Morphogenesis and Biomechanics of Engineered Skin Cultured Under Uniaxial Strain

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Background: Split-thickness autograft is the standard wound treatment for full-thickness burns. In large burns, sparse availability of uninjured skin prevents rapid closure of the wound, resulting in increased scar tissue formation or mortality. Tissue-engineered skin (ES) offers promise when autografts are not available.

The Problem: ES, constructed from a polymeric scaffold and skin cells, has been shown to reduce donor site area required to permanently close wounds, mortality, and morbidity from scarring but cannot restore all skin functions. Current generations of ES are orders of magnitude weaker than normal human skin, leading to difficulty in surgical application, greater susceptibility to mechanical damage during fabrication and application, and less elasticity and strength once engrafted.

Basic/Clinical Science Advances: Previous studies to improve ES biomechanics focus on altering the scaffolding material, which resulted in modest improvements but often inhibited proper skin development. As the skin is naturally under static strain, adding these mechanical cues to the culture environment is hypothesized to improve ES biomechanics. ES was cultured under applied static strains ranging from 0% to 40% strain for a total of 10 days. Strain magnitudes of 10% and 20% strain resulted in significantly stronger ES than unstrained controls, showed upregulation of many genes encoding structural extracellular matrix proteins, and exhibited increased epidermal cell proliferation and differentiation.

Clinical Care Relevance: Enhanced biomechanical properties of ES can allow for facile surgical application and less damage during dressing changes.

Conclusion: These findings suggest that mechanical cues play a significant role in skin development and should be further explored.

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Submitted for publication August 29, 2011.

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Abbreviations and Acronyms

ECM = extracellular matrix

ES = engineered skin

BACKGROUND

MORBIDITY AND MORTALITY of patients with massive, full-thickness burns is closely related to the amount of donor sites available.¹ Wound closure in these patients is conventionally achieved through harvesting and grafting split-thickness skin grafts.^{2,3} Unfortunately, sufficient donor sites are not available on severely burned patients; thus, alternate therapies must be utilized.

Engineered skin (ES), constructed from a polymer scaffold and skin cells, provides several advantages over conventional therapy including reduction in donor site area required to permanently close wounds.⁴ Therefore, ES is an ideal candidate for massive acute injuries where donor sites are limited.

Although a large number of advances have been made in the field of ES over the past two decades, no ES

models fully replicate the anatomy, physiology, and properties of native skin.^{5–8} Most models of ES are 1–2 orders of magnitude weaker than normal human skin.^{9–14} Insufficient ES biomechanics cause the ES to be difficult to surgically apply, subject to damage by mechanical shear, and can result in loss of grafts and additional regrafting procedures. Improvements in the design of ES are needed to enhance its biomechanical properties without any deleterious effects on biological function.

TARGET ARTICLES

1. Powell HM, McFarland KL, Butler DL, Supp DM, and Boyce ST: Uniaxial strain regulates gene expression, morphogenesis and tissue strength in engineered skin. *Tissue Eng A* 2010; **16**: 1083.
2. Ebersole GC, Anderson PM, and Powell HM: Epidermal differentiation governs engineered skin biomechanics. *J Biomech* 2010; **43**: 3183.
3. Powell HM and Boyce ST: Engineered human skin fabricated using electrospun collagen-PCL blends: morphogenesis and mechanical properties. *Tissue Eng A* 2009; **15**: 2177.

CLINICAL PROBLEM ADDRESSED

For widespread clinical use, ES must promote rapid wound closure, be easy to apply, and resistant to damage during fabrication, shipping, and application. To facilitate wound closure in large (>60% total body surface area), full-thickness burn wounds, autologous skin replacements have been engineered and have been shown to heal these wounds with far less donor skin than conventional split-thickness autograft.^{4,15,16} Unfortunately, ES fails to meet desired *in vitro* and *in vivo* mechanical properties. The manufacture of an ES model that possesses high strength, elasticity, and pliability after engraftment is necessary to provide biomimetic function and is the focus of recent studies.

