Aligned poly(ε-caprolactone) nanowire/fiber arrays: properties for drug delivery and control of cellular interactions

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Statement of Purpose: Nanostructured materials for applications in biology and medicine have gained interest with recent findings that cellular responses can be directed by nanotopography. Here the simple method of template synthesis[1,2] is used to create arrays of free-standing nanowires and flexible nanofibers from the biocompatible, biodegradable polymer poly(ε-caprolactone) (PCL). It is shown that nanostructure morphology can be controlled and complex patterning may be achieved. Furthermore, sustained release of therapeutics can be delivered by the nanowires, and cellular interactions can be controlled.

Methods: PCL disks were placed on top of clean and dry templates of nanoporous aluminum oxide. Template synthesis was carried out at varying temperatures and times. After cooling to ambient room temperatures, the template was selectively etched in sodium hydroxide solution. Hot-melt encapsulation was used to load PCL nanowires with model drugs, fluorescein and bovine serum albumin (BSA). BSA release was quantified using a MicroBCA Assay and fluorescein release was quantified using a fluorocount plate reader. Nanowire surfaces were characterized by SEM, AFM, and contact angle. Fibroblast (IMR-90) cell interactions with the PCL nanowire surface were examined using fluorescence microscopy and SEM.

Results/Discussion: In order to overcome the need of fabricating nanoporous aluminum oxide membranes of varying thickness to produce varying nanowire lengths, nanowire length as a function of melt time and temperature was instead studied. By varying either the melt temperature or time, the morphology of the nanowires could be changed. Nanowire arrays less than 10 μm in length were found to be freestanding (Fig. 1A). Nanowires greater than 10 μm in length, instead, folded over to form long strands of arrayed nanofibers layered over the substrate base (Fig. 1B). A combination of soft lithographic techniques and template synthesis can be used to form complex pattern structures of both nanowires and nanofibers (Fig. 1C).

Fibroblasts cells were found to adhere similarly on glass, flat PCL, and on the PCL nanowires (~12 μm in length) after 24 hours in culture. However, few of the cells on the PCL nanowire substrates spread (65.6% glass, 11.5% PCL, 0.6% PCL nanowires). Closer examination of cells after 72 hrs in culture showed two main cell morphologies on the PCL nanowires: spreading versus retracted. Spreading cells, though 2 to 3 times smaller than control cells, appeared to interconnect islands of nanowires, whereas retracted cells were seen to rest and conform to the shape of the nanowire islands (Fig. 1D).

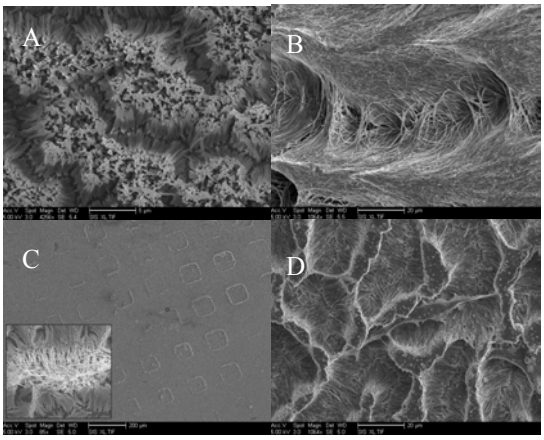

Figure 1. A) Free-standing PCL nanowires 2 μm in length. B) Flexible PCL nanofibers 27 μm in length. C) PCL nanofiber patterns (square outline) atop an array of nanowires. D) Endothelial cell cultured on PCL nanowires.

Model drug release from PCL nanowires was observed over a period of seven weeks at 37°C in phosphate buffered saline as a release medium. Fluorescein was found to release steadily over a period of a week. The release profile for BSA, however, was characterized as a short burst phase during the first 8 hours where approximately 30% of the cumulative protein released was delivered. Sustained release was achieved over a period of 21 days after which release leveled off (Fig. 2).

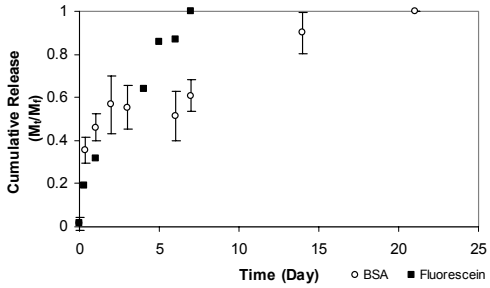

Figure 2. Release of BSA and fluorescein from PCL nanowires.

Conclusions: Combining techniques for nanowire fabrication, patterning at the micron level, and localized drug delivery capability will allow for the creation of sophisticated constructs to control cellular responses. The capability to control cell responses at both the nano- and microscale using material properties will be useful not only in the regeneration of hard and soft tissues, but also in device fabrication, and coatings for implantables such as stents, orthopedic implants, and biosensors.

References:

[1] Martin, C.R. *Science* 1994: **266**: 1961-1966.
[2} Steinhart, M., Wendorff, J.H., and Wehrspohn, R.B. *ChemPhysChem* 2003:**4**: 1171-1776.