Methods for the assembly and characterization of polyelectrolyte multilayers as microenvironments to modulate human mesenchymal stromal cell response

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Supporting information

An example spreadsheet used to calculate amounts of different reagents required for layer-by-layer assembly. *Procedure S1.* Media preparation & cell expansion. *Procedure S2*. Count cells. *Procedure S3.* Setting the parameters of the room and IR-VASE device. *Procedure S4.* Alignment of Samples. *Procedure S5.* Transmission test. *Procedure S6*. Determining the roughness of the coating.

Table S1. An example spreadsheet used to calculate amounts of different reagents required for layer-by-layer assembly. Green cells represent user-entered values and blue cells contain calculations.

	A	B	C
1	Number of substrates	2	
2	Number of bilayers	6	
3	Volume of each wash	10	mL
4			
5	PEI volume required	=B3*B1	mL
6	HEP volume required	=B2*B3*B1	mL
7	COL volume required	=B3*B2*B1	mL
8	Wash volume required	=B1*B3+2*B2*B1*B3	mL
9	Round up to account for error:		
10	25	mL	PEI
11	130	mL	HEP
12	130	mL	COL
13	280	mL	Wash
14	Buffer pH 4 required	=A12*2	mL
15	Buffer pH 5 required	=A13+A11+A10	mL
16			
17	**Recipe:**		
18			
19	pH4 solution	=3.1/1000*B14	mL Glacial Acetic Acid
20		=1.27/1000*B14	g Sodium Acetate
21		=B14	mL Ultrapure Water
22			
23	pH 5 solution (wash included)	=1.3/1000*B15	mL Glacial Acetic Acid
24		=5.29/1000*B15	g Sodium Acetate
25		=B15	mL Ultrapure Water
26			
27	COL	=0.05/50*A12	g Collagen
28		=B14	mL pH4 buffer
29	Note: Evaporate off until COL is	=B28/2	mL
30	HEP	=0.05/50*A11	g Heparin
31		=A11	mL pH5 buffer
32			
33	PEI	=0.05/50*A10	g PEI
34		=A10	mL pH 5 buffer
	Note: Double the mass of PEI if it is 50% solution in water (which is typical)		

Procedure S1. *Media preparation & cell expansion (30 min).*

Media preparation:

1. Obtain Alpha-minimum essential media (α-MEM) (1×) from Gibco (supplemented with L-glutamine, ribonucleosides, and deoxyribonucleosides) and immediately thaw at 37° C water bath. Then transferring into a biosafety cabinet.
2. Add 20% fetal bovine serum, 1.2% penicillin-streptomycin, and 1.2% L-glutamine to the media.

Cell expansion:

1. Obtain hMSCs vial from nitrogen tank and immediately thaw at 37° C water bath. Place the vial inside the small beaker then put it inside the water bath and remove from the water bath once a small bit of ice is remaining (2-3 min).
2. Spray vial well with 70% ethanol before transferring into a biosafety cabinet.
3. Open the vial cap then transfer cells with 5 ml pipette into a 15 mL centrifuge tube.
4. Slowly add 4 mL of culture media containing 20% fetal bovine serum, 1.2% penicillin-streptomycin, and 1.2% L-glutamine) into the cells.
5. Centrifuge the 15 ml tube containing the cells at 200 x g for 10 min.
6. Place the vial inside the biosafety cabinet and carefully remove the supernatant that has accumulated at the bottom of the tube without disturbing the cell pellet.
7. Add 5 mL of culture media to cells and resuspend the cells with 5 mL pipet for 20 times.
8. Add 45 mL culture media to the cells.
9. Mix well and seed the cells into the T75 equally.
10. Transfer vessels into 37° C in a humidified incubator with 5% CO_2 and ensure that the surfaces are covered with media for 6 days.

Procedure S2. *Count cells.*

Count cells Manually:

- Prepare a sample with 50% cells in medium and 50%Trypan Blue.
- Manually count the cells under the microscope using a hemacytometer (Neubauer's chambers).
- Determine the number of cells (cells/mL).
- Take several measurements and determine an average.

Countess™ II FL Automated Cell Counter:

- Prepare a sample with 50% cells in medium and 50%Trypan Blue.

- Add10 µL of sample to Countess counting chamber A and10 µL to chamber B in a reusable or disposable slide.
- Wait 30 seconds to let the cells settle on the slide.
- Turn on the cell counter.
- Select and load a profile from the starting screen(e.g.,"Default") and click Load.
- Insert side A of the slide in the slide port.
- Wait for the camera to focus.
- Select Count.
- Record the results or Click on Save to export to a USB device.
- Take out the slide.
- Reinsert side B of the slide and repeat the procedure. Take an average result.

Procedure S3. *Setting the parameters of the room and IR-VASE device.*

Verify-in the room that the following parameters meet the requirements stablished in **Table S2**:

Table S2. Optimal parameters of the room and IR-VASE device

Parameter	Accepted value or range
Room Temperature	65-75°F
Room Humidity	<60%
Air Compressor Dryer Dew Point	Green area
Air Compressor Pressure	90-115psi
Air Compressor Outlet Pressure	~75psi
IR Purge Gas Generator Air Pressure Inlet	~70psi
IR Purge Gas Generator Moisture Indicator	Green
IR-VASE IR Box Dry Air Indicator	Blue
IR-VASE IR Detetor Box Dry Air Indicator	Blue
IR-VASE Dry Air Inlet Pressure	~29 psi
Air Compressor Manual Condensate	Drain
Tripp-Lite Smart Online UPS Status	2 or 20%

Procedure S4. *Alignment of Samples.*

Open WVASE-IR software. Click the *Align* menu and then click on *Load Sample.* The equipment will automatically align at 90°. The *Quad Detector Alignment* window will appear. Use the tilt knobs to align X and Y until both are <1 and then click on *Close*. In the *Translate Sample Stage*

Alignment window, rotate the X and Y knobs until a graph with a peak shows off; the higher the number, the better and then click on *Close*.

Procedure S5. *Transmission test.*

Open WVASE-IR software and perform the following procedure:

- Without Sample.
  Click on Motors, Incidence Angle: 90°, and click on OK
  Click on Motors, Incidence Angle: 0°, and click on OK
  Click on System, Alignment, New Baseline, and click on Display Relative intensity
  The Intensity will be approximately 1.0%
- Place the sample.
  Click on Motors, Incidence Angle: 90°, and click on OK
  Click on Motors, Incidence Angle: 0°, and click on OK
  Click on System, Alignment, New Baseline, and click on Display Relative intensity
  The Intensity should be between 0% and 0.01%.

Procedure S6. *Determining the roughness of the coating (10 min).*

Since the procedure is very similar to the determination of thickness, it will be shown as a summary:

- Open WVASE32 software.
- In the *Experimental Data* window, do a right-click.
  Click on *File,* click on *Open exp. File* and choose the file to analyze.
- Right-click → Range select (Experiment Window) → Wavelength (300-4000 1/cm) → ok
- Change Data type → Backside corrected type → ok
- Model → Add layer → IR Materials → *si_ir* → set 1nm thickness → ok
- Right-click → add above *si_ir* → *sio2_ntve_ir_g* → Place a thickness of 2.583nm → ok
- Right-click → add above *sio2_ntve_ir_g* → default materials → select *srough.may* → enter
  thickness previously measured → Check Fit → ok
- Generate data window → right click → Generate data
- Fit → Normal fit
- Fit → Pt. by Pt.

The sample roughness will appear in the *Fit* window.