SUPPLEMENTAL INFORMATION

A Polyelectrolyte Platform for Investigating Growth Factor Delivery Modes in Human Liver Cultures

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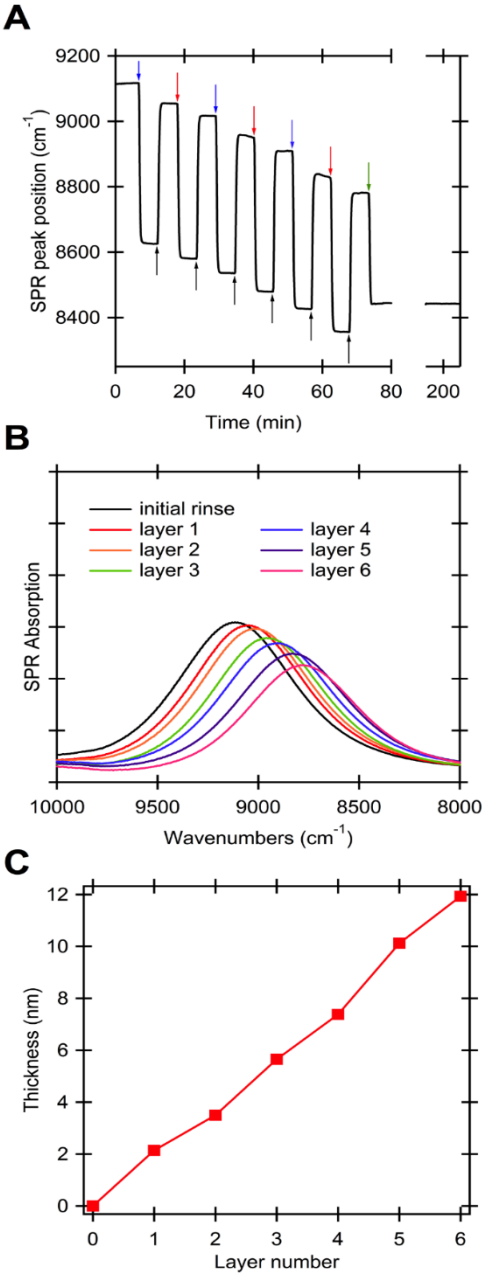
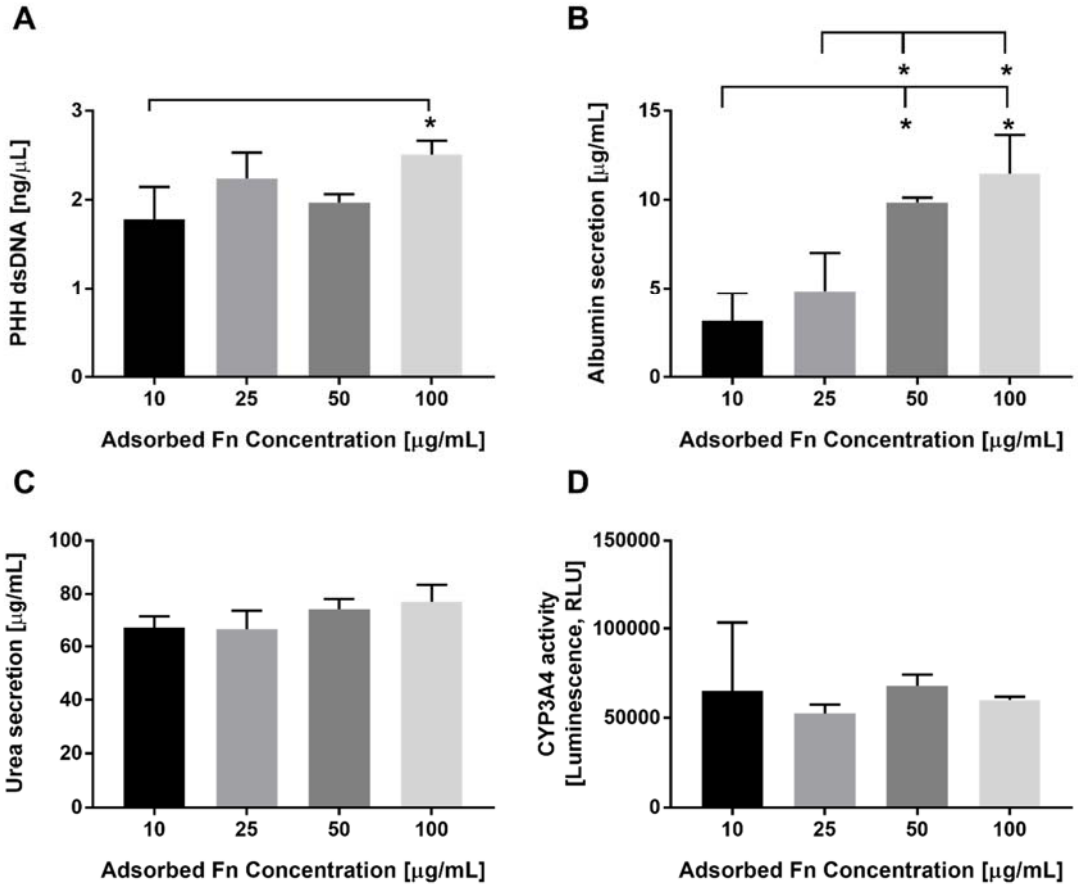


