In Vitro Culture of Chondrocyte in a Heparin-Based Hydrogel

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Statement of Purpose: Articular cartilage is an avascular, flexible connective tissue.[i] Once damaged, due to the very poor self-repair ability of cartilage, the injuries are retained and can subsequently lead to a more degenerated state. Thus, cartilage tissue engineering is regarded as a promising approach for regenerating articular. A main trend of tissue engineering has focused on isolated cells with a proper environment that can promote cellular functions and subsequently lead to the regeneration of new tissues. This approach typically requires a large number of cells to achieve functional tissue regeneration. However, the number of chondrocytes available from the patient is very limited, so the in vitro cell proliferation is essential to get the sufficient number of cells. Unfortunately, the expansion of chondrocytes in monolayer culture leads to dedifferentiation of chondrocytes in most of cell-adhesive environments. Therefore, hydrogels are of promising candidates for cartilage tissue engineering because their high water content and non-cell adhesive nature, mimicking the natural cartilage tissue ECM.[ii] Previously, we reported the development of an in situ forming heparin-based hydrogel formed by a Michael-type addition reaction between thiolated heparin and diacrylated poly (ethylene glycol).[iii] Heparin, which is one of the highly negatively charged natural polysaccharides, is capable of interacting with numerous proteins that have heparin-binding domain. The heparin-based hydrogels are injectable, biodegradable and can be crosslinked in the presence of biomolecules. The mechanical properties of the hydrogels can be controlled by crosslinking condition. In this study, we applied these heparin-based hydrogels for culturing chondrocytes, potentially applicable for cartilage tissue engineering.

Methods: Heparin-based hydrogels were prepared by a Michael-type addition reaction between thiolated heparin and diacrylated poly (ethylene glycol). Crosslinking of the hydrogel was characterized by the elastic modulus under physiological conditions. Chondrocytes were isolated from knee joint cartilage slices of New Zealand white rabbits by enzymatic digestion and cultured in the heparin-based hydrogels under various conditions for optimal culture.

Results: For gel preparation, PEGDA (6K) was used to react selectively with thiol groups of thiolated heparin at physiological conditions **(Figure 1)**. Most cells were alive after encapsulation in the heparin-based hydrogels, **(Figure 2)** and proliferated **(Figure 3-(A))**. Phenotypic analyses, such GAG contents and histological staining, also supported the proper role of the heparin-based hydrogel; a continuous increase in GAG amount was observed during the culture period **(Figure 3-(B)).**

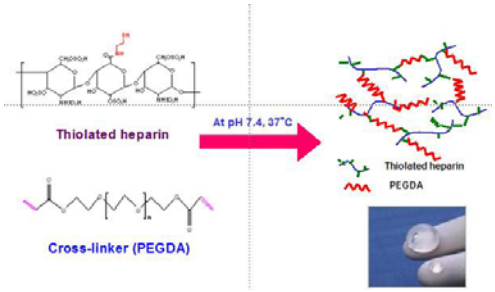

Figure 1. Reaction scheme for heparin-based hydrogel.

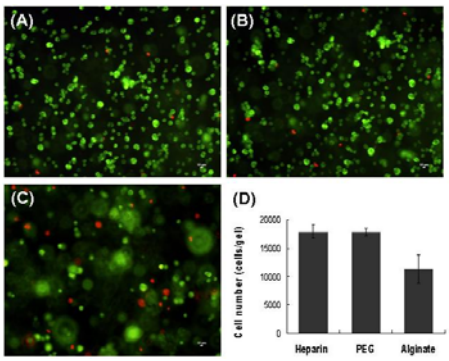

Figure 2. Chondrocytes viability in 2 hrs after encapsulation. (A) The heparin hydrogel at 10 (w/v) % total precursor concentration, (B) PEG hydrogel, (C) Alginate hydrogel. Scale bar: 20 μm, (D) The number of viable chondrocytes in each hydrogel by WST-1

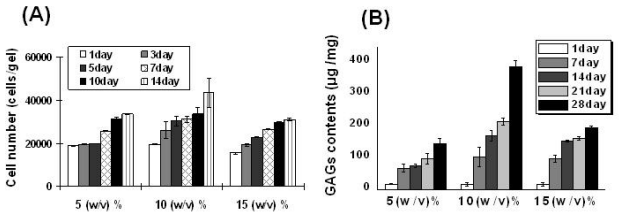

Figure 3. The effect of total precursor concentration, (A) chondrocyte proliferation in the hydrogel, measured by WST-1 assay. (B) GAG contents of the heparin hydrogels produced by chondrocytes normalized to the construct wet weights. Error bars represent means±standard deviation with n=4.

Conclusions: Chondrocytes were cultured in the heparin-based hydrogel. Most of chondrocytes were viable after encapsulation, and proliferated well inside the hydrogel. Also, there existed the optimum gel strength for cell proliferation and GAG production.

References:

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ii. Na, K. Biotechnol Lett 2007;29:1447-1452.
iii. Tae, G. Biomacromolecules 2007;8:1979-1986.