# Simultaneous biochemical and topographical patterning on curved surfaces using biocompatible sacrificial molds

**Javier G. Fernandez,[1,2] Josep Samitier,[1,2] Christopher A. Mills[1†]**

[1]Nanobioengineering group, Institute for Bioengineering of Catalonia (IBEC), c/ Baldiri Reixac 13, 08028 Barcelona, Spain
[2]Department of Electronics, University of Barcelona, c/ Martí i Franquès 1, 08028 Barcelona, Spain

Received 26 April 2010; revised 28 October 2010; accepted 29 November 2010
Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.33038

**Abstract:** A method for the simultaneous (bio)chemical and topographical patterning of enclosed structures in poly-(dimethyl siloxane) (PDMS) is presented. The simultaneous chemical and topography transference uses a water-soluble chitosan sacrificial mold to impart a predefined pattern with micrometric accuracy to a PDMS replica. The method is compared to conventional soft-lithography techniques on planar surfaces. Its functionality is demonstrated by the transference of streptavidin directly to the surface of the three-dimensional PDMS structures as well as indirectly using streptavidin-loaded latex nanoparticles. The streptavidin immobilized on the PDMS is tested for bioactivity by coupling with fluorescently labeled biotin. This proves that the streptavidin is immobilized on the PDMS surface, not in the bulk of the polymer, and is therefore accessible for use as signaling/binding element in micro and bioengineering. The use of a biocompatible polymer and processes enables the technique to be used for the chemical patterning of tissue constructions.  J Biomed Mater Res Part A: 00A: 000–000, 2011.

**Key Words:** biotechnology, chitosan, microfabrication, MEMs, soft lithography

## INTRODUCTION

The construction of artificial scaffolds for the conditioning of cellular development has been the focus of research in numerous biomedical research fields.[1–3] The scaffolds should possess a defined 3D shape as well as a complex topography and a chemical functionalization, defined at dimensions where cells interact with surfaces. For example, in their natural environment, cells interact with many micro and nanostructures, provided by such scaffolds as collagen fibrils or adjacent cells. Such an environment is also a complex map of chemical signals, which, separately or in combination, drive cellular development in terms of metabolism, differentiation, morphology, etc.

To construct 3D scaffolds with the complexity necessary for cell-culture interactions, microfabrication techniques inspired by those in electronic engineering have been developed. The production of structured and functionalized, enclosed structures, such as tubes,[4–6] would be particularly useful. The fabrication of such enclosed 3D structures and, in particular, the fabrication of microstructures inside tubes is possible using a layer-by-layer approach to build the mold.[7] This versatile technique, based on conventional lithography, is able to reproduce almost any solid volume. It caused great interest when it was first proposed for e-beam technologies 10 years ago, but fell out of favor due to the complexity of the method (requiring the correct alignment of many electron beam or photolithography masks), and the requirement for specific e-beam or UV resists as the structural material. In MEMS, layer-by-layer approaches are broadly used to fabricate multilevel structures, but the characteristics of the method made it suitable for only a few marginal applications in the fabrication of freestanding 3D structures.

An alternative method specifically for the fabrication of structured capillaries is the "Fabrication using Laminar Flow" technique.[8] This uses the interface of two fluids in the laminar flow regime to build 5 μm structures and paths along a microchannel. Here, production is cheap and straightforward, but the technique suffers from a lack of versatility, only being able to produce structures along the direction of the channel. Additionally, the positional control and reproducibility are almost null (i.e., structures are formed randomly in the middle of the channel).

Chemical functionalization inside cavities is a problem that has recently been addressed on planar structures with positive results.[9] The technique enables the functionalization of the bottom surface of large cavities ($\sim$1 mm$^2$) in flat surfaces fabricated by injection molding. However, to our knowledge, there is no reported information on techniques for the surface functionalization of enclosed 3D scaffolds or even open surfaces with structures smaller than 1 mm$^2$. Here, we detail a technique for room temperature production of enclosed 3D structures, in poly(dimethyl siloxane)

