Title: Towards a skin-material interface with vacuum-integrated capped macroporous scaffolds.

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Abstract

Avulsion, epidermal marsupialisation, and infection cause failure at the skin-material interface. A robust interface would permit implantable robotics, prosthetics, and other medical devices; reconstruction of surgical defects, and long-term access to blood vessels and body cavities. Torus-shaped cap-scaffold structures were designed to work in conjunction with negative pressure to address the three causes of failure. Six wounds were made on the backs of each of four three-month old pigs. Four unmodified (no caps) scaffolds were implanted along with twenty cap-scaffolds. Collagen type 4 was attached to twenty-one implants. Negative pressure then was applied. Structures were explanted and assessed histologically at day seven and day twenty-eight. At day twenty-eight there was close tissue apposition to scaffolds, without detectable reactions from defensive or interfering cells. Three cap-scaffolds explanted at day twenty-eight showed likely attachment of epidermis to the cap or cap-scaffold junction, without deeper marsupialisation. The combination of toric-shaped cap-scaffolds with negative pressure appears to be an intrinsically biocompatible system, enabling a robust skin-material interface.

Introduction

Skin fails to attach to materials because of epidermal marsupialisation, avulsion, and infection at the interface ¹ [Figure 1]. A robust skin-material interface would permit long-term implantation of robotics, prosthetics, intraosseous transcutaneous amputation prostheses (ITAPs), bone anchored hearing aids (BAHAs), catheters, and intravenous lines, and may enable the reconstruction of surgical defects with metals and polymers.

The interactions with materials of target, defensive, and interfering cells determine the intrinsic biocompatibility of systems ². For this study the “target cells” were keratinocytes (epidermal cells), specifically their interaction with implant surfaces, along with fibroblasts and their interaction with scaffolds. The “defensive cells” were lymphocytes and neutrophils. Fibroblasts also can act as “interfering cells,” forming capsules (connective tissue and fibroblasts surrounding implants) ³. Because wound healing involves an immune response, the involvement of defensive cells is inevitable. Excessive inflammation can lead to degradation of implanted materials, foreign body giant cell formation, capsules, and tissue necrosis. Hence, minimizing inflammation is critical ⁴.

Poor tissue integration into pores causes implant failure ^{5 6} and dynamic tissue integration is critical for preventing the three causes of failure at the skin-material interface ¹. A variety of porous scaffolds, rods with holes ⁷, rods with cuffs ⁸, and meshes ^{9 10} have increased the complication-free duration of skin implantation, but a long-term skin-material interface system has not been achieved because of poor attachment by target cells, inflammation mediated by defensive cells, infection,

and/or excessive connective tissue formation by fibroblasts.

Porous polymer scaffolds have been shown to integrate with tissue deep to epidermis, but optimising and investigating the critical epidermis-material interface was not part of these projects^{11 12 13 14 15}. Integration of tissue with high-density porous polyethylene has been shown to stabilise implants, protecting against extrusion^{13 14} and even supporting skin grafting¹⁶. Porous scaffolds are used in joint replacement surgery^{17 18} and have been shown to integrate well with bone. One such highly macroporous scaffold is Cytomatrix™¹⁹, made by chemical vapour deposition of tantalum onto an open pore carbon matrix [Figure 2].

Negative pressure ("vacuum") has been for over twenty years to improve wound healing²⁰. It has been shown to facilitate tissue ingrowth and vascularisation of a collagen and glycosaminoglycan matrix called Integra™²¹, but has not been tested in other materials research, in particular with tissue integration into macroporous scaffolds. Negative pressure improves wound healing, removes exudate, enhances angiogenesis, and restores the integrity of capillary basement membranes, thereby decreasing the permeability of blood vessels and improving wound oedema^{20 22}. Much of the early research on negative pressure and wound healing involved White-Landrace pigs because of anatomical and physiological similarities to human skin²³

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Along with the mechanical integration of tissues, the surface properties of materials are critical in determining implant performance and cell behavior. There are many methods in use to improve surface properties. For example, alterations in surface texture and the addition of chemicals such as hydroxyapatite²⁸ have been

shown to improve osseointegration. For keratinocytes, smoother surfaces have been shown to improve cellular attachment²⁹. Surface functionalisation (the addition of chemical functional groups) and the attachment (bioconjugation) of proteins are commonly used to improve cellular attachment and proliferation. Proteins used include collagen, fibronectin, albumin, and laminin-332. Collagen is most commonly conjugated to implanted materials, with many diverse biomedical applications^{30 31 32}. Collagen improves cellular adhesion, proliferation, and differentiation of many cell types in culture³⁴. Where exogenous collagen is used in clinical practice to optimize materials, it is invariably collagen type 1^{35 33 36 37 38 39 40 41 42 43 44 45 46 47 48 49}, including on polyurethane⁵⁰, polystyrene⁵¹, titanium⁵², titanium alloy⁵³, tantalum⁵⁴, and stainless steel⁵⁴. Covalent conjugation to materials is used to improve the stability of collagen^{55 56 57 58 59 60 61 62 63 64 54}. Similarly, cross-linking of collagen, after it has been adsorbed or otherwise conjugated to substrates, increases stability^{65 66}.

