Determining the Differential Effects of Stretch and Growth in Tissue-Expanded Skin: Combining Isogeometric Analysis and Continuum Mechanics in a Porcine Model

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BACKGROUND The relative effects of skin growth and stretch during tissue expansion have not been studied. The authors use novel analytic techniques that allow calculation of these factors at any point of a skin patch.

OBJECTIVE The authors sought to determine how stretch and growth change with different expansion rates and to correlate these values with histologic and cellular changes in skin.

MATERIALS AND METHODS Two minipigs were implanted with a total of 5 tissue expanders under tattooed skin grids. One pig was expanded over 35 days and the second over 15 days. Isogeometric analysis allowed calculation of growth and stretch. Expanders with similar total deformation were compared between protocols. Regression analysis determined predictive effects of stretch and growth on histologic data from the second animal.

RESULTS Deformation was more attributable to stretch in rapid than in slow expansion (1.40 vs1.12, $p < .001$). Growth was higher in slow expansion than in rapid (1.52 vs 1.07, $p < .001$). Both growth and stretch predicted epidermal thickness, dermal thinning, and keratinocyte proliferation. Growth predicted vascularity.

CONCLUSION Isogeometric analysis allows determination of precise surface area changes for correlation to microscopic-level data. Using the model, the authors identified that skin deformation in rapid expansion is more attributable to stretch.

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Tissue expansion is a commonly used technique in soft tissue reconstruction of burned skin, Mohs surgery defects, and alopecia.[1–3] Multiple expander filling protocols have been described, including several with quite rapid or even continuous filling.[4,5] Conversely, larger initial fill volume in combination with other risk factors has been identified as a predictor of tissue-expanded flap necrosis.[6] Further understanding is important to optimize expander placement and filling rate.

It is known that tissue expansion results in biological creep or true growth of new tissue.[7–9] It is also observed clinically that after an expander is removed, a certain amount of tissue recoils as a result of elastic deformation or stretch. Previously, there has been an inability to precisely measure these values. To address this, the study uses multiview stereo, isogeometric analysis, and continuum mechanics. Although these techniques have previously been applied to computerized simulation of skin expansion,[10–12] this study is the first to apply them to biologic models of tissue expansion. Integrating these 3 techniques provides a novel approach for the calculation of stretch and growth values at any point of an expanded skin

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patch.[13] The goals of this study are twofold: to examine how growth and stretch differ with filling protocol and to correlate stretch and growth with cellular-level processes. This will serve to guide further study to optimize filling protocols and placement.

Materials and Methods

Four 10 × 10 cm grids were permanently tattooed on 2 Yucatan miniature swine (Sinclair Bio-resources, Columbia, MO). On Pig #1, 2 textured rectangular 120 cc tissue expanders (PMT, Chanhassen, MN) were placed with contralateral grids as controls. In Pig #2, spherical-, rectangular-, and crescent-shaped expanders were placed under 3 grids, with a single control grid. It is important to note that because amounts of deformation are being compared between grids, the shape of the expander is controlled for as long as deformation is equal. In Pig #1, expanders were filled to 250 cc over 5 weekly fills of 50 cc (slow filling protocol). In Pig #2, expanders were filled to 225 cc over 2 weeks in 5 fills (rapid protocol). Grid areas were harvested 1 week after the final expansion.

At the time of expander placement and each fill, >10 photographs were taken of each skin patch at multiple angles. Skin patches were excised and then photographed again after animal sacrifice to assess the ex vivo skin under no tension. Three-dimensional models of each skin patch at each time point are created using Online Multiview Stereo (Autodesk 123D catch; Figure 1).

Isogeometric analysis allows the ability to calculate stretch and growth at any point on the tattooed grid.[14,15]

