Biomacromolecules

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Fully Biodegradable Self-Rolled Polymer Tubes: A Candidate for Tissue Engineering Scaffolds

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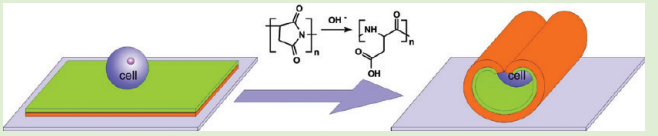
Supporting Information

ABSTRACT: We report an approach for the fabrication of fully biodegradable self-rolled tubes based on patterned polysuccinimide/polycaprolactone bilayers. These polymers are biocompatible, biodegradable, produced industrially, and are already approved for biomedical purposes. Both polycaprolactone and polysuccinimide are hydrophobic and intrinsically water-insoluble. Polysuccinimide, however, hydrolyzes in physiological buffer environment yielding water-swellable polyaspartic acid that causes the rolling of the polymer bilayer and formation of tubes. We demonstrate the possibility to encapsulate yeast cells using self-rolled tubes.



INTRODUCTION

The design of three-dimensional (3D) scaffolds that provide a suitable environment for the growth and division of cells is a keynote procedure for development of efficient approaches for regeneration of tissues and organs. To be suitable for tissue engineering, the scaffolds must fulfill many requirements such as biocompatibility, biodegradability, and porosity as well as proper chemical and mechanical environment mimicking properties of the extracellular matrix. Most of the developed approaches for fabrication of scaffolds can be classified either as "bottom-up" or "top-down" ones. "Bottom-up" ones include phase separation techniques and self-assembling materials.[1−3] Examples of "top-down" ones are electrospinning[4] and microfabrication.[5−9] Many "top-down" approaches suffer from inhomogeneous cells seeding caused by slow migration of cells inside the scaffold. As an alternative, cells might be seeded in situ during the formation of the scaffold. This, for example, can be done using self-assembling peptide hydrogels (molecular "bottom-up"). After self-assembly of the peptides, cells remain entrapped in the polymer network with the size of the mesh smaller than the cell size. The migration of cells inside such scaffolds is restricted.

Recently, a combination of "top-down" and "bottom-up" approaches was introduced for the design of scaffolds. Cells were encapsulated inside relatively large poly(ethylene glycol) (PEG) hydrogel microbricks which were allowed to self-assemble in 3D superstructures of defined shape.[10,11] The advantages of such mesoscale self-assembling systems are: (i) homogeneous seeding of the cells and (ii) simplicity to form large superstructures with different shapes. On the other hand, the applicability of the developed approach is limited due to the nonbiodegradability of the cross-linked PEG hydrogel and the small size of the pores, which is intrinsic to many hydrogel-based scaffolds.

Wrapping by thin films, which form self-rolled tubes[12] or self-folding particles,[13] is an alternative approach for the encapsulation of cells. Self-rolled tubes are thin bilayers which are able to roll due to internal stress produced as a result of thermal expansion, lattice mismatch, or swelling. The self-rolled tubes, being intrinsically anisotropic, are structurally close to muscle, nerve, and bone tissues. Moreover, microtubes form pores with sizes up to hundreds of micrometers, which is more suitable for the design of scaffolds due to free migration of cells. Encapsulation of cells using wrapping was recently realized on the example of self-rolling tubes made of inorganic materials.[14] Cell-seeded rolled tubes can be potentially assembled into complex submillimer- and microporous 3D structures[15] with homogenously distributed cells. It was demonstrated that cells can be encapsulated inside the tubes with diameter in the range of tens of micrometers without the use of harmful UV light. The use of inorganic materials for the fabrication of self-rolling tubes, however, does not allow their application for medical purposes. Recently, Luchnikov et al. demonstrated that self-rolling tubes could be designed using polymers.[16] They showed self-rolling of poly(4-vinylpyridine)/polystyrene bilayer film in acidic environment (pH = 2). On the other hand, Kalaitzidou et al. demonstrated thermoresponsive rolling-unrolling of PDMS/gold tubes at 60−70 °C.[17,18] Gracias et al. developed an approach for the design of self-folding particles made of patterned SU-8 photoresist-polycaprolactone films, which irreversibly fold at 60 °C.[19] The nonbiodegradability and nonbiocompatibility of these polymers as well as the difficulty to locally control such low pH values and the high temperature hamper the application of these systems in medicine as well.[20] More recently, we reported fabrication of partially biodegradable magneto-temperature sensitive microtubes and capsules able to capture and release cells in a response to change of temperature in the physiological range between 25 and 33 °C.[21,22] These tubes, based on the use of two polymers—one thermoresponsive poly(*N*-isopropylacrylamide)-based copolymer and hydrophobic

