Functional Biomaterials to Control the Immunosuppressive Capacity of Human Mesenchymal Stromal Cells

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Statement of Purpose: Human mesenchymal stromal cells (hMSCs) are a promising source for cell-based therapies as they secrete a myriad of reparative factors in response to inflammatory stimuli such as interferon-gamma (IFN- γ), a cytokine that has been demonstrated to enhance the hMSCs immunosuppressive capacity (José GR. Biomaterials. 2019; 220:119403), (David C. ACS Biomater. Sci. Eng. 2019; 5:2793-2803). In this study, we investigated the factors that control hMSC immunosuppressive capacity using two biomaterial substrates: 1) multilayers of heparin (HEP) and collagen (COL) (HEP/COL) formed via layer-by-layer assembly, and 2) peptoid-crosslinked hydrogels of norbornene-functionalized hyaluronic acid (NorHA). We specifically used the multilayers to investigate the effects of surface composition (HEP or COL) and the hydrogels to investigate the effect of mechanics.

Methods: Multilayer deposition was characterized using QCM-D, and surface topography was evaluated using atomic force microscopy (AFM). Chemical composition of the multilayer surface was assessed via X-ray photoelectron spectroscopy (XPS). The hydrogel storage moduli were collected via oscillatory rheometry. For all hMSC experiments, the culture medium was supplemented with IFN-γ (50 ng/ml) to enhance the immunomodulatory activity of hMSCs. We evaluated hMSCs growth, viability, and differentiation using standard assays. Immunomodulatory activity was assessed by measuring the level of indoleamine 2,3-dioxygenase (IDO) secretion and suppression of T-cell activity using peripheral blood mononuclear cells (PBMCs):hMSCs co-cultures.

Results: ***Multilayers*** Results of the in-situ assembly deposition monitored by QCM-D showed effective formation of the multilayers on the substrate (Fig. 1A). AFM measurements to evaluate the topography of dried and wet HEP/COL multilayers demonstrated that dried multilayers ending in HEP led to a rougher surface than the multilayers ending in COL, while all multilayers showed a smooth surface in wet conditions (Fig. 1B). XPS analysis confirmed the presence of more sulfur detected on the multilayers ending in HEP, indicating heparin's presence in the last layer while a higher carbon, oxygen, and nitrogen content confirmed the accumulation of collagen in layers ending in collagen. To investigate the effect of surface composition on hMSC immunosuppressive capacity, cells were seeded on top of the multilayers. A PrestoBlue assay indicated increased cell viability compared to tissue culture plastic, both with and without the IFN-γ supplementation. Real-time monitoring of cell behavior and proliferation confirmed that the multilayers improved the response of hMSCs to IFN-γ. In addition, the cultures on (HEP/COL) multilayers (without IFN-γ) also showed better proliferation compared to the controls on uncoated surfaces. IDO measurements showed that hMSCs

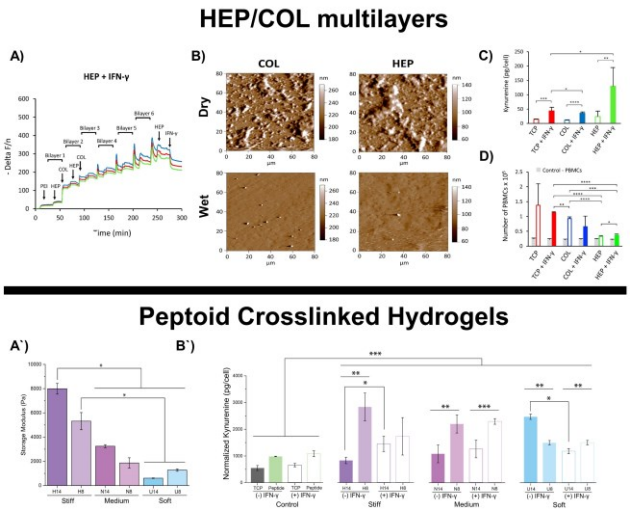

Figure 1. Multilayer surface characterization by A) QCM-D and B) AFM. Assessment of immunosuppressive capacity by C) IDO secretion and D) suppression of T-cell activity. A') Hydrogel elasticity. B') Assessment of immunosuppressive capacity on hydrogels by IDO secretion.

immunosuppressive capacity was significantly higher when cultured on multilayers ending in HEP supplemented with IFN-γ (Fig. 1C). PBMCs:hMSCs co-cultures directly seeded on multilayers demonstrated that HEP/COL surfaces in the presence of soluble IFN-γ have a greater capacity to suppress T-cell proliferation from CD3/CD28-activated PBMCs, which indicates that multilayers can promote the production of cells with pro-inflammatory and immunoregulatory capacities (Fig. 1D).

Hydrogels On the other hand, substrate stiffness was also observed to affect hMSC immunosuppressive capacity. Hydrogel substrate stiffness was controlled using the molecular rigidity of the peptoid crosslinkers to generate hydrogels of the same network connectivity but various elasticity (Fig. 1A'). For hMSCs seeded on top of these hydrogels, growth and cell spreading were modulated by substrate stiffness. Interestingly, all hydrogels upregulated IDO production over TCP culture (Fig. 1B`). In addition, IFN-γ stimulation upregulated IDO production on the hydrogels, except for the soft conditions (Fig. 1B`).

Conclusions: In summary, this study demonstrates that the surface composition and substrate stiffness impact the immunosuppressive capacity of attached hMSCs. For both of the platforms investigated here, hMSCs showed a higher IDO expression and differentiation markers than cells on TCP with IFN-γ supplemented in the medium. This work identifies biomaterial parameters that may lead to a general strategy to modulate hMSC response to soluble factors, thereby improving the immunosuppressive potential of hMSCs, which may lead to more efficient cell-based therapies aimed at treating several immune diseases.