During the vitrobiological phase of research, the results of which will be published in the near future⁶⁷, we demonstrated that collagen type 4 was significantly more stable than collagen type 1, both in adsorbed and covalently bound forms, with no degradation of covalently conjugated collagen type 4 noted after incubation for twenty days in 8 M urea. For percutaneous implants, we hypothesised that a two-pronged approach could lead to an intrinsically biocompatible skin-material interface system: covalently conjugating collagen type 4 to materials to optimise epidermal cell attachment, along with negative pressure enhanced sub-epidermal tissue integration into scaffolds to anchor overlying epidermis and to minimise inflammation.

Materials and Methods

Implant Preparation

“Cap-scaffold” implants were created by thermal melt-bonding torus-shaped “Elast-Eon™” (AorTech, Surrey, U.K.) caps to porous Cytomatrix™ (Cytomatrix Pty. Ltd., Singapore) scaffolds [Figure 3], [Figure 4]. Implants were sonicated in purified water with 2% phosphate-free detergent (Tween 20™, Sigma-Aldrich) for two 20 min periods, then in purified water for two 10 min periods, and then dried in laminar flow air at 20°C for seven days.

Implant types are shown in [Table 1]. The experimental data set was limited to six implants per pig (twenty-four implants in total). One pig died unexpectedly under general anaesthesia on day seven, further limiting data points to eighteen. Four of these remaining eighteen data points were unmodified scaffolds (without caps), leaving fourteen cap-scaffold implants for assessment of the epidermal interface. With this limitation, it only was possible to assess the effect of negative pressure on tissue integration into scaffolds and differences between full and partial skin thickness implants on scaffold integration and epidermal attachment. Other variations tested, but not in sufficient numbers to be statistically significant, were single vs. double stacked scaffolds, oblique vs. perpendicular skin incisions, and collagen type 4 treated vs. untreated specimens.

Collagen type 4 was bound covalently to implants after surface functionalisation via acetaldehyde plasma polymerisation (Aapp). Plasma

polymerisation of acetaldehyde (>99.5%, Sigma-Aldrich, Product #402788, North Ryde, Australia) was performed in a custom-built reactor, described in detail elsewhere⁶⁸, at CSIRO's Ian Wark Laboratory in Melbourne, Australia.

Specimens then were removed and placed into collagen type 4 (Collagen Type IV, Chemicon (Millipore), Product #CC076) solution for 2 hours at room temperature, at a concentration of 10µg/ml in PBS. Following incubation in the collagen solution, 20mg/ml of sodium cyanoborohydride (Sigma-Aldrich, Product #156159) was added to cause reductive amination. After incubating with sodium cyanoborohydride for 10 hours, specimens were washed with PBS five times and then stored in PBS at 4°C for thirty days.

Animal Care and Use

In vivo experimentation was performed over five weeks in 2011. Four female White-Landrace pigs, each weighing approximately 30 kg, were housed for one week pre-operatively. They were fed a cereal-based diet and were kept in a purpose-built shed at 18-25°C. After surgery, they were kept separately in custom-made stainless steel cages measuring 1_m x 1_m x 2_m, alongside one another. The operative procedure, use of anaesthesia, and animal care methods in the experiments were consistent with guidelines set by the Australian National Health and Medical Research Council (NHMRC). Experimentation was approved and limited by the School of Veterinary Science, Animal Ethics Committee, University of Melbourne, Victoria, Australia.