Received: March 3, 2011
Revised: April 26, 2011
Published: April 27, 2011

polycaprolactone (PCL)—can also hardly be used for the fabrication of tissue engineering scaffolds due to the nonbiodegradability and the poor biocompatibility of poly(*N*-isopropylacrylamide).

In this manuscript we report, for the first time, the fabrication of fully biodegradable self-rolled tubes. The tubes are based on cross-linked polysuccinimide/polycaprolactone bilayer. These polymers are biocompatible, biodegradable,[23,24] produced industrially, and were already approved for biomedical purposes. Both polycaprolactone and polysuccinimide are hydrophobic and intrinsically water-insoluble. Polysuccinimide, however, is able to hydrolyze in physiological buffer environment, yielding water-swellable biodegradable polyaspartic acid,[25] which leads to rolling of the tubes and encapsulation of cells (Figure 1).

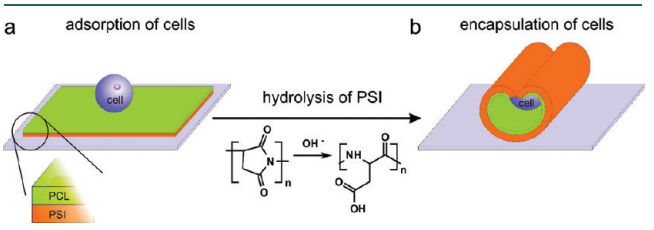


Figure 1. Scheme of the formation of self-rolled tubes. Polysuccinimide (PSI)/polycaprolactone (PCL) bilayer is deposited on a substrate. Slow hydrolysis of polysuccinimide in physiological buffer (pH = 7.4) yields polyaspartic acid, which leads to the rolling of the tubes and encapsulation of cells.

EXPERIMENTAL SECTION

Materials. L-Aspartic acid (Aldrich), 85% phosphoric acid (Aldrich), polycaprolactone (M_n = 70000−90000 g/mol, Aldrich), and 4-hydroxybenzophenone (Aldrich) were used as received.

Synthesis of Polysuccinimide (PSI). PSI was synthesized by acid-catalyzed thermal polycondensation of L-aspartic acid. A typical synthesis procedure was as follows. Powdery L-aspartic acid (20 g, 150 mmol) and 85% *o*-phosphoric acid (10 g, 87 mmol) were kept at 200 °C for 6.5 h under nitrogen flow. The mixture was cooled to room temperature and dissolved in *N*,*N*-dimethylformamide (DMF). The solution was poured into a large amount of deionized water, and the precipitate was washed several times with water until the filtered water became neutral. The obtained PSI was characterized by GPC: M_n = 15700 g/mol, M_w = 44900 g/mol, M_w/M_n = 2.86.

Fabrication of the Polymer Bilayers and Self-Rolled Tubes. The bilayers were produced by photolithography. For this, thin films (70−400 nm) of PSI containing 1 wt % of 4-hydroxybenzophenone with respect to the amount of the polymer were deposited on silicon wafer by spincoating from DMF solution. Next, thin films (70−500 nm) of PCL containing 4 wt % of 4-hydroxybenzophenone were deposited from toluene solution, which is a selective solvent, on top of the PSI film. The polymer bilayers were patterned by irradiation with UV light through a mask. UV irradiation activates benzophenone fragments, which produces free radicals and led to cross-linking in the irradiated areas. As a result, patterned bilayer films were formed on the substrate. The self-rolling tubes were fabricated by long-time exposure of the bilayer in PBS (0.15 M, pH = 7.4).

