Microfibers of a Hyaluronic Acid Derivative to Mimic Blood Vessels

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Abstract. Microfibers of a hyaluronic acid amphiphilic derivative (HA-EDA-C_{18}), were obtained by microfluidic technique. Exploiting the ionic strength sensible behavior of HA-EDA-C_{18}, microfibers were formed in a bath containing phosphate buffer saline. Microfibers were tethered with fibronectin and RGD peptide to control cell attachment and spreading. The morphology and stability of the microfibers were studied. Human umbilical vein endothelial cells (HUVECs) were cultured on microfibers. The results suggest that HA-EDA-C_{18} microfibers can be potentially used for the production of biomimetic vascular networks.

Keywords: hyaluronic acid, microfibers, microfluidic technique, HUVEC

INTRODUCTION

The fabrication of continuous polymer fibers with microfluidics is a simple process that has many advantages such as control of size and easy loading of biological effectors. Fiber-based systems are an ideal platform for mimicking biological materials and tissue constructs, and current research has begun to exploit fiber matrices for these biomedical applications [1, 2]. Microfluidic fiber fabrication is recently emerged as a very promising route to synthesize polymeric fibers at the micro and nanoscale, providing a fine control over fiber shape, size, chemical anisotropy and biological activity [3]. These fibers are useful for the creation of three-dimensional (3D) scaffolds, including clothes and other architectures by folding, bundling, reeling and weaving [4].These microfibers have also been attractive for creating complex 3D artificial extracellular matrix such as blood vessel networks [5], muscle fibers, nerve bundles [6] and other anatomical structures [7].

This work describes the production of microfibers made of a derivative of hyaluronic acid (HA-EDA-C_{18}) using a microfluidic technique. The introduction of octadecylamine (C_{18}-NH_2) and ethylenediamine (EDA) portions on hyaluronic acid backbone made the HA derivative sensible to the ionic strength of the surrounding media [8-9]; this particular behavior has been exploited to obtain physically crosslinked microfibers without using chemical crosslinkers. The microfiber versatility in terms of chemical and biological functionalization and ability to allow attachment and spreading of human umbilical vein endothelial cells has been studied.

RESULTS

HA-EDA-C_{18} has been synthetized as previously reported [8-9]. The derivative was dissolved (3.75% w/v) in a solution of 2-hydroxypropyl-β-cyclodextrin (3% w/v) in milliQ water; the vial was maintained at 37°C in orbital shaker all night to allow complete dissolution.

HA-EDA-C_{18} solution was placed into an Eppendorf connected to the microfluidic chip with 190 μm each depth (Dolomite microfluidics, UK). The chip was immersed in 10x PBS solution (chosen as a coagulating bath). When the polymer solution was extruded from the chip, it formed stable physical microfibers (**Fig.1**). These microfibers were left in PBS solution for one day to allow the polymer coacervation. Morphology of HA-EDA-C_{18} microfibers was analyzed with optical microscope and by SEM. The N-Hydroxysuccinimide ester of the maleimide butyric acid (MLB-NHS) has been employed to activate microfibers into a mixture DPBS pH 7.4/DMSO 16:1. Finally, fibronectin and RGD peptide functionalization of microfibers has been accomplished in DPBS pH 7.4. Human umbilical vein endothelial cells (HUVECs) were seeded on microfibers and their viability and adhesion were studied.

This study showed a simple, cost-effective, well-controlled and biologically compatible process for the production of uniform HA-EDA-C_{18} microfibers with controlled size, containing bioactive molecules and potentially able to control cell growth, adhesion, alignment, migration and to form an artificial vessel.

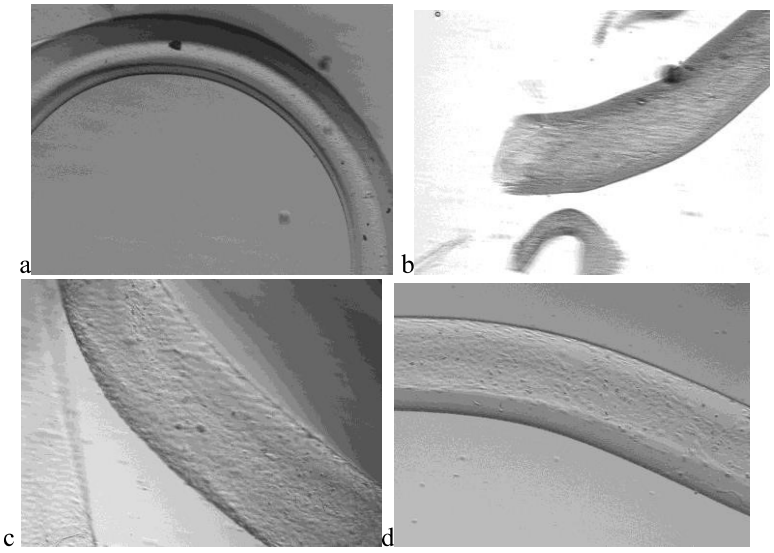

FIGURE 5. Optical microscope images of HA-EDA-C_{18} microfibers just obtained in PBS 10x (a and b). Microfiber functionalized with RGD with adherent HUVEC (c); fiber functionalized with fibronectin with adherent HUVEC.

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