Electrospun Fibers from Functional Polyglycidol/Poly(ε-caprolactone) Blends with Defined Surface Properties

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ABSTRACT: Highly hydrophilic but water insoluble fibers from star-shaped poly(ethoxyethyl glycidyl ether) and poly(ε-caprolactone) polymer blends in the submicron range were prepared using the electrospinning technique. The fibers were achieved in a smooth and homogeneous manner and do show tremendous decreased protein adsorption. Additionally, using alkyne- or vinyl sulfonate end capped polymer, fibers with the correspondent surface reactivity have been prepared. All fibers showed high biocompatibility and were highly hydrophilic but water stable. Furthermore, nonwovens based on functionalized poly(ethoxyethyl glycidyl ether) were equipped with small biofunctional molecules, e.g., the peptide sequence glycine-arginine-glycine-aspartate-serine (GRGDS). These fibers showed increased cell attachment compared with nonfunctionalized nonwovens. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: fibers; hydrophilic polymers; surface functionality; polyglycidol; tissue engineering

INTRODUCTION

Nonwoven fiber meshes of nanofibers for tissue engineering applications have gained great interest during the last decade. Especially bioresorbable materials, e.g., poly(α-hydroxy acids), are widely used in biomedical applications.[1] Poly(ε-caprolactone) (PCL) as a representative of this group is often used where slow degradation is favorable. Unfortunately pure PCL readily adsorbs proteins which influence strongly the biomaterial tissue interaction. One possibility to overcome this limitation is the increase of the hydrophilicity of the material to reduce the unspecific protein adsorption. A range of biomaterials can be tailored to be resistant to nonspecific protein adsorption by surface modification with poly(ethylene glycol) (PEG) to the substrate.[2–4] Ultrathin functional networks of star-shaped PEO were shown to be extremely resistant to unspecific adsorption of proteins.[5–7]

None the less, many polymers usable as an alternative to PEG are currently under investigation. Polyglycidols (PGs) fulfill all structural prerequisites to replace star-shaped PEGs in biomedical application and exceed the possibilities due to their high intrinsic functionality and the possibility to adjust the functionality to the demands of the application. As most synthetic polymers have no functional groups attached to the polymer main chain, PGs with hydroxyl side groups and its derivates are of great interest for applications in medicine because of their high functionality, solubility in aqueous media, and biocompatibility.[8–11] Therefore several groups have studied the ionic polymerization of glycidol leading to branched polymers in the last decades.[8,12–15] In general the microstructure of the hyperbranched PGs cannot be well controlled. To obtain architecturally well-defined PG, the hydroxyl group of the monomer has to be protected by a suitable protecting group leading to highly defined polymers with narrow distributions. Mostly ethoxyethyl glycidyl ether (EEGE) was used for the preparation of PG with controlled architecture since the protecting group is easily removed from PEEGE under acidic conditions. Therefore anionic polymerization of the protected monomer followed by removal of the

Additional Supporting Information may be found in the online version of this article.

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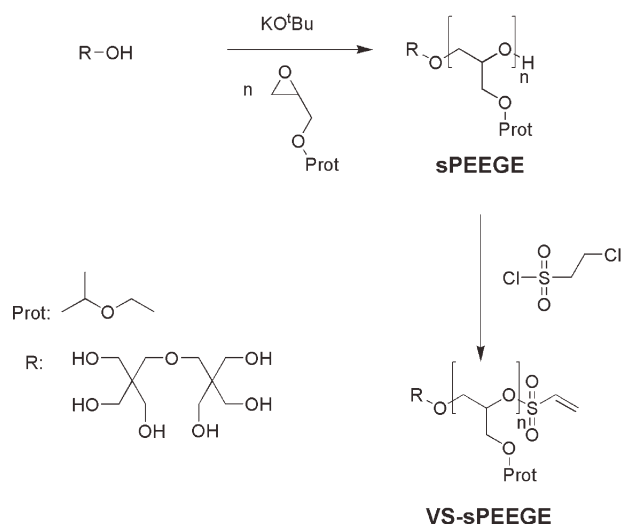

Figure 1 Overview of the synthesis route of sPEEGE followed by functionalization.[21]

protecting group yields polyglycidol with well-defined architecture.[11,16–20]

Most recently the synthesis of vinyl sulfonate end capped, star-shaped polyglycidols (VS-sPEEGE) with a high, unique reactivity towards different functional groups like amines in aqueous solutions has been reported.[21,22] Star-shaped polymers were used for an further increase of the functionality. The synthesis route of the used polymer is briefly shown in Figure 1.

These polymers have great potential towards their usage in polymer/protein or polymer/peptide conjugates due to their unique reactivity towards amines as reported previously.[21,22]

Furthermore, they can be used as precursors for the introduction of different moieties, e.g., alkyne groups, by reaction of the functionalized polymers with an alkyne-amine to synthesize alkyne functionalized PGs.

Electrospun nanofibers have attracted tremendous interest in biomedical applications. As this has been widely discussed during the last decade, detailed information on the electrospinning process can be found elsewhere.[23–25] As electrospun fibers mimic the dimension of fibrous proteins (fibrils) found in extracellular matrices, they allow infiltration of the cells into the matrix followed by proliferation.[26,27] Further functionalization of the fibers, e.g., with the bioactive peptide sequence glycine-arginine-glycine-aspartate-serine (GRGDS), led to a controlled cell interaction with the electrospun fibers.[28] The usage of end group functionalized polymers in the electrospinning process allows the preparation of reactive electrospun nonwovens, yet only few studies are dealing with the bioactivation of fully synthetic electrospun nonwovens.[25,29] While the usage of reactive PEO/PCL blends or PEO-*b*-PCL copolymers to produce hydrophilic electrospun nonwovens has already been studied, the use of polyglycidol for this purpose has not been reported yet.[30–32] Additionally, only few studies concerning the electrospinning of hyperbranched PG has been published till now.[33–36]

Therefore, the synthesis of nonwovens based on end-group functionalized, star-shaped PG and PCL by electrospinning is of great interest. Especially due to the possibility of direct functionalization with bioactive molecules these nonwovens are expected to be advantageous for many applications, e.g. for tissue engineering.

