Development and ultra-structure of an ultra-thin silicone epidermis of bioengineered alternative tissue

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Abstract

Burn wound care today has a primary objective of temporary or permanent wound closure. Commercially available engineered alternative tissues have become a valuable adjunct to the treatment of burn injuries. Their constituents can be biological, alloplastic or a combination of both. Here the authors describe the aspects of the development of a siloxane epidermis for a collagen-glycosaminoglycan and for nylon-based artificial skin replacement products. A method to fabricate an ultra-thin epidermal equivalent is described. Pores, to allow the escape of wound exudate, were punched and a tri-filament nylon mesh or collagen scaffold was imbedded and silicone polymerisation followed at 120°C for 5 minutes. The ultra-structure of these bilaminates was assessed through scanning electron microscopy. An ultra-thin biomedical grade siloxane film was reliably created through precision coating on a pre-treated polyethylene terephthalate carrier.

Introduction

Burn wound treatment has the primary objective of permanent wound closure which is reached through a sequence of events that include: resuscitation, wound cleansing, debridement, wound dressing and surgical intervention [1]. Bioengineered skin substitutes, although not perfect, have become an invaluable addition to the treatment of burn wounds of varying depths. These products, also known as bioengineered alternative tissue, provide wound closure that is temporary, semi-permanent or permanent. Furthermore, they also restore barrier function, facilitate wound healing, correct suboptimal healing and aid in pain management [2, 3]. Their constituents can be biologic, alloplastic or a combination of both. Commercially available products aim to replace or substitute the following biological components; epidermis (products such as Apligraf®, Biobrane® and now *AW*BAT® and CellSpray®), dermis (products such as Alloderm®, Dermagraft® and Integra®) or both layers of the skin also known as composite skin (products such as Apligraf®, OrCel® and PermaDerm®) [4-11].

Integra® and Biobrane® are both collagen-containing skin replacements and have become the benchmarks for current technologies. Biobrane® and *AW*BAT® (more recently) are indicated for superficial burns and both products were developed by Aubrey Woodroof [3, 4]. The efforts of Woodroof and his colleagues were concurrent with that of Burke and Yannas, which in turn resulted in the biologically active dermal regeneration template Integra® [3-5]. The authors published their findings in 1983 and described a cell-free construct consisting of an ultra-thin silicone film bound to a knitted nylon fabric. Within the

wound environment they demonstrated that the hydrophilic polyamide containing –CONH– linkages form hydrogen bonds with water and consequently alter the mechanical properties of this biomaterial. Nylons of clinical importance are nylon-6, nylon-6,6 and nylon-6,12 and these designated numbers indicate the amount of carbon atoms separating the amide linkages [12]. The nylon scaffold of Biobrane® is coated with collagen, thus rendering the product both biocompatible and hydrophilic [3, 4]. The epidermal portion of skin replacements generally consist of a biomedical grade siloxane available from Dow Corning Corporation (Midland, MI), Bayer or Nusil, to name a few. The backbone of this macromolecule is formed by repeating silicon units bound to oxygen.

Kipping's research laid the foundation of organosilicon chemistry and he coined the term 'silicone' upon recognition of the structural similarities with ketones [13]. 'Siloxane' denotes the basic repeating unit of silicones and numerous publications followed in the 1940s which illustrated the potential of siloxanes as biomaterials. For instance, Jaques *et al*. demonstrated in 1946 that silicone-coated glassware and needles delay blood coagulation [14].

The key features that make silicones suitable for long- and short-term implantation include elasticity and chemical stability [13]. Yet, despite the well-known use of silicones, little information exists on how to manipulate this biomaterial to form ultra-thin epidermal equivalents for use in wound care. Here the authors describe the development of bilaminar constructs to serve as temporary skin replacement for partial-thickness and full-thickness wounds. The feasibility to use one type of biomedical grade silicone as an artificial epidermis for two products is assessed and the ultra-structure of the laminates is described.

Materials and methods

Scaffold and mesh preparation

Extracted type I atelocollagen was combined with chondroitin-6-sulphate (Sigma-Aldrich Chemical Co., St. Louis, MO) to form a coprecipitate as previously described [15, 16]. Briefly, collagen suspensions were prepared in 0·05 M acetic acid, yielding 0·6% (w/w) and were subjected to high-speed blending for an hour at 4°C in order to prevent denaturation of the collagen. Chondroitin-6-sulphate in 0·05 M acetic acid was added to each of the prepared collagen suspensions, in a drop-wise manner. The coprecipitates were transferred to two 50 ml centrifuge tubes, centrifuged at 3336.11 *g* for 5 minutes to remove any trapped air bubbles, and slowly poured into Teflon-coated pans. Controlled freezing followed and Biofreeze DV 50, version 1.30.2 (CONSARCTIC®, GmbH; Schoellkrippen, Germany) with a pre-programmed freezing rate of 0·92°C/minute was used. The controlled freeze rate used was similar to that employed by O'Brien *et al*. [16]. Next, scaffolds were subjected to dehydrothermal treatment (DHT) at 105°C and 0·2 mbar for 24 hours once removed from the lyophilisation chamber [17, 18].

