Degradation and Cell Survival in a Fast-Degrading Thio-Acrylate based Hydrogel

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Statement of Purpose: The demand to successfully regenerate cranium tissue is an integral step to return the craniofacial functions. Recently, it has been shown that craniofacial tissue engineering by combining biological cells with scaffold biomaterials to be a favorable methodology to regenerate lost tissue by efficient cellular delivery. Potentially, a fast-degrading, mesenchymal stem cell (MSC)-encapsulating hydrogel can be used in conjunction with a 3D scaffold to provide efficient regeneration in the craniofacial area. Recently, Hao and Lin developed a photo-polymerize-able biodegradable hydrogel system based on thiol-acrylate for hepatic cell encapsulation [1]. This current hydrogel, however, has a longer degradation time and higher polymer content than we need for MSC delivery in cranial regeneration. To further evaluate the utility of this biodegradable hydrogels for MSC encapsulation for cranial regeneration applications, we used this present study to: (1) investigate the ability of tuning a biodegradable thio-acrylate hydrogel for faster degradation for MSC delivery; (2) to measure the MSC survival after encapsulation.

Materials and Methods: A biodegradable poly(ethylene glycol)-diacrylate (PEGDA) hydrogel was prepared by visible light initiated thiol-acrylate photopolymerization methods as described previously[1]. Eosin-Y was used a photo-sensitizer. Dithiothreitol (DTT) was used a dual-function co-initiator and cross-linker bi-functional thiol. N-vinylpyrrolidone (NVP) was used as a co-monomer. CRGD peptide was added to enhance cell attachment. PEGDA pre-polymer solutions were prepared at 3, 5, 7, and 9 wt%. To evaluate the degradation rate of these PEGDA wt% groups, DTT was added at 3, 5, 7, and 9 mM. The degradation of the hydrogel in DPBS up to 14 days was examined. Furthermore, to measure the mechanical properties of the hydrogel, oscillation viscometry analyses were performed on all groups. For in vitro study, MC3T3-E1 cells were encapsulated in the hydrogels at a final seeding density $12x10^6$ cell/mL. A PicoGreen dsDNA assay was performed to monitor cells viability and proliferation at 1, 7, 14, and 21 days. In addition, the differentiation of the encapsulated MC3T3-E1 cells were evaluated using a double staining assay from alkaline phosphatase (ALP) and Von Kossa.

Results and Discussion: PEGDA macromer at 3 wt% incorporated with 5mM of DTT was found to show fastest degradation rate and dissolved within four to five days *in vitro,* (**Fig. 1**). That is been demonstrated in histology sectioning of PEGDA specimens after 21 days culturing in osteogenic medium *in vitro* (**Fig. 2**). The live/dead staining images in all gel groups revealed that the cells were healthy, alive and there were almost no dead cells were detected during the studies (Fig. not shown). Furthermore, after 21 days culturing in osteogenic media *in vitro*, the specimens stained with Von Kossa demonstrated the mineralization activities in MC3T3-E1 cells. The results showed the cells encapsulated in PEGDA, 3, 5, 7, and 9 wt% can fully differentiated into osteoblasts lineage comparing to control groups cultured in basal media. An early detection of cells differentiation into osteoblasts was also noted in 7 and 9 wt% hydrogel groups cultured in basal medium, suggesting the effect of gel stiffness on cell differentiation.

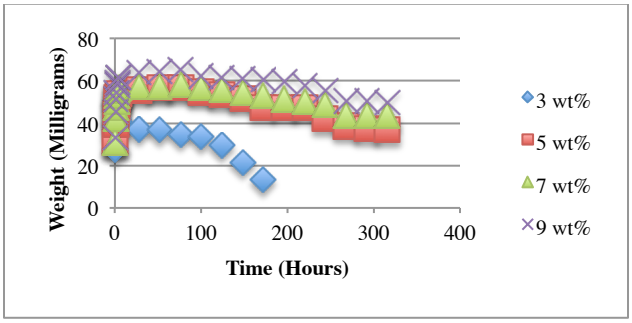

Figure 1: shows the degradation of 3-9 wt% hydrogel.

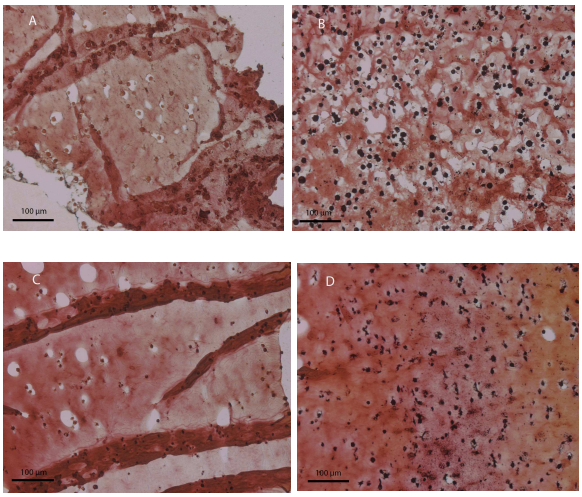

Figure 2: shows histology sectioning of PEGDA scaffolds, 5wt% A & B, 7wt% C & D, demonstrating Von Kossa positive staining of MC3T3-E1 cells cultured for 21 days in osteogenic medium.

Conclusion: This study describes the tuning of macromer formulation and bi-functional thiol concentration to achieve rapid degradation of the 3D matrix while maintaining the viability and differentiation potential of the pre-osteoblast cell line.

1. Hao Y, Lin CC. J Biomed Mater Res A 2014;102(11):3813-27.