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Nanofibrous poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)/collagen/graphene oxide scaffolds for wound coverage

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ABSTRACT

The purpose of wound management is to prevent wound from infection, increase the fibroblast cell growth, and preserve cellular function. The polymeric electrospun nanofiber scaffold made up of natural and/or synthetic polymer provides an extracellular matrix for support and initiates the growth, proliferation and differentiation of fibroblast cells. The present study deals with the development of poly3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid (PHBV) nanofibrous scaffold imbedded with graphene oxide (GO), and collagen. Nanofibrous PHBV offers advantages like structural resemblance to native extracellular matrix, high porosity and surface area to volume ratio. The nanofibrous mats were morphologically and chemically characterized by Field Emission Scanning Electron Microscopy (FESEM) and Fourier Transform Infrared (FTIR) Spectroscopy. FESEM images showed the nanofiber diameter was decreased and porosity increased by adding GO and collagen into the matrix without any chemical interaction among them. Incorporation of GO into the matrix increases mechanical strength of scaffold in addition to antibacterial activity against *E. coli* and *S. aureus* with decrease in pore size and hydrophilicity. In contrast, collagen addition into the nanofibers enhanced hydrophilicity without affecting mechanical strength and porosity significantly. Moreover, collagen enhanced cell proliferation capacity of nanofibers in comparison to the samples of PHBV + GO and virgin PHBV. The combination of collagen and GO with PHBV has balanced properties which can be utilised for the application.

1. Introduction

Wound is a disruption of the normal structure and function of skin and underlying soft tissue. The types of wound include cuts, scrapes, burn, scratches, and punctured skin. An ideal wound dressing should have the desired clinical properties such as, hemostatic, adherence to wound bed, non-inflammatory, protection against infectious agents, and maintain moist healing environment [1]. Also, it should mimic the structure and biological function of native extracellular matrix (ECM) proteins, which provide support and regulate cellular activities. To achieve this, engineered matrix must be biocompatible and/or biodegradable, and should not induce adverse effects on the surrounding tissue [2]. Recent progress in material science and polymer chemistry have given an entire class of new nanomaterials, ranging from bioactive tissue scaffold to novel electrospun polymer and hydrogel for tissue repair and wound management [3]. Nanoscale materials with high surface area, biodegradability and biocompatibility properties help to promote wound healing and target drug release with minimum side effects.

Many natural and synthetic polymers have been used in the preparation of artificial dressing material. Polyurethane, Teflon, silicon, and methyl methacrylate are some of the synthetic polymers used for this application [4]. The most widely used natural polymers for wound healing includes collagen, chitosan, flucoidan, poly-*N*-acetyl glucosamine, alginic acid and their salts, hyaluronic acid and its derivatives. Polycaprolactone/Gelatine composite scaffold has been found suitable for skin substitute [5]. Ciprofloxacin loaded chitosan porous scaffolds in combination with polyethylene glycol and polyvinyl alcohol have been studied. The *in-vivo* results showed that the scaffold is maintaining the cellular alignment during healing without any microbial growth to surrounding area [6]. The biopolymers are more effective as a wound healing accelerator than synthetic polymers because of biocompatibility and biodegradable property. Moreover, biopolymers structural arrangement is similar to ECM of normal skin [7].

It is well known that the collagen is a component and major protein of ECM. Collagen type I, II, and III are the main types of collagen found in connective tissue and constitute 90% of all collagen in the body. Nearly every tissue such as bone, skin, tendon, and ligament contain collagen. This natural material has been reported as scaffold modifiers [8]. However, collagen alone cannot be used for the application due to its poor

Abbreviations: PHBV, Poly3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid; GO, Graphene oxide; FESEM, Field Emission Scanning Electron Microscopy; FTIR, Fourier Transform Infrared; ECM, Extracellular matrix; *E. coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; FBS, Fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium.

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mechanical properties and rapid degradation behaviors [9]. Collagen in the form of gel has been tried for the application. A nanofibrous scaffold made up of Poly lactic-co glycolic acid/collagen is very effective as wound healing accelerators in early stage of wound healing [10,11].

Poly-3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid (PHBV) is resorbable thermoplastic polyester developed and approved by the Food and Drug Administration (FDA) for medical use [12]. PHBV is a biomaterial that is used for variety of applications including surgical sutures, wound dressing, vascular graft, drug delivery and tissue engineering [13]. It has specific properties such as biocompatibility, biodegradability, as well as piezoelectric nature [14]. However, the material is hydrophobic in nature which should be modified with other materials to improve its cell adhesion and water absorption properties [15,16]. Scaffold made up of collagen and Poly hydroxyl-4-butyrate with electrospun nanofibrous membrane has shown higher gas permeation and protection of wound from infection and dehydration [17].

Graphene is a planar, single layer of sp^2 hybridized nonaromatic carbon atoms in hexagonal arrangement. It is possesses a very high mechanical strength, surface area, electrical and thermal conductivity. It has immense applications ranging from transistors, transparent conducting electrodes, sensors, solar cells, electronic devices, lubricant additives, waterproof coating, structural materials, tissue engineering, biomaterial devices and drug delivery [18–20]. It shows excellent antimicrobial activity because of the ordered structure. The antimicrobial property is further enhanced in its oxidized form, graphene oxide (GO). GO sheet have its basal planes decorated mostly with epoxide and hydroxyl groups, in addition to carbonyl and carboxyl groups located at the edges. Antimicrobial actions are contributed by both membrane and oxidation stress. The three step antimicrobial mechanism is followed including initial cell deposition on graphene based materials, membrane stress caused by direct contact with sharp nanosheets, and at last superoxide anion-independent oxidation [21].

