

ORIGINAL RESEARCH ARTICLE – BASIC SCIENCE

Use of a novel porcine collagen paste as a dermal substitute in full-thickness wounds

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ABSTRACT

A commercially available porcine collagen sheet material has been found previously to be useful as an implant for reconstructive surgery. However, its use as a dermal substitute has been hindered by slow cell penetration and vascularization. A novel paste formulation of this material was investigated for its potential role as a dermal substitute in full-thickness wounds. A porcine punch biopsy model was initially used to assess the integration of a wide range of material formulations. Selected formulations were then assessed further in a larger wound-chamber model. Paste formulations were compared with those of sheet and another commercially available dermal regeneration template. The porcine collagen paste became integrated into full-thickness wounds without rejection and without excessive inflammation. It was detected in wounds up to day 27 postimplantation. Porcine collagen paste was readily infiltrated by host cells by day 2 and supported migrating keratinocytes on its surface. Staining for endothelial cells indicated neovascularity formation as early as day 4 and functional newly formed microvessels were noted at day 7. This was comparable with neovascularization of an alternative and clinically proven dermal regeneration template and was significantly superior to the sheet material formulation at the same time points. Our findings suggest that porcine collagen paste may be suitable as an alternative to current dermal substitutes in full-thickness wounds.

Currently, full-thickness extensive burns are most commonly treated by the application of split-thickness skin grafts to the prepared wound bed.¹ Autologous skin graft donor sites are, however, often insufficient for the treatment of relatively large wounds and meshing is the only technique available to enable the surgeon to cover larger areas of burns. Although this method allows coverage and subsequent healing of extensive burns, the mesh pattern is retained and can have a significant negative psychological impact on the burns patient. Other approaches have included the use of cultured keratinocytes either as a sheet or as a sprayed cell suspension. The results of such applications have been extensively reviewed,^{2,3} and the findings have led many to conclude that successful long-term healing and reduced scarring in full-thickness burns wounds are related to the presence of an adequate dermal layer.⁴⁻⁷

In physiologically normal skin the dermis is responsible for elasticity, strength and interacts with epithelial cells. Structural components for keratinocyte adhesion are also provided by the dermis, as shown by the application of cultured epithelial sheets onto partial thickness wounds with remnants of a dermis, where fast adherence and bonding between grafted and host tissues have been observed.³

A variety of dermal substitutes or skin equivalents have been investigated previously.⁸⁻¹¹ Many are based on

artificial materials such as polylactic or polyglycolic acid (Dermagraft®).^{7,12} Alternatively, biologically derived materials such as deepithelialized dermis,¹³ collagen-based (e.g., Integra® and Apligraf®)^{8,14} or fibrin-based (such as Beriplast, Hemaseel and Bioseed®-S)¹⁵⁻¹⁸ materials have been developed as wound repair biomaterials. Several skin equivalents only provide temporary wound coverage (Apligraf®, TransCyte®)^{7,19} and are usually applied to partial-thickness wounds that still retain dermal elements.

Some disadvantages of currently available biomaterials for skin reconstruction include the potential risk of transmissible diseases,⁷ the high cost of treatment^{10,20} as well as, in some cases, slower rates of neovascularization than is clinically desirable.²¹

Properties of the “ideal” skin or dermal substitute have been described and reviewed.²² In summary, such a biomaterial has to be safe for the patient, be clinically effective and convenient in handling and application. For any material to be safe, it must not cause any immunogenic response, transmit diseases, be cytotoxic or cause excessive inflammation. Such a material has to be biodegradable but stable enough to support normal tissue reconstruction, including reepithelialization. Long shelf life, immediate availability and easy handling also play an important role in making any material suitable for clinical use.

The porcine collagen material, Permacol™, in the form of a sheet, has been successfully used as a stable implant in cosmetic^{23,24} and reconstructive surgery.^{25,26} Previous studies have shown it is nonimmunogenic, nontoxic, and stable *in vivo*.^{21,27} It has also been found to integrate well and was detected *in vivo* after 20 weeks in a rodent model.²¹ When used clinically for abdominal wall hernia repair no recurrences were observed over 2 years, suggesting an implant persistence in the wound.²⁸ Permacol™ also has the advantage of being available “off-the-shelf” with a relatively long shelf life and does not demand special storage conditions. Its use in sheet form as a dermal substitute has, however, been hindered by slow cell penetration and vascularization^{21,29,30} despite the use of different treatments designed to encourage better cell penetration, such as incorporation of vascular endothelial growth factor and the use of laser perforations.²⁹

In this study, a new formulation of the commercially available porcine collagen biomaterial, Permacol™, was investigated for its potential role as a dermal substitute for the clinical treatment of full-thickness wounds. The material's ability to integrate into full-thickness wounds was evaluated by quantification of host cellular colonization and the progress of neovascularization formation, because the rate of vascular ingrowth plays an important role in the success of subsequent epithelial grafting. We hypothesize that the paste formulation, due to its particulate structure, would facilitate host cell infiltration, and a rapid biomaterial vascularization as a result.

The suitability of Permacol™ paste as a dermal substitute material was tested in an established porcine model.³¹ The pig was chosen due to the structural and physiological similarities between porcine and human skin.^{32–34}

MATERIALS AND METHODS

Dermal substitutes

Permacol™ sheet

Permacol™ sheet was supplied as a sterile surgical implant, 5×5 cm×0.75 mm, vacuum packed in sachets and kept moist in sterile saline at room temperature. The material appears as off white and is a tough but flexible sheet of diisocyanate cross-linked acellular porcine dermal collagen and its constituent elastin fibres (Tissue Science Laboratories plc., Aldershot, UK). Before use, the sheet was washed in fresh sterile saline, cut to the size of the wound and applied without further modifications.

