Can plantar fibroblast implantation protect amputees from skin injury? a recipe for skin augmentation

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Received: 5 May 2021 | Revised: 15 June 2021 | Accepted: 23 June 2021

DOI: 10.1111/exd.14419

HYPOTHESIS

Experimental Dermatology WILEY

Can plantar fibroblast implantation protect amputees from skin injury? A recipe for skin augmentation

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Funding information
Engineering and Physical Sciences Research Council, Grant/Award Number: EP/N026845/1; Marie Sklodowska-Curie EU Horizon 2020 grant, Grant/Award Number: 892407

Abstract

Skin injuries remain a persistent problem for users of lower-limb prostheses despite sustained progress in prosthesis design. One factor limiting the prevention of skin injuries is that skin on the residual limb is not suited to bear the mechanical loads of ambulation. One part of the body that is suited to this task is the sole of the foot. Here, we propose a novel strategy to actively augment skin's tolerance to load, increasing its resistance to mechanically induced injuries. We hypothesise that the load tolerance of skin can be augmented by autologous transplantation of plantar fibroblasts into the residual limb dermis. We expect that introducing plantar fibroblasts will induce the overlying keratinocytes to express plantar-specific keratins leading to a tougher epidermis. Using a computational finite element model of a weight-bearing residual limb, we estimate that skin deformation (a key driver of pressure ulcer injuries) could be halved by reprogramming skin to a plantar-like phenotype. We believe this strategy could yield new progress in pressure ulcer prevention for amputees, facilitating rehabilitation and improving quality of life for patients.

KEYWORDS
finite element model, keratin 9, load tolerance, pressure ulcer, residual limb

1 | INTRODUCTION

Skin mediates all physical interaction between the body and external objects/surfaces. Skin's ability to tolerate mechanical load is reflected in its structure, with a tough epidermis supported by a strong and flexible dermis.[1] This load tolerance has limits, however, and exposure to pathological mechanical loads leads to skin injuries such as tears,[2] blisters[3] and superficial pressure ulcers.[4] These injuries impose a huge burden on society, costing an estimated £5 billion annually in the UK.[5]

Skin's load tolerance is highly site specific[6] and reflects the function of the anatomical location.[7] The foot sole (plantar skin) can tolerate repeated pressures and shear stresses in excess of 1000 kPa[8] without injury, while 1/200th of those loads are sufficient to injure other parts of the body, such as the pelvis and the heel.[9]

When a normally non-load bearing body site is recruited to bear load, such as is required of the residual limb of lower-limb amputees, it contributes to a variety of contact-induced dermatoses such as eczema, irritation, callousing and pressure ulcers.[10] These injuries prevent prosthesis use which severely limits the quality of life of amputees and create a substantial roadblock to rehabilitation.[11]

Of these injuries, pressure ulcers are perhaps the most debilitating. One reason for the lack of progress on preventing prosthesis-related pressure ulcers is that we are rapidly reaching the limits of prosthesis design. Prostheses are increasingly high-tech[12] and are approaching a point where interface pressures can be minimised.[13] Ultimately, the issue of pressure ulcers in amputees remains because we are asking the skin of the residuum to perform the task of transferring ambulation loads—a task far beyond its normal function.

2 | PREMISES

The sole of the foot has a specialised structure that enables it to tolerate the mechanical forces of ambulation.[7] For example, the fat pad within the heel protects the skeleton from impact.[14] The skin is also specialised, with collagen fibres in the dermis poised to be load bearing[7] and cytoskeletal proteins such as Keratin 9 (K9) expressed in the epidermis[15] (Figure 1A). This distinct composition serves to protect skin on the sole of the foot from injuries such as superficial pressure ulcers.[7]

When plantar skin is used in the reconstruction of residual limbs, it performs better than skin from other donor sites.[16,17] The Syme amputation, named after the surgeon who pioneered it, uses heel pads (including the skin and fat pad) to reconstruct the lower residual limb; these individuals can walk directly on their stumps without incurring skin injury.[18] However, plantar skin is rarely available in sufficient quantities when reconstructing residual limbs. A novel approach to reducing skin injuries in prosthesis users is to stimulate vulnerable skin to enhance its load-tolerance. In practice, this would mean inducing a change in non-plantar skin to exhibit more plantar-like traits.

Skin from all body sites has an innate plasticity that enables it to adapt to a changing mechanical environment. The epidermis differentiates and thickens in response to shear[19] and can expand in response to stretch.[20] Indeed, the residual limb of many prosthesis users develop enhanced load tolerance given sufficient time and careful rehabilitation.[21,22] However, positional cues that confer 'skin identity' are not altered during rehabilitation and skin on the residual limb never acquires the positional information of distal sites such as the sole of the foot. A novel strategy to boost load tolerance is to transplant the positional information of the sole to the residual limb.

