Curvature facilitates podocyte culture in a biomimetic platform

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Supplementary Figure 1: Bubble topography may be hot embossed on standard polystyrene plastic. (a) Hot embossing conditions. (b) 24-well plate with topographic polystyrene base. (c) Immunofluorescent staining of podocytes cultured on hot embossed topographic plate. Nephrin (green), WGA (red), DAPI (blue). Scale bars 50 µm.
Supplementary Figure 2: Podocytes grown on the bubble substrate and with biochemical supplements express nephrin proteins. Immunofluorescent staining of podocytes cultured on flat or bubble substrates, before differentiation, and after differentiation with unsupplemented, ATRA-supplemented, or DEX-supplemented media. Rows 1 and 3 show low magnification with scale bar 100 µm, while rows 2 and 4 show high magnification images with scale bar 50 µm.
Supplementary Figure 3: Nephrin staining localizes in response to topographical and biochemical stimuli. (a) Total nephrin expression normalized to area of nuclei per image, for unsupplemented, ATRA-supplemented, and DEX-supplemented media groups on flat and bubble substrates. (b) Ratio of outer to central nephrin for three media groups on flat and bubble substrate. n=2-5, average ± s.d. shown. * p<0.05
Supplementary Figure 4: Nephrin proteins preferentially partition to the bubble structures in response to biochemical stimulation. (a) Distribution of nuclei in valleys or on bubble structures for three media conditions. (b) Percentage of total nephrin in a sample that partitions onto bubble structures. (c) Percentage of the bubble structure or valley areas that are covered with positive nephrin stain. * p<0.05, **** p<0.0001
Supplementary Figure 5: Cytoskeletal f-actin spreads radially in response to stimuli. Immunostaining for f-actin (green) of differentiated podocytes under various conditions. Rows 1 and 3 show low magnification with scale bar 30 µm, while rows 2 and 4 show high magnification images with scale bar 10 µm.
Supplementary Figure 6: Western blotting for nephrin. (a) Representative whole membrane with protein ladder and lanes for bubble and flat samples from DEX group demonstrating ~180 kDa nephrin band and other fragments. (b) Quantification of ~180 kDa bands for bubble and flat samples, normalized to GAPDH, revealing a trend for upregulated 180 kDa nephrin protein expression. n=3.
Supplementary Figure 7: Cytoplasmic extensions of podocytes and mesenchymal stem cells cultured on bubble substrates. Quantification of cytoplasmic extensions for (a) digitation ratio, (b) process length, and (c) process density, show that podocytes tend to form denser and more digitated extensions compared to MSCs. (d) Scanning electron micrographs show extensive branching and evidence of process interdigitation in podocytes but not in MSCs. Scale bar 2 µm.

* p<0.05, ** p<0.01
Supplementary Figure 8: Injection molding technique of designer polymers may be applied for topomembrane fabrication. (a) Schematic illustrating injection molding technique used for topomembrane fabrication. (b) Designer polymers assembled to a Transwell insert with a cap and glue system. (c) Standard 24-well plate with custom polymer topomembranes. SEM images showing (d) top view of polymer topomembrane with bubbles and with micropores, (e) curved view on topomembrane, (f) low magnification view of topomembrane with cells, and (g) high magnification view of podocytes growing over the top of the bubbles and covering the micropores.