Visible Light Crosslinkable Chitosan-based Hydrogels for Tissue Engineering Applications

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Statement of Purpose: Photopolymerizable hydrogels, irradiated by Ultra violet (UV) light or visible light have been extensively studied for tissue engineering [1]. Compared with UV light, visible light is less damaging to cells, enhances cure depth, and is safer for surgeons. Chitosan, a natural polymer of glucosamine and N-acetyl glucodamine, is attractive in the pharmaceutical and tissue engineering fields due to its biocompatibility, biodegradability, and antimicrobial properties [2]. Photocrosslinked chitosan hydrogel has been widely used in various biomedical applications such as drug delivery, wound healing and tissue repair [3]. In our previous study, new visible light crosslinkable chitosan hydrogels were generated with best cytocompatibility and highest stiffness. However, because chitosan does not contain specific cell binding domains, cells do not adhere directly to chitosan surface but rather bind through cell adhesion proteins, which adsorb onto chitosan. In this study, fibroncetin, collagen or arginine-glycine-aspartic acid peptide (RGD) was added to chitosan-based hydrogel to improve cell attachment of chitosan hydrogels.

Methods: Chitosan (molecular weight 400,000; 85% deacetylated), pentasodium tripolyphosphate (TPP), and chondroitin-4-sulfate (CHS) from bovine trachea were purchased from Sigma-Aldrich (St. Louis, MO). Sodium hyaluronic acid (molecular weight 350,000) was purchased from Lifecore Biomedical LLC (Chaska, MN). Nell-1 protein was produced and purified from Chinese hamster ovary cells (Aragen Bioscience, Morgan Hill, CA).

The viability of encapsulated cells was determined using a Live-dead assay (Invitrogen, Carlsbad, CA). The mechanical strength of the hydrogels was characterized using an Instron Electro-Mechanical Testing Machine. In this study, photopolymerizable chitosan was synthesized by graft methacrylation. Chitosan hydrogel was made from a mixture of chitosan (2%), HA (1%) and riboflavin (RF) (6.5 uM), which was then exposed to visible light. Fibronectin, collagen or RGD was added to chitosan solution to get modified chitosan hydrogel. To evaluate the cell adhesion to chitosan hydrogels, bone marrow stromal cells (BMSC, ATCC, Manassas, VA) were seeded on the surface of chitosan hydrogel coating layer. Morphology of cell attachment was observed with optical microscope (Fig. 1A). To evaluate the effect of incorporated adhesion molecules on encapsulated cells in the hydrogels, BMSC were suspended in chitosan solution and crosslinked by visible light illumination. Morphology of encapsulated cells was examined by labeling F-actin microfilaments with a fluorescent Phalloidin (Fig. 1B)

Results: Encapsulated cells using 6.5 uM RF at 40s exposure to blue light had 80% cell viability (Fig. 1A). Increasing of irradiation time significantly improved the mechanical strength of the gels (Fig. 1B). However, this condition reduced cell viability due to more free radicals produced.

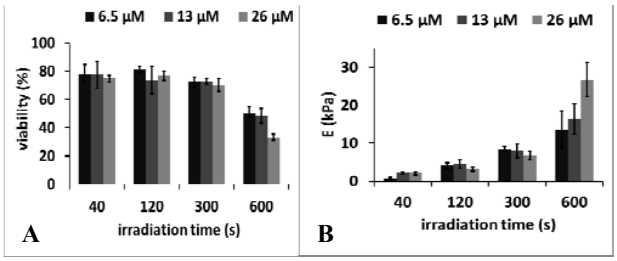

Figure 1. Chondrocyte viability (A) and Young's modulus (B) of photocrosslinkable chitosan hydrogels initiated with riboflavin of different concentration (6.5−26 μM) and irradiated by halogen blue light for different duration (40-600 s).

Chitosan hydrogels showed poor cell adhesion and most cells detached from the surface, forming aggregates (Fig. 2A: a, c, e). Addition of collagen, fibronectin or RGD in chitosan gels increased the attachment of cells. Cell attachment and encapsulation were shown (Fig. 2A: b, d, f). To further characterize the potential of cell attachment inside the chitosan gels, BMSC cells were incorporated into the gels during the gel preparation. Cells encapsulated in collagen-chitosan gels showed a bipolar, spindle shape morphology (Fig. 2B: b, d, f), indicating cell attachment on the materials after 7 days in culture, while cells inside chitosan gels remained in a round morphology (Fig. 2B: a, c, e).

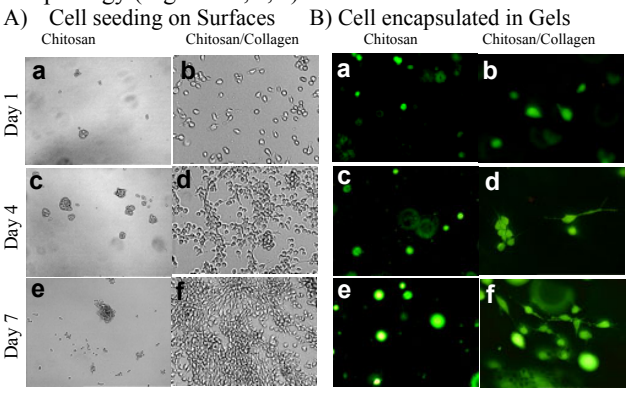

Fig. 2. A) BMSC cell adhesion on chitosan and collagen-chitosan hydrogel surfaces and B) BMSC cell encapsulation in chitosan hydrogels.

Conclusions: Modified photopolymerized chitosan hydrogels were proven to serve as a useful tool to both stimulate cell adhesion on the surface and cell proliferation in the extracellular matrix.

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