RELEVANT BASIC SCIENCE CONTEXT

As ES matures, proteins are formed within the epidermis and newly deposited collagen within the dermis undergoes fibrillogenesis, both of which alter skin biomechanics. Previous studies have shown that the strength and stiffness of the epidermis in ES significantly increases during the culture period, whereas the mechanical properties

of the dermis are virtually unchanged.¹³ As a result, a majority of studies have focused on altering the mechanical properties of the scaffold to improve the strength of ES.^{12,14,17} For example, chemical cross-linking methods have been shown to improve the mechanical properties of the scaffold but did not result in significant increases in ES strength.¹² Blended biopolymer–synthetic polymer scaffolds were also proposed to enhance scaffold mechanics and skin mechanics. These blended scaffolds showed marked improvements in scaffold mechanics but resulted in only modest benefits to skin mechanics, and in some polymer blends, the scaffolds inhibited epidermal differentiation.¹⁴ Thus, ES mechanics are not inherently correlated to the mechanical properties of the scaffold used. To utilize scaffold-only-based methods to improve tissue biomechanics, the scaffold must possess the desired mechanical properties and promote cell viability and proper tissue morphogenesis.

Mechanical stimulation, a biological approach to altering engineered tissue mechanics, has been widely used to promote the *in vitro* formation of engineered tissues such as blood vessels, heart patches, and bone.^{18–20} In dermal substitutes comprised of fibroblasts on collagen scaffolds, cyclic strain resulted in increased proliferation and thicker tissue constructs.²¹ Straining monolayer cultures of keratinocytes to 20% elongation increased proliferation and keratin 6 production and decreased keratin 10 production. Although mechanical stimulation of the proper magnitude and duration has been shown to have positive effects on many tissues, little is known regarding the effects of mechanical stimulation on ES. Additionally, as ES is comprised of cells and scaffold, the relationship between scaffold properties, mechanical stimulation, and tissue biomechanics is unknown and warrants further investigation.

EXPERIMENTAL MODEL OR MATERIAL: ADVANTAGES AND LIMITATIONS

The use of mechanical stimulation to modulate tissue mechanics is a significant advance over scaffold-based methods as it adds no additional chemicals or synthetic polymers into the ES, which may interfere with tissue development or may cause a strong immune response in the body. The static strain culture system utilizes a compression clamping system to hold the skin in place without tearing. Any level of static strain from 0% to 200% can be achieved by rotating a center threaded rod, which moves the free clamp with respect to the stationary clamp. The static strain devices are designed to hold the skin 5 mm above the bottom of

the clamps to maintain the air–liquid interface and are designed to test skin samples large enough for subsequent animal studies. The strain devices can be manufactured inexpensively and lend themselves to high-throughput experiments. Although the strain can be easily altered at any point during the culture period, no automation was incorporated into the design. As a result, complex dynamic stimulation cannot be performed using this generation of strain devices.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Mechanical forces are key regulators of tissue homeostasis and are essential for skin development and remodeling. The effect of mechanical strain on ES development, biomechanics, and gene expression was evaluated by Powell *et al.*¹² ES was fabricated and cultured at the air–liquid interface for 3 days. ES was then loaded into static strain devices, strained to 0% (restrained), 5%, 10%, 20%, or 40%, and held at these positions until culture day 10. Unstrained ES served as a control.

Uniaxial tensile testing of the control and mechanically stimulated ES after 10 days in culture indicated a significant improvement in ES strength when cultured under 10% and 20% static strain ($p < 0.05$ vs. static, 0% and 40%). As the magnitude of applied static strain increased, linear stiffness of the ES also increased with the 10%, 20%, and 40% strain groups exhibiting significant increases in stiffness compared with the static group ($p < 0.05$).

