Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Efficacy of Gelatin Hydrogel Impregnated with Concentrated Platelet Lysate in Murine Wound Healing

Sharon Claudia Notodihardjo, MD,[a] *Naoki Morimoto, MD, PhD,*[a,*] *Natsuko Kakudo, MD, PhD,*[a] *Toshihito Mitsui, MD,*[a] *Tien Minh Le, MD,*[a] *Yasuhiko Tabata, PhD, DMedSci, DPharm,*[b] *and Kenji Kusumoto, MD, PhD*[a]

[a] *Department of Plastic and Reconstructive Surgery, Kansai Medical University, Hirakata City, Osaka, Japan*

[b] *Laboratory of Biomaterials, Department of Regeneration Science and Engineering, Institute for Frontier Life and Medical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan*

ARTICLE INFO

Article history:
Received 9 January 2018
Received in revised form
29 June 2018
Accepted 11 September 2018
Available online xxx

Keywords:
Platelet lysate
Gelatin hydrogel
Long-term preservation
Growth factors
Biomaterial
Wound healing

ABSTRACT

Background: The efficacy of a gelatin hydrogel (GH) sheet impregnated with platelet-rich plasma in full-thickness wound healing has been reported. Human platelet lysate is another potential natural product for use in wound healing. The present study examined the effects of a GH sheet impregnated with concentrated freeze-dried platelet lysate on wound healing after storage for 9 mo.

Materials and methods: Platelet concentrates were subjected to three freeze-thaw cycles and freeze-dried then preserved at 4°C. Reconstitution with saline was then performed to produce 1-fold (hPL1), 2-fold (hPL2), and 3-fold (hPL3) concentrations of preserved platelet lysate. Full-thickness wounds were made on the back of male C57Bl6J/Jcl mice. Wounds were treated with saline, hPL1, or a GH sheet impregnated with saline, hPL1, hPL2, or hPL3. Histologic examinations using hematoxylin–eosin, Azan, and anti-CD31 staining were performed on days 4, 7, and 14 to assess neoepithelialization and granulation tissue and capillary formation.

Results: This study showed that the GH sheet itself or the simple administration of hPL1 did not accelerate the healing process. However, the GH sheet impregnated with hPL1 accelerated the granulation tissue formation to some extent, and the GH sheet impregnated with hPL2 or hPL3 clearly accelerated the capillary formation and the granulation tissue formation. In addition, the GH sheet impregnated with hPL3 had the longest epithelium formation.

Conclusions: A GH sheet impregnated with long-term preserved 2-fold or 3-fold concentrated platelet lysate enhances the wound healing process.

© 2018 Elsevier Inc. All rights reserved.

* *Corresponding author.* Department of Plastic and Reconstructive Surgery, Kansai Medical University, 2-5-1 Shin-machi, Hirakata City, Osaka 573-1010, Japan. Tel.: +81 72 804 0101; fax: +81 72 804 2031.

E-mail address: morimotn@hirakata.kmu.ac.jp (N. Morimoto).

0022-4804/$ – see front matter © 2018 Elsevier Inc. All rights reserved.

https://doi.org/10.1016/j.jss.2018.09.037

Introduction

Platelets are anucleated cells that initiate the wound healing process[1] by releasing growth factors from their alpha granules, such as platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), insulin-like growth factor I, and epidermal growth factor.[2,3] These growth factors are released from platelets when they are activated by stimuli or aggregated by some activators.[2] Many researchers and clinicians have adapted these roles of platelets for cell growth supplement and potential clinical treatment, such as wound healing.[4-6]

Platelet lysate is the cocktail of growth factors prepared from healthy donor human platelets and has been used mainly in *in vitro* experiments as supplements for cell culture.[4,7-10] The attractiveness of platelet lysate is that it can be prepared either from fresh platelets or expired platelet products and is superior to platelet-rich plasma (PRP) with respect to clinical versatility.[4,7] We previously focused on expired platelet concentrate products, as platelet concentrate products have a relatively short shelf life of around 5-7 d and a substantial amount of platelet concentrate products expire and must be discarded. Therefore, using expired platelet products as a source of growth factors is a good way to make use of a product that would otherwise be discarded.[4]

The concentrations of growth factors contained in platelet lysate can be altered in the reconstitution process after freeze-drying, and we used this method for platelet lysate for up to a 4-fold concentration in a previous study.[7] Regarding the inactivation of growth factors after the administration *in vivo*, bioscaffolds that can sustain the release of growth factors in the site have been reported.[11,12] We previously reported that a gelatin hydrogel (GH) sheet with an isoelectric point of 5.0 can ionically sustain the release of positively charged growth factors, such as PDGF, TGF-β, and bFGF, and the combination of a gelatin scaffold and growth factors can effectively promote wound healing in murine and rabbit models, as well as in clinical trials.[2,5-7,13-15]

In this study, we prepared preserved freeze-dried platelet lysate from expired irradiated platelet concentrate. We then evaluated their bioactivity and explored their optimum concentration in combination with a GH sheet to accelerate wound healing in a murine model.

