

Viability Characterization of CartiMax

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INTRODUCTION & BACKGROUND

CartiMax is a viable cryopreserved allograft putty with superior handling properties. This scaffold provides the foundation of matrix proteins, growth factors and adherent viable cells to facilitate articular cartilage repair of focal chondral defects. CartiMax consists of two components: Viable Cartilage Fibers and Cartilage Allograft Matrix (CAM). Viable cartilage fibers, contain viable chondrocytes within the cartilage matrix fibers and these fibers are cryopreserved to maintain optimal viability. CAM is lyophilized cartilage extracellular matrix that includes key endogenous growth factors and proteins. In this study, samples of cryopreserved viable cartilage fibers were thawed, rinsed of cryopreservation media and assessed for cartilage viability through live/dead staining, and assessing cell function through explant cell culture and the production of hyaline components.

CELL VIABILITY

To determine cell viability, samples of cryopreserved viable cartilage fibers were thawed as per the package insert. The viable cartilage fibers were subjected to an enzymatic digestion to isolate chondrocytes. The chondrocytes were then stained using the Live/Dead Viability Toxicity kit (Molecular Probes, Invitrogen). Stained sections were imaged under 10x magnification using a fluorescent microscope (Leica Microsystems). Viable (green stained) and dead (red stained) cells were counted in each field to establish the percentage of viable cells. Figure 1 illustrates a Viable Cartilage Fiber sample with 86.6% cell viability.

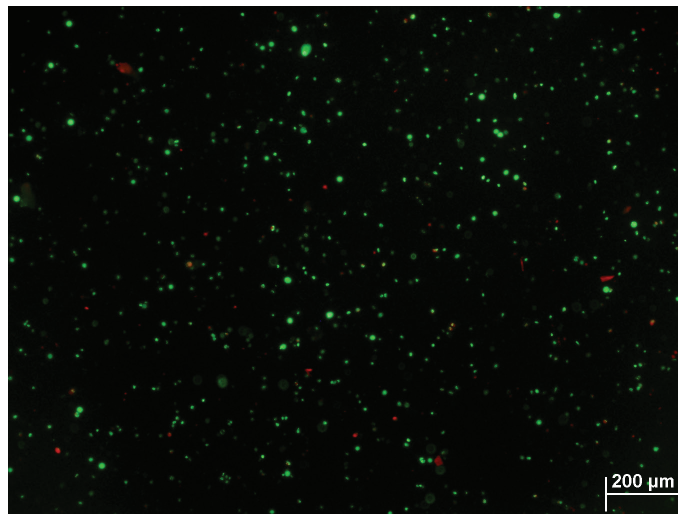


Figure 1. Representative live/dead staining image of viable chondrocytes. The percentage viability is 86.6%.

CELL MIGRATION & PROLIFERATION

To further evaluate extended shelf-life functionality, samples of cryopreserved viable cartilage fibers were stored for six months in vapor phase liquid nitrogen followed by six months in a -70°C freezer.

Those samples were then thawed per the package insert and cartilage fibers were placed onto tissue culture treated plates with culture medium (DMEM basal media with glucose). The media was exchanged every fourth day to allow cell migration and cell expansion. Figure 2 illustrates cells migrating from the cartilage fiber onto the tissue culture plate surface and cell proliferation occurring until confluency was attained.

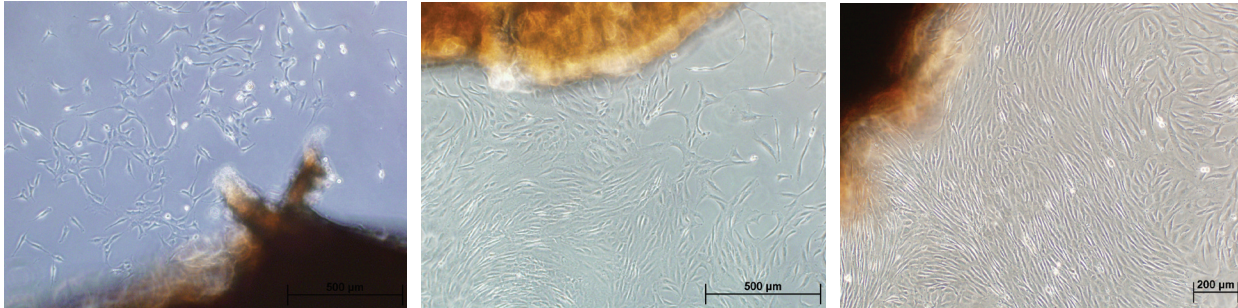


Figure 2. Viable chondrocytes are migrating from the cartilage fiber explant and proliferating confirming that the viable cells are functional post-thaw. (A) Chondrocytes migrating and growing out of the cartilage fiber (day 14), (B) cells proliferating (day 21) and (C) confluency attained (day 24).

PRODUCTION OF HYALINE COMPONENTS

To assess if the cells migrating out of the cartilage fibers are functional chondrocytes that can produce hyaline components, the explanted, confluent cells were trypsin-treated and passaged to expand cell number. Cell pellets were made by centrifuging 750,000 cells/0.5mL media in 15mL tubes and culturing for four days¹⁻³. The pellets were then transferred to 24-well non-coated plates to allow the pellets to grow. Pellets were fixed at day 14 with 10% formalin and stained for H&E, collagen type II and proteoglycans (safranin-O) at Premier Laboratories, Inc. (Boulder, CO).

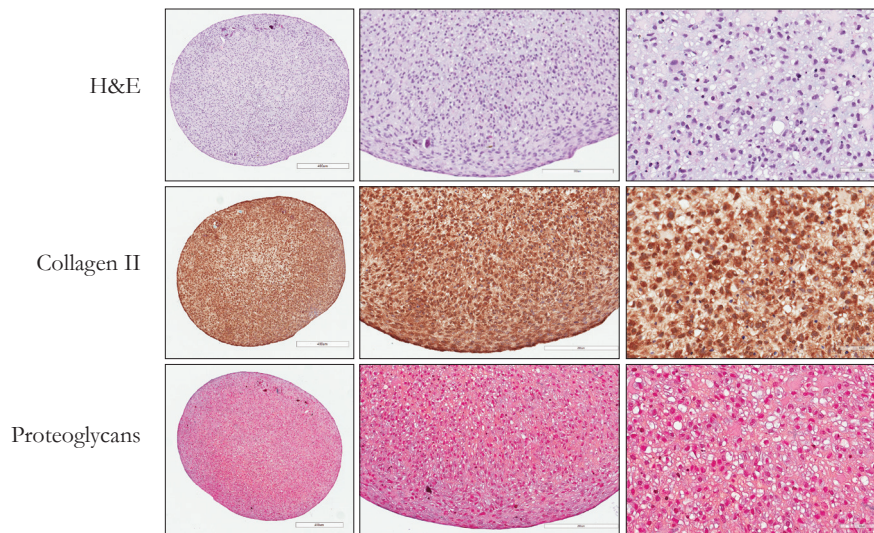


Figure 3. The viable cells that migrated and proliferated from the cartilage fiber explants are functional chondrocytes producing collagen type II and proteoglycans by day 14. These are classic hyaline components found in articular cartilage.

DISCUSSION

The data generated in this study demonstrates that cells from cryopreserved viable cartilage fibers are viable, have the ability to migrate out of the cartilage fiber and proliferate. They are also producing collagen type II and proteoglycans, which are components of hyaline cartilage. This study verifies that CartiMax contains functional, viable chondrocytes that secrete components found in articular cartilage.

REFERENCES

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