In this presentation we report the electrospinning of a blend containing star-shaped poly(ethoxyethyl glycidyl ether) and poly(ε-caprolactone) (sPEEGE/PCL) with up to 35 wt % sPEEGE to produce hydrophilic nonwovens with minimized unspecific protein adsorption. Furthermore, the use of alkyne or vinyl sulfonate end capped sPEEGEs lead to nonwovens with a high specific surface reactivity.

EXPERIMENTAL

Materials

Poly(ε-caprolactone) ($M_w = 65{,}000$ g mol^{-1}, PDI = 1.4) was purchased from Sigma-Aldrich GmbH (Steinheim, Germany) and used as received. Chloroform and methanol was purchased from VWR International GmbH (Darmstadt, German). Alexa Fluor® 488 azide, deuterochloroform, copper sulfate, dimethylsulfoxide, dipentaerythritol, potassium *tert*-butoxide, and PBS buffer solution were purchased from Sigma-Aldrich GmbH (Steinheim, Germany). (R)-(-)-4-(3-Aminopyrrolidino)-7-nitro-benzofuran was purchased from Fluka (Steinheim, Germany). 2-chloroethylsulfonylchloride was purchased from Alfa Aesar (Karlsruhe, Germany). BSA BODIPY® FL conjugate was purchased from Invitrogen GmbH (Karlsruhe, Germany) and sodium ascorbate was purchased from Merck KGaA (Darmstadt, Germany). All other chemicals were of high grade and purchased from different suppliers. All chemicals were used without any further purification.

Synthesis of sPEEGE, VS-sPEEGE, and alkyne-PEEGE

The synthesis of the vinyl sulfonate terminated, star-shaped polyglycidol has been described elsewhere.[21] Briefly, sPEEGE ($M_w = 14{,}500$ g mol^{-1}, PDI = 1.1) has been synthesized using dipentaerythritol as initiator, ethoxyethyl glycidyl ether as monomer, potassium tert-butoxide, and DMSO in an anionic polymerization procedure. Afterwards, sPEEGE has been reacted with 2-chloroethylsulfonylchloride to obtain VS-sPEEGE ($M_w = 14{,}800$ g mol^{-1}, PDI = 1.1). Alkyne terminated, star-shaped PEEGE ($M_w = 15{,}000$ g mol^{-1}, PDI = 1.1) was prepared by reaction of VS-sPEEGE

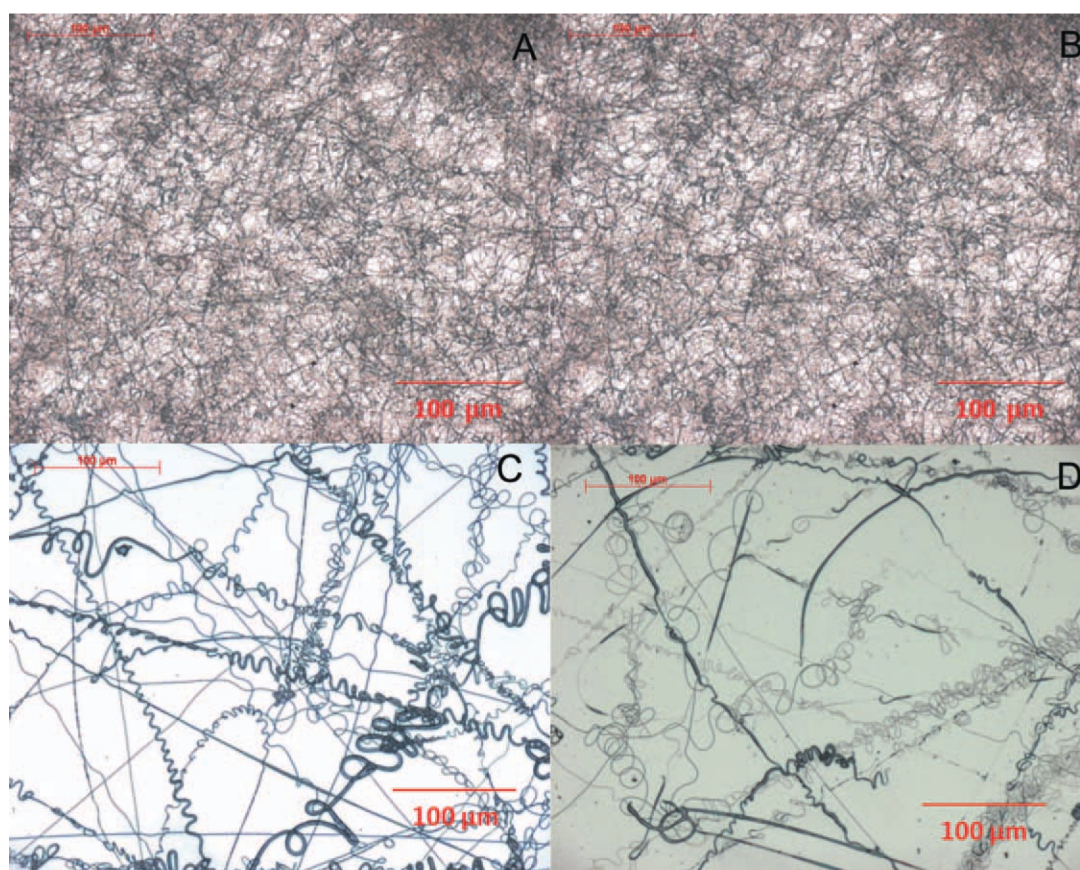

Figure 2 Optical microscope pictures of fibers from sPEEGE/PCL blends spun at an applied voltage of 15 kV and 15 cm distance with 15 wt.% (A), 25 wt.% (B) and 35 wt.% (C) sPEEGE amount and pure PCL fiber (D) as reference. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with 6 eq. Propargylamine under basic conditions similar to reported conjugation procedure for other amines.[21,22] All synthesized polymers were characterized by NMR and SEC prior to usage.