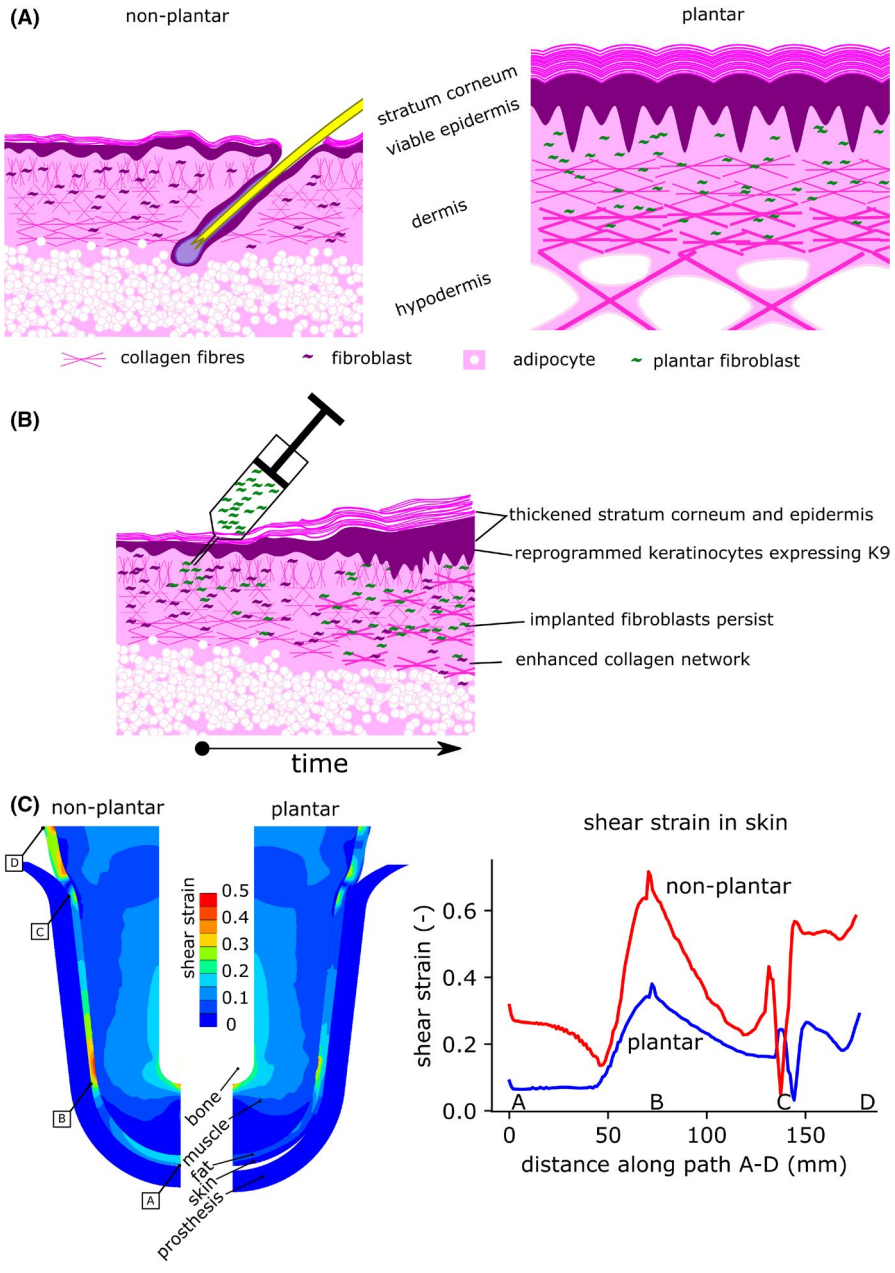

FIGURE 1 (A) Plantar skin has a structure distinct from other body sites that enable it to tolerate mechanical loads. (B) We hypothesise that by injecting fibroblasts from a plantar region, non-plantar skin will develop a plantar-like phenotype and become more load tolerant. (C) Simulations of a weight-bearing residual limb indicate reprogramming skin to plantar-like phenotype could reduce shear strains

Fibroblasts within the skin dermis are key orchestrators of both dermal and epidermal identity. In skin morphogenesis, signalling from the skin dermis directs epidermal development, while in adult skin cross talk between the epidermis and dermis facilitates skin homeostasis.[23] Fibroblasts carry positional information via site-specific HOX gene expression, and this information is retained over many passages in vitro.[24,25] When HOXA13 expressing plantar fibroblasts are cultured in the dermis of an in vitro skin construct, together with non-plantar keratinocytes forming the epidermis, *K9* expression is detected within non-planter keratinocytes in as little as 30 minutes.[25-27] These skin constructs also maintain expression of the cytoskeletal protein K9 after grafting onto nude mice.[26]

Fibroblasts have been shown to effect changes in vivo in the context of hair induction, where dermal papilla fibroblasts induce hair follicle neogenesis in recipient epithelia.[28] In the context of plantar skin in vivo, plantar dermis can reprogramme non-plantar epidermal sheets to become both thicker and express K9, two hallmarks of plantar identity.[26,29] There is also evidence to suggest that autologous fibroblast implantation can alter dermal composition by increasing dermal thickness and collagen content.[30,31]

Collectively, these studies support the concept that plantar fibroblasts can instruct changes in both the composition and morphology of the dermis and epidermis. A key question to ask now is whether implanted plantar dermal fibroblasts can also instruct reprogramming of non-plantar skin to acquire the crucial trait associated with a plantar identity: its load tolerance.

