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Heparin mimetic peptide nanofiber gel promotes regeneration of full thickness burn injury

Fatih Yergoz [a], Nurcan Hastar [a], Cagla Eren Cimenci [a], Alper Devrim Ozkan [a], Turgay Tekinay [b, c], Mustafa O. Guler [a, e, **], Ayse B. Tekinay [a, d, *]

[a] Institute of Materials Science and Nanotechnology, National Nanotechnology Research Center (UNAM), Bilkent University, Ankara, 06800, Turkey
[b] Life Sciences Application and Research Center, Gazi University, Ankara, 06830, Turkey
[c] Department of Medical Biology and Genetics, Faculty of Medicine, Gazi University, Ankara, 06500, Turkey
[d] Neuroscience Graduate Program, Bilkent University, Ankara, 06800, Turkey
[e] Institute for Molecular Engineering, University of Chicago, Chicago, IL, 60637, USA

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ABSTRACT

Burn injuries are one of the most common types of trauma worldwide, and their unique physiology requires the development of specialized therapeutic materials for their treatment. Here, we report the use of synthetic, functional and biodegradable peptide nanofiber gels for the improved healing of burn wounds to alleviate the progressive loss of tissue function at the post-burn wound site. These bioactive nanofiber gels form scaffolds that recapitulate the structure and function of the native extracellular matrix through signaling peptide epitopes, which can trigger angiogenesis through their affinity to basic growth factors. In this study, the angiogenesis-promoting properties of the bioactive scaffolds were utilized for the treatment of a thermal burn model. Following the excision of necrotic tissue, bioactive gels and control solutions were applied topically onto the wound area. The wound healing process was evaluated at 7, 14 and 21 days following injury through histological observations, immunostaining and marker RNA/protein analysis. Bioactive peptide nanofiber-treated burn wounds formed well-organized and collagen-rich granulation tissue layers, produced a greater density of newly formed blood vessels, and exhibited increased re-epithelialization and skin appendage development with minimal crust formation, while non-bioactive peptide nanofibers and the commercial wound dressing 3M™ Tegaderm™ did not exhibit significant efficiency over sucrose controls. Overall, the heparin-mimetic peptide nanofiber gels increased the rate of repair of burn injuries and can be used as an effective means of facilitating wound healing.

1. Introduction

Burn injuries are the fourth most common type of trauma worldwide, and third-degree burns constitute one of the most severe injuries of the skin. Although recent developments in post-injury care have greatly improved the survival and recovery rates of patients suffering from severe burns, the considerable length of the recovery process and the concurrent risk of infection contribute to patient mortality in burn injuries [23]. Even under ideal conditions, the formation of scar tissue follows wound closure, and may compromise the appearance and functionality of the healed tissue [12]. In addition, burn wounds are subject to a higher risk of necrosis compared to lacerations and blunt trauma injuries due to the oxidative and inflammatory stresses exerted by the zone of coagulation [25,30]. Consequently, untreated burn injuries often experience a period of progression where viable tissues adjacent to necrotic regions are themselves converted into necrotic tissue, increasing the depth and diameter of the injury [26,31]. Severe burn injuries are also associated with a rapid and systemic immune response that may result in septic shock due to a combination of increased capillary permeability, an excess of proteins in the interstitial space, and the overexpression of pro-inflammatory

* Corresponding author. Institute of Materials Science and Nanotechnology, Neuroscience Graduate Program, National Nanotechnology Research Center (UNAM), Bilkent University, Ankara, 06800, Turkey.
** Corresponding author. Institute for Molecular Engineering, University of Chicago, Chicago, IL, 60637, USA.
E-mail addresses: mguler@uchicago.edu (M.O. Guler), atekinay@bilkent.edu.tr (A.B. Tekinay).

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factors by macrophages [6]. Taken together, these factors necessitate the development of novel techniques that specifically address the unique pathophysiological needs of burn injuries, especially in the context of halting burn wound progression and facilitating tissue repair with minimal scar formation [29].

Hydrogels are effective materials for the treatment of burn injuries due to their water-retaining properties, which allow them to modulate the fluid balance at the site of injury and provide an environment similar to that present in native skin tissue. They can be functionalized with bioactive or antimicrobial agents to prevent secondary infections or accelerate the wound repair process [12]. Self-assembled peptide nanofiber gels contain bioactive sequences that effectively replicate the functions of many extracellular matrix proteins, allowing their use for the repair of a broad range of tissue injuries [10,22,24]. However, the function of peptide nanofiber systems depends strongly on their sequence, with minimal alterations resulting in major changes in toxicity, bioactivity and assembly kinetics [17,37]. Previously, peptide gels bearing the RGD-like RADA16 and ILVAGK/LIVAGK sequences were used as wound dressings for burn and laceration injuries [11,15], and we recently reported that heparin-mimetic peptide nanofibers (HM-PA, lauryl-VVAGEGD(K-psb)S-Am) increase re-epithelialization and granulation tissue formation in a rat acute skin wound model [35]. In contrast, a non-bioactive control gel (bearing a similar secondary structure but no heparin-mimetic motifs) provided no improvement over sucrose-treated controls, suggesting that the sequence (rather than the presence of the gel as a scaffold) was effective in promoting wound healing. HM-PA was shown to stimulate angiogenesis in vivo and exhibit a strong affinity to the growth factors VEGF, HGF, and FGF-2 in vitro, which is related to its ability to enhance wound repair [13]. However, post-injury progression of tissue necrosis, local accumulation of inflammatory cytokines, rapid fluid loss from blood vessels at the site of injury and an extended period of inflammation complicate the recovery of burn injuries, limiting the applicability of wound healing scaffolds in their treatment [34]. Consequently, it is interesting to use same peptide system for promotion of wound recovery under the unique microenvironmental conditions associated with burn wounds.

