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ORIGINAL ARTICLE

# Enabling mesenchymal stromal cell immunomodulatory analysis using scalable platforms

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## Abstract

Human mesenchymal stromal cells (hMSCs) are a promising cell source for numerous regenerative medicine and cell therapy-based applications. However, MSC-based therapies have faced challenges in translation to the clinic, in part due to the lack of sufficient technologies that accurately predict MSC potency and are viable in the context of cell manufacturing. Microfluidic platforms may provide an innovative opportunity to address these challenges by enabling multiparameter analyses of small sample sizes in a high throughput and cost-effective manner, and may provide a more predictive environment in which to analyze hMSC potency. To this end, we demonstrate the feasibility of incorporating 3D culture environments into microfluidic platforms for analysis of hMSC secretory response to inflammatory stimuli and multi-parameter testing using cost-effective and scalable approaches. We first find that the cytokine secretion profile for hMSCs cultured within synthetic poly(ethylene glycol)-based hydrogels is significantly different compared to those cultured on glass substrates, both in growth media and following stimulation with IFN-γ and TNF-α, for cells derived from two donors. For both donors, perfusion with IFN-γ and TNF-α leads to differences in secretion of interleukin 6 (IL-6), interleukin 8 (IL-8), monocyte chemoattractant protein 1 (MCP-1), macrophage colony-stimulating factor (M-CSF), and interleukin-1 receptor antagonist (IL-1ra) between hMSCs cultured in hydrogels and those cultured on glass substrates. We then demonstrate the feasibility of analyzing the response of hMSCs to a stable concentration gradient of soluble factors such as inflammatory stimuli for potential future use in potency analyses, minimizing the amount of sample required for dose-response testing.

**Keywords:** hydrogel, stem cells, in vitro potency, microfluidics

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## Insight

The translation of human mesenchymal stromal cell (hMSC)-based therapies has been hindered by the lack of sufficient technologies that accurately predict MSC potency and are viable in the context of cell manufacturing. Microfluidic platforms may provide an innovative opportunity to address these challenges by enabling multiparameter analyses of small sample sizes in a high throughput and cost-effective manner. We demonstrate the feasibility of incorporating 3D culture environments into microfluidics for analysis of hMSC response, and show a difference in immunomodulatory response between hMSCs cultured on conventional tissue culture substrates and those cultured in more clinically relevant environments.

## INTRODUCTION

Human mesenchymal stromal cells (hMSCs) are a promising cell source for many regenerative medicine and cell therapy based applications with more than 350 ongoing clinical trials in the US [1, 2]. hMSCs are hypo-immunogenic, have self-renewal and differentiation capacities, and can home to injured tissues [1, 3, 4]. Importantly, hMSCs secrete a variety of cytokines, growth factors, and metabolites that modulate the immune response [4]. Such paracrine factors, including indoleamine 2,3-dioxygenase (IDO) and a variety of interleukins and chemokines such as interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin-1 receptor antagonist (IL-1ra), and chemokine CXCL10 [5–8], are thought to primarily be responsible for hMSC immunosuppressive effect through regulation of immune cell proliferation, maturation and function [5, 9].

Moreover, it is due to these properties that hMSC-based therapies are under evaluation in clinical trials for treatment of diseases such as inflammatory bowel disease, graft-vs-host disease, and in myocardial injury [10–13]. Here, hMSC-based therapies may alleviate symptoms due to chronic inflammation of the digestive tract in the case of inflammatory bowel disease, graft immune response following allogeneic transplant in graft-vs-host disease, and improve tissue regeneration in the case of myocardial injury.

However, the translation of these therapies is severely hindered by a lack of reliable potency assays that are both predictive of clinical efficacy and are compatible with cell manufacturing processes. Classified by the FDA as more-than-minimally-manipulated cellular and gene therapy (CGT) products and by the European Medicines Agency as advanced therapeutic medicinal products (ATMP), hMSC-based therapies require the development of potency assays, or functional tests predictive of *in vivo* effect, for advanced phase clinical trials [14]. Development of these assays is particularly challenging for hMSC based therapies due to heterogeneity of source materials, variability in culture conditions [15], as well as complex and poorly defined mechanisms of action [5]. Moreover, conventional assays often rely on analysis of cells cultured on glass or plastic substrates, and often do not correlate well with *in vivo* performance, further impeding the development of predictive potency assays [14, 16].

In previously reported hMSC-based regenerative medicine applications, hMSCs are embedded within biomaterial scaffolds and implanted or inserted directly at the diseased or injured site. This change in the microenvironment from planar culture to a 3D scaffold may significantly impact hMSC activities, as it has been shown that hMSC differentiation and function are significantly influenced by environmental factors such as topography, adhesive signals, stiffness and porosity [17–20]. Furthermore, hMSCs cultured in 3D spheroids and scaffolds have been found to exhibit enhanced immunomodulatory potential compared to those cultured on conventional tissue culture substrates [21].

