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Carbohydrate-Decorated PCL Fibers for Specific Protein

Adhesion

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ABSTRACT: Ultra-fine biocompatible fibers decorated with carbohydrates were prepared by electrospinning. Both bulk- and surface- modification approaches have been devised and compared in terms of practicability and grafting density along the fibrous mats. On one hand, bulk-functionalized fibers were prepared by electrospinning of native and galactose-modified PCL polymers. The size and morphology of the resulting fibers was strongly influenced by the sugar-PCL content as observed by electron microscopy. Successful surface modification was evidenced by water contact angle measurements, but a rather low carbohydrate density was attained, as indicated by a colorimetric quantification. On the other hand, efficient and versatile surface-glycosylation was achieved after modification of azido-functionalized electrospun fibers by CuAAC click-chemistry. Homogeneous ultra-fine PCL fibers, decorated with azide functions, have been made highly hydrophilic upon coupling with propargyl-mannose and propargyl-galactose derivatives. Specific adhesion of lectins further attested good bioavailability of the

carbohydrate surface-residues, suggesting interesting perspectives of the latter approach in the development of bioactive materials for tissue engineering.

KEYWORDS: electrospinning, fibers, polycaprolactone, click chemistry, carbohydrates, lectins

1. INTRODUCTION

Electrospun nonwoven scaffolds represent advantageous materials for medical and bioengineering purposes due to their high surface area and small diameter fibers.^{1,2} As a consequence, electrospun fibers are often employed for wound dressing,³ drug delivery,⁴ sutures or tissue engineering.⁵⁻⁷ The application diversity of these fibrous mats is, however, often conditioned by their initial physico-chemical properties. Poly(ϵ -caprolactone) (PCL), as biodegradable and biocompatible polymer with low cytotoxicity, has been widely adopted as synthetic biopolymer for medical applications.⁸⁻¹⁰ Modification of PCL-based materials have also been reported in order to improve their hydrophilic properties and to achieve a friendly interface for living cells. Recent works emphasized that surface modification of electrospun fibers with chemical functions or biomolecules strongly influences protein binding and, therefore, cell-material interactions.^{11,12} Wet chemical methods, due to their simplicity and availability, have been often adopted for PCL fibers' surface modification. Mobarakeh et al.⁶ reported the surface modification of PCL fibers with Matrigel™ (a soluble sterile extract rich in laminin, collagen IV, fibronectin and heparin sulphate proteoglycans) by partial alkaline hydrolysis of the scaffold and subsequent covalent amide bond formation. Alternatively, gelatin-functionalized PCL film surfaces were developed by a “grafting-from” polymerization approach. Such modification required prior chemical activation of the PCL chains by aminolysis.¹⁰ Click chemistry has

recently received significant attention to modify material surfaces, films¹³ or fibers¹⁴, in order to generate a specific functionality. For example, cellulose surface modification has been efficiently achieved by means of thiol-ene reaction¹⁵ or azide-alkyne cycloaddition¹⁶ under heterogeneous conditions. Xu et al.¹⁷ highlight click chemistry as a convenient method for the synthesis of saccharide-terminated poly(ϵ -caprolactone)s as potential drug carriers.

Carbohydrates perform numerous roles in living organisms. They serve for the storage of energy, as structural components, but they are also involved in diverse cellular processes, enabling communication, proliferation, differentiation. Functionalization of polymers such as polyolefins with sugars has been explored periodically as a possible way to improve their biodegradability.¹⁸ Over time, carbohydrate-conjugated polymers have attracted attention for their biomedical applications. R. Gentsch et al.¹⁹ investigated the surface functionalization of PCL/PPfpMA fibers with monosaccharides and showed that these functionalized fibers trigger specific interactions with antigen-presenting cells, e.g., macrophages. K.-N. Chua et al.²⁰ demonstrated that galactose-conjugated nanofiber meshes promote cell-substrate interaction, suggesting potential scaffold application in liver tissue engineering. Equally, sugar-conjugated polymers were employed for immobilization of proteins,²¹ as cell's surface mimics,²² cell adhesion as well as for many pharmacological and biomedical applications.²³

Yet, only recently, attention has been paid to overcome the non-specific protein adsorption on electrospun fibers.^{12,24} A significant step towards specific protein adsorption using biofunctionalized polymeric fibers was made by D. Grafahrend and coworkers. They highlighted the importance of the polymer choice for electrospinning as well as the choice of active species at the fibrous surface.²⁴⁻²⁸ In the present study, we have investigated a versatile approach for surface functionalization of electrospun fibrous mats that would

allow efficient conjugation of carbohydrates, proteins and other biomolecules towards specific protein adhesion. Two different strategies have been devised. In a first approach, bulk fiber's glycosylation has been carried by electrospinning carbohydrate-modified α,ω -poly(ϵ -caprolactone)-diol M_n 2000 g mol⁻¹ (PCL₂-Gal) and native high molecular weight poly(ϵ -caprolactone) M_n 70000-90000 g mol⁻¹ (PCL₈₀). The second strategy relied on the surface functionalization by click chemistry of recently reported electrospun azido-fibers.²⁹ Hydrophilicity of the resulting fibers as well as bioavailability of the carbohydrates were evaluated by contact angle measurements and enzyme-linked lectin assays, respectively. A debate followed about the optimal path for obtaining carbohydrates-decorated ultra-fine fibers by comparing the bulk- and the surface- functionalization processes.

2. EXPERIMENTAL SECTION

Materials. Poly(ϵ -caprolactone) (PCL₈₀) M_n 70000-90000 g mol⁻¹, copper(II) sulfate pentahydrate (CuSO₄·5H₂O), sodium ascorbate, calcium chloride (CaCl₂), magnesium chloride (MgCl₂), manganese chloride (MnCl₂), TWEEN 20, phosphate buffered saline (PBS) 10x concentrate (pH=7.2-7.6 at 1:10 dilution), β -galactosidase (*Aspergillus oryzae*), 3-amino-9-ethylcarbazole (AEC) chromogen kit, Concanavalin A-peroxidase conjugate (HRP-ConA), *Arachis hypogaea*-peroxidase conjugate (HRP-PNA) and all organic solvents were purchased from Sigma Aldrich and used without further purification. α,ω -Poly(ϵ -caprolactone)-diol (PCL₂), M_n 2000 g mol⁻¹ (Sigma), was recrystallized from diethyl ether prior to use.

