Cellular Characterization of CartiMax

MTF Biologics, Research and Development Department

125 May Street, Edison, NJ 08837 USA

INTRODUCTION

CartiMax is a viable cryopreserved allograft putty with superior handling properties. This scaffold provides the foundation (matrix proteins, growth factors and adherent viable cells) to facilitate articular cartilage repair in treating focal chondral defects. CartiMax consists of two components: Viable Cartilage Fibers and Cartilage Allograft Matrix (CAM). Viable Cartilage Fibers, contain cryopreserved native, adherent viable chondrocytes within the cartilage matrix fibers. CAM is lyophilized cartilage extracellular matrix that includes key endogenous growth factors and proteins. In this study, samples of Viable Cartilage Fibers were thawed, digested to isolate cells, and stained with antibodies for specific cell surface markers present on the chondrocytes. Typically chondrocytes express CD44 and CD49e, but do not express CD45 (which is a blood cell marker). Flow cytometry with florescence activated cell sorting (FACS) was utilized to determine the specific cell populations. CD44 plays an important role in chondrogenesis and previous flow cytometry analysis showed that CD44 was expressed at significantly higher levels in chondrocytes with higher chondrogenic capacity.[1] It has also been found that chondrocytes express the cell surface marker CD49e (integrin α5), which is a primary chondrocyte fibronectin receptor. Integrins also play a key role in regulating cell proliferation, survival, differentiation, and matrix remodeling.[2, 3]

CELLULAR CHARACTERIZATION

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 the adherent cells. The isolated cells were re-suspended in a staining buffer containing bovine serum albumin (BSA), and stained with CD44 PE-Cy7 (BD Biosciences), CD49e PE (BD Biosciences), and CD45 APC (AbD Serotec) fluorescent antibodies to verify the presence of chondrocyte surface markers. The cells were incubated on ice (protected from light) for 30 minutes to allow the antibodies to attach. Then, the cells were washed and re-suspended in fresh buffer and kept on ice (maximum of 1 hour) until analysis with flow cytometry. *Figure 1* reveals that the isolated cells strongly express CD44 and CD49e, and do not express CD45.

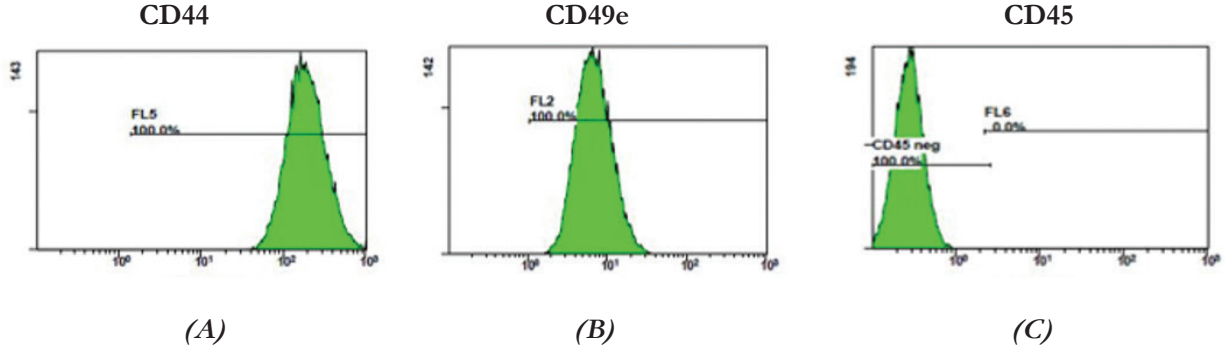

Figure 1. *FACS analysis of cells from viable cartilage fibers demonstrate, (A) presence of CD44 cell surface markers, (B) presence of CD49e cell surface markers, and (C) the absence of CD45 cell surface markers.*

Cell Surface Markers	Present
CD44	+
CD49e	+
CD45	-

Table 1. *Cell surface marker analysis of viable cartilage fibers verify they are CD44(+) and CD49e(+), and CD45(-). Presence of cell marker (+); Absence of cell marker (-).*

DISCUSSION

The data generated in this study demonstrated that the isolated cells from cryopreserved viable cartilage fibers expressed CD44 and CD49e cell surface markers, and did not express CD45. Chondrocytes express CD44 and CD49e[1-2]. This study verifies that the cells present on the viable cartilage fibers are a homogenous population of chondrocytes.

REFERENCES

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