Biodegradable Self-Folding Polymer Films with Controlled Thermo-Triggered Folding

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Self-folding films are a unique kind of thin film. They are able to deform in response to a change in environmental conditions or internal stress and form complex 3D structures. They are very promising candidates for the design of bioscaffolds, which resemble different kinds of biological tissues. In this paper, a very simple and cheap approach for the fabrication of fully biodegradable and biocompatible self-rolled tubes is reported. The tubes' folding can be triggered by temperature. A bilayer approach is used, where one component is active and another one is passive. The passive one can be any biocompatible, biodegradable, hydrophobic polymer. Gelatin is used as an active component: it allows the design of (i) self-folding polymer films, which fold at room temperature (22 °C) and irreversibly unfold at 37 °C, and (ii) films, which are unfolded at room temperature (22 °C), but irreversibly fold at 37 °C. The possibilities of encapsulation of neural stem cells are also demonstrated using self-folded tubes.

1. Introduction

Self-folding films are a unique kind of thin films, which are able to deform in response to a change of environmental conditions or internal stress and form complex 3D structures such as tubes, capsules, cubes, pyramids, etc.[1] Self-folding films are films with either vertical (bilayers)[2] or lateral (patterned films) inhomogenities,[3] which consist of two or more kinds of components with different volume expansion properties. Such films undergo deformation when the volume of one of the components increases. Self-folding films were demonstrated to be promising candidates for energy harvesting and storage,[4] design of porous materials,[5,6] sensors,[7] cell encapsulation,[2,8] microrobotic functionality,[9] design of bioscaffolds.[5,10] Among the variety of these applications the use of self-folding for biomaterials is especially promising.[11,12] In particular, it was shown that self-folded objects are promising candidates for the design of bioscaffolds, which resemble different kinds of biological tissues.[5]

In order to be suitable for biomaterial engineering, the materials which are used for fabrication of self-folding films must fulfill the following requirements: biocompatibility, biodegradability and sensitivity to stimuli in the physiological range. Metals and oxides demonstrate good biocompatibility although they are not biodegradable and their folding is spontaneous and not activated by stimuli in the physiological range. There are examples of polymer-based self-folding films with temperature-controlled folding based on poly(*N*-isopropylacrylamide).[2,13] These polymers demonstrate responsive properties in the physiological temperature range (25–37 °C), but are not biodegradable. Recently, we reported for the first time the design of biodegradable/biocompatible self-rolled tubes based on polycaprolactone and polysuccinimide, which roll due to slow hydrolysis of polysuccinimide in a physiological buffer environment.[12] The rolling of polysuccinimide-based bilayers is determined by the kinetics of hydrolysis and can hardly be controlled by external signals. Therefore, development of biodegradable/biocompatible self-folding polymer films, whose folding can be triggered by external signals, is strongly desirable. Among all possible signals, which can be used as a trigger, temperature appears to be the most favorable one. Indeed, pH and UV light can cause potential damage to cells, but they can usually tolerate a variation of temperature in the range between 4 °C and 37 °C.

Here, we report a very simple and cheap approach for fabrication of fully biodegradable and biocompatible self-rolled tubes, whose folding can be triggered by temperature. Moreover,

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these self-folding films demonstrate different folding behavior depending on the properties of the polymers. We used a bilayer approach where one component is active and another one is passive. The passive one can be any biocompatible, biodegradable hydrophobic polymer. Gelatin was used as an active component. Gelatin forms hydrogels upon cooling from an aqueous solution, due to helix-formation and association of the helices. These physically crosslinked hydrogels have a sol-gel transition temperature.[14] Chemically crosslinked gelatin undergoes one-way swelling in aqueous environment, wherein the degree of swelling strongly depends on the temperature. The use of gelatin as a thermoresponsive component is highly attractive since the polymer is cheap and produced in huge quantities by hydrolysis of collagen, which is the main component of connective tissue. The last point is very important because one can expect particularly favorable interactions with cells. Due to biocompatibility and biodegradability gelatin is already offered for application in tissue engineering, therapeutic angiogenesis, gene therapy, and drug delivery.[15] Moreover, as we show in this manuscript, gelatin allows the design of (i) self-folding polymer films, which fold at room temperature (22 °C) and irreversibly unfold at 37 °C and (ii) films, which are unfolded at room temperature (22 °C), but irreversibly fold at 37 °C. As a result, gelatin-based self-folding films can be used for both irreversible and reversible encapsulation of cells. Such complex thermoresponsive behavior cannot be achieved by, for example, polymers with LCST behavior, such as poly(N-isopropylacrylamide), which are unfolded and folded at elevated and reduced temperature, respectively.[2]

