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Bone marrow-impregnated collagen matrix for wound healing: experimental evaluation in a microcirculatory model of angiogenesis, and clinical experience

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Summary *Objectives*: This study aims to investigate the effect of collagen matrix impregnated with bone marrow on wound healing angiogenesis in an effective microcirculatory model and to describe our clinical experience.
Methods: We used a skinfold chamber of original design which visualises microcirculation following wound creation on the dorsal skin of the mouse to establish an in vivo experimental model to estimate angiogenesis. Animals were divided into two groups: a bone marrow group ($n=6$) in which bone marrow-impregnated collagen matrix was applied to the wound; and a control group ($n=7$), in which collagen immersed in saline was applied, and functional capillary density was quantified during the repair process.
Results: The increase rate in functional capillary density during wound healing significantly increased in the bone marrow group on days 3, 5 and 7 after creation of the wound but no significant difference was detected on day 10. A patient with a chronic leg ulcer that had not responded to conventional therapy for 1 year was treated with autogenous bone marrow-impregnated collagen matrix and successful wound closure was obtained.
Conclusion: The present study suggested that collagen matrix impregnated with bone marrow significantly promoted the repair process, especially in the early stage. The features of the treatment, including the possible use of a patient's own cells, simple method, immediate application without any processing procedure and preservation of the inclusive potentiality of bone marrow suspension, offer significant advantages in terms of the anticipated routine clinical use.

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Collagen matrix substitute dermis or artificial dermis has been developed and is commercially available for treating deep wounds in which bone and/or tendon are exposed.[1–3] When it is applied to a tissue defect, sprouting capillaries and fibroblasts migrate into the collagen, which acts as a scaffold for regeneration, resulting in induction of angiogenesis and fibroplasia. We have previously demonstrated the efficacy of the collagen matrix for the treatment of deep sacral ulcers.[4]

Several investigators have incorporated beneficial materials for wound healing into collagen matrix, including a pharmaceutical agent,[5] growth factors[6,7] and cultured cells.[8,9] Bone marrow attracted our attention as a possible beneficial material because it contains multipotential progenitor cells and produces growth factors. In the field of plastic surgery, we have already presented experimental evidence of the efficacy of therapeutic angiogenesis by bone marrow cells in salvaging flaps after ischemia-reperfusion injury.[10]

We therefore, planned to use collagen matrix impregnated with bone marrow for the promotion of wound healing. In this study, we developed an excellent in vivo experimental model that visualises microcirculation during wound healing angiogenesis. Employing this model, we assessed the efficacy of bone marrow-impregnated collagen matrix. We also described an experience with use of autogenous bone marrow incorporated in collagen for treatment of a chronic wound.

Materials and methods

Adult male ddy mice (30–40 g) were acclimatised to their holding facility for at least 5 days before the start of the experiment. Animals were handled according to the Principles of Laboratory Animal Care (NIH publication 86–23, revised 1985).

Establishment of the experimental model

We developed an experimental animal model of wound healing angiogenesis using skinfold chamber of our own design, which allows visualisation of the microcirculatory response to a wound.

Skinfold chamber implantation

We used an improved version of our original chamber previously reported.[11] The structure of the chamber (Yasuhisa-koki, Ltd., Japan) is shown in Fig. 1. The new chamber contains a plastic sheath (inside diameter: 8.0 mm) into which a transparent plastic cap can be inserted and fixed with screws.

The skinfold chamber was inserted under general anaesthesia with isoflurane (inhalation anaesthesia). The dorsal skin was pulled up and fixed to form a skinfold. A square area of one layer of skin (about 7 mm on one side) was removed, and the remaining layer was covered with the coverglass built into one of the frames of the chamber (Fig. 2). To allow the animals to become accustomed to the skinfold chamber, they were not used in experiments until after more than a 48-h recovery period.

Wound creation (Fig. 3)

The area selected for the wound on the intact side of the folded skin (opposite the coverglass) was marked with a round-shaped ink stamp having a diameter of 1.6 mm. A circular area of skin was then excised down to, but not including, the underlying striated skin muscle layer using microforceps and scissors under an operating microscope. The preparation provided a full-thickness dermal wound having a surface area approximately 2 mm^2 and a depth of approximately 0.3 mm. The wound was covered with a dressing and the plastic cap was used to fix the dressing in place throughout the experimental period.

Observation and recording of the microcirculation

The microcirculation in the chamber was inspected with a stereo intravital microscope (SZH10, Olympus, Japan) through a charged coupled device (CCD) colour video camera (DXC-107 a, Sony, Japan). To achieve sufficient transillumination of

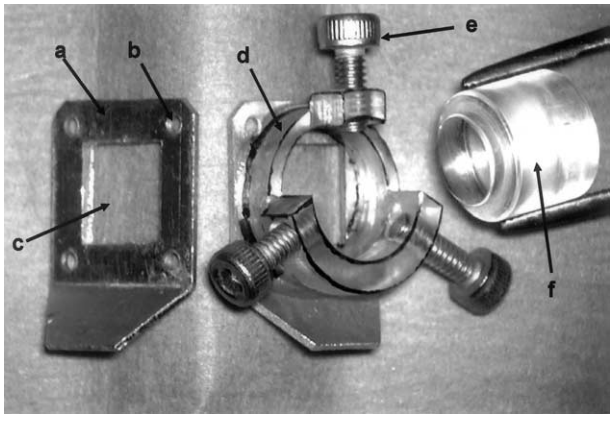

Figure 1 Appearance of the new chamber. The chamber consists of two aluminum frames held together by four polyethylene tubes. One frame is equipped with a plastic sheath and three small screws, and the other is furnished with a glass window for observation. (a) Aluminum frame. (b) Hole drilled for polyethylene tube to hold the two frames together. (c) Glass window for intravital microscopic observation. (d) Plastic sheath. (e) Fixing screw. (f) Plastic cap.