In the present study, we show that HM-PA nanofiber gels facilitate the recovery of burn injuries caused by a thermal burn model developed for the evaluation of topical treatment methods. Wound regeneration was monitored through histological observations, immunostaining and marker protein expressions across 21 days of recovery, and blood vessel densities, collagen organization and skin appendage formation were quantified to determine the effect of bioactive PA treatment on the repair process. HM-PA treatment was observed to enhance angiogenic activity during early regeneration; increase wound closure, re-epithelialization and granulation tissue formation rates, and promote the formation of skin appendages compared to non-bioactive peptide nanofibers, sucrose and 3M™ Tegaderm™ wound filler, suggesting that the HM-PA gel can be utilized as a potential platform for the treatment of burn wounds in addition to laceration injuries.

2. Materials and methods

2.1. Materials

9-Fluorenylmethoxycarbonyl (Fmoc) and tert-butoxycarbonyl (Boc) protected amino acids, lauric acid, 4-(2′,4′-dimethoxymethyl-Fmoc-aminomethyl)-phenoxyacetamido-norleucyl-MBHA resin (Rink amide MBHA resin), Fmoc-Asp(OtBu)-Wang resin, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and diisopropylethylamine (DIEA) were purchased from NovaBiochem (Merck) and all other chemicals are purchased from Sigma-Aldrich, Thermo scientific, Invitrogen and Fisher. Antibodies used in this study were purchased from Millipore and Abcam.

2.2. Synthesis and purification of molecules and gel formation

An Fmoc solid phase peptide synthesis method was followed for the synthesis of peptide amphiphile (PA) molecules. Two different solid supports were used for peptide synthesis: The positively-charged K-PA (lauryl-VVAGK-Am) and negatively-charged HM-PA (Lauryl-VVAGEGD(K-psb)S-Am) were fabricated using Rink amide MBHA resin, while the other negatively-charged PA (E-PA (lauryl-VVAGE)) was synthesized on Fmoc-Glu(OtBu)-Wang resin. Synthesis protocols were identical to these provided by Uzunalli et al. [35]. The mass and purity of molecules were assessed with liquid chromatography–mass spectroscopy (LC–MS). LC-MS data were obtained with an Agilent LC-MS equipped with Agilent 6530 Q-TOF with ESI source and Zorbax SB-C8 4.6 mm × 100 mm column for acidic conditions and a Zorbax Extend-C18 2.1 × 50 mm column for basic conditions. Mobile phases of each column were a gradient of water and acetonitrile containing 0.1% formic acid (for basic peptides) or 0.1% NH_4OH (for acidic peptides). Residual TFA adhering to positively charged PAs was removed by treatment with HCl solution, and the remaining peptide residue was lyophilized. An Agilent 1200 series preparative reverse-phase high performance liquid chromatography (HPLC) system was used for the purification of molecules and was equipped with a Zorbax Extend-C18 21.2 × 150 mm column for basic conditions and a Zorbax SB-C8 21.2 × 150 mm column for acidic conditions. As the mobile phases of each column, a gradient of water and acetonitrile containing 0.1% TFA or 0.1% NH_4OH was used according to charge of conditions.

In order to enable nanofibrous gel formation through charge neutralization, each oppositely-charged PA was reconstituted in 0.25 M sucrose solution prepared in ultrapure water and mixed at the molar ratio of 1:2 for the HM-PA/K-PA combination (resulting in a bioactive PA mixture) and 1:1 M ratio for the E-PA/K-PA (resulting in a non-bioactive control PA mixture). Total charges of both mixtures were −1. The pH of peptide solutions was adjusted to 7.4 and peptide mixtures were sterilized with UV light for 1 h prior to use.

2.3. Physical and mechanical characterization of self-assembled peptide nanofiber networks

2.3.1. Scanning electron microscopy (SEM)

Nanofiber networks formed by HM-PA/K-PA (1:2 M ratio) and E-PA/K-PA (1:1 M ratio) were inspected through SEM. Samples for SEM were prepared by incubating peptide nanofiber gels (1 wt %) with a final volume 60 μL for 20 min on silicon wafers. The peptide nanofiber gels were then dehydrated by sequential treatment with increasing concentrations of ethanol (20%, 40%, 60%, 80% v/v) for 2 min per wash and left in 100% absolute ethanol until drying in a critical point drier (Tousimis, Autosamdri-815B). Before the imaging process, samples were coated with 6 nm of Au/Pd layer and images were taken with a FEI Quanta 200 FEG scanning electron microscope under high vacuum with 5 kV beam energy.

2.3.2. Oscillatory rheology

An Anton Paar Physica RM301 rheometer operating with a 25 mm parallel plate at 21 °C was used for oscillatory rheology measurements. In order to measure storage (G′) and loss (G″) moduli, HM-PA or control nanofiber gels (1 wt %) with total volume of 300 μL were loaded on the center of the lower plate and incubated for 15 min prior to measurement while upper plate was brought to a 0.5 mm gap position. While taking storage moduli (G′) and loss moduli (G″) measurements, angular frequency was