Electrospinning. Electrospinning process was performed with a horizontal setup – a 5 mL syringe was filled with polymer solution slightly above the overlap concentration and placed on the syringe pump with the blunt 21-gauge needle attached. Flow rate was controlled by a syringe pump (KD Scientific series 200, USA) in the range from 0.01 to

0.03 mL/min. Fibers were collected directly on aluminum foil. The distance between needle tip and collector was fixed at 15 cm. Applied voltage (dual high voltage power supply, ± 30 kV, iseq GMBH Germany) ranged from 11 to 15 kV. All experiments were done at room temperature. The relative humidity noted was between 30 and 55%. For the bulk-functionalization purposes, PCL₂-Gal was blended with PCL₈₀ in ratio 20:80 and 40:60 in dichloromethane/methanol (DCM/MeOH 4/1 v/v) solvent mixture and electrospun. For the surface-functionalization, f-PCL-N₃ -20, -40 and -60 were prepared as reported previously.²⁹ Briefly, PCL₈₀ and PCL₂-N₃ were blended, using the same solvent mixture, in order to obtain 20, 40 or 60 wt% of PCL₂-N₃ in the blend and electrospun.

Synthesis of α,ω -Galactoside-poly(ϵ -caprolactone) (PCL₂-Gal) and its Electrospinning with PCL₈₀. α,ω -Azide-poly(ϵ -caprolactone) (PCL₂-N₃) was prepared as reported previously in the literature.²⁹ PCL₂-N₃ (1.5 g, 0.72 mmol) was then involved into click reaction with propargyl- β -D-galactoside (see Supporting Information for its detailed synthesis) (1.57 g, 7.2 mmol, 5 equiv. per azide group) in tetrahydrofuran/water (1/1 v/v) solvent mixture (100 mL) at 40 °C for 48 h in the presence of CuSO₄·5H₂O (0.36 g, 1.44 mmol, 1 equiv. per azide group) and sodium ascorbate (0.28 g, 1.44 mmol, 1 equiv. per azide group). Reaction mixture was concentrated in rotavapor, dissolved in 2 mL of *N,N*-dimethylformamide (DMF) and precipitated in 40 mL of toluene. The solid was filtrated and dried under vacuum to provide PCL₂-Gal (1.6 g) in 94% yield. PCL₂-Gal was then blended with PCL₈₀ in 20:80 and 40:60 w/w ratio in DCM/MeOH (4/1 v/v) solvent mixture and electrospun. Resulting fibers: f-PCL₂₀-Gal_B and f-PCL₄₀-Gal_B stand for a blend of bulk-functionalized PCL₂-Gal and PCL₈₀ polymers in ratios 20/80 and 40/60, respectively.

Surface-Grafting of Monosaccharides onto the f-PCL-N₃ Fibers Using Heterogeneous Click Chemistry. Monosaccharides, propargyl- α -D-mannoside (see Supporting Information for detailed synthesis) and propargyl- β -D-galactoside were conjugated onto

the surface of f-PCL-N₃-20, -40, and -60 fibers using CuAAC click chemistry coupling. Resulting fibers were labeled as f-PCL₂₀-Gal_S, f-PCL₄₀-Gal_S, and f-PCL₆₀-Gal_S for galactose surface-functionalized, while f-PCL₂₀-Man_S, f-PCL₄₀-Man_S, and f-PCL₆₀-Man_S correspond to the mannose surface-functionalized fibers from f-PCL-N₃-20, -40, and -60 fibrous mats, respectively. Click reaction between propargyl-monosaccharides and azido-fibers in heterogeneous phase is described on the example of f-PCL₂₀-Gal_S preparation. f-PCL-N₃-20 fibers (20 mg) were put in a microcentrifuge tube containing 4 mL of distilled water, and then 54.7 μL (10 equiv. per azide group on the surface, as estimated by the ninhydrin assay²⁹) of 0.1 M aqueous solution of propargyl-β-D-galactoside, 21.9 μL CuSO₄·5H₂O in distilled water (0.1 M, 4 equiv. per azide group) and 21.9 μL of sodium ascorbate in distilled water (0.1 M, 4 equiv. per azide group) were added. Reaction mixture was stirred for 24 h at room temperature and then fibers were thoroughly washed with distilled water. f-PCL₄₀-Gal_S and f-PCL₆₀-Gal_S were prepared from f-PCL-N₃-40 and f-PCL-N₃-60, respectively, following the same procedure while keeping the same molar ratio. f-PCL₂₀-Man_S, f-PCL₄₀-Man_S and f-PCL₆₀-Man_S functionalized fibers were prepared similarly as f-PCL-Gal_S fibers by replacing propargyl-β-D-galactoside with propargyl-α-D-mannoside.

Characterization. *Infrared Spectroscopy.* ATR-FTIR spectra of the fibers were recorded in the transmission mode on a Perkin-Elmer 1720X FTIR instrument using single reflection diamond ATR.

NMR Spectroscopy. ¹H and ¹³C NMR spectra were obtained with a Bruker AVANCE 400 MHz with 5mm QNP probe at 298 K.

Viscosity. Viscosity measurements of polymer solutions were done using HAAK MARS III controlled-stress rheometer equipped with cone-plate geometry (titan cone, characterized by a diameter of 60 mm, 1° angle and 53 μm gap). Flow measurements were

performed at 10 °C and an anti-evaporation system was used to reduce the solvent evaporation.

