Methylcellulose based injectable gel scaffolds incorporating BMP-9 coated chitosan microparticles for bone regeneration applications

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Statement of purpose: Injectable scaffolds in the form of gels, paste, and microparticles are considered advantageous especially in the regeneration of complex and irregular bone structures such as in craniofacial region, as they can be easily injected into those regions [1]. Methyl cellulose (MC), a water soluble polysaccharide, can undergo thermoreversible gelation at higher temperature and has been found to be non-toxic to the cells. The study of MC as the injectable scaffolds is limited, mostly due to its higher gelation temperature and limited physical strength. In this study, we developed a MC based gel by blending it with alginate (Alg) and Collagen I (Col) that can undergo gelation at body temperature (37 °C) with excellent physical stability. This gel was developed to provide the injectable medium for chitosan (CS)–tripolyphosphate (TPP) microparticles (MPs) loaded with recombinant BMP-9. BMP-9, a lesser known BMP, is considered to be most osteogenic and just few studies exist on the osteogenic behavior of recombinant BMP-9. We hypothesize that this injectable system constituting both gel and MPs, will undergo sol-gel transition at body temperature there by acting as a scaffold for hMSCs to grow and proliferate while also providing enough mechanical support.

Methods: 10% MC solution was prepared in 0.2% calcium chloride solution and stored in a refrigerator overnight. A polymer blend containing 70% MC, 15% alginate (Alg), and 15% neutralized collagen (Col) solution was prepared at 0 °C which formed a gel at 37 °C. CS-TPP MPs were prepared by simple coacervation technique where CS solution was added dropwise into TPP solution and air-dried overnight. 100 ng of BMP-9 was coated onto 100 mg of MPs at 4 oC and was added to the polymer mixture before gelation. The viscoelastic properties of the gel, with and without MPs, was measured using parallel plate rheometer. Frequency sweep test was performed at 37 °C from 1 to 100 rad/s at 20% strain. The release of BMP-9 from gel containing BMP-9 coated MPs into 1X PBS at 37 °C was determined using human BMP-9 DuoSet ELISA kit (R&D) according to manufacturer’s instruction. Human mesenchymal stem cells (hMSCs) were seeded into the gel to study the effects of BMP-9 released into the gel on cell viability and proliferation. Confocal laser scanning microscopy (CLSM) was done after staining the gel-cell constructs at day 3 and 5 with calcein AM to study the viability and proliferation of hMSCs within the gel scaffolds.

Results: The polymer blend of MC-Alg-Col underwent sol-gel transition at 37 °C within 5 min of incubation. This sol-gel transition is predominantly due to the property of MC and its solution in 0.2% CaCl2 was prepared to improve the strength of gel through ionic interaction with Alg. Col was added to improve the biocompatibility of gel as well as the stability of BMP-9. The incorporation of MPs into MC-Alg-Col system had no effects on the gelation time. The rheological study results (Fig.1) show the slight increase in G’ (storage modulus) and G” (loss modulus) at lower frequency and almost constant at higher frequencies indicating a stiff nature of gel in shearing deformation. The G’ and G” of MC-Alg-Col-MPs system was lower than that of MC-Alg-Col system. The release data showed that about 2% of BMP-9 originally added was released after 5h and the release increased to about 10% at day 7. CLSM images showing the proliferation of viable cells along the Z-depth of 300 µm within the gel are shown in Fig. 2. It can be observed that the proliferation was higher on the gel with BMP-9 coated MPs than within the gel containing non-coated MPs at both day 3 and 5.

![Figure 1. Storage (G’) and loss (G”) modulus of gel scaffolds.](image)

![Figure 2. CLSM images showing proliferation of hMSCs within the gel scaffolds incorporation BMP-9 coated (B & D) and non-coated (A & C) microparticles (Scale: 250 µm).](image)

Conclusions: In this study, we developed an injectable gel based scaffold that can undergo sol-gel transition with good strength at body temperature. When loaded with BMP-9 coated CS MPs, gel showed higher proliferation of hMSCs. This gel-MPs system containing BMP-9 can be used in the effective bone regeneration applications.

References: