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RAPID COMMUNICATION

A comparative evaluation of layer-by-layer assembly techniques for surface modification of microcarriers used in human mesenchymal stromal cell manufacturing

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Abstract

The demand for large quantities of highly potent human mesenchymal stromal cells (hMSCs) is growing given their therapeutic potential. To meet high production needs, suspension-based cell cultures using microcarriers are commonly used. Microcarriers are commonly made of or coated with extracellular matrix proteins or charged compounds to promote cell adhesion and proliferation. In this work, a simple method (draining filter) to perform layer by layer (LbL) assembly on microcarriers to create multilayers of heparin and collagen and further demonstrate that these multilayers have a positive effect on hMSC viability after 48 h of culture was demonstrated. The draining filter method is evaluated against two other methods found in literature—centrifugation and fluidized bed, showing that the draining filter method can perform the surface modification with greater efficiency and with less materials and steps needed in the coating process.

KEYWORDS
collagen, heparin, hMSCs, Layer-by-layer, microcarriers

1 | INTRODUCTION

Human mesenchymal stromal cells (hMSCs) are multipotent anchorage-dependent cells widely used in the medical field in applications such as treatment of chronic diseases, development of regenerative medicine, and drug delivery.[1–4] In 2020, 1138 clinical trials were registered worldwide to investigate the therapeutic potential of hMSCs for the treatment of multiple diseases.[5] Therefore, there is a growing demand for hMSCs—this need can be addressed with large-scale cell cultures. Microcarriers are support matrices whose large surface area to volume ratio makes them ideal in large-scale suspension-based bioreactor cell cultures for anchorage-dependent cells.[6,7] Their sizes range from 100 to 300 μm[6,8] and have been reported to yield anywhere from a tenfold to 500× increase in cell density at the end of a cell culture, depending on the type of cells, microcarriers, and bioreactors used.[9–11] A wide variety of microcarriers are available for use in mammalian cell cultures. A few examples include Cytodex 3 by Cytvia,[12] which has a cross-linked dextran matrix with a gelatin coating, Hillex II by SoloHill Engineering,[13] which has a crosslinked polystyrene matrix modified with cationic trimethyl ammonium, and Cultispher-S by Percell Biolytica AB,[13] which has a crosslinked porcine gelatin matrix—all three of these microcarriers are nonporous and are suitable for use in hMSC culture.[14–16] Surface modified microcarriers containing a positive surface charge or extracellular matrix-based material coating (such as collagen, laminin, and fibronectin) are common.[7,17–19] Typically, these coatings are necessary for cells to remain adherent during agitated bioreactor cell cultures, especially in polystyrene-based microcarriers—serum-containing media along with a chemically defined surface are necessary for cells to adhere.[19,20]

Layer by Layer (LbL) assembly is a surface modification technique that uses alternating charged polyelectrolytes to form dense films on a given substrate (Figure 1A).[21] A typical layer-by-layer assembly process uses an anchoring layer on a substrate to hold the multilayers—the

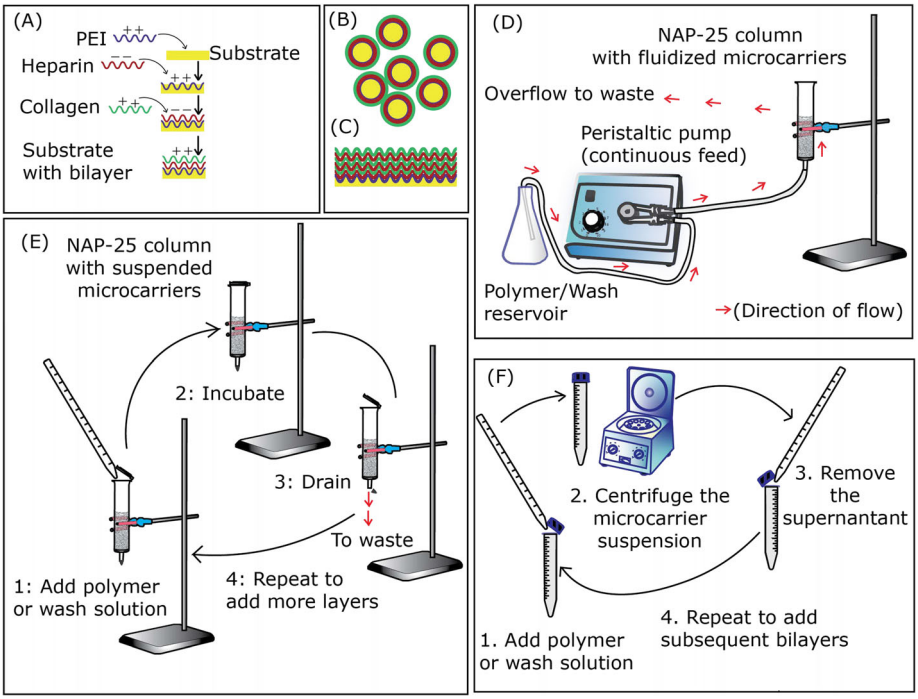

FIGURE 1 An overview of the layer-by-layer assembly process on microcarriers. (A) A molecular representation of the layer-by-layer process. A substrate is first modified with an anchoring layer (polyethyleneimine or PEI), followed by a polyanion (heparin) layer, and a polycation (collagen) layer. The polyanion and polycation layers can be repeated to add further bilayers to coat both spherical microcarriers (B) or flat surfaces (C). (D) Fluidized bed system—Here the microcarriers are resting on the NAP-25 column's bottom filter initially. Polymer or wash solutions are continuously fed to the NAP-25 column, passing through the filter, and fluidizing the microcarriers in the process. The microcarriers were fluidized for 15 min with PEI and then 10 min with a pH 5 acetate buffer. Heparin and collagen solutions were used to fluidize the column for 5 min each, with a 10-min buffer wash as with the PEI. (E) Draining filter system—Here the microcarriers are sitting on a porous filter in a NAP-25 column. Polymer or wash solutions are added to the sealed column, exposing the microcarriers to each solution—the exposure times were the same as those for the fluidized bed, except with a shorter acetate buffer wash of 3 min. Between each step, the column is uncapped to drain the solution. The microcarriers remain in the column and the next layer can be added when the column is fully drained. (F) Centrifugation system—Here the microcarriers are placed in a centrifuge tube, where the polyelectrolyte or wash solution is added and then the tube is centrifuged to force the microcarriers to settle. The supernatant may then be discarded for the application of the next layer. Incubation times are identical to the draining filter system

polyelectrolyte layers can be applied to the substrate by subjecting the substrate to a solution of the polyelectrolytes, and the substrate is typically washed with a buffer after the application of each polyelectrolyte layer.[22–24] LbL assembly offers many advantages in its simplicity—first, multilayers can be applied on a multitude of surface topographies; examples include flat surfaces, nanofibers, and colloidal particles.[23,25] Secondly, several different compounds may be incorporated onto the surface during the process.[26,27] Incorporation of heparin and collagen multilayers on cell culture surfaces has been shown to modulate soluble factors in hMSCs better than heparin or collagen alone.[28] Furthermore, since the layers deposited during LbL are on the nano scale,[29,30] the decrease in concentration in the polymer solution is negligible and the solution may be reused.

Our work uses heparin as the polyanion and type I collagen as the polycation. Heparin-collagen is a well-researched polymer pair in LbL assembly.[28,31–34] Our group has previously demonstrated the capacity of heparin/collagen multilayers deposited on flat surfaces in modulating hMSC response to soluble interferon gamma, improving cell proliferation and cytokine expression.[28,35,36] However, for large-scale manufacturing of hMSCs flat surfaces are undesirable given their small output, and thus suspension based bioreactors are preferred.[37] In suspension-based bioreactors, microcarriers are used as the adhesion surface for cell cultures.[6,38] While flat surfaces can be easily modified via LbL assembly via dip coating, the same approach cannot be used to modify the surface of microcarriers. Microcarriers are spherical particles that will become suspended when exposed to polymeric or wash solutions.

Several methods have been reported in literature for LbL film deposition on microcarriers and other particles, including centrifugation (Figure 1F),[39–42] fluidized bed (Figure 1D),[43–46] and tangential flow filtration.[47] Here, we investigate two of these methods—centrifugation and fluidized bed, against a novel yet simple method—draining filter (Figure 1E), to assess their efficacy in creating LbL films of heparin/collagen on polystyrene microcarriers. In this study, polystyrene microcarriers were modified using the three methods, and several characterization methods were used to analyze and compare the efficacy of the three methods in creating polymeric multilayers of heparin and collagen on microcarrier surfaces. Furthermore, hMSCs were cultured on the microcarriers for 48 hours to observe the effect of the multilayers on cell viability and cell harvesting.

2 | RESULTS

2.1 | Quantitative characterization shows the formation of polymeric multilayers on microcarriers

Corning untreated (polystyrene) microcarriers were surface-modified using the three methods—centrifugation, fluidized bed, and draining filter. Particle size analysis of the microcarriers after LbL assembly showed a shift in the distribution as a function of modification method. Mean particle size increased from 142.79 ± 1.01 μm to 145.95 ± 1.01 μm for the fluidized bed system, 147.39 + 1.01 μm for the centrifugation system, and 147.07 ± 1.01 μm for the draining filter system. A graph of the particle size distribution can be seen in Figure 2F.

Additionally, Zeta potential analysis was performed on the microcarriers to monitor changes in surface charge as a function of deposited layer. Since the polyelectrolytes carry an electric charge, their accumulation on the microcarrier surfaces were expected to change the mobility of the microcarriers in an electric field. The application of the anchoring PEI layer showed a positive surface charge and the polyanion (heparin) layer a negative charge as expected. However, the polycation (collagen) layer and any subsequent layers resulted in errors in measurement, since the microcarrier sizes are on the upper end of the recommended particle diameter. The PEI layer showed a surface charge of 26.7 ± 11.2 mV while the heparin layer showed a surface charge of -22.5 ± 7.18 mV.

X-ray photoelectron spectroscopy (XPS) was used to evaluate the surface chemistry of the plain and modified microcarriers. The most notable difference that can be observed between the plain and modified microcarriers, is the presence of a nitrogen peak (Figure 2A,B), which can be seen in more detail in the high-resolution nitrogen spectra in Figure 2C,D. LbL modified microcarriers showed a nitrogen peak that was absent in the plain microcarriers. Since collagen and heparin both contain nitrogen groups, the presence of this nitrogen peak indicates the incorporation of the layers onto the microcarrier surface. Figure 2C,D show high resolution spectra for Carbon, Oxygen, Nitrogen, and Sulfur. The change in shape of the carbon bump at binding energy = 288 mV shows a higher quantity of C═O bonds in the modified microcarriers. C═O bonds are present in both heparin and collagen. Additionally, a sulfur peak was observed on the modified microcarriers while the unmodified microcarriers only showed noise, confirming the presence of heparin.

2.2 | Qualitative characterization shows uniform formation of polymeric multilayers on microcarrier surfaces

Microcarriers were surface-modified using the three methods—draining filter, centrifugation, and fluidized bed. After the application of 6 bilayers using these three methods, the modified microcarriers were incubated with a fluoresently labeled collagen solution and subsequently rinsed with Phosphate-Buffered Saline (PBS) by centrifugation. The microcarriers were transferred to a 96-well plate to be observed under a fluorescent microscope. The results are shown in Figure 3A–C. Fluoresence imaging confirms that all three methods can be used to modify the microcarriers. However, the draining filter method shows more fluoresence intensity, indicating that more amount of collagen and heparin was deposited on the microcarriers. The method that produced the weakest fluoresence was the centrifuge method.

Azure A staining was performed on microcarriers that were LbL modified using the draining filter method. Azure A dye is a histological dye that changes color in the presence of heparin. After LbL assembly, the microcarriers were washed twice with PBS, and a solution containing the Azure A dye was added to two different NAP-25 columns, one with plain microcarriers and the other with LbL modified microcarriers. A color change—deep blue to purple, was observed in the modified microcarriers, confirming the presence of heparin, as can be seen in Figure 3D.

Scanning electron microscopy was performed on LbL-modified microcarriers (using the draining filter method). Increased roughness was observed on the modified microcarrier surface when compared to unmodified microcarriers, attributed to the accumulation of polyelectrolyte multilayers on the surface, as seen in Figure 3F,G.

2.3 | Cell viability assay shows a positive effect of the polymeric multilayers on microcarriers cultured with human mesenchymal stromal cells

A PrestoBlue cell viability assay was performed on the microcarriers. Plain and modified microcarriers were added to separate low-adhesion 96-well plates. After 12 h, cells were added to the wells containing the microcarriers and the viability assay was performed at 24 and 48 h. Though the results showed no change in cell viability after 24 h, a two tailed *t*-test showed a statistically significant difference (at the 0.05 level) between the modified and plain microcarriers when the cells were cultured on them for 48 h (Figure 3H).

Additionally, the plain and modified microcarriers were investigated for their cell harvesting efficiency. Plain and modified microcarriers were added to separate low-adhesion 96-well plates in cell media. Cells were seeded after 12 h and were incubated for another 48 h. The cells were then trypsinized and a cell count performed, showing that the modified microcarriers were able to promote higher attachment of the cells. Figure 3E shows the results—harvesting efficiency is shown as a fraction of the cells recovered from the 48-h incubation period.

3 | DISCUSSION

Particle size analysis of the LbL modified microcarriers against unmodified microcarriers shows that all three systems—centrifugation, fluidized bed, and draining filter, can be used to apply polyelectrolyte multilayers to the microcarrier surfaces. All three methods showed a small, yet consistent shift in particle diameter, showing a greater

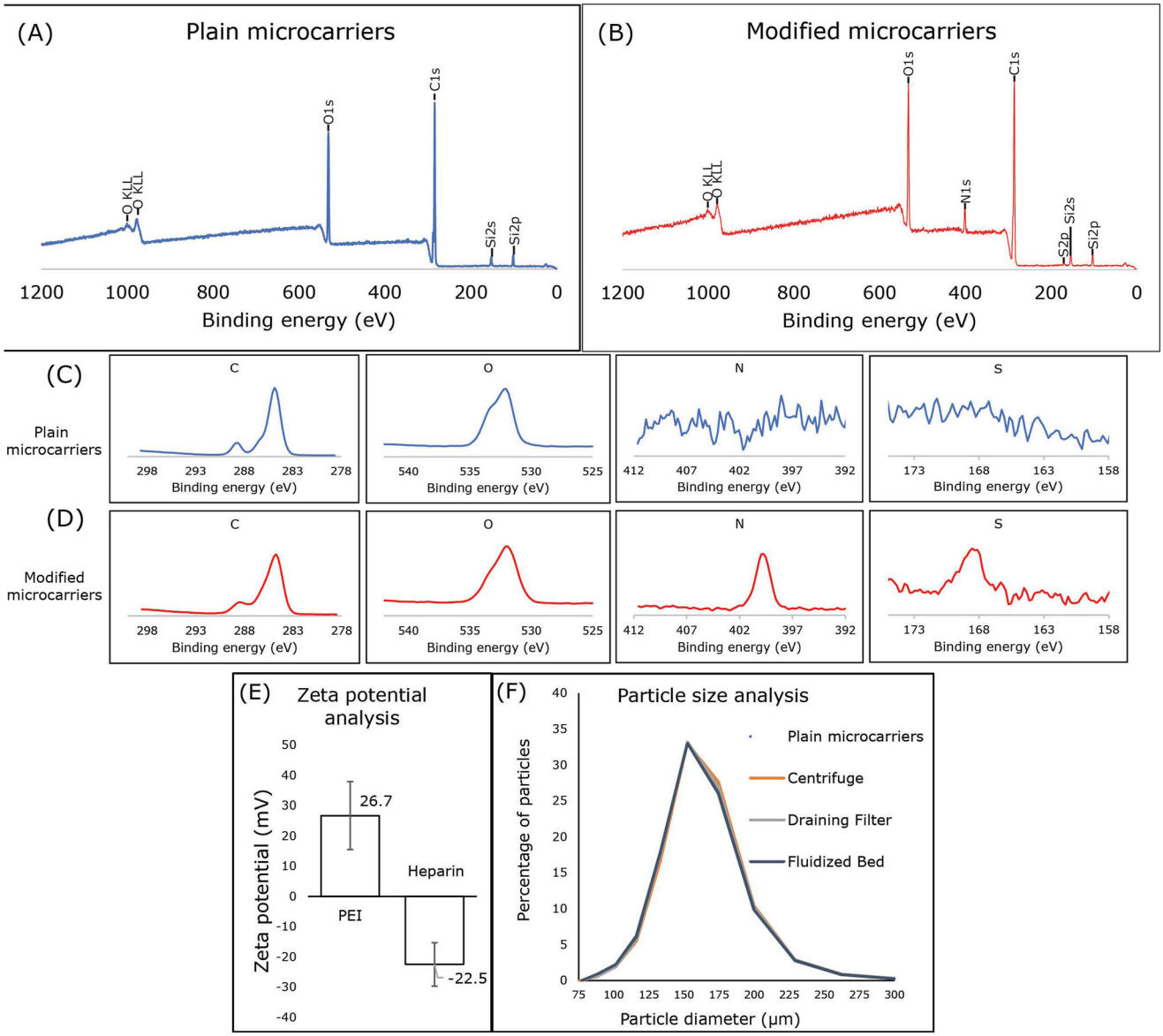

FIGURE 2 A quantitative assessment of the polymeric multilayers on microcarrier surfaces. Note that silicon was present in the tape used to affix the microcarriers to the sample holder (A) A full-spectrum XPS of plain microcarriers (B) A full-spectrum XPS of a microcarrier with surface modification. Carbon, oxygen, nitrogen, and sulfur high-resolution XPS spectra for plain (C) and LbL-modified (D) microcarriers. Notable differences can be observed on the nitrogen and sulfur, indicating the presence of heparin and collagen. (E) Zeta potential analysis of the modified microcarriers. Application of the PEI layers produced a positive charge on the microcarrier surface and heparin shows a negative charge as expected. (F) A particle size analysis of the microcarriers before and after LbL assembly using the three methods. All three methods for LbL assembly show growth in particle diameter over the plain microcarriers

proportion of higher-diameter particles after LbL modification. Zeta potential analysis was performed on microcarriers that were LbL-modified using the draining filter technique. PEI and heparin layers showed a positive and a negative charge, as expected. The results suggest that the coatings are nonuniform. The results from the XPS show that heparin is incorporated onto the microcarriers. A definite peak for sulfur around 168 eV is observed on the modified microcarriers.

The results from the fluorescence experiment show that the draining filter creates the most uniform polymeric multilayers on the surface of microcarriers, while centrifugation creates the least uniform multilayers. All microcarriers were treated with fluorescently-labeled collagen after LbL assembly in a similar fashion, and the microcarriers treated in the draining filter show the highest fluorescence. We theorize that the result is from a better drainage/wash process in the draining filter, as well as a better system for incubation. The draining filter does not forcefully settle the microcarriers like the centrifuge and offers a better removal of the polyelectrolyte and wash solutions than the fluidized bed, where the column is never drained. The purpose of the wash between polyelectrolyte layers is to wash away weakly adhered polymer molecules in preparation for the next polyelectrolyte,

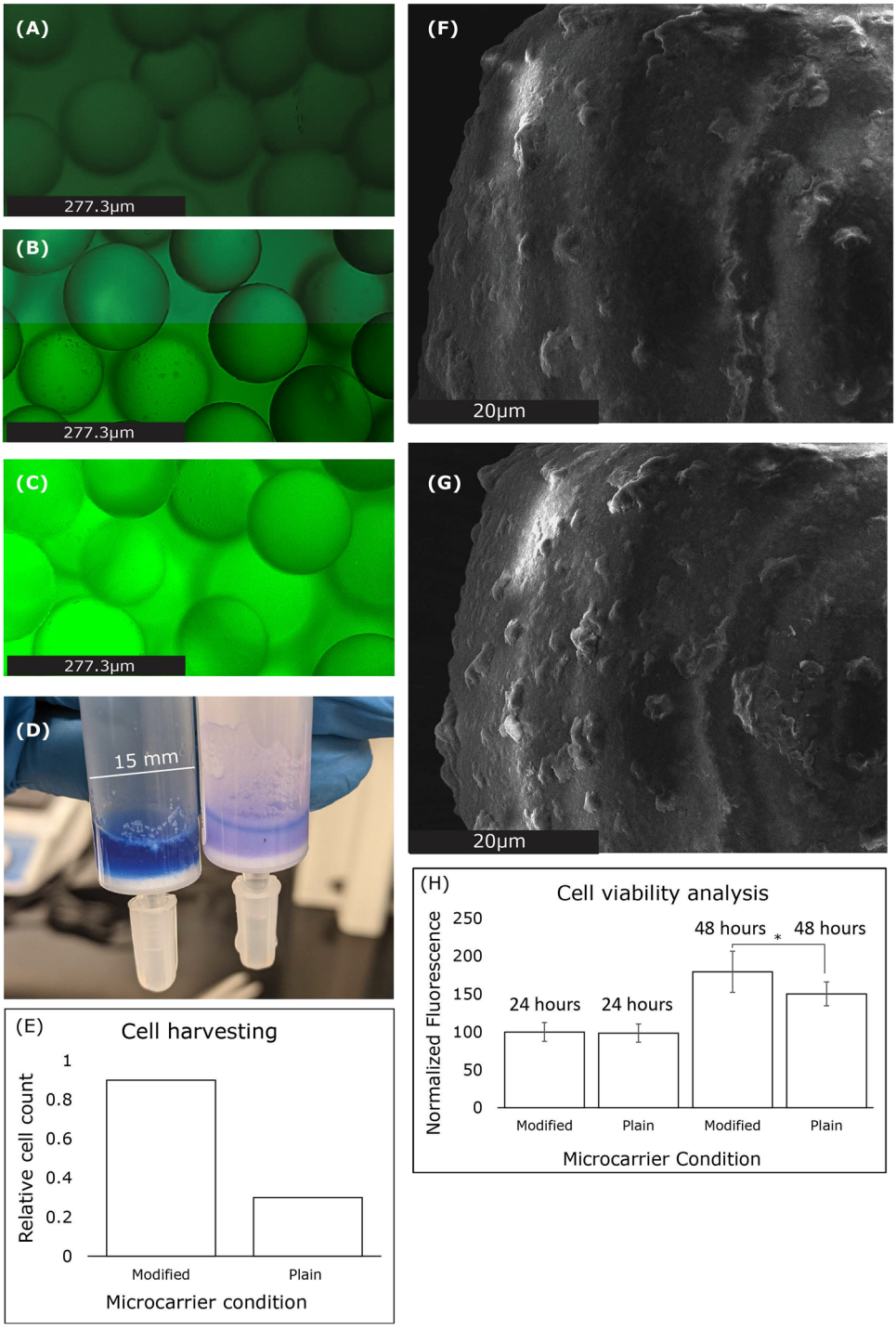

FIGURE 3 Qualitative assessment of the multilayers on microcarrier surfaces and cell culture. Fluorescent images of LbL modified microcarriers using the centrifugation method (A), the fluidized bed method (B), and the draining filter method (C). The modified microcarriers were stained using a fluorescently labeled collagen. (D) Azure A staining of microcarriers. Addition of the LbL microcarriers to Azure A dye changed the dye color to a purple hue (right) while the Azure A was unaffected by plain microcarriers (left) and remained a shade of deep blue. (E) Results from a cell harvesting experiment. Relative cell count is shown as a fraction of the cells that were recovered after hMSCs were cultured on the respective microcarriers for 48 h. Scanning Electron Microscopy of plain microcarrier surface (F) and LbL modified microcarriers (G). Increased roughness can be observed on the microcarrier surface, attributed to the accumulation of polyelectrolytes. (H) Results from a PrestoBlue cell viability assay. Variables include time (24 vs. 48 h) and microcarrier type (plain vs. modified) A higher fluorescence value indicates a higher cell viability. Results are shown as an average of 12 readings ± standard deviation. $^{*}p < 0.05$

and since the draining filter provides a near-complete drainage of the solutions, we expected the multilayers to be better adhered. Additionally, Azure A dye was used to stain the microcarriers after LbL assembly in a draining filter system—since two washes with PBS were performed at the end of the LbL process, the change in color of the dye shows that the heparin is strongly adhered to the microcarriers. Increased roughness is seen on the microcarriers under a scanning electron microscope, showing a visual change in the microcarrier's surface following LbL assembly. In addition, hMSCs cultured on well plates with the microcarriers were unaffected