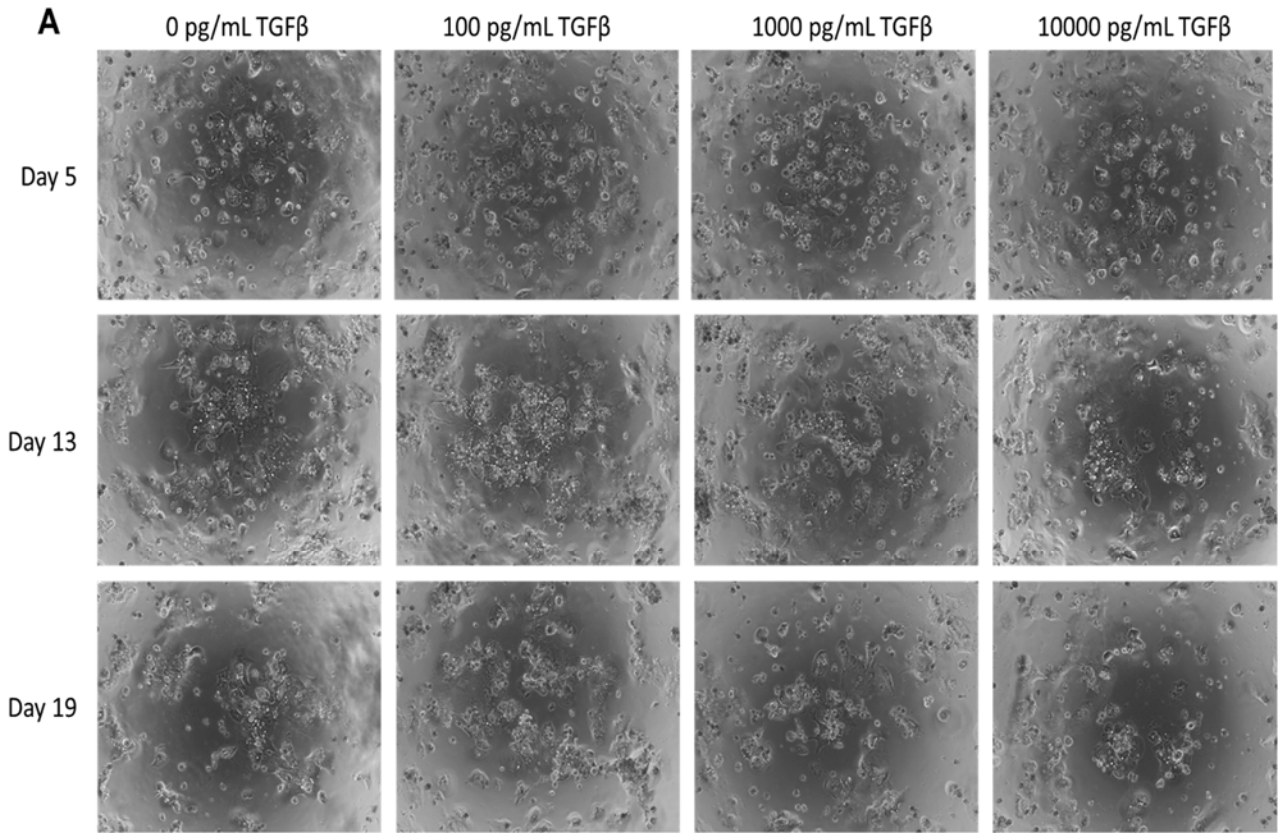
Supplemental Figure 1. Characterization of polyelectrolyte multilayer (PEM) substrate via Fourier-transform surface plasmon resonance (FT-SPR). FT-SPR was used to characterize the PEM-modified surfaces. PEMs were prepared on gold-coated glass surfaces as previously described.[1-3] Gold-coated substrates were first modified with a self-assembled monolayer (SAM) of mercaptoundecanoic acid (MUA). PEMs were constructed on MUA-modified gold films in the flow-cell of an SPR-100 module on a Nicolet 8700 FT-IR spectrometer (Thermo Electron, Madison, WI). The surface was exposed to an acidified water rinse, chitosan solution, acidified water rinse, and heparin solution. Each step was 5-6 minutes, and the sequence was repeated three times, followed by an additional acidified water rinse, to prepare six-layer PEMs (3 bilayers). For subsequent modification with TGFβ, the

