Engineered alternative skin for partial and full-thickness burns

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Engineered alternative skin in all its forms and shapes serve to provide temporary or permanent wound closure such as in the case of partial and full-thickness burns. The need for collagen-based regeneration templates is motivated by the fact that dermal regeneration of full-thickness injuries does not occur spontaneously and is inducted by contraction and scarring. Partial-thickness burns in turn can regress as a result of infection and improper treatment and require appropriate treatment. Nylon-silicone laminates such as Biobrane®, and more recently AWBAT®, address this by serving as a temporary barrier. Enhanced collagen-based scaffolds today, although not perfect, remain invaluable. Our initial approach was to characterize the design considerations and explore the use of collagen in the fabrication of a dermal regeneration matrix and a silicone-nylon bilaminate. Here we expand our initial research on scaffold fabrication and explore possible strategies to improve the outcome of collagen-scaffold medicated wound healing.

Burn treatment and the correction of skin defects remains a challenge. Current treatment approaches rely on engineered skin substitutes or alternative tissue, and these products have become important in the management of partial and full-thickness burns. Products like these aim to restore barrier function, facilitate wound healing, and manage pain and can either be temporary, semi-permanent, or permanent.1-2 Their building blocks can be classified as biological (natural or artificial) or alloplastic, and some employ a combination of both in order to substitute or replace the epidermis, dermis, and both the epidermis and dermis.3-6 The pioneering work of Burke and Yannas7-8 and Woodroof9 in the 1980s led to the development of the first commercially available products, Integra and Biobrane®, respectively, and thus paved the way for the array of skin substitutes that are available today as summarized in references 3–6 and 10.

The use of pre-fabricated collagen-based scaffolds will prevail and, although not perfect, they are currently the most successful option.9 Total skin replacement remains out of reach even with the advances in tissue engineering. Cellular based skin substitutes, regardless of the origin of the cells, are dependent on either discreet delivery systems or sophisticated tissue engineering scaffolds. One drawback of this approach is the extensive amount of time required to culture the cells in vitro and allow for the modeling of an extracellular matrix (ECM).10,11 Alterations in the biochemical composition of engineered matrices might hold the key toward improved and accelerated wound healing. Four major scaffolding approaches are currently employed in tissue engineering and include pre-made porous matrices, which include collagen-based scaffolds for partial and full-thickness burns (Fig. 1B–D); tissue ECMs and the ECM they form; decellularized ECM (Fig. 1A); and 3D cultures in a hydrogel.12

The design considerations and development of pre-made skin replacements are vast and well documented. It is currently accepted that the design considerations should include biocompatibility, controlled biodegradability, low or no antigenicity, a suitable micro-structure or architecture, and resistance to shear forces.5,13,14 The micro-structure relies on the pore structure and size and the total surface area of the scaffold.12,14 The micro-architecture, pore diameter ranging between 20 and 120 µm, and resistance against enzymatic degradation for at least 21 days in vivo allows for neo-collagenesis.15 These attributes can be achieved through tried and tested protocols and can also be manipulated through changes in the collagen concentration, rate of freezing and method of cross-linking.14-19 A typical protocol to construct a collagen-based extracellular matrix relies of the following sequential steps: type I atelocollagen extraction from bovine Achilles tendons, coprecipitate formation with a final collagen:chondroitin-6-sulfate ratio of 92:8, homogenization under controlled temperature, degassing, controlled freezing, lyophilization, dehydrothermal treatment (DHT) at 105 °C and 0.2 mbar for 24 h, silicone coating (such as the use of Dow Corning, Silastic®, Q7-4840), chemical cross-linking, and final washing step.14-18 Highly porous matrices were obtained through the use of a 0.6% (w/w) collagen concentration and a controlled rate of freezing of 0.92 °C/min. Morphologically, the scaffolds presented with a large amount of collagen sheets and polygonal pores with an average diameter of 62.18 µm (SD = 34.55) (Fig. 1B). These matrices were formed by using a 0.6% (w/w) collagen concentration and a controlled freeze rate of 0.92 °C/min. An exact mimic of normal dermal architecture as depicted in Figure 1A is difficult to obtain and the described methodology of fabrication can at best address aspects of bioactivity.12 The fabricated scaffolds demonstrated sufficient resistance against enzymatic degradation in vitro and follow on previous work by the author.18,19 The in vivo integrity of the matrices was...
confirmed through the use of an animal model. Scaffolds were implanted after surgical excision of normal skin on the dorsum of female Sprague-Dawley rats (South African Vaccine Producers) after obtaining ethical approval (AUCC approval No. H005-10). Data confirmed cellular infiltration on day 7 while scaffold integrity was retained. The cell population consisted typically of fibroblasts, lymphocytes, and a few neutrophils (Fig. 2A). The H&E stained sections (Fig. 2B) confirm the bioactivity and the scaffold afforded interaction with cellular components. Wound healing on day 28 (Fig. 2C) presented with a superficial layer having a scar tissue appearance (rich in type III collagen fibers) and a deeper neodermis (dominated by type I collagen fibers). Deeper collagen deposition typically demonstrates a basket weave configuration with associated fibroblasts and evidence of neovascularization.