The pigs were sedated, anaesthetised, given analgesia, and cared for postoperatively, described in detail elsewhere by the authors⁶⁹. In brief,

Cephazolin 12mg/kg (Cefazolin, Pfizer, West Ryde, Australia) antibiotic prophylaxis was administered intravenously at induction and for the first four post-operative days. One pig died unexpectedly of unknown cause at day seven during anaesthesia. The remaining three pigs were euthanised with pentobarbital (Pentobarb, Akorn, Lake Forest, Illinois, U.S.A.) intravenously 100mg/kg during general anaesthesia at day twenty-eight. Back hair was clipped and shaved in the sternal recumbency position. Skin was prepared by washing with Chlorhexidine and Cetrimide Irrigation Solution (Pfizer, West Ryde, Australia). Povidone iodine scrub was followed by a 70% alcohol rinse that was allowed to evaporate before wounding. Adhesive plastic drapes then were applied followed by draping with autoclaved linen. Six wound sites were marked 6 cm lateral to the midline of the back and 6 cm apart. Skin holes were punched using 8 mm diameter biopsy punches (Kai Medical, North Ryde, Australia); 4 mm long scalpel incisions were made in four quadrants to help fit the implants. Haemostasis was obtained by pressure with sterile gauze sponges. Electrocautery was not used. Implants were placed at partial (mid dermis) and full skin thickness (onto subcutaneous fat), with single or double-stacked scaffolds, and using perpendicular and oblique skin incisions abutting the cap [Figure 3]. After placement of implants, the four quadrant incisions were closed with 5/0 polyglactin 910 sutures (Vicryl™, Johnson and Johnson – Ethicon, North Ryde, Australia). Wounds and implants were covered with paraffin gauze dressings (Adaptic, Johnson and Johnson, North Ryde, Australia) followed by sterilised dressing foam (V.A.C.® GranuFoam™). Foam and surrounding skin were covered with adhesive-bound

plastic sheets (V.A.C.® Advanced Drape™). Tubing (SensaT.R.A.C.™ Pad with pre-attached tubing) connected the foam to negative pressure-delivering machines V.A.C. ATS® Therapy Units (KCI Medical, Lane Cove West, Australia) which delivered negative pressure of 125 mmHg for 10 min periods interspersed with 2 min zero pressure periods. Tubing was interposed with fibre-reinforced PVC garden hose to protect against chewing and other physical damage. The PVC tube was attached to the dressing tubing with a 12.7 mm plastic hose coupler to enable untwisting. Alarms built into the V.A.C. ATS® Therapy Units sounded in the event of air leaks from the dressings or connecting tubes. An alarm sounded on one occasion, revealing a tear in the adhesive-bound plastic wound dressing sheeting, which was rectified immediately.

The design and use of custom-made polyethylene covers to protect the wounds and dressings is described in detail elsewhere by the authors⁶⁹. In brief, a thermoplastic polyethylene cover was fashioned to the dimensions of a three-month old pig and fixed with 38 mm wide hook-fastener material, “Velcro.” A 5 cm diameter hole in the midline of each cover allowed passage of tubing for negative pressure wound dressings. All dressing changes were performed aseptically, under general anaesthetic by the primary investigator, on days four, seven, fourteen, and twenty-one. At day twenty-eight the pigs were euthanised and the implants excised. Following anaesthetic induction, the rigid polyethylene covers were removed, followed by removal of the negative pressure wound dressings. Wounds were checked and gently cleaned with saline-moistened cotton swabs to remove exudate and slough. Hair regrowth was shaved with size 10 scalpels.

Each wound then was dressed and protected as described above.

Clinical Evaluation of Wounds

Wounds were assessed clinically for colour, drainage and exudate, and new connective tissue and blood vessels ("granulation tissue") at every dressing change and at day twenty-eight, just prior to explantation, by the principal investigator, without blinding. Wound assessment was based on criteria [Table 2] used previously to investigate porous cuffs for peritoneal dialysis catheters implanted in goat skin⁹.

Histology

Implants were excised with a 1 cm cuff of skin. Implants then were placed into 3.7 % paraformaldehyde (Sigma-Aldrich) in PBS and stored at 4°C for one month. Implants then were washed three times with PBS to remove fixative and then serially dehydrated in $\geq 99.5\%$ ethanol (Sigma-Aldrich) (10 %, 30 %, 50 %, 70 % in purified water), in each concentration for 12 - 24 hours.

Specimens then were embedded in polymethylmethacrylate. After curing for three days, excess polymethylmethacrylate was removed with a hacksaw.

Specimens then were sectioned transversely with a diamond saw (Leica SP1600), 15 μm thick, with at least a 1 mm index (width) between sections. Each 15 μm thick section then was etched for 2 min with acidic ethanol (2% HCl in 95% ethanol). Specimens then were rinsed in water before being incubated for 2 min with 0.3% Basic Fuchsin (Sigma-Aldrich). Sections then were placed onto slides, fixed with ultraviolet-activated PMMA glue (Acry-Bond UV3232, Acrylic

Technologies Australia), and coverslipped. Specimens were examined using an Olympus light microscope (model BH2-UMA).

