

# Fibroblast Cell Attachment and Growth on Nanoengineered Sculptured Thin Films

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**Abstract:** Nanoengineered parylene-C sculptured thin films (STFs) are deposited on glass and silicon substrates using a direct one-step growth technique. The deposited STFs support fibroblast cell attachment and proliferation *in vitro*, which is an early indication of biocompatibility and bioactivity of this emerging class of biomaterials. Surface modification of endoprostheses of the small joints of the hand, which heal with fibrous stabilization, may be greatly enhanced by such nanoengineered biomaterials. © 2006 Wiley Periodicals, Inc. *J Biomed Mater Res Part B: Appl Biomater* 81B: 219–223, 2007

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## INTRODUCTION

Implanted polymeric medical devices are essential components of modern reconstructive surgery in order to restore function and thus improve the quality of life. Although these devices are said to be *biocompatible*, every one of them essentially elicits a foreign-body reaction, which encapsulates and walls it off within the body. Surface modifications of existing polymeric implants may allow for devices that may heal more physiologically, thereby leading to better tissue integration of implants.<sup>1</sup>

Biological cells have been grown on nanostructured surfaces, which suggests the significance of nanomorphology for all surfaces of an implanted device that are going to be in contact with biological tissue. There is a growing realization that ongoing research in the areas of fundamental surface biology, nanofabrication, and recombinant DNA technologies will provide enhanced 3-dimensional tissues designed to accomplish specific biological and medical goals. Microfabricated surfaces have been used to control cell adhesion at the micron scale.<sup>2–7</sup> It is known that the topography of the surface alters proliferation<sup>8,9</sup> and differentiation.<sup>10–13</sup> It has been also shown that submicron-scale features activate macrophage cell adhesion and regulate the amount of F-actin in cells.<sup>14</sup> The notion that polymer surface topography influences corneal epithelialization was demonstrated convincingly by Dalton et al.,<sup>15</sup> who showed

that polycarbonate surfaces with pores in the range 0.1–0.8  $\mu\text{m}$  support superior epithelial tissue stratification and protein deposition when compared with those containing pores greater than 1  $\mu\text{m}$ . More recently, Karuri et al. showed that cell attachment on silicon columnar films can be dependent on nanoscale topography<sup>4</sup>; nanostructured surfaces affect the morphology of human corneal epithelial cells. However, the long-term degradation of silicon interacting with biofluids is problematic, and the structures made heretofore require complicated and expensive fabrication techniques (e.g., lithography and masking).

Sculptured thin films (STFs) are assemblies of upright, parallel-shaped nanowires generally grown by vapor deposition techniques.<sup>16</sup> These are deposited on a substrate from a directional vapor source, with the substrate orientation relative to vapor source dynamically manipulated during deposition. The morphology of STFs comprises clusters of 1–3 nm that coalesce into 30–50 nm diameter columns, and then form bundles of 300–500 nm diameter, depending on the deposition material.<sup>16</sup> Blending of nanoscale and microscale morphologies is possible.<sup>17</sup> As STFs are porous with densities as low as 20–30% of the bulk material, they can be infiltrated with gases, liquids, liquid crystals and organic monomers; these infiltrating materials can alter the host STF's properties.<sup>16</sup> Many biomedical applications are therefore possible.

The three major advantages of STFs as biomaterials are as follows: (i) Their surface-to-volume ratio is very high, the available surface area increasing by over two orders of magnitude in relation to the bulk material. It has been shown using a nitrogen adsorption technique that the surface area of STFs can be 375 times larger than that of a

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flat film,<sup>18</sup> which is significant as it has been shown for silicon<sup>4</sup> and polycarbonate<sup>15</sup> films that increased surface area promotes cell adhesion. (ii) Their porosity is controllable,<sup>16</sup> and so it is possible to engineer not only the surface properties of STFs but also 3-dimensional scaffolds. (iii) They can be made out of many FDA-approved polymeric materials and can be endowed with transverse architectures<sup>17</sup> to provide the best possible substrate and coating material for biological attachment at the nanoscale.

Direct one-step fabrication of chiral STFs of parylene-C that have 200-nm size columnar features was recently demonstrated.<sup>19</sup> Modification of implant surfaces with vapor deposition of parylene appears feasible, and will allow for inexpensive nanoengineered coatings to augment tissue integration. In light of biomaterials literature supporting tissue-growth enhancement at nanoscale grooves and pores, it is reasonable to hypothesize that nanostructures such as the parylene STFs will optimize tissue ingrowth and provide for improved implants and prosthetic devices.

