# Cell Morphogenesis and Organization on Electrospun Scaffolds is Guided by Varying Porosity and Fiber Diameter

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**Statement of Purpose:** Cellular permeability and host-tissue inflammatory response are critical to the effective use of electrospun fiber mats as biomedical implants. Specifically, when the mats are used as tissue engineering scaffolds, they must facilitate the infiltration of cells and the formation of functional tissue.[1] In contrast, for the prevention of surgical adhesions, the fiber mats must be impermeable to cells to prevent the formation of a continuous scar across the implant. Our goal was to study the role of fiber diameter and inter-fiber porosity of electrospun fiber mats on the morphogenesis and infiltration of primary macrophages and fibroblasts.

**Methods:** Poly(desaminotyrosyl tyrosine ethyl ester carbonate), abbreviated poly(DTE carbonate), was synthesized using a previously published method.[2,3] The polymer was electrospun to fiber diameters of 2.3±0.76, 1.0±0.41, 0.54±0.15, 0.29±0.11 µm (mean ± standard deviation, n=15). Residual solvents were removed by 20 hour exposure to vacuum at 76±2 °C. UV-sterilized scaffolds were wetted 2 hours using DMEM supplemented with 10% FBS, antibiotics, and L-glutamine. Transgenic GFP rat dermal fibroblasts or RAW 264.7 macrophages were plated at 10,000 cells/cm$^2$ and allowed to migrate and proliferate for 5 days. On day 5, samples were fixed with 4% paraformaldehyde.. Macrophages were permeabilized with 0.1% Triton, 1% fetal bovine serum in phosphate buffered saline. The cytoskeleton was then stained with fluorescein conjugated phalloidin 1:200 (Molecular Probes, Oregon). The cells were either mounted for confocal imaging (Leica TCS SP2) or dehydrated for SEM (AMRAY-1830I). SEM image analysis was performed with NIH ImageJ (http://rsb.info.nih.gov/ij/), and confocal image analysis was performed with Leica Confocal Software.

**Results / Discussion:** Rat dermal fibroblasts took on three distinct morphologies, Figure 1. On the 2.3 µm diameter electrospun scaffold, the fibroblasts interacted with 2-3 fibers, assuming an elongated morphology when conforming to the surface. The 1.0 µm fiber diameter scaffold caused the cells to assume a more rounded morphology, as they interacted with a pocket of approximately 10 fibers. On scaffolds with the finest fiber mat, 0.29 µm, cells were no longer able to penetrate below the surface. Instead, we only observed cell processes that extended slightly beneath the surface of the scaffold. The 0.54 µm scaffold caused the fibroblasts to obtain an intermediate morphology exhibiting a blend of the characteristics of the 1.0 and 0.29 µm diameter scaffolds.

Macrophage morphology varied in the same manner as fibroblast morphology: The macrophages adopted a shape to conform to the largest fiber diameter scaffolds, presented a more rounded morphology when adhering to the intermediate fiber diameter scaffolds, and assumed a more spread and bipolar morphology on the finest fiber diameter scaffolds.

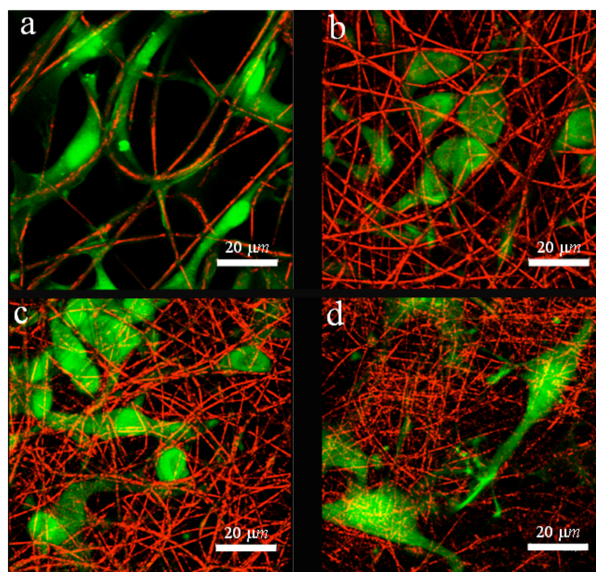


Figure 1: Fibroblast morphology at 5 days observed on a) 2.3 b) 1.0, c) 0.54, and d) 0.29 µm fiber diameter scaffolds

A lateral x-z scan of the cell-seeded scaffolds via confocal microscopy confirmed that cells were able to permeate into the depth of the 2.3 and 1.0 µm scaffolds; however, the 0.29 µm scaffold clearly size-excluded fibroblasts from permeating into the scaffold. Results for the 0.54 µm scaffold were inconclusive.

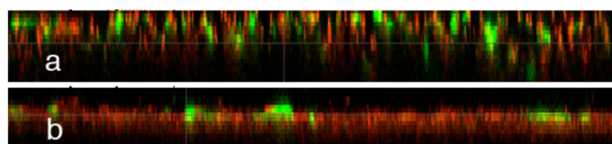


Figure 2: Day 5 fibroblast permeation observed on a) 2.3 and b) 0.29 µm fiber diameter scaffolds. Each cross section represents 27 microns of depth from the top surface of the scaffold.

**Conclusions:** The electrospun scaffolds had varying fiber diameter, which in turn controlled the inter-fiber porosity. Scaffolds of 0.29 µm clearly excluded firoblast infiltration. Results were inconclusive for the 0.54 µm fiber diameters, while the scaffolds with larger fiber diameters clearly facilitated the infiltration of cells into the scaffold. Our observations indicated that fiber mats must have fiber diameters of about 0.3 µm or below to be used for the prevention of surgical adhesions. Further work is under way to examine the degree of macrophage activation on the scaffolds with variable porosity.

**References:**

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