Permacol™ paste

This material was formulated from Permacol™ Injection, which was supplied as a permanent porcine collagen injection, 2.5 mL vials of a sterile suspension (60% wet weight collagen to saline volume) of diisocyanate cross-linked porcine dermal collagen in saline (Tissue Science Laboratories plc.). Permacol™ Injection is produced from Permacol™ sheet by cryogenic milling, and has a maximum particle size of 250 µm (manufacturer's information). The

paste was prepared by transferring the contents of the Permacol™ Injection vial, resuspended in RPMI-1640 (Sigma, Irvine, UK), to Eppendorf tubes and centrifugated at 3,500 g for 20 minutes. The resulting pellets of collagen were diluted with RPMI-1640 1:1 (v:v) to form 50% paste, 3:1 to form 75% paste or used without any dilution as a 100% paste. The dry weight of collagen made up 23% (SD=3.9) of the prepared porcine collagen 100% paste total weight. The particle size distribution of the paste was analyzed using a laser diffraction particle size analyzer (Malvern MasterSizer, Malvern Instruments Ltd., UK) and was found to range from 1.32 to 544 µm, mean=275 µm (SD=168). The larger size of the particles, when compared with Permacol™ Injection, was possibly due to particle aggregation during the paste preparation. Permacol™ paste was packed into sterile syringes, which were used for the storage, measurement and delivery of the material to the wound. 100 µL volumes of each paste formulation were measured in a syringe and applied to the experimental wounds in the punch biopsy model. 1.3 mL volumes of the 100% paste formulation were applied in the wound chamber model. Paste was extruded from the syringe and spread evenly over the wound with a spatula.

Integra® dermal regeneration template™

This was supplied as a 10×25 cm sheet of bilayer collagen and glycosaminoglycan membrane with a removable silicone outer layer and was stored in 70% isopropyl alcohol (Integra LifeSciences Corporation, Plainsboro, NJ). Before use, the isopropyl alcohol was removed by washing in sterile saline, according to the manufacturer's recommendations. For the punch biopsy model, described below, the silicone layer was removed before implantation; for the wound chamber model the silicone layer was left intact. The Integra® was cut to the size of the wound before application.

Experimental models

All experiments were approved by a local ethical review committee and carried out under Home Office Project Licence PPL 80/1735 according to the Animals Scientific Procedures Act (1986). Female Large White pigs, with an initial weight of 25–30 kg were used for this study. On arrival, pigs were placed into individual pens and allowed to acclimatize to their new surroundings for 7 days before initiation of any experiments. Animals were premedicated with an intramuscular injection of 1 mg/kg of xylazine (Bayer, Newbury, UK) and 5 mg/kg of ketamine (Pharmacia Animal Health Ltd., Corby, UK), and anaesthetized by administering 2–5% isoflurane (Baxter Healthcare Ltd., Thetford, UK) in oxygen:nitrous oxide mixture (50:50). After clipping and shaving the designated area each animal was washed with Chlorhexidine Gluconate 0.5% w/v in 70% v/v DEB (Adams Healthcare, Leeds, UK), followed by Povidone-Iodine USP 10% w/w (Adams Healthcare) and rinsed with I.M.S. (70% v/v, Adams Healthcare). Operation time did not exceed 3 hours. Postoperative analgesia was provided by a subcutaneous injection of 4 mg/kg of carprofen (Pfizer Ltd., Sandwich, UK) followed by further oral doses once daily for 2 days.

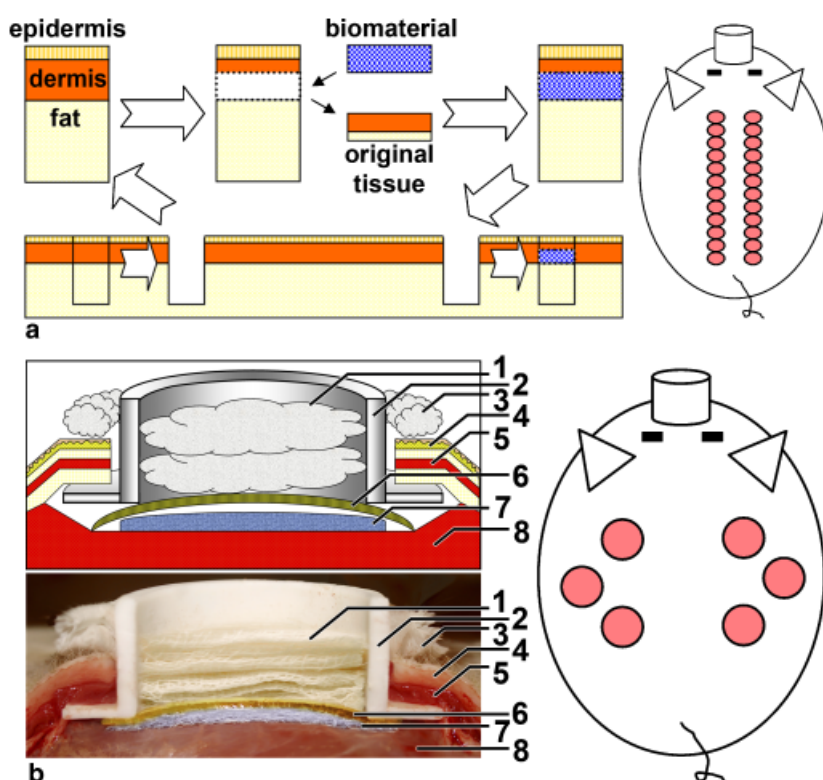


Figure 1. Schematic representation of the porcine *in vivo* full-thickness wound models. (a) Punch biopsy model. Part of the original dermis was replaced by the biomaterial being tested. (b) Wound chamber model. Bio-material (7) was applied on top of the fascia over the skeletal muscle (8), covered with silicone dressing (6) and further dressed with swabs (1). Flanges of the PTFE chamber (2) were inserted under the skin (4) and panniculus carnosus muscle layer (5), to restrict wound contraction and healing by epithelialization from the wound margins. The chamber was wrapped with povidone-iodine soaked gauze (3) to prevent wound infection. PTFE, polytetrafluoroethylene.

