

Introduction

Cartiform is a cryopreserved viable osteochondral allograft with pores and a reduced bone portion compared to traditional fresh stored osteochondral allografts. Cartiform contains tissue native viable chondrocytes, chondrogenic growth factors, and extracellular matrix proteins within the superficial, transitional, and radial zones of hyaline cartilage. Cartiform serves as an active biological matrix to promote articular cartilage repair to treat focal chondral defects. A detailed scientific characterization of Cartiform will be presented here.

Viable Chondrocytes are Critical to Repair

Cartiform is unique as it contains tissue native viable chondrocytes within a dense extracellular matrix (ECM), predominantly type II collagen and proteoglycans. Chondrocytes are integral in producing the dense ECM that provides the biomechanical stability necessary for withstanding high levels of physical stress and force, especially within high load-bearing joints such as the knee (Beccera et al., 2010). In addition to producing ECM, chondrocytes also express paracrine factors to promote chondrogenesis (Fischer et al., 2010). To protect this critical functionality, Cartiform manufacturing employs a proprietary process to preserve cell viability.

Cellular Composition of Cartiform

Confirmatory studies have shown the presence of chondrocytes within Cartiform through Cluster of Differentiation (CD) surface markers. CD markers are important tools for identifying and characterizing different types of cells. The cellular composition of Cartiform was assessed through an analysis of three key cell surface markers. CD44 is a marker that is consistently expressed on chondrocytes. CD44 is a hyaluronan receptor and is integral in mediating chondrocyte interactions with ECM (Grogan et al., 2007). CD49e (integrin α 5) is an ECM receptor found within chondrocyte populations that enables the chondrocytes to bind to fibronectin and fibrinogen (Diaz-Romero et al., 2005). Cartiform was also analyzed for the absence of CD45, a universal marker present on all hematopoietic cells, to ensure no contamination of potentially immunogenic cells within the product. Cells within Cartiform were analyzed in vitro using fluorescence activated cell sorting (FACS) for the presence of CD44 and CD49e and for the absence of CD45 to confirm the identity of chondrocytes and purity of the product (Figure 1).

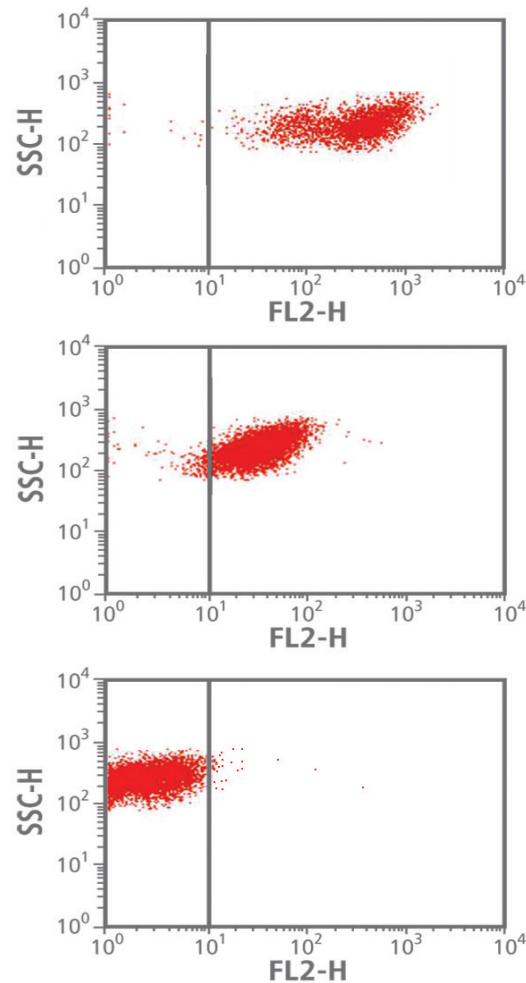


Figure 1. Analysis of cells from Cartiform demonstrating chondrocyte identity and purity. Representative FACS dot plots show the expression of CD44 (top) and CD49e (middle) cell surface markers and the absence of CD45 cell surface markers (bottom).

Cell Viability in Cartiform

Every lot is tested post-thaw to ensure that viable cells are delivered at the time of implantation. **Figure 2** illustrates *in situ* fluorescent staining showing the high density of viable chondrocytes and preservation of cellular organization within Cartiform.

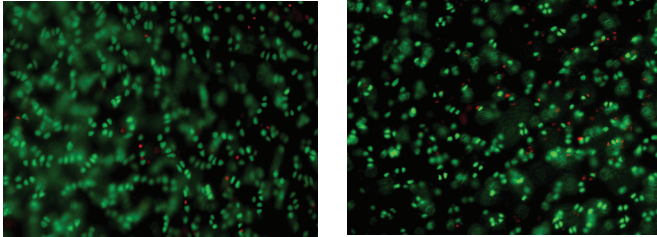


Figure 2. Live/dead fluorescent staining of Cartiform. Left: fresh Cartiform prior to cryopreservation; Right: thawed final product. Live cells are stained with a fluorescent green cytoplasmic dye, and dead cells are stained with a fluorescent red dye.