in terms of viability in the first 24 h and an increase in viability was observed after 48 h on the modified microcarriers over the plain microcarriers. A higher cell count from the harvesting experiment shows that the cells are adhering to and proliferating on the modified microcarriers better than the unmodified microcarriers. Since a bioreactor culture involves an initial incubation time where the cells and microcarriers remain unagitated in the vessel for a time, these results show that the multilayers may help improve suspension-based cell cultures.

There are several advantages that the draining filter offers over the other two systems that are not apparent only from the characterization results. First, there are virtually zero losses in the microcarriers when using the draining filter system. The microcarriers are too large to permeate the filter at the bottom of the NAP-25 column, and since there is only one-way flow (downwards through the filter), no microcarriers are lost during the LbL process. In contrast, in the fluidized bed, some microcarriers manage to reach the top of the column and drip out onto the waste container even when the flow rate of the polyelectrolyte or wash layers corresponds to a number very close to the minimum fluidization velocity. Though microcarrier losses are minimal during each step, compounding losses due to repeated addition of multilayers have been reported to decrease counts by an order of magnitude in literature.[43,47]

However, the most important advantage the draining filter offers is the ease with which the LbL process may be performed aseptically. Since the setup only consists of a small column and a clamp stand, the process can be easily carried out in a biosafety cabinet, while it would be difficult to do so using a centrifuge. An additional component, the peristaltic pump, is necessary in the case of the fluidized bed, which increases the contamination risk.

