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Evaluation of proanthocyanidin-crosslinked electrospun gelatin nanofibers for drug delivering system

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ABSTRACT

Electrospun nanofibers are excellent candidates for various biomedical applications. We successfully fabricated proanthocyanidin-crosslinked gelatin electrospun nanofibers. Proanthocyanidin, a low cytotoxic collagen crosslinking reagent, increased the gelatin crosslinking percentage in the nanofibers from 53% to 64%. The addition of proanthocyanidin kept the nanofibers from swelling, and, thus, made the fibers more stable in the aqueous state. The compatibility and the release behavior of the drug in the nanofibers were examined using magnesium ascorbyl phosphate as the model drug. Proanthocyanidin also promoted drug loading and kept the drug release rate constant. These properties make the proanthocyanidin-crosslinked gelatin nanofibers an excellent material for drug delivery. In the cell culture study, L929 fibroblast cells had a significantly higher proliferation rate when cultured with the gelatin/proanthocyanidin blended nanofibers. This characteristic showed that proanthocyanidin-crosslinked gelatin electrospun nanofibers could potentially be employed as a wound healing material by increasing cell spreading and proliferation.

1. Introduction

The process of utilizing electrostatic forces to form synthetic fibers is known as electrospinning. It is a preferred method for making nanofibers because it can produce fibers ranging from nanometer to sub-micrometer in diameter, depending on the solution and process parameters. These important parameters that influence fiber formation and structure are applied voltage, polymer flow rate, and capillary-collector distance [1,2]. The electrospun fibers form a porous scaffold which has a large surface area-to-volume ratio. Therefore, nanofibers are a promising material for many biomedical applications, such as wound dressings, drug delivery, and serving as a matrix template for tissue engineering [3,4].

Various materials can be electrospun, including biodegradable, non-biodegradable, and the natural materials. Most importantly, the material must be biocompatible. Biodegradable materials are a more popular choice due to the elimination of a second surgery to remove the implanted scaffold [1]. Gelatin is a protein that contains high contents of glycine, proline, and hydroxyproline. Gelatin fiber is a biodegradable polymer with excellent biocompatibility, plasticity, adhesiveness, cell adhesion and growth promotion, and low cost, making it ideal for use as a biomaterial. The use of a proper crosslinking agent to modulate the mechanical–chemical characteristics of gelatin is desirable for preventing toxicity and generating stable materials for biomedical applications [5,6]. The proanthocyanidin (PA) hydroxyl groups can interact with the gelatin carboxyl groups. Therefore, the PA compound appears to be a good candidate for such a role [7–10].

Proanthocyanidin (PA), a naturally occurring plant metabolite, has been adopted as an antioxidant, free-radical scavenger, and cardiovascular protector [11,12]. Proanthocyanidin (PA) is part of a group of polyphenolic compounds known as condensed tannins. They can stimulate normal skin fibroblast proliferation and increase the synthesis of an extracellular matrix, including collagen and fibronectin [13]. PA can be used to fix biologic tissues and biomaterials as well as stabilize biomaterials without cytotoxicity.

Conventional drug delivery systems such as nano or microspheres, liposomes, and hydrogels often initially have a burst drug-release problem. The drug release from electrospun fibers occurs via diffusion alone or diffusion and fiber degradation. The profile can be tailored by changing the content of the polymer mixture and the crosslinking time [14]. Our previous study has developed gelatin nanofibrous