†*Present address:* University of Surrey, Guildford, Surrey, GU2 7XH, UK; e-mail: C.Mills@surrey.ac.uk
**Correspondence to:** Javier A. Fernandez, Harvard University CLSB 5th floor, 3 Blackfan Circle Boston, MA 02115; e-mail: javier.fernandez@wyss.harvard.edu
Contract grant sponsor: European Community (CellPROM project); contract grant number: NMP4-CT-2004-500039
Contract grant sponsor: Spanish Ministry for Science and Education (FPU and Ramon y Cajal grants)

 

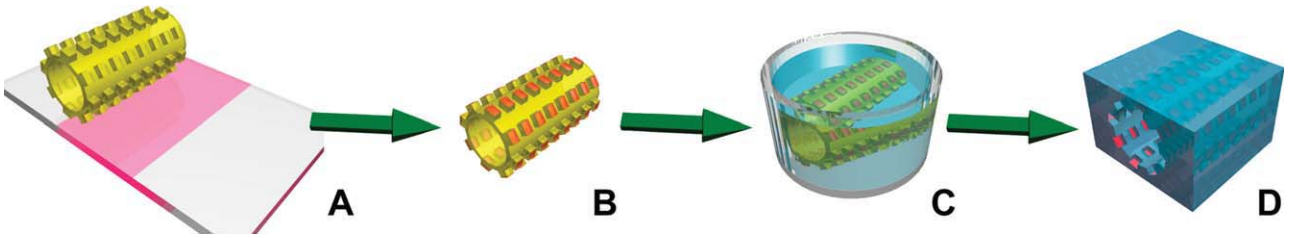


**FIGURE 1.** PDMS structure processing. (A) A structured chitosan tube is functionalized by rolling it over a PDMS surface coated with streptavidin or streptavidin-functionalized latex particles. (B) The protein/beads are transferred to the raised areas of the chitosan. (C) The mold is immersed in liquid PDMS, which is allowed to cure. (D) The chitosan mold is then exposed to water, in which it dissolves, transferring the protein to the PDMS, and giving rise simultaneously to a structured and functionalized PDMS replica. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(PDMS), simultaneously patterned with a predefined micrometric topography and chemical functionalization.

## RESULTS AND DISCUSSION

### Microtopography transfer from 3D chitosan scaffolds

Tubular chitosan scaffolds, incorporating microstructures on the outer surface, have been used to produce tubular channels in PDMS, which are completely covered with microstructures (Fig. 1). The technique to produce the chitosan scaffolds has been reported previously.[10] Briefly, topography-patterned chitosan films are produced by polymer casting of 2% (w/v) chitosan solution in 1% (v/v) acetic acid over a microstructured silicon mold. After solvent evaporation, the films are neutralized in a 4% (v/v) solution of NaOH to avoid further dissolution. The resulting chitosan films are hydrated to a point where they lose rigidity while retaining their topography. The flexible chitosan sheet is then molded over the desired shape (a needle in this case) and dried. This results in a scaffold that retains the microstructured 3D shape, while also being water soluble.

The PDMS is cured around the chitosan mold at room temperature for ~36 h. The rate of cross-linking, and hence the speed of curing, can be increased by heating the PDMS at ~80°C. However, our requirement to simultaneously transfer a protein limits the process to room temperature curing to conserve the protein bioactivity. After PDMS curing, the chitosan mold is rinsed with water in which it dissolves [Fig. 1(C,D)].

Figure 2 presents images, of increasing magnification, of a PDMS tube (1 mm in diameter and 2 cm long) with holes (5 × 5 $\mu m^2$ and 1 μm deep) on its inner wall. The images show a section, made by a scalpel, which was used to expose the chitosan to water for dissolution. At low magnification (A), the circular opening of the tube can be seen: the vertical marks are the result of the scalpel cut. At higher magnifications (B–D), the wall of the tube can be seen to fully replicate the 1 μm tall posts on the chitosan mold. The replication results are similar to those obtained previously on planar and "open" 3D structures, where the limit in structural resolution is given by the topography of the chitosan mold. This has been demonstrated to be possible below 100 nm.[11]

### Protein transfer to microstructured PDMS

Transfer of chemical species to the surface of the structured PDMS can be completed in two different ways; by direct transfer of the species to the PDMS surface or by using a carrier particle, which is itself coated with the molecule of interest. The first case is commonly used in microcontact-printing processes, where an inked mold is driven into conformal contact with the surface to be functionalized.[12] The transfer occurs, because the ink finds the adhesion to the substrate more energetically favorable than to the stamp.