Electron-Microscopy Measurement. Field Emission Scanning Electron Microscope (FESEM ZEISS ULTRA55) was used for observing the morphology of the fibers at 1 kV accelerating voltage, 5 mm of working distance and at magnifications of 500, 1000 and 2000 times. All samples were sputter coated with Pt of 1 nm thickness. Average fiber diameters of the electrospun fibers, were obtained as a mean value of 150 different diameters measured by ImageJ software.

Water Contact Angle (WCA). WCA measurements were done in the sessile-drop mode at 20 °C using Dataphysics Instruments Gmb goniometer. Nonwoven fibrous meshes were fixed onto an object slide using adhesive tapes at the sides of the sample. The volume of the applied droplet is 1 μ L. The resulting value of each measurement represents the average value of the left and the right contact angle. The images of the water droplet on electrospun fiber meshes and the corresponding contact angle were recorded from droplet deposition onto the fibers until its stabilization.

Dynamic Light Scattering (DLS). DLS measurements were performed using a ALV/CGS-8F goniometer, equipped with a linearly polarized He/Ne laser ($\lambda=632.8$ nm, P=35 mW) and an ALV multiple τ correlator with a 125 ns initial sampling time. The unfiltered mixtures were measured at 25 °C for a typical counting time of 200 s at a scattering angle of 90°. The size distributions were obtained with the CONTIN analysis of the autocorrelation functions and particularly with the Stokes-Einstein equation as detailed elsewhere.^{30,31} The viscosity and refractive index of the DCM/MeOH 4/1 solvent mixture alone are calculated to be 0.466 cP and 1.398, respectively.³²⁻³⁴

Carbohydrate quantification. Fiber surface carbohydrates were quantified by the Dubois assay.³⁵ This assay was carried out under heterogeneous conditions directly on the fibers

for surface-functionalized ones. For the bulk-modified fibers, Dubois assay was carried out on the supernatant after enzymatic hydrolysis of the surface carbohydrates.

Quantification of the surface carbohydrates on f-PCL-Gal_S and f-PCL-Man_S. Sugar-decorated fibers (2 mg) were dispersed in 100 μ L of distilled water and 100 μ L of phenol solution (5 w/v %) was added followed by addition of 1000 μ L of 96% of sulfuric acid. The solution was vigorously agitated for 15 min at room temperature and absorbance at 490 nm was measured with UVIKON 810 UV-vis spectrophotometer. The concentration of sugar was determined by reference to a calibration curve with galactose as a standard for f-PCL-Gal_S fibers and mannose as a standard for f-PCL-Man_S fibers.

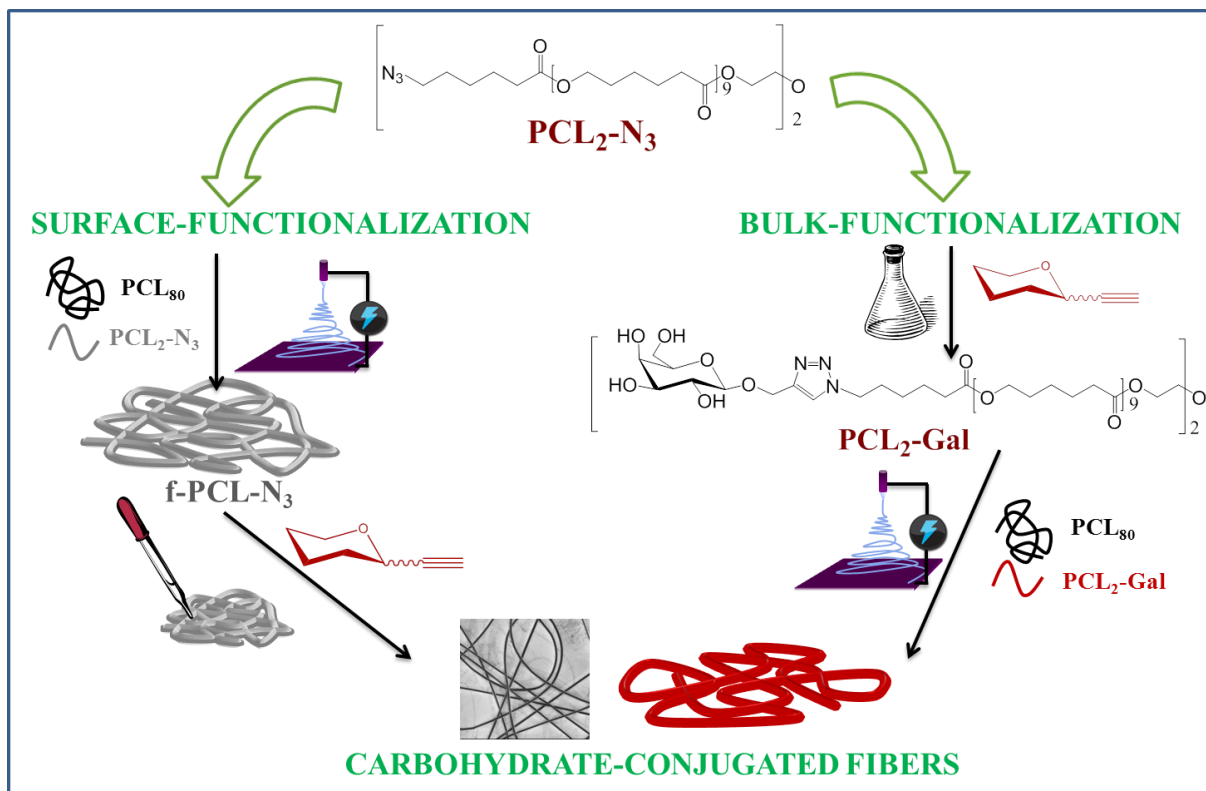
Quantification of surface carbohydrates on f-PCL-Gal_B. f-PCL-Gal_B fibers (4 mg) were put in a microcentrifuge tube containing 500 μ L acetate buffer (0.2 M, pH=4.50) and carbohydrate groups were hydrolyzed by a large excess of β -galactosidase from *Aspergillus oryzae* (Sigma) (25 μ L, 200 units/mL in PBS 1:10 dilution), at 30 °C with gentle stirring. Quantification of carbohydrates released from the fiber surface was achieved by standard Dubois assay at different times (4, 24, 48 and 72 h) on 50 μ L of supernatant.

