Potential of N-Methacrylate Glycol Chitosan As A Matrix For Adipose-Derived Stem Cells Encapsulation

Abby Sukarto, Brian G. Amsden.
Department of Chemical Engineering, Queen's University, Kingston, ON, Canada.

Statement of Purpose:
Glycol chitosan is a chitosan derivative that is soluble in water at pH 2 to 12. Methacrylate groups grafted to the backbone of glycol chitosan via free amine groups can be photopolymerized to form a hydrogel.[1] Such photocrosslinking is advantageous as it allows cell or drug entrapment at room temperature. Moreover, this derivatized chitosan and the hydrogel formed has been shown to be cytocompatible[1], but without cell incorporation during photocrosslinking. In this work, the viability of adipose-derived stem cells (ASCs) encapsulated in photocrosslinked methacrylated glycol chitosan (MGC) hydrogel was studied.

Materials: Glycol chitosan was purchased from Wako Chemical USA, Inc. Glycidyl methcarylate, Dulbecco's Modified Eagle's Medium/ Nutrient mixture F-12 Ham (DMEM/ F12), antibiotic antimycotic solution, calcein AM and ethidium homodimer-1 were obtained from Sigma-Aldrich. 12-kDa seamless cellulose dialysis tubing was purchased from Fisher Scientific Canada. Deuterium oxide was obtained from Cambridge Isotope Laboratories Inc. ASCs were harvested from the breast tissue of a patient undergoing elective surgery at the Kingston General Hospital, Kingston, ON, Canada.

Methods: Purified 2 w/v % glycol chitosan dissolved in distilled water was reacted with glycidyl methacrylate for 24 hrs at pH 9. The reacted solution was neutralized with 1M hydrochloric acid, dialyzed against distilled water in 12-kDa dialysis tubing twice for 4 hrs and lyophilized. The degree of *N*-methacrylation was measured using ^{1}H NMR (20 mg/ml in deuterium oxide at 90^0C). To incorporate ASCs within MGC gel, 6 w/v % of the lyophilized MGC solid dissolved in cell media (DMEM/F12, 10% fetal bovine serum and 1% antibiotic antimycotic solution) was well mixed with 10% v/v of 5 mg/ml Irgacure 2959 (I2959) photoinitator and ASCs at a seeding density of 150,000. The ASCs were isolated using the method established by Flynn et al.[2] 0.1 ml of this solution was transferred into a cylindrical Teflon mold and exposed to UV light (320 to 480 nm) on the top and bottom of the construct (8 mm D x 2 mm H) at an intensity of 10.8 mW/cm^2 for 3 mins. The encapsulated ASCs in MGC gels were transferred into a 24-well plate and fed with cell media. The viability of the ASCs was quantified using calcein AM (live cells) and ethidium homodimer-1 (dead cells) staining and imaged using an Olympus FV 1000 confocal scanning laser microscope.

Results: Figure 1A shows that the viability of ASCs encapsulated in MGC gels was not significantly different for gels prepared with MGC of high and low percent *N*-methacrylation on day 1. However, ASC viability was significantly lower on days 4 and 7 for gels with the lowest percent of *N*-methacrylation. Gels prepared with MGC of 11.6 and 24.9% *N*-methacrylation maintained a similar cell viability at each time point and this slightly decreased with time. Figure 1B indicates that the number of cells in MGC gel with 5.9% degree of methacrylation was lower on day 1. The number of ASCs retained in all the gels decreased between day 4 and 7, regardless of the *N*-methacrylation of the MGC used.

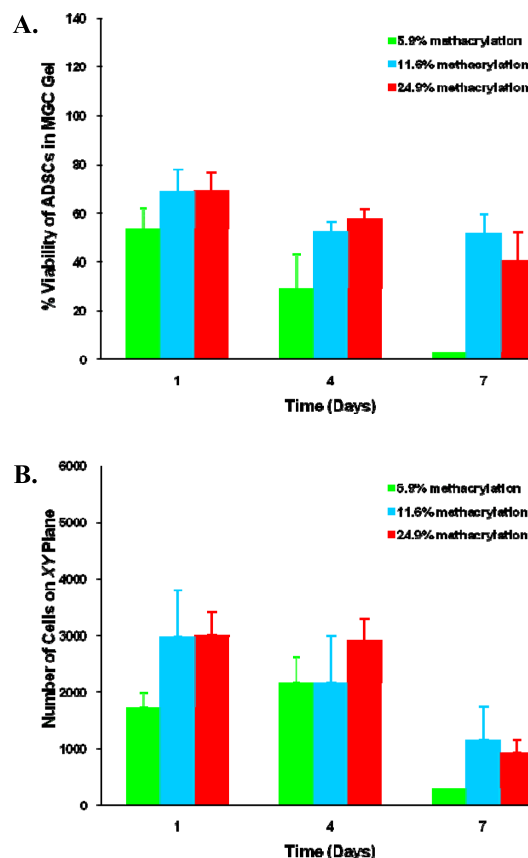

Figure 1. Time dependence of (**A**) viability and (**B**) number of ASCs encapsulated in MGC gels prepared with MGC of varying percent *N*-methacrylation.

Conclusions: The *N*-methacrylation of the MGC used had no effect on the number of ASCs surviving the initial photocrosslinking conditions, but did influence ASC number within the gel and their viability as time progressed. As gel stiffness increases with degree of *N*-methacrylation[1], the ASCs are possibly responding to these differences in gel stiffness.

References:

1. Amsden B.G. Biomacromolecules. 2007;8 (12): 3758-3766.
2. Flynn L., J Biomed Mater Res A. 2006;79 (2): 359-69.