

Suppression of α Smooth Muscle Actin Accumulation by Bovine Fetal Dermal Collagen Matrix in Full Thickness Skin Wounds

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Abstract: The suppression of elements associated with wound contracture and unfavorable scarring is a potentially important strategy in clinical wound management. In this study, the presence of α smooth muscle actin (α SMA), a protein involved in wound contraction, was analyzed in a series of wounds in which bovine fetal collagen (BFC) acellular dermal matrix (PriMatrix) was used in staged split thickness skin graft procedures. The results obtained through histological and quantitative image analyses of incidental biopsies from these wounds demonstrated a suppression of α SMA in the wound regions occupied by assimilated BFC relative to increased levels of α SMA found in other areas of the wound. The α SMA levels found in assimilated BFC were similar to α SMA levels in uninjured human dermis. These findings suggest a mechanism by which application of BFC could decrease contraction of full thickness skin wounds.

Key Words: fetal bovine collagen, dermal tissue generation, full thickness skin reconstruction

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Clinical use of bovine fetal collagen (BFC) acellular dermal matrix has included BFC as an initial coverage during 2-stage split thickness skin graft (STSG) reconstructions of scar contractures.¹ These procedures have resulted in functional contracture releases and durable, supple coverage. Analyses of wound bed tissue at the time of second-stage STSG application have shown cellular repopulation and revascularization of the BFC. The BFC implant has been found to mature into a tissue with identifiable characteristics of dermis, namely, a dermal collagen extracellular matrix (ECM) architecture, fibroblast density, and vasculature.^{1–3}

In further study of the effects of BFC on tissue generation in the wound bed, we have analyzed the cellular expression of the contractile protein α smooth muscle actin (α SMA) in biopsies of wounds initially covered with BFC as a first stage leading to secondary STSG coverage.

MATERIALS AND METHODS

In this clinical series, patients with complex wounds including scar contractures, failed skin grafts, and chronic wounds underwent full thickness excision with the resulting raw surfaces covered with BFC (PriMatrix; TEI Biosciences, Boston, MA). The BFC was meshed at a 2:1 ratio, expanded, and fixed to the wound margins with chromic sutures. Simple dressings of nonadherent gauze and antibiotic ointment, secured with gauze and net wrapping, were initially changed 1 to 2 days

after surgery and twice daily thereafter. Range of motion exercises and corrective splinting were instituted for a period of 7 or more days followed by second stage STSG coverage. Marginal wound bed tissues excised at the time of STSG application were processed for analyses.

Histological Staining

Tissue biopsies were fixed and embedded in paraffin. Slides were serially cut for analysis. Hematoxylin-eosin (Polyscientific, Bay Shore, NY) staining was performed to examine the newly generated tissue. Immunohistochemistry was used to distinguish between human ECM and the implanted BFC dermal fibers. Tissue sections were stained with an antibody specific to bovine collagen type I and an FITC-conjugated secondary antibody (Millipore, Billerica, MA).^{4,5} A fluorescent cellular counterstain was used to visualize nuclei within the tissue sections (Hoeschst 34580; Life Technologies, Grand Island, NY). To detect myofibroblasts, an antibody for α SMA (Biocare Medical, Concord, CA) and the Bond Polymer Refine Detection Kit (Leica, United Kingdom) were used and counterstained with hematoxylin.

Histological Analysis

To quantify the presence of α SMA, microscopic images were taken and analyzed using Image J software (<http://imagej.nih.gov/ij/>). Three images in the area of regions having high α SMA expression, and 3 images in the positively identified BFC dermal tissue were taken at $\times 20$ magnification and converted to a binary image using the same thresholding set value. The presence of α SMA was then quantified based on percent of α SMA in the region of interest (0.21 mm^2) for each image and averaged with standard deviation reported. Three controls containing uninjured human skin were also analyzed for the presence of α SMA. Similar to the wound site, 3 images from each control sample were analyzed and the value was reported as an average with standard deviation.

RESULTS

Biopsies from 5 patients were taken before the application of a STSG at 7 to 20 days post-BFC implantation. Hematoxylin-eosin examination showed up to 3 tissue types present within each biopsy (Fig. 1A): (1) dermal ECM repopulated with cells and vasculature, approximately 1 mm thick; (2) granulation tissue containing a high number of thin walled vessels; and (3) bands of aligned connective tissue. No foreign body reaction was noted in all biopsies evaluated. The use of the bovine collagen antibody positively identified the dermal ECM as BFC (Fig. 1). This immunohistochemistry staining revealed a blending of the implanted BFC fibers with newly deposited human ECM (Fig. 1B and C). In the BFC tissue, the collagen fiber architecture and distribution of patient cells and vasculature were comparable to control human dermis.