When a carrier (e.g., a micro or nanobead) is used, two advantages present themselves. First, considerations about the affinity between the species to be transferred and the substrate surface may to some extent be neglected, because the molecules to be transferred are held on the carrier surface and this to the scaffold surface. Second, the use of a carrier opens the possibility of including engineered physical properties in the carrier particle (e.g., electrical conductivity or magnetic susceptability) or a drug-delivery element for a controlled chemical release.

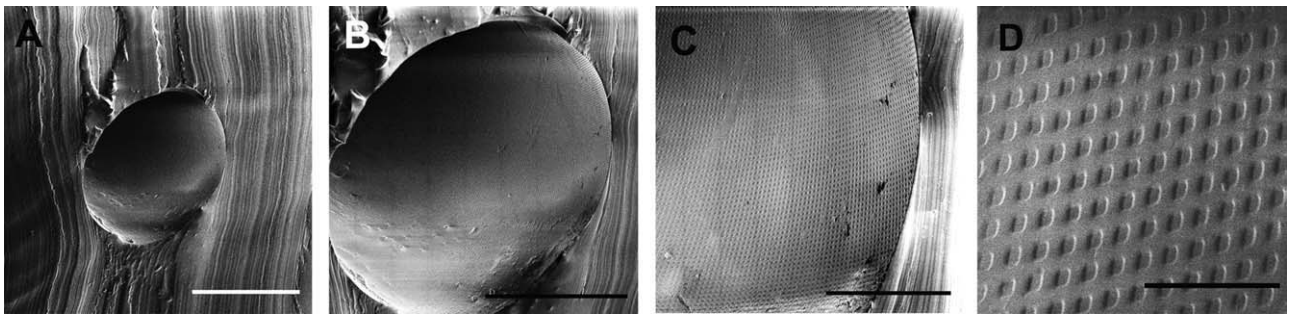


**FIGURE 2.** Tubular PDMS replica. SEM images of increasing magnification of a 1 mm diameter PDMS tube microstructured throughout with 25 $\mu m^2$, 1 μm deep holes. Scale bars are (A) 600, (B) 500, (C) 300, and (D) 50 μm, respectively.