To that end, we begin to address this challenge by developing and analyzing hMSC responses in scalable tissue-on-a-chip platforms. In these platforms, hMSCs are embedded in synthetic polyethylene glycol (PEG)-based hydrogels and subjected to a continuum of signals after which their phenotype can be assessed (Fig. 1). PEG-based systems have proven to be particularly advantageous for cell therapy and regenerative medicine applications due to their support of cell functions, proven safety *in vivo*, and tunable biochemical and mechanical properties [22–25]. The PEG-4MAL system in particular enables extensive plug-and-play modality due to its enhanced reaction efficiency compared to other PEG-based systems [26]. Moreover, these hMSC-laden hydrogels are incorporated into scalable microfluidic platforms fabricated without the need for extensive engineering training or state-of-the-art microfabrication facilities typical of traditional photolithography-based microfluidic techniques. Reducing sample size and increasing throughput of such assays, while minimizing cost, simplifies and further enables their manufacture and scale-up.

Using these platforms, we first find a differential in response to specific inflammatory stimuli between hMSCs cultured within PEG-based hydrogels and those cultured on planar glass slides by comparing cytokine secretion profiles. Frequently used assays to evaluate hMSC immunomodulatory function examine measures of T-cell activation in response to hMSC co-culture [5, 27, 28]. Surrogate measures of function have also been proposed via analysis of hMSC response to inflammatory stimuli such as interferon-γ (IFN-γ), which has been found to be an important licensing cytokine *in vitro* and at sites of inflammation [5, 29]. Moreover, recent reports have suggested that hMSC secretome response after stimulation by IFN-γ and after stimulation by activated peripheral blood mononuclear cells is highly correlated [29, 30]. This motivates our use of inflammatory stimuli to analyze differences between hMSCs cultured on planar substrates and those encapsulated in PEG-based hydrogels. Additionally, IDO is increasingly identified as a key mediator of hMSC induced inhibition of T-cell proliferation *in vitro* [30, 31], and is chosen here for further exploration as an endpoint analysis for use in potential tissue-on-a-chip platforms in which hMSCs cultured within PEG based hydrogels are exposed to controlled, stable concentration gradients of inflammatory stimuli. These studies support the need for further characterization of the effect of different parameters of the microenvironment on hMSC immunomodulatory properties, and indicate the potential to improve hMSC potency tests via tissue-on-chip platforms compatible with cell manufacturing processes.

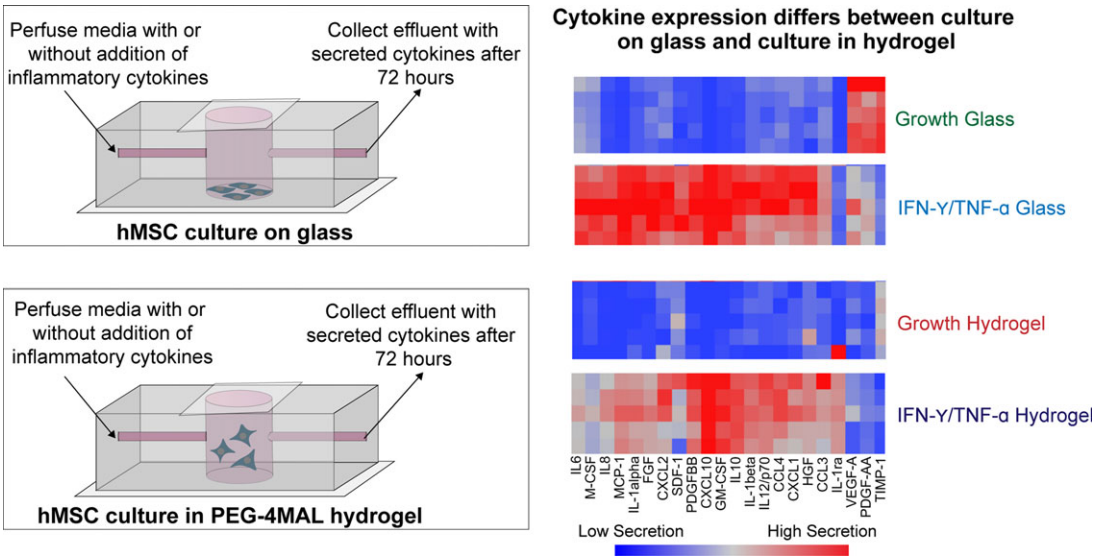


**Figure 1.** Mesenchymal stromal cells (MSCs) cultured on planar glass respond differently compared to those cultured in a tissue on a chip platform when perfused with growth media alone or stimulated with inflammatory cytokines.