Enzyme-Linked Lectin Assay (ELLA). ELLA test was done to determine the presence and bioavailability of the carbohydrates on the fiber surface. Horseradish peroxidase-labeled lectin (HRP) from *Canavalia ensiformis* (HRP-ConA, mannose-binding lectin) was dissolved in PBS (1:10 dilution) in order to obtain 250 mg/mL solution. Horseradish peroxidase-labeled lectin from *Arachis hypogaea* (HRP-PNA, galactoside-binding lectin) was dissolved in PBS (1:10) to obtain 200 mg/mL solution. Carbohydrate bulk- and surface-functionalized fibers, f-PCL-Gal_B and f-PCL-Gal_S as well as native fibers, f-PCL₈₀, were placed separately in screw-capped test tubes and left for 5 min at room temperature in PBS (1:10 dilution, pH=7.2-7.6) containing 2 v/v% of TWEEN 20 for blocking extra

binding sites. The fibers were washed in PBS (1:10 dilution) and then put in a 1 mL of fresh PBS (1:10 dilution) containing 25 μ L of HRP-PNA solution (200 μ g/mL), with 1 mM MnCl₂, 1 mM MgCl₂ and 1 mM CaCl₂ for 16 h at 20 °C. Fibers were then thoroughly washed with PBS (1:10 dilution) and then put in 4 mL of deionized water where 2 drops of (2.5 M, pH=5.0) acetate buffer, 1 drop of AEC Chromogen Sigma (3-amino-9-ethylcarbazole), a perceptible peroxidase substrate, provides red chromogen deposition on lectin-modified surfaces) and 1 drop of 3% hydrogen peroxide were added. After 10-15 minutes, the reddish coloration of the f-PCL-Gal_B and f-PCL-Gal_S fibers was observed. Similar procedure was followed for f-PCL-Man_S fibers with the difference of the lectin used. For α -mannose recognition, 25 μ L of HRP-ConA solution (200 μ g/mL) was used.

3. RESULTS AND DISCUSSION

The present study was focused on the functionalization of electrospun fibers with carbohydrates, by bulk and surface modification approaches (Scheme 1), and on the evaluation of their protein adhesion properties. Bulk glycosylated fibers were envisaged by electrospinning of galactosylated poly(ϵ -caprolactone) (PCL₂-Gal) and bare PCL₈₀ while surface-modified fibers were considered by click chemistry conjugation of the sugar groups onto electrospun azido-PCL fibers.



Scheme 1. Schematic representation of the bulk- and surface-functionalization processes towards carbohydrate-decorated fibers

3.1. Bulk Functionalization of PCL Electrospun Fibers

Prior to electrospinning, PCL_2 chains were activated at both ends by azido groups as recently reported in the literature²⁹ and functionalized with galactosyl ligands through copper-catalyzed Huisgen cycloaddition. Successful formation of $\text{PCL}_2\text{-Gal}$ was confirmed by mass spectrometry and by ^1H NMR with characteristic signals of the triazolyl group and of the sugar anomeric proton at 8.03 and 4.81 ppm respectively (Figure 1).

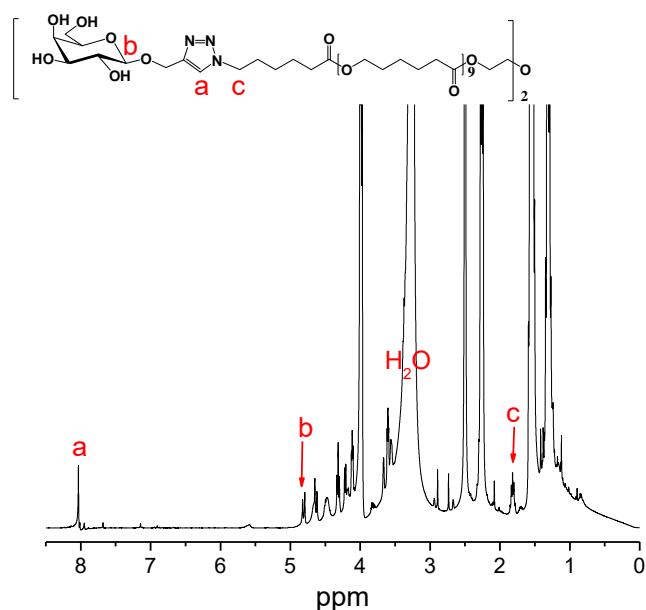


Figure 1. ^1H spectrum of the $\text{PCL}_2\text{-Gal}$ in $\text{DMSO-}d_6$

Electrospinning, in dichloromethane/methanol (DCM/MeOH 4/1 v/v), of 20:80 and 40:60 (wt/wt) mixtures of $\text{PCL}_2\text{-Gal}$ and PCL_{80} respectively afforded f- $\text{PCL}_{20}\text{-Gal}_B$ and f- $\text{PCL}_{40}\text{-Gal}_B$ fibrous mats. DCM is a good solvent for poly(ϵ -caprolactone); volatile and thus advantageous for electrospinning purposes. However, in order to increase the conductivity of the electrospinning solution and solubility of monosaccharides, a small amount of conducting solvent (herein methanol) is usually used.³⁶