4 | CONCLUSIONS

Three different systems were investigated for their efficacy in creating polymeric multilayers on microcarrier surfaces—a centrifugation method, a fluidized bed method, and a draining filter method. Quantitative characterization techniques—particle size analysis, zeta potential analysis, and X-ray Photoelectron Spectra of the modified microcarriers show the formation of the multilayers. Similarly, qualitative characterization techniques show the formation of the multilayers as well as highlight the efficacy of the draining filter system over centrifugation and fluidized bed systems.

The draining filter system is a simple yet novel system for performing LbL assembly on microcarriers. Compared to the fluidized bed system, the draining filter system eliminates the need for long wash times necessary to flush out the previous polyelectrolyte solutions before application of the next layer. When compared to the centrifugation system, the draining filter requires fewer manual steps for separation of the supernatant and microcarriers. Another thing of note is that less microcarriers are lost in the draining filter—since the filter is impermeable to microcarriers, there are virtually zero losses during the entire LbL process. Minor microcarrier losses are inevitable due to the design of the other two systems—some microcarriers are removed with the supernatant in the centrifugation system while some leave the top of the column with the waste in the fluidized bed system. In addition, because the draining filter system requires less equipment and steps, the system is more appropriate when performing the LbL process aseptically. It is important that the process be performed in a sterile environment because the microcarriers must be free of any contaminants if they are to be used in cell culture applications.

Though all three systems were effective at creating multilayers on the microcarrier surfaces, it is apparent through the fluorescent staining of the microcarriers that the draining filter system can create more uniform surfaces. Aseptically modified microcarriers using the draining filter system were used in cell culture, showing that they have a positive effect on cell viability after 48 h. Since the initial incubation time where the cells attach to microcarriers is crucial to the rest of the cell culture process, our work shows that LbL films of heparin and collagen have a potential to improve cell cultures.

Our future work will focus on using the modified microcarriers with hMSCs in an agitated bioreactor and observing the effect of the coatings on cells more in-depth, including monitoring the bioreactors for glucose, ammonia, lactate, and glutamine levels, and testing the cells for modulation of interferon gamma through indoleamine 2,3-dioxygenase secretion. Furthermore, we will evaluate the integrity of the coatings after being exposed to the agitated environment of a bioreactor. We expect that the results from the proliferation and cell harvesting experiments will translate to the agitated culture.

5 | EXPERIMENTAL SECTION

5.1 | Layer-by-layer assembly

Sodium heparin (Celsius laboratories Inc. PH3005), poly(ethyleneimine) 50% weight solution in water (Sigma-Aldrich P3143), sodium acetate anhydrous (Fisher Bioreagents BP333-500), glacial acetic acid (Fisher Chemical A38-21, lyophilized type I collagen sponges (Integra Life-sciences Holdings Corporation, Añasco PR), and ultrapure water at 18 MΩ cm were used. All polyelectrolyte and buffer/wash solutions were prepared using a recipe from our previous work.[32] Polyelectrolyte solutions were prepared at a concentration of 1 mg ml^{-1}

0.1 g of Corning untreated microcarriers (Fisher Scientific 13-700-508) were used for each LbL technique. Prior to LbL assembly, microcarriers were hydrated in a 15 ml Fisherbrand centrifuge tube (Fisher Scientific 05-539-12). Ultrapure water was added to the centrifuge tube and the tube was vortexed to hydrate the microcarriers. The tube was then centrifuged at 400 rpm for 3 min to settle the microcarriers and the LbL process was started after removing the supernatant

The three different methods used for LbL assembly are outlined below:

Centrifugation: A centrifuge tube containing the hydrated microcarriers was filled to the 10 ml mark with a PEI solution and centrifuged at 400 rpm for 15 min. The supernatant was removed, and a pH 5 acetate buffer (wash) solution added. The tube was again centrifuged for 3 min, removing the supernatant afterward. This concluded the addition of an anchoring layer. A heparin solution was then added, and the tube centrifuged for 5 min, discarding the supernatant afterward, and the wash with the pH 5 acetate buffer was repeated. A collagen solution was added, the tube centrifuged for 5 min, and the supernatant removed. A wash with the acetate buffer was repeated, concluding the addition of a bilayer. The process was then repeated until six bilayers were formed (skipping the PEI step, which is only used to add an initial anchoring layer).