In this communication, we present the bioactivity of nanoengineered polymeric sculptured thin films (STFs) for COS-7 fibroblast cells. The deposition process is robust, inexpensive, and does not require any template, mask, or lithography technique.<sup>16</sup>

## METHODS

### Deposition of Polymeric Sculptured Thin Films

The type-C parylene is the polymer form of the low-molecular-weight dimer of para-chloro-xylylene.<sup>20</sup> Parylene-C is a biocompatible polymer<sup>21</sup> that is widely used in biomedical coatings. The chemical formula of the parylene-C monomer is C<sub>8</sub>H<sub>7</sub>Cl.

A flow chart of the fabrication process, including structural formulas of the dimer and the monomer and the resulting STF, is shown in Figure 1. Depositions were made in a commercial parylene reactor that had been modified to combine chemical and physical vapor deposition processes specifically for direct one-step fabrication of polymeric STFs.<sup>19</sup> The parylene dimer was placed in a vacuum system and converted to a reactive monomer vapor by pyrolysis. The amount of the dimer was the only adjustable deposition parameter. The monomer vapor was then directed through a nozzle towards a rotating substrate, on which it condensed and polymerized. The deposition rate and the deposition pressure were controlled by the evapo-

ration temperature (155°C) of the dimer and the pyrolyzing temperature (690°C). 0.7 g of parylene-C dimer was inserted into the vaporizer for each deposition, during which process the vapor pressure was maintained at approximately 10 Torr. The thickness for all chiral STFs deposited was found to vary between 15 and 30 μm. Uniformity of STF patches within an individual batch and between identical batches (e.g., for deposition onto different substrates) was qualitatively assessed by scanning electron microscopy.

### Fibroblast Cell Growth

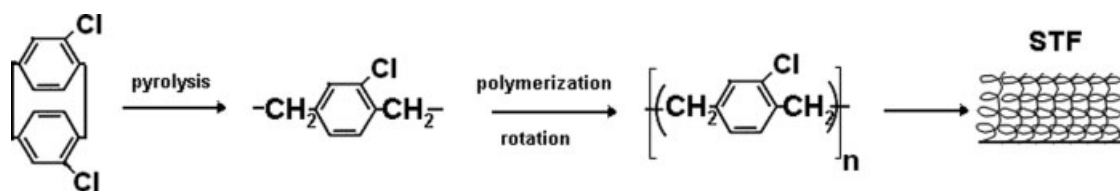
The COS-7 cells, originating from an African Green Monkey kidney fibroblast cell line (ATCC 1651 CRL) that has been immortalized by the expression of the large T antigen from the SV40 virus, were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 0.1 mM nonessential amino acids, and 1.0 mM sodium pyruvate at 37°C in a humidified 5% CO<sub>2</sub> environment. STFs were sterilized by ethylene oxide.

### Cell Imaging and Fixation

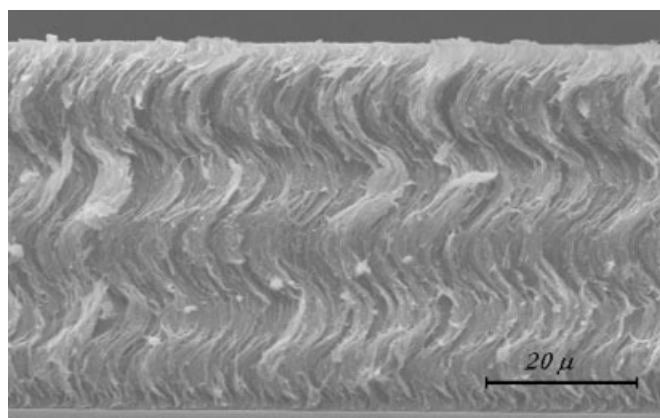
The Olympus Fluoview 300 confocal laser scanning microscope with two single-line lasers (Blue argon, 488 nm, and Red HeNe, 633 nm) was used with two different objectives (PlanApo 60× and UplanFL 40×) for imaging. Chiral STFs were cultured in 10 cm Petri dishes with 3 × 10<sup>6</sup> COS-7 cells. After 72 h of growth, the cultures were washed with PBS and stained with 10 mM CFDA-SE (Molecular Probes) in PBS for 10 min at room temperature. The cells were then fixed in 2% fresh paraformaldehyde in PBS at 4°C for 48 h, and imaged by confocal microscopy. Cell nuclei were localized by counterstaining DRAQ5 (Bioss, UK) diluted 1:500 in PBS.