The integration of the dermal substitute materials was assessed in two different animal models.

Punch biopsy model

This model was used for the preliminary screening of several material formulations and allowed the dermal substitutes to be completely enclosed within the wound (Figure 1a). In this model, the dermal substitutes were completely isolated from the external environment thus eliminating adverse effects such as microbial contamination and the drying out of both wounds and the implanted biomaterials. Full-thickness punch biopsies, 24 per animal, 12 on each psoas muscle ridge, were created in three animals to produce wounds of 8.0 mm diameter and 0.5–1.0 cm depths. The deepest part of the original dermis measuring 1.0–1.5 mm in thickness was removed from the excised biopsy plug and replaced with the material of interest according to randomized positions. The epidermal portion of the excised biopsy plug was reapplied to close the wound. Excision biopsies were taken with a 10.0 mm biopsy punch at days 8, 15, and 27.

Wound chamber model

Full-thickness circular wounds 4.5 cm in diameter (six per animal), were created between scapula and iliac fossa on the dorsal area of two animals (Figure 1b). The wound edges were undermined by approximately 1 cm to allow polytetrafluoroethylene (PTFE) chambers (Northwick Park Institute for Medical Research, Harrow, UK) to be

inserted under the skin and stitched in place with four silk 2.0 sutures (Ethicon Ltd, Livingston, UK). These chambers were inserted to restrict wound contraction and to prevent healing by epithelialization from the wound margins. The selected biomaterials were placed within the chambers. The fascia over the skeletal muscle acted as the wound bed. With the exception of Integra[®], which was already complemented with a protective silicone sheet, each biomaterial was covered with a silicone dressing (Advasil, Advansis Medical, Kirkby-in-Ashfield, UK), which was fenestrated using a scalpel blade to allow exudate evacuation. The silicone dressings and Integra[®] were secured in place with surgical staples (Appose[®] ULC AutoSuture, Tyco Healthcare UK Ltd., Gosport, UK). Each chamber was packed with dry swabs, dressed around with swabs soaked in povidone-iodine solution to exclude microbial contamination and covered with Velband[®] orthopaedic wool (BSN medical Ltd., UK), secured with Mefix[®] (Mölnlycke Health Care AB (Publ), Göteborg, Sweden) and Elastoplast[®] (BSN medical Ltd.) adhesive tapes. Custom-made plastic rigid jackets were applied and secured in place with Velcro[®] straps to protect the experimental wounds from abrasion and accidental displacement. Intra-chamber 6.0 mm punch biopsies were taken at days 2, 4 and 7, with final excision biopsies taken on day 14.

Histomorphometry and immunohistochemistry

Upon removal, each biopsy was bisected vertically, one half was processed to paraffin wax embedding by a routine automated procedure and the other half was frozen for cryosectioning.

Paraffin wax-embedded tissue

Immediately after excision, tissue was placed in 10% (v/v) neutral-buffered formol saline (Genta Medical, York, UK) for 24 hours. Fixed tissue was processed overnight (VIP-3000 processor, Sakura Finetechnical Co. Ltd., Tokyo, Japan), and embedded in paraffin wax. Five-micrometer sections were cut, deparaffinized, rehydrated, and used for morphological analysis. The sections were stained with hematoxylin & eosin (H&E) to show the tissue structure; and with picrosirius red for collagen visualization. Picrosirius red enhances the natural birefringence of normal collagen fibers and indicates their thickness and quality when the stained tissue is viewed microscopically under polarized light.^{35,36} When collagen is denatured it loses its natural birefringence and appears darker than undenatured collagen.^{37,38}

Frozen tissue

Tissue biopsies were embedded in cryoprotective O.C.T.[™] Compound (Sakura Tissue-Tek, Zoeterwoude, Europe) and snap-frozen in liquid nitrogen. Fifteen-micrometer sections were cut using a -20°C cryostat microtome and allowed to air-dry for 24 hours. Slides with tissue sections were incubated in a humidified chamber for 20 minutes with normal goat serum (Sigma-Aldrich Company Ltd., Ely, UK) and a further hour with primary rabbit anti-human von Willebrand Factor polyclonal antibody (anti-vWF, 1 : 200 concentrate, DakoCytomation, Ely, UK) used at a 1 : 200 dilution of the supplied stock to identify blood vessel formation. Tissue was washed with phosphate-buffered saline (PBS) and incubated for a further hour with secondary antibody Fluorescein-isothiocyanate (FITC)-labeled goat anti-rabbit IgG (MP Biomedicals, Aurora, OH) at a 1 : 100 dilution as recommended by the supplier.

Slides were examined with an Olympus BX2 microscope and captured digitally with an Olympus DP-70 digital camera (Olympus, Watford, UK). Image analysis was per-

formed using the Image-Pro Plus software (Media Cybernetics Inc., Bethesda, MD).

Statistical analysis was performed in SigmaStat 3.5 (Systat Software Inc., Chicago, IL) using ANOVA or Student's *t* test as appropriate, with significance set at $p < 0.05$.

