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# Surface biofunctional drug-loaded electrospun fibrous scaffolds for comprehensive repairing hypertrophic scars

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ABSTRACT

Incorporation of bioactive drugs and biofunctionalization of polyester fibrous scaffolds are essential means to improve their bio-functions and histocompatibility for regenerative medicine. However, it is still a challenge to biofunctionalize such drug carriers via traditional biochemical methods while maintaining their properties without changes in drug activity and loading ratio. Here, we demonstrated a facile approach for biofunctionalization of PLGA fibrous scaffolds with various molecules (i.e., PEG polymer, RGD peptide and bFGF growth factor for cell repellent, adhesion and proliferation, respectively) via mussel-Inspired poly(dopamine) (PDA) coating in aqueous solution. By virtue of the mild and efficient nature of this approach, the drug-loaded PLGA fibers could be easily biofunctionalized and showed negligible effects on the scaffold properties, especially drug activity and loading ratio. Further, *in vivo* study showed that, a ginsenoside-Rg3-loaded fibrous scaffold functionalized with bFGF growth factor could not only promote the early-stage wound healing in rabbit ear wounds (bio-signal from bFGF), but also inhibit later-stage hypertrophic scars formation (release of Rg3 drug). Therefore, the mussel-inspired method for bio-modification provides a facile and effective strategy to combine drug and bio-function in one system, thus facilitating a synergistic effect of drug-therapy and bio-signal when such biomaterial is used for regenerative medicine.



## 1. Introduction

Electrospun polyester fibrous scaffolds possess excellent characteris tics such as adjustable biodegradability, appropriate mechanical properties, and high porosity for cell growth, thus holds great promise in regenerative medicine. Moreover, the flexibility for drug-loading and the local, controllable release features also make these fibrous scaffolds as promising implantable, degradable drug carriers for treating disease and repairing tissue [1,2]. Despite of the tremendous progress made in this field, the low-cytocompatibility and low-bioactivity of these polyester fibrous scaffolds remain two general problems to be addressed [3]. As known, this drawback is mainly due to the super-hydrophobic surface and non-bioactive components of the polyesters which is not conducive for cell adhesion, growth, specific biological functions, and more seriously, it may disrupt or delay the tissue healing process. Therefore, surface biofunctionalization of the fibrous drug carriers with bioactive molecules, such as bioactive peptide or growth factors, is essential for an improved cytocompatibility and even specific biological functions *in vivo* [4,5].

However, surface biofunctionalization of the fibrous scaffolds can be quite complicated because the surfaces of these fibrous scaffolds are chemically “inert”, i.e., lack of reactive functional groups. Although physical adsorption (e.g., surface coating with chitosan, collagen) [6,7] are simple for surface functionalization, this kind of temporary surface coating and unstable molecular immobilization greatly limited their applications. Alternatively, co-

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solvent electrospinning (e.g., gelatin, Pluronic F-127) [8,9], plasma treatment (e.g., $O_2$, $NH_3$) [10] and surface graft polymerization (e.g. methacrylic acid) [11] showed more stable molecular immobilization. These methods, however, are procedurally hard to adjust for bio-modification of these drug delivery vehicles, probably due to the following reasons: (1) excessive organic solvent dissolving may lead to drug leakage or drug degeneration; (2) multistep procedures may reduce the mechanical strength of fibrous scaffolds; (3) the rigorous reactions of these methods are not available for unstable biomacromolecules, such as growth factors.

To overcome these challenges, we intend to apply a simple and versatile surface modification method based on a mussel-inspired polydopamine (PDA) coating in aqueous condition [12–14]. PDA is easy to deposit via the oxidative self-polymerization of dopamine at slightly basic pH (8–8.5) onto virtually any type and shape of surface [15–17]. Under slightly basic conditions, the catechol groups (in dopamine) equilibrate to o-quinones that are extremely reactive to nucleophilic functional groups such as amines and thiols via Schiff base or Michael addition reactions [18]. By using this mild method, various ligand molecules with amine and thiol groups have been steady immobilized on the surface including cellulose papers [13], metals [19], glass [20], polymer membrane [21,22], nanoparticles [23] and even yeast cells [24]. Due to the simplicity, versatility and tenderness, we hypothesized that the mussel-inspired polymerization method can be employed to bio-functionalize the surface of drug-loaded fibrous scaffolds, which will maximally maintain the properties of the scaffolds as well as the drug bioactivity and loading ratio, and thus such method could overcome the drawbacks involved in the traditional bio-functionalization processes.