Field Emission Scanning Electron Microscopy (FE-SEM) observations showed rather interesting fiber-diameter trend (Figure 2, A-C). While the average diameter of electrospun PCL_{80} fiber was 591 nm, it increased to 1.1 μm for f- $\text{PCL}_{20}\text{-Gal}_B$ and reached 2.4 μm for f- $\text{PCL}_{40}\text{-Gal}_B$ fibers. Concurrently, the increasing content of $\text{PCL}_2\text{-Gal}$ reduced the ability to electrospin the blend and led to heterogeneous fiber diameters (Figure 2D). Water contact angles (WCA), represented as insets in Figure 2, showed significant decrease from 130° to 90° , for f- PCL_{80} and f- $\text{PCL}_{20}\text{-Gal}_B$ fibers, respectively. These findings support the presence of galactose groups on the surface of the fibers. Unfortunately, f- $\text{PCL}_{40}\text{-Gal}_B$ fibers did not

allow precise contact angle measurements because of inhomogeneous layer deposition resulting from a poor electrospinnability. Nevertheless, surface carbohydrates of bulk-functionalized fibers were further quantified by colorimetric assay. f-PCL₂₀-Gal_B and f-PCL₄₀-Gal_B fibers were exposed to enzymatic treatment with a β -galactosidase (*Aspergillus oryzae*) and the concentration of sugar released in the supernatant was determined by the Dubois assay. Surprisingly, a rather small amount (2-3 wt%) of the initial galactose content was found to be present on the fiber's surface. To exclude possible degradation of carbohydrates during the electrospinning process, a total sugar analysis in the fibers has been carried out and confirmed that about 97% of the galactose initially introduced was confined in the fiber's core.

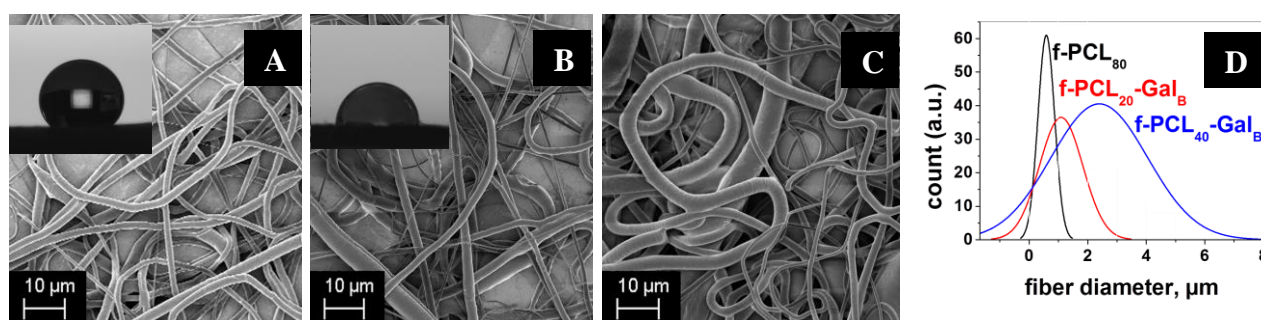


Figure 2. (A-C) FE-SEM images of Pt-coated: (A) f-PCL₈₀ non-derived fibers, (B) f-PCL₂₀-Gal_B and (C) f-PCL₄₀-Gal_B fibers and (D) graphical representation of their fiber diameter distributions. The insets A and B represent the water sessile drops onto the PCL₈₀ and f-PCL₂₀-Gal_B fibers, respectively.

The modest surface functionalization of f-PCL-Gal_B fibers as well as their large diameter distributions opened the question whether this trend is related to inter-, intra-molecular interactions or rather polymer-solvent interactions? To interpret these results, dynamic light scattering (DLS) and viscosity measurements were carried out. On the Figure 3, representing the DLS size distributions, objects with a radius of few tens of

micrometers are observed for the solvent mixture DCM/MeOH 4/1 alone. It alludes to the emulsion character of the two partially miscible solvents, with MeOH droplets dispersed in the DCM medium. Upon addition of PCL₂-Gal in the solvent mixture, an additional pic at $R_h \sim 350$ nm is observed, indicating the formation of PCL₂-Gal aggregates. The DLS size distribution shown on the Figure 3 is mass-weighted; thus larger objects seem to be more present in the solution. When considering the number of particles in solution (see Supporting Information), one could realize that polymers aggregates are in fact one million times more abundant than MeOH droplets.

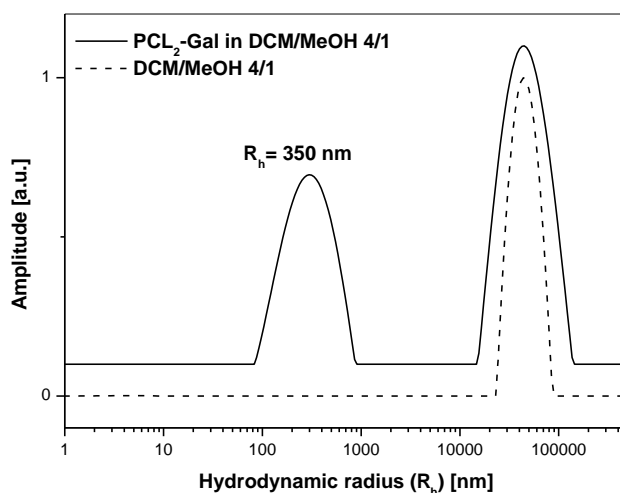


Figure 3. Size distribution of DCM/MeOH 4/1 solvent mixture alone (----) and with PCL₂-Gal dissolved in it (—), at 90°

Viscosity measurements were performed on polymer solutions of PCL₈₀ (8 wt%) and on blends PCL₈₀/PCL₂, PCL₈₀/PCL₂-N₃ and PCL₈₀/PCL₂-Gal at ratio of 80:20 and total polymer concentration of 10 wt%. Figure 4 shows the viscosity of polymer solutions as a function of the shear rate. PCL₈₀ and blends of PCL₈₀/PCL₂ and PCL₈₀/PCL₂-N₃ are Newtonian in the shear-rate range explored. As expected, the viscosity of blends is higher than the pure PCL₈₀. The slight decrease of PCL₈₀/PCL₂-N₃ viscosity compared to that of PCL₈₀/PCL₂ could be assigned to a decrease of the density of intermolecular hydrogen

bonding induced by the presence of N_3 groups. However, the flow behavior of the sugar-conjugated PCL_{80}/PCL_2 -Gal solution is extremely different, exhibiting a significant shear-thinning behavior at low shear rates.

