Photo-Curing 3D Printing and Innovative Design of Porous Composite Structures for Biomedical Applications

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Light-activated resins and composites are used in conjunction with a light curing unit and allow an on-demand process of polymerization. These kinds of materials usually represent the most popular choice in the restorative dental practice. Some works have already highlighted contemporary tendencies in the use of nondegradable scaffolds and mesenchymal stem cells in regenerative medicine. Accordingly, the aim of the current research is to develop 3D porous and light-activated composite structures with optimized functional properties. Preliminary mechanical and biological tests are carried out.

1. Introduction

Tissue engineering and stem cell-based therapies represent the most challenging fields in regenerative medicine.[1–4]

Tissue engineering approach is characterized by the synergistic combination of cells and suitable 3D structures (i.e., scaffolds), which can act as functionally supportive biomolecules.

A scaffold may be considered as an interconnected porous structure, which is able to support cell adhesion, proliferation, and differentiation, also promoting the extracellular matrix analogue deposition necessary for tissue regeneration.[4–7]

Synthetic [i.e., poly(ϵ-caprolactone)] and natural polymers (i.e., chitosan, collagen, alginate, agarose, hyaluronic acid, and fibrin) have been largely proposed to manufacture scaffolds for hard and soft tissue regeneration.[4–7]

In this scenario, mechanical features are crucial, as hard tissues (i.e., bone) are stronger (higher strength) and stiffer (higher elastic modulus) in comparison to soft tissues.[4,7]

Composite devices consisting of polymers reinforced with inorganic nanoparticles were developed.[4,7–9] The inclusion of nanofillers in the polymeric matrix up to a threshold concentration generally provides an enhancement of the mechanical properties.[7–9]

On the other hand, light-activated resins and composites are employed in conjunction with a light curing unit, thus allowing an on-demand process of polymerization.

They generally represent the most popular choice in the restorative dental practice. Over the past years, the use of non-degradable scaffolds and mesenchymal stem cells has also been proposed in regenerative medicine.[10]

With regard to the repair, regeneration, and reconstruction of damaged tissues, 3D porous structures have been developed as biodegradable and non-biodegradable scaffolds. In the latter case, the designed pore geometry and porosity can allow to modulate the structure stiffness and to promote tissue ingrowth, thus stabilizing the implanted device.[1–3,10]

The advances in methodologies of analysis[11–14] and design strategies[4,14–16] have pushed the research towards the development of novel materials and devices for different applications. Although over the past years great efforts have been made in engineering biomedical devices, with a special focus on several materials,[4,7,17–19] as well as on experimental[13,7–9] and theoretical[14–16] analyses, in the current research design, strategies and photo-curing 3D printing were employed to fabricate 3D porous composite structures with optimized functional properties. The mechanical and biological performances of the manufactured devices were preliminarily evaluated.

2. Experimental Section

An additive manufacturing technique based on injection/extrusion methods (i.e., 3D fiber deposition/fused deposition modeling) was integrated with a blue light-emitting diode (LED) curing unit. Specifically, a three-axis computer-aided design (CAD)/computer-aided manufacturing (CAM) system was implemented to manufacture 3D light activated composite devices with controlled architectures, pore geometry, and porosity for biomedical applications. A precise spatial positioning

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of the light curing unit was obtained using the three-axis CAD/CAM system.

The material consisted of an organic matrix and a reinforcement. The polymeric matrix was mainly based on bisphenol A glycidyl methacrylate (Bis-GMA), ethoxylated bisphenol A dimethacrylate (EBPADMA) and TEGMA. In the hybrid composite material, three different kinds of fillers (72.5% by weight) were combined: barium glass, prepolymerized particles, and silica nanoparticles. The initiator system was camphorquinone-amine.

The material was injected/extruded through a nozzle with an inner diameter of 800 µm at room temperature. The composite fibers/filaments were deposited according to the selected lay-down pattern (i.e., 0/90°). A pressure of 2.0 bar and a deposition speed of 250–300 mm/min were employed. Different values of filament distance (i.e., center-to-center distance, 1400–1600 µm) and slice thickness (600–640 µm) were considered.

Three-point bending tests were carried out using an INSTRON 5566 testing machine (Norwood, MA, USA). Each specimen was tested in flexure as a beam supported at two points and loaded at the midpoint. Specimens were kept in dark environment before performing three-point bending tests. All the tests were carried out using a support span (L) of 20 mm and a crosshead speed (i.e., the rate of crosshead motion) of 1 mm min^{-1}. Thus, the maximum stress in the outer surface of the specimen occurred at the midpoint. The stress-strain curves were evaluated from the load-deflection curves. The "apparent" stress (σ) and strain (ϵ) were calculated according to the following equations:

$$\sigma = \frac{3FL}{2bd^2} \tag{1}$$

$$\varepsilon = \frac{6Dd}{L^2} \tag{2}$$

where F, D, b, and d represent the value of the load at a given point on the load-deflection curve, the maximum deflection of the center of the specimen, the width and the depth of the specimen, respectively. The slope of the initial linear portion of the stress-strain curve was considered to determine the flexural modulus.