## MATERIALS AND METHODS

### Cell culture

Human mesenchymal stromal cells (hMSCs) were acquired from the NIH Resource Center at Texas A&M University and confirmed as hMSC [32]. Briefly, cells were obtained from healthy donors, a 22 year-old male (8013L) and a 23-year-old female (8011L), via bone marrow aspirate, followed by density centrifugation for mononuclear cells and selected for adherent culture. Cells were screened for colony forming units, cell growth, and differentiation into fat and bone using standard assays. Flow cytometry analyses confirmed that cells were positive for CD90, CD105, CD73a and negative for CD34, CD11b, CD45, CD19. Received frozen stocks were thawed and grown in MEM-α (ThermoFisher) containing 16% fetal bovine serum (FBS), 2 mM L-glutamine and 100 U/mL penicillin/streptomycin (ThermoFisher). hMSCs were seeded at 1500 cells/cm$^2$ in a 15 cm, sterile polystyrene dish (Corning), and dissociated using 0.25% trypsin/EDTA. Cells were used between passage 3–5.

### Preparation of PEG-based hydrogel

Hydrogels used for hMSC culture were prepared by mixing 4-arm poly(ethylene glycol)-maleimide (PEG-4MAL) (>95% purity, Laysan Bio) with the cross-linkers GCRDVPMS↓MRGGDRCG (VPM) (>95% purity, Genscript) and dithiothreitol (DTT) (Sigma-Aldrich)), and were functionalized with the adhesive peptide GRGDSPC (RGD) (>95% purity, Genscript) [33]. Briefly, RGD, VPM and DTT were dissolved in 50 mM HEPES buffer at 5.0 mM, 17.95 mM, and 24.75 mM, respectively, and PEG-4MAL in 1X PBS at 6.82 mM. Before use, components were filter-sterilized using sterile Corning®, Costar®, Spin-X® 0.22 μm pore, centrifuge tube filters (Sigma). Functionalization was performed by mixing PEG-4MAL with RGD in a 2:1 ratio at room temperature for 20 minutes. VPM and DTT were combined to provide a final cross-linking solution composed of 80% VPM and 20% DTT. hMSCs were added to the PEG-4MAL/RGD solution and combined with the crosslinker at a final ratio of 3:1:1 of PEG-MAL/RGD: hMSCs: crosslinker, for a final PEG-MAL weight percent of 6.0% and RGD density of 1.0 mM.

### Measurement of inflammatory cytokine secretion

Secreted inflammatory biomarkers were first measured and compared to glass slide controls using a device fabricated with cost-effective and accessible materials, as previously described [34, 35]. Brass wires (500 μm diameter) were cast in polydimethylsiloxane (PDMS). Once cured, a 6 mm hole was punched through the PDMS and around the wire. The wires were then removed, generating a channel traversing the PDMS through which inflammatory stimuli could be perfused. The hole was then filled with PEG-4MAL hydrogel containing hMSCs, and enclosed via plasma-bonded glass coverslip (Fig. 2).

Inflammatory biomarker expression by hMSCs cultured in PEG-4MAL hydrogel or on planar glass slide controls in the microfluidic chip were analyzed using a custom Luminex kit (R&D) for cultures treated with inflammatory stimuli IFN-γ (50 ng/mL) and TNF-α (15 ng/mL) or unstimulated controls at 96 hours post-stimulation.

### Design and fabrication of device for concentration gradient generation

A device to analyze exposure of hMSCs to a concentration gradient of inflammatory stimuli was also developed to demonstrate the feasibility of using microfluidics for high throughput potency testing. We propose this platform as a more efficient way to analyze hMSC response to a range of inflammatory stimuli concentrations for relative comparison, with an increased length scale to reduce cross-talk and minimize the concentration gradient observed by the cell. Here, microfluidic channels were formed using double sided adhesive and polydimethylsiloxane (PDMS). Specifically, a craft cutter (Silhouette) was used to cut channels in double-sided tape, consisting of silicon adhesive coated on both sides of a polyethylene terephthalate (PET) carrier, and combined with a polyester secondary liner (3M$^{TM}$). The top and bottom of these channels were formed by sandwiching the double-sided adhesive between two PDMS layers.

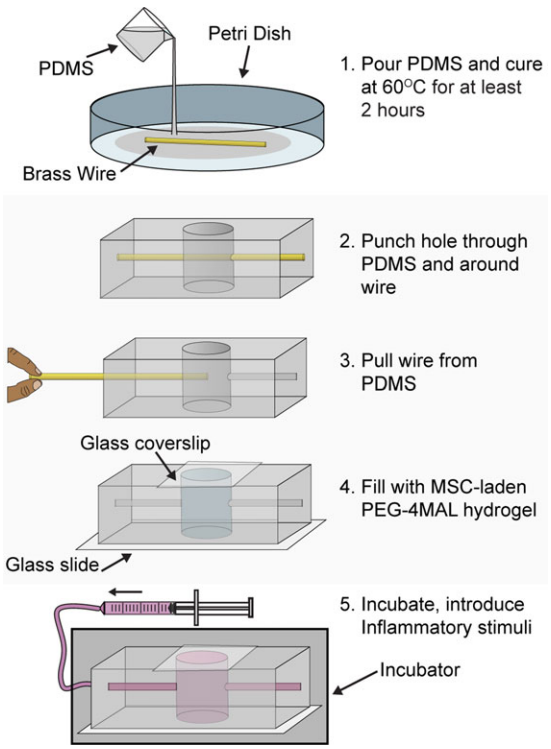


**Figure 2.** Fabrication of microfluidic enabling perfusion and cytokine expression in PEG-4MAL hydrogels [34, 35].

4 mm and 1 mm biopsy punches were used to form holes for the hydrogel and reservoirs, respectively, and a handheld corona gun was used for bonding all other layers. The device was assembled immediately before initiation of the experiment (Fig. 4A).