RESULTS

Optimization of Permacol[™] paste concentrations (punch biopsy model)

The wound biopsy model allowed rapid assessment of different formulations of Permacol[™] paste enabling the elimination of those, which we determined not to fulfil the requirements of a dermal substitute, outlined earlier. The three formulations of Permacol[™] paste, 100%, 75%, and 50% concentrations were compared in randomized, quadruplicate wounds. Figure 2 shows representative sections taken from each formulation for comparison.

Picrosirius red staining and polarized light microscopy allowed the visualization of the Permacol[™] paste collagen within the tissue sections. The histology of the wounds where the 100% Permacol[™] paste formulation was applied, showed retention of the paste collagen, surrounded by the native dermis without any microscopically detectable contraction (Figure 2a). In the wounds where 75% Permacol[™] paste was applied some paste disintegration was seen. A compact layer of the paste infiltrated by the host cells was noted at the very bottom of the wound, but dispersion of Permacol[™] paste particles with cell infiltrate appeared to occur in the uppermost layers (Figure 2b). Biopsies showed an almost complete disintegration of the 50% Permacol[™] paste bulk, small islands of which were present in some parts of the wound at the site of implantation (Figure 2c) after 27 days.

Picrosirius red staining also revealed no Permacol[™] collagen denaturation in either paste or sheet forms in any of the experimental wounds.

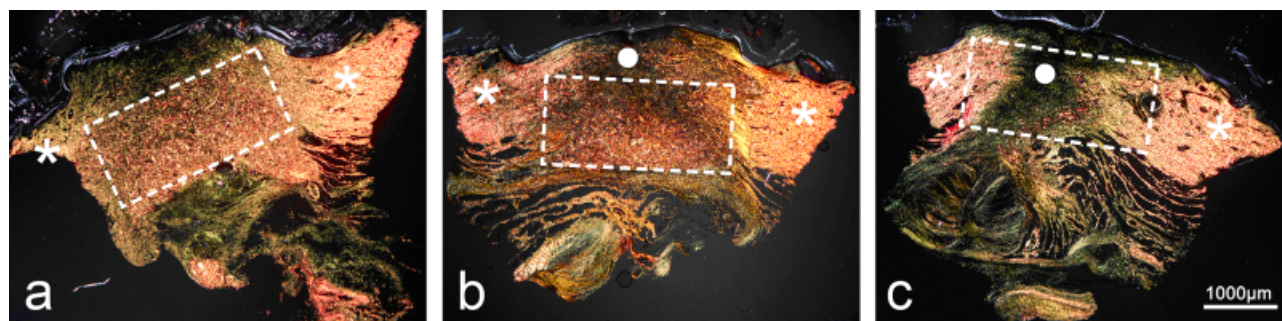


Figure 2. Punch biopsies implanted with Permacol[™] paste formulations excised on day 27, $\times 20$, scale bar 1,000 μm . Birefringence of collagen could be seen when picrosirius red-stained tissue sections were examined using polarizing microscope: (a) 100% v : v paste implant. This showed the retention of the Permacol[™] paste within the site of implantation represented by the dotted box. (b) 75% v : v paste implant. Collagen of this formulation was retained in only the lower part of the wound, but was lost from the upper part, which allowed native dermis (*) to contract from the wound margins. (c) 50% v : v paste implant. Only remnants of the Permacol[™] paste collagen were seen in this wound as it was replaced by granulation tissue (•). Extensive contraction of the wound was seen.

In general, the collagen content of the well-defined site of implantation of 100% Permacol™ paste appeared less dense and organized compared with native dermis at the 27 day time point. The 50% and 75% Permacol™ paste formulations were deemed not suitable for dermal replacement and consequently eliminated from further studies. One hundred percent Permacol™ paste appeared to be the most promising formulation and was assessed further in the wound chamber model.

***In vivo* integration of Permacol™ paste (punch biopsy and wound chamber models)**

Permacol™ paste allowed good host cell infiltration and supported growth of keratinocytes (Figure 3a), which migrated from the wound margins in the punch biopsy model. In this instance the epidermal part of the plug showed poor bonding to the wound and allowed keratinocytes to migrate from the edges of the wound over the implanted material. The keratinocyte layer formed on top of the Permacol™ paste was well structured, had an undulating basal layer which appeared to be firmly attached to the Permacol™ paste (Figure 3a, arrows). No seromas, which could indicate an ongoing inflammatory reaction, were noted in any of Permacol™ paste samples over the course of the studies. Two days postoperatively, the cells had penetrated the Permacol™ paste (Figure 4IIb) but not the Permacol™ sheet (Figure 4IIIb). At day 14 this trend continued with only a few cells visible in the basal part of the Permacol™ sheet (Figure 4IIc). Significantly greater number of cells was detected within the paste formulation (Figure 4IIc). Cytomorphological observations did not show any excessive cellular infiltration or giant cells (Figures 3b, 4IIc) which would normally be associated with an inflammatory response to a foreign material, thus indicating successful biointegration of the collagen paste. Overall, the host cellular infiltration of Permacol™ paste assessed by

histological observations (Figure 4II) was superior to Permacol™ sheet (Figure 4III) and similar to Integra® (Figure 4I) at all time points.

These findings were corroborated by cell counts performed on histological sections for each investigated biomaterial and are summarized in Figure 5. Levels of cellular infiltration were comparable for Permacol™ paste and Integra® but significantly lower for the Permacol™ sheet (ANOVA, all pairwise multiple comparison procedures (Tukey's test), $p < 0.05$).

The Permacol™ paste was still visible, maintaining its shape and consistency at the termination of the experiment at day 14 in the wound chamber model (Figure 4IIc) and at day 27 in the punch biopsy model (Figure 2a), indicating the presence of a stable biomaterial, at least within the 27-day timescale. Longer-term studies using the pig model would provide further information regarding the long-term stability of the material *in vivo*.

