

Free Session

Biomaterials for tissue engineering applications

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Tunable Thiol/Ene Gelatin Hydrogels via Crosslinker Variation To Optimize Laser-Based Biofabrication

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Symposium: Advanced bioinks for biofabrication and tissue regeneration: from lab printing to patient

Introduction: Photo-crosslinkable gelatin hydrogels have proven to be very suitable bioink or biomaterial ink components for biofabrication applications^[1]. Especially the widely investigated gelatin-methacryloyl (gel-MA) holds an impressive track record^[2]. However, currently, increasing attention is being paid towards thiol-ene photo-click chemistry to obtain hydrogel networks benefitting from a higher reactivity, superior biocompatibility and processability while providing an additional handle to tune the hydrogel properties. In order to employ this chemistry, often an ene-functionality is introduced onto gelatin which is crosslinked in the presence of a thiolated crosslinker^[1]. However, to date, limited research has been performed on the influence of the applied crosslinker on the hydrogel properties.

Experimental methods: To apply this chemistry to gelatin, its primary amines were functionalized with 5-norbornene-2-carboxylic acid via conventional carbodiimide coupling chemistry yielding gel-NB with a degree of substitution of 89%^[3]. These hydrogel precursors were photo-crosslinked using 2 mol% LAP or Irgacure 2959 in an equimolar thiol-ene ratio using a range of multifunctional, thiolated crosslinkers including dithiotreitol (DTT), tetraethylene glycol dithiol (TEG2SH), polyethylene glycol dithiol (Mn 3400 g/mol, PEG2SH 3400), 4-arm polyethylene glycol tetrathiol (Mn 10000 g/mol, PEG4SH 10000), 4-arm polyethylene glycol tetrathiol (Mn 20000 g/mol, PEG4SH 20000) and thiolated gelatin with a degree of substitution of 72% prepared following a previously reported protocol^[4]. The influence of the different crosslinkers was assessed in depth using photo-rheology, gravimetric swelling measurements, laser-based processing (using 2 mol% P2CK as photoinitiator^[5]) and cell encapsulation experiments using gel-MA as benchmark.

Results and discussions: The high molecular weight, multifunctional crosslinkers resulted in hydrogels which exhibit superior mechanical properties, along with a lower swelling degree. However, in general, the thiol-ene systems exhibit higher swelling ratios in comparison to the gel-MA hydrogels. Furthermore, the cytotoxicity of the different crosslinkers was assessed using an immortalized human adipose-derived stem cell line. Additionally, cell encapsulation experiments were performed using the different hydrogel formulations indicating that the cell morphology over time can be influenced by changing the applied crosslinker as this will influence the mesh size and mechanical properties.

Finally, the laser-based (two-photon polymerization) structuring range and concomitant swelling behaviour of the materials was assessed as a function of the applied laser power (at 100 mm/s scan speed) using 2 mol% of P2CK as photoinitiator. The swelling of these microstructures as a function of laser power provides insights in the degree of conversion. In this respect, besides differences in reactivity and swelling ratio for the different hydrogel formulations, also a previously unreported phenomenon was observed. At high intensities, cleaving of the gelatin backbone occurred even in the absence of photo-cleavable functionalities, which was substantiated by photo-cleavage experiments in the presence of a photosensitizer.

Conclusions: Varying the thiolated crosslinker in thiol/norbornene gelatin hydrogels proves to be a suitable handle to tune the physico-chemical properties, the *in vitro* biological response and the laser-based processing potential of the hydrogel. Furthermore, at high laser intensities, a previously unreported competitive photocleavage reaction can occur within the gelatin derivative.

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