In the present work, we hypothesized that the polymeric electrospun nanofiber scaffold of PHBV in combination with GO and collagen may provide an extracellular matrix to support and initiate the growth, proliferation and differentiation of fibroblast cells including better strength and antibacterial properties. The developed nanofibrous mat was characterized and studied for *in-vitro* and cell proliferation properties.

2. Materials and methods

2.1. Materials

Poly (3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid) (PHBV) of PHV content 12 mol% of medium molar mass, collagen type 1 from calf skin, Docusate sodium salt and 2,2,2-Trifluoroethanol (TFE) were purchased from Sigma Aldrich. Exfoliated GO received as gift sample from School of Chemical Engineering, South Korea. Fibroblast cell line (3 T3-L), Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), antibiotics, and trypsin-EDTA were purchased from Hi-media. All solvents used were of analytical grade.

2.2. Preparation of electrospunnanofibers

The nanofibers were prepared by electrospinning technique. PHBV 16% wt was dissolved in Chloroform:TFE in 50:50 ratio and the polymeric solution subjected to electrospinning (E-Spin, India) at 25 °C. The polymer solution was placed in a 10 ml glass syringe fitted with a 25G needle. A clamp connected to a high voltage power supply, 25 kV was attached to the needle. A collector wrapped with aluminum foil was placed at a distance of 15 cm apart from the needle tip. The polymer jets generated from the needle by high voltage flow and formed the nanofiber mesh onto collector at flow rate 1 ml/h. GO, at 0.3% wt, was mixed in PHBV solution by adding 0.0025% sodium lauryl sulfate. The solution was mixed and homogenized in ultrasonic bath for 2 h and then subjected to electrospinning experiment at the same parameters. For collagen loaded samples, collagen was added to PHBV + GO solution at 7.25 mg/ml and the solution was stirred overnight at 30 °C up to complete dissolution and electrospinning was done at the same parameters. The electrospun samples were vacuum dried overnight at 40 °C.

2.3. Characterization of electrospunnanofibers

Structure of prepared nanofibers was initially observed by optical microscope (MOTIC image plus 2.0). All images were taken at 40× objective to observe the morphology of nanofibers. Later, the morphology of the electrospun fibers was studied by Field Emission Scanning Electron Microscope (FESEM EDAX XL-30, Philips, Netherlands) at 5 kV. Samples were analysed at different magnifying power. For each sample, images were taken and their diameters were measured to calculate an average fiber diameter. The IR spectrum of samples was recorded using Fourier Transform Infra-Red (FTIR) Spectrophotometer (DRL 8000, Shimadzu, Japan) within the range of 400 to 4000 cm^{-1}. The nanofibrous scaffold placed over the surface and spectra were recorded.

2.4. In-vitro studies

2.4.1. Porosity analysis

Porosity analysis of the samples was done by gravimetric method [22]. Volume and thickness of the nanofibrous samples were measured. The experiment was performed in triplicate. The porosity of the samples was estimated through the Eqs. (1) and (2):

$$\text{PHBV NF Scaffold density}\left(\frac{\text{g}}{\text{cm3}}\right) = \frac{\text{Mass of scaffold (g)}}{\text{Volume of scaffold}} \tag{1}$$

$$\text{PHBV NFs scaffold porosity}(\%) = \frac{(1-\text{scaffold density (g/cm3)})}{\text{Material density (g)}} \times 100 \tag{2}$$

The pore size and surface area of the samples were analysed through automated gas sorption analyser (Autosorb iQ3, Quantachrome, USA). The samples were degassed at 60 °C for 30 min. Adsorption- desorption isotherm was performed. The Brumauer–Emmett–Teller (BET) surface area was calculated using experimental points at a relative pressure (P/P_0) of 0.05–0.3. The total pore volume was calculated from the nitrogen amount adsorbed at the P/P0 of 0.99 for each sample. Barrett–Joyner–Halanda (BJH) model from a 20-point BET surface area plot was used for the calculation of average pore size distribution in the nanofibers. All the calculations were done by AsiQwin software (Quantachrome, USA).

2.4.2. Water uptake capacity

To determine the water uptake capacity of nanofibrous scaffolds the samples were weighed and immersed in distill water. The samples were removed from the water at predefined intervals of 1, 24, 48 and 72 h and weighed after removing the surface water with a blotting paper. The percent water content (WC%) was calculated according to the Eq. (3) as follows:

$$\text{WC}(\%) = \frac{\text{W}-\text{Wo}}{\text{Wo}} \times 100 \tag{3}$$

where, Wo and W are the weight of the samples before and after immersion in water for different time intervals, respectively. Hydrophilicity was measured at 25 °C using contact angle measurement instrument (Dataphysics, Netherlands) followed by image processing of sessile drop with OCA software.