POLYSACCHARIDES HYDROGEL - RADIATION INDUCED FORMATION AND MEDICAL APPLICATIONS

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[Engineering of Biomaterials 143 (2017) 38]

Introduction

Permanent hydrogels of synthetic or natural polymers can be formed by chemical methods with utilisation of crosslinking agents. Crosslinkers, typically bi-functional and highly reactive compounds, are often toxic, similarly to initiators or catalysts, thus any traces of such residues may be harmful for the living organism. Moreover, thermal initiation usually excludes possibility of inclusion biologically active molecules due to thermal instability. Radiation method can be employed in order to circumvent those disadvantages, therefore it is especially suitable for manufacturing of hydrogels for biomedical applications. Polysaccharides, despite their general tendency for radiation-induced degradation, can be converted into permanent hydrogels when irradiated with or without additives under certain conditions [1]. The scission of glycosidic bonds is predominantly responsible for the reduction in the molecular weight of macromolecules. However, during irradiation of polysaccharides in an aqueous solution, and when applying specific irradiation conditions, scission and crosslinking take place simultaneously. Whether the outcome is a degraded polymer or a gel is determined by the prevailing mechanism of radicals reactions. Thus, it was demonstrated that polysaccharide derivatives having high molar concentration of substituent side groups prone to create stable carbon-centered radicals, irradiated in highly concentrated solutions or in solutions of specific pH (in the case of ionic polysaccharides) [2]. In this report, a short review of current approaches to crosslink polysaccharides will be reviewed and followed by an exemplary application of carboxymethylchitosan hydrogel for nerve regeneration scaffold.

Materials and Methods

Carboxymethylchitosan (CMCS) of the deacetylation degree (DDA) 93.8%, DS 96% and intrinsic viscosity in 0.1 mol dm^{-3} NaCl of η = 2.77 dm^3 g^{-1} was obtained from Kraeber & Co. GmbH (Germany). Aqueous solutions of CMCS were irradiated by electron beam (EB) with and without a crosslinking agent of poly(ethylene glycol) diacrylate (PEGDA, M_W = 700 g mol^{-1}, Sigma-Aldrich). Obtained gels were evaluated by standard sol-gel analysis. Nerve regeneration conduits were fabricated from a solution of poly(lactic acid) and poly(trimethylene carbonate) mixture by phase-inversion method. LDH and XTT cytotoxicity, and *in vivo* biocompatibility and functional studies were conducted using rat animal model.

Results and Discussion

Results of this study indicated that ionizing radiation is a convenient tool to synthetize hydrogels based on CMCS when irradiated in highly concentrated aqueous solutions. Irradiation of 12% CMCS, as the optimum concentration, leads to formation of hydrogel scaffold at a dose of 25 kGy. Since not all macromolecules form the network, which is due to partial degradation of the polysaccharide, the gel fraction (GF, mass of insoluble fraction per mass of used polymer) was evaluated along with the determination of the equilibrium degree of swelling (EDS, grams of water per gram of gel). The GF and EDS of the gels were ca. 30% and 50 g/g.

Results of LDH and XTT cytotoxicity tests and *in vivo* examination of local tissue response were an indicator of the good biocompatibility of CMCS hydrogel. The CMCS gels were manufactured *in situ* inside the lumen of nerve guidance tube by irradiation of the tube prefilled with CMCS solution/paste, (the physical gel). The internal gel is strong enough to support regenerating nerve, but also its softness will not obstruct the regrowing cone of the nerve to reach its distal part.

Preliminary animal studies, involving discontinued femoral nerve of a rat showed positive results of nerve regeneration. The nerves were reconnected, as observed in histopathological analysis. Number of neuroma occurrence at the reconnection site was reduced as compared to simple ends suturing (end-to-end connection as a control). Functioning of the nerve resulted in gradual return of motoric functions.

Conclusions

The study highlights the potential of carboxymethylchitosan hydrogel as lumen filling of nerve regeneration channel based on biodegradable polymer blends of poly(lactic acid) and poly (trimethylene carbonate). After *in situ* synthesis of the gel inside the tube, the product is ready for immediate use, because applied technology combines gel formation and sterilization into a single process. This can be accomplished if the dose applied for CMCS hydrogel formation is of 25 kGy or more, as in the present studies. Preliminary animal studies demonstrated biological safety of the CMCS hydrogel, and functional evaluation using rat model revealed positive results of regeneration of femoral nerve.

References

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