Visible light inducible chitosan composite hydrogel containing collagen or chondroitin sulfate for cartilage tissue engineering

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Statement of Purpose: In an articular cartilage, chondrocytes are surrounded by extracellular matrices (ECM), which are highly hydrated complex networks of biomolecules.[1] Hydrogels, macromolecular networks swollen in water or biological fluids,[2] have been widely used as three-dimensional (3D) tissue engineering scaffolds due to their high water content, controllable physical properties, effective encapsulation of cells and bioactive molecules, and efficient mass transfer.[3]

Chitosan has been used in many regenerative studies because it is a naturally derived polysaccharide and a biocompatible polycationic natural polymer as well. The free amino groups in chitosan allow ionic interaction with anionic polymers or chemical modification. Recently, we reported visible light inducible chitosan (methacrylated glycol chitosan, MeGC) hydrogel systems for tissue engineering.[4] Type II collagen (Col) and chondroitin sulfate (CS) are two major components of the hyaline cartilage ECM that are commonly used for cartilage regenerative materials. Col can be gelled without chemical modifications. Polyanionic CS can form a polyelecrolyte complex (PEC) hydrogel with polycations. The Col and PEC hydrogels have a limitation of weak mechanical strength.[5] Here, we are reporting visible light crosslinkable chondrogenic microenvironments using MeGC/Col and MeGC/CS composite hydrogel systems.

Methods: The MeGC was prepared as previously described.[4] Degree of deacetylation (DDA) of glycol chitosan (GC; Sigma, St Louis, MO) and degree of substitution (DS) of MeGC were analyzed using ^{1}H-NMR. MeGC/Col-L (2%/0.1%), MeGC/Col-H (2%/0.2%), MeGC/CS-L (2%/0.5%), and MeGC/CS-H (2%/1%) hydrogels were used for the composite systems. Composite solutions were prepared by mixing stock solutions of MeGC (4%, in PBS) with Col (0.5%, in PBS) or CS (2%, in 0.05% acetic acid). The hydrogel was formed by exposing the solution to visible blue light (Bisco Inc., Schaumburg, IL) in the presence of riboflavin (RF; Sigma, St Louis, MO), vitamin B2, as a photoinitiator. Hydrogels were characterized using SEM (FEI, Hillsboro, OR) and electromechanical testing machine (Instron, Norwood, MA).

For 3D culture of chondrocytes in the hydrogels, chondrocytes were isolated from the knees of 3-month-old New Zealand white rabbits and expanded (passage 2). Harvested chondrocytes were suspended in a 100 μL of polymer solutions. This mixture was exposed to visible blue light for 40 s in the presence of 6 μM RF. The crosslinked hydrogel was cultured in 1 mL of culture medium up to 6 weeks. The medium was replaced twice per week. The 3D cultured samples were analyzed for chondrocytes proliferation, viability, and differentiation.

Results: The DDA of GC and DS of MeGC were 93% and 26%, respectively. The sol-to-gel transition time of MeGC, MeGC/Col, and MeGC/CS solutions decreased RF concentration dependent manner. Cross-sectional SEM images of hydrogels showed porous structures.

Chondrocytes cultured in all hydrogel systems developed spherical phenotypes, a natural phenotype of hyaline chondrocytes. In composite hydrogels, cells formed larger aggregates compared with MeGC (Figure 1). The size of cell aggregation was larger in the hydrogels with higher concentration of Col or CS. Cell proliferation increased as the Col concentration increased. The cross sections of cultured hydrogels were stained with hematoxylin & eosin (H&E), safranin-O for sulfated glycosaminoglycan (sGAG), and immunohistochemistry (IHC) for Col. Secretion of cartilage specific ECMs, such as sGAG and Col, was higher in composite hydrogels than MeGC control hydrogel (Figure 1d & 1e). In addition, more uniform distribution and higher intense of sGAG were found in MeGC/Col-H and MeGC/CS-H hydrogels. Significantly higher sGAG and Col were synthesized in MeGC/Col-H hydrogel. These results suggest that Col activated chondrocytes proliferation and both Col and CS enhanced chondrocytes differentiation.

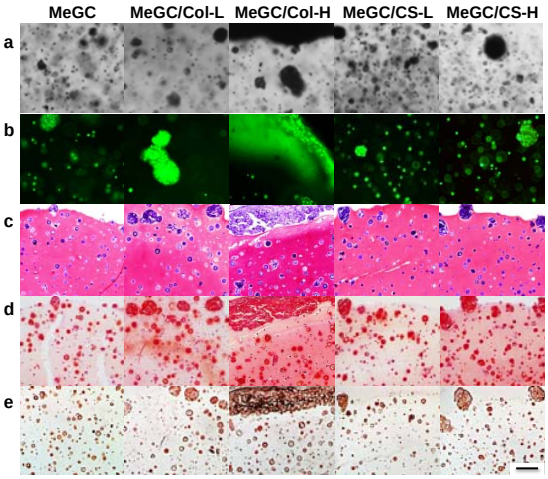

Figure 1. Bright field (a), Live/Dead (b), H&E (c), safranin-O (d), and IHC (Col) (e) images of 6-week cultured chondrocytes in hydrogels. Scale bar, 200 μm.

Conclusions: In this study we investigated a visible blue light crosslinkable composite hydrogels. Incorporation of Col or CS in the crosslinkable chitosan network greatly facilitated cell proliferation and cartilaginous ECM production. These blue light inducible chitosan composite hydrogels showed high potential as advanced injectable scaffolding systems for chondrogenic regenerative.

References:

1. Woodfield TBF. Tissue Eng. 2005;11:1297-1311.
2. Berger J. Eur J Pharm Biopharm. 2004;57:19-34.
3. Hoffman AS. Adv Drug Deliv Rev. 2012;64:18-23.
4. Hu J. Acta Biomater. 2012;8:1730–1738.
5. Fratzl P. Crit Rev Oral Biol Med. 2003;8:32–39.