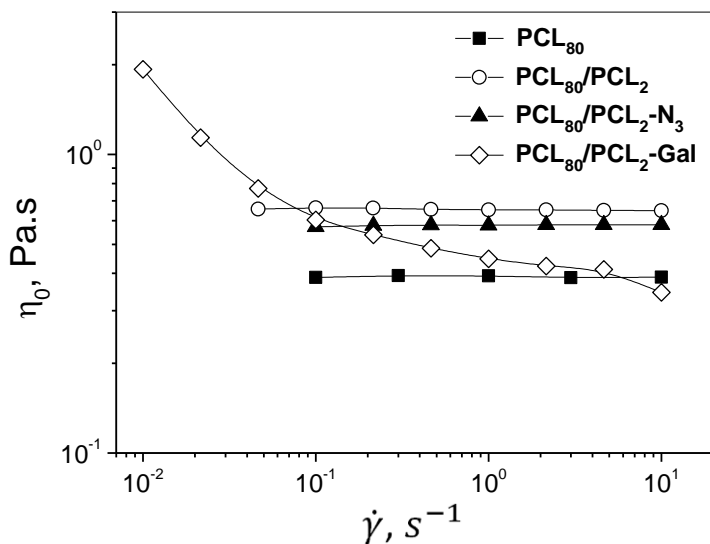


Figure 4. Zero-shear viscosity/shear rate profiles for polymer mixtures of 8 wt % PCL_{80} and: (■) without PCL_2 , (○) 2 wt % of PCL_2 , (▲) 2 wt % PCL_2-N_3 and (◇) 2 wt % of PCL_2-Gal

Accordingly, it was hypothesized that the amphiphilic structure of PCL_2-Gal might lead to the formation of a transient network of PLC chains. The presence of inter-chain complexes could then induce the steric interactions responsible for the significant increase in the solution viscosity and the polymer fibers' diameter, as already observed by Yu et al.³⁷ with a mixture of phosphatidyl choline (PC) surfactant and polyvinylpyrrolidone (PVP) in chloroform. Indeed, in the solvent system of DCM (polar, aprotic, good solvent for PCL) – MeOH (polar, protic, non-solvent for PCL but good solvent for galactose) (4/1), galactosyl units of PCL_2-Gal might tend to aggregate inside the methanol micro-emulsions, forming aggregated galactose domains in MeOH and PCL chain domains in

DCM, as schematically represented in the Figure 5. Gentsch et al.³⁸ already observed the aggregation phenomenon of the low-molecular-weight peptide-PLLA amphiphile in chlorinated solvents. As a consequence, most of the galactose content is certainly confined in the core of the fibers to minimize interaction with DMC thus explaining their irregular morphologies and micrometric diameters as observed by FE-SEM. Enzyme hydrolysis and water-contact-angle tests also go in behalf of such hypothesis, confirming that the sugar density on the fiber's surface is rather limited.

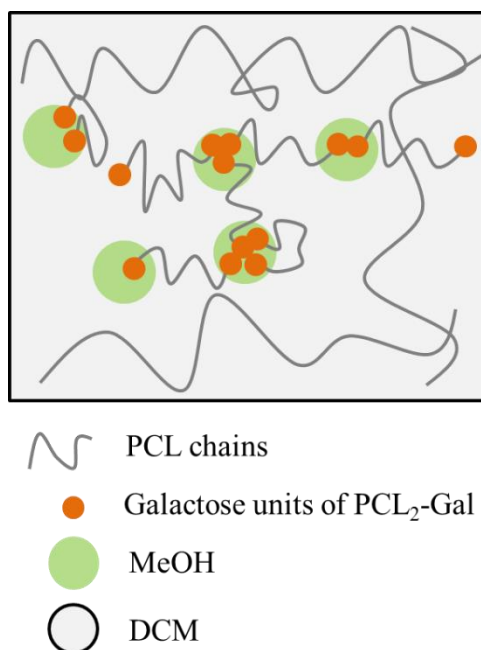


Figure 5. Schematic representation of the PCL₂-Gal/PCL₈₀ polymer organization in the DCM/MeOH 4/1 solvent mixture

In order to reduce or avoid the carbohydrate confinement in the fibers during electrospinning, alternative solvent systems should be employed. However few of the usual solvents combine *i)* a good solubility of both the carbohydrate and the PCL parts and *ii)* a good processing regarding electrospinning. Hence, an alternative approach to draw highly surface-decorated PCL fibers was further investigated by means of selective surface modification.

3.2. Surface-Functionalization of the f-PCL-N₃ Fibers

In a recent work we reported a simple and elegant way to obtain ultra-fine surface-decorated azido-fibers.²⁹ Briefly, using a classical electrospinning setup, a blend of azide-functionalized low-molecular-weight PCL₂-N₃ and non-derived PCL₈₀ in DCM/MeOH 4/1 solvent mixture was electrospun. The high electric field induced migration of the azide group to the surface of the fibers. These findings opened exciting perspectives towards versatile surface functionalization of the fibers by click chemistry.

Herein, we investigated the effectiveness of such surface functionalization with monosaccharides – galactose (Gal) and mannose (Man) – to access to new biomaterials with protein adhesion capacities. f-PCL-N₃-20, -40, and -60 fibers were functionalized by heterogeneous click chemistry with propargyl- α -D-mannoside and propargyl- β -D-galactoside affording f-PCL₂₀-Mans, f-PCL₄₀-Mans and f-PCL₆₀-Mans, and f-PCL₂₀-Gals, f-PCL₄₀-Gals and f-PCL₆₀-Gals, respectively. ATR-FTIR spectra of the azido fiber f-PCL-N₃-20 and its galactosylated counterpart f-PCL₂₀-Gals are illustrated in Figure 6. The presence of sugars on the fibers is evidenced by the large peak at 3300 cm⁻¹ assigned to stretching vibrations of hydroxyl groups as well as vibrations of -N-H at 3744 cm⁻¹. A peak at ~1642 cm⁻¹, corresponding to -C-O- stretching groups of monosaccharide, absent in native PCL₈₀, was also noted. The characteristic peak of azide groups, at 2100 cm⁻¹, indicates some remaining azido groups on the surface as well as inside the fibers after the sugar coupling.