### Characterization of concentration gradient and CFD modeling

FITC- Dextran (20 kDa, Sigma Aldrich) at 1 mg/mL in PBS was used to visualize and characterize the formation of a concentration gradient through the hydrogel in the microfluidic device. Images were acquired using Nikon TE 2000 over a period of 96 hours, and identical acquisition parameters were used throughout the experiment (Fig. 4B). Computational fluid dynamics simulations were conducted using COMSOL Multiphysics (COMSOL, Inc., Burlington, MA, USA). A 3D model of the device was developed, and time-dependent diffusion through the hydrogel evaluated using the transport of diluted species module. Here, an effective diffusion coefficient relative to that of water, calculated using Stokes Einstein, ($D/D_0$) was used to model diffusion through the hydrogel. A value of 0.3 was used here based on diffusion measurements performed in other PEG-based hydrogels [36].

### Immunocytochemistry and image analysis

To analyze the effect of the inflammatory stimuli gradient on cells encapsulated in the PEG-4MAL hydrogel, encapsulated cells were fixed and stained for indoleamine 2,3-dioxygenase (IDO). After 96 hours of exposure to the inflammatory stimuli gradient, cells encapsulated in the gel were washed with PBS for 1 hour and fixed with 4% paraformaldehyde for 3 hours. After fixation, cells were washed for 3 hours with PBS, permeabilized, and blocked overnight with 0.2% Triton-X in 1% BSA. Cells were then washed with PBS again for 3 hours, after which gels were incubated with 50 μg/mL of AlexaFluor 488-conjugated IDO antibody (R&D Systems) diluted 1:11 in 1% BSA for 2 days. Before imaging, gels were washed overnight with PBS, additionally stained for 2 hours with Hoechst to visualize the nuclei, and washed for 1 hour with PBS. Images were acquired using a Zeiss Laser Scanning Confocal Microscope mounted on an AxioObserver Z1 inverted microscope stage, and analyzed using CellProfiler 3.0.0 (Broad Institute) [37] and MATLAB R2017a.

Stitched 10x images were analyzed by correlating the average IDO fluorescence intensity to the distance from each inlet. Linear regression was used to model the relationship between the average intensity and distance, and correlation coefficients ($R^2$) found for each. It is expected that the IDO intensity profile moving away from the inflammatory stimuli inlet will more closely match a linear profile compared to that moving away from inlets perfusing growth media alone, where no change in IDO intensity is expected.

Images were further analyzed by identifying the mean IDO intensity per cell from z stacks taken at the inflammatory inlet (0 microns) and at the opposing edge of the gel (4000 microns) using CellProfiler. Briefly, images of the stained nuclei were thresholded and identified as the primary objects. Associated IDO stains were subsequently identified for each of the nuclei using the propagation method, and the intensity measured for each object.

### Statistical Analysis

Cytokine secretion data comparing hMSCs cultured in different environments are presented as mean ± SEM, with 4-5 samples per group. Differences between groups were analyzed by one-way ANOVA followed by Tukey post hoc test using GraphPad Prism. Two-way unsupervised hierarchical clustering was performed on Luminex mean fluorescence intensities (MFI) using JMP Pro.

Analysis of IDO expression after exposure to concentration gradients was performed by correlating the average IDO fluorescence intensity to the distance from each inlet and determining correlation coefficients ($R^2$) for each. The distribution of the mean IDO intensity per cell from z stacks taken at the inflammatory inlet (0 microns) and at the opposing edge of the gel (4000 microns) were additionally compared using one-way ANOVA.

## RESULTS AND DISCUSSION

### Functional differences observed between hMSCs cultured in the 3D hydrogel-based tissue-on-a-chip platform compared to on planar culture surface

We first evaluated the differences in cytokine secretion profile between hMSCs cultured on glass slides and in PEG-4MAL hydrogels, both when perfusing with growth media and media containing inflammatory stimuli. hMSCs were either allowed to adhere to a glass slide or encapsulated within PEG-4MAL hydrogels and loaded into the described microfluidic device. After perfusing with growth or inflammatory stimuli media for 72 hours, which has been shown in previous reports to provide sufficient stimulation [30], the effluent from the following 24 hours was collected and analyzed for their cytokine secretion profile. Live/dead staining (Calcein AM/Toto-3 Iodide) on the first and