Histological analysis of scaffold integration consisted of an evaluation the presence of inflammation, the quality of tissue within the scaffold, and apposition of tissue to the scaffold, based on a histologic rating scale for soft-tissue implants⁷⁰ [Table 3]. The interstitial tissue response was quantified by numerical rating of tissue morphology and cellularity. Capsules were not detected around any implants so the rating scale was altered to remove qualitative and quantitative capsule assessments and, instead, a rating scale for tissue integration into the scaffolds was added. Epidermal marsupialisation into scaffolds from the wound edges was deemed to exclude assessment of dermal and subcutaneous approximation to scaffolds.

Epidermal apposition to implants occurred at the “upper cap” (A), part way down the cap (“partial cap”) (B), “upper scaffold” (C), or within the scaffold (“into scaffold”) [Figure 4]. Each “cap-scaffold” was transversely sectioned four times; being disc shaped, there were two epidermal-implant interfaces for each transverse section. Owing to the unexpected death of one pig at day seven, six “cap-scaffold” implants were explanted after day seven (yielding twenty-four sections with forty-eight epidermal interfaces) and fourteen “cap-scaffold” implants were explanted on day twenty-eight (yielding fifty-six sections with a theoretical 112 interfaces, but in practice limited to 106 interfaces because of complete epidermal overgrowth across the Elast-Eon™ torus in three sections). Each implant was assessed for extent of epidermal marsupialisation on each of

each of its eight interfaces.

Statistics

The number of animals requested was based on a power analysis of numbers required for detailed histological analysis of the epidermis-implant interface.

Funding limited the number of pigs to four and statistical analysis indicated that twenty implants were required in each pig. Ethics approval was obtained for only six implants per pig. One pig died unexpectedly under general anaesthesia on day seven, further limiting twenty-eight day data points to eighteen. Four of these eighteen data points were “scaffolds” only (without caps), leaving fourteen cap-scaffolds for assessment of the epidermal interface. Full-thickness vs. partial skin thickness implantation of cap-scaffolds was analysed by Fisher’s exact test.

There were insufficient numbers to express statistical differences amongst collagen-treated vs. untreated, perpendicular vs. oblique cut skin edges, and single vs. double-layered scaffolds.

Results

Clinical evaluation of wounds

At day seven there was normal surrounding skin colour, no drainage or crust, granulation tissue extended through the centre of the caps, and skin closely apposed to the outer edge of the caps (clinical appearance grade scale “1” for each criteria). Four unmodified scaffolds and fourteen cap-scaffolds were explanted at day twenty-eight. All showed normal surrounding skin colour, normal granulation tissue, and skin closely apposed to the implants [Figure 5]. Full-

thickness implants showed no drainage or crust formation (clinical appearance grade scale “1” for each criteria). The seven partial-thickness implants showed scaffolds protruding through the skin, with a small amount of crust admixed with scaffolds (clinical appearance grade scale “2” for each criteria). There were no signs of infection.

Histological Findings

At day seven there was no capsule formation around any of the six scaffolds. Beneath regions of epidermal marsupialisation and/or attachment, there was close apposition of dermal and subcutaneous tissue to all six scaffolds with few gaps between scaffolds and tissue (histologic grading scale “3”). Blood vessels and fibroblasts in interstitial tissue were seen in all six scaffolds (histologic grading scale “3”). No inflammatory cells were observed around or within the scaffolds. Epidermal marsupialisation was not associated with inflammatory cells. [Figure 6].

At day twenty-eight, beneath regions of epidermal apposition, there was close approximation of tissue to scaffolds, with no gaps between scaffolds and tissue (histologic grading scale 4) and the tissue appeared mature and resembled tissue in non-injured regions (histologic grading scale 4). There were no dead or inflammatory cells. The three uncapped scaffolds implanted at full-thickness showed no inflammatory cells and tissue within scaffold pores resembled tissue in non-injured regions (histologic grading scale 4). Two of these scaffolds were integrated completely [Figure 7], with overlying reconstitution of glands and hair follicles, along with overgrowth of epidermis. The third unmodified scaffold, while

showing close, mature tissue approximation (histologic grading scale 4), also showed epidermal marsupialisation along the healed wound incision and into the scaffold. In this specimen, no inflammatory cells were seen [Figure 8].

For the day seven implants, epidermis apposed the cap or upper scaffold [Figure 6] (not into the scaffold) in the three full-thickness implanted cap-scaffolds. In the partial-thickness implanted cap-scaffolds, epidermis marsupialised into the scaffold in two and reached the upper scaffold in one. Well-defined epithelial junctions with upper scaffold and/or cap were observed in the three full-thickness implanted specimens [Figure 6]. Clear junctions were not seen in the partial-thickness implanted specimens. [Table 4].