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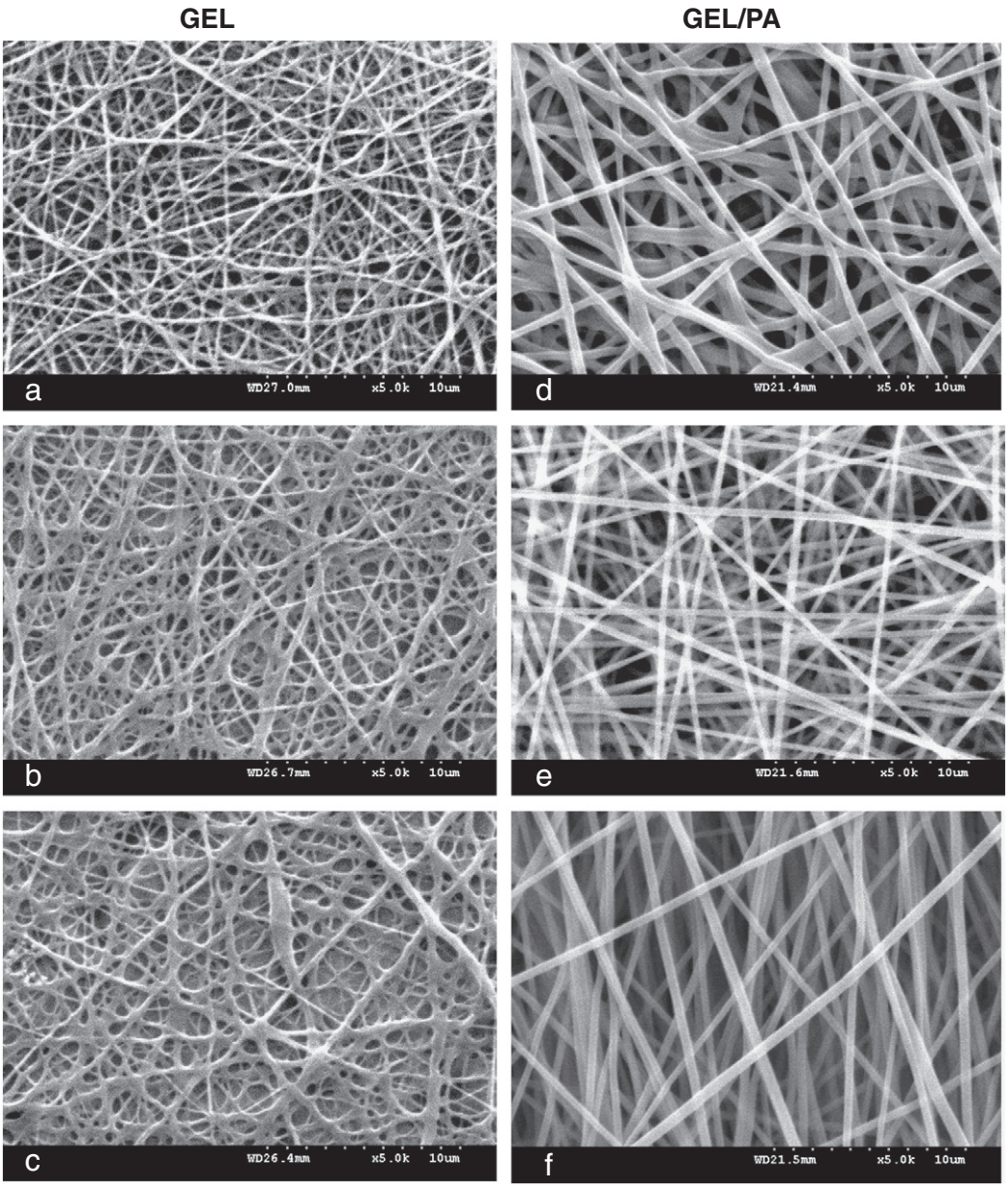

Fig. 1. SEM photographs of the electrospun GEL and GEL/PA nanofibers crosslinked by 50 wt.% glutaraldehyde vapor for various periods of time. (a) GEL, 15 min. (b) GEL, 45 min. (c) GEL, 90 min. (d) GEL/PA, 15 min. (e) GEL/PA, 45 min. (f) GEL/PA, 90 min.

matrix that was prepared by electrospinning with glutaraldehyde crosslinking treatment. The results of in vitro biological evaluations demonstrated that the gelatin nanofibrous matrix fabricated by an electrospinning technique was found to enhance cell adhesion and proliferation [15].

This gives electrospun fibers a potential application for controlled drug delivery. The high surface to volume ratio of electrospun fibers can enhance drug loading. This property makes electrospun fibers good candidates for topical/transdermal drug delivery [16–18].

In this study, the drug loading and release behavior of the Gel/PA blend fibers were examined by using magnesium ascorbyl phosphate (MAP) as a model drug. MAP, a derivative of ascorbic acid, is stable in water. MAP can be hydrolyzed to ascorbic acid by phosphatases in the liver or skin. Thus MAP exhibits vitamin C-reducing activity [19]. MAP can reduce melanin synthesis by inhibiting tyrosinase activity, which is why it has been widely prescribed as a skin-lighting and depigmenting agent [20,21]. We also evaluated the feasibility of adding PA to the gelatin electrospun nano-fibers. The improved mechanical properties and stable structure of the GEL/PA blend nanofibers were characterized by physical–chemical, biochemical, and cell culture studies.

2. Materials and methods

2.1. Materials

MAP, DPPH, and gelatin powders (Bloom number 300, Sigma Chemical Co., USA) from porcine skin were purchased from Sigma. PVA (MW = 1400) was supplied by SHOWA (Japan) (Mw ~ 1400, Showa Co., Japan). L-929 mouse fibroblast cells (Food Industry Research and Development Institute, Taiwan) and human melanoma cells (RPMI-7951, BCRC no. 60274, Food Industry Research and Development Institute, Taiwan) were cultured in 10% fetal bovine serum (FBS)/DMEM. All cell culture reagents were purchased from Invitrogen. The other agents were analytically pure. The polymer and the solvent were used without further purification.