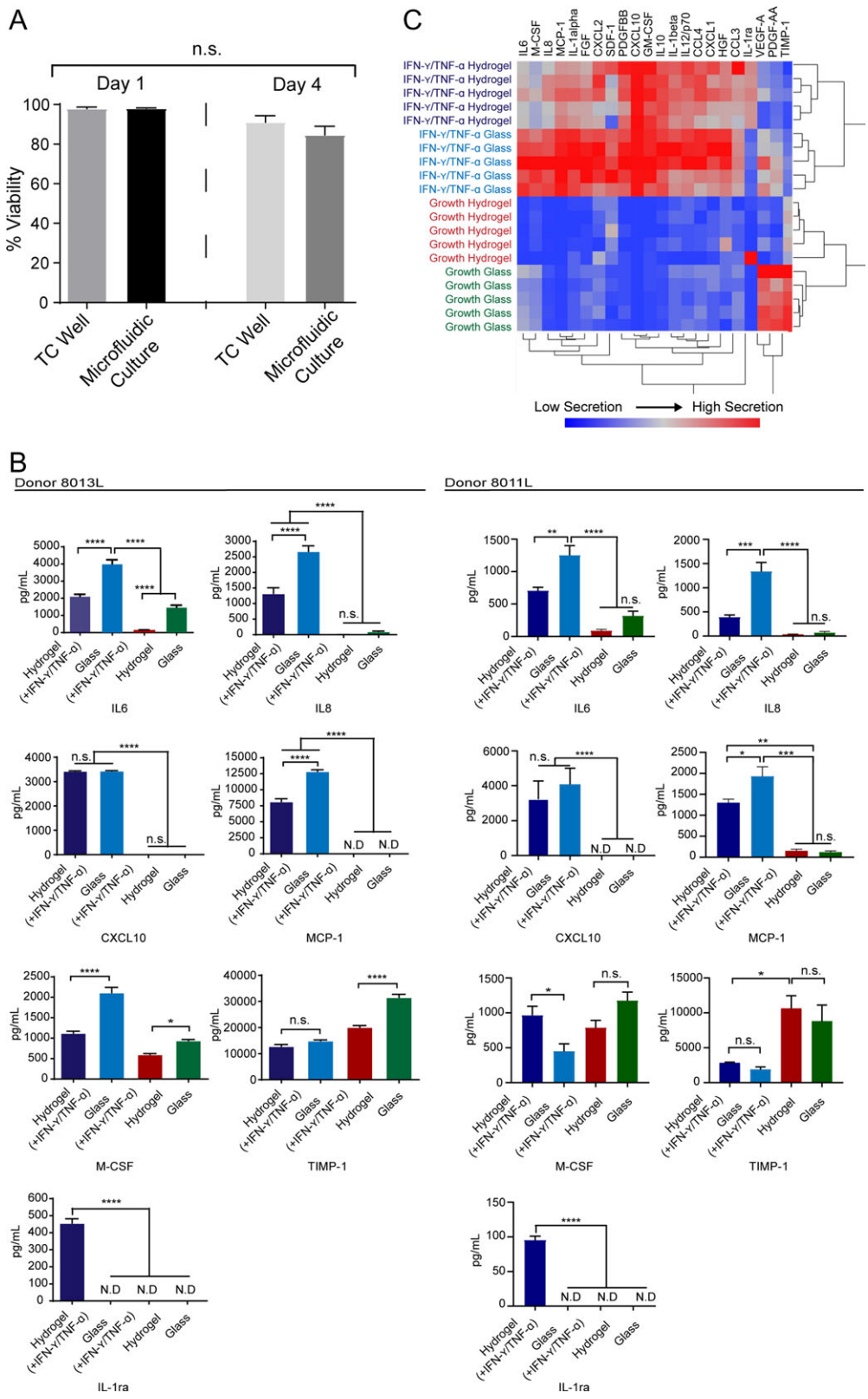


**Figure 3.** Cytokine secretion profiles for MSCs cultured on planar glass slides or in PEG-4MAL hydrogels, after perfusion with growth media alone, and after perfusion with inflammatory stimuli. (A) Live/Dead staining reveals no statistical difference in viability between conventional tissue culture well and microfluidic cultures at Day 1 and Day 4 of the experiment. (B) Differences in secretion of highlighted individual cytokines. (C) Hierarchical clustering analysis group unstimulated and stimulated samples as well as according to microenvironment based on secretion profiles (results shown for Donor 8013L).