2. Results and Discussion

In this paper, we demonstrate two approaches for the design of thermoresponsive gelatin-based self-folding films (**Figure 1**). For the first one, not-crosslinked biodegradable polycaprolactone and gelatin were used (Figure 1a,b). In the second approach, we used UV- and VIS- photocrosslinked polymer bilayers. VIS curable system (gelatin-F/PHF-Q) was designed using furfuryl modified gelatin (gelatin-F), which contains a small amount of Rose Bengal as photoinitiator (Figure 1c) and a copolymer of hexanediol and fumaryl chloride (PHF) containing camphorquinone as photoinitiator (Figure 1d). The first UV curable system (gelatin/PHF-Q) was designed using pure gelatin (Figure 1e) and hydrophobic PHF with camphorquinone as photoinitiator (Figure 1d). The second UV curable system was designed using pure gelatin (Figure 1e) and polycaprolactone with 4-hydroxybenzophenone as photoinitiator (Figure 1f). In fact, the use of deep UV irradiation (254 nm) can be considered an advantage: first it allows to minimize the number of modifications required to make polymers photosensitive; second, it allows to avoid water-soluble Rose Bengal as initiator for gelatin; and third, deep UV irradiation is typically used for disinfection of surfaces and allows reduction of biological contamination of materials.

The polymer bilayers, which consist of a bottom gelatin and top hydrophobic polymer, were prepared by sequential dip-coating. All polymers, which were used in this work, are biocompatible and biodegradable: gelatin is a natural polymer, derived from collagen, PCL is a synthetic biocompatible/biodegradable polyester, which is already approved for medical applications,[16] PHF, as it is shown below, is also biocompatible. Moreover, since PHF is a linear aliphatic polyester, it is expected to be biodegradable.[17] The expected products of the degradation of PHF—hexanediol (used in cosmetics) and fumaric acid[18]—are not toxic. The photoinitiators, which are used to induce photocrosslinking of polymers, were already used for bio-related purposes. For example, benzophenone derivatives are used for preparation of photocrosslinked hydrogels, camphoroquinone is the photoinitiator for tooth PMMA-based cement, derivative of diazostilbene is antioxidant, Rose

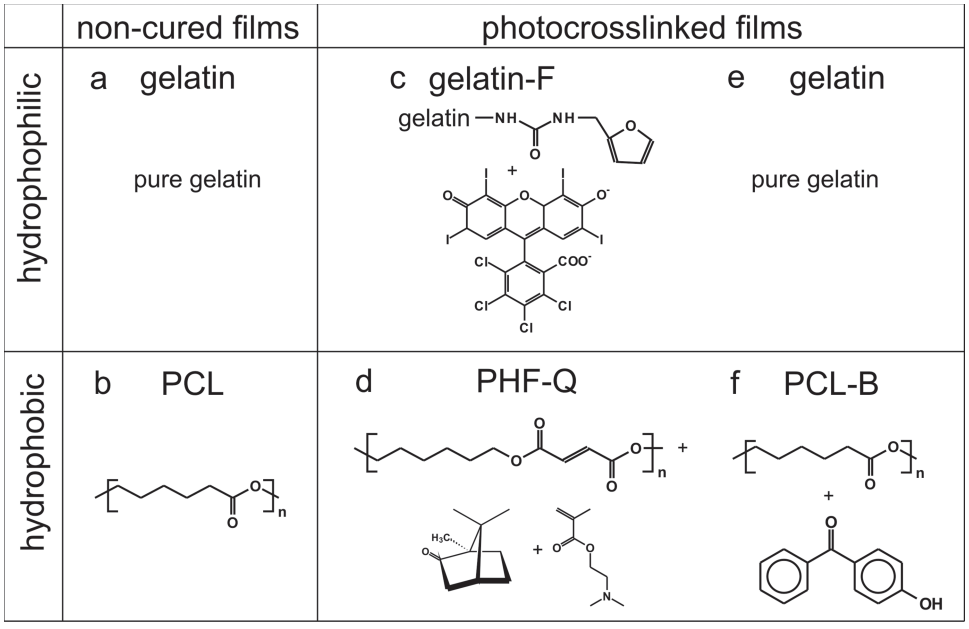

Figure 1. Chemical formulas of polymers which are used for the design of thermoresponsive biodegradable/biocompatible self-folding films.

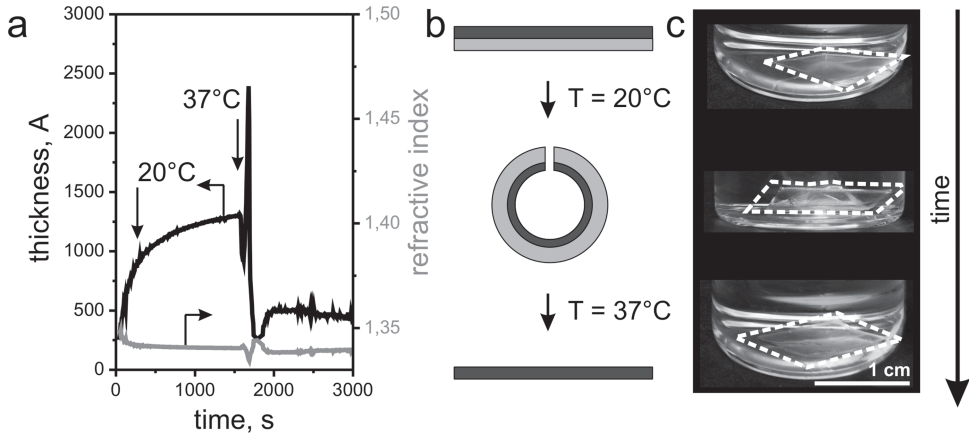

Figure 2. Behavior of non-crosslinked gelatin/polycaprolactone system: (a) Swelling and dissolving of non-crosslinked gelatin film (thickness in a dry state 20 nm) in PBS buffer (0.15 M, pH = 7.4) first at T = 22 °C (0–1500 s) and then at T = 37 °C (1500–3000 s). Scheme (b) and experimental (c) observation of folding/unfolding of the non-crosslinked gelatin/PCL bilayer in water. The thickness of the gelatin is 200 μm; the thickness of the PCL is 20 μm.