Fluidized bed: A NAP-25 column (Millipore Sigma GE17-0852-01) was modified by removing the upper screen and the resin inside. The column was washed several times with water and the tip severed with a pair of scissors. The column was affixed to a clamp stand. The hydrated microcarriers were added to the column and the water was allowed to drain. Next, a Fisherbrand Variable-Flow Peristaltic pump (Fisher Scientific 13-876-1) was used to continuously supply polymer/wash solutions at a rate of 1 ml min^{-1} through the severed tip in the same order as with the centrifugation system. The wash times were increased to 10 minutes (keeping PEI, Heparin, and Collagen at 15, 5 and 5 min still) to allow for complete removal of polymer solutions. A waste beaker was placed below the NAP-25 column to collect the liquid leaving the column.

Draining filter: The NAP-25 column was prepared similarly as with the fluidized bed. Polymer/wash solutions were added in the same order through the top of the column. A cap was placed over the severed tip during the incubation time (PEI for 15, wash for 3, and heparin and collagen for 5 min). The cap was removed after the incubation time, draining the column, and the polymer/wash solutions were added in sequence to form the desired number of bilayers.

All microcarriers were washed with Dulbecco's phosphate-buffered saline (Corning 14190250) after the LbL process. All surface modifications were done with 13 polyelectrolyte layers (starting and ending with heparin) except for the microcarriers used in particle size analysis and fluorescence microscopy, where 12 polyelectrolyte layers (starting with heparin and ending with collagen) were applied.

Fluorescent labeling of collagen was performed using an Invitrogen Oregon 488 labeling kit (Fisher Scientific O10241) according to the manufacturer's instructions. A Leica DM IL LED Inverted Laboratory Microscope was used to obtain the fluorescence images.

Two milliliters of Azure A (Fisher Scientific AAJ6134614) at a concentration 80 μg ml^{-1} was used to perform Azure staining of microcarriers in a modified NAP-25 column.

5.2 | Particle physical and chemical characterization

A Horiba LA-950 particle size analyzer was used to obtain particle size distribution for the microcarriers prior to and after LbL assembly.

A Malvern Analytical Zetasizer Nano ZS90 was used to perform the zeta potential analysis. Microcarriers were placed in DTS1070 folded capillary cells (Fisher Scientific NC0491866) in a 3% glycerol solution to keep them suspended.

Scanning electron microscope (SEM) images were obtained using an FEI Tital 80–300 Scanning/Transmission Electron Microscope.

X-ray Photoelectron Spectroscopy was performed using PHI VersaProbe scanning X-ray monochromator XPS.

5.3 | Cell viability assay and harvesting

Adult bone marrow-derived human mesenchymal stromal cells (Rooster Bio, United States) at passage 6 were used in the assay. MEM Alpha 1× (Gibco 12561056) supplemented with 20% Fetal Bovine Serum (Gibco 12662029), 1.2% L-Glutamine (Corning 25005CI), and 1.2% Penicillin-streptomycin (Corning 3002CI) was used as cell culture media.

Thirteen polyelectrolyte layers were added to the microcarriers using the Draining filter system in a biosafety cabinet. All equipment in the process was disinfected using 70% ethanol and UV treatment. After LbL assembly, the microcarriers were added to a Corning Ultralow Attachment 96-well plate (Sigma–Aldrich CLS3474) along with cell culture media and human mesenchymal stromal cells, filled to 200 μl. After 24 h, 50% of the media was replaced with a 20% solution of PrestoBlue (Fisher Scientific P50200) in hMSC culture media. The plate was incubated for 1 h and the fluorescence read using a Synergy LX multimode reader (BioTek SLXFA). Similarly, another plate containing the microcarriers and cells was tested for cell viability after 48 h, along with a plate with microcarriers and no cells as control. A two-tailed *t*-test was used to check for statistical differences between the samples at the 0.05 significance level.

To perform the harvesting experiment, microcarriers were similarly modified with 13 polyelectrolyte layers, conditioned in cell culture media for 12 h in a Corning Ultralow Attachment plate, and seeded with hMSCs from the same donor at passage 8. The plate was incubated for 48 h at 37°C. Afterwards, the microcarriers were washed twice with PBS, and then subsequently trypsinized using 0.25% Trypsin (Corning 25-050-CI) under gentle agitation for 10 minutes. The trypsinization was halted by adding the cell culture medium and cells were separated from the microcarriers using a cell strainer (Foxx Life Sciences 410-0001-OEM). A cell count was performed using a

hemacytometer. Unmodified microcarriers were used as control in both the PrestoBlue and harvesting experiments.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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