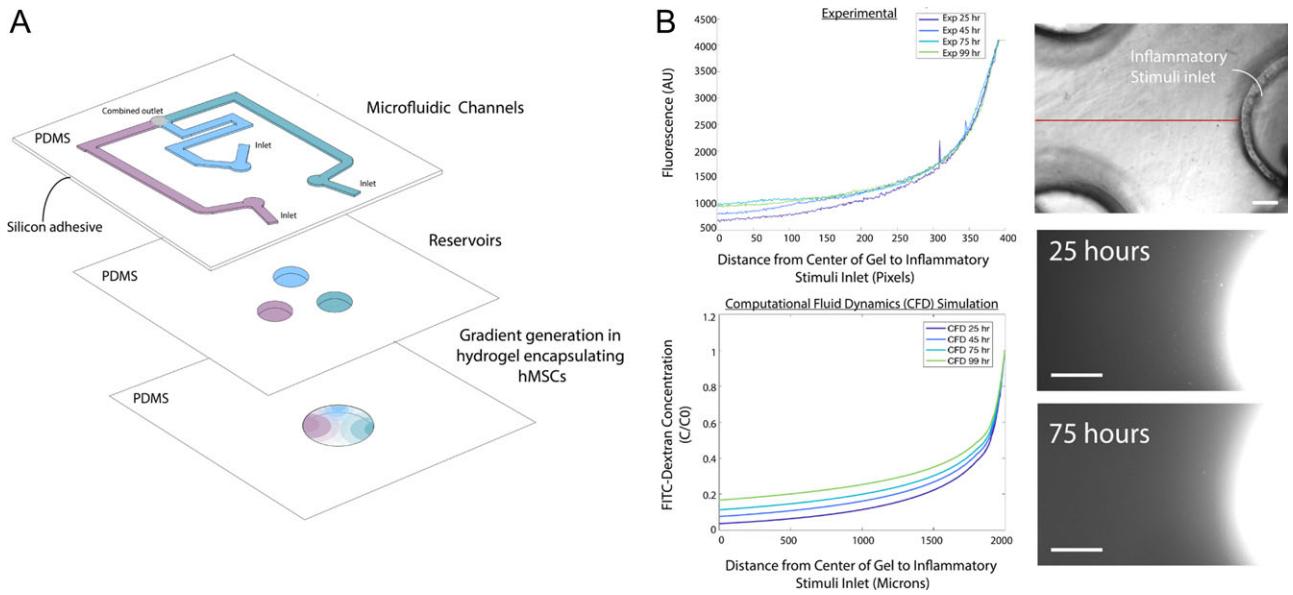


**Figure 4.** (A) Schematic of microfluidic design for generating gradients in hydrogel. Fluidic channels in the top layer deliver inflammatory stimuli as well as growth media to the hydrogel in the bottom layer. (B) FITC-Dextran (20 kDa) was used to visualize the development of the gradient in the hydrogel over time, and compared to modeling results. Scale bars are 200 μm.

last day of the experiment verified that there was no difference in viability between cells cultured in tissue culture wells and those cultured in the microfluidic device (Fig. 3A).

Without the addition of inflammatory cytokines, hMSCs within hydrogels and those seeded on glass substrates exhibited significant differences in their cytokine profiles. hMSCs isolated from donor 8013L secreted significantly lower levels of IL-6, tissue inhibitor of metalloproteinases 1 (TIMP-1) and macrophage colony-stimulating factor (M-CSF) when encapsulated in PEG-4MAL hydrogels compared to cells cultured on glass (IL-6 and TIMP-1 $P < 0.0001$, M-CSF $P < 0.05$ Fig. 3B). We did not find significant differences in cytokine secretion between conditions after perfusion of growth media alone for hMSCs isolated from donor 8011L.

Whereas certain differences were seen when hMSCs were perfused with growth media, perfusion with media containing IFN-γ and TNF-α, both key pro-inflammatory cytokines secreted by activated T-cells, caused further differences between hMSCs in hydrogels and those on glass. Unsupervised hierarchical clustering demonstrates that much of the variability in the data is described by differences between stimulated and unstimulated conditions, but also show that samples cluster according to their microenvironment (results shown for donor 8013L, Fig. 3C). Specifically, for both donors tested, we found that hMSCs cultured in the hydrogel displayed significant differences in their levels of secretion of IL-6, IL-8, monocyte chemoattractant protein 1 (MCP-1), M-CSF, and IL-1ra compared to on planar glass controls. IL-6, MCP-1, and IL-8 secretion were found to decrease (all $P < 0.0001$ for donor 8013L; IL-6 $P < 0.01$, MCP-1 $P < 0.05$, IL-8 $P < 0.001$ for donor 8011L, Fig. 3B), while secretion of IL-1ra was found to increase ($P < 0.0001$ for both donors, Fig. 3B) when cultured in hydrogels compared to on glass substrates. No difference was observed in CXCL10 secretion and in TIMP-1 secretion after stimulation by IFN-γ and TNF-α (n.s. for both donors, Fig. 3B).

Importantly, the influence of donor variability and cell number on these results, and on the predictive power and reliability of these assays, was not specifically assessed, and represents a limitation of these studies. However, these results are consistent with recent reports comparing the secretory activity of hMSCs in other biomaterials to planar controls [38, 39]. Reduced IL-6 secretion and minimal change in secretion of M-CSF after just culturing hMSCs in 3D, for example, was also observed by a previous study in which hMSCs were cultured in 3D polystyrene [38]. Similarly, this study also showed reduced secretion of MCP-1 and IL-6 compared to planar controls after exposure to inflammatory stimuli via macrophage like cells (dTHP-1), which is in full agreement with our observations. hMSCs encapsulated in alginate microspheres also displayed similar results regarding MCP-1 and IL-6 changes after exposure to inflammatory stimuli through co-culture with LPS stimulated hippocampal slices; however, IL-1ra was found to decrease in microspheres relative to 2D culture [39]. These modulated cytokines include a range of factors known to be involved in immunomodulation. M-CSF is involved in regulation of monocyte and macrophage lineage maturation [40], while IL-8, IL-6 and IL-1ra are known inflammatory mediators and MCP-1 is a chemokine involved in recruitment of monocytes. Whereas it is yet unclear whether these results correlate with hMSC function and further, clinical outcome, it is worthwhile to consider differences in response between hMSCs cultured on conventional tissue culture substrates and those cultured within more clinically relevant environments.

