P008 Time-dependent 3D printing of chemically crosslinked gelatin hydrogels

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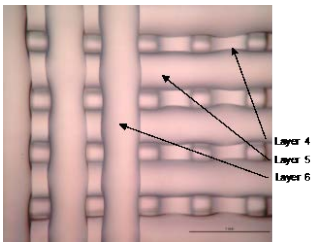
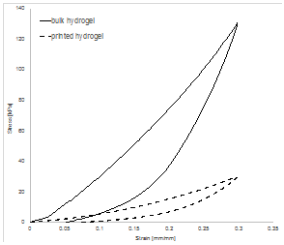
Hydrogels 3D printing has recently gained tremendous interest in tissue engineering thanks to the unique possibility of printing patient-customized scaffolds with controlled porosity, pore size and pore distribution. The successful 3D printing of a hydrogel is strictly related to the chosen printing strategy since it affects the accuracy of the printed material and the printed scaffold chemical, physical and mechanical properties.

In this study, we propose an innovative time-dependent 3D printing strategy of a chemically crosslinked gelatin hydrogel, printed by tuning the 3D printing process with the crosslinking kinetic of the hydrogel, without the need of post-curing or external treatments.

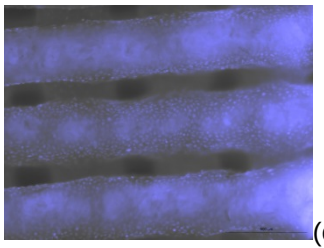
The hydrogel was prepared by a patented [1] reaction based on a Michael type addition, by mixing type A gelatin from porcine skin with *N,N'*-methylenebis(acrylamide) (MBA) as crosslinker. The 3D printing was performed by loading the gelatin/MBA reaction solution in the printer pneumatic dispensing cartridge, kept at 35 °C, and by printing on a glass substrate, kept at 4 °C to allow the shape retain of the printed scaffold. After the printing, to simultaneously promote the deposited filaments shape maintenance at low temperature (i.e., $T < T_{gelation}$) while allowing the crosslinking reaction to complete, scaffolds were sealed in petri dishes and kept at 20 °C for 48 h.



(a)



(b)



(c)

The rheological properties of the hydrogel during the crosslinking reaction allow to determine that the hydrogel is printable until 60 min after the gelatin/MBA mixing (printability time window at G'' > G', constant viscosity value). Printing parameters were optimized by using a 25 G needle and by setting 200 μm as layer thickness; 2 x 2 cm scaffolds were printed by 0 – 90° layer-by-layer deposition; filaments with 500 μm diameter were obtained and structures with more than 8 layers were successfully printed, with a final scaffold thickness > 1 mm (Fig.a). Printed hydrogels swollen in distilled water at 37 °C showed to be stable for more than 2 weeks, confirming the effective crosslinking. Mechanical compressive properties (Fig.b) were lower for the printed scaffolds compared to compact hydrogels, due to the porous structure and higher water content, suitable for soft tissue regeneration. Preliminary *in vitro* tests were performed on lyophilized printed scaffolds by dynamically seeding them in a L929 cell suspension, achieving a uniform distribution of cells (Fig.c). These preliminary results exhibit the success and efficiency of the printed strategy adopted, showing good printing shape fidelity and cell distribution.

[1] M.C. Tanzi, S. Farè, I. Gerges, PCT/EP2012/060277