Dense collagen/chitosan hybrid gels as scaffolds for cartilage tissue engineering

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Statement of Purpose: Articular cartilage exhibits limited potential for self-repair and current procedures for restoration include auto- and allo-graft replacements, which are frequently unsatisfactory (Mathew et al. Biomaterials, 2000;21:2589-2598). Tissue engineering offers the potential as an alternative approach to promote tissue repair by using three-dimensional scaffolds that mimic the extracellular matrix (ECM). The ECM is comprised of collagen (Coll) and glycosaminoglycans (GAGs), among other components, which play a key role in stimulating chondrogenesis (Li Z. Biomed. Mat. Res. A, 2005;75:485-493). Chitosan (CTS) is a natural polysaccharide that is structurally similar to GAGs. To date, scaffolds based on Coll/CTS hybrid hydrogels have exhibited low solid volume fraction when compared to native ECM attributable to their highly-hydrated nature (>99% fluid). An approach to engineer tissue-equivalent scaffolds can be achieved through controlled unconfined plastic compression (PC) (Brown et al. Adv. Funct. Mat. 2005;15:1762-1770). ECM-like scaffolds can be rapidly produced by removal of fluid from gels via PC. This study investigates the interactions of seeded chondrocytes and dense Coll/CTS hybrids by evaluating cell viability, proliferation, morphology, matrix contraction and GAG synthesis. The morphological and mechanical properties of the scaffolds were also investigated.

Methods: Sterile, rat tail tendon-derived type I collagen (2.11 mg/ml protein, First Link Ltd., UK) and ultrapure CTS with a 79.8% degree of deacetylation (Ultrasan™, BioSyntech, Canada) were used as starting materials. Two different Coll/CTS hybrid scaffolds with relative compositions of 2:1 and 1:1 were developed and compared to dense Coll scaffolds. Co-gelling of the two systems were prepared by neutralization with 5 N NaOH. Dense cellular scaffolds were prepared by seeding RCJ3.1C5.18 fetal rat calvarial cells ($2.4x10^3$ cells/ml pre PC) and cultured in complete α-minimum essential medium (Gibco-Invitrogen, USA) containing 1% penicillin/streptomycin (Gibco), 10^{-7} M dexamethasone (Sigma) and 15% v/v of fetal bovine serum (PAA Laboratories, CA). Scaffolds were allowed to gel for 30 mins at 37ºC, followed by PC (0.5 kPa for 5 mins), and cultured for 3 weeks.

Results: Cell viability within scaffolds was evaluated using confocal laser scanning microscopy of Live/Dead reagent-stained cells. Chondrocyte viability was maintained in all materials, immediately after gelation, PC and subsequent culture for up to day 21 (*Fig. 1*). Moreover, cells seeded in hybrid scaffolds showed cartilage-like nodules (indicated by white arrows), as early as day 7 after seeding, with spherical cell morphology. Cell metabolic activity was assessed by AlamarBlue™, which showed a significant increase up to day 5 in dense Coll/CTS scaffolds followed by a plateau at day 14. In contrast, the proliferation of cells seeded in dense Coll decreased post day 5. In addition, total GAG content (scaffold and surrounding medium) was quantitatively determined by biochemical analysis (*Fig. 2*). After 7 days in culture, significantly higher levels of total production of GAGs normalized to DNA were observed in the hybrids as compared to dense Coll scaffolds (*$p<0.05$; significantly different compared to Coll at the indicated time point using ANOVA followed by Tukey *post-hoc* test). These results were confirmed by histological sections stained with Safranin O/Fast Green.

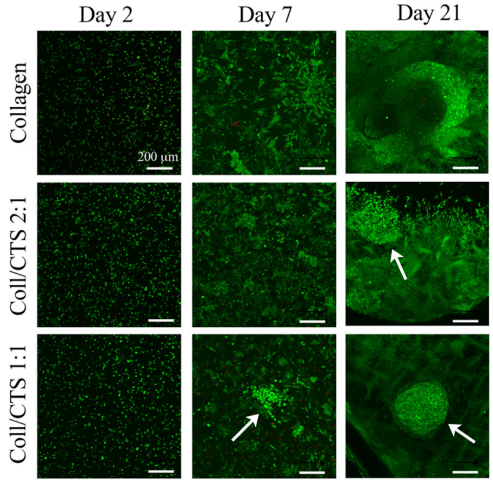

Fig. 1. Live/Dead cell assay showing cell viability and nodule formation up to day 21.

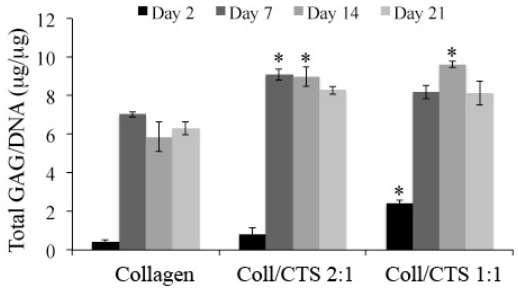

Fig. 2. Total GAG synthesis normalized to DNA content over 21 days.

Conclusions: Dense collagen/chitosan hybrid gels support nodule formation and GAG production without compromising cell viability and proliferation, indicating their suitability as ECM-like scaffolds for cartilage tissue engineering.

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