Visual assessment of the biopsies stained for α SMA revealed differences between the 3 tissue types. The highest expression was noted in the tissue with bands of aligned connective tissue, indicating a contractile phenotype (Figs. 2 and 3). Quantification of the images showed 2 to 5 times more α SMA in the contractile tissue regions than in the regions of assimilated BFC matrix (Fig. 4). Similar levels of α SMA were found in the BFC dermal tissue and uninjured human

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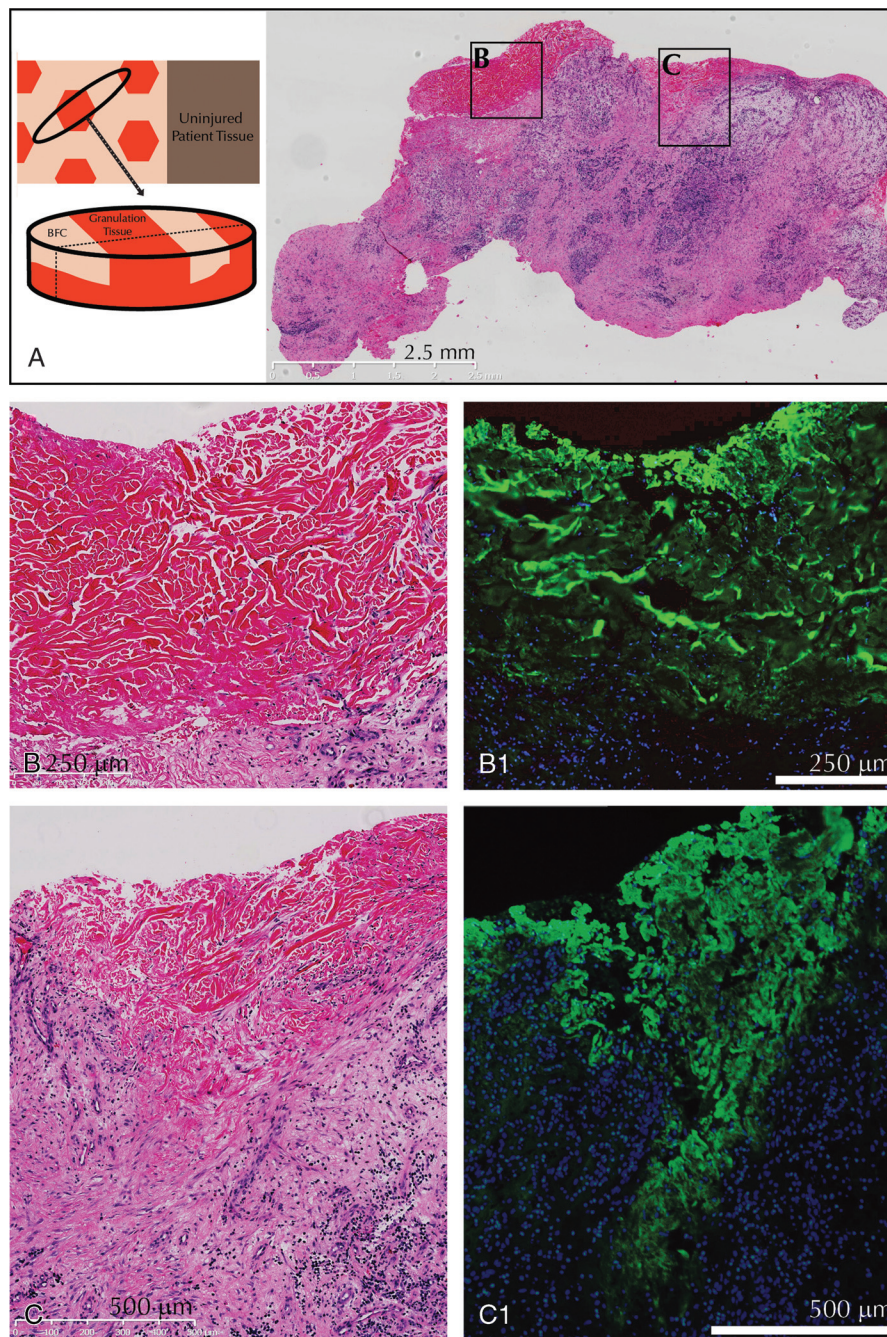