Cartiform Preserves Unique Microstructure of Hyaline Articular Cartilage

Hyaline cartilage is organized in a unique structure that supports its function of lubrication and load-bearing capacity within joints. This complex organization includes three distinct layers within the cartilage tissue – the superficial, transitional (middle) and radial (deep) zones. These layers exhibit variations in extracellular matrix biochemical composition and cell morphology, which display different biomechanical properties and rates of cellular activities supporting normal articular cartilage function (Quinn et al, 2005). Cartiform preserves the microstructural organization of normal articular cartilage to promote proper repair (**Figure 3**).

Cartiform Contains Key Extracellular Matrix Proteins

While the general structural organization of the ECM within Cartiform was confirmed to closely resemble that of native articular cartilage through H&E staining, the exact contents of the ECM within Cartiform were further investigated by measuring ECM protein content within Cartiform extracts using Enzyme-Linked Immunosorbent Assays (ELISAs). Hyaline articular cartilage ECM is composed primarily of aggrecan, hyaluronan (HA), and type II collagen, a unique combination that gives cartilage its tensile

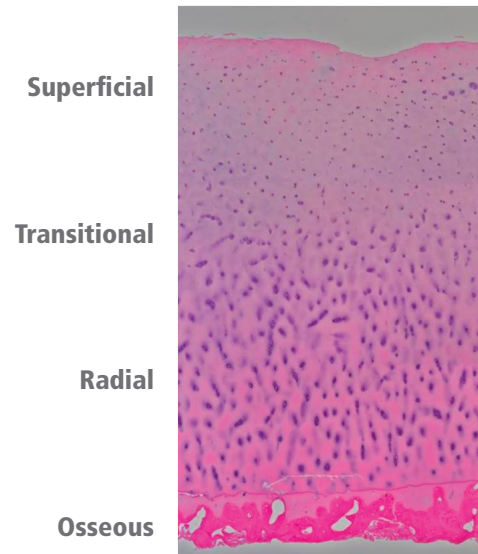


Figure 3. Structural Organization of Cartiform. Cartiform preserves the microstructure of 3 distinct cartilage zones (superficial, transitional or radial) and an osseous layer as evident on histological staining (H&E).

strength (due to collagen type II) and load absorption capabilities (due to HA and aggrecan complex). On the microscale, the ECM serves as a scaffold for cellular attachment, mediates cell-cell interactions, and acts as a reservoir for growth factors. To investigate the ECM composition within Cartiform, extracts were prepared by homogenizing cryopreserved Cartiform with PBS, centrifuging the homogenate, and collecting the supernatant. Analysis of protein content within the Cartiform extracts via ELISA revealed that Cartiform contains an abundance of aggrecan, hyaluronan, and type II collagen, the major components of healthy hyaline cartilage. Therefore, the ECM of Cartiform has the desired components to serve as an ideal scaffold for cartilage repair.

Cartiform Promotes Cartilage Repair

Bone marrow stimulation results in fibrocartilage repair tissue, which is predominantly made up of type I collagen. This repair tissue has poor biomechanical performance and leads to poor long-term clinical outcomes (Mithoefer et al., 2009). To enhance clinical outcomes of bone marrow stimulation, Cartiform contains factors that promote the formation of type II collagen-rich hyaline cartilage. Studies to demonstrate its chondrogenic potential are described below.

Cartiform Expresses Chondrogenic Factors

Growth factors are integral in creating the optimal microenvironment for effective cartilage repair (Fortier et al., 2011). Factors that regulate chondrocyte functionality in healthy cartilage also serve to recruit host mesenchymal stem cells (MSCs) to the repair site following injury and are capable of promoting chondrogenesis and matrix production within the host MSCs. To confirm that Cartiform provides the proper microenvironment to promote these functions, Cartiform was investigated by analyzing Cartiform extracts and cultured supernatants using ELISAs. Extracts were prepared as described above, and cultured supernatants were obtained by collecting media from Cartiform cultures at 7, 14, and 21 days. Protein content was measured in both the extracts and the cultured supernatants using ELISA and the results serve as estimates of the total protein content within Cartiform and the protein secretion from Cartiform into the surrounding microenvironment that can be expected *in vivo*. Growth factors detected in Cartiform are provided in **Table 1**. Results indicate that Cartiform expresses a variety of chondrogenic factors necessary for repair. Furthermore, Cartiform has the ability to produce and sustain chondrogenic growth factor levels over time due to the presence of viable chondrocytes and dense ECM. This is demonstrated in **Figure 4**, which shows ongoing release of the chondrogenic growth factor, TGF- β 1, over the three-week duration of the experiment.