Experiments with Yeast Cells. Yeast cells encapsulated inside the tube were incubated in Dulbecco's modified Eagle's medium: Nutrient Mixture F-12. All experiments with yeast cells were carried out at 25 °C.

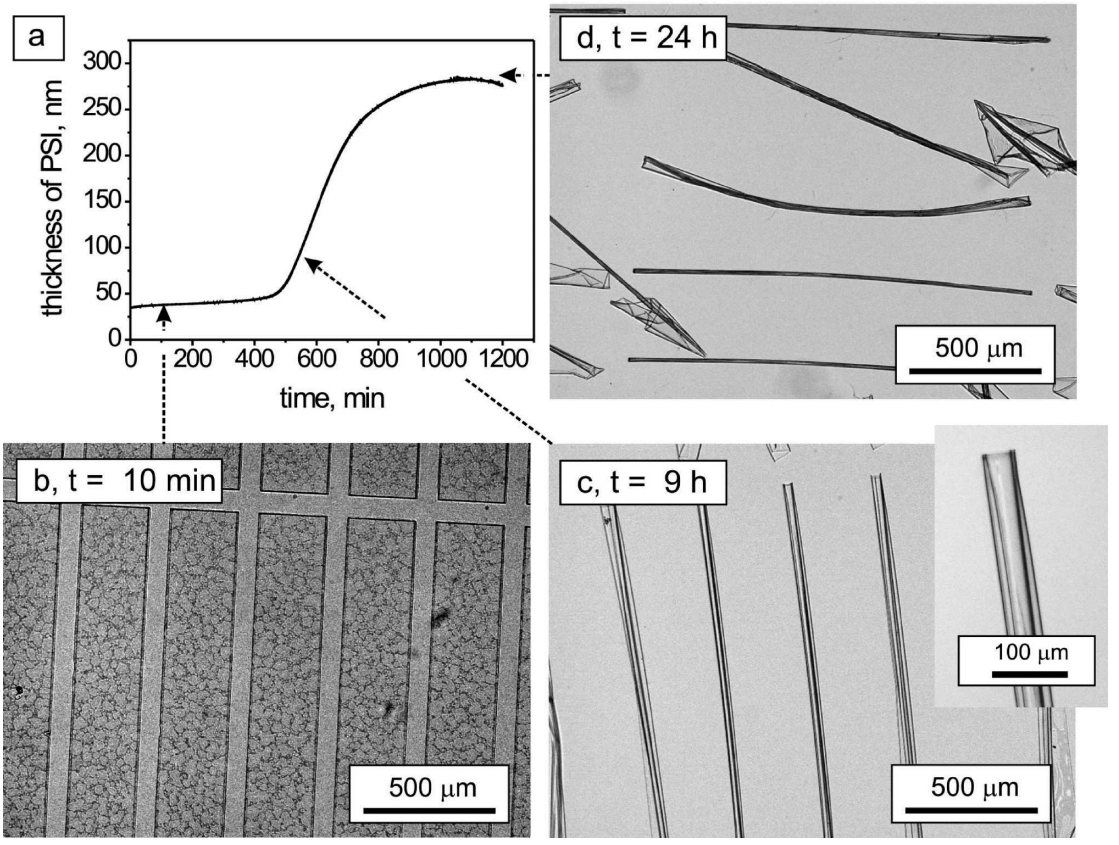


Figure 2. Swelling of photocross-linked polysuccinimide films (a) and morphologies of self-rolling tubes (th(PSI) = 200 nm; th(PCL) = 86 nm, pattern 1800 μm × 300 μm) on different stages of rolling; (b) after 10 min of incubation; (c) after 9 h of incubation, d = 55 μm; (d) after 24 h of incubation, d = 25 μm.

