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Novel electrospun gelatin/oxycellulose nanofibers as a suitable platform for lung disease modeling

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ABSTRACT

Novel hydrolytically stable gelatin nanofibers modified with sodium or calcium salt of oxycellulose were prepared by electrospinning method. The unique inhibitory effect of these nanofibers against *Escherichia coli* bacteria was examined by luminometric method. Biocompatibility of these gelatin/oxycellulose nanofibers with eukaryotic cells was tested using human lung adenocarcinoma cell line NCI-H441. Cells firmly adhered to nanofiber surface, as determined by scanning electron microscopy, and no signs of cell dying were detected by fluorescent live/dead assay. We propose that the newly developed gelatin/oxycellulose nanofibers could be used as promising scaffold for lung disease modeling and anti-cancer drug testing.

1. Introduction

Oxidized cellulose (OC), known also as oxycellulose and/or 6-carboxycellulose, is biocompatible, biodegradable, bioresorbable and non-immunogenic polymer with bactericidal activity against wide spectrum of pathogenic microorganisms including *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa* [1]. Thanks to these properties, OC has found its way to clinical medicine as a component of hemostatic agents, wound dressings, absorbable surgical suture materials, medical lubricants for surgical gloves, drug carriers, superabsorbents, and others [2]. Still, although OC may offer tangible benefits to also "more sophisticated" biomedical applications, with engineering of tissues and organs in particular, this area of OC utility has not yet been systematically explored. One of the reasons seems to be an inability to produce nanofibrous 3D structures (scaffolds) from "pure" OC using current electrospinning-based methodologies. Although this inability can be overcome by first producing nanofibers from cellulose acetate and then oxidizing them by various techniques [3–18], this strategy is not well suited for effective production of larger 3D structures.

Electrospinning, a simple process producing fibers with diameters ranging from tens of nanometers to several micrometers [19–21], is applicable to many different polymers, including those that possess properties favorable for application in biomedical research and clinical medicine [22–28]. One of such polymers is gelatin, natural protein, which is produced by partial hydrolysis of collagen. Gelatin is biocompatible, biodegradable, with high water absorption and local hemostatic capacity [29,30], making it promising for regenerative medicine as a structural material for cell attachment either in the form of 3D flexible foams or nanofibers. Electrospinning of gelatin has been achieved using several different solvents starting from organic 2,2,2-trifluoroethanol [31], through formic and/or acetic acid [32,33], to recent preparation of gelatin nanofibers by Zha and coworkers [34] using "green chemistry" from ethanol/phosphate buffer saline mixture. Still, gelatin nanofibers dissolve in water so that they require crosslinking process to improve their hydrolytic stability along with enhancing their mechanical properties [35]. Variety of chemical and physical modalities are available to achieve crosslinking of gelatin including aldehydes (formaldehyde, glutaraldehyde, and glyceraldehyde) [36],

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Table 1
The composition of prepared polymer mixtures and suitable electrospinning parameters.

Gel/OC weight ratio	Sample labeling	Voltage [kV]	Product/outcome
1:0	Gel	57	Nanofibers
1:1	Gel/$CaOC_{1:1}$	65	Beads and defects
	Gel/$NaOC_{1:1}$	48	
2:1	**Gel/$CaOC_{2:1}$**	**60**	**Nanofibers***
	Gel/$NaOC_{2:1}$	**48**	
3:1	Gel/$CaOC_{3:1}$	58	Nanofibers
	Gel/$NaOC_{3:1}$	45	
1:2	Gel/$CaOC_{1:2}$	68	Electrospraying
	Gel/$NaOC_{1:2}$	56	
1:3	Gel/$CaOC_{1:3}$	70	N. A.**
	Gel/$NaOC_{1:3}$	70	

* Best samples which were then analyzed and tested.
** N. A. — not analyzed — no fiber formation.

genipin [37], polyepoxides [38], acyl azide [39], isocyanates [40], carbodiimides [41,42], enzymatically or naturally derived crosslinking agents [43], dehydrothermal treatment (DHT) and ultraviolet or gamma irradiation [44].

In this study we have combined oxycellulose with gelatin to develop nanofibrous materials that can be readily produced from acetic acid solution by electrospinning technology [45] and exert the medically-relevant properties, which are inherent to OC. To do so, we have used both water-soluble Na^+ salt (NaOC) and Ca^{2+} salt (CaOC) that is insoluble in most solvents. The reason for this is the fact that these two chemical variants of OC differ in their biological properties. They both have well pronounced hemostatic activity [46–48] and capability to decrease pH in a wound [49]. However, CaOC is more efficient bactericide [6] and also pain-relieving agent due to attaching Ca^{2+} to pain receptors [48]. This study seeks to develop and evaluate, in terms of their structure-property relationship, thoroughly new nanofiber materials with medical applicability.

Indeed, there is unlimited spectrum of potential medical applications, with each expectably posting specific requirements for biomaterial properties. We are particularly interested in materials that may serve as carriers resembling basement membrane of epithelial cells of distal portion of lung. Since we aim at this goal, to evaluate properties of new biomaterials, here we have used human lung adenocarcinoma cell line (NCI-H441) that is being considered as suitable model for human distal lung epithelial barrier [50].