Any additions aimed at enhancing wound healing in vivo can only be added during the coprecipitate formation and/or after final cross-linking. These two steps serve as the points of transformation where the matrix can become a delivery system in order to promote wound healing.20 Cells are typically seeded after the final fabrication and this is also true for nylon-based laminates.19,21 This is typically after the final coating of the knitted tri-filament mesh which is coated with an ultra-thin layer of biomedical grade silicone as reported by Wessels and Pretorius in 2013 (Fig. 1C and D).19 Constructs like these consist of biomaterials with mechanical properties that allows wound adhesion through the added collagen, promotes cell migration along the nylon filaments, and simultaneously serve as a physical defense against bacterial infection while permitting the flow of wound exudate.10,19,21 The nylon tri-filament mesh allows for the covalent addition of collagen, coating with a hydrogel, or cellular seeding in vitro. TransCyte®, originally termed Dermagraft-TC, relies on a similar construct and is seeded with neonatal dermal fibroblasts.21

The application of biomaterials has evolved from the development of medical devices and prostheses, to its current
applications through the study of their biological interaction and intended purpose. Biomaterial selection is determined by the tissue that is targeted for replacement or augmentation. Tissue engineering strategies aimed skin replacement primarily employs biological and synthetic polymers such as collagen, nylon, siloxanes, and many others. The safe use of these biomaterials, especially collagen, has been proven over time and their application has had a significant impact on soft tissue repair. Alternative skin for partial-thickness burns described here has become a valuable aid to current treatment regimens and demonstrate satisfactory clinical results. However, currently available collagen-based scaffolds for full-thickness injuries fail to yield perfect results. These porous constructs allow for cell migration and nutrient and metabolite diffusion, resist biodegradation, afford appropriate biological signaling, and serve as a vehicle for extrinsic biologically active components aimed at improved healing. The shortfall, despite these favorable attributes, could be ascribed to the fact that they are molecularly flawed as ECM mimics. Our understanding of the structure and function of the ECM and its components has advanced over the past 20 years as illustrated by the review of Eckes et al., in 2010. The interactions between cells and the ECM affects gene transcription, modifies growth factor signaling, and this in turn has an effect on ECM deposition by fibroblasts. Work by Fisher and colleagues in 2009 and more recently in 2013 demonstrated that age-related dermal collagen fragmentation negatively impacts fibroblast activity. Their findings suggest that the ECM of normal skin permits integrin-mediated fibroblast contact, which in turn regulates cell shape and normal fibroblast collagen production through mechanical interaction. This relates to their earlier work that reported de novo fibroblast collagen synthesis induced by mechanical force on the dermal ECM and cells after cross-linked hyaluronic acid has been injected to correct facial wrinkles. Aging-related collagen fragmentation according to Fisher and colleagues has been shown to reduce fibroblast stretch. This in turn leads to an increase in oxidative stress and results in an elevation in MMP-1 (matrix metalloproteinases-1) expression. The latter further aggravates ECM breakdown and thus has
Further deleterious effects on fibroblast function and ECM integrity. The underlying principle could relate to scaffolding approaches in tissue engineering. Meticulous assembly through 3D-printing of biomaterials and scaffolds might address this and has become more feasible with advances in research.

Conclusions

Skin substitutes or engineered alternative skin remain invaluable regardless of their shortcomings. They are known to facilitate wound healing, improve patient survival, and produce satisfactory functional and cosmetic results. The use collagen, more specifically atelocollagen, as a biomaterial has led to major advances in the development of engineered alternative skin and subsequent soft tissue repair. This is largely attributed to its favorable biological and physicochemical properties. Collagen can easily be transformed, as described by the author, into porous scaffolds and combined with other biomaterials such as nylon-silicone laminates. Porous dermal scaffolds can be obtained through a sequence of steps that includes the formation of a coprecipitate, controlled freezing, lyophilization, and crosslinking. Yet, current off-the-shelf full-thickness substitutes fail to regenerate accessory skin structures, create neurovascular bundles, and deliver scars healing. Biomaterials and tissue engineering scaffolds produced through rapid prototyping and 3D-printing will replace those developed through conventional fabrication strategies. These engineered alternative skin replacements will have the potential to yield improved results both clinically and cosmetically. This is due to the fact that perfect ECM mimics require the exact molecular composition as the healthy in vivo counterpart they aim to reproduce.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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