It is well known that properties of the microenvironment, including porosity, topography, and stiffness among others, can modulate the function and response of interacting cells. Specifically, topographical cues have been shown to be important regulators of hMSC function, and have been primarily investigated in the context of promoting differentiation for cell replacement at the site of injury [41–43]. However, the effects of these microenvironmental cues on paracrine and immunomodulatory function has yet to be fully explored.

Our results in combination with studies conducted by others have shown that the hMSC microenvironment can influence immunomodulatory function, and have demonstrated differences in secretion profiles between hMSCs cultured on planar

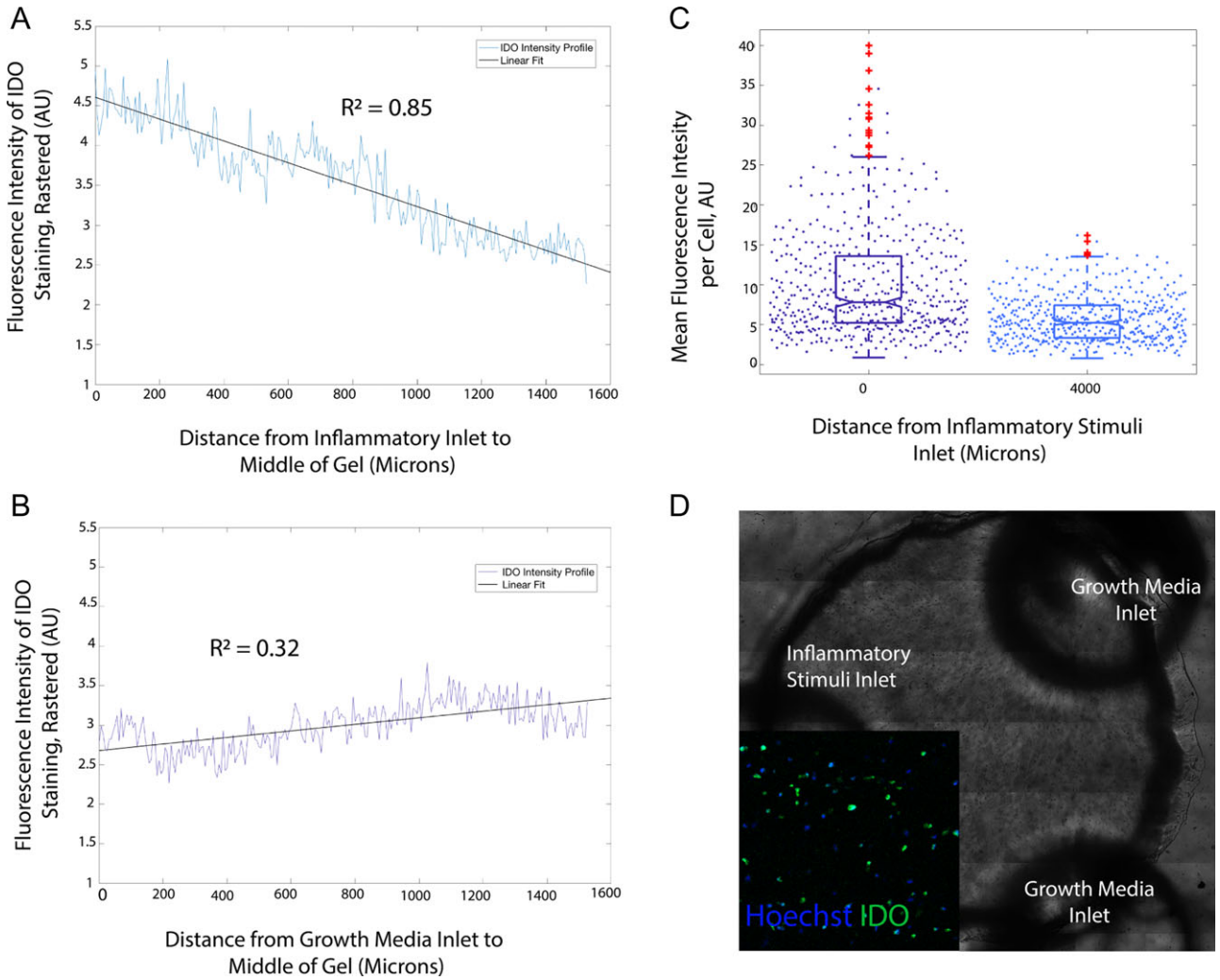


**Figure 5.** (A–B) MSC response to a gradient of inflammatory stimuli was evaluated by examining averaged intensity profiles of IDO immunostaining from the inflammatory stimuli inlet to the middle of the gel compared to those from the growth media inlets to the middle of the gel, and (C) by comparing the mean intensity per cell at 0 micron and 4000 micron positions at various z planes throughout the gel. (D) Brightfield image of the device with the inset displaying representative image of IDO immunostaining and Hoechst labeling.

surfaces and those cultured in scaffold materials as well as in spheroids. Compared to on planar surfaces, where apical basal polarity is artificially induced, adhesions are altered, and the mechanical environment is not representative of what would be found *in vivo*, hydrogel matrices can provide a more physiologically relevant environment, where gradients of soluble factors are possible and mechanical properties are tunable.