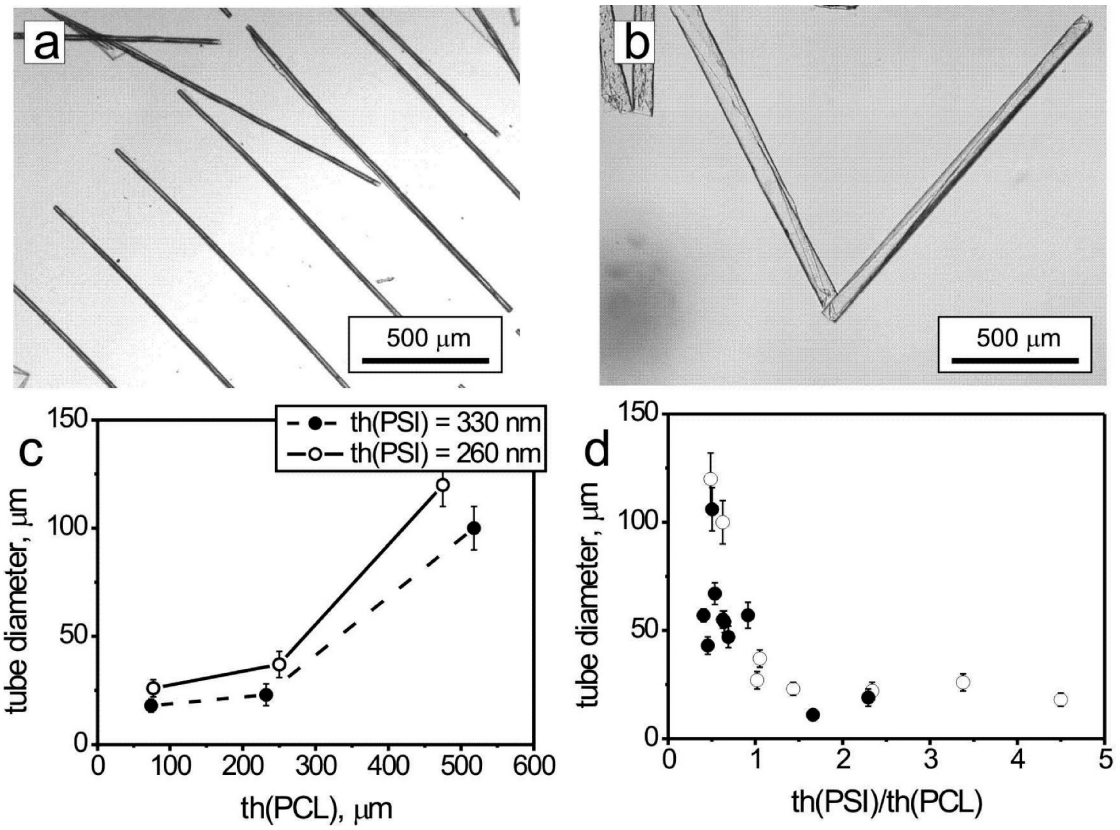


Figure 3. Examples of microtubes with different diameters of (a) 22 μm or (b) 100 μm as well as the dependence of the tube diameter on the thickness of PCL (c) and the th(PSI)/th(PCL) ratio (d). The empty circles in (d) correspond to samples prepared by dipcoating without further treatment, solid circles correspond to PSI/PCL films annealed at 60 °C and cooled down to −196 °C.

RESULTS AND DISCUSSION

First the swelling of photo-cross-linked PSI thin film in physiological buffer (PBS 0.15 M, pH = 7.4) at T = 25 °C was investigated. Considering the fact that pH of culture media is often very close to pH of PBS, the swelling behavior of PSI is expected to be similar in both cases. The PSI film was deposited on silica wafer from DMF solution by spin-coating. To avoid dewetting of the polymer film caused by slow evaporation of the solvent, the samples were quickly heated up to 170 °C on a heating plate. The obtained PSI films were very smooth, and the rms roughness was below 1 nm. In PBS buffer at 25 °C PSI film swelled that resulted in increase of its thickness by ca. 8−10 times: from 35 to 283 nm after 24 h of hydrolysis. Considering the fact that the PSI layer is homogenously cross-linked, the final degree of swelling of the thicker layers is expected to be similar: 8−10 times.