Materials and methods

Preparation of GH sheets

GH sheets were prepared by the Department of Regeneration Science and Engineering Lab. of Biomaterials, Institute for Frontier Medical Sciences, Kyoto University. In brief, acidic gelatin with an isoelectric point 5.0 and molecular weight of 99,000, isolated from bovine bone (Nitta Gelatin Co, Osaka, Japan) was used.[6,16] First, gelatin in 5% (w/v) aqueous solution was cross-linked with glutaraldehyde at 4°C for 12 h in polystyrene dishes. The resulting hydrogel sheets were then immersed in a glycine aqueous solution for 1 h at 37°C to block the residual aldehyde groups of glutaraldehyde. The hydrogel sheets were then rinsed with double-distilled water followed by freeze-drying. The dried hydrogels were sterilized by ethylene oxide gas.

Preparation of concentrated hPL

We prepared platelet lysate from expired irradiated platelet concentrate, leukocytes reduced (Japanese Red Cross Society).[4] The expired platelet concentrate was supplied by Kansai Medical University Hospital at the expiration date. Our experiment protocol was approved by the ethics committee of Kansai Medical University with approval No. 1427.

Platelet concentrate was snap-frozen to −80°C and then incubated at 37°C. We performed a freeze-thaw cycle three times to lyse the platelets and release the growth factors.[8] We then centrifuged the lysate at 3000 *g* for 30 min and filtered the supernatant through a 0.22-μm filter unit (Millex-GP; Merck Millipore Ltd, Bedford, MA). After filtration, 2 U/mL of heparin sodium (5000 units/5 mL; Mochida Pharmaceutical Co, Tokyo, Japan) was added to prevent gelatinization, and then the solution was stored at −80°C.[7] Freeze-dried platelet lysate was prepared using a freeze-dryer (FDU 2200; Tokyo Rikakikai Co, Ltd, Tokyo, Japan) according to the manufacturer's instructions and stored at 4°C for 9 mo until use. Freeze-dried platelet lysate that had been preserved for 9 mo then reconstituted to its original volume and was referred to as hPL1 in this study. Besides the original concentration (hPL1), 2-fold concentration (hPL2), and 3-fold concentration (hPL3) were prepared by adjusting the volume of saline for reconstitution, based on the original volume before freeze-drying.[7]

A quantitative analysis of growth factors in concentrated platelet lysate

We evaluated the concentrations of PDGF-BB, VEGF, and TGF-β1 in each reconstituted platelet lysate solution using an enzyme-linked immunosorbent assay (ELISA). We used human PDGF-BB, VEGF, and TGF-β1 Quantikine ELISA kits (DBB00, DVE00, DB100B; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. We prepared 5 μL of samples for PDGF-BB and VEGF and 0.125 μL of samples for TGF-β1 ($n = 8$). Samples for TGF-β1 were acid-activated by 1N HCl and neutralized by 1.2 N NaOH/0.5 M HEPES before the evaluation. Each sample was added to each well of provided 96-well plates coated with antibodies. They were then incubated, washed, and secondary antibody was added to the wells. After adding substrate solution, the optical densities were evaluated at a test wavelength of 450 nm and the reference wavelength at 540 nm using Enspire 2300 Multilabel Reader spectrophotometer (PerkinElmer Japan Co, Ltd, Yokohama, Japan) to evaluate the concentrations.

The evaluation of the bioactivity of platelet lysate

To evaluate the bioactivity of freeze-dried platelet lysate after preservation, we compared the growth of fibroblasts using Dulbecco's modified eagle medium (DMEM) with fetal bovine serum (FBS, Hyclone, Logan, UT) or DMEM with hPL1. We prepared 1000 mL DMEM ("*Nissui*"1; Nissui Pharmaceutical Co, Ltd,

Download English Version:

https://daneshyari.com/en/article/11025256

Download Persian Version:

https://daneshyari.com/article/11025256

Daneshyari.com