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Electro-spun hydroxyethyl cellulose nanofibers: the relationship between structure and process

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Electrospinning is a unique and versatile technique for the fabrication of nanofibers that mimics the native extracellular matrix for tissue engineering. In this paper, hydroxyethyl cellulose (HEC) has been electrospun. Selected parameters of spinning solutions (viscosity, conductivity and surface tension) and process parameters (applied voltage, needle-to-collector distance and collector type) were studied. The formation of nanofibers was found to depend absolutely on the right combination of voltage and distance. HEC concentration was varied from 0.25 to 2.0 % (w/w) and various additives – Tween 80, sodium chloride and organic solvents – were tested. SEM micrographs showed that HEC nanofibers with large numbers of beads could be reduced by addition of Tween 80 and sodium chloride. Addition of organic solvents did not improve nanofiber formation. It was shown that the key solution properties influencing nanofiber morphology were the polymer concentration, the viscosity and surface tension of its solution. Additionally, there are no methods or procedures for electrospinning that are universally applicable. Each polymer needs to be studied individually and the respective electrospinning conditions determined experimentally. Understanding the process and solution properties influencing the electrospun fibers is helpful in the design of tissue scaffolds. HEC nanofibers could offer advantages in the development of modern wound dressings.

Key words: Electrospinning – Nanofiber – Hydroxyethyl cellulose – Morphology – Scanning electron microscopy.

The broad range of potential applications of nanotechnology envisaged in the last decade has raised the need for developing new nanotechnological methods, as well as new nanosystems, one such being electrospun nanofibers. Publications on "electrospun nanofibers" in 1990-2000 numbered only six, compared to 2291 in the last decade, indicating the steeply increasing interest in research on nanofibers.

Nanofibers are solid fibers with several remarkable nanoscale features, among them a very large surface area mass ratio, a porous structure, a theoretically unlimited length together with better mechanical performance and flexibility than any other form of the same material. All these characteristics make nanofibers promising candidates for biomedical applications, provided they are prepared from biocompatible materials [1-4]. One important area of interest in nanomedicine is the development of extracellular matrix (ECM) analogues that contain micro- and nanoscale fibers for application in tissue engineering [2, 5]. Cells respond to the nano-topography of synthetic substrates in terms of adhesion, proliferation, migration and gene expression. Beachley and Wen demonstrated that the nanofibrous topography itself, independent of fiber composition, has the potential to favorably modulate cell behavior such as unidirectional alignment, increased viability, attachment, ECM production, guided migration, and controlled differentiation [6].

A versatile and popular method for producing ultrafine fibers is electrospinning. The process is governed by strong electric forces, which overcome the weaker forces of surface tension in the polymer solution [1, 7, 8]. A fine, charged jet is ejected from the tip of a capillary tube at a certain threshold voltage. The jet flows in the direction of an applied external electric field, causing elongation and orientation of polymer chains within the electric field [8]. As the jet moves towards a grounded metal collector, the solvent evaporates, and random, nonwoven nanofibers are formed. These nanofibers provide topographical cues that resemble the filamentary structure of ECM [2, 8, 9].

For the preparation of electrospun nanofibers, a conductive polymer solution is needed [10]. Polymer chain entanglement in solution results in complex, non-linear rheological behavior that complicates the nanofiber formation [11]. This complex rheology, coupled with the strength of extended polymer chains (covalent versus non-covalent bonds) suppress varicose instability and prevent break-up of the jet into droplets [12]. The process is characterized by jet instabilities that are persistent and responsible for jet thinning. Electrospinning is thus a highly coupled process involving high-speed, nonlinear electrohydrodynamics, complex rheology of the polymer solution throughout the process, and transport of charge, mass and heat within the jet.

The applicability of electrospinning is not straightforwardly transferable from known polymers to new materials, therefore appropriate electrospinning parameters have been determined individually for successful formation of nanofibers from more than 200 known natural, semi-synthetic and synthetic polymers [13]. Previous studies have shown that system configuration and operational conditions differ vastly from one material to another. Moreover, solution properties and process parameters are the main factors influencing the transition of a »normal« polymer solution into one of ultrafine fibers [14]. Viscosity, surface tension and conductivity of the solution are the key parameters governing the electrospinning process.