The swelling of the PSI film had a step-like character (Figure 2a). The thickness of PSI increased slightly during the first period (0−9 h), and strong swelling started after 8−9 h of incubation in buffer. The swelling was nearly completed after 24 h. The observed step-like characters of the swelling are most probably due to diffusion-limited penetration of water in the hydrophobic PSI layer. In the beginning, water starts to diffuse slowly in the hydrophobic PSI layer hydrolyzing it. As soon as a threshold amount of succinimide groups is hydrolyzed, the PSI layer starts to swell to a higher degree due to repulsion between the formed negatively charged carboxylic groups. As a result, the diffusion of water in the polymer layer increases that leads to a faster hydrolysis of the remaining succinimide groups. In fact, the delayed hydrolysis of PSI films can be considered as an advantage because cells might have enough time to bind to the surface of the polymer bilayer and spread before the tube is formed.

The PSI/PCL bilayers were produced using photolithography. First, the PSI was deposited from its DMF solution using spincoating. PCL was deposited in the same manner from toluene solution. A small amount of 4-hydroxybenzophenone was added to both polymers to provide possibility to cross-link them using UV light. The benzophenone derivatives, which generates free radicals upon irradiation with UV light,[26] have already been applied for photo-cross-linking of biodegradable biomaterials.[27] Notably, other similar compounds including benzophenone, 4-carboxybenzophenone, and 2,4-dihydroxybenzophenone were found to be almost inefficient for the cross-linking of the polymers. This may be related to a difference in the adsorption spectra or the quantum yield. After illumination with UV light, the noncross-linked polymer was removed by sequential rinsing in chloroform and DMF where PCL and PSI are respectively soluble. To avoid cytotoxicity, the rest of the DMF were removed by rinsing in large amount of ethanol. The cross-linked polymers are expected to be biodegradable as well since they still contain the carbon−oxygen and carbon−nitrogen bonds of the original polymers. The obtained patterned bilayers were used for rolling experiments.

The obtained PSI/PCL bilayers were incubated in PBS. The bilayers, which appeared patchy due to the crystalline PCL, remain unchanged during the first 9 h at T = 25 °C (Figure 2b). The rolling started after 9 h of incubation and incompletely rolled tubes (d = 55 μm, Figure 2c) were formed. Further incubation led to a shrinkage of the tubes and a decrease of their diameter

(d = 25 μm, Figure 2d). The formed tubes could be easily detached from the silicon substrate due to the swelling of the polyaspartic acid (hydrolyzed PSI). The decrease of the tube diameter with time is in qualitative agreement with the Timoshenko equation.[28] According to the Timoshenko equation, higher stress in the film, which is proportional to the swelling degree, leads to a smaller diameter of the tubes. The step-like rolling behavior of PSI/PCL bilayer correlates very well with the step-like character of swelling of the PSI layer.