FIGURE 1. Positive identification of BFC fibers. Each biopsy was H&E stained, and IHC was performed to positively identify BFC using an antibody specific to bovine collagen type I. (A) A cartoon of the biopsy location and the appearance of tissue biopsied along with the H&E-stained section demonstrating the presence of 3 tissue types within each biopsy: (1) dermal ECM repopulated with cells and vasculature, approximately 1 mm thick; (2) granulation tissue containing a high number of thin walled vessels; and (3) bands of aligned connective tissue. Regions having a dermal extracellular matrix were confirmed as BFC with positive identification seen in B1 and C1 (green: bovine collagen type I). Patient cells (blue: cellular nuclei) were seen distributed throughout the implanted collagen fibers. H&E indicates hematoxylin-eosin; IHC, immunohistochemistry.

dermis with the expression of the protein primarily associated with the vasculature found within the dermal collagen fibers (Figs. 3 and 4).

DISCUSSION

Histological characterization of tissue generated post-BFC implantation in this and other studies demonstrate the ability of the BFC

matrix to be repopulated with patient cells and vasculature producing a dermal tissue similar to native human dermis.¹⁻³ In addition to evaluating this BFC dermal tissue, this study analyzed the granulation and contractile tissue that developed within the interstices of the meshed BFC as well as deep to the BFC implant. Quantification of these tissues revealed that the BFC dermal tissue had similar levels of the contractile

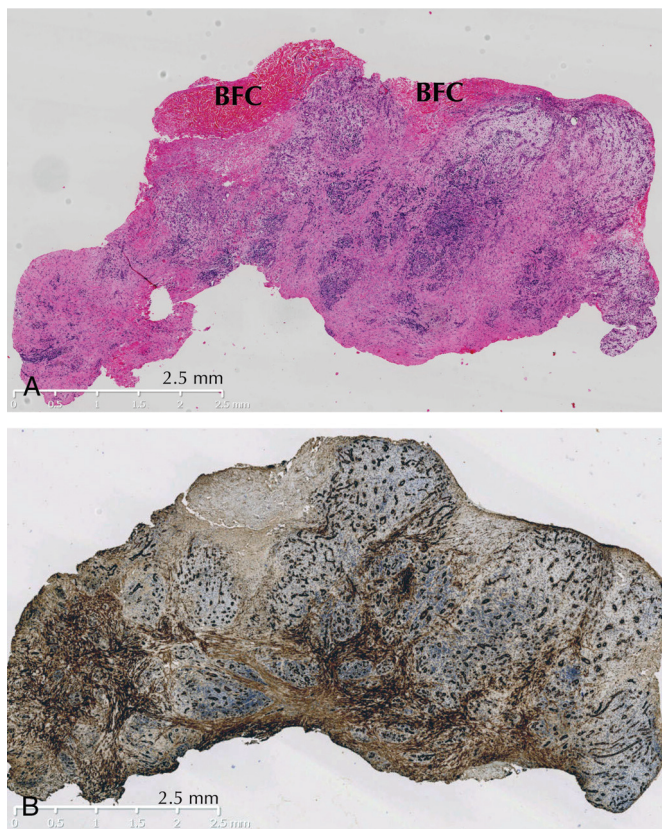


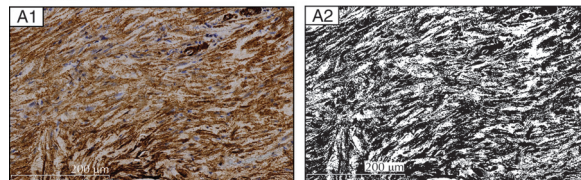
FIGURE 2. α -smooth muscle actin IHC staining. Sections from each biopsy were H&E stained, and IHC was performed to identify α SMA. (A) Representative H&E-stained section. (B) Corresponding biopsy section stained using IHC for α SMA (brown) and cellular nuclei (blue). Low accumulation of α SMA was observed in BFC regions and high accumulation of α SMA was observed in the bands of aligned connective tissue indicating contractile tissue.

protein α SMA as uninjured human dermis with expression localized to the blood vessels found within the dermal collagen fibers. Regions of the generated tissue not containing BFC had elevated levels of the contractile protein 2 to 5 times greater than BFC dermal tissue and uninjured human dermis.