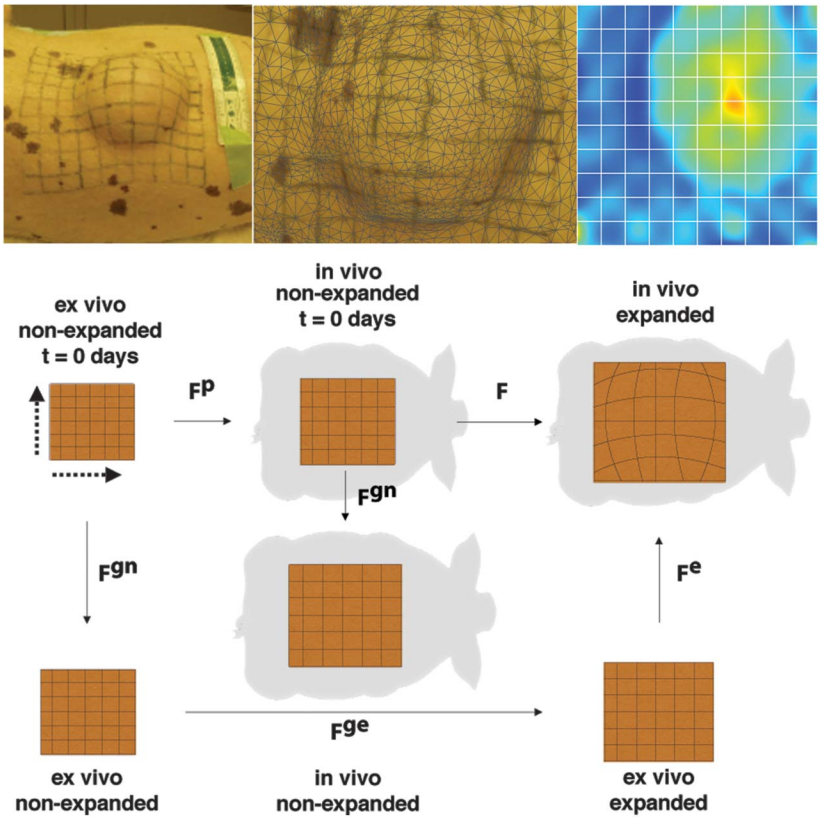

Figure 1. Experimental design. (Above, left) Subcutaneous expanders are filled under a tattooed 10 × 10 cm grid. (Above, middle) Multiview stereo photography is used to convert photographs into a 3-dimensional model of the skin surface. (Above, right) Isogeometric analysis calculates deformation across the grid area. (Below) Different parameters calculated from deformation at each stage. In control patches, prestrain (F^p) is calculated from the elastic recoil of skin ex vivo. Natural growth (F^{gn}) is calculated from increase in size of patch over time. In expanded patches, both prestrain and natural growth are controlled for. Stretch (F^e) is calculated from elastic recoil of skin ex vivo, and growth (F^{ge}) is calculated from the increase in size of the skin.

These values were compared between slow and rapid expansion protocols. Tissue harvested from Pig #2 was subjected to histologic evaluation. Immunohistochemistry was performed for Ki-67 and PECAM-1.

Results

Deformation values are reported as coefficients. For example, a deformation of 1.5 signifies that the area increased 1.5× its baseline size. One expander failed in each animal. Total deformation for Pig #1 rectangular expander (slow) and Pig #2 spherical expander (rapid) was similar (1.76 for slow, 1.66 for rapid, $p = .271$) Therefore, stretch and growth values were directly compared between these (Figure 2). Stretch was significantly greater in rapidly expanded skin than in slowly expanded skin (1.40 vs 1.12, $p < .001$). Growth was significantly decreased in rapidly expanded versus slowly expanded skin (1.07 vs 1.52, $p < .001$).

Epidermal thickness increased with stretch (beta = 0.529, $R^2 = 0.26$, $p < .001$) and also increased with growth (beta = 0.127, $R^2 = 0.016$, $p = .005$). Dermal thickness decreased with both stretch (beta = −0.661, $R^2 = 0.451$, $p < .001$) and growth (beta = −0.414, $R^2 = 0.172$, $p < .001$). Stretch was a better predictor of both histologic values.

In the epidermis, proliferating cells (number of Ki-67–positive cells per 100 interfollicular basal cells) increased with both stretch (beta = 0.390, $R^2 = 0.145$, $p < .001$) and growth (beta = 0.508, $R^2 = 0.181$, $p < .001$), as shown in Figure 3. In the dermis, however, the number of proliferating cells/high power field increased with stretch (beta = 0.280, $R^2 = 0.079$, $p = .035$). Growth did not predict proliferation in the dermis ($p = .41$). Tissue vascularity (PECAM-1–positive pixels/high power field) increased with growth, (beta = 0.458, $R^2 = 0.21$, $p < .001$), as shown in Figure 4. Stretch did not predict vascularity ($p = .085$).

