# The Effect of Chondroitin Sulfate Glycosaminoglycan Substrates on the Immunosuppressive Capacity of Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs) are multipotent stem cells that possess promising therapeutic potential for immunomodulation, tumor targeting, and regenerative medicine. The therapeutic potential of MSCs is largely regulated through paracrine signaling mechanisms including binding interactions with the extracellular matrix (ECM). Since sulfated glycosaminoglycans are an important ECM constituent in the bone marrow stem cell niche with binding affinities reliant on sulfation patterns, we hypothesized that collagen bound chondroitin sulfate glycosaminoglycans (CS-GAGs) possessing different sulfation patterns, will induce changes in MSC immunosuppressive potency and morphology. Collagen provides distinct binding sites for CS-GAGs, hence we employed a layer-by-layer strategy to immobilize CS-GAGs to the underlying collagen substrates, and assessed the effects of mono-sulfated CS-GAGs (CS-A) and over-sulfated CS-GAGs (CS-E) on MSC Indoleamine 2,3-dioxygenase (IDO) activity. Standard tissue culture plates were coated with collagen 50ug/mL overnight prior to CS-GAG coating. High (2mg/mL) and low (1mg/mL) concentrations of CS-GAGs diluted in PBS were overlaid on top of the collagen coating overnight. Then, MSCs were cultured on CS-GAG immobilized culture plates for 24 hours in culture media supplemented with 50ng/ml INF-γ. MSCs from multiple donors were used to detect any donor-to-donor variability. IDO assay was used to analyze how much immunomodulated activity took place through the detection of kynurenine (KYN) production. MSC morphology on collagen bound CS-GAG substrates was imaged using *Array Scan VTI HCS Reader* and analyzed using Partial Least Squares Discriminant analysis (PLS-DA). Our study demonstrated that IFN-γ stimulated MSCs cultured on collagen bound over-sulfated CS-GAG substrates displayed distinct morphology and expressed higher IDO activity indicating higher immunosuppressive capacity. With this study, the aim is to enhance the biomanufacturing of MSCs through the manipulation of the CS-GAG surface. Further information about the adhesion, migration, and proliferation of MSCs can be applied towards broad industry and clinical use.