Highly Tunable, Rapidly Covalently Gelling Hydrogels for Cell Encapsulation

Bakaic, E; Smeets, N.M.B.; Hoare, T.+
McMaster University, Hamilton, Ontario, Canada L8S 4L7
bakaice@mcmaster.ca

ABSTRACT SUMMARY

A highly tunable, rapidly covalently gelling composite hydrogel platform based on poly(oligoethylene glycol) methacrylate (POEGMA) and dextran (DEX) has been developed. The POEGMA-DEX hydrogel composition was systematically varied to achieve control over the mechanical and physiochemical properties while maintaining high cell viability. These composite hydrogels could provide as an advantageous alternative biomaterial scaffold for use in cell encapsulation.

INTRODUCTION

Cell encapsulation, in which cells are entrapped within a protective matrix, offers a way of overcoming limitations of cell sourcing and immune activation in clinical applications as well as enabling the use of cells as natural "factories" for the production of biomass, the processing of waste products, or the detection of a targeted analyte (even in harsh environments). However, the success of this technology is severely governed by the types of encapsulating materials available. Therapies based on adaptations of natural polymers, such as alginate, agarose, chitosan, and cellulose have limitations in terms of mechanical strength and potential contamination and thus pose issues to the host, such as rejection and immune response[1]. Conversely, solely synthetic materials can pose cytotoxicity issues and have poor degradability. An optimum balance between the mechanical strength of the capsule and the mass transport, and the combination of advantageous natural and synthetic polymer properties of the membrane is key to successful cell encapsulation. Design of composite hydrogels allows these requirements of extracellular matrix development to be met.

To address this challenge, we have designed composite hydrogels based on a combination of synthetic poly(oligoethylene glycol) methacrylate (POEGMA), known to exhibit cytocompatibility, bio-repellent ,and thermoresponsive properties as a function of the OEGMA side-chain length (a potentially useful property to dynamically tune capsule pore size), and naturally-occurring dextran to produce a novel material with tunable properties [2].

EXPERIMENTAL METHODS

POEGMA-DEX hydrogels are prepared by co-extrusion of a hydrazide-functionalized POEGMA and aldehyde modified dextran (DEX) to form a covalent hydrazone bond. The hydrazone bond, which forms quickly under physiological conditions, is reversible in aqueous media demonstrating a successful injectable and degradable system. Gelation occurs in the absence of heat or irradiation, ideal for cell encapsulation. Degradation of this hydrazone bond through hydrolysis produces polymer precursor chains below the 38kDa renal clearance limit [3].

Hydrazide-functionalized poly(oligo ethylene glycol) methyacrylate-hydrazide (PEGMA-hzd) polymers are prepared from the free-radical copolymerization of diethylene glycol methacrylate ($M(EO)_2MA$ n=2 EO units), oligo ethylene glycol methacrylate ($OEGMA_{475}$, n = 6-7 EO units), and acrylic acid (AA), followed by EDC coupling of adipic acid dihydrazide to AA residues. Dextran-aldehyde (DEX-ald) is synthesized through periodate oxidation of DEX. Gelation was performed using polymer concentrations between 4-6 wt% in saline.

RESULTS AND DISCUSSION

Control over volume phase transition temperature (VPTT) of the polymers can be achieved by varying the ratios of the component OEGMA monomers used (between n=2 and n=6-7 ethylene glycol repeat units). The facile tunability and large VPTT range that can be achieved using OEGMA monomers provides an advantage over the use of e.g. *N*-isopropylacrylamide (NIPAM) based polymers. The effect of the composition on the thermosensitivity of the POEGMA-DEX hydrogel is shown in Fig. 1. It can be seen that all POEGMA-DEX hydrogels display a clear volume phase temperature transition (VPTT).