Of the eight full-thickness cap-scaffolds explanted at day twenty-eight, four showed mixed epidermal apposition to the Elast-Eon™ cap and upper scaffold [Figure 9], with no marsupialisation into the scaffold. Of these, three (specimens 1, 5, and 8 in [Table 5]) showed close epidermal apposition to the upper cap [Figure 10]. Well-defined epithelial junctions with upper scaffold and/or cap were observed in these implants. Clear epidermis-implant interfaces were not seen in the partial-thickness implanted specimens. Four of these eight full-thickness implanted cap-scaffolds showed marsupialisation into the scaffolds [Figure 11] (specimens 2, 3, 4, and 6 in [Table 5]).

Of note, one implant showed epidermis bridging the central zone of granulation tissue in the middle of the torus and apposing the inner aspect of the scaffold on the contralateral side from which the epidermis originated [Figure 11].

All six partial-thickness cap-scaffolds showed epidermal marsupialisation into

the scaffolds. All cap-scaffolds showing epidermal apposition to the Elast-Eon™ cap or upper scaffold were implanted at full-thickness. Statistical analysis, comparing marsupialisation into the scaffold for full-thickness vs. partial-thickness implanted cap-scaffolds, using Fisher's Exact Test, showed a two-tailed P value < 0.0001 ($P < 0.05$, statistically significant) [Table 6].

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Discussion

With limited numbers, controls (in particular, implants without negative pressure) and statistical significance were lost, but some qualitative assessment was possible. With only fourteen cap-scaffolds for assessment of the epidermal interface at day twenty-eight, only differences between full and partial skin thickness implants on scaffold integration and epidermal attachment could be assessed. Differences between collagen-bound vs. non collagen-bound implants, types of skin incision, and single vs. double-layered scaffolds, could not be assessed.

The complete integration of tissue with scaffolds at day twenty-eight and nearly complete tissue integration at day seven suggested that negative pressure application for several more days might have ensured complete tissue integration. The lack of defensive and interfering cells in tissues integrated with scaffolds may be explained by the known high compatibility of tantalum^{71 72} and enhanced wound healing properties of negative pressure²⁰.

Without negative controls (implants without negative pressure), it was difficult to assess the improvement conferred by negative pressure, but previous research^{14 16} on tissue-implanted porous polyethylene showed poor quality tissue integration into more centrally located pores, these being mostly filled with defensive cells. Similarly, skin-implanted titanium mesh⁹ showed capsule formation, defensive cells, and incomplete tissue integration.

Pigs were chosen as the experimental model because of similarities between porcine and human skin. While the clinical assessments of pig wounds described

herein were based on criteria used previously to investigate peritoneal dialysis catheters implanted in goat skin⁹, there are significant anatomical differences between goat and pig skin^{73 74 75 76 77}. Nevertheless, the chosen assessment parameters (colour, drainage and exudate, granulation tissue, and skin apposition) are common indicators of inflammation, infection, and healing in most mammals. Blinded wound assessments would have been ideal but were not practical because of time, cost, and staffing issues. While wound assessments were not blinded, the uniformity of findings for all implants suggests minimal observation bias amongst specimens and the lack of observed markers for inflammation or infection was corroborated by histology findings and is seen in clinical practice with the use of negative pressure wound dressings on human patients.

The torus-shaped cap-scaffold structure appeared to facilitate a robust interface with skin by three mechanisms: (1) transmission of negative pressure via the central hole to underlying scaffold; (2) tissue incorporation into the scaffold, providing a bed and anchor for overlying epidermis, thereby preventing avulsion at the cap-epidermis interface; (3) provision of a physical point of attachment for epidermis (the cap or cap-scaffold junction).

The complete integration of unmodified scaffolds (no caps), with healing of overlying skin, suggested that negative pressure might be used in future to integrate macroporous scaffolds into tissues such as bone, fat, or muscle. Also, the effectiveness of tissue integration and skin growth over scaffolds without caps suggested that a central hole might not be necessary; a skin-material interface may be possible with solid caps. However, cap-scaffolds lacking central holes are likely to

show size limitation because, beyond a certain diameter, there will be space immediately deep to the cap, unreachable by negative pressure. Previous experience with porous scaffolds, without negative pressure, has show distance and size limitation for tissue integration ¹⁴. Pores that are not connected to other pores are termed “dead spaces” and suffer from poor nutrient flow and gas exchange leading to cellular death. In addition to the transmission of negative pressure, the central hole could be used as a portal for instrument or tube insertion while the cap could act as a prosthetic point of attachment.

