Integra® dermal regenerative template application on exposed tendon

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Published online: 25 March 2014
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Abstract

Background Tendon without paratenon presents the reconstructive surgeon with a tissue coverage challenge. Integra® dermal regenerative template has been shown to initiate a stable, vascularized bed for skin grafting over tendon. However, histological processes that occur during incorporation have not been described. The purpose of this study is to characterize the pattern of changes that occur when Integra® is applied to an avascular tendon. We hypothesize that vascular incorporation will originate from the wound periphery and proceed toward the tendon center.

Methods A full-thickness defect was created over a denuded Achilles tendon in a single hind limb in eight New Zealand white rabbits. Integra was placed over the avascular tendon, and the limb was dressed and splinted. Two animals were euthanized at each timepoint (weeks 1, 2, 3, and 4), and hematoxylin and eosin (H&E)-stained tissue specimens were microscopically evaluated.

Results Week 1 specimens demonstrated limited adherence between Integra and the tendon, while myofibroblasts were found encircling the tendon. No cellularity was noted centrally. At week 2, the dermis–Integra junction had increasing vascularity and the central portion developed increasing cellularity. By week 3, Integra was completely revascularized. At

week 4, Integra had the histological appearance of normal dermis.

Conclusion Neovascularization of Integra® over exposed tendon occurs from the peripheral tissue. Ingrowth proceeds from the dermis–Integra interface toward the center of the graft. Four weeks after application to the denuded tendon, Integra has the histological appearance of native dermis.

Keywords Integra · Dermal regeneration template · Tendon

Introduction

Tendon can become exposed in many ways including trauma, burns, and oncological resection. Subsequent coverage presents the reconstructive surgeon with a challenging soft tissue deficit. An intact paratenon is required as skin grafts will not incorporate over an avascular wound bed. Durable coverage of denuded tendon on the extremities is sometimes difficult due to a paucity of local and regional flaps. Free flaps, while a viable option, require long operative times and healthy recipient vessels and create donor site morbidity.

Integra® dermal regeneration template was introduced in 1981 by Integra Life Sciences Corporation (Plainsboro, NJ). It is a dual-layer, nonviable matrix which enables ingrowth of host fibroblasts and endothelial cells through imbibition, fibroblast migration, neovascularization, and remodeling/maturation [3, 12]. The dermal layer is composed of cross-linked bovine tendon collagen and chondroitin-6-sulfate [1, 3]. The epidermal layer consists of a temporary silicone sheet that limits moisture loss and covers the wound bed [1]. When placed over a well-debrided area, the silicone epidermal layer is shed as the dermal substitute is incorporated and vascularized by the surrounding tissue [9]. This process is usually complete in 2–4 weeks [6, 8, 12, 15] but may take longer depending on the size and type of defect [9, 16].

This study was presented at the ASPS Senior Residents Conference, January 2011, Nashville, TN, and Midwestern Association of Plastic Surgeons Annual Meeting, May 2011, Chicago, IL.

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The successful reconstruction of a defect that includes exposed tendon must achieve multiple goals. The coverage material must provide vascular tissue for wound closure, adequate tendon glide, and an expeditious return of acceptable function [14]. Clinical studies indicate that Integra placed over exposed tendon will incorporate and provide a stable, vascularized bed for skin grafting [9, 14, 17]. However, no study to date has examined the histology of this process. We undertook this study to characterize the tissue incorporation process of Integra dermal regenerative template at the cellular level when it is applied over an avascular tendon.

Materials and methods

This study was reviewed and approved by the Laboratory Animal Care and Use Committee at the Southern Illinois University School of Medicine.

Eight female New Zealand white rabbits ($n=8$) were anesthetized and prophylactically given antibiotics. A randomly selected hind limb from each animal was shaved and aseptically prepared. A 4 cm × 6 cm full-thickness defect was created on the posterior aspect of the distal leg directly over the Achilles tendon. All paratenon was sharply debrided creating a soft tissue defect with exposed avascular tendon. Integra was placed over the wound bed and secured to the defect using 6-0 nylon suture. Acticoat was placed over Integra and moistened with sterile water. Layers of cotton Webril were wrapped over the entire lower extremity, and a long fiberglass splint was fashioned. Splints were secured to the lower extremity using Webril, Coban, and tape.

Animals were given butorphanol (0.1 mg/kg IM) as needed for pain relief. Dressing changes were performed twice a week while animals were sedated. Animals were euthanized and wound specimens harvested at each time interval [week 1 ($n=2$), week 2 ($n=2$), week 3 ($n=2$), and week 4 ($n=2$)]. Specimens were cut transversely to include Integra and tendon in each sample (Fig. 1). Hematoxylin and eosin (H&E)-stained tissue slides were created to identify cellular ingrowth. Specimen slides were assessed visually by a trained pathologist to characterize patterns of cellular change.

Results

All animals tolerated the surgery, splinting, and dressing changes well. All animals demonstrated normal healing prior to sample collection. No Integra® needed to be replaced, and no surgical site infections occurred. Five to seven H&E slides were made from each specimen selected from various points along the Integra–tendon interface.