Bengal is a food dye. The biocompatibility and biodegradability of furfuryl modified gelatin Rose Bengal photoinitiator was previously demonstrated.[19] Moreover, furfuryl modified gelatin showed no cytotoxicity, even with more than 20% Rose Bengal. Therefore, we expect that these systems can be applied for bio-applications.

2.1. Non-Crosslinked Bilayer

We started from the investigation of the swelling properties of native non-crosslinked gelatin films. Exposure of thin gelatin film to water at room temperature (T = 22 °C) led to its swelling from 20 nm (as measured in a dry state) up to about 120 nm (**Figure 2**a). Increasing the temperature to body temperature (T = 37 °C) resulted in a sharp increase of the film thickness followed by its abrupt decrease. Obviously, gelatin swells moderately in cold PBS 0.15 M (pH = 7.4) buffer, still staying in a gel form, while further increase of temperature to 37 °C results in a stronger swelling of the film, accompanied by the polymer dissolution. The residual thickness of gelatin film was 50 nm in a swollen state at 37 °C and 6 nm after drying, which was found to be independent of the initial thickness of gelatin (either it was 50 nm or 2 μm). This residual layer most probably originates from adsorption of gelatin on the charged silica wafer. Very thick gelatin films demonstrated similar temperature-dependent swelling behavior. For example, a gelatin film with a thickness of around 200 μm swelled up to 1000% in water at room temperature after 2 h of swelling and up to 1900% after 24 h as measured by the mass change. Heating up to 37 °C led to the complete dissolution of the swollen gelatin films. Interestingly, dissolution of gelatin occurred exactly at 37 °C which can be used for temperature controlled release in the human body.

We fabricated a non-crosslinked gelatin-polycarolactone bilayer by deposition of a 50 μm layer of polycaprolactone on a 220 μm thick layer of gelatin. The sample was annealed at 60 °C for 30 s in order to melt polycaprolactone, to fuse it to the gelatin layer and to make the bilayer more stable. The film was immersed in cold water. Correspondingly to the swelling scenario of gelatin, the film slowly deformed and rolled up into a tube (Figure 1b,c). The inner diameter of the tube was around 1–2 mm. An increase of temperature led to the unfolding of the film, which was caused by the dissolution of the gelatin. Finally, an unfolded PCL film was left.

Thus, the non-crosslinked PCL-gelatin system undergoes folding at low temperature and unfolding at higher temperature. Since the polymers are not photocrosslinkable, bilayers with different shape can be prepared by knife cutting or by cutting with an IR heating laser. Both polymers used in this system are biocompatible and biodegradable which allows the non-crosslinked PCL-gelatin system to be employed in bio-related applications.

2.2. Crosslinked Films

Next, we investigated folding of four photocrosslinked bilayer, where gelatin is the bottom layer and the hydrophobic polymer is the top layer. Similar to native gelatin, all photocrosslinked gelatin films swelled in cold water (T = 24 °C) up to 1000% of its mass after 2 h of swelling. On the other hand, since the film was crosslinked, heating to 37 °C did not lead to its dissolution and the swelling degree increased up to 1200%.

The polymer bilayers were prepared by sequential deposition of the respective polymers. The bilayers were photocrosslinked by irradiation through a photomask by using either UV light (254 nm) in the case of gelatin/PHF-Q and gelatin/PCL-B films or blue light (405 nm) in the case of gelatin-F/PHF-Q films. After being photocrosslinked, the bilayer was rinsed in chloroform in order to remove the non-crosslinked hydrophobic polymer leading to the formation of a structured bilayer formed by the bottom layer of the crosslinked and non-crosslinked gelatins and top layer of crosslinked hydrophobic polymers (**Figure 3**a,d,h).