Pro-inflammatory signaling, specifically IFN-γ signaling through the JAK-STAT pathway, has been shown in other cells to be regulated by actin cytoskeletal changes as well as by cell adhesion signaling [29, 44], both of which can be modulated by the microenvironment. The microenvironment may then play an important role in determining the immunosuppressive function of hMSCs. Indeed, a recent study found that culture on electrospun fibers of differing orientations altered hMSC morphology and resulted in increased immunosuppressive capacity compared to those on flat substrates [45]. Cell manufacturing processes developed in the future to evaluate hMSC immunomodulatory potency may then need to be performed in more physiologically relevant environments to obtain accurate evaluations of their potency.

## A concentration gradient of inflammatory stimuli demonstrates potential for multi-parameter testing

To further demonstrate the feasibility of evaluating hMSC response while cultured in hydrogels in a high-throughput and scalable manner, a platform was developed to analyze the response of encapsulated hMSCs to a range of inflammatory stimuli for relative comparison. This type of platform could reduce the amount of sample required for dose-response testing, and could enable higher throughput potency analysis.

First, a concentration gradient was generated in the hydrogel using a system of opposing sources and sinks (Fig. 4A). A fluorescently labeled molecule, similar in size to IFN-γ and TNF-α, (FITC-Dextran, 20 kDa) was used to visualize the temporal evolution of the concentration gradient. Within the time frame of this study, the gradient was found to be relatively stable and mirrored computational fluid dynamics modeling results (Fig. 4B).

The use of this device for potential hMSC potency testing was then investigated. hMSCs were encapsulated in the PEG-MAL hydrogel, and loaded into the microfluidic device directly

after plasma treatment. The inflammatory stimuli IFN-γ and TNF-α in growth media were perfused through one inlet, while growth media alone was perfused through the others. Given that the gradient was found to stabilize within 25 hours, the device was perfused for a total of 96 hours to allow for a full 72 hour exposure to the gradient.

hMSCs were then stained for IDO, a tryptophan-catabolizing enzyme necessary for MSC immunosuppression upon IFN-γ stimulation. IDO is increasingly identified as a key paracrine factor involved in hMSC immunosuppressive function [5, 9, 30], and was used here as an example endpoint analysis. Low levels of IDO are typically expressed in unstimulated MSCs, while IDO expression increases in hMSCs in response to IFN-γ stimulation. Here, changes in IDO expression were evaluated in response to a gradient of IFN-γ and TNF-α by immunostaining. Linear regression was performed on the averaged intensity profiles from each inlet to the middle of the gel. Compared to the intensity profiles from the two growth media inlets ($R^2 = 0.32$ and $R^2 = 0.56$, respectively), the averaged intensity profile from the inflammatory stimuli inlet to the middle of the gel was found to more closely fit a linear profile ($R^2 = 0.85$) (Fig. 5A and B). The averaged intensity profile from the inflammatory inlet also displayed higher linearity compared to the isotype control profiles at all three inlets ($R^2 = 0.0006$, $R^2 = 0.12$, and $R^2 = 0.29$, respectively). Moreover, the distribution of the mean IDO intensity per cell differs when examining cells at the inflammatory stimuli inlet compared to a distance of 4000 microns away from the inflammatory stimuli (Fig. 5C). These results thus indicate a potential for using this platform for multi-parameter analyses, evaluating response to superimposed concentration profiles of relevant stimuli and morphogens. Further experiments may determine to what extent such measurements correlate with hMSC function.

## CONCLUSION

These results further indicate a role for the hMSC microenvironment in determining immunomodulatory function by demonstrating differences in cytokine secretion between hMSCs cultured in RGD-presenting PEG hydrogels within tissue-on-a-chip platforms and those cultured on planar controls. More predictive assays to evaluate hMSC immunomodulatory potency may then incorporate 3D hydrogels that recapitulate important aspects of the *in vivo* environment. Moreover, we demonstrate the feasibility of developing platforms that are compatible with cell manufacturing processes while using cost-effective and scalable approaches. Future improvement and integration of these assays could allow for correlation with hMSC function *in vitro* and *in vivo*, comparison of response across different populations and sample types, increased compatibility with cell manufacturing processes, and ultimately aid in translation of MSC based therapies to the clinic.

## Conflicts of interest

There are no conflicts to declare.

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