On the other hand, the design and selection of a biomaterial play a pivotal role in tissue engineering. The ideal biomaterial should to be nontoxic and biocompatible if it is to support the reconstruction of new tissue without inflammation. In this regard, various polymers have been studied, for instance chitosan and alginate. With the exception of cellulose acetate [15-17], electrospinning of other cellulose derivatives has been studied to only a limited extent. We have focused on hydroxyethyl cellulose (HEC), which has not previously been electrospun. HEC is a non-ionic, partially substituted poly(hydroxyethyl) ether of cellulose that is widely used in pharmaceutical formulations for thickening, binding and film coating. It is water-soluble, since the small substituent groups present only a minor steric barrier for the access of water molecules to the free hydroxyl groups in the polymer, resulting in a rapid, high degree of swelling in a short time [18, 19]. With its biocompatibility, hydrophilicity, free radical scavenging ability, electrostatic and hydrogen bonding ability and order of polymer chains, HEC has the characteristics of a polymer recommended for modern wound dressings [20]. Transforming HEC into nanofibers

could therefore unite the benefits of the properties of the material with those of its physical structure, offering an advantageous approach to wound healing.

The aim of our research, therefore, was to formulate nanofibers in a form suitable for bioapplication. The key properties of the spinning solution (viscosity, conductivity and surface tension) were first evaluated and optimized. The process parameters of electrospinning were then studied and finally, the effects of various additives (salt, surface-active substance, organic solvent) on the spinnability of HEC were investigated. The morphology of the produced electrospun samples was analyzed using scanning electron microscopy.

I. MATERIALS AND METHODS

1. Materials

Hydroxyethyl cellulose (Natrosol Pharm, type 250 HHX - Pharm, Lot: X-01017, $M_w \approx 1\,200\,000$ g/mol) was supplied by Aqualon, Hercules, Rijswijk, The Netherlands. Poly-(vinyl alcohol) (PVA) (Mowiol 10-98, $M_w = 61\,000$ g/mol, degree of polymerization ≈ 1400, degree of hydrolysis ≈ 98.4 ± 0.4 %) was from Clariant GmbH, Frankfurt am Main, Germany. Sodium chloride, ethanol, acetone, and isopropanol were supplied by Merck kGaA, Darmstadt, Germany, and Tween 80 from Fluka, Buchs, Switzerland.

2. Methods

2.1. Preparation of the polymeric solution

HEC spinning solutions in the concentration range of 0.25 to 2.0 % (w/w) were prepared by hydration of weighted amounts of HEC powder with distilled water, with gentle stirring overnight, to yield a homogenous viscous dispersion. PVA was dissolved in distilled water at 90 ºC with gentle stirring for 1 h to make a 10 % (w/w) spinning solution used for comparison.

Additives for spinning solutions, sodium chloride and/or surfactant Tween 80, were first dissolved in water and then mixed with HEC powder to a concentration of the latter of 0.75 % (w/w). The concentration of NaCl in the final solution was in the range of 0.005 to 4.0 % (w/w), and of Tween 80 in the range of 0.001 to 1 % (w/w). Compositions of solutions are shown in *Table I*.

HEC spinning solutions with organic solvents were prepared utilizing the same procedure, with the difference that HEC was hydrated in distilled water containing 10 to 40 % (w/w) of acetone, ethanol or isopropanol.

2.2. Characterization of polymeric solutions

Prior to electrospinning, the viscosity, conductivity and surface tension of the HEC solutions were determined. The viscosity was measured at 22 ± 1 ºC with an SV-10 Vibro Viscosimeter (A&D Company, Japan) at a vibration frequency of 30 Hz (viscosity measuring range 0.3-10 000 mPas, repeatability 1 %, accuracy ± 3 %). The conductivity was determined at room temperature using an Iskra Conductivity meter MA 5964 (Ljubljana, Slovenia) having an electrode conductivity constant 0.7265 cm^{-1}. Surface tension was measured at 25 ºC by the plate method with a Processor Tensiometer K-12, Version 5.05 (Kruess GMBH, Hamburg, Germany). Dynamic light scattering measurements of selected samples were performed at 22 °C using a Nano Zetasizer 3000 (Malvern Instruments, Worcestershire, United Kingdom). For each solution, average values of parameters from at least three measurements are reported.