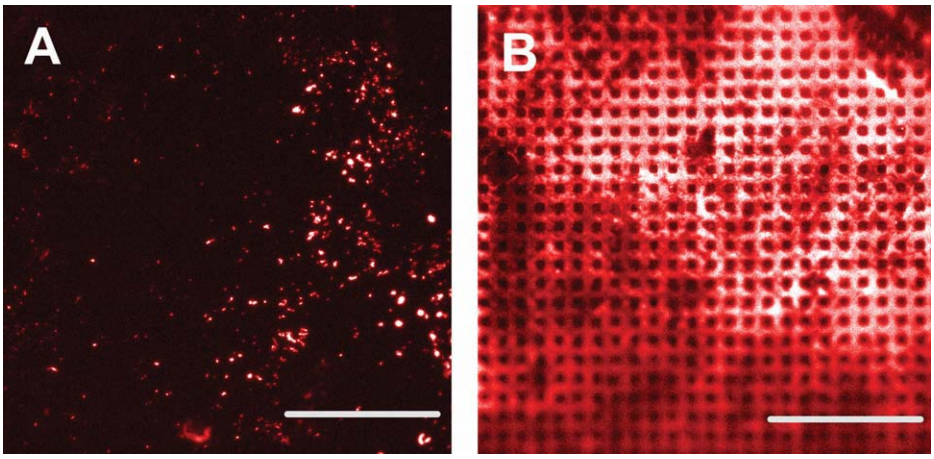


**FIGURE 3.** Conventional and sacrificial transfer. Fluorescence microscope images showing the simultaneous transfer of Texas-Red labeled streptavidin and topography to PDMS using chitosan molds. The images show PDMS surfaces patterned using (A) conventional soft lithography, where the chitosan mold is simply peeled off the PDMS and (B) dissolution of the mold. In each case, 1 μm tall, 5 × 5 $\mu m^2$ posts are faithfully transferred to the PDMS surface (Bars = 80 μm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 3 compares the results of the simultaneous chemical and topographic transfer (SCTT) method on PDMS using (A) conventional soft lithography, where the microstructured mold and the replica are mechanically separated (i.e., by lift-off) and (B) the dissolution of the mold. In each case, the chitosan mold is structured with 1 μm deep, 25 $\mu m^2$ holes, giving rise to PDMS posts of the same dimensions. The mold is also used to transfer Texas-Red-labeled streptavidin. Figure 3(A) shows the result of conventional soft lithography in an area where some transfer occurred; no clear pattern can be seen, and only a small amount of randomly distributed aggregates of fluorescent streptavidin are observed. The streptavidin has a greater affinity for the mold than the replica, and most of the protein remains on the mold surface. In contrast to the lack of protein transfer, however, optical examination of the PDMS surface reveals a well-defined topography.

Figure 3(B) (where the chitosan mold was dissolved) shows a faithful transfer of the streptavidin to the PDMS surface. The nonuniform protein transfer (i.e., variations in the intensity) is caused by an irregular coating of the chitosan mold as a consequence of an inhomogeneous primary coating or a defect on the chitosan or silicon mold (e.g., a blocked cavity or a missing post). The posts are faithfully fabricated on the PDMS surface over an area of 1 $cm^2$. The lower surface of the PDMS is coated with fluorescent streptavidin while the posts themselves are free of any functionalization [dark spots at Fig. 3(B)]. This raises the possibility of incorporating a secondary functionalization (e.g., a different protein), on the top surface of the posts, using inverted microcontact printing.[13]

The results of protein transfer using the dissolved chitosan suggest that the transfer does not occur until the mold is dissolved. Rather, transfer occurs by surface absorption to the PDMS when the chitosan dissolves, a process that could be defined as the delivery of the functionalization by the pH-sensitive mold. This process has a high-spatial resolution and, due to the close proximity of the protein to the PDMS surface, minimizes surface diffusion.

Figure 1 shows how the chitosan is functionalized by contact printing onto a structured chitosan scaffold. Transfer of the topography and the chemical functionalization to the PDMS is similarly completed in the same step, but at different points in time. Transfer of the topography occurs when the PDMS is cured [Fig. 1(C)], whereas the final transfer of the chemical species occurs when the chitosan mold is dissolved [Fig. 1(D)]. This process prevents the chemical species to be transferred from being immersed in the curing liquid PDMS. Such immersion could mask its chemical characteristics (i.e., reactivity). The immobilized streptavidin molecules on the chitosan (and possibly penetrating into the polymer matrix) are transferred only when the chitosan is dissolved and immobilized on the adjacent surface of the PDMS. This provides functionalized surfaces that may be used for *in vitro* experiments involving drug delivery or for studies on cell-surface interactions.

**Protein bioactivity**

We have previously reported on the process of constructing microstructured 3D chitosan scaffolds,[10] where the chitosan was used directly as a scaffold for cell-culture experiments in aqueous environments. In that case, to prevent premature dissolution of the chitosan in the culture medium, the positive charge of the polymer was neutralized. Here, we use the chemical resistance of the PDMS to allow us to selectively dissolve the embedded chitosan. The chitosan is used as a sacrificial mold for simultaneously transferring topography, shape, and (bio)chemical functionalization to the PDMS.

Because of the chemical inertness of the PDMS to a variety of solvents,[14] there exist a number of options for selectively dissolving the chitosan, which is pH sensitive. However, this variety is limited by our wish to transfer biochemical species to the surface of the PDMS at the same time. To ensure the bioactivity of the molecules, and the generalization of the technique to other molecular species, water has been used as the chitosan solvent. The chitosan scaffolds used here were not neutralized and consequently