Gene expression of extracellular matrix (ECM) proteins and adhesion molecules in ES was assessed at the end of the 10-day culture period in the static, 0%, 20%, and 40% strain as the 5% and 10% groups' mechanical properties were not significantly different than the 0% and 20% groups, respectively. The application of 0% and 20% strain resulted in an upregulation of many genes for ECM and adhesion molecules including collagen type I alpha 1, laminin alpha 2–5, and integrins alpha 3, 4 and 5, compared with nonstrained controls; however, fold-changes in gene expression observed in the 20% group were, on average, 50% greater than the 0% group. In contrast, application of 40% strain downregulated or maintained equivalent expression of these genes compared with the static group. For example, COL1A1 was upregulated 2.8-fold in the 20% group and downregulated 2.4-fold in the 40% group relative to the static group. The relative change in gene expression between the control and stimulated groups was modest and

typically ranged between a two-fold upregulation or a two-fold downregulation.

Epidermal differentiation was also strongly related to the presence and magnitude of applied strain. Loricrin, a major component of the cornified envelope of terminally differentiated keratinocytes, was either not present or localized in small areas in the static, 0%, and 40% strain groups. Staining for loricrin was positive in a continuous layer of keratinocytes within the upper epidermis of the 5%, 10%, and 20% groups.

Increases in ECM synthesis as a result of mechanical stimulation have been seen in a variety of cell types. An increase in collagen type I gene expression was previously observed in mechanically stimulated fibroblasts cultured on flexible membranes.^{21,22} Although the magnitude of ECM gene upregulation was small (between 1.3 and 2.8 times the control), the additive effect these changes may have resulted in a stronger, more dense ECM. It is proposed that increased ECM production is one of the contributing factors to the increase in mechanical strength seen in the 10% and 20% groups. However, it is unlikely that it is the sole contributing factor.

As much of the tensile strength of ES is attributed to the presence of structural proteins within a highly differentiated epidermis,^{23,24} the enhanced epidermal maturation observed in the 5%–20% strain groups may have contributed to their improved biomechanics. For example, when loricrin is absent from the cornified envelope in loricrin-deficient mice, the skin is more susceptible to damage by mechanical stress.^{25,26} A possible mechanism for changes in loricrin production in response to mid-level strain (5%–20%) may be an increase in ion channel activity. Mechanical strain has been shown to increase ion channel activity during intervals of stretch.^{27,28} Increases in external Ca^{2+} concentration induce increases in intracellular Ca^{2+} concentrations, which are required for keratinocyte differentiation.²⁹ Thus, mechanical stretch may activate Ca^{+2} ion channels, leading to more rapid keratinocyte differentiation.

In contrast, the application of 40% strain to the ES did not result in a significant increase in loricrin production or an increase in mechanical properties. Immunostaining of the 40% ES for loricrin revealed that loricrin was present at far lower levels than the mid-level strains and exhibited noncontinuous, punctate staining. A shift in balance between differentiation and proliferation in the ES may be responsible for this phenomenon. The application of 40% strain likely generated significantly greater tension than that of normal resting skin. In

normal human skin, increases in skin tension activate a complex series of events resulting in cell growth and tissue regeneration.³⁰ It is possible that the application of 40% strain to the newly forming ES caused the keratinocytes to proliferate greatly to restore physiologic levels of tension. This could result in a delay of keratinocyte differentiation, which, when coupled with lower ECM protein production, could result in weaker ES.

The current data suggest that static strain during culture can improve the strength of ES. Although the increased strength of the ES may facilitate surgical application by improving handability and reduce graft failure due to mechanical damage, additional studies are required to fully understand and exploit the potential of mechanical stimulation in ES.

INNOVATION

Many studies focus on developing high-strength scaffold materials to improve ES mechanics. These strategies often incorporate cytotoxic factors or nonbiological polymers into ES, which can result in poor functional properties^{12,14,17} and prolonged inflammation in the body. The development of an inexpensive system to deliver biomimetic mechanical cues is a significant advance in the design and manufacturing of ES. Although the static strain device is simple in construction and function, it

allows for intricate studies of mechanosignal transduction in a well-controlled environment.

