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Drug depot-anchoring hydrogel: A self-assembling scaffold for localized drug release and enhanced stem cell differentiation

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ABSTRACT

Localized and long-term delivery of growth factors has been a long-standing challenge for stem cell-based tissue engineering. In the current study, a polymeric drug depot-anchoring hydrogel scaffold was developed for the sustained release of macromolecules to enhance the differentiation of stem cells. Self-assembling peptide (RADA16)-modified drug depots (RDDs) were prepared and anchored to a RADA16 hydrogel. The anchoring effect of RADA16 modification on the RDDs was tested both *in vitro* and *in vivo*. It was shown that the *in vitro* leakage of RDDs from the RADA16 hydrogel was significantly less than that of the unmodified drug depots (DDs). In addition, the *in vivo* retention of injected hydrogel-incorporated RDDs was significantly longer than that of hydrogel-incorporated unmodified DDs. A model drug, vascular endothelial growth factor (VEGF), was encapsulated in RDDs (V-RDDs) as drug depot that was then anchored to the hydrogel. The release of VEGF could be sustained for 4 weeks. Endothelial progenitor cells (EPCs) were cultured on the V-RDDs-anchoring scaffold and enhanced cell proliferation and differentiation were observed, compared with a VEGF-loaded scaffold. Furthermore, this scaffold laden with EPCs promoted neovascularization in an animal model of hind limb ischemia. These results demonstrate that self-assembling hydrogel-anchored drug-loaded RDDs are promising for localized and sustained drug release, and can effectively enhance the proliferation and differentiation of resident stem cells, thus lead to successful tissue regeneration.

1. Introduction

Stem cell based tissue engineering is regarded as a promising therapy for impaired organs [1]. Although some studies have demonstrated successful regeneration effects, there are still obstacles limiting the translation of stem cell-based tissue engineering from bench to bedside. The most common problems are poor cell proliferation and meager differentiation after transplantation [2,3]. These problems are chiefly caused by an insufficiency of growth factors at defect sites in the host body. Moreover, the regeneration process requires a sustained supply of growth factors for a considerable period of time. Therefore, long-term delivery of growth factors is urgently required for successful tissue engineering [4–6].

There is general agreement that growth factors serve as the most powerful impetus for the proliferation and differentiation of stem cells [7,8]. As tissue engineering scaffolds accommodate stem cells, a localized supply of growth factors will exert considerable influence on resident cells. Researchers have strived to develop strategies to deliver growth factors in a sustained and localized manner. The original drug-loaded scaffolds were engineered by simply spraying or encapsulating drug in polymeric or hydrogel scaffolds [9]. Drug release was based on the solubility and diffusion of the drug, which suffered from a burst release and could only be maintained for a few days. Through aptamer or heparin-binding, growth factors could be released in a significantly sustained manner [10,11]. However, because of the intrinsic instability of growth factors, exposure to the scaffold environment might attenuate their bioactivity. Polymeric drug carriers have been widely used as drug depot to encapsulate proteins and control their release at an

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appropriate rate [12,13]. The combination of polymeric particles and scaffolds has received much attention, and has shown great promise in terms of the sustained and localized delivery of proteins. Although numerous hybrid scaffolds, especially polymeric particle/hydrogel composites, have been developed, very few studies have investigated the retention of polymeric particles in a hydrogel [14–16]. Owing to the highly porous structure of hydrogel scaffolds, polymeric particle-based drug depot may be leaked from them [17–19]. For tissue engineering, the leakage of drug-loaded polymeric particles directly depletes the drug reservoir, which attenuates local and effective drug delivery. Therefore, the ideal drug depot/hydrogel hybrid scaffolds should demonstrate only a tiny leakage of the drug carriers.

Various hydrogels have been exploited as scaffolds to encapsulate stem cells or deliver drugs. Among them, self-assembling peptide hydrogels, such as the RADA16 peptide hydrogel, have drawn increasing attention in recent years, because they can closely mimic the natural physiological structure of the extracellular matrix, supporting stem cell proliferation and migration in their microenvironment. Moreover, the RADA16 peptide hydrogel has some unique attributes that make it ideal for tissue engineering: it is generally non-immunogenic, demonstrates gelation through self-assembly in a physiological environment, maintains high water content (up to 99.5% w/v), is suitable for non-invasive administration (*i.e.* injection or void filling), demonstrates low toxicity and explicit composition, and is commercially available (named Puramatrix) [20,21]. Inspired by the self-assembly of the RADA16 peptide, the current study hypothesized that RADA16-modified drug depot will have some interaction with the RADA16 monomer and assemble together with RADA16 fibers to form a hydrogel skeleton, so as to anchor the drug depot to the hydrogel. So far, there have been few studies utilizing the strategy for localized and sustained drug delivery in tissue engineering.

In the present study, a polymeric drug depot was prepared by poly (lactic-*co*-glycolic acid) (PLGA) and a RADA16-modified drug depots (RDDs)-anchoring hydrogel was developed (Fig. 1). To assess the anchoring effect, the leakage of RDDs was assessed *in vitro* and the retention of RDDs was evaluated *in vivo*, compared with that of unmodified drug depots (DDs). The structure of the RDDs-anchoring hydrogel was observed using scanning electron microscopy (SEM). To apply this hybrid scaffold for drug delivery, vascular endothelial growth factor (VEGF) was encapsulated in the RDDs as a model drug, and the long-term release of VEGF was determined within 28 days. As VEGF is a key regulator for the maturation of endothelial progenitor cells (EPCs), EPCs were cultured on these VEGF-loaded hybrid scaffolds in 3-D mode *in vitro* and their proliferation and differentiation of the EPCs were observed. Furthermore, the VEGF-loaded scaffolds combined with EPCs were applied for enhanced neovascularization in a mouse model of hind limb ischemia.

