating the feasibility of single piece 3D-printed constructs integrating multiple porosity and multiple composites.

**IV. CONCLUSION**

In this paper, we systematically studied the influence of the composition and porosity of FDM 3D-printed PCL/b-TCP scaffolds on properties that are of interest for bone tissue engineering applications, namely surface morphology and hydrophilicity, degradation, impact on cell behavior, and mechanical performances. We first considered the influence of the composition alone, and then the influence of both parameters. We
demonstrated that the composition affects surface properties, degradation rates, and mechanical performance, and as a result, impacts cell attachment, proliferation, and osteogenic differentiation. When combined with the porosity for 3D constructs, a synergistic effect of both parameters can be highlighted on the mechanical performances. However, based on this study, no optimal composition or porosity can be identified, and both parameters should be selected regarding the application. In this regard, the results of this study can provide guidance during the design process. As a proof of concept, we finally designed a construct for intervertebral disc replacement that integrates multiple compositions and porosities. Because FDM 3D printing is used, its manufacturing is possible in a single piece, the feasibility being illustrated with a prototype.

In the end, this study enlarges the scope of FDM 3D printing for bone tissue engineering applications, by providing guidance for the design of PCL/β-TCP constructs and proving the feasibility of single piece constructs integrating multiple porosities and composite compositions. It potentially leads the way toward the development of novel designs, for instance implants specific not only to the patient but also to the type of bone.

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**Supplementary Material**

To view supplementary material for this article, please visit https://doi.org/10.1557/jmr.2018.112.