# Differential MHC Class Receptor Expression in *In Vitro* Human Mesenchymal Stem Cells is Mediated by Scaffold Material

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INTRODUCTION: MSCs have long been considered immunoprivileged, with the ability to down-regulate the immune response of allogeneic and even xenogeneic hosts [1,2]. However, more recent studies have found that mesenchymal stem cells harvested from bone marrow are highly heterogeneous and expression profiles of major histocompatibility complex (MHC) proteins vary not only between subjects but between different harvests of the same subject[3]. Certain of these MHC receptors in human cells have been classified as immunosuppressive (MHC Class Ib) while others have been classified as immunogeneic (MHC Class Ia, and MHC Class II)[4]. We have previously demonstrated differential expression of these receptors by human MSCs in tissue engineered constructs based on their location within the constructs[5,6]. The role of cell encapsulating scaffold materials in modifying the basal expression of these molecules has not been explored. This study aims to determine the effects of different scaffold materials on the expression of both immunogenic and immunosuppressive MHC class receptors by human MSCs in tissue engineered constructs.

METHODS: *Cell Culture:* Human MSCs (RoosterBio, Inc) at population doubling level (PDL) 7-9 were cultured in hBM-MSC High Performance Media (RoosterBio, Inc) to PDL 14-15 before being harvested for construct manufacture. *Construct Manufacture:* To create the alginate discs, 3% alginate was seeded with hMSCs ($25x10^6$ cells/ml), crosslinked using 2% $CaSO_4$, and injected between two glass plates. Discs 6 mm in diameter were cut from the sheet gel and placed in wells of a 24-well plate. Collagen discs were created by pipetting 500 µl of type I collagen gel (4 mg/ml) seeded with hMSCs ($1x10^6$ cells/ml) into wells of a 24-well plate. The constructs were cultured for 2 weeks in DMEM media with 10% FBS, 2.5% HEPES, and 1% antibiotics. Discs were collected at days 4, 7, 9, 11, and 14 for histological processing. *Analysis:* Immunohistochemistry (IHC) was performed to determine the expression of MHC class markers Ia, Ib, and II. Slides were counterstained with DAPI to identify nuclei. IHC images were converted to faux color images and overlaid with DAPI images to determine co-localization.

RESULTS: After 14 days in culture, the expression of MHC Class receptors on the surface of the human MSCs in the different scaffold materials had diverged (Figure 1A). The cells within the alginate constructs expressed higher levels of the Class Ia receptor which is immunogenic, as well as higher levels of the Class Ib receptor which is immunosuppressive. MSCs within the collagen constructs expressed higher levels of Class II receptors which are immunogenic. Time sequence images show the change in the expression of the Class II receptor in both alginate constructs and collagen constructs from day 4 to day 14 (Figure 1B). From day 4 to day 14, the expression of the immunogenic Class II receptor decreases in the alginate constructs while increasing significantly in the collagen constructs.

DISCUSSION: These results indicate that the scaffold material in which MSCs are cultured has an effect on the expression of MHC class receptors. Collagen, in comparison to alginate, had a greater up regulation of an immunogenic receptor at day 14 and cells encapsulated in collagen had increased expression of the immunogenic receptor Class II over time. This may be due to increases in cell adhesion and cell spreading between days 4 and 14.

SIGNIFICANCE: These data demonstrate that the immunological profile of human MSCs can be manipulated using appropriate scaffold materials. This sets the stage for the design of biomaterials specifically engineered to modify MHC Class receptor expression.

REFERENCES: [1] Leveque+, *J. Cell.Mol.Med.*, 2015 [2] Rasmusson+, *Exp. Cell Res.*, 2006 [3] Schnabel+, *Stem Cell Res.Ther.*, 2014 [4] Huang+, *Circulation*, 2010 [5] Hudson+, *ORS 2015*, 2015 [5] Hudson+ (2), *ORS 2015*, 2015

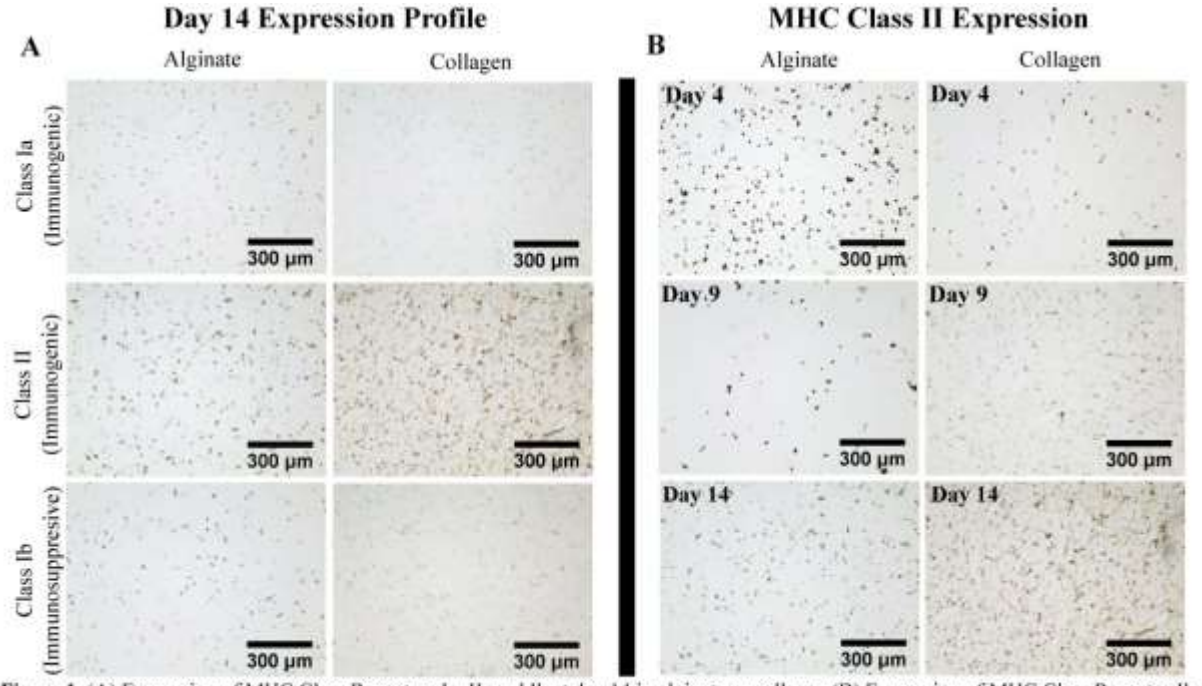


**Figure 1.** (A) Expression of MHC Class Receptors Ia, II, and Ib at day 14 in alginate or collagen (B) Expression of MHC Class Receptor Ib at days 4, 9, and 14 in alginate and collagen