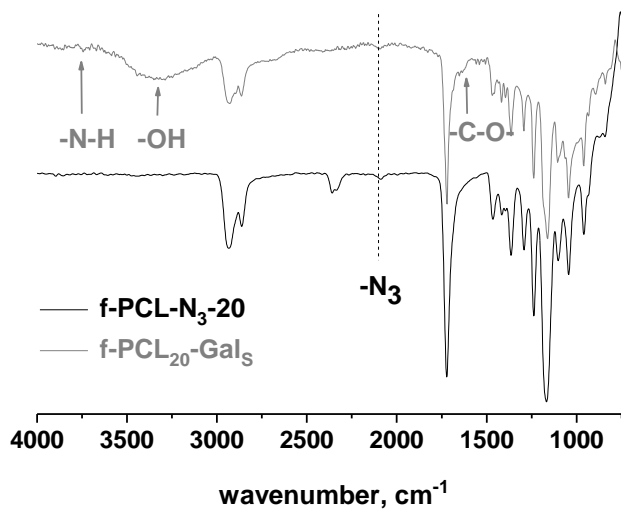


Figure 6. ATR-FTIR spectra of f-PCL-N₃-20 (black line) and f-PCL₂₀-Gal_S (grey line) fibers

The sugar content introduced on the surface of the f-PCL-Gal_S and f-PCL-Man_S fibers was determined by the Dubois phenol-sulfuric acid assay.^{18,39} (see Table S1 Supplementary Information). As one would expect, the carbohydrate density at the surface increased with the amount of available azide groups in the f-PCL-N₃ fibers. Interestingly, it was however observed that the overall coupling yield remained constant, about 20 wt%, for both galactose- and mannose- surface-functionalized fibers. In comparison with the bulk functionalization approach where 2-3 wt% only of the initial sugar amount was displayed at the surface of the fibers, click chemistry modification of azido-decorated fibers allows significantly higher functionalization with 20 wt% of grafted carbohydrate.

Wettability and hydrophilicity of the surface-functionalized f-PCL-Gal_S and f-PCL-Man_S fibers were investigated using dynamic water-contact-angle measurements. As shown in Figure 7, the contact angle of f-PCL-Gal_S fibers is decreasing over time. In addition, the soaking rate of f-PCL-Gal_S fibrous membranes increased together with the galactose content at their surface. While the WCA of f-PCL₂₀-Gal_S reached zero in about

60 s, f-PCL₆₀-Gal_S fibers' WCA attained 0° value in less than 3 s. The gradual difference in the soaking rate was more accentuated for f-PCL-Man_S samples where complete wettability was reached in 40, 12 and 7 s for f-PCL₂₀-Man_S, f-PCL₄₀-Man_S and f-PCL₆₀-Man_S fibers, respectively. These results, again support the efficient glycosylation of the fiber's surface. In addition, this glycosylation allowed to turn a hydrophobic material into a highly hydrophilic one. This point is very important in perspective of biological applications since one could expect hydrophilic materials to be more biocompatible and to favor interactions with proteins and cells.

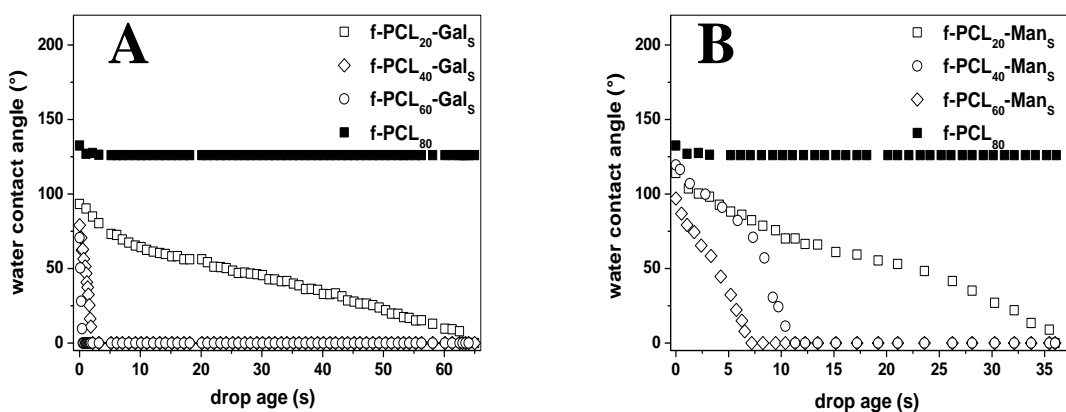


Figure 7. Dynamic water contact angles of (A): (□) f-PCL₂₀-Gal_S, (◇) f-PCL₄₀-Gal_S, and (○) f-PCL₆₀-Gal_S and (B): (□) f-PCL₂₀-Man_S, (◇) f-PCL₄₀-Man_S, and (○) f-PCL₆₀-Man_S surface-functionalized fibers together with (■) f-PCL₈₀ as a reference

Bioavailability of carbohydrates was next investigated by enzyme-linked lectin assay (ELLA). Galactose and mannose-decorated fibers were respectively exposed to solutions of Concanavalin A-peroxidase conjugate (HRP-ConA) and *Arachis hypogaea*-peroxidase conjugate (HRP-PNA). Otman et al.⁴⁰ reported specific recognition of α -D-mannose at the surface of polymeric nanoparticles by ConA, demonstrating that mannose groups, conjugated to the poly(ϵ -caprolactone), could bind the lectin. In this study, upon staining

with the AEC chromogenic substrate, both f-PCL-Gals and f-PCL-Mans fibrous mats exhibited a positive coloration in presence of HRP-PNA and HRP-ConA lectins respectively (Figure 8). f-PCL-Mans fibers showed a very intense red color while f-PCL-Gals staining was less pronounced (see Figure 8). Such a difference between ConA and PNA binding efficiencies is not unexpected and Wu et al.⁴¹ previously observed that PNA lectin binds more strongly to lactose than to galactose, suggesting that this lectin needs a longer arm-spacer for a better carbohydrate recognition. Nonetheless, the control samples with non-functionalized f-PCL-N₃ fibers showed no significant coloration under the same treatment, demonstrating that ELLA labeling is highly carbohydrate-specific.