The effect of crosslink density was also investigated in the POEGMA-DEX composites. The reversible swelling properties of the POEGMA-DEX hydrogels, based on PO9, PO2, and P08 (11, 25, and 50 functional groups/chain) respectively can be seen in Fig. 2. An increase in crosslink density greatly decreases the reversible swelling capabilities of the gels. Control over crosslink density may also allow for pore size control within the polymer matrix.

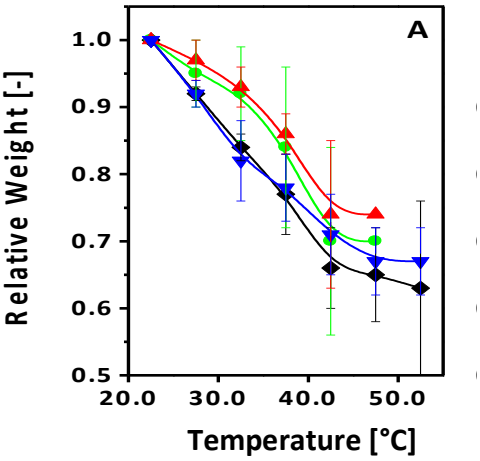

Fig. 1: De-swelling of the POEGMA-DEX hydrogels as a function of the temperature. A) Effect of the $M(EO)_2MA$: $OEGMA_{475}$ composition (◆, black) PO1 = 61.9°C, (●, green) PO2 = 64.1°C, (▲, red) PO3 = 67.7°C and (▼, blue) PO4 = 75.9°C. B) Effect of the average number of functional groups per polymer chain (●, grey) PO9 = 10, (●, green) PO2 = 25 and (●, black) PO8 = 50.

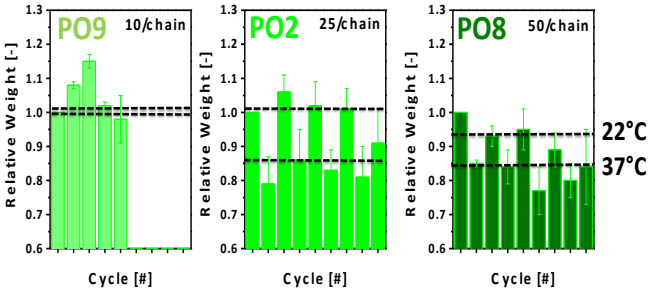

Fig. 2: Reversibility of the temperature phase transition of the POEGMA/DEX hydrogels.

Hydrogel composite degradation is controlled by both crosslink density and precursor polymer concentration. For example, a PO2-DEX hydrogel (6 wt%/6wt%) degrades in 120 min in 1M HCl, while lower concentrations eg. PO2 with 4 or 2 wt% DEX-ald, completely degrade in 75 and 45 min, respectively. In PBS however, all hydrogels show very slow degradation kinetics and are stable for at least 3 weeks

The elastic moduli of the POEGMA-DEX hydrogels were within the range of 1.8-2.3 kPa, which is also the range of neural and adipose tissue within the body. However, we feel that further tuning of polymer concentration and/or crosslinking density will allow us to mimic muscles, cartilage, and bone tissues.

MTT assays performed on 3T3 fibroblasts grown in the presence of POEGMA-DEX gels show high cell viability and non-cytotoxicity over a range of concentrations with 1.30 ± 0.20 and 1.06 ± 0.14 percent relative viability based on gel location.

CONCLUSION

Combining synthetic (POEGMA) and natural (dextran) polymers allows for precise control over parameters such as pore size, elastic modulus, and cell viability, as required for successful cell encapsulation.

ACKNOWLEDGMENTS: Funding from NSERC CREATE-IDEM is acknowledged.

REFERENCES:

1. Zimmermann, H.; Zimmermann, D.; Reuss, R.; Feilen, PJ.; Manz, B.; et al., 2005, 16, 491–501
2. Lutz, J. Journal of Polymer Chemistry, 2008, 46, 3459-3470.
3. Patenaude, M.; Hoare, T. Biomacromolecules, 2012, 13 (2), 369–378