surfaces were then exposed to either phosphate buffered saline (PBS) without TGFβ, or PBS with TGFβ at concentrations between 100 pg/mL and 300 pg/mL for 2 hours. Each experiment was repeated three times. FT-SPR spectra were collected during the entire surface modification procedure using Omnic 7.3 software (Thermo Electron) at 8 cm^{-1} resolution from 6000 to 12000 cm^{-1}. An FT-SPR spectrum was obtained every 4.7 seconds by co-adding 16 scans at each time point. The SPR peak position as a function of time was computed from the center of gravity, and the film thickness was determined from the rinse following each adsorption step as described previously.[3] **(A)** SPR peak position as a function of time during PEM assembly and a 2-hour PBS rinse. During the first five minutes, the surface is exposed to an acidified water rinse to establish a baseline peak position. Arrows indicate the introduction of a new solution into the flow cell: blue (chitosan), black (acidified rinse), red (heparin), and green (PBS). Each time a chitosan or heparin solution is introduced, the peak position shifts by nearly 500 cm^{-1}. This shift is due to differences in the refractive index of the rinse and polymer solutions and indicates the adsorption of the polyelectrolyte on the surface. When the subsequent acid rinse is introduced, the peak position does not return to the previous value, because the surface has been modified with adsorbed polyelectrolyte. After the final acidified water rinse, the surface is exposed to PBS. The signal is stable for the subsequent 2 hours, indicating that there are no apparent changes in the optical properties of the film during the PBS rinse. **(B)** Representative FT-SPR spectra during the PEM deposition experiment shown in panel A; each spectrum was taken during an acidified water rinse following the indicated layer. From the relative positions of the center of gravity of these peaks, the film thickness can be calculated. **(C)** PEM thickness determined by the FT-SPR peak shift during the rinse following each of the layer deposition steps, relative to the initial rinse. The chitosan layers (odd-numbered) contribute slightly more

incremental thickness than heparin layers (even-numbered) (~2.3 nm compared to ~1.6 nm), as we have previously observed under similar conditions.[2]