Week 1 samples ($n=2$) demonstrated limited adherence between Integra and tendon. Near the dermis–Integra

Fig. 1 Image of how the Integra/tendon was cut for processing

interface, we began to see actively dividing myofibroblasts encircling the tendon, along with PMNs, giant cells, and macrophages. However, there is an absence of blood vessels at this location (Fig. 2). No blood vessels or cellularity was noted over the central Integra–tendon segment.

In week 2 samples ($n=2$), ectatic, immature vessels are now present at the dermis–Integra junction, which were not present in week 1 (Fig. 3). A substantial number of eosinophils and multinucleated giant cells were present at this site. Similarly, adherence between Integra and tendon and the density of blood vessels were greatest near the native dermal edge and diminished toward the repair's center. Blood vessels remain absent at the most central segment. A surprising finding was increased vascular channels and nervous tissue noted within the tendon substance at week 2 (Fig. 4).

By week 3, specimens ($n=2$) demonstrated complete revascularization of Integra. There was good adherence of Integra to a myofibroblastic layer surrounding the tendon. Additionally, there were less inflammatory cells and a more mature appearance of the dermal matrix at the dermis–Integra junction.

Week 4 samples ($n=2$) demonstrated full incorporation of Integra. The regeneration template had a histological appearance that was indistinguishable from native dermis with the exception of absent rete ridges and fur. The overall cellularity decreased and blood vessel walls had a more mature appearance (Fig. 5).

Discussion

Several clinical studies have shown that Integra® dermal regenerative template can be used to repair avascular wounds, such as exposed tendon [2, 4, 6, 8, 9, 14]. However, no histological study documenting this healing process was identified in the current literature. Understanding the cellular process by which this acellular dermal matrix is incorporated and vascularized can aid the reconstructive surgeon who often encounters complex soft tissue defects.

Fig. 2 H&E of the dermis–Integra junction at week 1 demonstrating fibroblast activity. Note the actively dividing fibroblast in the *middle* of the image

Integra has been shown to be useful in the treatment of primary acute full-thickness and partial-thickness injuries [1, 5, 7, 10, 12]. Skin grafts require vascularized tissue with little motion to be successful [2, 7, 11, 12]. Alternatives to dermal substitutes have been employed. Negative-pressure therapy with a Vacuum Assisted Closure (VAC; Kinetic Concepts Incorporated, San Antonio, TX) has been used to provide a stable granulation tissue bed over exposed tendon to allow for skin grafting. However, this approach has limitations as the development of an adequate recipient bed may take up to 12 weeks prior to skin grafting [13]. Integra with a skin graft may also offer the benefits of decreased contracture compared to split-thickness autograft alone and improved contour compared to free flaps.

Stern et al. noted vascularization of the entire thickness of the Integra matrix by 2 weeks, while Moiemien et al. found nearly complete vascularization of Integra® with a 95 % split-thickness autograft take rate by 4 weeks [12, 15]. Because Integra will be grafted over an avascular tendon, we elected to evaluate integration in our study over a full 4-week period to allow for sufficient vascular ingrowth.

Our results show a clear and orderly progression of Integra tissue incorporation. First, there is the appearance of macrophages, polymorphonuclear leukocytes, giant cells, and fibroblasts. This is followed by immature, ectatic vascular channels. Next, remodeling occurs with a further increase in

Fig. 3 Ectatic, immature blood vessels at the dermis–Integra junction at week 2

Fig. 4 Increased vascular and nerve tissue noted within the tendon proper at week 2

cellularity and maturation of the vascular channels. Finally, the dermal matrix becomes nearly indistinguishable from native dermis in its architecture, vascularity, and cellularity. The data supports a neovascularization of Integra dermal regenerative template from the peripheral tissue as hypothesized. The process begins at the acellular dermal matrix margins in contact with native vascular tissue. Subsequently,

Fig. 5 Integra over exposed tendon at week 4. It is nearly indistinguishable from native dermis. (Note the lack of fur follicles and rete ridges)

ingrowth occurs from the native dermis–Integra interface toward the center of the graft in an orderly fashion.

A limitation of the study is the quality of specimens, with early samples showing limited acellular dermal matrix adherence to the native tendon and tissue fracturing. We propose three possible explanations for this. First, this may be related to difficulties processing such a sample with the silicone sheeting intact. As described previously, Integra® is provided with a thin silicone layer on the superficial surface as a carrier and moisture barrier and to protect the matrix while vascularization proceeds. During tissue processing, silicone can limit the ability to create fine cuts by adhering to the blade while the tissue is sectioned. Second, during processing, the slides were made with a thickness of 3–5 µm. Processing the slides with thicker cuts such as 10–12 µm may also limit this separation. A final possible explanation for separation and fracturing may be related to limited cellularity and vascularity. Avascular dermal matrix would have limited fibroblast activity at this interface. It would be suspected that as fibroblasts migrate into the matrix and become active, there would be greater adherence of acellular dermal matrix to the tendon. This is supported by the later specimens with improved cellularity and vascularity at this interface as well and greater adherence.