Immersion of the crosslinked bilayers in cold water (24 °C) led to swelling of both crosslinked and non-crosslinked gelatin (Figure 3a,b) that resulted in slight wrinkling of the bilayer (Figure 3e,i). As it was shown above, non-crosslinked gelatin is not soluble in cold water. Formed gel of non-crosslinked gelatin

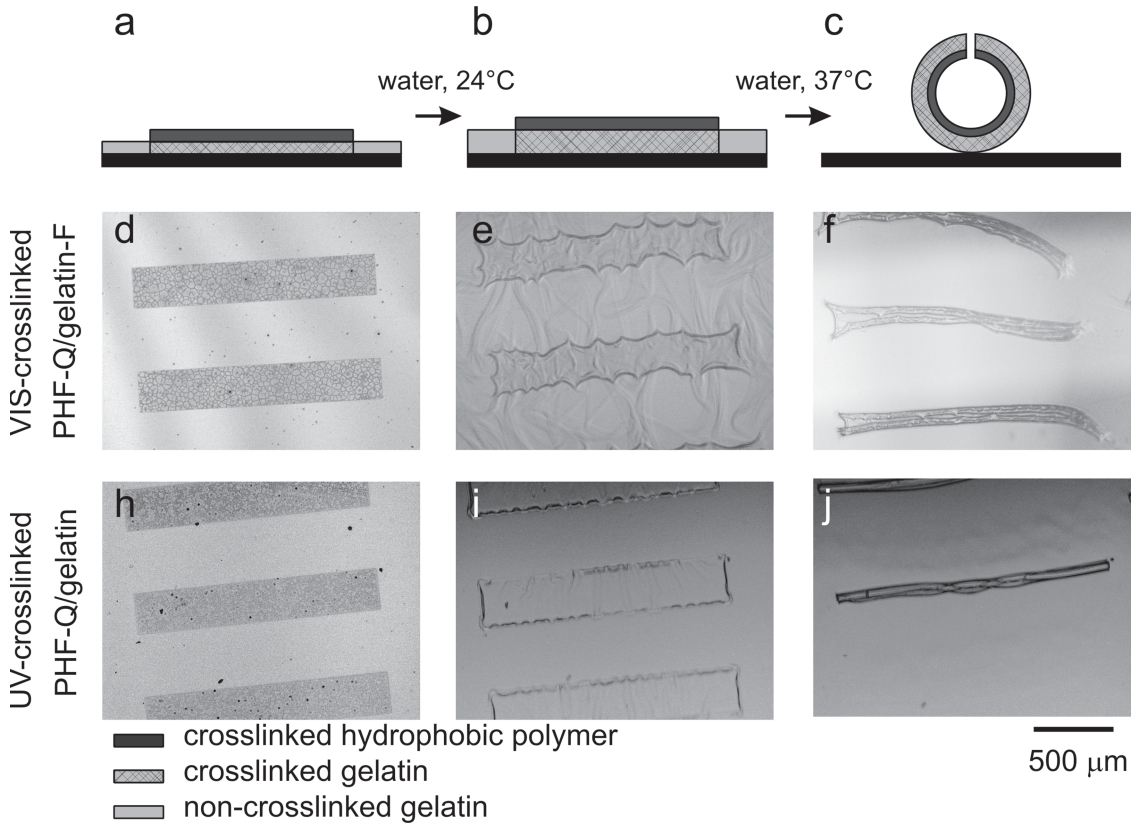

Figure 3. Folding of crosslinked bilayers. (a–c) Schematic scenario of temperature-dependent behavior of the bilayer; (d–f) microscopy snapshots of the gelatin-F/PHF-Q bilayer at different temperatures. (h–j) – microscopy snapshots of the gelatin/PCL-Q bilayer at different temperatures. Panels (d) and (h) correspond to panel (a). Panels (e) and (i) correspond to panel (b). Panels (f) and (j) correspond to panel (c). The thickness of the gelatin in both cases is ca 1.7 μm, the thickness of PHF is 100 nm. Gelatin/PCL-Q films demonstrate similar behavior.

merely held photocrosslinked bilayer and prevented its folding. An increase of temperature to T = 37 °C led to the dissolution of the non-crosslinked gelatin. As a result, the photocrosslinked bilayer film rolled due to stress produced by swelling of the photocrosslinked gelatin (Figure 3c,f,j).

We investigated the effect of the thickness of each layer on the radius of the formed tubes. It was found that 1.7 μm gelatin and 100 nm PHF-Q layers form tubes with diameters around 10–20 μm. Interestingly, an increase of the thickness of the PHF-Q layer does not lead to an increase of the diameter but results in the inability of the bilayer to fold. The origin of this effect is most probably the high stiffness of the PHF-Q layer. Tubes with different diameters can be however easily prepared using another UV-crosslinkable film gelatin/PCL-Q (**Figure 4**a,b). The PCL is a relatively soft polymer and the diameter of tubes can easily be adjusted by changing the thickness of each layer. In particular, an increase of the thickness of both polymers results in a diameter increase of the tubes (Figure 4c) which is in qualitative consistence with the Timoshenko equation.[20]

2.3. Encapsulation of Cells

Finally, we demonstrate possibilities to encapsulate cells using gelatin-based self-folding films and investigate the behavior of neural stem cells for the example of one of the UV-crosslinked thermoresponsive gelatin-based systems, which contains the minimal number of additives and modifications, namely gelatin/PHF-Q. The behavior of cells was first investigated on individual polymer films: gelatin and PHF (**Figure 5**). After 66 h the cells were adsorbed on the previously crosslinked polymer films and started to form agglomerates indicating that cells well adhere to both hydrophobic and hydrophilic polymers, as well as that cells are alive and are able to divide. It is interesting to observe that the behavior of cells on both polymers (natural gelatin and synthetic PHF) is almost identical. Next, we adsorbed primary fetal mouse neural stem from their dispersion in serum-free media on the top of unfolded gelatin/PHF-Q bilayer at room temperature and allowed them to settle down for 10 min until a considerable amount was accumulated on the polymer surface. Similar to the previous observations, the increase of the temperature led to rolling of the bilayer and formation of tubes filled with cells (Figure 5).