For the day seven cap-scaffold explants, tissue integration appeared almost complete and epidermis apposed the cap or upper scaffold in the three full-thickness implanted cap-scaffolds, with clearly defined demarcations between the implants and epidermis. These findings suggest that epidermal migration was mostly complete by day seven. At day twenty-eight, marsupialisation into the scaffold was less likely in full-thickness implanted scaffolds compared to partial-thickness implanted scaffolds ($P < 0.0001$). Of the eight cap-scaffolds implanted at full-thickness, four showed epidermal apposition to the cap, but also varying degrees of epidermal migration down to but not into the scaffold. Future research may favour epidermal-material interfacing at the scaffold-cap junction rather than the cap itself. If this is the case, the surface of the cap could be designed to facilitate epidermal marsupialisation down to the cap-scaffold junction. Of the four cap-scaffolds showing epidermal apposition to the cap, three showed close epidermal apposition to the upper cap with well-defined epithelial-cap interfaces. Of note, these three implants also showed shearing damage of the thin and delicate edge of epidermis apposing the cap,

possibly consequent to firm attachment of the epidermis. The diamond saw is designed to section metals and may have been too coarse for sectioning the delicate epidermis-material interface. Future experimentation with cap-scaffolds made entirely from polymer, followed by conventional histological sectioning, may minimise damage to, and permit closer inspection of, the epidermis-cap interface.

In future, marsupialisation might be minimised by removing a cuff of epidermis prior to implantation [Figure 4], enabling tissue integration throughout the scaffold, prior to the arrival of the advancing edge of epidermis. Such de-epithelialised zones were proposed in the initial research plan, before implant numbers were reduced.

One day twenty-eight implant showed epidermis bridging the central zone of granulation tissue in the middle of the torus and attaching to the inner aspect of the scaffold on the contralateral side [Figure 11]. This finding supports the de-epithelialisation of a cuff of skin around the implant wound, followed by the application of negative pressure, i.e. facilitating tissue ingress before re-epithelialisation. In addition, this finding suggests epidermis may attach preferentially to the cap-scaffold junction, rather than the cap.

Aapp has been used previously^{78 79 80 81 82 83} for bioconjugation to materials. Conjugation of collagen type 4 (skin basement membrane collagen) to the cap-scaffolds may improve epidermal attachment, but numbers were insufficient to show this. Nevertheless, given the improved attachment of epidermal cells to contact lenses seen after bioconjugation of collagen type 1⁸⁴ and the resistance to degradation of collagen type 4 observed in vitrobiological research by the authors, to be published later this year, surface optimisation with covalently bound collagen type

4 may facilitate epidermal attachment and warrants further investigation.

Previous research on skin-implanted materials involved periods of fewer than four weeks^{85 8 15} and full vascularisation of Integra™ was observed by four weeks⁸⁶.

Negative pressure is likely to have shortened the time required for vascularisation; the average time taken for vascularisation of Integra™ with negative pressure was reported as 7.25 days²¹. Hence, twenty-eight days is likely to have been sufficient time for assessing tissue integration and vascularisation of scaffolds.

Materials that breach skin inevitably become colonised with bacteria at the skin-material interface. While epidermal attachment to the cap or cap-scaffold junction may present a barrier to bacteria, twenty-eight days of implantation may be insufficient to assess long-term infection risk. Clinical experience with intravenous lines, endotracheal tubes, dialysis catheters, dental implants, BAHAs, ITAPs, among other percutaneous devices, provides a bank of information about infection risk and prevalence. Serious bloodstream infections are associated with central venous catheters (CVCs), particularly those placed in an intensive care unit (ICU)⁸⁷. CVC infection increases significantly after seven days of implantation⁸⁸. BAHA removal is quoted at 0.5%⁸⁹ and 0.75%⁹⁰ due to infection, usually occurring after several months. Most skin reactions around BAHA's are secondary to inflammation caused by bacteria at the skin-implant interface⁹¹. To minimise the risk of avulsion, skin abutting the BAHA is immobilised⁸⁹. There is a "race" between tissue apposition to the implant and biofilm adhesion to the implant's surface^{92 93}. If the race is won by tissue, dead spaces are eliminated, media favouring bacterial growth is displaced, and endogenous immunologically competent tissue is placed in close proximity to

