3D printed bone tissue-mimicking constructs as in vitro preclinical testing platform

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INTRODUCTION

To reduce the need of revision surgeries of bone implants ascribed to loosening phenomena and implant instability, functional coatings are under investigation, requiring standard/reproducible procedures for their preclinical evaluation in a physiological-mimicking environment. In vitro bone tissue models represent a powerful tool for predictive in vitro screening, resulting in a reduction of animal experimentation in agreement with the 3Rs principle. 3D bioprinting represents a promising technique for the fabrication of 3D cellularized in vitro models. Indeed, besides its environmental-friendly approach (i.e., no solvents required), 3D bioprinting opens the way to the extrusion of constructs with shape and format properly engineered to fulfil the needs required by the specific testing set-up. In this perspective, this work was aimed at developing a 3D-printed bone tissue-like models through micro-extrusion additive manufacturing of scaffolds from a customized bio-ink. An in-depth investigation of construct morphology and printing parameters was conducted to develop scaffolds with optimal contact area with the surface of prosthesis prototypes.

MATERIALS AND METHODS

Gelatin methacryloyl (GelMA) was synthesized according to [1]. Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectroscopies were used to assess synthesis success. Then, the bio-ink was developed by combining the polymeric GelMA (7%w/V) component and the inorganic rod-like nano-hydroxyapatite (nHA, 3%w/V) to mimic the bone mineral content. Bio-ink responsiveness to temperature and light irradiation (UV, 365nm) was assessed through thermo- and photo-rheology. Different CAD models were developed using Rhinoceros and converted in g.codes through Slic3r. Lastly, NIH-3T3 murine fibroblasts were preliminarily loaded in the bio-ink. Cell dispersion and viability/proliferation after extrusion were assessed through commercial assays.

RESULTS AND DISCUSSION

IR and NMR spectra proved the successful GelMA synthesis with a 99% degree of methacryloylation. Thermo-rheology evidenced that the addition of nHA to GelMA solution did not alter bio-ink thermo-responsiveness as demonstrated by unchanged gelation onset temperature (i.e., 24°C) and kinetics. Differently, differences in the storage modulus before and after irradiation at 365nm (ΔG') showed remarkably higher values for GelMA/nHA compared to GelMA (i.e., ΔG'=5.3kPa vs. 3.7kPa), suggesting the role of nanoparticles as reinforcement filler. Subsequently, by exploiting ink thermo-responsiveness and photo-crosslinking ability, GelMA/nHA formulations were micro-extruded in mild conditions into square meshed structures with high shape fidelity to identify the optimal printing window (Fig. 1). Then, printed geometry was refined to morphologically reproduce the cortical/cancellous bone tissues and to maximize cell response (Fig. 2). 3D scaffolds (i.e., 25 layers in cylinder-shape constructs with 400 μm pore size) were mechanically characterized showing adequate properties for bone tissue engineering/modelling. No cell sedimentation phenomena as well as damages induced by shear stresses during scaffold fabrication were observed after scaffold extrusion. Furthermore, strong proliferation capability within GelMA/nHA matrices was observed up to 7 days of culture. Lastly, the system was validated as in vitro testing platform by testing the cytocompatibility of ultra-high molecular weight poly(ethylene) and 0.1% ZDEC polyurethane films, as negative and positive control, respectively. Results were in accordance with 2D in vitro tests.

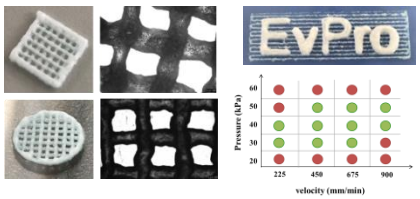

Figure 1. Representative images of bi-layer structures of different shapes, project LOGO and printability window.

Figure 2. Optimized 3D model: CAD, slicing,3D printed structure and magnification of cell distribution.

CONCLUSIONS

This work evidenced promising results towards the extrusion of GelMA-based 3D constructs as platforms for in vitro preclinical screening.

REFERENCES

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