Supplemental Figure 2. Comparison of adsorbed fibronectin (Fn) concentrations on heparin-terminated polyelectrolyte multilayers (PEMs) for attachment and functionality of primary human hepatocyte (PHH) monocultures. (A) Double-stranded DNA (dsDNA) quantification from adherent PHHs (9 days after cell seeding) on heparin-terminated PEMs modified with 10, 25, 50, or 100 μg/mL of fibronectin. **(B)** Cultures as in panel 'A' except albumin secretion after 2 days is shown. **(C)** Cultures as in panel 'A' except urea synthesis after 2 days is shown. **(D)** Cultures as in panel 'A' except cytochrome P450 3A4 (CYP3A4) after 6 days is shown. *P<0.05 (one-way ANOVA and Tukey's *post-hoc* test).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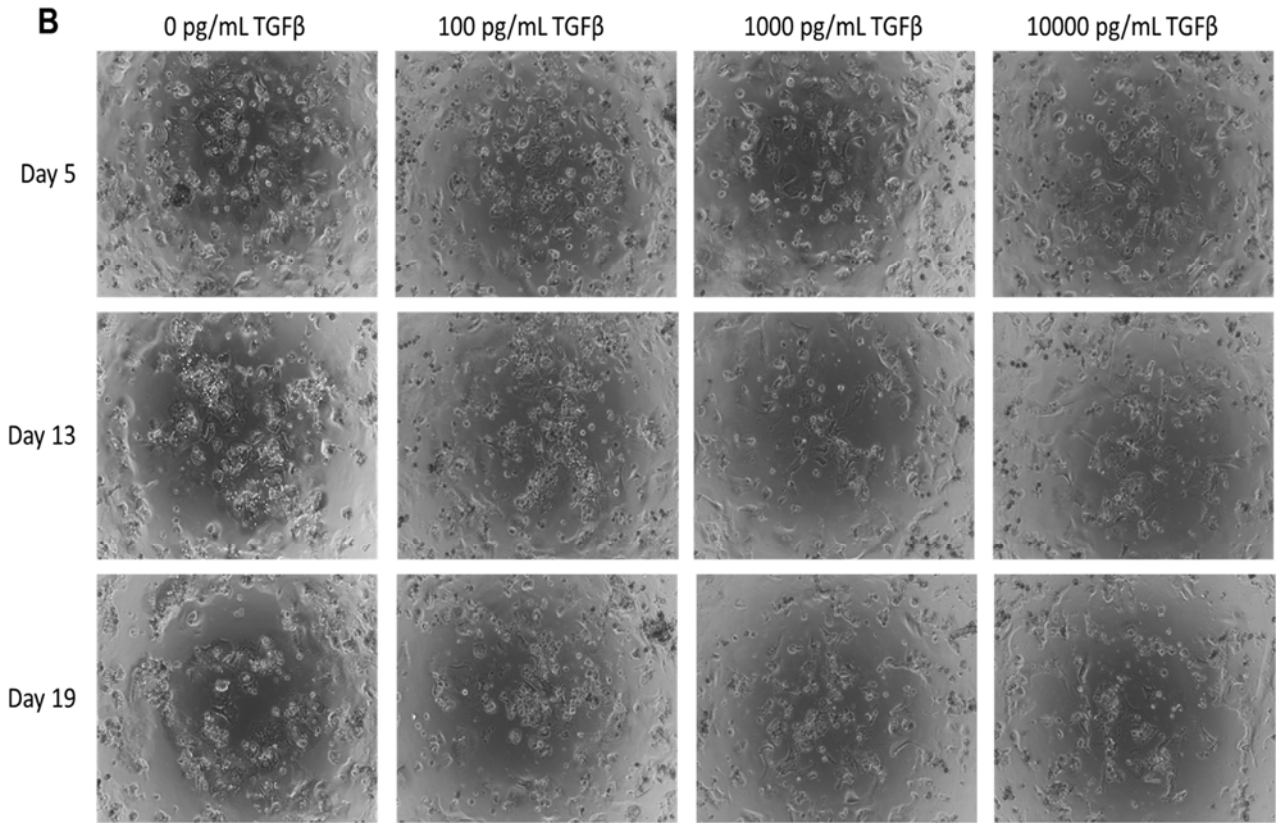
100 pg/mL TGFβ
1000 pg/mL TGFβ
10000 pg/mL TGFβ
Day 5
Day 13
Day 19


