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Efficacy of gelatin gel sheets in sustaining the release of basic fibroblast growth factor for murine skin defects

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ABSTRACT

Background: Gelatin has been used as a material sustaining the release of basic fibroblast growth factor (bFGF), which promotes fibroblast proliferation and capillary formation and accelerates wound healing. In the application of these materials, bFGF is impregnated immediately before application, and it is difficult to conform the shape to the wound. In this study, we prepared a pliable and plastic gelatin gel sheet (GGS) that sustains bFGF and conforms to the shape of the wound as a result of cross-linking just before application. In addition, we examined the sustained release profile of bFGF from GGS and its effect on wound healing in murine skin defects.

Materials and methods: A 13-wt% gelatin solution was mixed with bFGF before cross-linking with 1% glutaraldehyde solution. GGSs impregnated with 7 μg/cm^2 of bFGF were incubated in phosphate-buffered saline and collagenase solution, and GGS degradation and bFGF release were evaluated. In the murine experiments, GGSs treated without bFGF and GGSs impregnated with 1, 3.5, 7, or 14 μg/cm^2 of bFGF were applied to full-thickness skin defects created on the backs of C57BL/6JJcl mice, and the wound closure, epithelial length, extent of granulation tissue and capillary formation were compared.

Results: bFGF was released according to the degradation of GGS in phosphate-buffered saline, and the remaining bFGF was released in collagenase solution. In the animal studies, epithelialization was accelerated in the GGSs treated with 1 and 3.5 μg/cm^2 of bFGF, and granulation tissue formation and angiogenesis were promoted based on the amount of bFGF impregnated into the GGS.

Conclusions: GGS impregnated with bFGF is capable of sustaining the release of bFGF, with consequent accelerated epithelialization, granulation tissue formation, and angiogenesis *in vivo*. GGS is a novel and promising wound dressing that sustains bFGF and can be adapted to the shape of various wounds in the treatment of both acute and chronic wounds.

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1. Introduction

Although wound treatment has made progress in recent years, chronic skin ulcers, such as diabetic foot ulcers, venous leg ulcers, ischemic ulcers, and decubitus, are still difficult to treat and are costly problems in health care (Table) [1,2]. In the United States alone, an excess of US $25 billion is spent annually on the treatment of chronic skin ulcers [3]. At present, several growth factors are clinically available for the treatment of chronic skin ulcers [4–6].

Basic fibroblast growth factor (bFGF), first identified in 1974 by Gospodarowicz, promotes fibroblast proliferation and capillary formation and accelerates wound healing (Fig. 1) [7–10]. In Japan, human recombinant bFGF (FIBRAST SPRAY; Kaken Pharmaceutical, Tokyo, Japan) has been used clinically for the treatment of chronic skin ulcers since 2001, and its clinical effectiveness has been demonstrated [6]. However, this therapy must be applied daily because of the short half-life and inactivation of bFGF *in vivo*, which is a burden for both the medical staff and patient.

To overcome this issue, various sustained-release systems for delivering bFGF have been developed [11–14]. Among them, gelatin is one of the most common materials used for the sustained release of various growth factors, including bFGF [15–17]. We previously developed a novel scaffold, the collagen/gelatin sponge (CGS), which is capable of providing the sustained release of bFGF, platelet-derived growth factor-BB, and transforming growth factor (TGF)-β and reported the safety and efficacy of CGSs impregnated with bFGF for the treatment of chronic skin ulcers [18–25].

With previous methods, the gelatin carriers are prepared in advance and growth factors, including bFGF, are impregnated just before application *in vivo* or clinically. Therefore, it is difficult to conform the shape of the material to the applied wound. Recently, a novel technique for preparing gelatin gel was reported [26]. In this method, growth factors are incorporated into the gelatin solution, after which gelatin gel containing growth factors is cross-linked with glutaraldehyde (GA). The gelatin gel made using this technique may be an ideal wound dressing, as the gelatin solution is pliable and plastic enough to conform to any shape of wound and/or other sites, including tissue defects, before cross-linking.

Table – The characteristics of acute and chronic wounds.

Characteristics	Wound closure time	Growth factors in wound bed tissue	Cell proliferation and migration
Acute wounds	Rapid	↑	↑
Chronic wounds			
Diabetic foot ulcers			
Venous leg ulcers	Prolonged	↓	↓
Ischemic ulcers			
Decubitus			

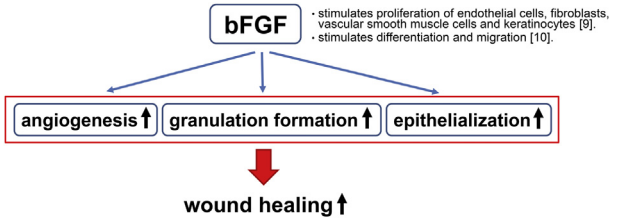

Fig. 1 – The acceleration of wound healing by bFGF. (Color version of figure is available online.)

In this study, we examined the sustained release profile of bFGF in our gelatin gel sheet (GGS) using the previously mentioned method. We then evaluated the effects of the GGSs incorporated with bFGF in treating murine skin defects and explored the appropriate concentrations of bFGF required for wound healing.

2. Materials and methods

2.1. Ethics statement

The animals were maintained at the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University. The number of animals used in this study was kept to a minimum, and all possible efforts were made to reduce their suffering in compliance with the protocols established by the Animal Research Committee of Kyoto University. The animals were anesthetized with isoflurane when they underwent painful procedures. They were sacrificed at the end of the experiment via the inhalation of carbon dioxide gas. Our experimental protocol was approved by the Animal Research Committee (permit number: Med Kyo 14569).

2.2. Preparation of gelatin gels

We used gelatin isolated from the pig dermis with an isoelectric point (IEP) of 5.0 and molecular weight of 99,000 (Nippi, Inc, Tokyo, Japan). Gelatin solutions at concentrations of 6.5, 13, and 26 wt% were prepared by dissolving the gelatin in phosphate-buffered saline (PBS; Life Technologies, Tokyo, Japan) kept at 50°C. The mixture of gelatin solution (300 μL; 6.5, 13, or 26 wt%) and GA solution (20 μL; 0.5, 1, or 2%, respectively) was poured, at a temperature of 50°C, into a cast measuring 12 mm in diameter (Yahata Neji Co, Ltd, Aichi, Japan) and stirred immediately using a pipet tip, after which gelation formation occurred, usually within 20 s after mixing to form a sheet-shaped gelatin gel 12 mm in diameter and approximately 3 mm in thickness, called a GGS in this study (Fig. 2).

The GGSs were evaluated in an elasticity test, bFGF release study, and *in vivo* study; however, they were too thin to measure the breaking strength. Therefore, column-shaped gelatin gels 5 mm in diameter and 12 mm in height were prepared for the compression test.

GGSs of 13 wt% in gelatin concentration were impregnated with 1, 3.5, 7, and 14 μg/cm^2 of bFGF (FIBRAST SPRAY; Kaken