Vascularization of the dermal matrix occurred at a much faster rate than initially anticipated as well. At week 1, some early cellularity was seen, and quickly by week 2, the vascular channels were present and cellularity had spread to the central portions. Taking additional samples at various times during weeks 1 and 2 would be useful to further characterize the changes during this critical point.

In considering the use of Integra® for coverage of exposed avascular tendon in reconstructive extremity surgery, the authors continue to follow the recommended staged treatment protocol with placement of split-thickness skin graft after 3 weeks. Based on the findings from this study, the authors do consider the vascularity of the surrounding tissue when evaluating a wound with exposed tendon and potential coverage with Integra®. Since neovascularization occurs from the peripheral tissue, Integra® is more likely to be considered as an option for wound coverage if there is healthy granulation tissue surrounding the exposed tendon. Use of Integra® in these situations provides coverage of the tendon and avoids the need for a pedicled flap or free tissue transfer and their resultant donor site morbidity.

Acknowledgments Funding was provided by the Memorial Medical Center Foundation, Springfield, IL.

Conflict of Interest John Hulsen declares that he has no conflict of interest.

Ryan Diederich declares that he has no conflict of interest.

Michael W. Neumeister declares that he has no conflict of interest.

Reuben A. Bueno, Jr. declares that he has no conflict of interest.

Statement of Animal Rights The authors, JH, RD, MN, RB, Jr., certify that all institutional and national guidelines for the care and use of laboratory animals were followed in this study.

References

- Burke JF, Yannas IV, Quinby WC, Bondoc CC, Jung WK. Successful use of physiologically acceptable artificial skin in the treatment of extensive burn injury. *Ann Surg.* 1981;194:413–28.
- Chan ES, Lam PK, Liew CT, Lau HC, Yen RS, King WW. A new technique to resurface wounds with composite biocompatible epidermal graft and artificial skin. *J Trauma.* 2001;50:358–62.
- Gottlieb ME. Management of complex and pathological wounds with Integra. In: Lee BY, editor. *The wound management manual.* New York: McGraw-Hill; 2004. p. 226–89.
- Graham PG, Helmer SD, Haan JM, et al. The use of Integra dermal regeneration template in the reconstruction of traumatic degloving injuries. *J Burn Care Res.* 2013;34:261–6.
- Heimbach D, Lutermaier A, Burke J, et al. Artificial dermis for major burns. A multicenter randomized clinical trial. *Ann Surg.* 1988;208:313–20.
- Helgeson MD, Potter BK, Evans KN, et al. Bioartificial dermal substitute: a preliminary report on its use for the management of complex combat-related soft tissue wounds. *J Orthop Trauma.* 2007;21:394–9.
- Hunt JA, Moisidis E, Haertsch P. Initial experience of Integra in the treatment of post-burn anterior cervical neck contracture. *Br J Plast Surg.* 2000;53:652–8.
- Jeng JC, Fidler PE, Sokolich JC, et al. Seven years' experience with Integra as a reconstructive tool. *J Burn Care Res.* 2007;28:120–6.
- Lee LF, Porch JV, Spenler W, Garner WL. Integra in lower extremity reconstruction after burn injury. *Plast Reconstr Surg.* 2008;121:1256–62.
- Lorenz C, Petraccic A, Hohl HP, Wessel L, Waag KL. Early wound closure and early reconstruction. Experience with a dermal substitute in a child with 60 percent surface area burn. *Burns.* 1997;23:505–8.
- Loss M, Wedler V, Kunzi W, Meuli-Simmen C, Meyer VE. Artificial skin, split-thickness autograft and cultured autologous keratinocytes combined to treat a severe burn injury of 93% of TBSA. *Burns.* 2000;26:644–52.
- Moiemen NS, Staiano JJ, Ojeh NO, Thway Y, Frame JD. Reconstructive surgery with a dermal regeneration template: clinical and histologic study. *Plast Reconstr Surg.* 2001;108:93–103.
- Repta R, Ford R, Hoberman L, Rechner B. The use of negative-pressure therapy and skin grafting in the treatment of soft-tissue defects over the Achilles tendon. *Ann Plast Surg.* 2005;55:367–70.
- Shores JT, Hiersche M, Gabriel A, et al. Tendon coverage using artificial skin substitute. *J Plast Reconstr Aesthet Surg.* 2012;65:1544–50.
- Stern R, McPherson M, Longaker MT. Histologic study of artificial skin used in the treatment of full-thickness thermal injury. *J Burn Care Rehabil.* 1990;11:7–13.
- Tompkins RG, Hilton JF, Burke JF, et al. Increased survival after massive thermal injuries in adults: preliminary report using artificial skin. *Crit Care Med.* 1989;17:734–40.
- Weigert R, Choughri H, Casoli V. Management of severe hand wounds with Integra dermal regeneration template. *J Hand Surg Eur Vol.* 2011;36:185–93.