Neovascularization of Permacol™ paste (chamber model)

Early neovascularization of the Permacol™ paste was observed at day 4 by the presence of vascular elements, which could be recognized on H&E-stained tissue sections (Figure 3c, arrows). Newly formed microvessels appeared to be functional due to the presence of circulating blood cells, which were observed in some samples at day 7 (Figure 3d, arrows).

Immunostaining for vascular endothelium (anti-vWF, Figure 6) confirmed vascularization of the Permacol™ paste (Figure 6II), which was minimal at day 4 (Figure 6IIa), and increased over days 7 and 14 (Figure 6IIb and IIc). The level of neovascularization at days 4 and 14 (Figure 6IIa and IIc) was visibly comparable with that seen in Integra® (Figure 6Ia and Ic). At day 7,

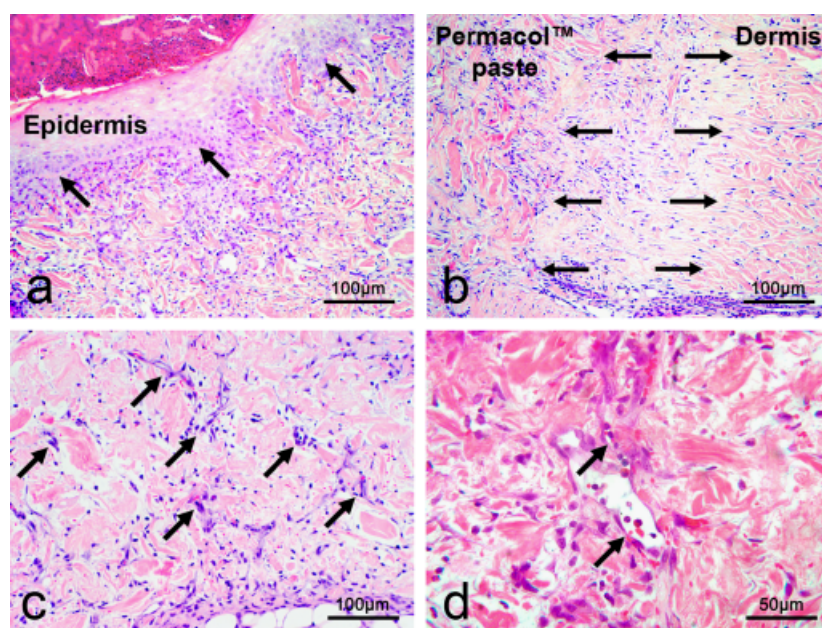


Figure 3. Hematoxylin & eosin-stained tissue sections showing the biointegration of Permacol™ paste in full-thickness punch biopsy (a, b) and wound chamber (c, d) porcine models: (a) Permacol™ paste was well-infiltrated with host cells and covered with an epidermis, formed from keratinocytes migrating from the wound margin. Note the well-structured epidermis with an undulating basal layer (arrows) intimately attached to the Permacol™ paste collagen bulk. Day 15, $\times 200$, scale bar 100 μm ; (b) No significant inflammatory cellular infiltration as an indicator of acute rejection or chronic inflammation was seen in the Permacol™ paste (left) and porcine dermis (right) interface (in between the arrows). Day 15, $\times 200$, scale bar 100 μm ; (c) Early neovascularization as indicated by the capillary formation (arrows) was seen as early as day 4, $\times 200$, scale bar 100 μm ; (d) Figure showing functional microvessels with circulating blood cells within the bulk of the paste (arrows) at day 7, $\times 400$, scale bar 50 μm .