Knitted tri-filament nylon 6 was purchased from Falke Group (Pretoria, South Africa) and processed. Briefly, the nylon was cut opened, washed in 70% isopropyl-alcohol and partially stretched over a template in order to keep it in place. The next step entailed the formation of the silicone epidermis for both nylon and collagen-based matrices as described below.

Formation of artificial epidermal layers

Dry collagen-glycosaminoglycan (GAG) sponges were formed once lyophilisation was completed and silicone epidermal portion of each of the scaffolds was prepared as described below. Biomedical grade silicone rubber (Dow Corning, Silastic®, Q7-4840) was used to form a circa 0·25 mm thick film on a polyethylene terephthalate carrier. Precision coating was achieved by setting the aperture at 0·2 mm above the polyethylene terephthalate carrier. The thickness was selected due to the fact that the polymerisation will result in a circa 10% loss in thickness which will result in a final film of circa 0·22 mm according to the product specifications. Equal amounts of solutions A and B were thoroughly mixed prior to application. The biomedical grade silicone selected has tear strength of 26·9 kN/m, a tensile strength 9·4 MPa and elongation capacity of 540%. The polyethylene terephthalate carrier ensured easy removal after the silicone had polymerised. Punching of pores was done by means of a manual press with uniformly (1 cm) spaced (1651·0 µm diameter) pins. The collagen scaffolds and stretched nylon fabric were separately and carefully placed onto the wet unpolymerised silicone. Polymerisation was followed at 105°C for 30 minutes. This temperature will not denature the collagen as long as all the moisture has been removed during lyophilisation [15]. The samples were then removed and allowed to cool, separated from the polyethylene terephthalate carrier and prepared for scanning electron microscopy (SEM).

Electron microscopic analysis

SEM was performed on both the nylon-based and collagen-GAG scaffolds. The architecture of the ultra-thin artificial cuticles was compared. The 'pan-side' surfaces of the collagen-based scaffolds were prepared as described by Doillon *et al*. [19]. DHT collagen-based as well as nylon-based scaffolds were prepared by gold coating and the samples were viewed and photographed through the use of a Carl Zeiss field-emission scanning electron microscopy (FE-SEM) Ultra 55 (Carl Zeiss Microscopy GmbH; Jena, Germany) [20, 21]. Image analysis followed using the UTHSCSA *ImageTool* program (developed at the University of Texas Health Science Centre at San Antonio, TX maxrad6.uthscsa.edu). The obtained data sets were compared with each other and any statistical significance was assessed through a Student's *t*-test. The probability value used to determine statistical significance was 95% ($P < 0·05$). Errors in both the text and figures were reported as standard deviation.

Results and discussion

Results indicate that ultra-thin silicone epidermises (E) for both the collagen-based and nylon-based matrices were produced. Image analysis of the micrographs (Figure 1) showed collagen-based scaffolds with a mean silicone epidermal thickness of 198·36 (± 25·8) µm. Furthermore, the controlled freeze rate of 0·92°C/minute resulted in a porous scaffold with numerous collagenous sheets, a porous structure and fibre aggregates as indicated in Figure 1. The collagen elements were embedded up to a depth of 8·83 (± 4·56) µm. Image and data analysis from the nylon-based scaffolds, show nylon filaments (F) of a thickness of 28·33 µm and penetrated the silicone up to a mean depth of 47·77 µm (Figure 2). The membrane thicknesses were more constant with a mean of 195·08 (± 3·70) µm (Figure 2). The superior surface clearly demonstrated the presence of irregular pores (P) (Figure 3) with an average

diameter of 1125·19 (± 26·42) µm. The differences in thickness between the epidermal portions of both the collagen-based and nylon-based matrices were found to be insignificant (P = 0·51). From the data it can be deduced that the methodology employed resulted in consistency in the thickness of the epidermal portions of both matrices. The variability of the collagen-based film thickness resulted from the uneven surface of the collagen matrices and pressure applied during the union of the two components. This can be considered as a possible shortfall for this method in this instance and the impact on the biomechanical properties of the film requires further investigation. However, the artificial barrier function and pliability of the film remained intact and this was considered sufficient for the potential application.

