Selective Adhesion of Mesenchymal Stem Cells to Extracellular Matrix Molecules
Janice O’Sullivan, Cynthia M. Coleman, Mary Murphy and Frank Barry
Corresponding author: frank.barry@nuigalway.ie
Regenerative Medicine Institute, National University of Ireland, Galway, Ireland

Introduction Arthritis is a chronic disease of the joints characterized by progressive destruction of articular cartilage. As cartilage is composed primarily of extracellular matrix (ECM) with limited capacity for self-repair, the therapeutic application of mesenchymal stem cells (MSCs) offers potential to regenerate healthy cartilage. It was therefore hypothesized that a therapeutic, chondrogenic population of MSCs within the bone marrow can be isolated by their adhesion to cartilaginous ECM proteins.

Methods One day before MSC isolation, culture flasks were coated with either hyaluronan (HA) or chondrotin sulphate (CS) overnight. Bone marrow from the iliac crest of consenting human donors was exposed to uncoated, HA coated or CS coated plates for 24 hours to isolate early adherent MSCs or for 5 days as per traditional methods. Confluent MSCs were further expanded on uncoated plastic. At P2, MSCs were induced to differentiate using previously reported methods.

Results All isolated cells retained fibroblastic morphology and had comparable expansion characteristics. Chondrogenic differentiation of MSCs was enhanced in all ECM isolated groups when compared to the traditionally isolated controls as indicated by an increase in GAG/DNA content (Fig 1). Pellet size and Safranin-O staining intensity increased in pellets derived from ECM isolated cells. Adipogenic differentiation was significantly increased in all ECM isolated MSCs, especially in the early adherent HA and CS isolated cells (Fig 2A). Osteogenic potential was enhanced in all ECM selected MSCs with the largest increase in calcium deposition in cells adherent to CS after 24 hour exposure (Fig 2B).

Discussion The development of therapies that promote chondrogenesis is of particular importance due to the limited capacity of cartilage to repair. Cartilaginous ECM molecules, HA and CS, were utilized to isolate both early and late adherent MSC subpopulations from the bone marrow. Early ECM adherent cells had greater capacity for tri-lineage differentiation suggesting cells expressing receptors for HA or CS may be more potent progenitors. Specific isolation of these subpopulations for clinical application will enable a reduction in the number of MSCs required for clinical efficacy and potentially enhanced tissue regeneration.

Disclosures The authors have nothing to disclose.

Fig 1: Chondrogenic capacity of HA and CS isolated MSCs. Chondrogenesis was assessed using DMMB and picogreen assays to quantify the GAG:DNA ratio of each pellet. Histological analysis by Safranin O staining confirmed enhanced deposition of GAGs in the ECM.

Fig 2: Adipogenic (A) and osteogenic (B) differentiation of HA and CS adherent hMSCs indicating a significant increase in adipogenic potential in early adherent cells, especially those bound to HA (A). Increased osteogenic potential in both HA and CS isolated cells (B).