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Comparative histologic and immunologic evaluation of 1,4-butanediol diglycidyl ether crosslinked *versus* noncrosslinked acellular swim bladder matrix for healing of full-thickness skin wounds in rabbits

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ABSTRACT

Background: Collagen-rich extracellular matrix from land-based mammalian tissues is increasingly used in regenerative medicine. However, its uses are associated with risk of disease transfer and may carry an ethnocultural stigma. In the present study, collagen-rich acellular swim bladder matrix (ASBM) from Rohu fish was prepared using sodium deoxycholate and crosslinked with 1,4-butanediol diglycidyl ether (BDDGE). Wound healing potential of ASBM and ASBM-BDDGE was compared in full-thickness skin wounds in rabbits.
Materials and methods: Four full-thickness skin wounds (20×20 mm^2 each) were created on the dorsum of 18 rabbits and randomly divided into three equal groups. Wounds were left open, repaired with ASBM and ASBM-BDDGE in groups sham (I), ASBM (II), and ASBM-BDDGE (III), respectively. Planimetry, contracture, immunologic, and histologic observations were carried out to evaluate wound healing.
Results: Significantly ($P < 0.05$) lesser wound contraction was observed in ASBM (II) and ASBM-BDDGE (III) groups compared with sham (I) group. Total immunoglobulin G response in rabbit sera was decreased significantly ($P < 0.05$) in the ASBM-BDDGE (III) group compared with ASBM (II) group by enzyme-linked immunosorbent assay. Stimulation index of peripheral blood lymphocytes was decreased significantly ($P < 0.05$) in the ASBM-BDDGE (III) group compared with ASBM (II) group by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Histologically, improved epithelialization, neovascularization, fibroplasia, and best arranged collagen fibers were observed in ASBM (II) and ASBM-BDDGE (III) groups as early as on postimplantation day 21.
Conclusions: Findings of this study indicate that BDDGE crosslinked ASBM derived from Rohu fish has potential for the clinical applications. Furthermore, it is expected that their clinical applications will not be limited by ethnocultural stigma.

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1. Introduction

Full-thickness skin wounds resulting from various pathologies and trauma pose a formidable challenge to the clinician [1]. They cannot get repaired autologously in adult mammal because of lack of the dermis [2,3]. Autologous skin remains the treatment of choice for permanent closure in such wounds [4]. They are the most accurate means of recapitulating both the biologic and mechanical properties of lost skin. However, limited availability of autologous skin in large injuries and donor site morbidity after skin excision limit their use [5–7]. Allografts and xenografts are thus opened up as alternate options for closure of such wound cases. But they are restricted because of immunologic rejections and chances of disease transfer [1]. To overcome such problems, synthetic matrices have been investigated but failed to elicit biological cues similar to biological matrices. Besides, synthetic matrices are also nonbiodegradable and their mechanical properties are different from the skin [6]. Hence, there is a need of a proper dermal substitute that functions as a temporary template during healing of full-thickness skin wounds.

Collagen-rich matrices prepared after removal of cellular components from native tissues are currently being used to facilitate wound healing and tissue regeneration. Those matrices provide a native framework for cell adhesion at the site of tissue defect and allow local cells to migrate into the matrix and adhere before undergoing differentiation [8]. Collagen-rich matrices are slowly degraded by cellular proteases at the implantation site and are replaced by new endogenous extracellular matrix (ECM) proteins secreted by ingrowing fibroblasts. Furthermore, the matrices stimulate rapid neovascularization during tissue regeneration [9] and are relatively inert immunologically [10]. They are resistant to infections [11–14], a feature that may be attributable to their inherent antimicrobial activity [15] and their ability to rapidly vascularize, therefore clear bacteria. They have been traditionally prepared from native tissues of land-based mammalian species [12–14,16–19]. However, its biomedical uses are associated with a risk of disease transfer [20] and may also carry an ethnocultural stigma. Fish processing wastes such as swim bladder may serve as economical, viable, and safer alternative for collagen-rich matrices to mammalian sources. Moreover, the environmental issue related to pollution from fish wastes can also be addressed [21].

The swim bladder tissues are mainly composed of fibrillar or type I collagen (80%) [22] and multilayered transitional epithelial cells. Previously, antigenic epitopes associated with the cellular elements have been shown to elicit proinflammatory response and overt immune–mediated rejection of the tissues [23]. Hence, the removal of antigenic epitopes is necessary to minimize or avoid an adverse immunologic response by xenogeneic recipients [24]. However, even after a complete extraction of cellular proteins, a cross-species response was appreciated after use of xenogenic acellular tissues [23]. Crosslinking is another technique that is used to reduce the cross-species immune response toward the structural proteins [10,25]. Despite having high collagen content, the swim bladder tissue has not been investigated till date to the best of our knowledge.

The wound healing effects of biologic scaffolds are generally evaluated in full-thickness skin animal models including rabbits [18,26,27]. Rabbits possess a subcutaneous panniculus carnosus muscle that contributes to skin wound healing by both contraction and reepithelialization, whereas reepithelialization is the only mechanism of skin wound healing in humans. Despite differences in wound healing pattern with humans, rabbits are often used as full-thickness skin wound model for its ready availability, low costs, ease of handling, and postoperative care [28]. To mimic the skin wound healing pattern in rabbits, panniculus carnosus muscle is generally being excised [18,26,27].

Considering the previously mentioned reports, in the present study, we investigated the healing potential of collagen-rich acellular swim bladder matrix (ASBM) in tissue repair and regeneration in rabbit. We also crosslinked ASBM with epoxy compounds to examine whether it reduces immunogenicity further. Besides, we compared the healing potential between ASBM and 1,4-butanediol diglycidyl ether (BDDGE) crosslinked ASBM–implanted full-thickness skin wounds in a rabbit model.

2. Materials and methods

2.1. Chemicals and reagents

Standard chemicals and reagents were obtained from Sigma–Aldrich (St. Louis, MO) unless otherwise noted.

2.2. Animals and ethics statement

The protocols used in this study were approved by the Institute Animal Ethics Committee of the Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. The National Institutes of Health "Guide for the Care and use of Laboratory Animals" was followed in all animal experiments. All efforts were made to reduce the number of animals and minimize the animal suffering. Eighteen New Zealand white rabbits (*Oryctolagus cuniculus*) of either sex (aged 5–6 mo, 1.3–2.2 kg) were procured from the Laboratory Animals Resource section of the Indian Veterinary Research Institute. Animals were housed individually in rabbit cages maintained at 24°C and 55%–65% humidity with 12–12 h light–dark cycle. The rabbits had free access to food and water. The animals were acclimatized to laboratory conditions for 10 d before wounding.

2.3. Preparation of ASBM and crosslinking with epoxy compounds

Preparation and crosslinking of ASBM from Rohu fish (*Labeo rohita*) were based on previous reports [29,30]. The representative images of Rohu fish, native swim bladder, and hematoxylin and eosin (H&E)-stained section of native swim bladder and ASBM (×40 magnification and scale bar 100 μm) are presented in Figure 1. Briefly, swim bladder of Rohu fish (a fresh water fish) was collected under clean conditions, transferred to the laboratory in chilled (4°C) sterile phosphate-buffered saline (PBS, pH 7.4) supplemented with 0.1% amikacin and 0.02% ethylenediaminetetraacetic acid, and immediately processed.

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