Discussion

The continued high complication rates of tissue expansion in clinical situations such as preoperatively irradiated, burned, or scarred tissue suggest a need for further optimization of this technique.[1,10,16,17] Early studies revealed that epidermal thickening and dermal thinning were present in expanded skin, and that there was an increase in epidermal mitotic activity after each expansion.[7,11] Further study revealed that these changes persist

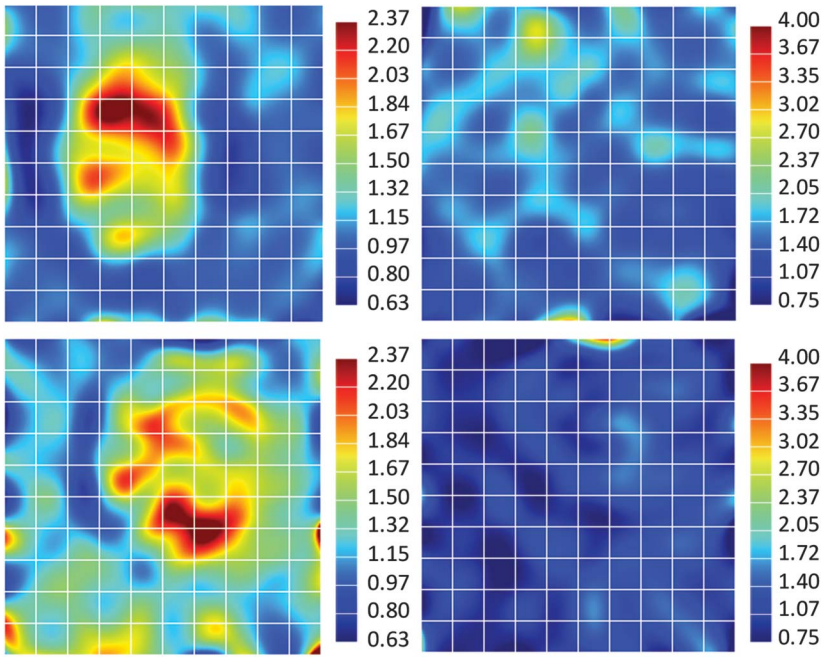

Figure 2. Comparison of stretch and growth in slow (top) and rapid (bottom) filling protocols. (Left) Stretch is significantly higher in rapid versus slow protocol ($p < .001$). (Right) Growth is significantly higher in slow versus rapid protocol ($p < .001$).