Cell viability and proliferation were analyzed at different time points using the Alamar Blue assay (AbD Serotec Ltd, UK) and the results were reported as a percentage of Alamar Blue reduction.

In brief, the porous structures were prepared for cell seeding following a reported protocol.[7,8] They were seeded with bone marrow-derived human mesenchymal stem cells (hMSCs) using 1×10^4 cells/sample.

The experiments were done at least three times in triplicate. The data obtained at different time points were analyzed by ANOVA followed by Bonferroni post hoc test. A value of $p < 0.05$ was considered statistically significant.

3. Results and Discussion

Design strategies together with the structure-property relationship play a fundamental role in developing advanced materials.[7,8] As frequently reported, the technology process influences the material structure and properties as well as the final application.[7,8]

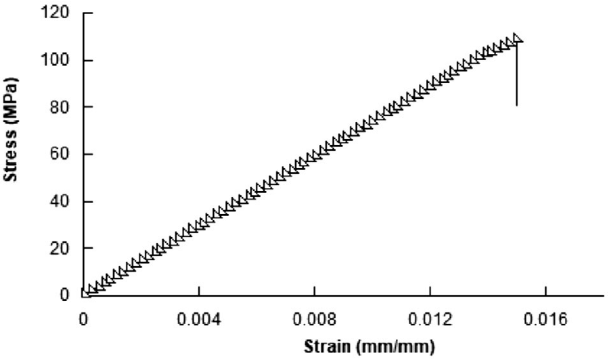

Figure 1. Results from three-point bending tests: typical stress-strain curve obtained for 3D porous structures (filament distance of 1600 µm, slice thickness of 600 µm) manufactured by photo-curing 3D printing.

Table 1. Results from three-point bending tests performed on 3D porous structures (filament distance of 1600 µm, slice thickness of 600 µm) manufactured by photo-curing 3D printing: modulus (E) and maximum stress (σ_{max}). The results are reported as mean value ± standard deviation.

E (GPa)	σ_{max} (MPa)
7.4 ± 0.8	109.7 ± 10.1

Three-point bending tests can be considered one of the most popular test methods to evaluate the mechanical properties and, in particular, the elastic modulus.

Figure 1 reports a typical stress-strain curve obtained from three-point bending tests.

The maximum flexural stress sustained by the test specimen during the bending tests (i.e., the flexural strength) was measured, whereas the bending modulus was evaluated from the slope of the linear region of the stress-strain curve.

Typical values of bending modulus (E) and maximum stress (σ_{max}) are reported in **Table 1**.

On the other hand, in vitro biological tests were performed to evaluate the behavior of hMSCs. **Figure 2** reports the results as percentage of Alamar Blue reduction over time.

The values of Alamar Blue reduction significantly increased ($p < 0.05$) over the investigated time period (from 1 to 7 days), thus indicating cells survival and proliferation.

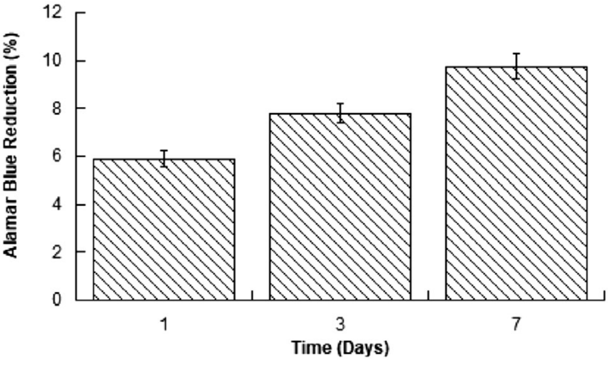

Figure 2. Results from in vitro biological tests: percentage of Alamar Blue reduction evaluated for the cell-laden constructs. Data are reported as mean value and error bar represents the standard deviation.

4. Conclusion

Within the limitations of the current research, the following conclusions were reached:

1) A systematic study on photo-curing 3D printing and a novel design of porous composite structures were reported. In particular, an additive manufacturing technique based on injection/extrusion methods was integrated with a light curing unit.
2) The reported strategy also aimed at suggesting the potential to tailor the performances of the additively manufactured structures through the material-design combination.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

composite structure design, computer-aided design, design for photo-curing 3D printing, mechanical and functional properties

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