Finally, we investigated the viability of the cells adsorbed on individual polymers as well as cells encapsulated in the tubes (**Figure 6**a,b). Polystyrene and fibronectine-coated surfaces were used as negative and positive control, respectively. It was found that cells on gelatin and PHF showed viability close to the positive control – fibronectin. The viability of the cells in the tubes was reduced, possibly due to confinement, but still remained above the values for the negative control. The most important

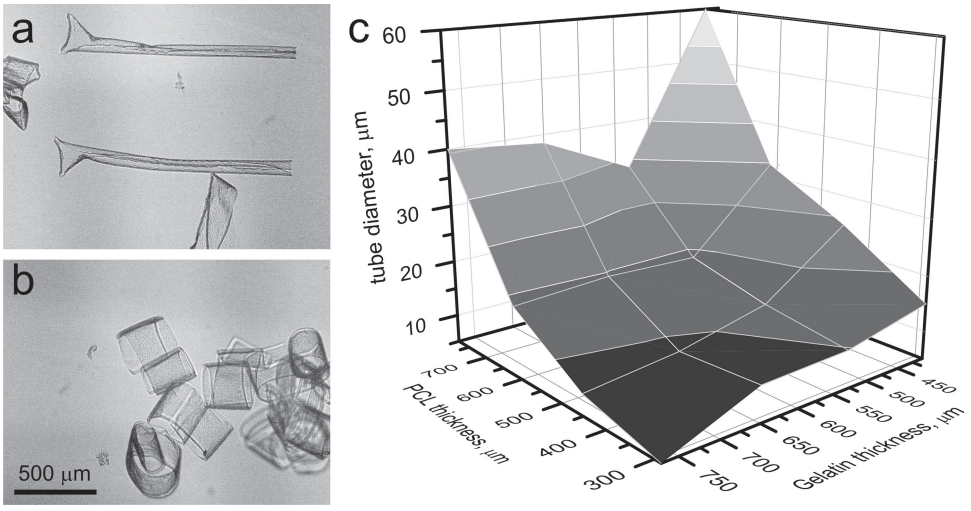

Figure 4. Optical microscopy images of tubes (a,b) obtained by folding of gelatin/PCL-Q; Dependence of tube diameter of gelatin/PCL-Q bilayers on the thickness of each layer (c).

aspect was, however, that cells in the tubes remained stable for at least 7 days thus implying that polymers are non-toxic and the tubular environment does not cause apoptosis of the cells.

3. Conclusions

In conclusion, we demonstrated two approaches for the design of fully biodegradable and biocompatible self-folding films with temperature-triggered folding. Both approaches are based on gelatin as active component, which can be either native or photocrosslinked. Depending on the properties of gelatin (either crosslinked or not) the films can either fold at room temperature and unfold at 37 °C or remain undeformed at room temperature and fold at 37 °C. Both these scenarios are useful for encapsulation and release of the cells as well as for the design of bioscaffolds for tissue engineering. We demonstrated that neural stem cells can be encapsulated in rolled-up tubes during their formation and they remain alive for a considerable period of time.

4. Experimental Section

Synthesis of Visible Light Crosslinkable Gelatin: 1.25 g of gelatin (porcine skin, 300 Bloom) were dissolved in 125 mL of warm (~40 °C) water in 250 mL flask equipped with a magnetic stirrer. Then, 500 μL of furfuryl isocyanate were dissolved in 10 mL of DMSO and added dropwise to the gelatin solution. The reaction lasted 24 h under room temperature and constant stirring. In order to remove DMSO and unreacted furfuryl isocianate, dialysis of the resulting mixture against distilled water was made. After dialysis, the solution of modified gelatin was reduced to the jelly-state via rotor evaporation. Obtained substance was dried in vacuum under 60 °C temperature for 24 h. 1 g of modified gelatin was