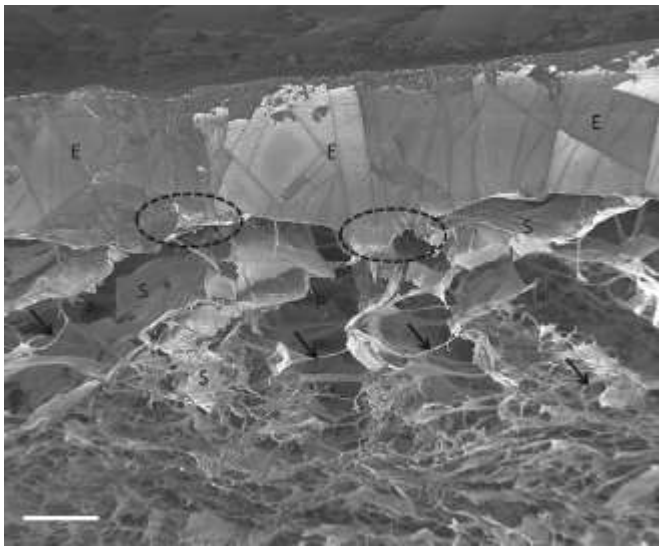

Figure 1. Scanning electron microscopy-micrograph of the lateral aspect of a collagen-based scaffold demonstrating the silicone epidermis (E) with a thickness of 198·36 (± 25·8) µm. The encircled areas demonstrate the embedding of the collagen into the silicone. Controlled freezing (rate of 0·92°C/minute) resulted in a porous scaffold with numerous collagenous sheets (S), pores (P) and fibre aggregates (arrows). Scale bar = 100 µm.

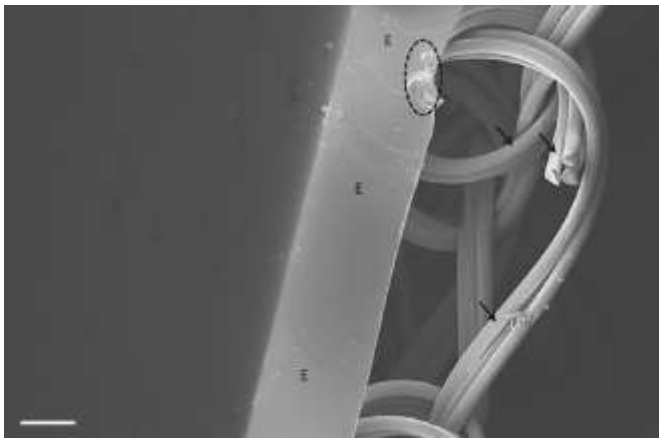

Figure 2. Scanning electron microscopy-micrograph of the lateral aspect of a nylon-based scaffold demonstrating the silicone epidermis (E) with a thickness of 195·08 (±3·70) µm. The encircled area shows the cut surface of the nylon filaments (arrows) embedded into the silicone epidermal portion. Scale bar = 100 µm.

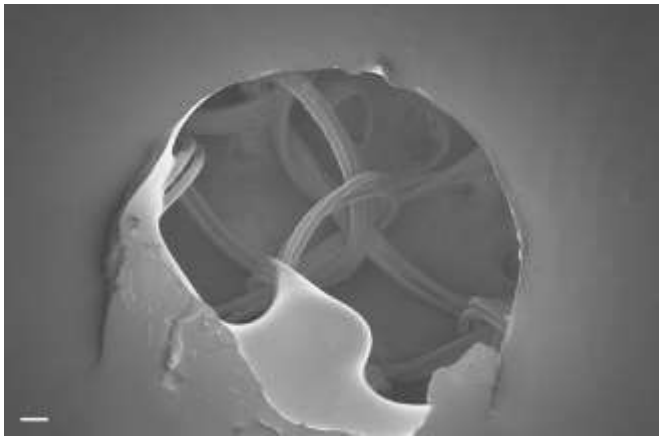

Figure 3. Superior aspect of the silicone epidermis demonstrating the punched pore and revealing the nylon filaments underneath. Scale bar = 100 µm.

Conclusion

Loss of skin can result from thermal burns, traumatic avulsion of portions of the skin, defects caused by surgery and from diseases. The restoration of the defect, and therefore wound closure, temporary or permanent and the restoration of barrier function are characteristically achieved through the application of engineered alternative tissue or skin substitutes. Advances in technology has allowed for the replacement allograft and xenografts with engineered collagen-based dermal analogues and skin substitutes. These engineered alternative tissues rely on biomaterials such as poly(lactic-coglycolic acid); fibronectin functional domains; silk fibroin; bovine collagen and recombinant technology derived gelatine and hyaluronan [22]. Some skin substitution products rely on an epidermal layer fabricated from biomedical grade silicone in order to control evaporative water loss and prevent the permeation of bacteria and toxins. Here the authors describe the development as well as the ultra-structure of an ultra-thin silicone epidermis of bioengineered alternative tissue. The methodology employed ensures a reliable method to develop an artificial epidermis for both collagen- and nylon-based skin substitutes.

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