

## Introduction

Cartiform is a cryopreserved viable osteochondral allograft designed to treat focal chondral defects. Cartiform is processed to preserve all cartilage components in their native state including endogenous viable chondrocytes. Chondrocytes within hyaline cartilage produce extracellular matrix (ECM) proteins and growth factors that maintain tissue integrity and support chondrogenesis required for cartilage repair.<sup>1,2</sup> Cell viability in fresh stored (4°C) allograft cartilage decreases over time and limits the shelf life of fresh stored products to approximately 30 days.<sup>3</sup> To address this limitation, different preservation methods have been developed, but preservation of endogenous viable chondrocytes in cartilage remains challenging. We developed Cartiform, a cryopreserved osteochondral allograft, in which endogenous viable chondrocytes are successfully retained. The goal of this study was to assess cell viability in Cartiform stored at -80°C for up to two years.

## Methods

Cell viability within fresh and cryopreserved Cartiform from three donors was evaluated using live/dead cell-staining. Cryopreserved units stored for both short (<1 week) and long (>2 years) periods of time at -80°C were thawed and tested for each donor. A scalpel blade was used to cut thin tissue sections from the superficial end of each Cartiform unit. The sections were incubated with a live/dead viability/cytotoxicity staining solution (Molecular Probes, Inc., Eugene, OR) according to the manufacturer's instructions. Stained sections were photographed at 100x magnification using a fluorescent microscope. Viable and dead cells were counted as indicated by either green or red fluorescence, respectively, within three random fields of the known area (0.38 mm<sup>2</sup>). The counts from the three fields were averaged and the percentage of viable cells was calculated for each unit. Comparisons between groups were made using Student's t-test with significance at  $p < 0.05$ .

## Results

On average, fresh Cartiform contained 83.4% viable cells, cryopreserved Cartiform that was stored at -80°C for less than one week contained 74.7% viable cells, and cryopreserved Cartiform that was stored at -80°C for greater than two years contained 72.6% viable cells. No statistically significant differences in cell viability between fresh and cryopreserved Cartiform samples were found. Representative images of live/dead stained Cartiform sections are shown in **Figures 1 and 2**.

## Discussion

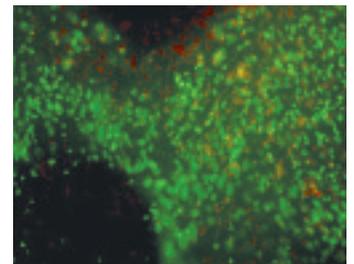
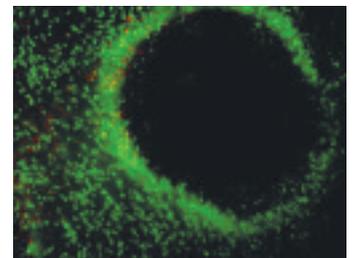
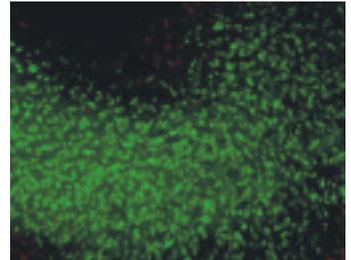
Experimental data from three donors confirm retention of endogenous viable cells within Cartiform after storage at -80°C longer than two years. Upon implantation, the viable chondrocytes within Cartiform are expected to aid in the repair process through ECM production, growth factor secretion and induction of MSC (Mesenchymal Stem Cell) chondrogenesis.

## Significance

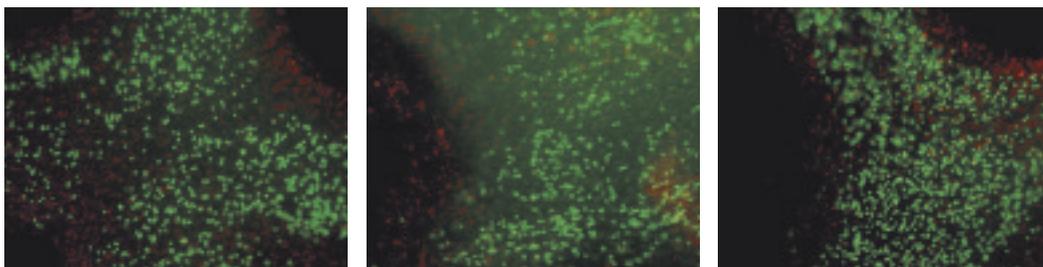
Commercially available fresh stored cartilage allografts (osteochondral allografts and particulated juvenile allograft cartilage) have a limited shelf life due to the decline in cell viability with time.<sup>3</sup> In contrast, Cartiform preserves endogenous chondrocytes that remain viable after two years of storage at -80°C. The ability to retain cellular integrity within fresh tissue over a prolonged storage time is a unique feature of Cartiform. Cartiform provides surgeons with viable allograft cartilage that is available on demand.

## References

- 1 Becerra J, Andrades JA, Guerado E, Zamora-Navas P, Lopez-Puertas JM, Reddi AH. Articular cartilage: structure and regeneration. *Tissue Eng Part B Rev.* 2010;16(6):617-627.
- 2 Fischer, J, Dickhut A, Rickert M, Richter W. Human articular chondrocytes secrete parathyroid hormone-related protein and inhibit hypertrophy of mesenchymal stem cells in coculture during chondrogenesis. *Arthritis Rheum.* 2010;62(9):2696-2706.
- 3 Williams, RJ 3rd, Drees JC, Chen CT. Chondrocyte survival and material properties of hypothermally stored cartilage: an evaluation of tissue used for osteochondral allograft transplantation. *Am J Sports Med.* 2004;32(1):132-139.



**Figure 1.** Live (green) and dead (red) cell-staining of Cartiform units derived from one donor. Images show: fresh Cartiform, prior to cryopreservation (top), cryopreserved Cartiform, post-thaw after six days storage at -80°C (middle), and cryopreserved Cartiform, post-thaw after 2.7 years storage at -80°C (bottom).



**Figure 2.** Live (green) and dead (red) cell-staining of cryopreserved Cartiform units derived from three different donors, post-thaw after >2 years storage at -80°C.