Electrospinning

For electrospinning sPEEGE/PCL polymer blends with varying sPEEGE amount (0, 15, 25, 35 wt %) were dissolved in a mixture of chloroform and methanol (75/25, v/v) to produce 15 wt % solutions (10 wt % for the pure PCL solution). Unless otherwise noted, the polymer solution was pumped to the 18-gauge, flat-tipped, stainless steel spinneret at a rate of 0.4 mL h^{-1} connected to a voltage source of 15 kV. The fibers were either collected on silicon wafers fixed to an aluminum SEM stup (diameter 12 mm) or on a grounded aluminum cylinder (diameter 80 mm, length 25 mm), rotating at 200 rpm, both at a 150 mm distance from the tip of the spinneret.

Proof of surface reactivity

Electrospun fibers produced from alkyne-functionalized sPEEGE were incubated for 24 h at room temperature in an aqueous solution of an azide-functionalized Dye (Alexa Fluor® 488 azide) (50 nmol mL^{-1}), copper(II) sulfate (2.5 pmol mL^{-1}) and sodium ascorbate (10 pmol mL^{-1}) according to the standard Huisgen-Sharpless-Meldal reaction.[37,38] Afterwards the samples were washed twice with distilled water. For the proof of the reactivity of the vinyl sulfonate end groups electrospun nonwovens were incubated in a solution of (R)-(-)-4-(3-Amino-pyrrolidino)-7-nitro-benzofuran (50 µg mL^{-1}) in water for 1 h at room temperature. Afterwards the samples were washed twice with distilled water and then twice with ethanol. All samples were kept in the dark during all incubation and washing steps prior to analysis with fluorescence microscopy.

Contact angle measurement

Contact angle were determined by sessile drop measurements with a geniometer G40 (Krüss, Hamburg, Germany), using electrospun meshes collected on rotating drum. The volume of the applied droplet is 5 µL. The resulting value of each single measurement is the average value of the left and the right contact angle. For each sample, five droplets had

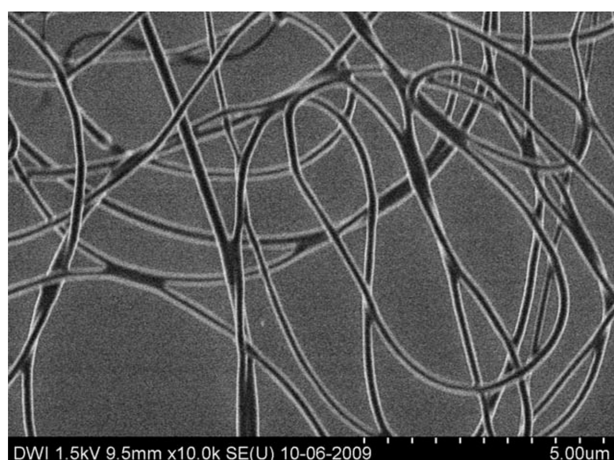

Figure 3 SEM-image of fibers from sPEEGE/PCL blends with 25 wt.% sPEEGE spun at an applied voltage of 15 kV and 15 cm distance.

been measured for determination of the contact angle. The presented data are the average values of five measurements. Errors were determined through evaluation of the standard derivation of the measurements.

Protein adsorption

Electrospun fibers were incubated for 20 min in a solution of BODIPY labeled bovine serum albumin (50 μg mL^{-1}) in PBS buffer solution. Afterwards the samples have been washed twice with PBS buffer solution and then washed twice with distilled water. All samples were kept in the dark during all incubation and washing steps prior to analysis with fluorescence microscopy.

NMR analysis

NMR spectra of the fibers were collected on a Varian Mercury 300 MHz NMR spectrometer. For the measurements 8 mg of the electrospun nonwovens were solved in 0.7 mL deuterochloroform containing TMS as internal standard.

SEM and optical microscopy

The samples were imaged with SEM (S-4800 Ultra High Resolution Scanning Electron Microscope, Hitachi, Tokyo, Japan) using an accelerating voltage of 1 kV. Microscope images were taken with an Axioplan 2 (Zeiss, Oberkochen, Germany).