3 | HYPOTHESIS

We hypothesise that the load tolerance of skin on the stump can be augmented by autologous transplantation of plantar fibroblasts into the residual limb dermis (Figure 1B).

3.1 | How to test the hypothesis

In skin, both autologous and allogeneic fibroblasts and keratinocytes have been trialled extensively for wounds such as burns and ulcers where they serve to replace lost cells. We hypothesise that autologous plantar skin fibroblasts transplanted into the dermis on a residual limb of an amputee will operate via a different mode of action, and act as signalling conductors to reprogramme adjacent epithelium to a plantar identity. This will serve to protect the skin from injury such as ulceration. Our hypothesis can be tested within a cell-therapy framework by implanting autologous plantar fibroblasts into injury 'hotspots' on the residual limb of amputees.

The experimental approach would include expansion of sufficient cell numbers in vitro, identification of appropriate injection sites, and quantification of changes to the skin load tolerance. There are well-defined protocols for fibroblast expansion in vitro that fully comply with GMP clinical requirements. Cells would be injected into the papillary dermis to maximise their paracrine effects on the adjacent epidermis, or throughout the papillary and reticular dermis for collagen synthesis and dermal remodelling.

The stratum corneum thickness can be quantified non-invasively using OCT imaging,[21] while skin elasticity can be quantified using a suction cup elastomer.[32] Tissue distress can be quantified by monitoring transcutaneous O_2 and CO_2 measurements,[33,34] and this monitoring has been successfully coupled with controlled loading devices to quantify tissue load tolerance.[35] The ultimate measure of success would be fewer incidences of skin injury in treated patients compared to an untreated control group.

3.2 | Preliminary supporting evidence

We have begun to test the feasibility of reprogramming skin as a therapy to reduce injuries by estimating how altering skin's mechanical properties might improve the biomechanics of prosthesis use. We previously compared the mechanical properties of plantar and non-plantar skin,[7] showing that plantar skin has a greater resistance to deformation (a key driver of ulceration). To estimate how plantar-like skin would perform on a residual limb, we developed a computational model of a residual limb interacting with a prosthesis (Figure 1C and Supplementary Material). This model enabled us to estimate the shear strains (a measure of deformation) in skin under typical loading (standing) in a prosthesis, and how these strains would change if we achieved 100% reprogramming after autologous cell therapy (ie plantar skin). We found that reprogramming non-plantar skin to a plantar-like phenotype could reduce shear strains by 47% (from 0.71 to 0.38) at high-pressure locations. This reduction would offer substantial protection to the cells and microvasculature of skin. While quantitative data on skin load tolerance is lacking, the strain tolerance of skeletal muscle cells was estimated at 0.65 for short-term loads.[36] These results therefore support the concept of skin augmentation as a preventative strategy. The next step is to determine to what extent residual-limb skin can be re-engineered.

4 | RELEVANCE AND PERSPECTIVES

Prostheses have become high-tech medical devices incorporating advances in materials and computational modelling. Despite these advances, pressure ulcers and other skin injuries are still a major limitation for prosthesis users. We believe that this is because ultimately, we are asking the skin of the residual limb perform a function that it is not specialised to do—to transfer the loads experienced from ambulation. We propose that instead of reengineering the prosthesis to alter the skin-socket interface, we should exploit the instructive nature of fibroblasts to reengineer the skin to become load tolerant. We believe this strategy could yield new progress in pressure ulcer prevention in amputees, facilitating rehabilitation and improving quality of life for patients.

ACKNOWLEDGEMENTS

This work was funded by a project grant from the EPSRC to CAH (EP/N026845/1) and by a Marie Sklodowska-Curie EU Horizon 2020 grant (agreement No 892407) to CJB.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

CAH devised the hypothesis and conceptualised the research plan. CJB developed the computational model and analysed the data. Both CJB and CAH wrote and approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Supplementary Material Methods
Figure S1 Finite element model geometry representing a trans-tibial residual limb interacting with a prosthesis (left). Finite element mesh used to discretise the domain (right)
Supplementary Material References

How to cite this article: Boyle CJ, Higgins CA. Can plantar fibroblast implantation protect amputees from skin injury? A recipe for skin augmentation.. *Exp Dermatol*. 2021;00:1–5. https://doi.org/10.1111/exd.14419