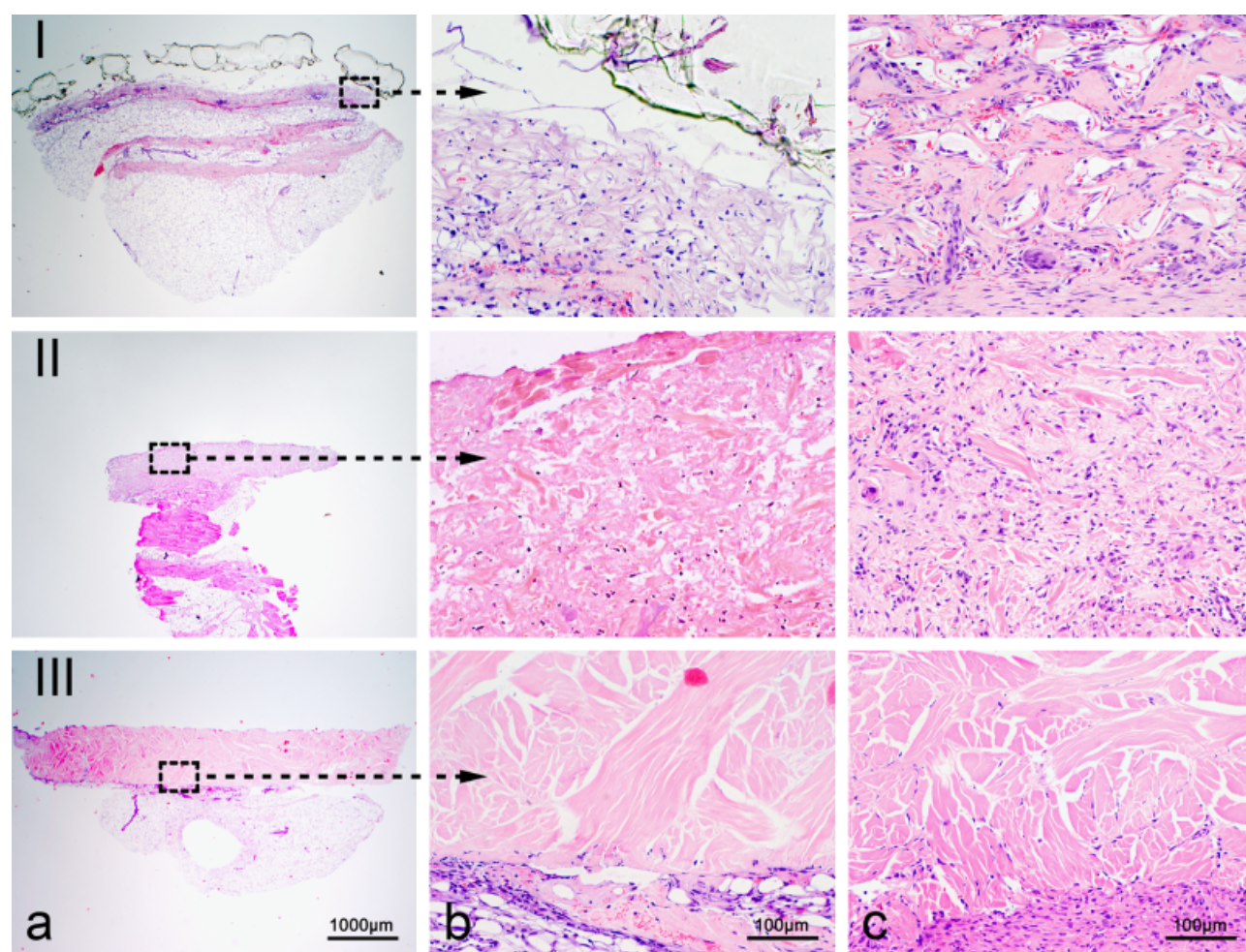


Figure 4. Hematoxylin & eosin-stained sections of biopsies to assess the host cellular infiltration of the implanted materials in the wound chamber porcine model: (I) Integra[®], as a control material, showed good host cell infiltration as on day 2 (a, $\times 20$, scale bar 1,000 μm ; b, $\times 200$, scale bar 100 μm), as well as on day 14 (c, $\times 200$, scale bar 100 μm); (II) Permacol[™] paste was well infiltrated with host cells throughout the entire thickness of the material on day 2 (a, $\times 20$, scale bar 1,000 μm ; b, $\times 200$, scale bar 100 μm), with increased cellular infiltration on day 14 (c, $\times 200$, scale bar 100 μm) which was comparable with that seen in Integra[®] (Ic); (III) Permacol[™] sheet showed poor biointegration and host cellular infiltration: very few cells were seen within the material on day 2 (a, $\times 20$, scale bar 1,000 μm ; b, $\times 200$, scale bar 100 μm), and only the lower compartment, closest to the wound bed, showed some cellular infiltration by day 14 (c, $\times 200$, scale bar 100 μm).

however, the Permacol[™] paste (Figure 6IIb) appeared to be vascularized to a greater degree than Integra[®] (Figure 6Ib).

No vascularization was detected in the Permacol[™] sheet over the period of the study (Figure 6III). The absolute depth of vascularization was measured by image analysis software. The difference in the median values between groups at each time point was assessed by *t* test. Results are summarized in Figure 7. There was no significant difference in neovascularization of Integra[®] and Permacol[™] paste at day 4 (0.01 and 0.16 mm, $p=0.364$). At day 7, although visually there seemed to be a difference in depth of vascular ingrowth (Integra[®]: 0.25 mm, SD=0.18;

Permacol[™] paste: 0.54 mm, SD=0.39), this difference was not statistically significant ($p=0.075$). By day 14 the depth of neovascularization of Permacol[™] paste reached 2.12 mm (SD=0.37), which was significantly greater ($p=0.002$) when compared with vascular penetration into the Integra[®] (1.52 mm, SD=0.29).

DISCUSSION

In this study, a novel paste formulation of Permacol[™] was tested for its suitability as a dermal substitute. The use of a paste formulation may have advantages in treating areas that would be difficult to treat with a sheet

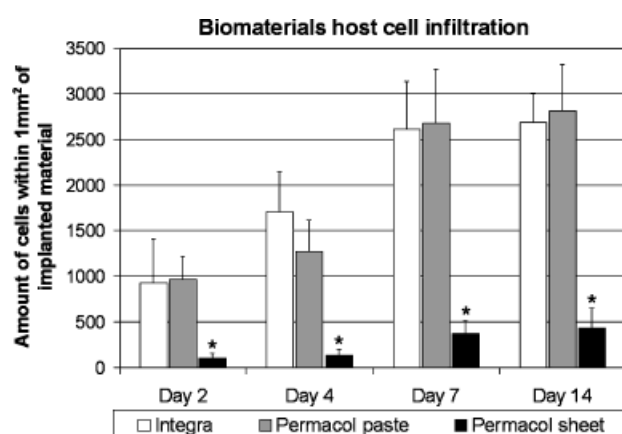


Figure 5. Graph, showing the number of host cells infiltrating each biomaterial (Integra[®], Permacol[™] paste, and Permacol[™] sheet) in the wound chamber porcine model. Permacol[™] paste (gray) and Integra[®] (white) showed similar rates of host cellular infiltration, whereas the infiltration of Permacol[™] sheet (black) was significantly lower at all time points, as assessed by ANOVA, All Pairwise Multiple Comparison Procedures (Tukey's test). Error bars represent standard deviation, *Significant difference between control material (Integra[®]) and Permacol[™] sheet ($p < 0.05$).

material. For example, areas where variations exist in body contour or wound surface roughness may be better treated with a material which can be spread over an uneven surface. In such cases sheet materials may fail to adhere and "take" due to limited contact with the underlying wound topography. Permacol[™] paste may also result in a more cosmetically acceptable appearance when compared with sheet biomaterials, where close approximation is needed to eliminate scarring along the edges of applied sheets.⁸

The current study indicates that a paste formulation of Permacol[™] can successfully integrate into full-thickness wounds without rejection or excessive inflammation.