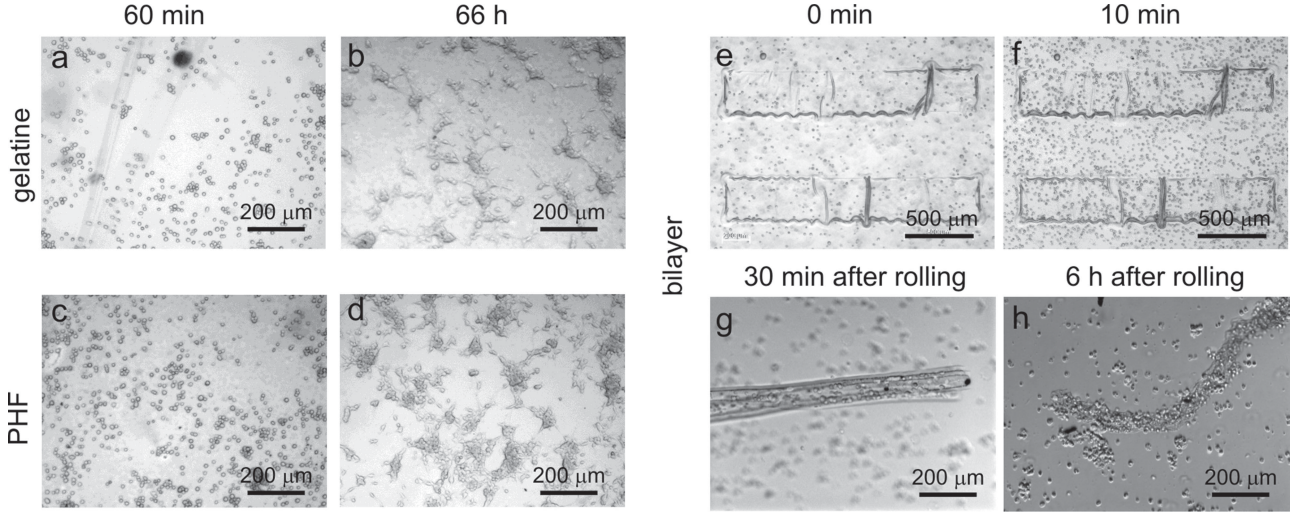

Figure 5. Encapsulation of neural stem cells using UV crosslinked gelatin/PHF-Q bilayer. (a,c) – cells on gelatin and PHF directly after seeding; (b,d) – cells on gelatin and PHF after 66 h of incubation. (e,f) – cells on gelatin/PHF-Q bilayer directly after seeding and after 10 min at room temperature (g,h) – cells in the gelatin/PHF-Q.

