Meso-scale topological cues to promote endothelial cell proliferation on macro-scale, blood-contacting polymeric substrates

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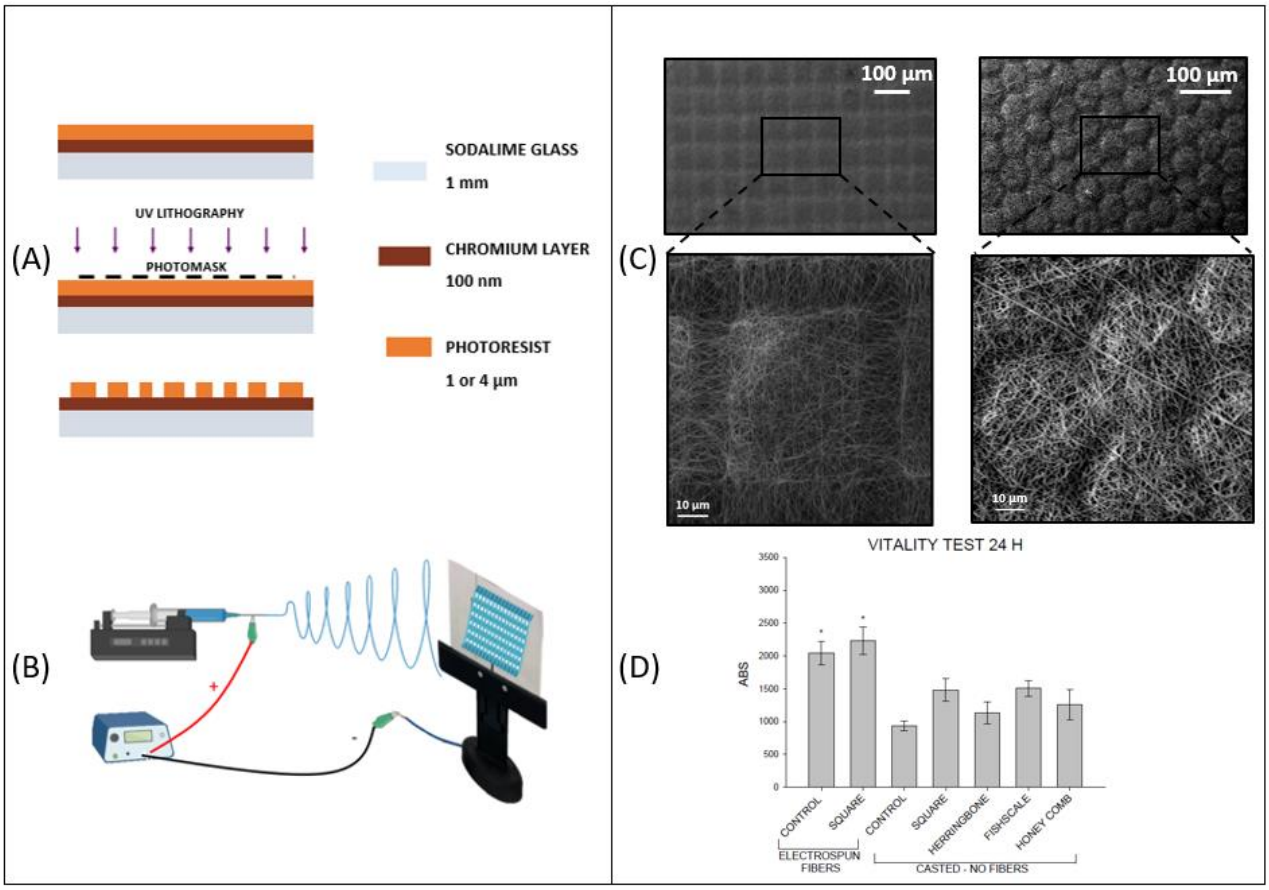

Figure 1. Schematic of the polymer processing method developed to prescribe specific mesoscale topologies: (A) The polymer collecting target was produced via photolithography with steps including: a glass-chromium substrate + AZ1518 photoresist laser patterned with the desired mesoscale geometry; (B) The patterned wafer acted as a collector for the electrodeposition with processing variables properly selected to transfer the pattern from the wafer to the electrospun substrate. (C) SEM images with two mesoscale patterns successfully transferred on a large scale (4x4cm) with a depth of 4 µm: on the left square topology with a side of 60µm and 20µm gaps; on the right honeycomb topology with a side of 60µm and 20µm gaps; (D) Vascular smooth muscle cell (VSMC) Alamar Blue vitality test at 24 hours (One Way ANOVA * = $p < 0.05$ vs CASTED – NO FIBERS SAMPLES).

Keywords: mesoscale topological cues; endothelial cell proliferation; polymer electrodeposition.

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Objective: Mesoscopic topological cues are a challenging and promising alternative to promote the formation of a stable endothelial cell layer able, long-term, to impact the thrombogenicity of medical devices. The use of polydimethylsiloxane (PDMS) micropatterned films and substrates has shown limitations, such as slow degradation rate, low surface-to-volume ratio, low permeability, and a rather limited scalability which all affected efficacy and biocompatibility. In this preliminary study, we introduce a hybrid lithography and electrodeposition method to fabricate permeable, large, fiber-based substrates with mesoscale patterns putatively able to mimic the basement membrane and promote functional endothelium formation.

Methods: Lithography was performed through a direct laser writing system(LW405E, Microtech srl) that selectively removed the photoresist and engraved the prescribed pattern on a three-layer substrate composed of soda-lime glass, AZ1518 photoresist, and chrome. The conductive chrome layer enabled the wafer to serve directly as a collector for the electrodeposition process. The alternated conductive/non-conductive regions of the target were designed to attract the electrospun fibers selectively, processing values were investigated in the range V(Δ14kV),d(10cm), t(30min), T(22°C), HR(35%), Q(0,4ml/hr), needle(21G). Two different patterns depths were evaluated: 1µm, and 4µm.

Patterns were investigated via scanning electron microscopy (SEM) and quantitative analysis (%patterns on polymer substrate/patterns on CAD model) to prove the capacity of the method to prescribe a mesoscale pattern regardless of its shape: square and honeycombs both with a side of 60µm and 20µm gaps. Scaffolds were seeded with vascular smooth muscle cells (VSMCs) to assess the impact of the topological cues on cellular metabolic activity via Alamar blue test.

Results: Qualitative and quantitative analysis of SEM images confirmed the ability to transfer a pattern geometry regardless of its shape with an accuracy of 84% and 98% respectively for 1µm and 4µm depth wafers. This proprietary technique(US2024/0016983A1) demonstrates higher cellular activity than casted non-fibrous patterns.

Conclusions: This study showed how photolithography and electrodeposition can be combined to process micro-fiber-based substrates 4cmx4cm with mesoscale patterns of desired shape feasible for applications at organ level scale enhancing cellular viability.