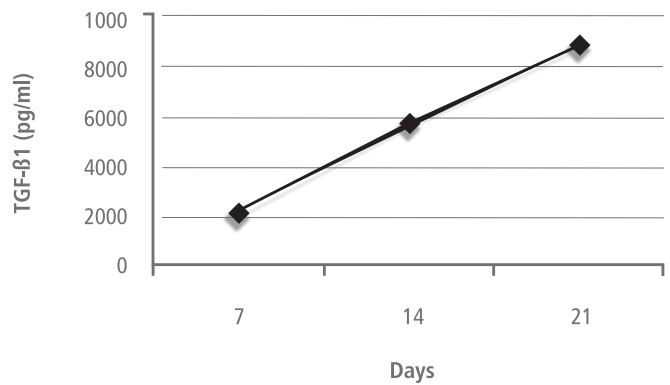


Figure 4. Cartiform releases TGF- β 1 through three weeks.

Cartiform Promotes Host MSC Recruitment and Migration

Host MSCs are key to tissue regeneration following bone marrow stimulation surgery. Published reports have demonstrated the importance of host MSCs in proper cartilage repair, primarily through differentiation of MSCs into functional chondrocytes (English and Islam, 2009), as well as by regulating production of ECM by chondrocytes (Bian et al., 2011). To demonstrate that Cartiform can actively recruit host MSCs to support better cartilage repair, an *in vitro* chemotaxis assay was performed. Briefly, MSCs were plated onto the surface of transwell filters and stimulated by conditioned media produced by Cartiform adherent to the bottom of the chamber. DMEM was used as a negative control and DMEM +10% FBS was used as a positive control. Cells that migrated onto the underside of the filter were visualized by staining with a cellular dye. As **Figure 5** depicts, conditioned media from Cartiform recruits MSCs to migrate across the boundary (filter) to an extent comparable to the positive control.

Chondrogenic Factors	Function
TGF- β 1, 3	Promotes chondrogenic differentiation and regulates type II collagen expression
BMP-2, 4, 7	Induces chondrogenesis of MSCs and stimulates ECM production by chondrocytes
bFGF	Stimulates proliferation of chondrocytes
IGF-1	Induces ECM synthesis

Table 1. Chondrogenic factors present within Cartiform

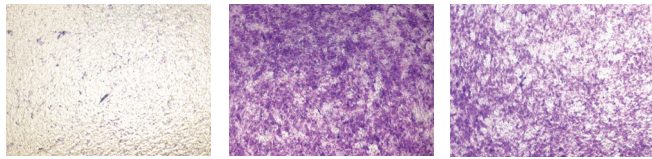


Figure 5. Cartiform induced recruitment and migration of MSCs. Representative images of MSC chemotaxis studies demonstrating the chemoattractive effects of Cartiform on MSC migration. (Left) Negative control illustrating no migration. (Center) Positive control and (Right) Cartiform demonstrating active MSC migration at comparable levels.

Cartiform Induces Chondrogenesis

Chondrogenesis of host MSCs is critical for effective bone marrow stimulation-induced cartilage repair; however, MSC differentiation into chondrocytes following bone marrow stimulation is ineffective, leading to inferior fibrocartilage repair. To determine if factors from Cartiform promote chondrogenesis of MSCs, Cartiform was tested in an established *in vitro* model of chondrogenesis. Briefly, MSC pellets were cultured with or without Cartiform for 21 days and analyzed for type II collagen staining via immunohistochemistry. **Figure 6** demonstrates that Cartiform induces chondrogenesis of MSCs as indicated by strong type II collagen staining. Together these data show that Cartiform has the ability to stimulate chondrogenesis.

Summary

Cartiform is an osteochondral allograft composed primarily of viable chondrocytes within a type II collagen matrix that structurally resembles native articular cartilage. Cartiform provides all the growth factors and ECM proteins necessary to recruit and promote chondrogenesis of the host MSCs that enter the defect site following bone marrow stimulation surgery. As such, the use of Cartiform in conjunction with bone marrow stimulation surgery is expected to result in hyaline articular cartilage repair tissue rather than fibrocartilage.

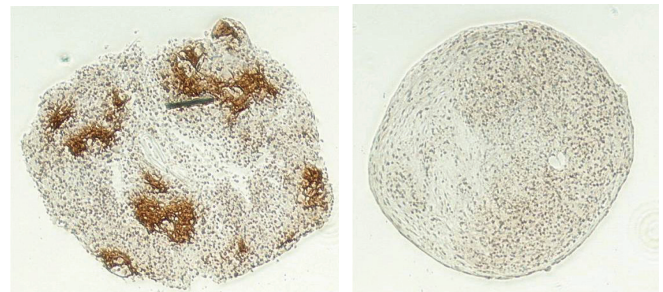


Figure 6. Cartiform induces chondrogenesis of MSCs. MSC pellets were co-cultured with Cartiform (left) or media alone (right) for 21 days and analyzed for type II collagen staining. MSC pellets co-cultured with Cartiform display positive staining for type II collagen, indicating MSCs have differentiated into chondrocytes.

References

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