SUMMARY ILLUSTRATION

(A) Schematic of the experimental design for the inoculation, application of uniaxial strain, and subsequent culture of ES. Inset: Photograph of custom-designed static strain devices. Scale bar = 2 cm. (B) Ultimate tensile strength of ES at culture day 10 as a function of applied strain. (C) Immunostaining for epidermal marker of differentiation, loricrin, in ES at culture day 10. Dashed white line in each panel indicates the dermal–epidermal junction. Scale bar = 100 μ m. Modified from Ref.¹²

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

The use of mechanical stimulation during culture shows great promise as a method to create ES with increased mechanical homology to native skin. The addition of simple static strain to the culture environment resulted in statistically significant increases in skin strength and stiffness compared with static culture. However, the mechanical properties of ES remain far inferior to that of native human skin; thus, static strain alone is not sufficient. These studies also focused on failure properties of the skin, not low-load biomechanics

characteristic of everyday use. For ES to match the function of native human skin, both low-load and failure properties must be matched and these properties are being incorporated into the most recent ES models. To reach the ultimate goal of complete structural and functional homology to native skin, significant advances in the understanding of *in vitro* tissue development are needed with a focus on epidermal–dermal interactions and ECM production and reorganization.

FUTURE DEVELOPMENT OF INTEREST

To more faithfully recapitulate the *in vivo* mechanical environment, far more complex strain patterns must be applied to ES during development. Most recently, a mechanical bioreactor has been constructed in our laboratory, which is capable of dynamic strain application and is also outfitted with an inline load cell to probe low-load skin mechanics throughout the culture period. This new tool will allow more intricate mechanical stimulation studies and provide the ability to directly match the strains applied to skin during rest and during physical activity. Additional studies focusing on the molecular mechanisms that control the cross-talk between the epidermis and dermis are needed to gain better control over skin morphogenesis and properties. As the role of mechanical stimulation and cell–scaffold/cell–cell interaction in tissue development are more fully understood, it is anticipated that far more tissue-engineered products will be translated into clinical use.

ACKNOWLEDGMENTS AND FUNDING SOURCES

The authors thank the Shriners Hospitals for Children for a Postdoctoral Research Fellowship (grant no. 8450) and The Ohio State University for supporting these studies.

TAKE-HOME MESSAGE

Basic science advances

- ES is orders of magnitude weaker than normal human skin.
- Scaffold-based approaches to improve the mechanical properties of ES result in modest increases in tissue strength and often inhibit proper tissue development.
- The addition of biomimetic mechanical cues to the culture environment provides a method to alter cell function and regulate skin mechanics without the addition of toxic or nonbiological materials to the system.
- Normal human skin experiences constant static strain of varying magnitudes dependent on the anatomical site.
- ES cultured under 10% and 20% strain were significantly stronger than unstrained controls.
- ES cultured under 20% strain showed upregulation of many genes encoding structural ECM proteins, including collagen type I alpha 1 and fibronectin 1.
- Mechanical stimulation at 5%, 10%, and 20% strain significantly increased epidermal cell proliferation and enhanced epidermal differentiation.

Clinical science advances

- ES can be used as both a therapeutic device and an *in vitro* tool for the study of tissue development and wound healing.
- A culture device that can precisely deliver mechanical signals to ES represents a technology that can be further explored to improve the function and mechanical properties of tissue-engineered products and, ideally, patient outcomes.
- A greater understanding of the role of mechanical signals in skin development and resultant mechanical properties can be utilized to engineer new generations of skin substitutes but may also be used to regulate the wound healing process.

Relevance to clinical care

- ES provides an alternate therapy to close large wounds when donor skin is not available.
- Improvements to ES mechanics may facilitate surgical application, prevent damage during transplantation, and result in improved functional outcomes after engraftment.

AUTHOR DISCLOSURE AND GHOSTWRITING

The authors have no competing interests or disclosures relevant to this work, and no persons other than the authors contributed to the writing of this article.

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