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Biomimetic electrospun scaffolds from main extracellular matrix components for skin tissue engineering application – The role of chondroitin sulfate and sulfated hyaluronan

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ABSTRACT

Incorporation of bioactive components like glycosaminoglycans (GAGs) into tissue engineering scaffolds, is a promising approach towards developing new generation functional biomaterial. Here, we have designed electrospun nanofibrous scaffolds made of gelatin and different concentrations of chemically sulfated or non-sulfated hyaluronan (sHA or HA) and chondroitin sulfate (CS). Evenly distributed fiber morphology was observed with no differences between varying concentrations and types of GAGs. *In vitro* release kinetics revealed that GAGs release is driven by diffusion. The effects of these scaffolds were analyzed on human keratinocyte (HaCaT), fibroblast (Hs27) and mesenchymal stem cells (hMSCs) adhesion and proliferation. A significant increase in cell number (~5 fold) was observed when cultivating all three cell types alone on scaffolds containing sHA and CS. These findings suggest that sulfated GAG-containing electrospun nanofibrous scaffolds might be beneficial for the development of effective skin tissue engineered constructs by stimulating cellular performance and therefore accelerate epidermal-dermal regeneration processes.

1. Introduction

The development of artificial scaffolds has long been achieved, while those for specific applications and functions, *e.g.* reducing the infections and scarring are now in focus. Tissue-engineered skin replacement can be used for a wide range of therapeutical application *viz.*, (a) acute, chronic and trauma skin wounds/ulcer treatment, (b) diabetic ulcer and venous stasis and (c) surgical injury and abrasion. The major goal of skin substitutes is not only to functionally support the wounded tissue and promote healing but also to be straightforwardly applicable under emergency/clinical circumstances [1].

The extracellular matrix (ECM) is a principal constituent of the intracellular-microenvironment, playing a pivotal role of maintaining and regulating tissue function [2,3]. Thus, incorporating essential components of ECM in biologically functional scaffolds to mimic this microenvironment is an efficient approach to control cellular proliferation process in tissue regeneration [3,4]. Except for hyaluronan (HA), the native GAGs are always present in sulfated form [5]. GAGs play a crucial role in different stages of skin tissue regeneration and maturation, being an important component of its ECM [6] and able to bind a number of proteins including several chemokines and growth factors [1]. The carbohydrate backbone of GAGs as well as the position and number of sulfate groups within the polymer chain plays an important role on these GAG-protein interactions [7]. Incorporation of HA/chondroitin sulfate (CS) combination into tissue engineered scaffold is widely reported in literature [8–13]. Fabrication of electrospun based scaffold for tissue engineering application is well known for its native tissue mimicking properties [14–19]. Recently, we have reported that sericin loaded cationic gelatin composite electrospun nanofibrous scaffold (cationic gelatin/HA/CS) stimulates epithelial differentiation of hMSCs in keratinocyte-hMSC co-culture model in terms of various epithelial markers (pan-cytokeratin, keratin 14 and p63) [20]. Previously, our group have also explored the interactions of native as well as chemically modified GAGs with different sugar backbones and variable types of functional groups for their binding affinities to growth factors relevant in skin and bone healing. These studies demonstrated that chemically modified hyaluronan derivatives with high sulfation degree (sHA, average sulfation degree per repeating-disaccharide unit ((DS_S) ~3) displayed strong interaction with TGF-β1, BMP-2 and -4 in 2D cell culture [7,21] and can be considered a promising support for regenerative medicine for epithelial damage treatments. Other research

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group like Van der Smissen et al. demonstrated that degree of sulfation of GAGs influences the initial cellular attachment of human dermal fibroblasts (hDF) on collagen I/sulfated hyaluronan scaffolds after 24 h [1]. Similarly Salbach-Hirsch et al. showed that the sulfated HA significantly control osteoclastogenesis process significantly by interfering the formation of RANKL/OPG complex [22].

Against these background chemically sulfated sHA and naturally sulfated CS as functional constituents and gelatin as a cellular substrate were used to fabricate biomimetic nanofibrous 3D scaffold platform, which could act as a skin wound dressing, mimicking the extracellular microenvironment and thereby promoting wound healing process.

2. Materials and methods

2.1. Materials

2,2,2-Trifluoroethanol (TFE), mitomycin C and gelatin from porcine skin gel strength 300, Type A were obtained from Sigma-Aldrich, USA. Chondroitin sulfate from porcine trachea (a combination of 30% chondroitin-6-sulfate and 70% chondroitin-4-sulfate, sulfation degree of 0.9, Mw = 20,000 g/mol) was acquired from Kraeber (Ellerbek, Germany). Hyaluronan (Mw $1.1 \cdot 10^6$ g/mol; polydispersity 4.8) from *Streptococcus* was bought from Aqua Biochem (Dessau, Germany).