the material. The close and rapid tissue integration into scaffolds seen with negative pressure may protect against infection by favouring tissue apposition ahead of biofilm adhesion. The rod-skin interface for ITAP's remains problematic⁹⁴. Skin can move freely against the rods leading to epidermal marsupialisation and a persistent layer of pus at the interface between the skin and rod⁹⁵. Most ITAP failures are attributable to infection, with infection rates of up to 18% at 3 years reported⁹⁶. ITAP's have been modified with surface roughening^{97 98}, pores^{99 100} and porous cuffs^{8 101} in an attempt to reduce infection. Cuffs drilled with 7 mm holes in rods implanted in mice for up to 6 months appeared to be resistant to infection¹⁰¹. Porosity appeared to prevent infection in rods implanted sheep up to 9 months¹⁰⁰, but epidermal marsupialisation still occurred. For skin-interfaced materials, the presence of epidermal marsupialisation down to deeper tissues opens a portal for bacteria and suggests the possibility of long-term infection.

Conclusions

Limited to six implants per pig, this research became a pilot study primarily to assess the wellbeing of experimental pigs to inform a bigger study. Nevertheless, some qualitative analysis was possible. Negative pressure appeared to assist tissue in the race with bacteria to adhere to material surfaces and tissue apposition to scaffolds. In addition, the lack of inflammation and infection at day twenty-eight was likely to be consequent to negative pressure and suggested the possibility of long-term stability. Epidermal marsupialisation appeared to be prevented by three mechanisms: subepithelial tissue integrated into scaffolds presenting a physical

barrier; preferential epithelial attachment to the caps or cap-scaffold junctions; and scaffold-integrated subepithelial tissue anchoring overlying epithelium, minimising avulsion at the epidermis-material interface.

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Declaration of interest

Associate Professor Mark Kirkland is CEO of Cytomatrix, the company that provided the scaffolds and helped to fund the project. The inventor, Dr. Gil Stynes, has submitted a provisional patent relating to this research.

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Marsupialisation. Epidermis migrates around, rather than attaching to, percutaneous implants.
458x328mm (72 x 72 DPI)

Accept

Cytomatrix™. These highly interconnected scaffolds enabled tissue ingrowth facilitated by negative pressure.
Note the large pore size (“macroporous”) and absence of “dead” spaces.
155x117mm (150 x 150 DPI)

Accep

Full thickness cap-scaffold implantation. In retrospect, approximating epidermis to the cap at the time of insertion was counterproductive and probably promoted marsupialisation. A - epidermis; B - dermis; C - central hole in cap; D - radial incision to facilitate implantation.
100x87mm (150 x 150 DPI)

Accepted

A “successful” epidermal interface was defined as epidermis apposing the upper cap (A), mid cap (B), or cap-scaffold junction (C). Marsupialisation into the scaffold (D) was regarded as “failure.” Future research will investigate implantation after removal of epidermal cuffs; this may enable negative pressure-assisted ingress of tissue into scaffolds, prior to the arrival of the advancing edge of epidermis, thereby facilitating epidermal attachment to the cap or cap-scaffold junction.

245x91mm (72 x 72 DPI)

Accepted

Day 28 cap-scaffold immediately prior to explantation. Healthy granulation tissue is seen in the central hole (A). The wound site is of normal colour, with no exudate or crust formation, and with epidermis closely apposed to the cap (B).
106x97mm (150 x 150 DPI)

ACC

Day 7 cap-scaffold, 4x magnification. A - tantalum scaffold; B - epithelium bridging sections of scaffold has stopped marsupialising; C - close tissue integration with scaffold.
479x361mm (72 x 72 DPI)

Accep

Day 28 scaffold integration, 2x magnification. A - transected hair follicle; B - sebaceous gland. Connective tissue is well integrated into the scaffold and there are no lymphocytes or neutrophils.
479x361mm (72 x 72 DPI)

Accep

Day 28 scaffold, 2x magnification. Epidermis marsupialising along wound line into scaffold. A - epidermis and keratin surrounding scaffold; B - epidermis attaching to scaffold. The integration of tissue with uncapped scaffolds buried deeply in skin suggests the possibility of negative pressure assisted integration of scaffolds into other deep tissues, including bone, connective tissue, muscle, and glands.
408x301mm (72 x 72 DPI)

Accel

Day 28 cap-scaffold, 2x magnification. A - epidermal attachment to upper scaffold. Future research may show that the best location for epidermal attachment is at the cap-scaffold junction rather than the upper cap.