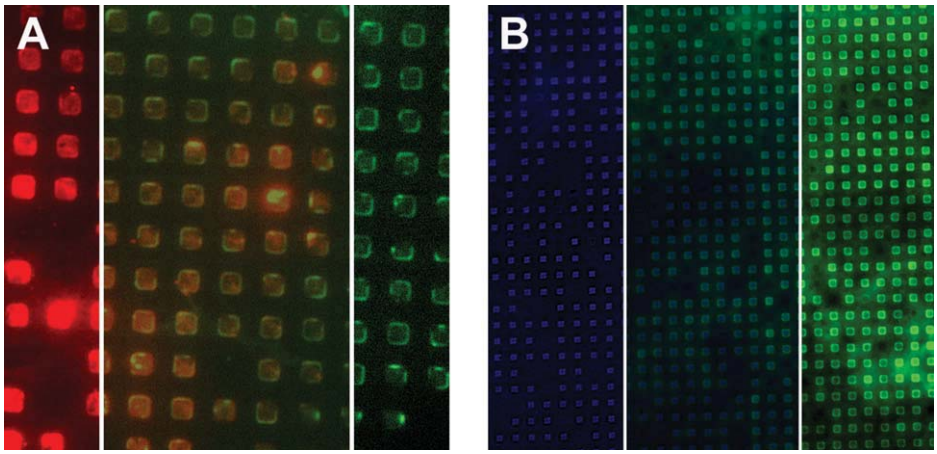


**FIGURE 4.** Topography and chemical transfer to PDMS replicas. In both cases, fluorescence microscopy images are presented using different excitation wavelengths to highlight the position of the fluorescent species on a PDMS surface containing 25 $\mu m^2$, 1 $\mu m$ deep square holes. The left panel shows the position of the transferred species, and the right panel shows the position of the Oregon green-labeled biotin after incubation. The center image shows superimposed images of the molecules and their correlated positions. (A) Direct transfer of streptavidin (Texas red). Image area = 100 × 100 $\mu m^2$. (B) Transfer of latex beads coated with Nile blue labeled streptavidin. Image area = 300 × 300 $\mu m^2$. The bioactivity of the streptavidin after the transfer is clearly observed by the co-localized attachment of the green-labeled biotin. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

have an overall positive charge, which makes them soluble at neutral pH.

Although the transfer method has shown to precisely locate the streptavidin within the PDMS microstructures, the functionality of the protein must not be compromised by the transfer. During the molding and transfer process, the conformation of the protein may be lost, or the protein may be buried in the PDMS, making it inaccessible. A solution for the potential problem of immersion of the transferred species has been examined with the transfer of streptavidin-loaded nanoparticles, rather than direct transfer of the streptavidin.

The bioactivity of the transferred streptavidin, using both of these methods, has been tested by the introduction of fluorescently labeled biotin. The biotin–streptavidin couple is one of the strongest, noncovalent bond systems found in nature, and it is broadly used in purification or detection of various biomolecules, including DNA.[15]

PDMS surfaces containing 1 μm deep, 25 $\mu m^2$ holes (in contrast with the posts produced in Fig. 3) with Texas red-labeled streptavidin or Nile blue-labeled streptavidin-loaded nanoparticles immobilized on the bottom surface of the holes have been immersed in a solution of Oregon green-labeled biotin. Nonspecific biotin attachment was prevented by blocking unspecific binding sites with bovine serum albumin (BSA). Figure 4 shows the co-localization of the fluorescence of each molecule. The left panel in each case shows the immobilized streptavidin, while the right panel shows the immobilized biotin. The center panel shows the co-localization of the fluorescent labels, and hence the biotin and streptavidin. The well-defined pattern found for the biotin immobilization demonstrates the bioactivity of the streptavidin, to the point that, in all cases, where the biotin is not observed (i.e., there is an absence of a green fluorescent square), streptavidin is similarly absent [Fig. 4(A)].

In the case of the transfer of nanoparticle-coated streptavidin [Fig. 4(B)], even though the majority of the protein is in the holes, the surrounding area is partially covered by the streptavidin, possibly due to a loose attachment between the beads and the surface of the chitosan mold and/or the PDMS replica, or between the streptavidin and the beads. In the central image (where the images are superposed), some fluorescent beads are present where there is no correlation with biotin fluorescence. This phenomena, not observed in the direct transfer case, is the result of a failure in the streptavidin functionality, which is either inaccessible or nonreactive with the biotin.

