BioPapers: An Innovative Approach to 3D Cell Scaffolds

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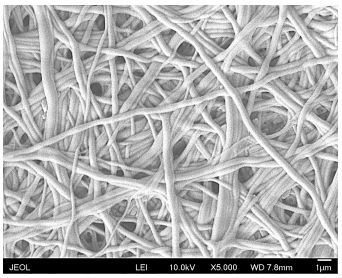
Statement of purpose: Cell-based assays are an important pillar in the discovery phase for early, cost-effective testing of pharmaceuticals, biologics, and scaffolds prior to large-scale and expensive *in vivo* studies [1]. Systems such as 3D cell and tissue culture and cell-based biosensing are yet to be adopted by the pharmaceutical industry. Limitations of traditional 2D cell-based assays in pharmaceutical research and development may account for a portion of the 90% of compounds that fail during clinical development [2]. 3D cell-based assays and sensing of the microenvironment are likely more analogous to the *in vivo* extracellular matrix (ECM). These platform studies could bridge the gap between monolayer cultures and animal studies [4]. They can also serve as platforms for fine-tuned tissue engineering matrices. Unfortunately, *in vitro* culture and technology has changed little over the last few decades, and newly available techniques are not implemented as industry standards to date [1]. One emerging approach to solving this problem is the generation of 3D nanofibrous scaffolds via electrospinning. Electrospinning materials offers the ability to control BioPaper architecture, fiber size, alignment, porosity, mechanical properties, depth, and degradation rate [4].

Methods: Gelatin was dissolved in 70% acetic acid and electrospun into nanofibrous constructs using a needless electrospinning system. The nanofibers were set into 12-well transwell cell crowns and crosslinked in EDC/NHS solution, then coated with collagen at 3mg/ml. The surface morphology and fiber diameter of the nanofibrous constructs were observed using SEM and analyzed via ImageJ. Tensile properties of uncrosslinked and crosslinked constructs were measured. PC-12 cells were cultured in DMEM w/ 10% calf serum in an incubator at 37°C with 5% CO2. Constructs were seeded at 2×10^6 cells/mL on nanofibers, and on flat polystyrene as control. Cells were visualized and analyzed by two molecular probes, calcein-AM (Invitrogen) and ethidium homodimer-1 (EthD-1) to determine cytocompatibility. Stained PC-12 cells were imaged by confocal microscopy. Cells were fixed with 4% formaldehyde and then permeabilized by immersion in 0.2% Triton for 5 min. Afterwards, the nuclei was stained with 40 ,6-diamino-20 -phenylindole. Cell viability and adhesion were calculated from cell counts of images obtained through raster patterning of three specimens. Scaffolds were held at -20°C and room temperature for 3 months and re-imaged via SEM.

Results: Our work has demonstrated that nanofiber gelatin mats, BioPapers, can be electrospun at scale and mounted into standard 6, 12 and 24-well transwell holders. Additionally, we demonstrate BioPapers can be crosslinked and retain nanofiber structure while also becoming insoluble in culture medium, thereby making them useful for cell-based assays. The average fiber diameter was determined to be ~650nm with SEM analysis (**Fig 1**). Pore size was calculated to be 1-15μm. We also found that crosslinking increases the mechanical strength of the BioPapers from 0.1 to 1.8 GPa. We demonstrated stability of the BioPaper scaffolds after 3 months at <-20°C. Fiber analysis was also performed and significant fiber fusion was see at 30 days when scaffolds were stored at room temperature, while scaffolds stored at lower temperatures maintained fiber integrity. We next sought to determine BioPaper compatibility with cell-based assays. Our immunostaining data demonstrates that BioPapers can support growth of cells in culture as well as support confocal analysis. Compatibility with confocal imaging is further supported via live/dead staining assays **(Fig 2)** We further assessed viability of cells grown on BioPapers, demonstrating that cells have above 95% viability in culture over a 10 day growth period.



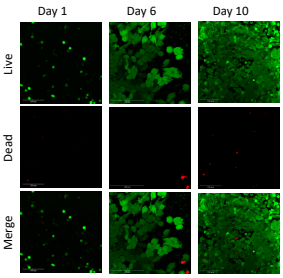


Figure 1 (L). Crosslinked Gelatin BioPaper
Figure 2 (R). Live/Dead staining of PC-12 cells on BioPapers. Nuclei are stained with DAPI (blue). Actin fibers are stained with Alexa 488 Fluorophore (green).

Conclusions: We have shown that BioPapers can be formatted for multiple tissue culture plate sizes. We have also found that crosslinking improves usability and stability of BioPapers in cell-based assays and improves mechanical strength. The achieved fiber diameter of ~650nm supports cellular attachment and growth. BioPapers provide a 3D environment that allow for high cell viability and can be used for confocal analysis.

References: [1] Edmondson, R., et al., Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay Drug Dev Technol, 2014. 12(4): p. 207-18. [2] Hait, W.N., Anticancer drug development: the grand challenges. Nat Rev Drug Discov, 2010. 9(4): p. 253-4. [3] Yamada, K.M. and E. Cukierman, Modeling tissue morphogenesis and cancer in 3D. Cell, 2007. 130(4): p. 601-10. [4] Bhardwaj, N. and S.C. Kundu, Electrospinning: a fascinating fiber fabrication technique. Biotechnol Adv, 2010. 28(3): p. 325-47.