B
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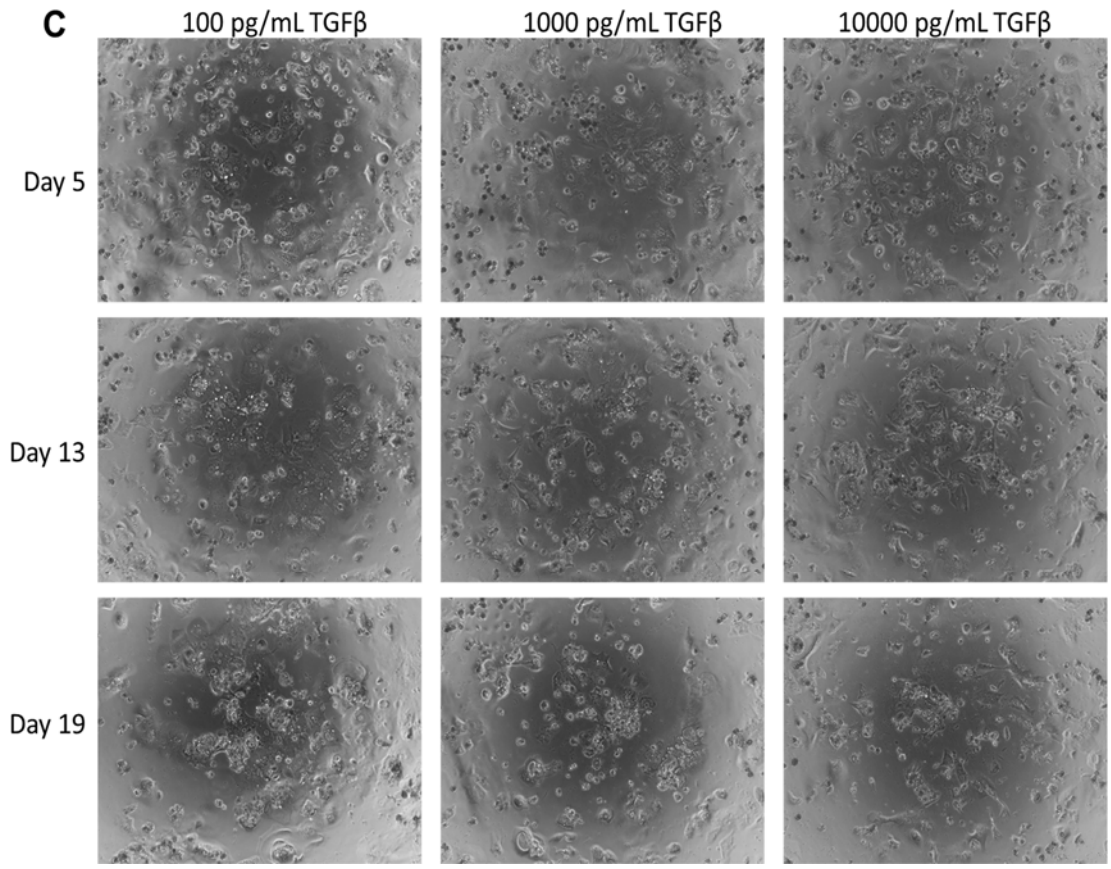
Day 5
Day 13
Day 19