2. Materials and methods

2.1. Materials

Gelatin (Gel, powder from bovine skin, Type B, BioReagent), 1-ethyl-3-(3-dimethyl-aminopropyl)-1-carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), acridine orange, ethidium bromide, 4′,6-diamidino-2-phenylindole (DAPI) (all purchased from Sigma-Aldrich, Germany), acetic acid (99% p.a., Penta, Czech Republic), sodium salt of oxidized cellulose (NaOC) (degree of oxidation 16–24%, Synthesia, a.s., Czech Republic), calcium salt of oxidized cellulose (CaOC) (degree of oxidation 16–24%, Lifeline Plus, s. r. o., Czech Republic), RPMI 1640 medium, phalloidin rhodamine (all purchased by Life Technologies, Czech Republic), Trypsin-EDTA solution (Biosera, France), fetal bovine serum (FBS), L-glutamine, Penicillin/Streptomycin (GE Healthcare Life Sciences, USA), ethanol, paraformaldehyde (PENTA s.r.o.), Triton TX-100 (CARL ROTH GMBH + CO. KG, Austria), glutaraldehyde (Polysciences, USA), cacodylate buffer (Spi supplies/Structure Probe, USA) and liquid carbon dioxide (Messer Technogas s.r.o., Czech Republic) were used as received without further purification. Genetically modified *Escherichia coli* K-12 was kindly provided by the Department of Biochemistry, University of Turku, Finland. Nutrient agar (Mo-Bio, USA) for *E. coli* cultivation contained 100 μg/mL of ampicillin (Biotika a.s., Slovakia) in order to sustain the selection. Bacterial cells for luminometric measurements were cultivated in phosphate buffer (solution A: 0.91 g KH_2PO_4 in 100 mL H_2O; solution B: 2.39 g $Na_2HPO_4 \cdot 12H_2O$ in 100 mL H_2O; 41 mL of solution A was mixed with 59 mL of solution B; pH was adjusted to 7.0). For cytotoxicity tests, human adenocarcinoma cell line NCI-H441 purchased from the American Type Culture Collection (ATCC, USA) was used. Ultrapure water (type II according to ISO 3696) was prepared on our Elix 5 UV Water Purification System (Merck spol. s r. o.).

2.2. Methods

2.2.1. Electrospinning process

Electrospinning was carried out using laboratory machine Nanospider™ NS LAB 500S from Elmarco s.r.o. (Czech Republic) at room temperature, relative humidity of 60% using rotary spinning electrode with the speed of 5 rpm. The distance between spinning and collecting electrode was kept at 150 mm. Nanospider™ technology is scalable, needle-free, high voltage (up to 80 kV) and free liquid surface electrospinning process that produces uniform non-woven nanofiber mats from wide spectrum of polymers [51,52]. Therefore, for each sample the applied voltage was adjusted in a range 40–70 kV, the voltage lower than 40 kV has not led to spinning process (see Table 1).

Gelatin (8 wt% in acetic acid) was slowly mixed in a ratio of 1:0; 1:1; 1:2; 1:3; 2:1 and 3:1 with either CaOC or NaOC powder and stirred overnight. Sample labeling, applied voltage and process results are described in Table 1. Sample labeling (abbreviations) of prepared nanofibers were formed by the abbreviations of used polymers with their weight ratios being provided in subscript (e.g. Gel/$NaOC_{2:1}$).

2.2.2. Hydrolytical stability

Nanofibers made from Gel/$CaOC_{2:1}$ and Gel/$NaOC_{2:1}$ samples were crosslinked either by dehydrothermal treatment (DHT) at 100 °C in vacuum oven (Vacucell, BMT, Czech Republic) for 10 h and/or in situ with 1-ethyl-3-(3-dimethyl-aminopropyl)-1-carbodiimide hydrochloride and N-hydroxysuccinimide (EDC/NHS) in molar ratio 1:2 (20 mM/40 mM, respectively). The use of NHS in the crosslinking reaction gives an NHS-activated carboxylic acid group, which is very reactive

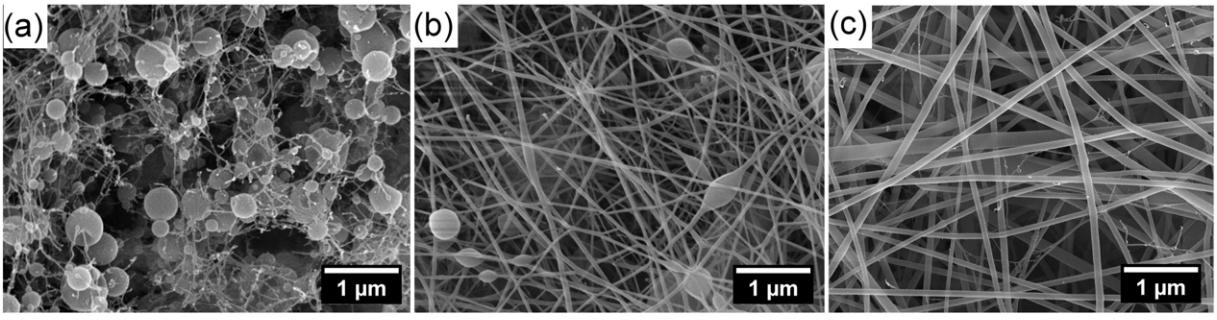

Fig. 1. Representative SEM images characterizing three types of obtained structures formed by different Gel/OC weight ratio: (a) electrospraying – sample Gel/$NaOC_{1:2}$, (b) fibrous products with many beads – sample Gel/$NaOC_{1:1}$, (c) homogeneous nanofibers – sample Gel/$NaOC_{2:1}$. Similar structures were observed with the CaOC modifications.