2. Materials and methods

2.1. Materials and animals

Poly (lactic-*co*-glycolic acid) (PLGA, lactic acid:glycolic acid = 50:50; molecular weight 20 kDa) was kindly provided by Evonik, GmbH. (Evonik, Germany). Maleimide-polyethylene glycol (PEG)-PLGA (molecular weight of 22 kDa) and FPR648-labelled PLGA (lactic acid:glycolic acid = 50:50; molecular weight 20 kDa) were purchased from PolySciTech (West Lafayette, IN, USA). Emprove exp. poly (vinyl alcohol) (PVA) 4-88 was provided by Merck, GmbH. (Merck, Darmstadt, Germany). The RADA16 peptide (AcN-RADARADARADARADA-$CONH_2$) and RADA16GGC (RADARADARADARADAGGC) were synthesized by ChinaPeptides, Co., Ltd. (Shanghai, China). VEGF was purchased from Sino Biological Inc. (Beijing, China). A VEGF enzyme linked immunosorbent assay (ELISA) kit was purchased from MultiSciences Biotech Co., Ltd. (Hangzhou, China), while 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR) was obtained from Fanbo biochemical Co., Ltd. (Beijing, China). CD31 and endothelial nitric oxide synthase (eNOS) antibodies were purchased from Abcam Co., Ltd. (UK). CD34, von Willebrand factor (vWF), and α-smooth muscle actin (α-SMA) antibodies were provided by Goodbio Technology Co., Ltd. (Wuhan, China). CyQUANT® NF Cell Proliferation Assay Kit was purchased from Thermo Fisher Scientific (USA). Cell Counting Kit-8 (CCK-8) was obtained from Beyotime (China). Other chemical regents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Human umbilical vein endothelial cells (HUVECs) were obtained from ATCC (USA) and were cultured in Dulbecco's minimum essential medium (Corning) supplemented with 20% fetal bovine serum (Gibco), 1% penicillin (100 IU/mL, Corning), and streptomycin (100 μg/mL, Corning). Four-week-old female BALB/c mice and nude mice weighing 20 ± 2 g were obtained from Sino-British SIPPR/BK Lab. Animal Co., Ltd. (Shanghai, China). All animal experiments were performed according to the Guiding Principles for the Care and Use of Experimental Animals in Fudan University (Shanghai, China). The protocols of the study were evaluated and approved by the Ethical Committee of Fudan University.

2.2. Preparation and characterization of RADA16 peptide-modified VEGF-loaded drug depots

2.2.1. Synthesis and characterization of RADA16-PEG-PLGA

The RADA16GGC peptide was conjugated to PLGA-PEG-maleimide through the reaction of the maleimide group from the PLGA-PEG-maleimide with the SH group from the RADA16 GGC peptide, as described previously [13]. Briefly, 5 mg of RADA16GGC peptide was dissolved in 5 mL of deionized water and 50 mg of PLGA-PEG-maleimide was dissolved in 5 mL of *N*, *N*-dimethylformamide (DMF). Then, the RADA16GGC solution and the PLGA-PEG-maleimide solution were mixed together followed by magnetic stirring at 500 rpm overnight. The resulting RADA-PEG-PLGA solution was dialyzed using deionized water and a dialysis bag (cut-off molecular weight: 5 kDa) to remove the unconjugated RADA16GGC peptide and DMF. Then the RADA-PEG-PLGA was extracted using chloroform and dried using vacuum evaporation. To characterize the synthesized RADA-PEG-PLGA, RADA-PEG-PLGA and PLGA-PEG-maleimide were dissolved in deuterated chloroform and characterized using H Nuclear Magnetic Resonance (HNMR, Ascend 600 MHz, Bruker, Germany).

2.2.2. Preparation and characterization of the V-RDDs

VEGF-loaded RDDs (V-RDDs) were prepared using a double

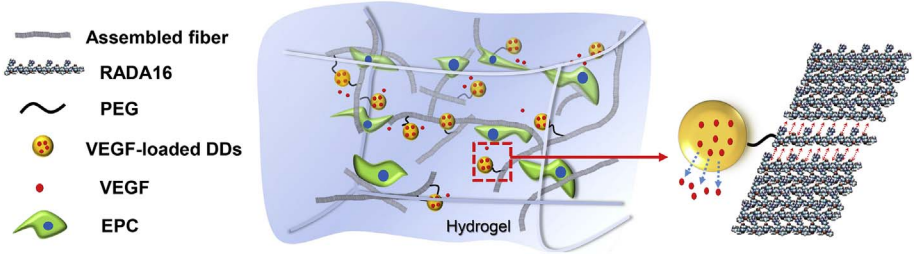

Fig. 1. Schematic illustration of a vascular endothelial growth factor (VEGF)-loaded RDDs-anchoring hydrogel. The RADA16 peptide is the basic self-assembling unit forming fiber and constructing hydrogel; poly (lactic-*co*-glycolic acid) (PLGA) based, VEGF-loaded drug depots (DDs) were modified using the RADA16 peptide (V-RDDs) to anchor them to the skeleton of the hydrogel; PEG was applied as a spacer to ensure the full stretch of the RADA16 peptide. VEGF demonstrated sustained release into the hydrogel to enhance the proliferation and differentiation of resident EPCs.