Osteogenic Differentiation of Human Mesenchymal Stem Cells in Injectable In situ Thermogelling Chitosan Solutions

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Statement of Purpose: Injectable in situ setting hydrogels are of great interest for the development of therapeutic implants and as drug and cell delivery vehicles [1]. Injectable gelling systems allow for introduction in a minimally invasive manner, a conformed fit of the implant, and delivery of bioactive molecules or cells to the defect site. We have recently developed a novel chitosan-ammonium hydrogen phosphate (AHP) solution as an in situ thermogelling system [2]. Chitosan is a biodegradable, biocompatible natural polymer derived from chitin, which is a structural component of crustacean shells. The addition of AHP to chitosan results in the formation of near neutral solution that can gel at physiological temperature in clinically relevant time periods of 8-10 minutes [2]. This study was aimed to evaluate the feasibility of chitosan-AHP as a stem cell delivery vehicle. Human mesenchymal stem cells (hMSC) are multipotent cells derived from bone marrow that can be induced to differentiate into various cell lineages depending on exposure to cytokines, molecules, and growth factors. The goal of this study is to demonstrate that the chitosan-AHP gel can support the growth of hMSCs as well as their osteogenic differentiation when exposed to the proper growth factors.

Methods: Chitosan (85% deacetylation) and AHP was obtained from Sigma-Aldrich (St. Louis, MO). hMSCs, basal mesenchymal stem cell media, and osteogenic differentiation media was obtained from Cambrex (East Rutherford, NJ).

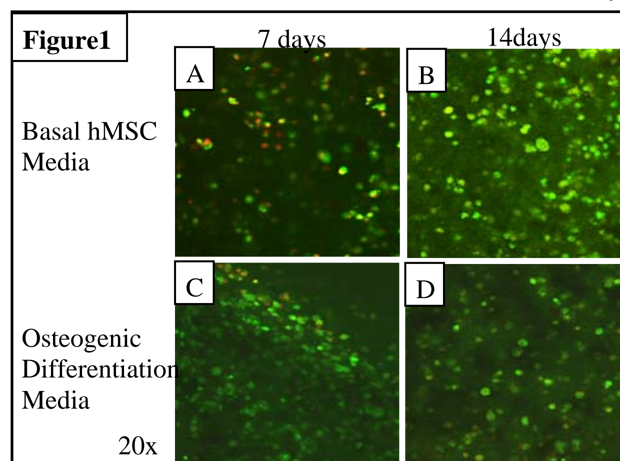
Preparation of Thermogelling Solution: 80 µL of filter sterilized AHP (60% solution) was added to autoclaved chitosan solution (2.8% in 0.5% acetic acid) in a glass vial at 4ºC with vigorous magnetic stirring in an icebath .

hMSC Encapsulation: The above mixture was equilibrated at 37ºC and hMSCs (9.2 x 10^6/mL of the gelling solution) were added. The cells were uniformly suspended in the chitosan-AHP mixture, and poured into sterile 35mm diameter plates. The solution was allowed to gel at 37ºC in a humidified incubator with 5% CO_2. Circular disks were bored from the gel and placed into 24 well plates and cultured in either basal mesenchymal stem cell media or osteogenic differentiation media. Control gels without cells were prepared in a similar manner.

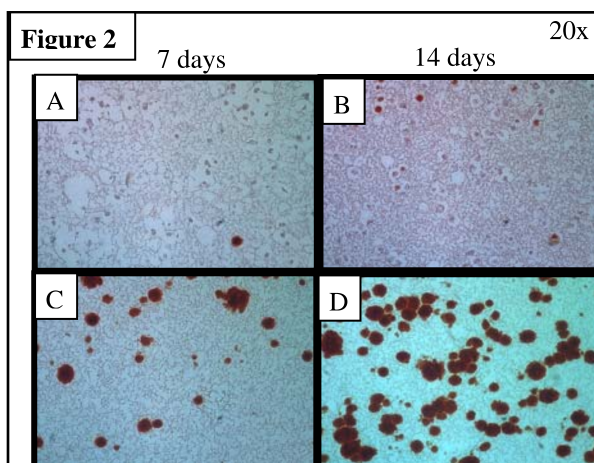
Live/Dead viability assay: The viability of cells encapsulated within the gels after 7 and 14 days were followed by the Live/Dead assay (Invitrogen, Carlsbad, CA) Briefly, at predetermined time points the media was removed and circular disks were washed with D-PBS. The disks were then stained with Calcein AM and Ethidium homodimer-1 per the manufacturer's protocol.

Histology: Media was removed and circular disks were washed with D-PBS and fixed with 1% glutaraldehyde. The gels were then paraffin embedded, sectioned, and then stained with alizarin red for the presence of calcium per Armed Forces Institute of Pathology protocols.

Results / Discussion: Figure 1 shows the Live/Dead staining at 7 (A and C) and 14 days (B and D). It is evident that almost all of the hMSCs are alive at 14 days



in both the basal hMSC media and the osteogenic differentiation media showing that hMSCs could survive the cell encapsulation procedure. **Figure 2** shows alizarin red staining of the histological sections of the hMSC encapsulation at 7 (A and C) and 14 (B and D) days. Basal media (A and B) shows minimal differentiation whereas the osteogenic differentiation media (C and D)



shows a time dependent increase in osteoblasts that are mineralizing.

Conclusions: These results demonstrate the potential for injectable chitosan matrices to be used to deliver hMSCs to an osseous defect and differentiate into osteoblasts allowing for filling of the defect. This system could be applied to any surgery currently requiring autograft, subsequently eliminating the morbidity associated with autograft harvest.

References:

1. Di Martino et al. Biomaterials 26, 5983-90, 2005.
2. Nair LS & Laurencin CT, US Patent Appl. 60/705,812