### 3D simultaneous chemical and topography transfer

The transference of functionalization and topography is an independent process that can be produced separately. However, 3D SCTT has been attempted to produce forms of PDMS containing structured surfaces and patterns of streptavidin in a single process. Unfortunately, the complexity of the scaffolds and the dimensions of the structures, ranging from the micrometer scale of the topography to the centimeter scale of the whole scaffold, do not allow the imaging of the results in a single image. Figure 5 presents several confocal microscopy images of a 1 mm diameter PDMS tube, with 1 μm deep, 25 $\mu m^2$ holes on its inner wall, and functionalized with Texas red-labeled streptavidin at the base of the holes [the full PDMS structure is depicted in Fig. 1(D)].

A low-magnification image of the structured PDMS tube is given in Figure 5(A). Background fluorescence appears to be high because of the diffusion of the light through the surrounding PDMS; however, the patterned streptavidin is clearly observable. When the structures are examined at higher magnification, the discrete fluorescent spots are easily differentiated [Fig. 5(B)]. The image is focused on the top of the tube through the upper portion of PDMS, which

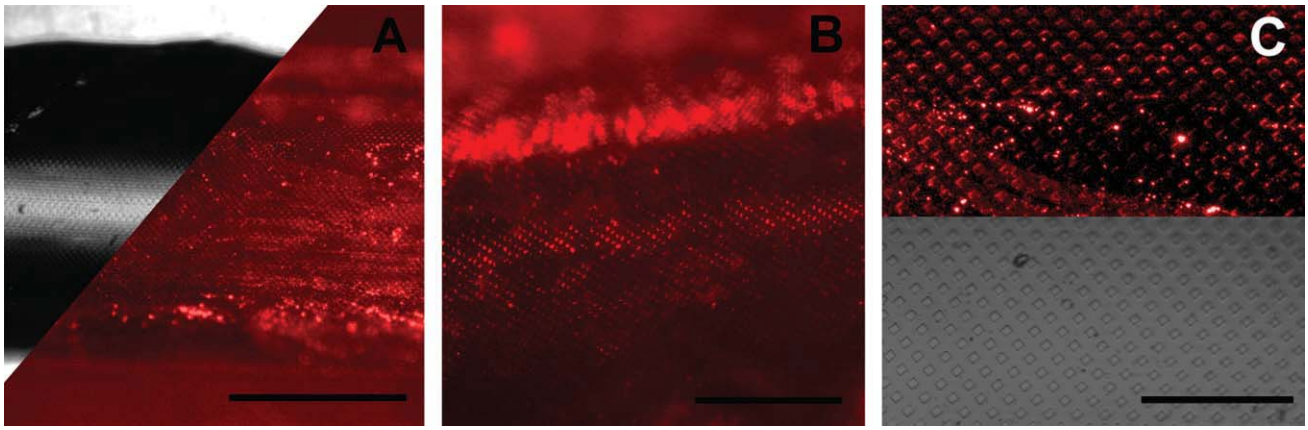


**FIGURE 5.** Optical and fluorescence images of a PDMS tube. The tube has 1 μm deep, 25 $\mu m^2$ holes on its inner wall, functionalized with Texas red-labeled streptavidin at the base of the holes. (A) White-light optical image (left) aligned with a fluorescence image of the adjacent area (bar = 500 μm). (B) Magnified fluorescence image of the tube showing the pattern produced by the streptavidin immobilized in the topography (bar = 200 μm). (C) Magnified white-light optical image (bottom) aligned with a corresponding fluorescence image (top) highlighting the spatial relation between the functionalization and the topography (bar = 70 μm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

is the reason why, using a high-magnification lens, most of the tube is out of focus. Consequently, the image blurs, at the top and bottom of the image, as the distance between the topography and the focal plane increases. Using a higher magnification, and compiling several images through the focal plane of the structures, it is possible to obtain clear images of topography and fluorescence from the streptavidin, which, when placed side by side, confirms the spatial correlation between the fluorescent species and the topography [Fig. 5(C)].