200x186mm (72 x 72 DPI)

Acc

Day 28 cap-scaffold, 4x magnification. A - epidermal apposition to upper cap. There is no evidence of marsupialisation and epidermis appears to be attaching to the upper cap.
406x327mm (72 x 72 DPI)

Accepted

Day 28 cap-scaffold, 2x magnification. Epidermis marsupialising under the cap and through the scaffold (C), over granulation tissue in the centre of the torus (B), before ceasing migration at the junction of the cap and scaffold on the contralateral side (A). This finding supports the hypothesis that cap-scaffolds should be implanted with a margin of de-epithelialised skin to allow tissue integration into the scaffold, prior to epithelial migration and attachment.

345x189mm (72 x 72 DPI)

Accepted

Table 1: Implant types

Pig 1 (7 days)	Pig 2 (28 days)	Pig 3 (28 days)	Pig 4 (28 days)
PT O S	PT O D	PT P S	PT S (scaffold only)
FT O S	PT O D	PT P D	PT O S (No C4)
PT O S	FT O D (scaffold only)	PT P S	FT S (scaffold only)
FT O S	FT O D	FT P D	FT O S (No C4)
PT O S	FT O D	FT P S	FT S (scaffold only)
FT O S	FT O D	FT P D	FT O S (No C4)
<p>Key:</p> <p>All are cap-scaffolds unless specified as "scaffold only". All are treated with collagen type 4 unless specified as "No C4".</p> <p>FT = Full skin thickness</p> <p>PT = Partial skin thickness</p> <p>O = Oblique cut skin edge</p> <p>P = Perpendicular cut skin edge</p> <p>S = Single scaffold</p> <p>D = Double stacked scaffold</p>			

Table 2: Scale for Clinical Appearance of Implant Wound

Implant Wound	Score
Skin Colour	
Normal	1
Pink	2
Red indurated	3
Drainage	
No drainage/crust formation	1
Little exudate	2
Significant exudate	3
Granulation tissue	
Normal	1
Surface slough	2
Red indurated	3
Skin Retraction	
Absent, skin closely apposed	1
Present, scaffold partly visible	2
Scaffold completely visible	3

Table 3: Histologic Grading Scale for Implants

Response	Score
Capsule, quantitative evaluation (Thickness rating)	
1-4 fibroblasts	4
5-9 fibroblasts	3
10-30 fibroblasts	2
>30 fibroblasts	1
Not applicable	0
Capsule, qualitative evaluation	
Capsule tissue is fibrous, mature, not dense, resembling connective tissue or fat tissue in the non-injured regions	4
Capsule tissue is fibrous but immature, showing fibroblasts and little collagen	3
Capsule tissue is granulomatous and dense, containing both fibroblasts and many inflammatory cells	2
Capsule consists of masses of inflammatory cells with little or no signs of connective tissue organisation	1
Cannot be evaluated because of infection or other factors not necessarily related to the material	0
Interstitium, qualitative evaluation	
Tissue in interstitium is fibrous, mature, not dense, resembling connective tissue or fat tissue in the non-injured regions	4
Tissue in interstitium shows blood vessels and young fibroblasts invading the spaces; few macrophages may be present	3
Tissue in interstitium shows giant cells and other inflammatory cells in abundance, but connective tissue in between	2
Tissue in interstitium is dense and of inflammatory type exclusively	1
Implant cannot be evaluated because of problems not related to the material tested	0
Interstitium, apposition to scaffold	
Tissue in interstitium is closely applied to scaffold with no gaps between scaffold and tissue	4
Tissue in interstitium is applied to scaffold with few gaps between scaffold and tissue	3
Tissue in interstitium is loosely applied to scaffold with many gaps between scaffold and tissue	2
Tissue in interstitium shows few or no signs of apposition to the scaffold	1
Apposition of tissue to scaffold cannot be evaluated because of epidermal marsupialisation throughout the scaffold	0

Table 4: Day 7 implants and the level of apposition seen on interfaces

Specimen	Upper cap	Partial cap	Upper scaffold	Into scaffold
1	0	3	0	5
2	0	6	2	
3	0	6	0	2
4	0	2	6	
5	2	3	3	
6	2	3	2	

Table 5: Day 28 full thickness implants and the level of apposition seen on interfaces. Where indicated sections could not be assessed because epidermis had overgrown the Elast-Eon™ cap

Specimen	Upper cap	Partial cap	Upper scaffold	Into scaffold
1	2		6	
2			4	4
3			5	3
4				8
5	1 (4 interfaces overgrown)		3	
6			5	3
7		3	5	
8	4 (2 interfaces overgrown)	2		

Table 6: Partial thickness vs. full thickness cap-scaffolds' epidermal level

	Into scaffold	To scaffold	To cap
6 Partial thickness cap-scaffold implants	48 (i.e. All interfaces)		
8 Full thickness cap-scaffold implant	18	28	12

Accepted Article

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