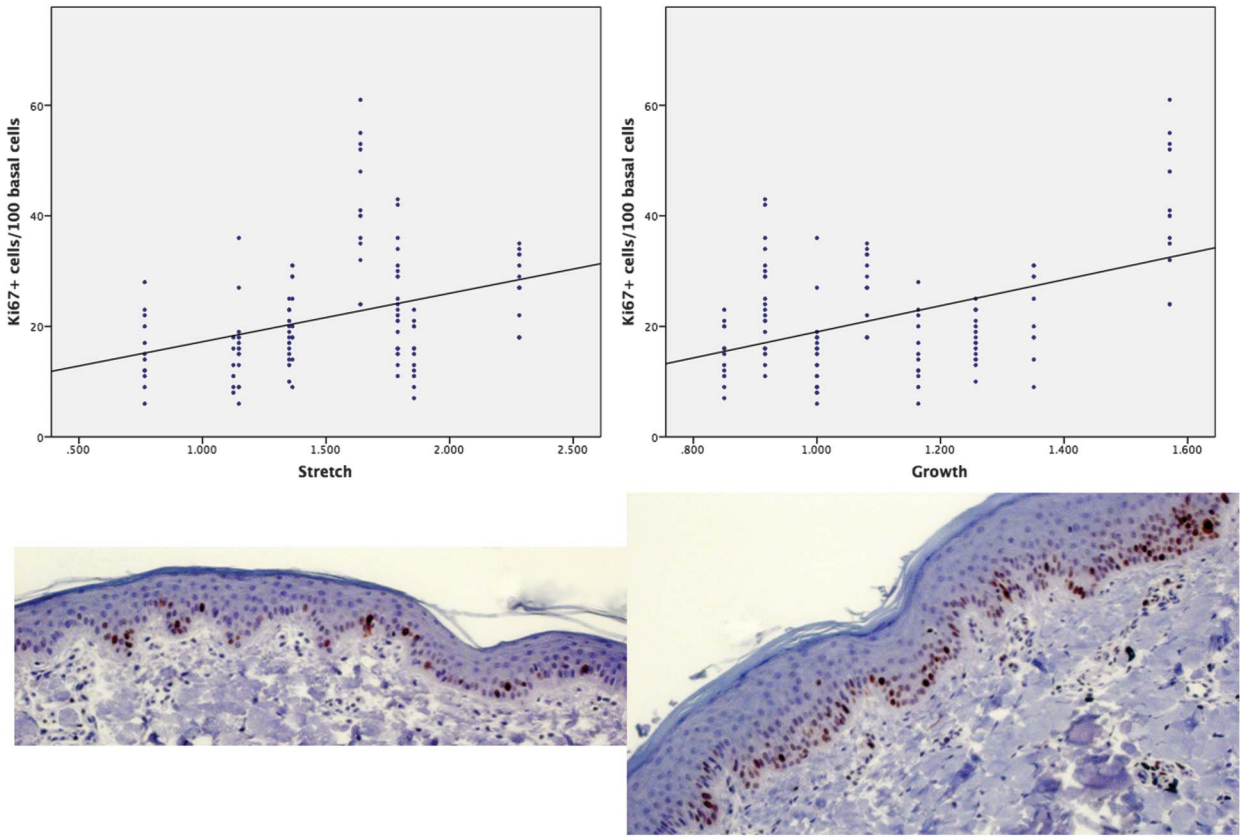

Figure 3. Proliferating keratinocytes in the epidermis in relation to stretch and growth. (Above, left) Scatterplot of epidermal labeling index (Ki-67–positive keratinocytes/100 interfollicular basal cells) related to stretch. (Above, right) Scatterplot of epidermal labeling index related to growth. These plots illustrate that both are similar predictors. Equal magnification views of control epidermis stained for Ki-67 (lower, left) versus highly stretched (lower, right) epidermis. Also note significantly thickened epidermis in highly stretched skin.

for weeks after expansion is complete.[8] The effect of tissue expansion to increase vascularity has also been described and used clinically.[12,18,19] Although these changes have been known for some time, it has never been determined whether these are related to stretch or growth.

Most skin deformation in this study was due to growth in a slower filling protocol, and mainly attributable to stretch in a rapid protocol. This illustrates that completely filling an expander may not imply growth of new tissue. This introduces an additional consideration in tissue expansion protocols: the innate rate of growth of tissue in response to stretch (i.e., biologic creep rate).[9] In reconstruction after Mohs surgery, congenital nevi, or for alopecia, a large amount of additional skin is needed and growth is potentially

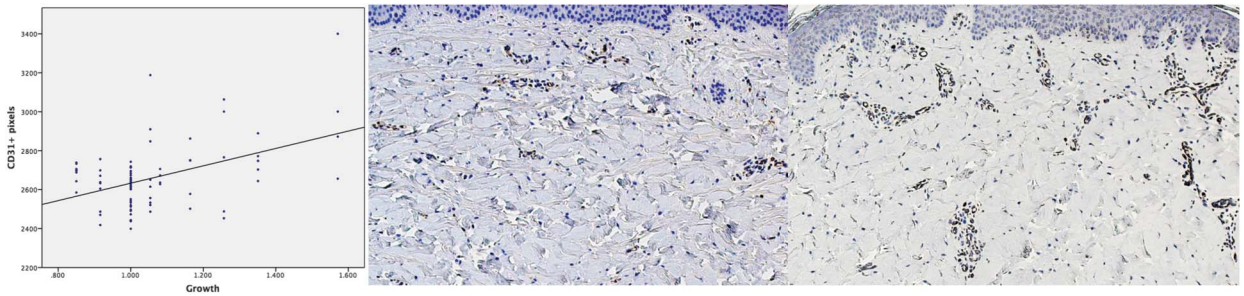

Figure 4. Vascular density (measured as PECAM-1–positive pixels/high power field) in relation to growth. (Left) Scatterplot of vascular density in relation to growth. (Middle) Control skin stained for PECAM-1. (Right) Same magnification view of highly grown skin. Contrast enhanced in both images to highlight immunostaining.

quite important.[2,3] Tissue measurements in this study represent one time point in the expansion process, taken 7 days after final fill in 2 separate expansion protocols. From these data, the authors cannot calculate the biologic creep rate. This will serve as a continued area of research using our model.

Dermal thinning and epidermal thickening seem to be related to stretch more than growth in the evaluation. The data also seem to demonstrate that keratinocyte proliferation is associated with both stretch and growth processes.[7]

Conclusions

Rapid expansion protocols can produce similar overall deformation of skin, but a slower protocol grows more additional tissue. Growth and stretch are associated with the different classic histologic findings of tissue expansion. The data provided will help to guide further research in understanding the mechanisms of growth in tissue expansion.

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