## EXPERIMENTAL

### Materials

Medium molecular weight chitosan (75–85% deacetylated and 200–800 cps viscosity), derived from crab shell, was purchased from Aldrich (Sigma-Aldrich Chemical Co., USA) and prepared in dilute glacial acetic acid solution as previously reported.[10] PDMS (Sylgard 184) was purchased from Dow Corning Co. (USA) and prepared in a 10:1 (base:cross-linker ratio) as per the manufacturers specifications. Streptavidin and biotin were purchased from Aldrich () and prepared into 100 and 250 μg/mL PBS solutions, respectively. Streptavidin-functionalized latex beads (250 nm diameter, Nile-blue labeled) in 1% v/v solution were purchased from Aldrich and diluted at 0.2% for the experiments.

### Chitosan scaffold preparation

Microstructured, three-dimensional (3D) chitosan scaffolds based on tubular structures were prepared using a method described previously.[10,16] The tubes were structured on their outer walls with 25 $\mu m^2$, 1 μm tall/deep microstructures. The structures were selectively coated with streptavidin or streptavidin-functionalized latex beads, using a roll-on inverse microcontact-printing technique [Fig. 1(A)]. Streptavidin solution or streptavidin-functionalized latex bead solution was deposited on a flat piece of PDMS and allowed to dry. The structured chitosan tube was then lightly rolled over the PDMS, causing the upper surfaces of the structures on the chitosan surface to become coated with the streptavidin or the functionalized latex particles [Fig. 1(B)].

### Simultaneous chemical and topography transfer to PDMS

The chitosan tubes are immobilized ~1 mm from the base of a Petri dish using cured poly(dimethyl siloxane) (PDMS) pieces, and the entirety is submerged in liquid PDMS [Fig. 1(C)]. The PDMS is cured at room temperature for ~36 h, after which the solid PDMS, containing the chitosan scaffold is removed from the container. The PDMS is then cut with a scalpel to expose part of the chitosan. The entirety is subsequently immersed in water, which dissolves the chitosan, and leaves a PDMS replica of the tube containing the microstructures and transferred chemical functionalization [Fig. 1(D)]. The period of time that the polymers need to be immersed depends on the dimensions of the chitosan exposed to the water. The streptavidin can be transferred directly to the PDMS surface or by using the protein-loaded nanoparticles. To transfer the beads to the surface of the chitosan, 200 μL of diluted bead solution (0.2% v/v in water) was deposited on a flat PDMS surface and transferred in the same way [Fig. 1(A)]. The bioactivity of the transferred streptavidin has been tested by immersion of the patterned PDMS, for 10 min, in a solution of Oregon green-labeled biotin (0.25 mg/mL). Nonspecific attachment of the biotin was prevented by blocking unspecific binding sites with BSA (2.5% v/v in the biotin solution).

## CONCLUSIONS

The enclosed microstructured and chemically patterned structures produced here in PDMS can be used in a variety of new applications in both engineering and biomedicine applications.[17,18] It may also be used as a method to improve current technologies, where topographical and chemical patterning is limited to a single face of the

microengineered device. In addition, the possibility of immobilizing beads allows the possibility of spatially controlled transfer of localized electrical and magnetic characteristics as well as drug-delivery elements.

The use of a biocompatible material (broadly used as drug carrier) and water as solvent make the STCC technique a reliable method for the micrometric patterning of biochemical signals/drug delivery, for example, in tissue constructs.

The internal topography and chemical functionalization may be used to disturb laminar flow regimes,[19] or guide cell-culture growth independently from the flow direction.[20] The fabrication of such structures from a single piece of PDMS simplifies the fabrication, improves the mechanical properties of such microfluidic devices, and reduces leakage. Sensor devices may achieve improved efficiency by the use of multiwall patterning to increase surface area. Direct immobilization of DNA samples (via the biotin/streptavidin bonding) enables the fabrication of microfluidic polymer chips for the self-assembly of DNA conjugates.[21]


## ACKNOWLEDGMENTS

The authors thank Drs. C. Moormann and T. Wahlbrink (AMO Gesellschaft für Angewandte Mikro- und Optoelektronik GmbH, Germany) for supplying the microstructured silicon molds used here. JGF and CAM conceived the fabrication strategy. CAM and JS supervised the project. JGF designed and performed experiments and analysed data. CAM and JGF wrote the paper.


