ABSTRACT INTRODUCTION:

Hyaline cartilage is a very hard and resilient tissue; however, it has a very limited healing capacity after damage due to osteoarthritis or injury. The objective of this project is to determine the suitability of modified fibrin hydrogels as scaffolds for autologous human articular cartilage tissue engineering. The attractive feature of the fibrin gel system is that the precursors of the gel are available in autologous form. We have previously shown that genipin, which is a naturally occurring cross-linking agent used in herbal medicine as an anti-phlogistic and for its anti-inflammatory properties, stabilizes the fibrin gels.

METHODS:

Human articular chondrocytes were isolated from cartilage harvested from consenting patients undergoing total knee arthroplasty. The chondrocytes were encapsulated into fibrin gels (10^7 cells/ml) consisting of fibrinogen (120 mg/ml), and thrombin (2.2 U/ml), with or without genipin (0.045 mg/ml). Cell viability in the gels was measured at 24 hours and 1 week by staining gels with Calcein-AM (live) and Ethidium Homodimer-1 (dead).

Fibrin gel mechanical properties were evaluated in unconfined dynamic compression for 20 cycles at 0.1 Hz with a MTS Sintech 1/G material testing machine and a Mach-1™ mechanical testing machine. Dynamic modulus was calculated at 15% strain.

The gels were cryosectioned to 10 µm thickness and stained with Alcian Blue, Safranin O, and Haematoxylin and Eosin (H&E). Immunohistochemistry staining was performed with sections using primary antibodies against collagen II, collagen I, and antigens with secondary Cy2 labelled antibody and DAPI to visualize cell nuclei.

Relative gene expression was measured with Real-Time RT-PCR with the comparative C_t method for encapsulated chondrocytes for collagen II, collagen I, aggrecan, and Sox9 genes with expression normalized to G4PDH and to chondrocytes in monolayer at confluence.

The fibrin gel was also implanted subcutaneously into rats and after 30 days the material was removed, and stained with H&E.

RESULTS SECTION:

No breakdown of the gels was detected during 5 weeks of culture. Cell viability was calculated to be >50% after 24 hours and at 1 week in culture. Genipin cross-linking did not significantly affect cell viability.

Dynamic compression tests performed at 0.1 Hz for 10 cycles indicate that the dynamic compression modulus was significantly greater (p<0.01) in genipin cross-linked gels (0.040 ± 0.003 MPa, n = 18) compared to uncross-linked gels (0.028 ± 0.003 MPa, n = 16).

After 5 weeks in culture, the cells secreted an extracellular matrix (ECM) that stained with Alcian Blue (Fig. 1a) and Safranin O (Fig. 1b), indicating the presence of cartilage specific proteoglycan production similar to the native cartilage tissue (Fig. 1d,e). Haematoxylin and Eosin (H&E) staining indicated that the cell morphology appeared more round like that of the native articular chondrocytes. The secreted ECM also stained positively for collagen II (Fig. 2a) and aggrecan (Fig. 2b) with little staining for collagen I (Fig. 2c). This staining was similar to that observed for control articular cartilage (Fig. 2d-f).

Real-Time RT-PCR (Fig. 3) indicated that encapsulated chondrocytes in genipin cross-linked gels showed an increase in Sox9, collagen II, collagen I and aggrecan expression over 2.5 and 5 weeks of culture (total of n=6). Significant differences were calculated for all genes at both time points and for both gel types (p<0.01).

H&E staining of the subcutaneous implants indicated that while there was some gel breakdown, there was no evidence of an immune response. A thin capsule formed in some circumstances but no immune cells were found near or within the material (data not shown).

DISCUSSION:

While many researchers are developing successful systems for articular cartilage tissue engineering, we have opted for an autologous gel system where the gel components (fibrinogen, thrombin) and cells can be isolated from the patient ahead of time allowing for complete biocompatibility and minimal risk of infection.

The fibrin gel and genipin cross-linking were relatively non-toxic to cells and the genipin also enhanced the mechanical properties of the gel. The encapsulated cells secreted an ECM that stained positively for collagen II and aggrecan with little staining for collagen I, indicating articular versus fibrocartilage matrix production. The chondrocytes also expressed very high collagen II compared to collagen I, which is indicative of the articular chondrogenic phenotype. We have also shown that the material is biodegradable and non-immunogenic. Therefore, modified fibrin hydrogels show potential as cellular scaffolds for articular cartilage tissue engineering. An in vivo orthopaedic model must now be developed to fully evaluate the potential of the fibrin gel.

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REFERENCES


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