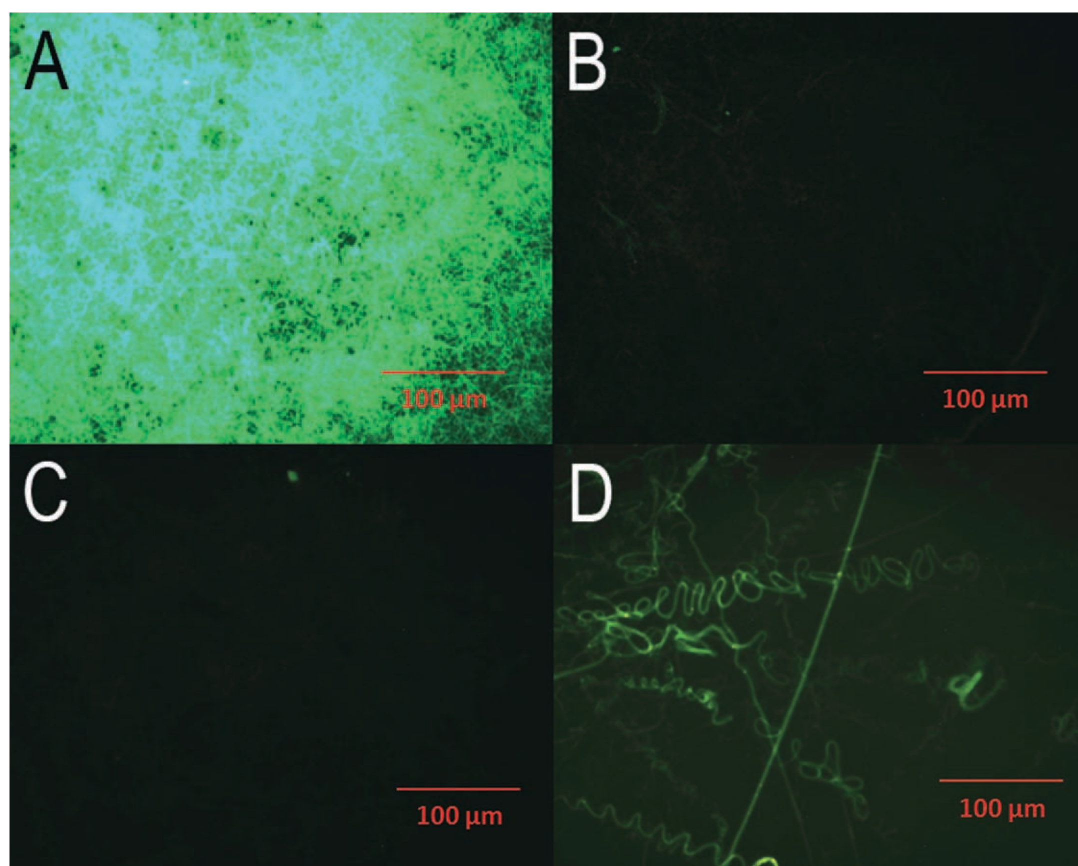

Figure 4 Fluorescence images of electrospun sPEEGE/PCL blends with 0 wt % (A), 15 wt % (B), 25 wt % (C), and 35 wt % (D) sPEEGE amount after incubation with BODIPY® labeled BSA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

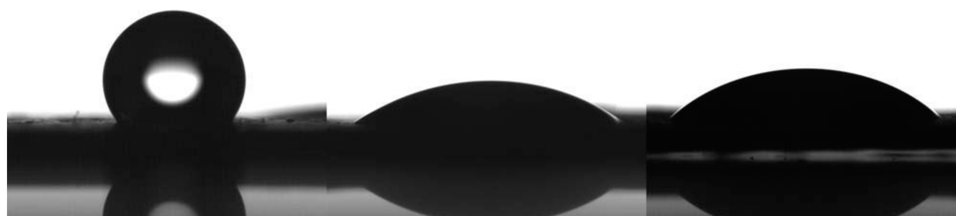

Figure 5 Images of contact angle measurements on electrospun PCL nonwoven (left), sPEEGE/PCL (15 : 85) (middle) and sPEEGE/PCL (25 : 75) (right) nonwoven.

Cytotoxicity assay

The potential cytotoxicity of the nonwoven was evaluated against primary human skin fibroblasts. The cytotoxicity assay was performed incubation of electrospun nonwoven within a fibroblast cell culture in a 6-well plate. The investigation of the cell growth had been performed as follows: first, 15,000 cells/well were incubated in RPMI1640 medium containing 10% FCS for 4 h. Afterwards, new cell medium as well as the electrospun nonwoven (~1 cm^2) had been added to the cells and they were incubated at 37°C for 48 h. As control, cell medium without nonwoven has been used. Alamarblue was added (100 μL mL^{-1} medium) and incubation was continued for another 2 h at 37°C. Totally, 2 × 100 μL of the medium were transferred to a 96-well plate and fluorescence of the samples was measured using an Optima Fluorescence Reader at 544 nm.

Immobilization of GRGDS on electrospun fibers and Investigation of cell behavior

For investigation of the cell growth glass samples were coated with NCO-sP(EO-*stat*-PO) by literature known procedure to suppress cell growth on the substrate.[28,39] Afterwards, fibers from vinyl sulfonate terminated sPEEGE/PCL blends (25/75 w/w) were electrospun on coated glass slides. The fibers were incubated 30 min in H_2O and then in an aqueous solution of 50 μg/mL GRGDS for 1 h. Nonbound GRGDS was removed by washing twice with PBS buffer solution followed by washing twice with H_2O.

To investigate the cell behavior, 20,000 human dermal fibroblast cells in 1 mL Dulbecco modified eagle medium (DMEM) with 1% fetal bovine serum were seeded on the fibers and then incubated up to 96 h at 37°C and 95% humidity. Cell growth was then investigated using an optical microscope.

RESULTS AND DISCUSSION

The morphology and quality of the fibers strongly depends on the processing parameters (e.g., flow rate or voltage), the ambient parameters (e.g., air humidity or pressure) and the solution parameters.[40] Especially the latter ones influence the interaction of the polymer chains during the electrospinning process and therefore the quality of the electrospun fiber. Furthermore, as sPEEGE is a highly viscous but liquid polymer, the ratio between PCL and sPEEGE does strongly determine the solution parameter and the quality of the formed fibers.

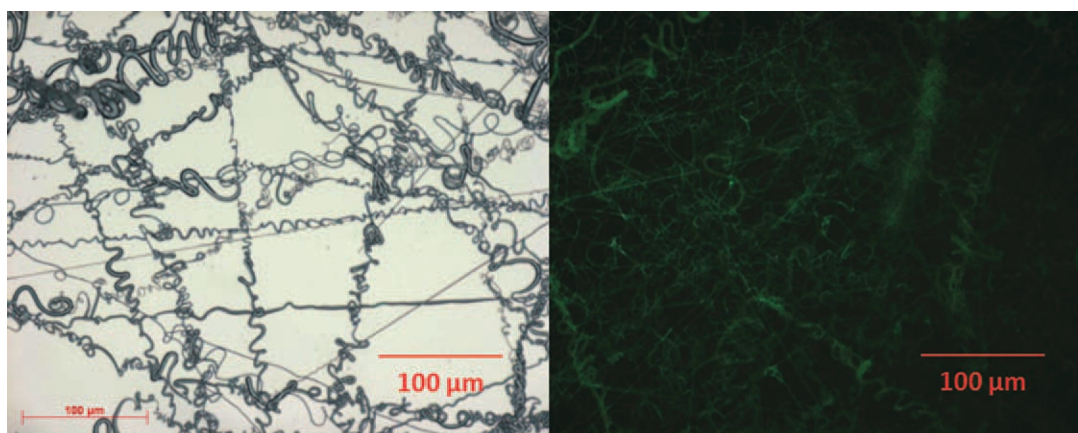

Figure 6 Left: optical microscope picture of fibers from sPEEGE/PCL blend with 25 wt % alkyne-end capped sPEEGE electrospun at an applied voltage of 15 kV and 15 cm distance. Right: fluorescence images of the same fibers after incubation with BODIPY® labeled BSA. [Color figure can be viewed in the online issue, which is available at wileyonline library.com.]