2.2. Synthesis of low molecular weight HA and sulfated HA

Sulfated HA and low molecular weight HA was synthesized and evaluated as described previously [21,23,24]. Concisely, the tetrabutylammonium salt of hyaluronan (TeBA-HA) was used as reactant for the sulfation reaction. The procedure is described as follows:

General procedure: 2.0 g (4.98 mmol) of HA was dissolved in 400 ml of double distilled water by stirring continuously for overnight at room temperature. Afterwards, 20 g of Dowex WX 8 ion exchanger (tetrabutylammonium-form) was mixed with the solution and kept overnight in continuous stirring. The final solution was filtered and lyophilized overnight and kept in vacuum oven at 40 °C for drying. Yield = 90%.

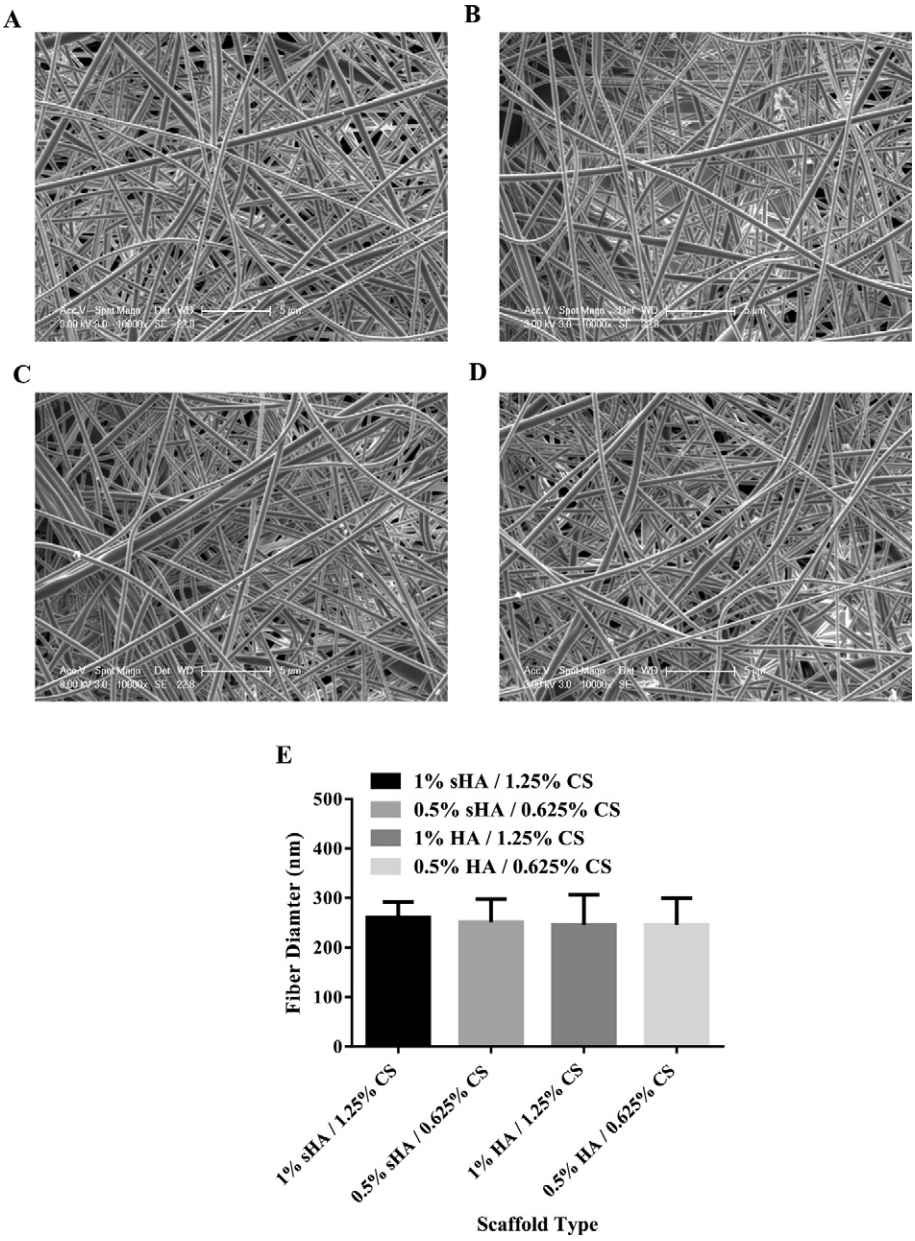

Fig. 1. Characterization of electrospun scaffolds; SEM image of electrospun scaffold (A) 1% sHA/1.25% CS, (B) 0.5% sHA/0.625% CS, (C) 1% HA/1.25% CS and, (D) 0.5% HA/0.625% CS and (E) Fiber diameter distribution of the scaffolds in SEM image.