Histology of the wounds, where 100% Permacol[™] paste was applied, showed a biomaterial which offered the suitable consistency to fulfil both surgical needs (i.e., handling and adaptability to the wound) and tissue regeneration: the biomaterial was stable enough to stay as a single piece, but flexible enough to take the shape of the wound and fill any cavities and inner undulations of the wound.

Failure of the 50% and 75% Permacol[™] paste to remain at the wound site may be attributed to the effect caused by the wound shifting when the whole skin, with underlying fat and superficial panniculus carnosus muscle layer, is moved over the skeletal muscles due to the growth of the animal. This phenomenon has been described as the "chamber shift effect"³⁹ and could be responsible for leaving particulate biomaterial dispersed over the skeletal muscle beneath the skin layer, as seen in some wounds. Wound bleeding, which could cause material loss, was not detected during the initial operation or during any of the dressing changing procedures. Alternatively, the 50% and 75%

pastes may not have been compact enough to stop the expansion of granulation tissue. Being composed of discrete particles rather than a layer, the pastes were dispersed within the tissue or expelled from the biopsy site during the wound healing and contraction process.

Host cells populated the Permacol[™] paste at much earlier time point when compared with the Permacol[™] sheet. This outcome was attributed to the particulate structure of the paste, which ensured a more porous structure than that seen in the sheet material and provided potential routes for cell migration. The reduced stiffness of the Permacol[™] paste matrix in contrast to the stiffer collagen sheet could also potentiate cell migration. It is known that the mechanical properties of the bioengineered matrices play a crucial role in wound healing and modulate cell behavior.⁴⁰

Permacol[™] paste, consisting of diisocyanate cross-linked collagen, resisted degradation and provided the wound with a stable, but flexible implant. In the initial stages of wound healing it would seem possible that Permacol[™] particles are held together by extracellular matrix (ECM) produced by host cells, which is subsequently remodeled in the later stages of wound healing. Integra[®] is reconstituted collagen sponge, which relies on infiltrating fibroblasts to deposit new collagen Type III within its structure. This subsequently matures to collagen Type I producing a viable neodermis. This is a lengthy process and in human can take in excess of 4 weeks. Permacol[™] paste, however, consisting of collagen Type I, already has material properties more akin to native dermis and, as such, only vascular ingrowth and minimal ECM deposition are required to establish a viable dermis.

Fibrovascular infiltration into the 100% Permacol[™] paste formulation and the dermal substitute Integra[®] was comparable. Granulation tissue formation was controlled in a similar manner to that seen in Integra[®], but allowed faster neovascularization of the collagen implant. The paste formulation negates the need to wait for silicone membrane detachment, as is the case with Integra[®], and may also allow for earlier permanent wound closure.

A well-developed microvasculature network had started to form in Permacol[™] paste as early as day 4, unlike the sheet formulation where no vascularization was observed by day 14. Similar rates of neovascularization were observed in Permacol[™] paste when compared with Integra[®], although it was also noted that the penetration of neovascular elements into the paste material at day 7 appeared to be superior to that observed in Integra[®]. This may be advantageous in the clinic as it may allow an earlier application of an extra-thin split-thickness skin graft or sprayed keratinocyte suspension in severely burned patients. The combination of properties, mentioned above, makes Permacol[™] paste more attractive for clinical use.

Dermal substitute biomaterials can achieve definite wound closure only in combination with epithelial elements, e.g., split-thickness skin graft or cultured keratinocytes. The porous structure of materials such as Integra[®] allows the rapid application of cultured epithelial cells by techniques such as centrifugation⁶ or by direct application⁴¹ to the underside of the matrix. This is