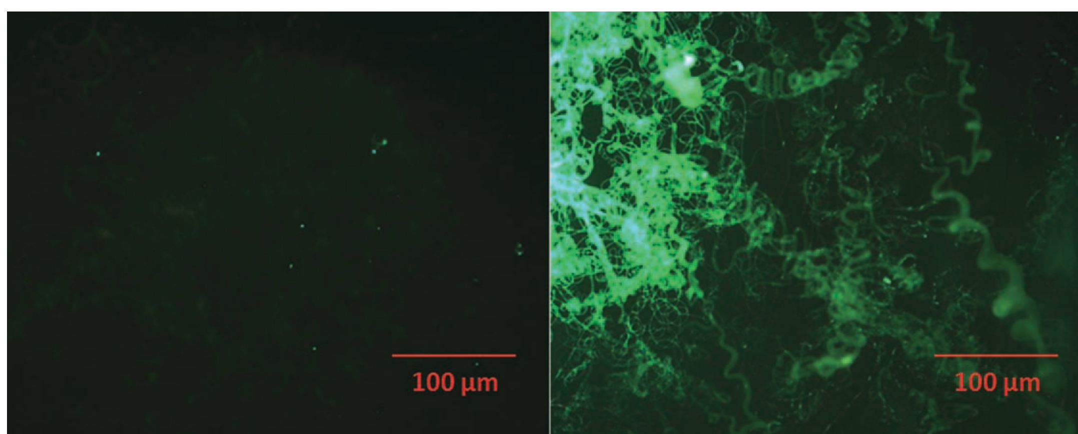

Figure 7 Fluorescence image of fibers from sPEEGE/PCL blends with no specific end groups (left) and alkyne end groups (right) at the sPEEGE after click reaction. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In the first part of this work by variation of the ratio between the polymers the influence on the fiber quality and their ability to minimize protein adsorption is under investigation. In the second part surface reactive nanofibers are prepared using functionalized sPEEGEs followed by conjugation reaction with GRGDS as well as the cell behavior is under research.

Electrospinning of sPEEGE/PCL Blends with different sPEEGE amount

To investigate the influence of the sPEEGE amount on the fiber formation, three different blends with 15, 25, and 35 wt % sPEEGE have been successfully electrospun and compared with pure PCL fibers. The obtained fibers were analyzed by optical microscopy (Fig. 2) and electron microscopy (Fig. 3) as well as by NMR spectroscopy (Supporting Information).

All blends could be easily electrospun and resulted in smooth and homogeneous fibers. Investigation of the composition of the nonwovens by NMR spectroscopy showed good correspondence between the fibers and the spinning solution with slightly less amount of sPEEGE in the fiber.

For investigation of the protein repelling properties of the fibers, the electrospun nonwovens were incubated in a solution of fluorescently labeled bovine serum albumin (BODIPY® BSA), washed thoroughly and then the remaining fluorescence was determined by fluorescent microscopy as shown in Figure 4.

All fibers with different sPEEGE amount show a tremendously decreased fluorescence compared with pure PCL fibers indicating a decreased protein adsorption on fibers. Fibers with 15 wt %, respectively 25 wt % sPEEGE amount showed no detec0 protein adsorption while fibers with 35 wt % showed minor

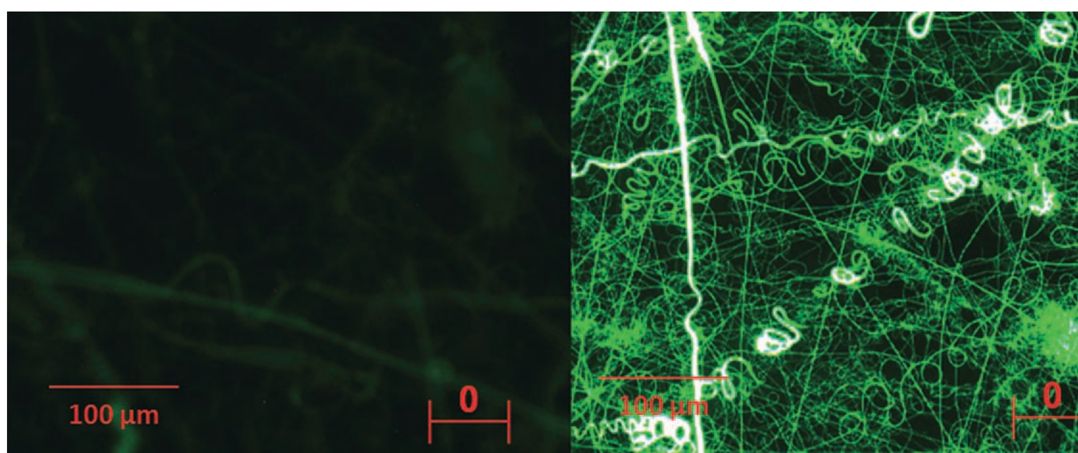

Figure 8 Fluorescence image of fibers from sPEEGE/PCL blends with no specific end groups (left) and vinyl sulfonate end groups (right) at the sPEEGE after incubation with (R)-(-)-4-(3-Aminopyrrolidino)-7-nitro-benzofuran in water. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

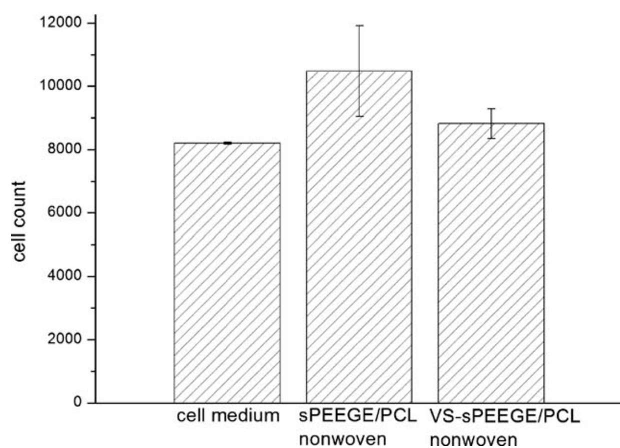

Figure 9 Results of the cytotoxicity assay of the functionalized and nonfunctionalized nonwoven extracts against primary human fibroblasts.

protein adsorption. This might be due to the increased fiber diameter and therefore higher surface area. Because of the lack of protein adsorption on the sPEEGE/PCL electrospun fiber surfaces, we believe that sPEEGE is enriched at the fiber surface. This effect was already observed for different copolymers, e.g., PEG-*b*-PDLLA or PEG-*b*-PCL.[30,31]

Therefore, if sPEEGE is enriched at the surface, the hydrophilicity of the electrospun nonwovens should be significantly raised compared with pure PCL fiber meshes. For quantification of the hydrophilicity, the contact angle of the fiber meshes against water has been determined (Fig. 5).

