

Incorporating Decellularized Cartilage in Injectable Colloidal Gel Pastes: Evaluation of Cellular Response
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Statement of Purpose: Hydrogels are promising materials for cartilage regeneration. However, hydrogels are prone to leaking from the defect site if polymerized *in situ*.¹ Our research team is addressing this drawback by designing colloidal gel pastes that exhibit a yield stress prior to crosslinking.² We have already established gels that exhibit a yield stress by incorporating decellularized cartilage (DCC) particles in traditional methacrylated hyaluronic acid (MeHA) gels. Additionally, we have determined that incorporating DCC reduces gel swelling and increases the elastic modulus compared to MeHA controls. Although our group has also shown that DCC is a chondroinductive material for rat bone marrow stem cells (rBMSC), we have yet to test the cellular response to DCC in a hydrogel microenvironment. Therefore, we hypothesized that these DCC colloidal gels would facilitate cellular attachment and influence chondrogenic gene expression.

Methods: MeHA was prepared by reacting hyaluronic acid (57 kDa) with glycidyl methacrylate. DCC particles were fabricated from fresh porcine articular cartilage. The cartilage was then decellularized using alternating detergent washes and osmotic shocks. To make the colloidal gel solution, MeHA and DCC particles were mixed in 0.01M PBS containing 0.05% (w/v) Irgacure photoinitiator. Polymerized gels were fabricated by placing the gel solution in a mold between glass slides and exposing the molds to 312 nm UV light (Spectrolinker XL-100; Spectronics Corp.) for 15 min on each side. Rat bone marrow stem cells (P2) were seeded onto DCC gels at a density of 100,000 cells/cm² in a 96 well plate and fed with DMEM. One day after seeding, gel samples were removed from the well and cell attachment was observed with scanning electron microscopy (SEM). After 21 days of culture, cell attachment was quantified by DNA content using the PicoGreen Assay (Molecular Probes).

Results: SEM and bright field images indicated that gels incorporating DCC had increased cell attachment and promoted more cell growth overall than gels containing only MeHA (Fig. 1).

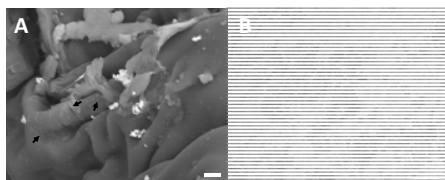


Figure 1. Cell Attachment on Hydrogel Formulations. A) SEM image of hBMSCs seeded on 12.5% MeHA/12.5% DCC gel (arrows indicate regions of cellular attachment). The scale bar is 10 μm. B) PlasDIC image of hBMSCs seeded on 25% MeHA gels after 1 day of culture. Scale bar is 100 μm.

Cells seeded on DCC-incorporated gels had a more flattened and spread out morphology than cells seeded on gels containing only MeHA. Analysis of the DNA content revealed that gels seeded on DCC incorporated hydrogels contained at least double the amount of DNA compared to control MeHA gels. The gels were seeded with 32,000 cells initially, which corresponds to approximately 0.27 μg DNA and the horizontal black line on the graph in Figure 2.

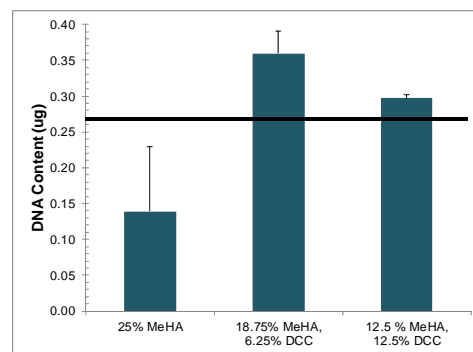


Figure 2. DNA content for Hydrogel Formulations after 21 days of culture. Data are reported as Mean ± SD.

Conclusions: Materials that can be easily implemented by physicians have the greatest potential to be successful in the clinic. In this regard, our team has fabricated colloidal gel pastes that exhibit a yield stress (paste-like behavior) while maintaining mechanical integrity *in vitro*. In this work we have shown that incorporating DCC into the gels allowed cells to spread out and attach to the hydrogel, which is an important first step in evaluating the use of these gels as cartilage scaffolds. Additionally, this increase in cell attachment occurred without the presence of incorporated cell adhesive peptides. We are currently in the process of investigating gene expression of cells encapsulated within DCC colloidal gel pastes. However, because we have previously shown DCC alone induces rBMSCs along a chondrogenic lineage (unpublished), we anticipate the DCC colloidal gels will do the same. Thus, DCC colloidal gels not only have the potential to be an easy-to-implement and moldable material that will stay in the defect site, but they may also induce chondrogenesis, making them a highly desirable material for cartilage defects.

References:

1. Rughani R. *Macromolecules*. 2010; 43: 7924-7930.
2. Wang Q. *Adv Mater*. 2008;20:236-239.