## RESULTS

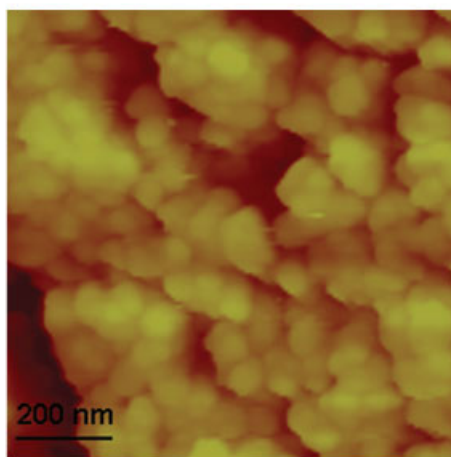
The concept of cell ingrowth within the 3-D textured scaffold was supported by an *in vitro* study of fibroblast cell proliferation on the surfaces of parylene-C chiral STFs. An array of microscopic techniques was employed. Scanning electron microscopy and atomic force microscopy were first used to characterize the parylene-C STF. A confocal laser microscope and image processing were then used in conjunction with fluorescent probes.



**Figure 1.** Flow chart of parylene-STF deposition: The parylene process starts with a dimer, which is converted to a monomer in a vacuum chamber. The monomer vapor is directed through a nozzle towards a rotating substrate on which the parylene STF is formed.



(a)



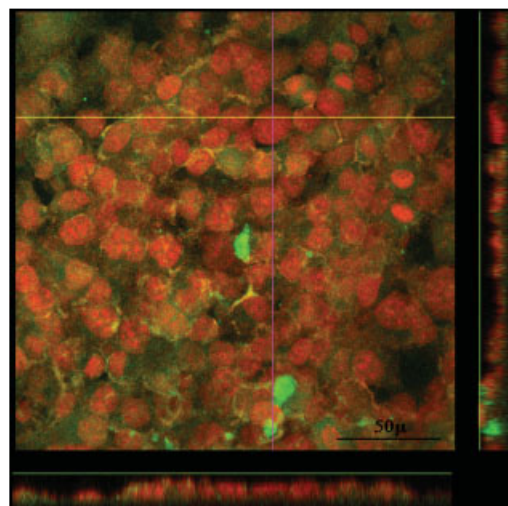
(b)

**Figure 2.** (a) Cross-section SEM micrograph of a parylene-C chiral STF evaluated for cell attachment and growth. (b) Top-surface AFM image of the same film showing that 200 nm columns comprise 50 nm nanowires (which are, in turn, made of even smaller clusters that cannot be seen at the chosen level of magnification). [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

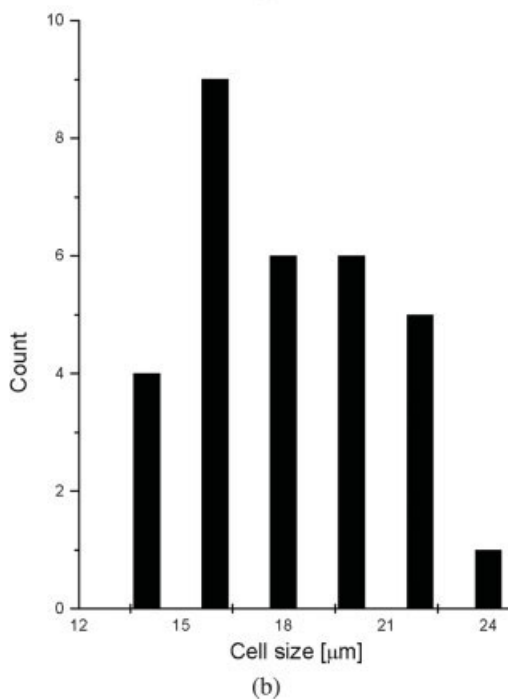
Figure 2 shows a cross-section SEM micrograph and a top-surface AFM image of a parylene-C chiral STF deposited for 10 min on a silicon substrate, this type of substrate being compatible with scanning electron microscopy. These images confirm that parylene-C STFs are assemblies of shaped nanowires that provide enhanced surface-to-volume ratios required of biocompatible substrates. Furthermore, the AFM image [Figure 2(b)] shows that the 200-nm diameter columns are made of 50 nm nanowires.

