Cartiform®
Viable Osteochondral Allograft

Cartiform® Promotes Mesenchymal Stem Cell Migration and Chondrogenesis

Osiris Therapeutics, Inc.

Introduction

Host mesenchymal stem cells (MSCs) are the key to cartilage regeneration following bone marrow stimulation. Published reports have demonstrated the importance of MSCs in proper cartilage repair through differentiation into functional chondrocytes and by stimulating synthesis of ECM by chondrocytes.[1-4] Cartiform is a cryopreserved viable osteochondral allograft that can be implanted following bone marrow stimulation. When used with marrow stimulation, the growth factors and extracellular matrix (ECM) proteins secreted by Cartiform may increase host MSC migration into the lesion and to enhance chondrogenesis of the recruited MSCs. The goal of this *in vitro* study was to assess the effects of Cartiform on MSC migration and chondrogenesis.

Methods

MSC migration was evaluated using a chemotaxis assay. MSCs were placed on the top of a transwell filter with 8 µm pores and the cells were allowed to migrate for 4 hours into basal media (negative control), basal media + 10% FBS (positive control), or Cartiform-derived conditioned media. The conditioned media was generated by culturing Cartiform in basal media for 7 days. The MSCs that had migrated to the bottom of the transwell filter after the 4 hour culture were fixed and stained in a 4% paraformaldehyde/ 1% gentian violet solution and analyzed using light microscopy. MSC chondrogenesis was evaluated using a MSC pellet and Cartiform co-culture assay. MSCs were centrifuged to form pellets and cultured in basal media. Cartiform was added to the culture so it was submerged in the media but not in direct contact with the MSC pellet. Cell pellets cultured without Cartiform served as negative controls. After 3 weeks, the MSC pellets were fixed, sectioned and stained for type II collagen, a marker of chondrogenesis.

Results

Cartiform-conditioned media induced MSC migration across the transwell filter **Figure 1.** Without the presence of chemoattractive factors, no MSC migration was observed (negative control). In the presence of FBS, a known MSC chemoattractant, a high number of MSCs migrated across the filter (positive control). These results confirm that the conditioned media contained MSC chemoattractive factors secreted by Cartiform. MSC pellets that were co-cultured with Cartiform were positive for type II collagen, indicating that Cartiform released factors that induced MSC chondrogenesis **Figure 2.** In contrast, MSC pellets that were cultured in basal media alone were negative for type II collagen.

Discussion

Successful articular cartilage repair following marrow stimulation requires MSCs to migrate to the lesion site and undergo chondrogenesis. This process is mediated by the presence of chondrogenic growth factors and ECM proteins provided by healthy cartilage surrounding the lesion. When these signals are lacking, the MSCs often fail to differentiate into chondrocytes and fibrocartilage is generated instead of hyaline cartilage. Cartiform contains allograft articular cartilage that secretes these factors and was found to induce MSC migration and chondrogenesis *in vitro*. These data indicate that Cartiform has the potential to recruit MSCs and induce chondrogenesis after implantation into a cartilage lesion following bone marrow stimulation *in vivo*, a requirement for the generation of hyaline cartilage.

Significance

This study demonstrates that Cartiform, a cryopreserved viable osteochondral allograft, secretes MSC chemoattractive and chondrogenic factors, which are critical for articular cartilage repair following a marrow stimulation procedure.

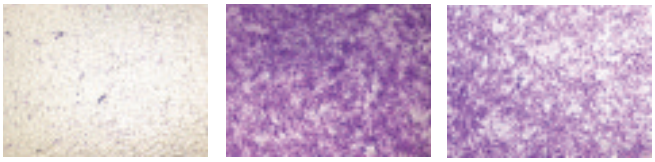

Figure 1. *Cartiform-derived soluble factors recruit MSCs. Representative images of gentian violet-stained MSCs that migrated to the bottom side of the transwell filters after a 4 hour culture. Negative control (basal media) does not support migration (left). Positive control media supplemented with FBS (middle) and Cartiform-conditioned media (right) induce MSC migration in comparable magnitudes.*

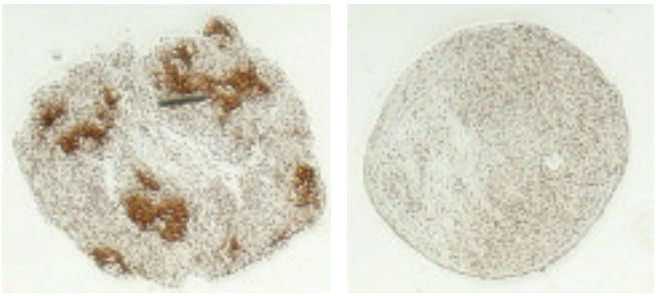

Figure 2. *Soluble factors released from Cartiform induce MSC chondrogenesis. Representative images of type II collagen staining of MSC pellets after culture with Cartiform (left) or media alone (right). MSC pellets co-cultured with Cartiform are positive for type II collagen (brown staining), indicating MSC differentiation into chondrocytes.*

References

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