Thiolated Crosslinker effect on Physico-Chemical properties and Laser-Based Processing of Gelatin Thiol-ene Hydrogels.

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Gelatin hydrogels have been frequently applied in the fields of biofabrication and regenerative medicine, due to their close resemblance to the natural extracellular matrix (ECM) since it is derived from collagen, the main constituent of the ECM. As a result, it exhibits favourable cell-interactivity due to the presence of arginylglycyl aspartic acid (RGD) sequences along its backbone. Furthermore, it is biodegradable, low-cost and FDA-approved with a long track record in the food and pharmaceutical industry. Despite all these benefits towards biofabrication, the material also exhibits an upper critical solution temperature (around 30°C) resulting in dissolution at physiological conditions. To overcome this limitation, the material can be modified chemically to introduce photo-crosslinkable functionalities resulting in a covalent network upon UV irradiation in the presence of a photoinitiator.

In this respect, the most frequently reported modification is the introduction of methacrylamides by reaction of the primary amines in gelatin with methacrylic anhydride1,3,4. These methacrylamides can subsequently be polymerized using UV-irradiation following a radically induced chain growth polymerization mechanism. Although this material has a proven track record in the field of tissue engineering, throughout the past decade, the interest has shifted more towards thiol-ene photoclick crosslinking chemistry for multifunctional, thiolated crosslinkers with different molecular weights and thiol functionalities including dithiolothreitol (DTT), tetraethylene glycol dithiol (TEG2SH), polyethylene glycol dithiol (Mn 3400 g/mol, PEG2SH 3400), 4-arm polyethylene glycol tetrahedral (Mn 10000 g/mol, PEG4SH 10000), 4-arm polyethylene glycol tetrahedral (Mn 20000 g/mol, PEG4SH 20000) and thiolated gelatin with a degree of substitution of 72% prepared following a previously reported protocol5.

The influence of these different crosslinkers was assessed in terms of mechanical properties, swelling degree, network density and crosslinking kinetics using photorheological measurements. Furthermore, the cytotoxicity of the different crosslinkers was assessed using adipose tissue-derived stem cells, as well as preliminary cell encapsulation experiments were performed.

Finally, the 2PP structuring range and concomitant swelling behaviour of the materials was assessed as a function of required laser power at a constant scanning speed (100 mm/s). The observed swelling degree of these microstructures provides insight with respect to degree of conversion.

References:


Keywords:
Gelatin, Thiol-ene Photoclick, Two-photon Polymerization, Rheology, Adipose-derived stem cells