Thermosensitive bioartificial hydrogels as smart injectable and biocompatible systems allowing post-injection chemical crosslinking

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Introduction

Injectable hydrogels as carriers for drugs and/or cells have gained increasing interest in the last years due to the possibility to vehicle their payload in the desired loco through mini-invasive procedures [1, 2]. In this work, a new library of bioartificial hydrogels was designed combining the chemical versatility and reproducible physicochemical properties of a synthetic polymer with the enhanced cell adhesiveness of a natural polymer. Specifically, an amphiphilic polyurethane (PEU) bearing amino groups was first synthesised and then blended with hyaluronic acid (HA) to obtain thermosensitive bioartificial formulations. The influence of HA molecular weight on polymers miscibility was investigated as well as the thermosensitivity and the injectability of the newly designed bioartificial systems. Modification of the PEU and the HA may allow post-injection chemical crosslinking enhancing the chemical stability of the hydrogel.

Experimental Methods

The amphiphilic PEU was synthesised in a two step procedure under nitrogen by reacting a commercial triblock copolymer (Poloxamer 407, Poly(ethylene oxide)-Poly(propylene oxide)-Poly(ethylene oxide)) with 1,6-hexamethylene diisocyanate. Then, the prepolymer was chain extended with a diol (N-Boc diethanolamine) containing protected secondary amino groups. Infrared (IR) spectroscopy and Size Exclusion Chromatography (SEC) were then performed to assess the success of the synthesis and to evaluate PEU molecular weight, respectively. Subsequently, the synthesised PEU was subjected to an acidic treatment in chloroform/trifluoroacetic acid 90/10 V/V to remove BOC-protecting groups and the exposed secondary amino groups were quantified through a colorimetric assay (Orange Sodium salt). The synthetic component (D- DHP407) was then blended with a high (HA_400kDa) and low (HA_82kDa) molecular weight HA, reaching different weight ratios. Lastly, formulations were prepared by dissolving both polymers in physiological saline solution and then characterized in terms of thermosensitivity by means of tube investing test and gelation time test at 37 °C; injectability in the sol state through needles of different diameters (G18, G21 and G22) and cytocompatibility according to ISO10993-5. Secondary amino groups in PEU and carboxyl groups in HA may be exploited to graft functional molecules for in situ post- injection crosslinking.

Results and Discussion

The successful PEU synthesis was proved through IR spectroscopy by the appearance of new bands ascribed to urethane bonds, while SEC analysis gave a molecular weight in the range 30000 – 35000 Da with 1.4 polydispersity index. Secondary amino groups were quantified to be 4.5×10^{20} groups/g of polymer by means of Orange II Sodium Salt assay. For what concerns hydrogel preparation, D-DHP407 and HA_400kDa were mixed at 98/2, 95/5, 92/8, 88/12 and 83/17 wt. ratios. All tested formulations formed compatible blends, but, due to the high molecular weight of HA_400kDa, further increase in the natural component content highly increased system viscosity affecting injectability. Hence, D-DHP407 was blended with a low molecular weight HA (HA_82kDa) obtaining compatible and injectable blends even at 50/50 wt. ratio. Subsequently, hydrogel temperature-driven gelation was tested and all considered formulations turned out to gel within few minutes at 37 °C, thus suggesting that HA introduction did not affect PEU thermosensitivity. Regarding hydrogel injectability, all blends could not be extruded through G22 needle, while they could be injected through larger needle diameters (G21 and G18). Finally, by increasing HA content, thus decreasing the synthetic component, an increase of hydrogel biocompatibility was observed.

Conclusion

A new platform of thermosensitive bioartificial hydrogels was developed by blending a custom-made amphiphilic polyurethane, which ensures hydrogel thermo-responsiveness, with hyaluronic acid, responsible for an improved cytocompatibility. Furthermore, the presence of exposed secondary amino groups along PEU chains and carboxylic groups in HA chains opens the possibility to graft functional moieties to both molecules for post-injection crosslinking. Such injectable system is under development as a matrix for *in situ* treatment of myocardial tissue, by releasing agents promoting direct reprogramming of cardiac fibroblasts into cardiomyocytes.

References

[1]Zhang Z., Expert. Opin. Biol. Ther., 17:1, 49-62, 2017
[2] Boffito M. et al., J. Biomed. Mater. Res. A, 103(3):1276-90, 2015.

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