Photo-Crosslinkable Chitosan Hydrogels for Neural Tissue Engineering

Chandra M. Valmikinathan, Vivek J. Mukhatyar, Ravi V. Bellamkonda

Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA

Statement of Purpose: Injuries created in the peripheral and central nervous systems create physical gaps and cavities that are irregular, thereby leading to challenges in restoring contiguity via scaffolds to engender repair. Photopolymerizable in-situ gelling hydrogels are a promising solution. Hydrogel scaffolds potentially provide an environment that allows cell adhesion, proliferation, differentiation and host tissue integration. Chitosan based hydrogels have been promising for nerve regeneration owing to their optimal positively charged amine groups generally supporting neurite extension. Photopolymerizable chitosan derivatives have been prepared previously; however have inherent disadvantages including loss of biodegradability, cytotoxicity and lack of control over pore sizes and mechanical properties.

In this study, we have shown the development of novel photocrosslinkable water soluble chitosan (carboxymethyl chitosan) based hydrogels using aminoethyl methacrylate. This approach retains the degradability of chitosan, produces non-toxic degradation products and allows for control over the degree of crosslinking, and therefore better control over the mechanical properties and scaffold pore size, thereby allowing survival and differentiation of cortical neurons and dorsal root ganglions.

Materials and Methods: Carboxymethyl chitosan (CM-Chitosan) was prepared by a modified method previously described, where acid end groups were added to the chitosan backbone. The purified CM-chitosan was further modified to present acrylate end groups suitable for photocrosslinking. 1g of the CM-chitosan was then dissolved in 80 mL 0.1 M morpholinoethano sulfonic acid (MES), and carbodiimide, followed by 1.2 g of AEMA. The reaction was allowed to proceed for 24 hours, followed by dialysis, and lyophilization overnight to obtain photocrosslinkable chitosan (CM-Acrylate). A 0.5 or 1% solution of CM-Acrylate was prepared by dissolving it in phosphate buffered saline (pH 7.4) containing 0.1% Irgacure 2959 (I2959) as photoinitiator. The solution was exposed to UV light for 3 minutes, causing the solution to gel. To characterize the hydrogel mechanical properties, the shear elastic modulus was measured using parallel plate geometry (20 mm diameter) rheology. The elastic stored modulus (G′), and viscous loss modulus (G″), were evaluated as a function of frequency on 1% CM-acrylate hydrogels.

Dorsal root ganglions (DRG) were explanted from P1 rat pups. The connective tissues and the nerve roots were trimmed off and the DRGs were placed in collagenase-dispase solution for 15 minutes at 37 ^{o}C. The DRGs were then placed in 400 µL of 0.5% (w/v) gel solution and were exposed to UV light for 3 minutes to cause gelation. As a control, DRGs were also placed in 1% Seaprep® agarose hydrogels. The gels were then transferred to an incubator and were cultured for 5 days prior to evaluation. Embryonic day 18 (E18) rat cortices were isolated and the cortical neurons were extracted and seeded in a similar approach as described above. 30000cells/gel was encapsulated in agarose as well as CM-acrylate chitosan hydrogels. Two days post implantation, neurites were imaged with anti-tubulin and the extent of neurite extent was evaluated.

Results and Discussion: Acrylate modified chitosan hydrogels were prepared using the techniques described above. A degree of methacrylation of 23±12 % was obtained as estimated by NMR spectroscopy. Figure 1 shows the chemistry of chitosan modification as well as the gross morphology and the pore architecture of the hydrogels prepared by photocrosslinking.

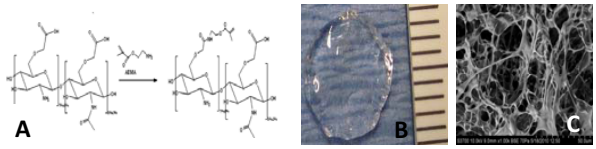

Figure 1: A) Acrylate modification of Chitosan Hydrogels B) image of hydrogel C) Scanning electron micrograph of hydrogel showing open pore architecture

Rheological measurements and estimation of storage modulus of agarose and chitosan hydrogels were estimated as described above. From the data, it can be observed that 1% (w/v) chitosan hydrogels had lower storage modulus as compared to 1% seaprep hydrogel, which have been reported earlier for supporting neurite outgrowth from DRGs. Also, the evaluation shows similar storage modulus as that of human brain or spinal cord tissues.

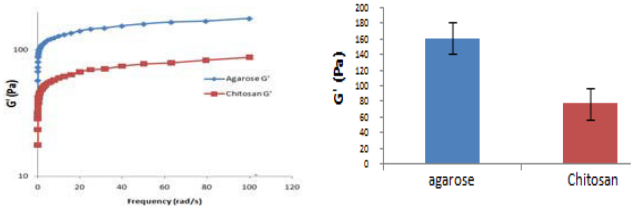

Figure 2: Rheological characterization of chitosan and agarose hydrogels

Neurite extension from DRGS (Fig 3A) and cortical neuron differentiation (Fig 3B) in photocrosslinkable chitosan hydrogel are shown below. Meanwhile, agarose based hydrogels do not support neurite outgrowth from cortical neurons (Fig 3C).

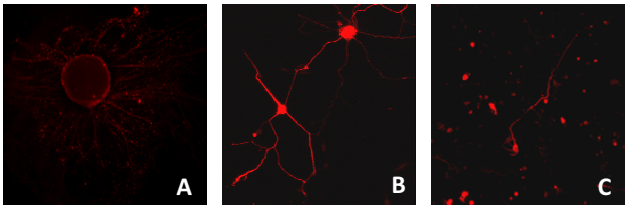

Figure 3: A) DRG neurite extension in CM-acrylate hydrogels B) Cortical neurons extending processes in CM-Acrylate C) 1% agarose hydrogels with encapsulated cortical neurons

Conclusions: The present study shows the fabrication and characterization of acrylate modified chitosan hydrogels. We have shown the application of such in-situ gelling systems for nerve regeneration. This approach allows for fabrication of chitosan hydrogels without any cytotoxic degradation byproducts and with control over pore size and mechanical properties. Also, this hydrogel system still retains its active amine groups, which can be further modified to include extracellular matrix proteins such as laminin or fibronectin.

References:

1. Mahoney J et.al., Biomaterials, 2006, 27, 265-74
2. Leach J et.al., Biotech & Bioengg, 2003, 82, 578-89
3. Dillion G.P. et.al., J. Biomedical Mat. Res., 2000, 51, 510-9
4. Yu L.M. et.al., J. Biomedical Mat. Res.,2007, 82, 243-55