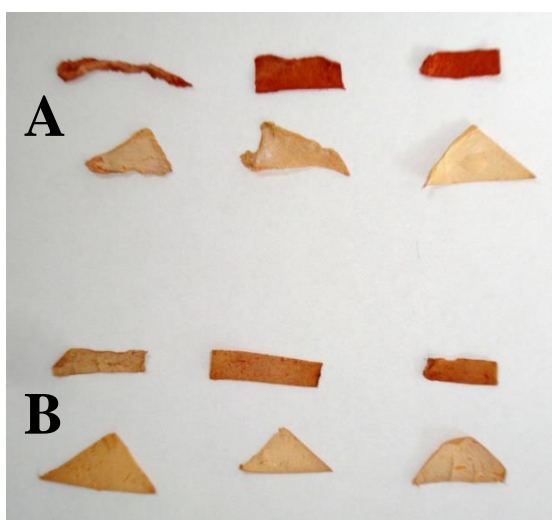


Figure 8. Image (from left to right) of ELLA assays on: (A) f-PCL₂₀-Mans, f-PCL₄₀-Mans, and f-PCL₆₀-Mans (B) f-PCL₂₀-Gals, f-PCL₄₀-Gals and f-PCL₆₀-Gals fibers. For each sample, top line corresponds to the positive test samples while bottom line matches the control samples.

Comparing the collected results of bulk- and surface-functionalized fibrous scaffolds, we could stress several important points. First of all, electrospun fibers derived from PCL₂-Gal and PCL₂-N₃ present quite different diameters and morphologies. f-PCL-N₃, exhibited

a regular diameter of 600 nm whatever the azide group content while the diameter of f-PCL-Gal_B fibers was rather irregular and increased with the galactose content in the electrospinning blend. In addition, bulk-functionalized f-PCL₂₀-Gal_B fibers had a rather small sugar content at their surface (~3 wt%) compared with the surface-functionalized f-PCL₂₀-Gal_S and f-PCL₂₀-Man_S fibers (~20 wt%). Such a difference might be explained by the different solubility of PCL₂-N₃ and PCL₂-Gal in the solvent system used for electrospinning. Indeed, PCL₂-N₃ is hydrophobic and totally soluble in DCM/MeOH. On the contrary, PCL₂-Gal is amphiphilic and forms aggregates in the same solvent system as evidenced by DLS. The polymer probably adopts a conformation in which the sugar is confined in the core of the aggregates to minimize interactions with the solvent. Accordingly, the galactose residues are predominantly entrapped in the core of the fiber and very few are available at the surface for protein binding.

On the contrary, f-PCL-N₃ electrospun fibers presented high density of azide groups at their surface as a response to the high electric field applied. Functionalization by click chemistry has further allowed the glycosylation of 20 wt% of sugar at the fiber surface and transformed hydrophobic PCL fibers into hydrophilic and bioactive PCL scaffolds. Despite the effectiveness of surface functionalization by CuAAC, the use of copper catalysts can be an obstacle for medical applications. To overcome this drawback, strained alkynes might be used to realize copper-free click conjugation.⁴²

4. CONCLUSIONS

Bulk- and surface-glycosylation of electrospun fibers were explored and their effectiveness was compared. Electrospun blend of PCL₂-Gal and PCL₈₀ resulted in poorly surface-decorated fibers with moderately increased hydrophilicity. Yet, surface functionalization by click chemistry of f-PCL-N₃ ultra-fine fibers with galactose and mannose moieties was

rewarded with successful sugar conjugation and high hydrophilicity of functionalized fibers. Enzyme-linked lectin assays of the fibrous mats confirmed the ability of carbohydrate to interact with specific lectins, indicating the biological potential of these scaffolds in tissue-engineering.

SUPPORTING INFORMATION

Additional information about synthesis of propargyl- β -D-galactose and propargyl- α -D-mannose accompanied with their NMR and MALDI-TOF mass spectroscopy, NMR and MALDI-TOF analyses of PCL₂-Gal, as well as tabulation of the Dubois colorimetric results of surface-functionalized f-PCL-Gal_S and f-PCL-Man_S fibers. This material is available free of charge via the Internet at <http://pubs.acs.org>

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ABBREVIATIONS

PCL₈₀ poly(ϵ -caprolactone) M_n 80 000 g/mol; **PCL₂** α,ω - diol-poly(ϵ -caprolactone) M_n 2 000 g/mol; **PCL₂-N₃** α,ω -azido-poly(ϵ -caprolactone); **PCL₂-Gal** α,ω -galactoside-poly(ϵ -caprolactone); **f-PCL₈₀** poly(ϵ -caprolactone) fibers; **f-PCL-N₃-X** fibers containing X=20, 40 and 60 wt% of PCL-N₃ and (100-X) wt% of PCL₈₀; **f-PCL_X-Gal_B** bulk-functionalized fibers containing X=20 and 40 wt% of PCL₂-Gal and (100-X) wt% of PCL₈₀; **f-PCL_X-Gal_S** surface-functionalized f-PCL-N₃-X fibers with propargyl- β -D-galactose; **f-PCL_X-Man_S** surface-functionalized f-PCL-N₃-X fibers with propargyl- α -D-mannose.

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