## REFERENCES


1. Engelmayr GC, Cheng M, Bettinger CJ, Borenstein JT, Langer R, Freed LE. Accordion-like honeycombs for tissue engineering of cardiac anisotropy. Nat Mater 2008;7:1003–1010.
2. Harrison C. Anticancer drugs: Scaffold to selectivity. Nat Rev Drug Discov 2008;7:390.
3. Dalby MJ, Gadegaard N, Tare R, Andar A, Riehle MO, Herzyk P, Wilkinson CDW, Oreffo ROC. The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. Nat Mater 2007;6:997–1003.
4. Bellan LM, Singh SP, Henderson PW, Porri TJ, Craighead HG, Spector JA. Fabrication of an artificial 3-dimensional vascular network using sacrificial sugar structures. Soft Matter 2009;5: 1354–1357.
5. Golden AP, Tien J. Fabrication of microfluidic hydrogels using molded gelatin as a sacrificial element. Lab Chip 2007;7:720–725.
6. Yun KS, Yoon EE. Fabrication of complex multilevel microchannels in PDMS by using three-dimensional photoresist masters. Lab Chip 2008;8:245–250.
7. Jacobsen SC, Wells DL, Davis CC, Wood JE. Fabrication of microstructures using non-planar lithography (NPL). In Micro Electro Mechanical Systems, 1991, MEMS '91, Proceedings. An Investigation of Micro Structures, Sensors, Actuators, Machines and Robots. IEEE: Nara, Japan; 1991. p 45–50.
8. Kenis PJ, Ismagilov RF, Whitesides GM. Microfabrication inside capillaries using multiphase laminar flow patterning. Science 1999;285:83–85.
9. Biancardo SBN, Pranov HJ, Larsen NB. Protein in-mold patterning. Adv Mater 2008;20:1825–1829.
10. Fernandez JG, Mills CA, Samitier J. Complex, microstructured, 3D surfaces using chitosan biopolymer. Small 2009;5:614–620.
11. Fernandez JG, Mills CA, Pla-Roca M, Samitier J. Forced soft lithography (FSL): Production of micro- and nanostructures in thin freestanding sheets of chitosan biopolymer. Adv Mater 2007;19: 3696–3701.
12. Xia Y, Whitesides GM. Soft lithography. Angew Chem Int Ed 1998;37:550–575.
13. Embrechts A, Feng CL, Mills CA, Lee M, Bredebusch I, Schnekenburger J, Domschke W, Vancso GJ, Schonherr H. Inverted microcontact printing on polystyrene-*block*-poly(*tert*-butyl acrylate) films: A versatile approach to fabricate structured biointerfaces across the length scales. Langmuir 2008;24:8841–8849.
14. Lee JN, Park C, Whitesides GM. Solvent compatibility of poly-(dimethylsiloxane)-based microfluidic devices. Anal Chem 2003; 75:6544–6554.
15. Green NM Jr, Anfinsen CB, Edsall JT, Richards FM. Advances in Protein Chemistry. New York: Academic Press; 1975.
16. Fernandez JG, Mills CA, Martinez E, Lopez-Bosque MJ, Sisquella X, Errachid A, Samitier J Micro- and nanostructuring of freestanding, biodegradable, thin sheets of chitosan via soft lithography. J Biomed Mater Res A 2008;85:242–247.
17. Weibel DB, Whitesides GM. Applications of microfluidics in chemical biology. Curr Opin Chem Biol 2006;10:584–591.
18. Sia SK, Whitesides GM. Microfluidic devices fabricated in poly-(dimethylsiloxane) for biological studies. Electrophoresis 2003;24: 3563–3576.
19. Stroock AD, Whitesides GM. Controlling flows in microchannels with patterned surface charge and topography. Acc Chem Res 2003;36:597–604.
20. Uttayarat P, Chen M, Li M, Allen FD, Composto RJ, Lelkes PI. Microtopography and flow modulate the direction of endothelial cell migration. Am J Physiol Heart Circ Physiol 2008;294: 1027–1035.
21. Schröder H, Hoffmann L, Müller J, Alhorn P, Fleger M, Neyer A, Niemeyer CM. Addressable microfluidic polymer chip for DNA-directed immobilization of oligonucleotide-tagged compounds. Small 2009;5:1547–1552.