The addition of 15 wt % sPEEGE decreases the contact angle of the electrospun nonwoven to 32° from 129° for the pure PCL fiber mesh indicating the existence of the hydrophilic sPEEGE on the surface on the fibers. Higher amounts of sPEEGE led to slightly higher contact angles (up to 42°) and therefore decreased hydrophilicity, which is another explanation for the increased protein adsorption on fibers with 35 wt % sPEEGE. In General, the high hydrophilicity of the electrospun fibers seems to be the major reason for the suppression of the protein adsorption.

Electrospun nonwovens with high surface functionality

To obtain surface reactive nonwovens, sPEEGE with alkyne and sPEEGE with vinyl sulfonate end groups had been electrospun. Afterwards, the fiber morphology was investigated by optical microscopy and the protein repelling properties were determined by incubation in BODIPY® BSA followed by fluorescent microscopy. In Figure 6, the results were shown exemplarily for alkyne-functionalized fibers.

For fibers with both alkyne and vinyl sulfonate end groups, nonwovens with homogeneous fibers and reduced protein adsorption were obtained.

The high reactivity of the alkyne functionalized fibers could be proved by a Huisgen-Sharpless-Meldal reaction (often referred to as click-reaction) using an azide-functionalized dye (commercially available Alexa Fluor® 488 azide) under standard click conditions with copper (II) sulfate as catalyst and sodium ascorbate in aqueous solution. Afterwards, the dye has been determined by fluorescence microscopy. The results are shown in Figure 7.

In contrast to fibers produced by using a sPEEGE/PCL blend with no functionality, the functionalized nonwoven does react readily with the azide dye resulting in strongly fluorescent fibers. The usage of the click reaction for further functionalization of electrospun fibers show tremendous advantages, but still there are some drawbacks. As the reaction is highly specific towards azides, no other functional groups can react with the fibers. Furthermore, copper as catalyst still remains an unsolved challenge for click reactions in life Science due to the high cytotoxicity of the catalyst. Recently a new ligation method using vinyl sulfonate terminated polyglycidol reacting readily with different small molecules bearing different functional groups, e.g., amines, in a Michael-type addition reaction has been reported.[21,22]

Nonwovens produced by electrospinning of vinyl sulfonate terminated sPEEGE (VS-sPEEGE) together with PCL should show a high reactivity towards small molecules carrying an amino moiety. This is proved by incubation of the nonwoven in an

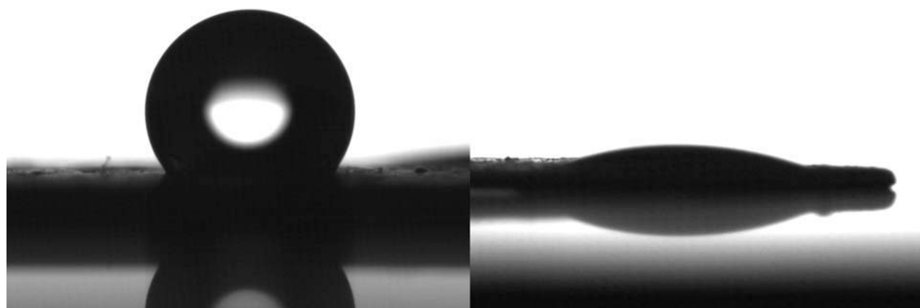

Figure 10 Images of contact angle measurements on electrospun PCL nonwoven (left) and GRGDS-sPEEGE-PCL (85 : 15 wt %) nonwoven (right).

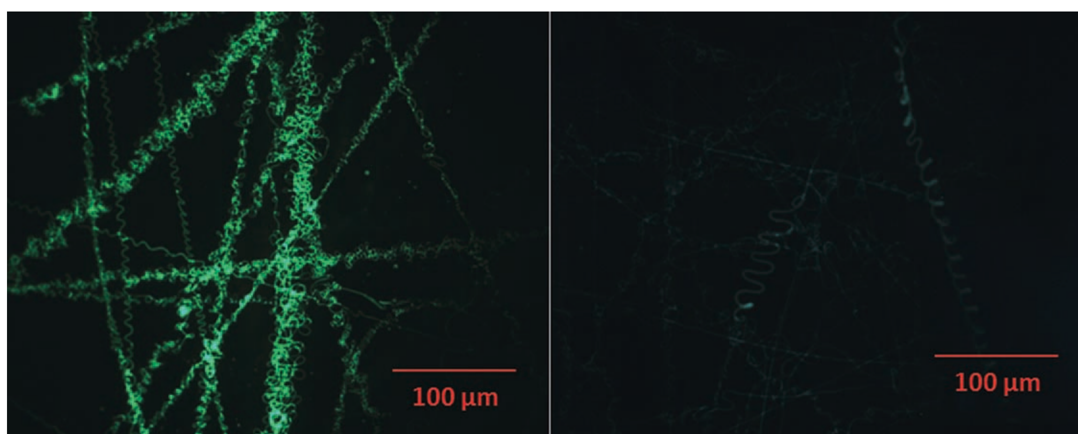

Figure 11 Fluorescence images of PCL (left) and GRGDS-sPEEGE/PCL fiber meshes (right) after incubation with BODIPY® labeled BSA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

aqueous solution of an amino-functionalized dye ((R)-(-)-4-(3-Aminopyrrolidino)-7-nitro-benzofuran) for 1 h, followed by washing and detection of the dye by fluorescence microscopy as shown in Figure 8.