The biocompatibility and bioactivity of these polymeric chiral STFs for COS-7 fibroblast cells was demonstrated by confocal microscopy. The cells were labeled with CFDA-SE and DRAQ5 dyes. Two-dimensional biofilm formation on chiral STFs was observed, as exemplified by the confocal laser microscope images in Figure 3(a), which shows the fluorescent localization of amines and nucleic DNA 72 h after seeding onto the STFs. This figure unambigu-

ously demonstrates the cell growth on and, thus, the bioactivity of our chiral STFs. Several fields showed nuclei (red) undergoing division, thereby indicating continuing cell growth. Cross sections in Figure 3(a) (right and bottom panels) demonstrate that cells formed monolayers on the STFs. Cells remained attached well to the chiral STFs despite numerous washing and staining steps. Furthermore,



(a)



**Figure 3.** (a) Confocal laser microscope image of COS-7 fibroblast cells grown on a parylene-C chiral STF: Cell nuclei are labeled red, whereas cellular amines are labeled green. A plane near the outer cell surface, 72 h after seeding on the film, shows the cells that formed monolayers on the film and remained well-attached. Right and bottom panels show the cross sections. (b) The histogram for the cell size of COS-7 cells on a parylene STF. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

**TABLE I. Properties of Parylene-C**

General Properties of Parylene-C	Properties of STFs for Medical Use
Excellent adhesion properties.	Shear resistance of >200 N can easily be obtained for implants.
Thermomechanically stable between $-200^{\circ}\text{C}$ and $150^{\circ}\text{C}$ .	Robust for sterilizing agents and detergents.
Fully conformal on any type of surface material or design.	Implants can be easily coated.
FDA approved, class VI biocompatibility rating.	Potential for animal and human use.

the cells adhered sufficiently well to neighbors that they could be released from the chiral STFs as single sheets after culturing under condition, which led to acidification and exhaustion of nutrients in the growth media.

COS-7 cells were labeled with DiI membrane stain (Invitrogen) and their sizes were quantified using an image software. COS-7 fibroblasts are on average 18.2 microns in size [Figure 3(b)] and formed two-dimensional biofilms on parylene-C STFs. We used  $1\text{ cm} \times 1\text{ cm}$  glass substrates for depositing STFs, a material surface of  $0.5\text{ mm}^2$  was evaluated, and 36% cell surface coverage area was observed in 72 h. Parenthetically, the production of STFs on  $10\text{ cm} \times 10\text{ cm}$  substrates (industrial scale) appears possible with current technology.<sup>17</sup>

## CONCLUSIONS

We have presented polymeric sculptured thin films that have 200 nm size features for fibroblast cell attachment and growth. The morphology of STFs comprises 50 nm nanowires that form 200-nm diameter bundles. Our data has shown early indication of biocompatibility and bioactivity. Nanoscale topography, especially when compared with flat surfaces of bulk films, affects the cell adhesion of fibroblast cells. Furthermore, the porosity of STFs is unique in being controllable (Lakhtakia and Messier,<sup>16</sup> Sec. 5.2) as well as possessing engineerable texture, in comparison with other types of nanoporous materials for biomedical use. Medically important properties of parylene are listed in Table I. We should also note that polymeric STFs are relatively inexpensive to deposit in comparison with e-beam lithography and other similar methods (i.e., just  $\$0.5/(\mu\text{m cm}^2)$  including nanofabrication utility charges).

Fibrous integration is crucial to the stability of silicone elastomer arthroplasty of small joints of the hand and feet; these implants currently fail because of soft tissue imbalance and lack of implant integration. Of all the various biomaterials available for reconstruction of the finger joints, elastomeric implants are the “gold standard” within the surgical community.<sup>22</sup> The use of parylene STFs to modify elastomeric prosthetic surfaces appears enticing in light of

the data presented in the previous section. Augmenting fibrous integration of the elastomeric implants will invariably lead to a more durable arthroplasty and a better clinical outcome.

For future studies, we shall focus on more detailed cell growth studies and the attachment mechanism of cells to STFs. The mechanism of cell attachment may be either through direct interaction with the parylene STF or mediated through attachment-facilitating serum proteins such as vitronectin and fibronectin. We shall also study the expression of genes involved in wound healing after cell attachment to STF. In addition, we plan to use other polymers that are well known for biocompatibility such as silicone elastomer, polyurethane, and teflon.

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