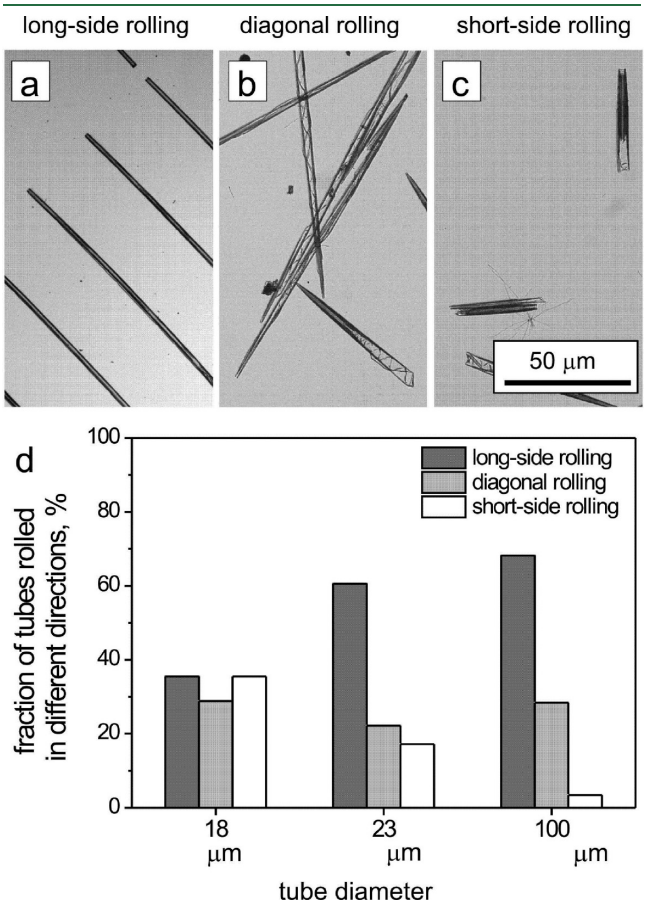


Figure 4. Different morphologies of tubes formed by a rolling PSI/PCL bilayer: (a) long-side rolling; (b) diagonal rolling; (c) short side rolling. Fraction of the microtubes rolled in different directions depending on the tube diameter (d, pattern 1800 μm × 300 μm). Approximately 50 microtubes were analyzed in each case.

It was found that the rolling of thick bilayers often results in peeling of the polymer films, which might be caused by the rigidity of the microcrystalline PCL layer. The microcrystalline structure of PCL was also most probably the origin of the defects of rolling (Figure 2d). To prevent the formation of large PCL crystallites, polymer bilayers were heated up to 60 °C to melt the PCL and then quickly cooled down by liquid nitrogen T = −196 °C. This thermal treatment resulted in a decrease of the size of the crystallites from tens of micrometers to less than a micrometer (images not shown) and improved the rolling in many cases.

Next, the effect of the thickness of the polymer layers on the diameter of the formed tubes was investigated. It was found that tubes diameter increased with the increase of thickness of the PCL layer as well as with the decrease of the thickness of the PSI layer (Figure 3a−c). These results were obtained for tubes after 24 h of rolling when no further change of the tube diameter with time was observed. Moreover, PSI/PCL bilayers prepared with and without thermal treatment demonstrated similar dependence of the tube diameter on the ratio between thicknesses of the polymers layers (Figure 3d). In general, the smaller was the ratio of the thickness of the PSI layer to the thickness of PCL layer (r = th(PSL)/th(PCL)) the larger the diameter of the formed tubes (Figure 3d). Since strain in the film is related to the difference between the thickness of the PSI in swollen and dry states, these findings are in a qualitatively agreement with the Timoshenko equation as well.[28] Using this principle, we explored the possibilities to fabricate tubes with a diameter more than 50 μm using both the decrease of the thickness of the PSI (th(PSI) = 67 nm; th(PCL) = 132 nm; r = 0.5) and the increase of thickness of the PCL layer (th(PSI) = 260 nm; th(PCL) = 473 nm; r = 0.54). Notably, tubes were formed only in the second case because very thin PSI layer made the tubes too soft and the tubes were easily deformed and smashed by the liquid stream. In fact, tubes of a diameter ranging from 10 μm up to more than 100 μm can be fabricated. Tubes with the size 100 μm or more are particularly promising for the design of porous scaffolds.[29]

Contrary to previously investigated PNIPAM/PCL system,[21] which rolled predominately in one direction, PSI/PCL bilayers were able to roll in different directions. We distinguished three cases: long-side rolling, diagonal rolling, and short-side rolling (Figure 4). The probability to roll in each of these directions depended on the ratio of the final diameter of the tubes to the size of the pattern and the number of the possible revolutions. The bilayer rolled in all directions equally if tubes with the smallest diameter were formed. The increase of the diameter led to the formation of tubes with predominately long-side rolling. To

