

Hydrophobic Ultrafine Hyaluronic Acid Nanofibers

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Extended Abstract

Hyaluronic acid (HA) is a naturally occurring biopolymer, which exhibits biocompatibility, ability to form hydrogels, has viscoelastic properties and is biodegradable. Thus HA is broadly used in applications based on healthcare, dermatology, pharmacy and tissue engineering [1], [2]. In recent years there has been a growing interest in preparing nanofibers based on HA as these fibers can act like a scaffold for regenerative medicine. Nanofibers are widely prepared by electrospinning, since it provides reproducible method for creating the uniform nanofibrous mats. However, the electrospinning of large molecular weight biopolymers such as HA is difficult due to its polyelectrolytic nature and high viscosity in aqueous solutions [3]. Also, the stability of HA nanofibrous mats *in vitro* or *in vivo* is limited, as HA dissolves immediately after contact with water. On the other hand, with commonly used techniques for crosslinking of polymers it is still difficult to maintain biocompatibility.

This paper introduces the conditions and methods for preparation of biocompatible, biodegradable, stable and uniform HA nanofibrous mats. In this study, the conditions for easy reproducible electrospinning of high molecular weight (1,2 MDa) HA in concentration 0,5% w/w in aqueous solutions were found. It was proven there is no need to add toxic solvents or blend HA with other polymers for enhancing the electrospinnability and that contrasts with other studies such as [3]–[5]. The ultrafine fibers of HA were prepared via needle-less method called Nanospider™. The morphology was carried out by the scanning electron microscope (SEM) and it was found that the bead free fibers with an average diameter of $(79,4 \pm 18,6)$ nm were prepared.

To increase the stability in aqueous solutions the plasma modification method was chosen. The surface treatment was performed by cold methane plasma, RF Frequency Plasma Assisted Chemical Vapor Deposition (PACVD) method. This technique is a chemical deposition method, chemical reactions were conducted in a high-frequency electric field in a vacuum chamber into which methane gas was injected. Other parameters included vacuum pressure [Pa], voltage [V], gas flow [scm] and time [min]. In all processes, the parameters were kept constant at the following values: $p = 20$ Pa; $U = 200$ V; $CH_4 = 20$ scm; $t = 3$ min. The SEM observation show that the diameter of nanofibers was increased to $(113,77 \pm 30,84)$ nm. The important result is, that the hydrophobicity of the HA nanofibers was rapidly increased. According to contact angle measurement via sessile drop technique, the contact angle was increased from $(46,59 \pm 15,19)^\circ$ to $(106,29 \pm 10,23)^\circ$. The FTIR spectra showed no difference before and after treatment and it means, that the chemical structure of HA remained unchanged after plasma exposure. The stability of HA layer was maintained during *in vitro* testing and no change in morphological structure was observed. The cytotoxicity of the HA nanofibrous mat after plasma treatment was assessed via *in vitro* testing according to ISO 10993-5:2009 Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity. It was found, that the treated HA nanofibers are cytocompatible and even promote cell adhesion and proliferation of mouse 3T3 fibroblasts.

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