C
100 pg/mL TGFβ
1000 pg/mL TGFβ
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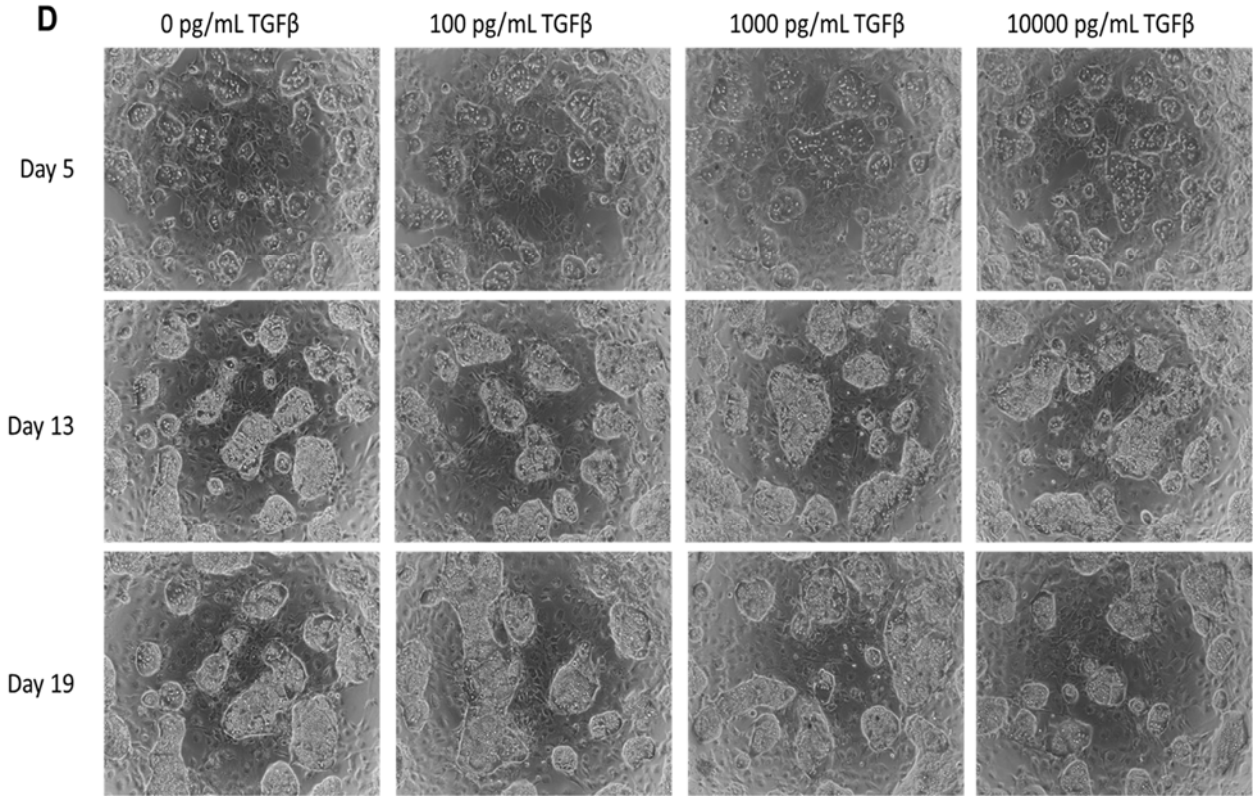
Day 5
Day 13
Day 19


D
0 pg/mL TGFβ
100 pg/mL TGFβ
1000 pg/mL TGFβ
10000 pg/mL TGFβ
Day 5
Day 13
Day 19

Supplemental Figure 3. Phase contrast images over time of primary human hepatocyte (PHH) monocultures and PHH and 3T3-J2 co-cultures treated with varying concentrations of transforming growth factor beta (TGFβ). (A) PHH monocultures were established on heparin-terminated PEMs modified with 100 μg/mL of fibronectin with varying concentrations of adsorbed TGFβ. **(B)** PHH monocultures were established on standard tissue culture polystyrene (TCPS) modified with 100 μg/mL of fibronectin and treated with varying concentrations of soluble TGFβ delivered via culture medium changes every other day in solution starting on day 5 of culture (continuous). **(C)** PHH monocultures were established on TCPS modified with 100 μg/mL of fibronectin and treated with varying concentrations of TGFβ delivered once in solution on day 5 of culture (1 soluble delivery). TGFβ-free controls are not shown since they are the same as those shown in panel B. **(D)** Co-cultures of PHH and 3T3-J2 murine embryonic fibroblasts were established on heparin-terminated PEMs modified with 100 μg/mL of fibronectin with varying concentrations of adsorbed TGFβ.

References

1. Boddohi S, Killingsworth CE, Kipper MJ. Polyelectrolyte multilayer assembly as a function of pH and ionic strength using the polysaccharides chitosan and heparin. Biomacromolecules 2008;9(7):2021-2028.
2. Almodóvar J, Bacon S, Gogolski J, Kisiday JD, Kipper MJ. Polysaccharide-based polyelectrolyte multilayer surface coatings can enhance mesenchymal stem cell response to adsorbed growth factors. Biomacromolecules 2010;11(10):2629-2639.
3. Almodóvar J, Place LW, Gogolski J, Erickson K, Kipper MJ. Layer-by-layer assembly of polysaccharide-based polyelectrolyte multilayers: a spectroscopic study of hydrophilicity, composition, and ion pairing. Biomacromolecules 2011;12(7):2755-2765.