Material properties and differentiation potential of collagen-II based 3D microbeads for cartilage tissue engineering

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Statement of Purpose: Repair and regeneration of thick cartilage defects remains a difficult orthopedic problem. Biomaterial-based tissue engineering has shown promise in cartilage repair. Bone marrow-derived mesenchymal stem cells (MSC) have played a critical role in this approach due to their wide availability, tissue-specific differentiation, and immunomodulatory properties. In the present study, agarose (AG) and collagen Type II (C2) were used to mimic the extracellular matrix (ECM) of cartilage. AG is a polysaccharide used to mimic the proteoglycan component of cartilage, and C2 is the main protein component of the cartilage ECM. We analyzed the differentiation potential of bone marrow derived mesenchymal stem cells in AG- and C2-based 3D microbeads. The long-term goal of this work is to create modular microtissues for therapeutic delivery of cells in orthopaedic repair applications.

Methods: Microbeads were made through water-in-oil emulsification method. Initial screening of the microbeads formulation was done by combining C2 and AG at different mass ratios. Human MSC were encapsulated in 0.64 wt% AG/0.07wt% C2 (AGC2) (1:9 mass ratio) hydrogels at a density of $5X10^5$ cells/ml and cultured in vented 15 ml tubes. The osteogenic, adipogenic and chondrogenic potential of MSC in AC2 microbeads were compared to that of AG only microbeads using Standard differentiation supplements. Monolayer and pellet cultures served as positive controls. Differentiation was quantified through lineage specific markers such as osteocalcin, sulfated glycosaminoglycans (sGAG) and standard staining methods including Alizarin red for calcium phosphate and oil-red-O for lipid droplets. Cell viability, morphology and proliferation were monitored using confocal microscopy and DNA quantification methods.

Results: MSC encapsulated in the AG only beads maintained round morphology due to the lack of any attachment ligands. Although MSC started spreading when C2 was included (Fig.1), the integrity of the beads decreased with increasing C2 concentration. After extensive screening of different mass ratios, 1:9 (C2:AG) was found suitable for our study.

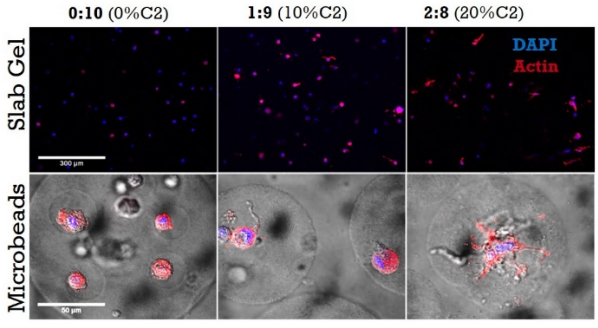
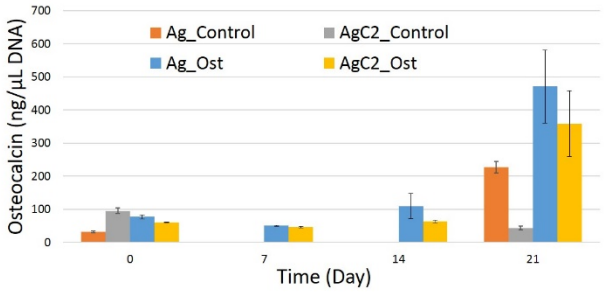


Fig. 1: Cell morphology in AG and AGC2 gel and beads

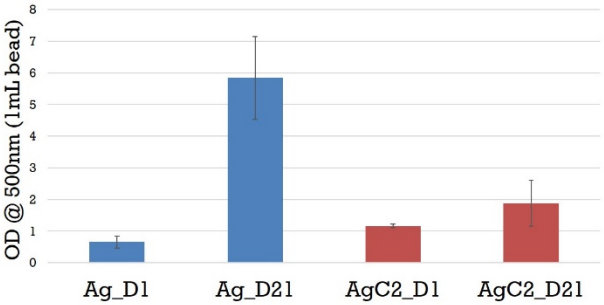
Osteogenic differentiation of MSC, as evident from osteocalcin quantification (Fig.2A) and Alizarin red staining at day 21show no significant difference in AgC2 microbeads compared to that of AG beads.

Fig.2A: Osteocalcin ELISA - Osteogenesis



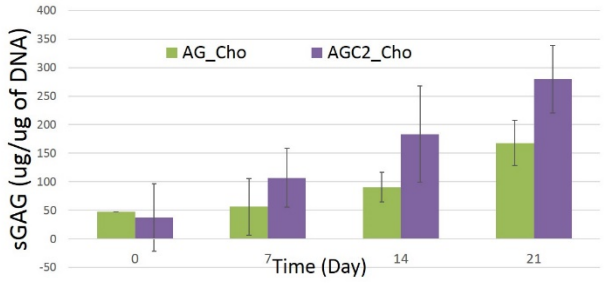
However, significant decrease in adipogenesis was seen in AGC2 beads compared to that of AG beads, quantified by oil-red-O staining of the intracellular lipid droplets (Fig.2B).

Fig.2B: Oil-red-O - Adipogenesis



More importantly, chondrogenesis of MSC assessed by the quantification of sGAG (Fig.2C) showed a significant increase ($p<0.05$ at day 21.), when C2 was included in the AG beads. This response can be attributed to the 3D microenvironment rich in large diameter C2 fibrils that provided additional strength and stiffness for promoting chondrocytic phenotype.

Fig.2C: sGAG Assay- Chondrogenesis



Conclusions: Our data show that AGC2 microbeads promote chondrogenesis of MSC, while suppressing adipogenesis. Such microbeads could be used to augment the differentiated function of MSC, and to provide a suitable extracellular environment to promote tissue repair and integration.