Compared with a nonwoven consisting of non-functionalized sPEEGE, the one containing vinyl sulfonate terminated sPEEGE does show high fluorescence after incubation indicating the conjugate addition of the dye to the nonwoven. In both cases, the electrospun fiber meshes maintain the functionality of the deployed sPEEGE. Together with the minimized protein adsorption of the fibers, this offers a lot of different possible applications for such fiber meshes.

Cytotoxicity assay of functionalized and nonfunctionalized nonwovens

Especially for the application in tissue engineering, it is extremely important that the nonwovens are

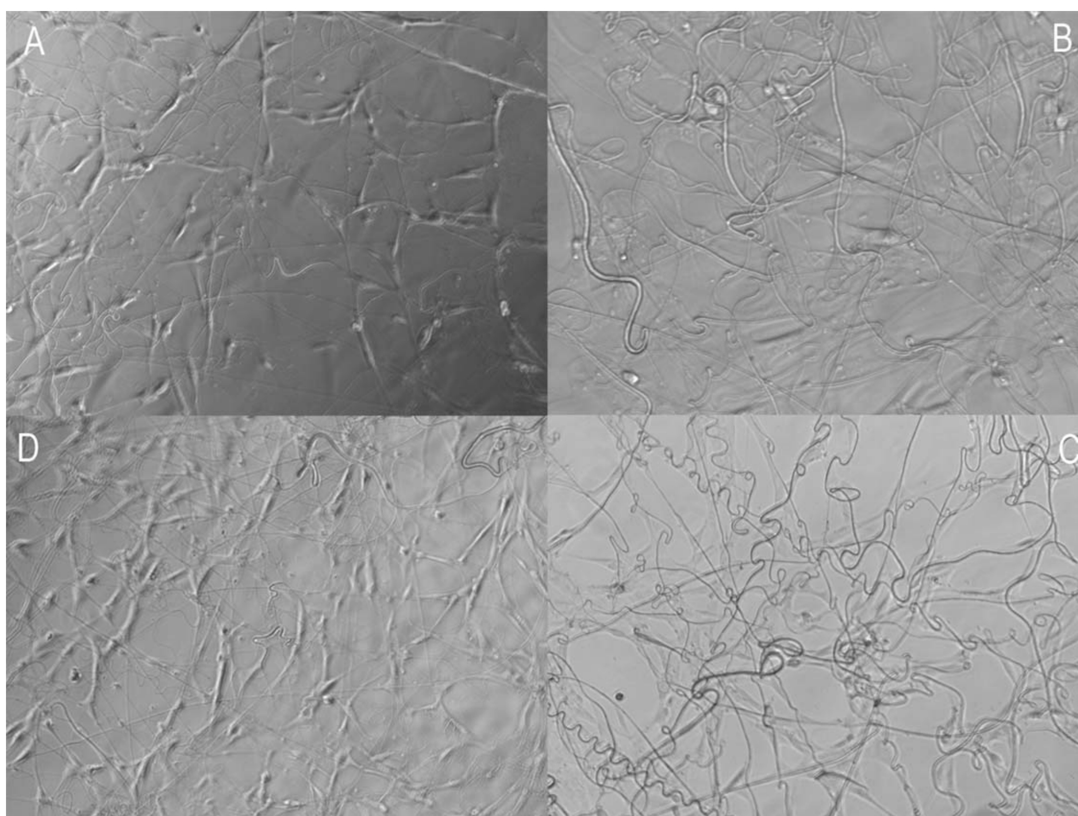

Figure 12 Optical microscope pictures of the growth of human fibroblast cells on electrospun fibers with (A,D) and without (B,C) GRGDS immobilized on the surface after 48 h (A,B), and 72 h (C,D) growth time.

highly biocompatible and do not show any influence on the growth of human cells. This has been investigated by incubation of human fibroblasts with the nonwoven for 48 h. The results of the *in vitro* cytotoxicity assay are shown in Figure 9.

In general, both the extract of the nonwoven containing vinyl sulfonate terminated polyglycidol as well as the nonfunctionalized one, did not induce any toxic effects on the human fibroblast cells, showing a high cell viability.

Biofunctionalization of the electrospun nonwoven and their cell behavior

To control the adsorption of cells on the electrospun fibers, the nonwovens were further functionalized by immobilizing GRGDS on the surface of the fibers. For this purpose, surface reactive fibers based on vinyl sulfonate terminated polyglycidols were incubated in an aqueous solution of GRGDS overnight and then excess of GRGDS had been removed by washing. To investigate the influence of the attached GRGDS on the properties of the electrospun fibers, the contact angle of the nonwoven against water has been determined (Fig. 10).

Furthermore, the unspecific protein adsorption has been investigated by incubation in a solution of BODIPY® BSA, followed by determination of the fluorescence by microscopy (Fig. 11).

Both the results for the contact angle measurements as well as the results for the unspecific protein adsorption of fibers containing GRGDS are comparable to the results of fibers without GRGDS: Therefore, the conjugation of GRGDS at the reactive end groups does not influence the hydrophilic surface properties of the fibers.

To investigate the cell behavior on the nonwovens, both GRGDS-functionalized and nonfunctionalized electrospun nonwovens were incubated with human fibroblasts for up to 72 h. The growth of the cells was determined by optical microscopy as seen in Figure 12.

Only minor cell growth was observed on nonfunctionalized fibers. In contrast, on fibers equipped with GRGDS strong cell adhesion has been observed indicating the successful immobilization of the peptide on the surface of the fiber.

CONCLUSIONS

Electrospun fibers containing high amounts of star-shaped poly(ethoxyethyl glycidyl ether) can be produced using a polymer blend together with poly(ε-caprolactone). Using an applied voltage of 15 kV and a distance of 15 cm, smooth and homogenous fibers can be produced. The obtained electrospun fibers are water insoluble but hydrophilic. It could be shown that, depending on the amount of sPEEGE, the fibers show a significant reduction of the unspecific protein adsorption.