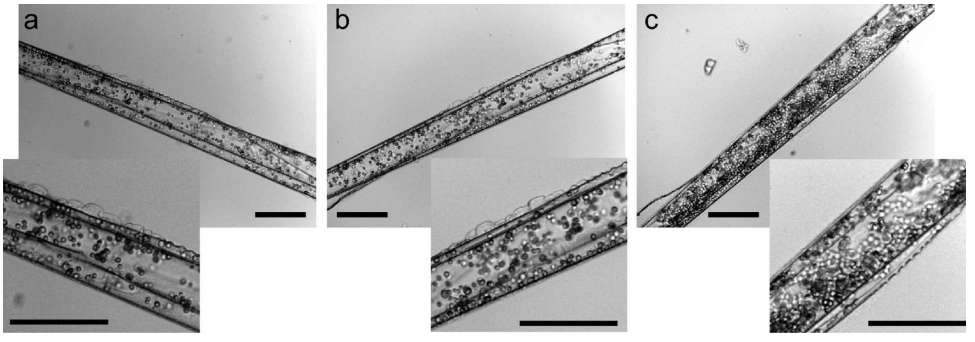


Figure 5. PSI/PCL self-rolled tube (a) after encapsulation of yeast cells in PBS buffer, (b) directly after the transfer to the nutrition media, and (c) after 14 h of incubation in the nutrition media. Scale bar is 100 μm.

explain the obtained results we assign the following scenario of rolling. Water starts to diffuse and hydrolyzes PSI along the perimeter of the patterned polymer bilayer. The bilayer loses its contact with the substrate at the point where adhesion to the substrate is the smallest, starts to roll, and makes several revolutions. Considering the fact that the tubes are unable to unroll or change their morphology if they are produced by multiple revolutions,[21] the direction of rolling of the whole thin tube is determined by the direction of rolling in the initial period of time. As a result, tubes with a small diameter roll in all directions with similar probability. On the other hand, tubes with larger diameter are formed by single revolution. These tubes have more possibilities to change their morphology and to find energetically more favorable state during the rolling.[30]

Finally, we tested the possibility to encapsulate yeast cells using biodegradable self-rolled tubes. The bakery yeast cells were adsorbed on patterned PSI/PCL bilayer from PBS pH = 7.4 buffer. After 27 h of incubation, the PSI/PCL bilayer formed tubes with diameter 80−100 μm with encapsulated yeast cells (Figure 5a). The number of cells remained approximately constant in PBS. One microtube with encapsulated yeast cells was transferred into the nutrition media (Figure 5b) and further incubated for 14 h. Incubation in nutrition media led to proliferation and division of the yeast cells that increased their number. This was an indication of nontoxicity and availability of free space for new cells as well as on the permeability of the tubes for nutrition.

CONCLUSIONS AND OUTLOOK

We reported an approach for fabrication the fully biodegradable self-rolled tubes, which are suitable for the encapsulation of cells. Our approach is based on the fabrication of polymer bilayers where both components are water-insoluble polymers: polycaprolactone and polysuccinimide. Polysuccinimide is able to slowly hydrolyze and swell in a physiological buffer environment leading to self-rolling of the polymer bilayer and the formation of microtubes. We also demonstrated that the self-rolled tubes can be used for encapsulation of cells, which proliferate and divide inside the tube. Since the used polymers are biocompatible, biodegradable, and produced industrially and are approved for biomedical purposes, the proposed approach is of practical interest for controlled cell delivery and the design of scaffolds for tissue engineering.

ASSOCIATED CONTENT

Supporting Information. Movie of self-rolled tube with encapsulated yeast cells (avi format). This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

The authors are grateful to Manfred Stamm and Georgi Stoychev for fruitful discussions. This work was supported by the Leibniz Institute of Polymer Research Dresden.

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