In this study, we demonstrated that the surface of electrospun PLGA fibrous scaffold loading with drugs could be efficiently modified with various biomolecules (i.e., biocompatible polymer PEG, basic fibroblast growth factor bFGF and cell adhesive peptide RGD) via the mussel-Inspired PDA coating (Scheme 1). By virtue of the mild and efficient nature of this approach, the original properties of the fibrous scaffolds, e.g., the mechanical properties, chemical structures, and the morphology, did not show significant changes. Also, cell behaviors on the fibrous scaffold could be flexibly regulated according to the immobilization of biomolecules, for example, PEG for cell repellent, RGD peptide for cell adhesion and bFGF for cell proliferation (Scheme 1). Using this method, a bioactive drug 20(R)-ginsenoside Rg3-loaded PLGA fibrous scaffold with surface immobilized growth factor bFGF were easily obtained. Moreover there were only negligible changes in the drug activity and drug loading ratio. Our previous studies showed that the drug Rg3 has shown a property to inhibit hypertrophic scars (HS) formation [25–27] and bFGF has the potential to accelerate wound healing and consequently be beneficial to prevention of HS formation [28,29]. But the combination of drug therapy and bio-signal stimulation in one scaffold for medical application has not yet been studied, probably because there is no efficient method for bio-functionalization of a drug-loaded scaffold currently. Therefore in this study, besides the versatility of the mussel-inspired PDA coating for biofunctionalization, we also explored a medical practicability of this method for inhibiting HS formation and hyperplasia in a rabbit ear model *in vivo* by using a bFGF-modified and Rg3-loaded PLGA fibrous scaffold (Scheme 1). As expected, the detailed investigations in cell biology, histology, and molecular biology confirmed that the synergistic effect of Rg3 drug and bFGF growth factor *in vivo* definitively showed enhanced inhibition of HS formation as compared to a single stimulus by drug or growth factor. The results in this study indicated that the mussel-Inspired approach would provide a facile and effective strategy to combine drug-loading and biofunctionalization in one system, thus facilitating a synergistic effect of drug-therapy and immediate signal stimulation when such kind of biomaterials is used for regenerative medicine.

## 2. Materials and methods

### 2.1. Materials

PLGA (Mw = 100 kDa, Mw/Mn = 1.83) was prepared by bulk ring-opening polymerization of L-lactide using stannous chloride as an initiator (Jinan Daigang Co., Jinan, China). Arg-Gly-Asp (RGD, >97%, CAS: 99896-85-2) and Poly(ethylene glycol) methyl ether amine (PEG-$NH_2$, Mn = 500, CAS: 80506-64-5) were purchased from Sigma–Aldrich company (China). Dopamine hydrochloride (98%) was purchased from Aldrich. Rg3 was purchased from Fusheng Pharmaceutical Ltd. (Dalian, China). Recombinant murine bFGF was purchased from PeproTech Inc. (Rocky Hill, NJ). Ultrapure water (18.3 MΩ/cm) was obtained from a Human Ultrapure System (Human Corp., Korea). 1,1,1,2,2,2-Hexafluoro-2-propanol (HFIP) was purchased from Sigma–Aldrich (USA). All the other chemicals and reagents were of reagent grade, they were all purchased from Guoyao Regents Company (Shanghai, China).

### 2.2. Electrospinning

Rg3 (60 mg) was dissolved in HFIP (2 g) for fabricating drug solution, and PLGA (1 g) was dissolved in dichloromethane (2.5 g) for fabricating polymer solution. The drug solution and the polymer solution were then further mixed to prepare the electrospinning solutions. The electrospinning processes were performed as described previously [1]. Briefly, the electrospinning apparatus was mainly equipped with high-voltage equipments (Tianjing High Voltage Power Supply Co., Tianjing, China) and a precision pump (Lange Supply Co., Baoding, China). A grounded aluminum foil was used as a collector. The electrospun solution was loaded in a 2 mL syringe, which was attached to a circular-shaped metal syringe needle as the nozzle. The parameters of electrospinning were 0.6 mL/h for flow rate, 15 kV for high-votage, 12 cm of collecting distance. All samples were dried with vacuum at room temperature for 24 h before using.

### 2.3. Surface modifying of fibers

PLGA electrospun fibrous scaffolds were immersed in 75% ethanol for 5 h at 25 °C for sterilization. All other solutions were filtrated for sterilization. The PLGA electrospun fibers were pre-coated with PDA through incubating PDA tris-buffer solution using 10 mM Tris, pH 8.5 at 25 °C for 12 h. For surface functionalization, the P–P fibers were immersed into RGD tris-buffer solution (10 mg/mL), PEG-$NH_2$ tris-buffer solution (10 mg/mL) and bFGF tris-buffer solution (10 mg/mL) for 12 h at 4 °C, respectively. The concentration of RGD, PEG-$NH_2$ and bFGF were chosen according to previously published studies [30,31]. The modified electrospun fibrous scaffolds were rinsed with deionized water for 5 times. They were then dried under a stream of argon. The functionalized PLGA fibers were designated as P-P-RGD, P-P-PEG and P-P-bFGF.

Tris-buffer solution was fabricated using 10 mM Tris, pH 8.5 at 25 °C, and series concentrations of PDA solution were fabricated using Tri-buffer solution. Rg3-loaded PLGA electrospun fibers were immersed in mixture solution of 95% PDA solution (different concentration and time) and 5% ethanol (v/v) at 25 °C for grafting PDA on the surface of fibers, and these fibers were rinsed with deionized water for 5 times for further use. bFGF solution were fabricated using Tri-buffer solution at 4 °C. The PDA-modified Rg3-loaded PLGA electrospun fibers were subsequently transferred into a bFGF solution (different concentration and time) at 4 °C. The