2.3. Electrospinning of the polymeric solutions

The electrospinning setup utilized to obtain nanofibers is shown schematically in *Figure 1*.

The polymer solution was placed in a 20 mL plastic syringe fitted with a metallic needle with inner diameter 0.8 mm. A syringe pump (Model R-99E, RazelTM) was used to feed a constant flow rate (1 mL/min). A high voltage of 5 to 40 kV at the needle was achieved by connection to a voltage generator (model HVG-P60-R-EU, Linari Engineering s.r.l., Italy) capable of generating voltages in the range of 0 to 60 kV. The grounded collector, covered with aluminum foil, was located at a distance 10 to 25 cm from the needle tip. Deposition of the HEC nanofibers was observed as a gradual change of the color of the aluminum foil from grey to white during electrospinning. To obtain an insulating gap and a rough surface, the aluminum foil was cut. Additionally, a metallic wire net was used as collector.

2.4. SEM characterization of nanofibers

The diameter and morphology of electrospun nanofibers were examined using a scanning electron microscope (SEM, 235 Supra 35VP-24-13, Carl Zeiss, Germany) operated at an accelerating voltage

Table I - Composition of the solutions used in this study and their characteristics.

HEC % (w/w)	NaCl % (w/w)	Tween 80 % (w/w)	Viscosity (mPas)	Conductivity (μS/cm)	Surface tension (mN/m)
0.25			7.7 ± 1.2	19.9 ± 0.6	58.7 ± 1.3
0.5			38.1 ± 0.3	39.5 ± 1.8	65.6 ± 2.4
0.75			110.0 ± 7.9	55.7 ± 0.5	68.2 ± 0.6
1			204.8 ± 7.4	71.4 ± 3.1	68.5 ± 2.0
1.25			333.3 ± 2.9	90.7 ± 0.1	71.8 ± 2.7
1.5			512.2 ± 20.3	106.3 ± 0.4	68.8 ± 2.9
2			815.7 ± 1.2	135.7 ± 1.0	67.2 ± 0.2
0.75	0.005		117.0 ± 2.0	159.8 ± 2.0	65.6 ± 2.0
0.75	0.05		113.8 ± 3.6	1419.0 ± 37.2	69.0 ± 0.8
0.75	0.1		114.8 ± 7.5	2023.0 ± 109.7	69.3 ± 0.9
0.75	0.2		112.5 ± 7.5	3948.0 ± 15.8	68.9 ± 0.9
0.75	0.5		114.8 ± 11.6	9424.5 ± 74.7	67.5 ± 0.5
0.75	1		132.5 ± 6.5	16626.0 ± 51.2	68.0 ± 0.7
0.75	2		120.8 ± 11.0	31880.0 ± 216.6	68.5 ± 0.2
0.75	4		135.8 ± 4.8	59526.7 ± 785.0	68.3 ± 0.3
0.75		0.001	123.0 ± 12.3	56.2 ± 0.1	64.8 ± 0.4
0.75		0.005	128.0 ± 11.4	55.9 ± 0.1	54.5 ± 0.4
0.75		0.01	123.0 ± 3.6	54.5 ± 1.2	46.9 ± 0.8
0.75		0.1	123.7 ± 8.1	55.5 ± 1.1	47.4 ± 1.1
0.75		0.5	126.7 ± 12.1	57.4 ± 0.9	48.1 ± 0.4
0.75		1	122.0 ± 18.5	59.2 ± 0.8	48.3 ± 0.7
100 % PVA			105.7 ± 3.3	103.8 ± 6.2	56.6 ± 4.1