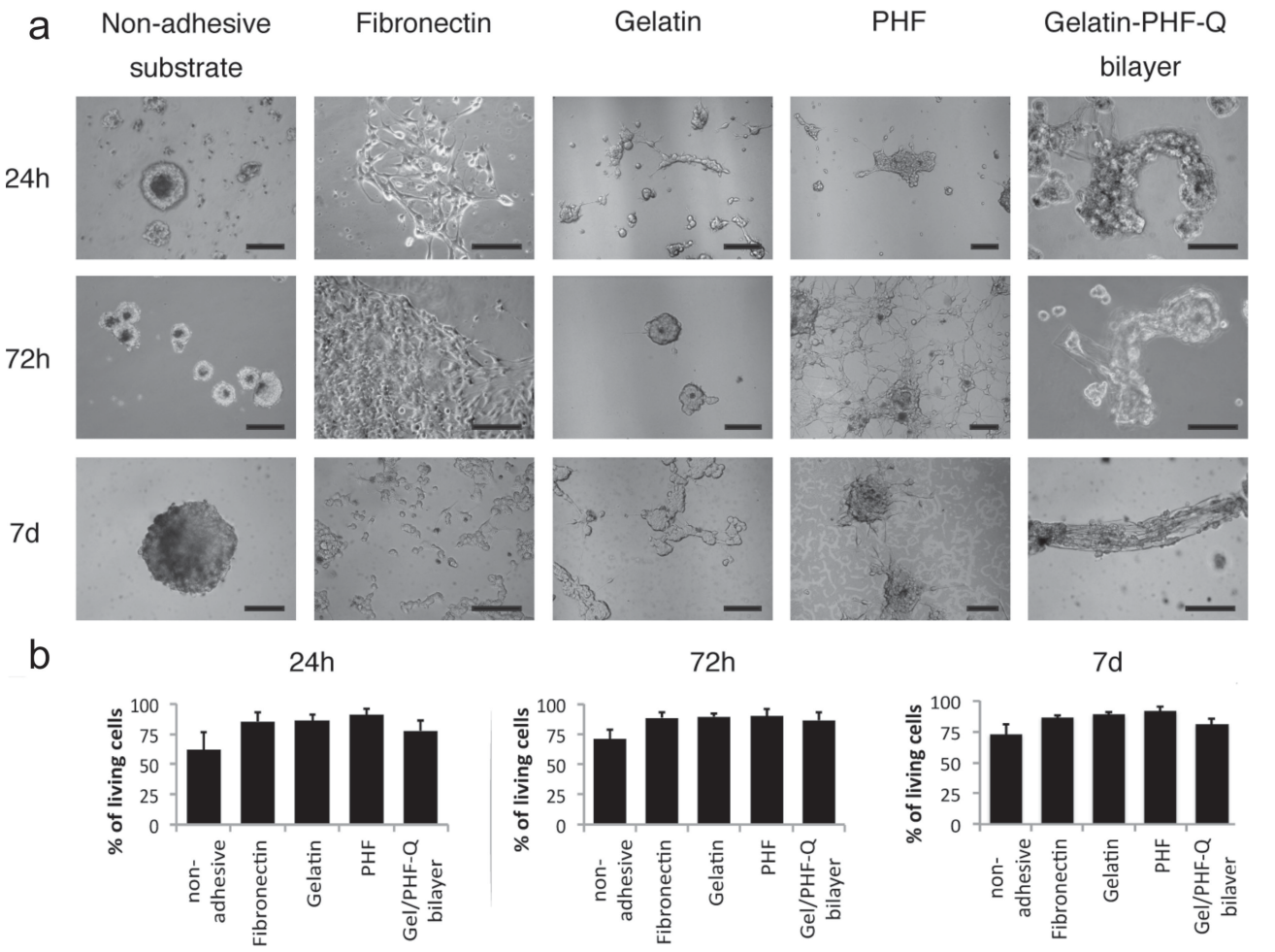

Figure 6. Viability of Neural Stem Cells (a) Microscopy images of Neural Stem Cells in gelatin/PHF-Q bilayer tubes, on gelatin, PHF, fibronectin functionalized substrate (positive control) and negative control (non-adhesive substrate). Trypan blue staining shows dead cells. Scale bar = 100 μm; (b) Cell counts of trypan-blue stained Neural Stem Cell cultures demonstrate high biocompatibility of gelatin and PHF).

dissolved in 10 mL of warm water. Then, 50 mg of Rose Bengal were added to the gelatin solution. We also added 100 U/mL of penicillin and 100 μg/mL of streptomycin to gelatin solution.

Synthesis of Biodegradable Hydrophobic Visible Light Crosslinkable Polymer – PHF: PHF were synthesized by the reaction of polycondensation between 1,6-hexanediol and fumaryl chloride. Before the reaction 1,6-hexanediol was dried in vacuum under 60 °C temperature for 24 h. Fumaryl chloride was distilled in vacuum in order to remove fumaric acid.

2.615 g of dried 1,6-hexanediol were dissolved in 20 mL of dehydrated THF in dry two-necked 50 ml flask equipped with a magnetic stirrer and $CaCl_2$ tube. Then 2.4 mL of distilled fumaryl chloride were dissolved in 5 mL of dehydrated THF and added dropwise to the solution of 1,6-hexanediol. After reagents were mixed, the temperature was raised up to 80 °C and all THF was evaporated from the mixture. The reaction lasted until the mixture became solid. Then 20 mL of CH_2Cl_2 were added to dissolve it. The final polymer was obtained by precipitation in 1 L of petroleum ether. 1H NMR ($CDCl_3$, 500 MHz): 6.84 (s, 2H), 4.20 (s, 4H), 1.71 (s, 4H), 1.42 (s, 4H).

Solution of the polymer was prepared as follows: 350 mg of PHF were dissolved in 10 mL of $CHCl_3$. Then 50 μL of DMAEMA and 50 mg of Camphorquinone were added to the solution.

Fabrication of Self-Rolled Tubes: For the preparation of gelatin-based bilayers, polymers were sequentially deposited on a cleaned silicon wafer substrates with a typical size of 11 mm × 25 mm using dip-coating. First, gelatin layer was deposited from its warm (37–40 °C) water solution. Passive polymer was then deposited from the selective solvent on the top of the first layer. In the case of crosslinkable systems, resulting bilayers were illuminated either with UV- or with VIS-light through a photomask. Then, uncrosslinked polymers were fully or partially developed using corresponding solvents. For non-crosslinkable gelatin-based system bilayer patterning was achieved by cutting.

Neural Stem Cell Culture and Encapsulation: Primary fetal mouse neural stem cells were a gift of A. Storch (University Clinic Carl Gustav Carus Dresden) Stem cell culture was done as described previously.[21] In short, neural stem cells were maintained in serum-free media comprising a DMEM (high glucose)/F-12 mixture (2:1), supplemented with 20 ng/mL of Egf and Fgf-2 (Sigma) and 2% B-27 supplement (Gibco/Invitrogen, Carlsbad, CA, USA).

Cell Survival of Neural Stem Cells: Trypan blue cell survival method is based on the principle that live (viable) cells do not take up trypan blue, whereas dead (non-viable) cells do. Neural stem cells were cultivated on a substrate functionalized with fibronectin as a positive control, non-adhesive substrate as a negative control, PHF, gelatin and in bilayer tubes. After 24 h, 72 h and 7 d medium was aspirated from cultures and replaced by phosphate buffered saline (pH 7.4). The same amount of 0.4% Trypan Blue solution (w/v) was added, and the sample