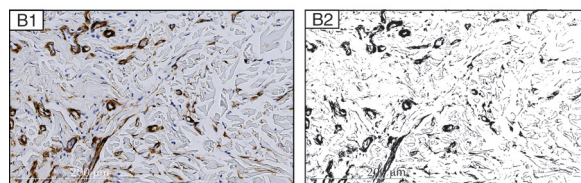
In wound healing, the myofibroblast, identified by the presence of α SMA, is a key element in the remodeling of newly deposited connective tissue after injury. Myofibroblasts are related to wound contraction and scar tissue formation, with contractility correlating with the expression of α SMA.⁵⁻⁹ The differentiation of fibroblasts into myofibroblasts occurs in response to changes in the composition, organization, and mechanical properties of the ECM as well as to growth factors and cytokines locally released by inflammatory and resident cells.¹⁰⁻¹² In early forming granulation tissue, few myofibroblasts are present, but this cell type becomes numerous during the remodeling and contraction of the wound.¹³

The suppression of myofibroblasts in the wound environment has become a focus for interventions aimed at decreasing scar contracture after cutaneous injury. A range of biological molecules aimed at modulating fibroblasts or myofibroblasts by altering α SMA expression have proved promising in animal wound healing models but have yet to reach the clinic.^{14,15} The clinical data presented in this work on modulating α SMA with an acellular dermal matrix correlates with findings reported on the use of acellular dermal matrices to decrease α SMA in

Contractile Tissue



BFC Dermal Tissue



Normal Human Dermis

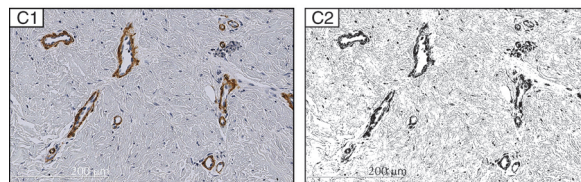


FIGURE 3. Expression of α SMA in contractile tissue, BFC dermal tissue, and normal human dermis. Representative images of α SMA (brown) expression in contractile tissue, BFC dermal tissue, and normal human dermis (bright field A1, B1, and C1). These regions, 0.21 mm² in area, were digitally processed to binary images under the same threshold settings using Image J software (A2, B2, and C2), and α SMA (black) could be quantified as a percentage of the region of interest.

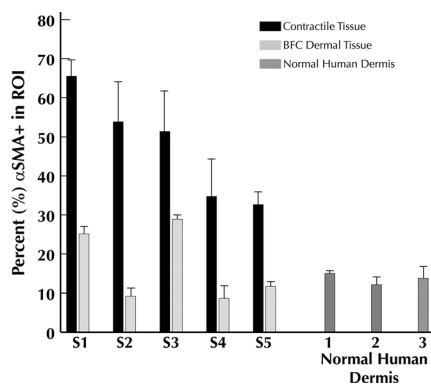


FIGURE 4. Quantification of α SMA in contractile tissue, BFC dermal tissue, and normal human dermis. Quantitative image analysis was used to determine the percent α SMA present in contractile tissue, BFC dermal tissue, and normal human dermis. For each sample, 3 images for each region of interest (ROI) were captured and converted to a binary image using Image J software. The presence of α SMA (black) was reported as a percent of the region of interest (0.21 mm²). For each sample, the average percent α SMA is reported with standard deviation. Similar to the reconstructed site, 3 images from each control sample were analyzed and the value was reported as an average with standard deviation. Quantification of the images revealed similar levels of α SMA in the BFC tissue and uninjured human dermis. The contractile protein was found at levels approximately 2- to 5-fold higher in the contractile tissue than in the BFC dermal tissue or normal human dermis.

animal studies evaluating contracture.^{14,16} Clinically, acellular dermal matrices are being studied as a means to decrease capsular contraction when used in implant-based breast reconstruction procedures.¹⁷ For example, Moyer et al¹⁸ reported a decrease in α SMA expression in peri-implant tissue of irradiated patients where an acellular dermal matrix had been implanted. The authors suggest that this contractile protein suppression could correlate with a decrease in capsular contraction.

All these observations contribute to a tentative profile of ways in which acellular dermal matrices could be manipulated toward more favorable wound healing outcomes. The results presented in this study demonstrate the ability of BFC to generate dermal tissue, rather than granulation and contractile tissue, where it is implanted. These findings suggest a mechanism by which BFC implantation before application of a STSG could decrease contraction in full thickness skin wounds.

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