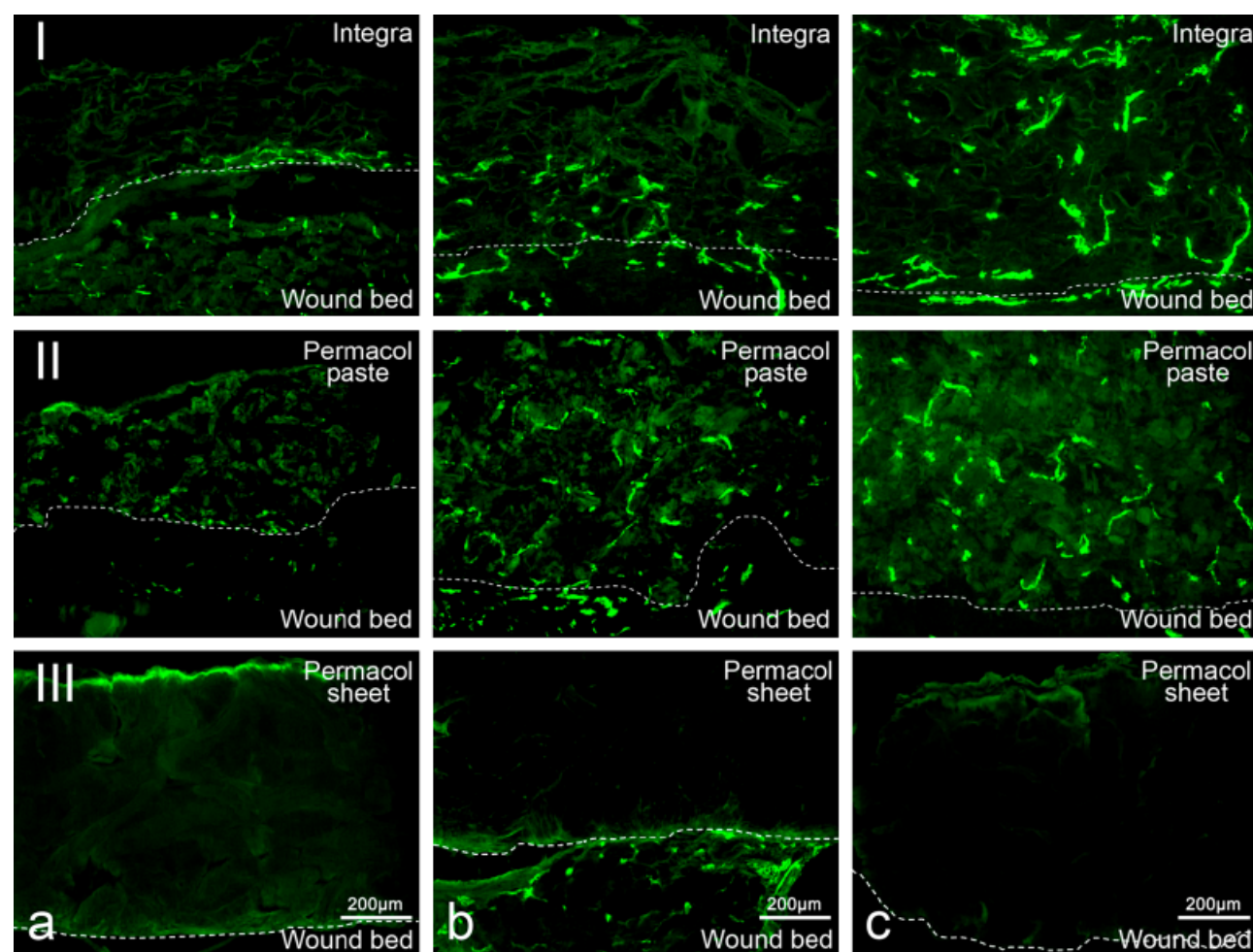


Figure 6. Neovascularization of the Integra[®] (I), Permacol[™] paste (II) and Permacol[™] sheet (III) in the wound chamber model at days 4 (a), 7 (b), and 14 (c), as revealed by staining of endothelial cells with FITC-labeled anti-vWF polyclonal antibody, $\times 100$, scale bar 200 μm . At day 4 only minimal vessel ingrowth was seen at the implanted biomaterial/wound bed interface (dotted line) in Integra[®] and Permacol[™] paste (Ia and IIa). By day 7 neovascutature formed in Permacol[™] paste (IIb) was seemingly more extensive when compared with Integra[®] (Ib). Similar level of neovascularization was seen in both Integra[®] and Permacol[™] paste by day 14 (Ic and IIc). No vasculature ingrowth was seen in Permacol[™] sheet at any time points. FITC, fluorescein isothiocyanate.

not possible with the solid structure of the Permacol[™] sheet. More complex, expensive and time-consuming techniques such as *in vitro* co-culture would need to be applied, and may only achieve limited success. The paste formulation as described here would be easy to combine with cultured dermal fibroblasts or keratinocytes, thus opening up its use to wider aspects within the field of tissue engineering.

Permacol[™] is xenogenic by its nature, which may bring concerns about safety. However, porcine-derived materials are widely used in the clinic for numerous applications, and have been proven to be safe and efficient,^{23,42,43} they also have advantages over bovine-derived products due to the increased resistance of the pigs to prion disease.⁴⁴ Permacol[™] sheet has been used clinically for different purposes for a number of years^{25,26,28} with no evidence

of an adverse reaction. This track record can be extrapolated to the Permacol[™] paste formulation, which is chemically identical to the sheet. In addition, the benefits described in this report are likely to widen the fields of clinical application, including the treatment of deep extensive burns.

The treatment of burn injuries can be protracted and expensive, and the high cost of dermal substitute materials, which are currently available can be prohibitive. Any new dermal substitute biomaterial needs to perform at least as well as materials that are currently available; however, a substantial reduction in cost would make them even more attractive for wider adoption in clinical use.

In conclusion, our findings suggest that Permacol[™] in the form of a paste may act as a suitable alternative to current dermal substitutes for full-thickness wounds.

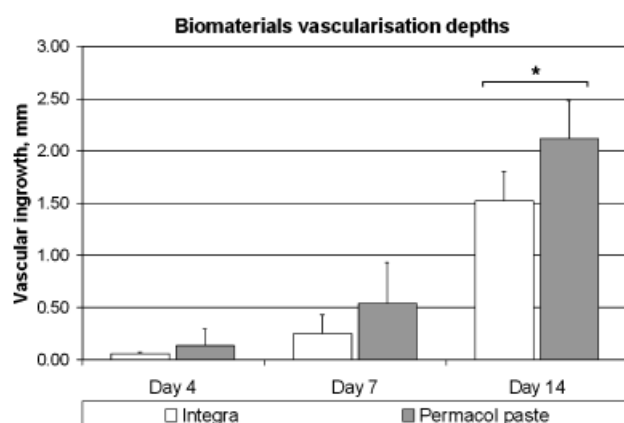


Figure 7. Graph showing neovascularization depth of Integra® (white) and Permacol™ paste (grey) in porcine wound chamber model. The depth of vascular ingrowth was comparable at days 4 and 7, but significantly higher in Permacol™ paste at day 14 ($p=0.002$, t test). Error bars represent standard deviation. *Significant difference between studied groups.

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