was allowed to stand for 5 to 15 min. Viable and non-viable cells were counted separately.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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[1] a) J. S. Randhawa, K. E. Laflin, N. Seelam, D. H. Gracias, *Adv. Funct. Mater.* **2011**, *21*, 2395; b) L. Ionov, *Soft Matter* **2011**, *7*, 6786.
[2] S. Zakharchenko, N. Puretskiy, G. Stoychev, M. Stamm, L. Ionov, *Soft Matter* **2010**, *6*, 2633.
[3] a) H. Thérien-Aubin, Z. L. Wu, Z. Nie, E. Kumacheva, *J. Am. Chem. Soc.* **2013**, *135*(12), 4834; b) J. Kim, J. A. Hanna, M. Byun, C. D. Santangelo, R. C. Hayward, *Science* **2012**, *335*, 1201.
[4] a) X. Y. Guo, H. Li, B. Y. Ahn, E. B. Duoss, K. J. Hsia, J. A. Lewis, R. G. Nuzzo, *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 20149; b) J. Deng, H. Ji, C. Yan, J. Zhang, W. Si, S. Baunack, S. Oswald, Y. Mei, O. G. Schmidt, *Angew. Chem. Int. Ed.* **2013**, *52*, 2326.
[5] S. Zakharchenko, N. Puretskiy, G. Stoychev, C. Waurisch, S. G. Hickey, A. Eychmuller, J.-U. Sommer, L. Ionov, *J. Mater. Chem. B* **2013**, *1*, 1786.
[6] Y. Mei, D. J. Thurmer, C. Deneke, S. Kiravittaya, Y.-F. Chen, A. Dadgar, F. Bertram, B. Bastek, A. Krost, J. r. Christen, T. Reindl, M. Stoffel, E. Coric, O. G. Schmidt, *ACS Nano* **2009**, *3*, 1663.
[7] E. J. Smith, S. Schulze, S. Kiravittaya, Y. Mei, S. Sanchez, O. G. Schmidt, *Nano Lett.* **2010**, *11*, 4037.
[8] a) G. S. Huang, Y. F. Mei, D. J. Thurmer, E. Coric, O. G. Schmidt, *Lab Chip* **2009**, *9*, 263; b) S. Schulze, G. Huang, M. Krause, D. Aubyn, V. A. B. Quiñones, C. K. Schmidt, Y. Mei, O. G. Schmidt, *Adv. Eng. Mater.* **2010**, *12*, B558.
[9] a) A. A. Solovev, W. Xi, D. H. Gracias, S. M. Harazim, C. Deneke, S. Sanchez, O. G. Schmidt, *ACS Nano* **2012**, *6*, 1751; b) W. Xi, A. A. Solovev, A. N. Ananth, D. H. Gracias, S. Sanchez, O. G. Schmidt, *Nanoscale* **2013**, *5*, 1294.
[10] a) E. Gultepe, J. S. Randhawa, S. Kadam, S. Yamanaka, F. M. Selaru, E. J. Shin, A. N. Kalloo, D. H. Gracias, *Adv. Mater.* **2012**, *25*, 514; b) C. L. Randall, Y. V. Kalinin, M. Jamal, A. Shah, D. H. Gracias, *Nanomed.-Nanotechnol.* **2011**, *7*, 686.
[11] a) M. Jamal, S. S. Kadam, R. Xiao, F. Jivan, T.-M. Onn, R. Fernandes, T. D. Nguyen, D. H. Gracias, *Adv. Healthcare Mater.* **2013**, *2*, 1142; b) M. Jamal, N. Bassik, J. H. Cho, C. L. Randall, D. H. Gracias, *Biomaterials* **2010**, *31*, 1683.
[12] S. Zakharchenko, E. Sperling, L. Ionov, *Biomacromolecules* **2011**, *12*, 2211.
[13] G. Stoychev, N. Puretskiy, L. Ionov, *Soft Matter* **2011**, *7*, 3277.
[14] E. H. Schacht, *J. Phys: Conference Series* **2004**, *3*, 22.
[15] a) X. Wu, Y. Liu, X. Li, P. Wen, Y. Zhang, Y. Long, X. Wang, Y. Guo, F. Xing, J. Gao, *Acta Biomater.* **2010**, *6*, 1167; b) S. Young, M. Wong, Y. Tabata, A. G. Mikos, *J. Control. Release* **2005**, *109*, 256.
[16] a) C. Chiari, U. Koller, R. Dorotka, C. Eder, R. Plasenzotti, S. Lang, L. Ambrosio, E. Tognana, E. Kon, D. Salter, S. Nehrer, *Osteoarthritis Cartilage* **2006**, *14*, 1056; b) J. M. Williams, A. Adewunmi, R. M. Schek, C. L. Flanagan, P. H. Krebsbach, S. E. Feinberg, S. J. Hollister, S. Das, *Biomaterials* **2005**, *26*, 4817.
[17] M. A. Rice, J. Sanchez-Adams, K. S. Anseth, *Biomacromolecules* **2006**, *7*, 1968.
[18] S. Wang, L. Lu, M. J. Yaszemski, *Biomacromolecules* **2006**, *7*, 1976.
[19] T. I. Son, M. Sakuragi, S. Takahashi, S. Obuse, J. Kang, M. Fujishiro, H. Matsushita, J. Gong, S. Shimizu, Y. Tajima, Y. Yoshida, K. Suzuki, T. Yamamoto, M. Nakamura, Y. Ito, *Acta Biomater* **2010**, *6*, 4005.
[20] S. Timoshenko, *J. Opt. Soc. Am. Rev. Sci. Instrum.* **1925**, *11*, 233.
[21] A. K. Meyer, A. Jarosch, K. Schurig, I. Nuesslein, S. Kissenkoetter, A. Storch, *Brain Res.* **2012**, *1474*, 8.