The usage of reactive, end-group functionalized sPEEGE in the polymer blend lead to fibers with high surface reactivity. These fibers can be further linked with small molecules depending on their introduced functionality. All produced fiber nonwovens do not show any influence of the growth of human fibrozytes. Surface reactive electrospun nonwovens were successfully equipped with GRGDS showing an increased cell attachment on the fibers compared with nonfunctionalized ones.

References

1. Navarro, M.; Aparicio, C.; Charles-Harris, M.; Ginebra, M. P.; Engel, E.; Planell, J. A. Adv Polym Sci 2006, 200, 209.
2. Harris, J. M. Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications; Plenum Press: New York, 1992.
3. Harder, P.; Grunze, M.; Dahint, R.; Whitesides, G. M.; Laibnis, P. E. J Phys Chem B 1998, 102, 426.
4. Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. Langmuir 2001, 17, 5605.
5. Groll, J.; Ademovic, Z.; Ameringer, T.; Klee, D.; Moeller, M. Biomacromolecules 2005, 6, 956.
6. Groll, J.; Amirgoulova, E. V.; Ameringer, T.; Heyes, C. D.; Röcker, C.; Nienhaus, G. U.; Moeller, M. J Am Chem Soc 2004, 126, 4234.
7. Groll, J.; Fiedler, J.; Engelhard, E.; Ameringer, T.; Tugulu, S.; Klok, H.-A.; Brenner, R. E.; Moeller, M. J Biomed Mater Res A 2005, 74, 607.
8. Sunder, A.; Hanselmann, R.; Frey, H.; Mülhaupt, R. Macromolecules 1999, 32, 4240.
9. Kainthan, R. K.; Janzen, J.; Levin, E.; Devine, D. V.; Brooks, D. E. Biomacromolecules 2006, 7, 703.
10. Frey, H.; Haag, R. Rev Mol Biotech 2002, 90, 257.
11. Keul, H.; Moeller, M. J Polym Sci Part A: Polym Chem 2009, 47, 3209.
12. Sandler, S. R.; Berg, F. R. J Pol Sci: Part A-1 1966, 4, 1253.
13. Tokar, R.; Kubisa, P.; Penczek, S.; Dworak, A. Macromolecules 1994, 27, 320.
14. Sunder, A.; Mülhaupt, R.; Haag, R.; Frey, H. Adv Mater 2000, 12, 235.
15. Sunder, A.; Mülhaupt, R.; Haag, R.; Frey, H. Macromolecules 2000, 33, 253.
16. Lapienis, G.; Penczek, S. Biomacromolecules 2005, 6, 752.
17. Hans, M.; Gasteier, P.; Keul, H.; Moeller, M. Macromolecules 2006, 39, 3184.
18. Dworak, A.; Baran, G.; Trzebicka, B.; Walach, W. React Funct Polym 1999, 42, 31.
19. Taton, D.; Le Borgne, A.; Sepulchre, M.; Spassky, N. Macromol Chem Phys 1994, 195, 139.
20. Walach, W.; Kowalczuk, A.; Trzebicka, B.; Dworak, A. Macromol Rapid Commun 2001, 22, 1272.
21. Haamann, D.; Keul, H.; Klee, D.; Moeller, M. Macromolecules 2010, 43, 6295.
22. Haamann, D.; Keul, H.; Klee, D.; Moeller, M. Macromolecular Symposia 2010, 296, 1.
23. Agarwal, S.; Wendorff, J. H.; Greiner, A. Polymer 2008, 49, 5603.
24. Dersch, R.; Graeser, M.; Greiner, A.; Wendorff, J. H. Aust J Chem 2007, 60, 719.
25. Greiner, A.; Wendorff, J. H. Angew Chem 2007, 119, 5770.
26. Matthews, J. A.; Wnek, G. E.; Simpson, D. G.; Bowlin, G. L. Biomacromolecules 2002, 3, 232.

27. Zhang, Y.; Ouyang, H.; Lim, C. T.; Ramakrishna, S.; Huang, Z.-M. J Biomed Mater Res Part B: Appl Biomater B 2005, 72, 156.
28. Klinkhammer, K.; Bockelmann, J.; Simitzis, C.; Brook, G. A.; Grafahrend, D.; Groll, J.; Moeller, M.; Mey, J.; Klee, D. J Mater Sci Mater Med 2010, 21, 2637.
29. Agarwal, S.; Wendorff, J. H.; Greiner, A. Macromol Rapid Commun 2010, 31, 1317.
30. Grafahrend, D.; Lleixa Calvet, J.; Salber, J.; Dalton, P. D.; Moeller, M.; Klee, D. J Mater Sci Mater Med 2008, 19, 1479.
31. Grafahrend, D.; Lleixa Calvet, J.; Klinkhammer, K.; Salber, J.; Dalton, P. D.; Moeller, M.; Klee, D. Biotechnol Bioeng 2008, 101, 609.
32. Heffels, K.-H.; Gasteier, P.; Grafahrend, D.; Salber, J.; Dalton, P. D.; Moeller, M.; Groll, J. Proceedings to 2nd Aachen-Dresden International Textile Conference, 2008.
33. Ifkovits, J. L.; Devlin, J. J.; Eng, G.; Martens, T. P.; Vunjak-Novakovic, G.; Burdick, J. A. ACS Appl Mater Interfaces 2009, 1, 1878.
34. de Queiroz, A. A. A.; Bressiani, J. C.; Bressiani, A. H.; Higa, O. Z.; Abraham, G. A. Key Eng Mater 2009,396–398,633.
35. Yi, F.; LaVan, D. A. Macromol Biosci 2008, 8, 803.
36. Torres Vargas, E. A.; do Vale Baracho, N. C.; de Brito, J.; De Queiroz, A. A. A. Acta Biomater 2010, 6, 1069.
37. Torne, C. W.; Christensen, C.; Meldal, M. J Org Chem 2002, 67, 3057.
38. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. Angew Chem 2002, 114, 2708.
39. Klinkhammer, K.; Seiler, N.; Grafahrend, D.; Gerardo-Nava, J.; Mey, J.; Brook, G. A.; Moeller, M.; Dalton, P. D.; Klee, D. Tissue Eng C 2009,15.
40. Fong, H.; Chun, I.; Reneker, D. H. Polymer 1999, 40, 4585.