Epidermal differentiation governs engineered skin biomechanics

G.C. Ebersole a, P.M. Anderson a, H.M. Powell a,b,*

a Department of Materials Science and Engineering, The Ohio State University, 116 W. 19th Ave., 243C Fontana Labs, Columbus, OH 43210, USA
b Department of Biomedical Engineering, The Ohio State University, The Ohio State University, 116 W. 19th Ave., 243C Fontana Labs, Columbus, OH 43210, USA

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Engineered skin must be mechanically strong to facilitate surgical application and prevent damage during the early stages of engraftment. However, the evolution of structural properties during culture, the relative contributions of the epidermis and dermis, and any correlation with tissue morphogenesis are not well known. These aspects are investigated by assessing the mechanical properties of engineered skin (ES) and engineered dermis (ED) during a 21-day culture period, including correlations with cellular metabolism, cellular organization and epidermal differentiation. During culture, the epidermis differentiates and begins to cornify, as evidenced by immunostaining and surface electrical capacitance. Tensile testing reveals that the ultimate tensile strength and linear stiffness increase linearly with time for ES, but are relatively unchanged for ED. ES strength correlates significantly with epidermal differentiation ($p < 0.001$) and a composite strength model indicates that strength is largely determined by the epidermis. These data suggest that strategies to improve ES biomechanics should target the dermis. Additionally, time-dependant changes in average ES strength and percent elongation can be used to set upper bound limits on mechanical stimulation profiles to avoid tissue damage.

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1. Introduction

Tissue engineered skin (ES) offers promise as an alternate wound healing treatment when sufficient amounts of autograft are not available, such as in massive burns. Numerous tissue ES replacements have been created (Ehrenreich and Ruszczak, 2006; Bello et al., 2001; Eaglstein et al., 1999; Berthod and Damour, 1997; Purdue et al., 1997; Clark et al., 1996; Wainwright et al., 1996; Cooper et al., 1991; Boyce et al., 1988; Heimbach et al., 1988) and clinically utilized ES has been shown to reduce the donor site area required to permanently close wounds, mortality, and morbidity from scarring (Boyce et al., 2006, 1995). Unfortunately, ES, with published values of ultimate tensile strength between 0.01 and 0.7 MPa (Powell and Boyce, 2009, 2006; Powell et al., 2009; LaFrance et al., 1995), is orders of magnitude weaker than normal human skin. As a result, it is difficult to apply surgically and subject to damage by mechanical shear (Powell et al., 2009; Boyce et al., 2005).

Mechanical stimulation has been widely used to promote the in vitro formation of engineered tissues such as blood vessels, heart patches, bone and cartilage (Datta et al., 2006; De Croos et al., 2006; Isenberg et al., 2006; Freyria et al., 2005; Stegemann and Nerem, 2003). For instance, mechanically stimulated mesenchymal stem cell-seeded collagen constructs were significantly stiffer than their non-stimulated counterparts (Nirmalanandhan et al., 2008). In fibroblasts on collagen scaffolds, cyclic strain produced increased proliferation and thicker tissue constructs (Eastwood et al., 1998). For ES, a 20% static strain during culture significantly increased ultimate tensile strength and promoted epidermal differentiation (Powell et al., 2009). Although mechanical stimulation can have positive effects on engineered tissues, little is known regarding the optimal magnitude of external mechanical stimuli for skin. Such optimization requires better understanding and prediction of the mechanical properties of ES as a function of time.

The goal of this study was to investigate the evolution of mechanical properties in ES and correlate changes in mechanical strength and stiffness with biological activity, including differentiation and proliferation. The relative contribution of the epidermis and dermis to the tissue properties was also investigated. From this data, mathematical relations were developed to describe the time dependant-behavior of ES. These relations could be utilized to set load and strain limitations for dynamic mechanical stimulation of ES.

2. Materials and methods

2.1. Scaffold preparation

Electrospun collagen scaffolds were fabricated using a 10% w/v solution of acid-soluble collagen (SEMED S) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, Sigma, St. Louis, MO). Matrices were spun at a potential of 30 kV onto an 8.5 cm² grounding plate at a distance of 20 cm. The electrospun scaffolds were physically
crosslinked by vacuum dehydration at 140 °C for 24 h, then chemically crosslinked in a solution of 5 mM 1-ethyl-3-3-dimethylaminopropylcarbodiimide hydrochloride (EDC, Sigma, St. Louis, MO) in 100% ethanol for 24 h (Powell et al., 2008). The scaffolds were then disinfected in 70% ethanol for 24 h and rinsed thoroughly to prepare for cell inoculation (Powell et al., 2008).

2.2. Engineered skin

Engineered human skin was prepared on the collagen scaffolds using human dermal fibroblasts (HF) and epidermal keratinocytes (HK) following a procedure previously described (Powell and Boyce, 2008). Briefly, fibroblasts were inoculated onto the scaffolds at a density of 5 × 10^5 cells/cm² and incubated at 37 °C and 5% CO₂ for 1 day. Subsequently, HKs were inoculated onto the cell-scaffold constructs at a density of 1.0 × 10^6 cells/cm². The day of keratinocyte inoculation corresponds to day 0 of ES fabrication. The following day, the ES was lifted onto a perforated stainless steel platform covered by a piece of sterile cotton to form an air–liquid interface for the ES. The cells were incubated for 21 days with growth media changed every 48 h.

2.3. Engineered dermis

To independently determine the properties of the dermis and epidermis, dermal replacements were fabricated following the same protocol for ES without an epidermal layer added on top of the dermis. HFs were inoculated into collagen scaffolds at a density of 5 × 10^5 cells/cm² and incubated in a 150 mm tissue culture dish at 37 °C, 5% CO₂ in UCMC 160 medium (Swope et al., 2006). As the interaction between the fibroblasts and keratinocytes is essential for proper tissue development, HFs were inoculated onto the bottom of the 150 mm tissue culture dish at 1 × 10^6 cells/cm² at day 0 to more closely mimic the ES environment and allow for chemical communication between the two cell types. The dermal constructs were incubated for 21 days with growth medium exchanged every 48 h.

2.4. Surface electrical capacitance

Previous studies show that surface electrical capacitance (SEC) is inversely related to barrier function in skin, and is a convenient, non-destructive measure of composite theory in which the dermis and the epidermis serve as parallel, mechanically distinct layers (Matthews and Rawlings, 2003): SEC = σₐsec(t) + σₑsec(t)

(2)

The quantities σₐ and σₑ are the stress in the epidermis and dermis, respectively, when the tissue reaches the ultimate tensile strength, and fₑ(t) and fₛ(t) are the corresponding epidermal and dermal volume fractions. Thus, Eq. (2) is a statement of mechanical equilibrium along the in-plane direction of loading. It was applied at five time points: t = 4, 7, 10, 14, and 21 days by replacing S(t), fₑ(t), fₛ(t), with experimentally measured values of the UTS and constituent volume fractions of engineered skin. The volume fraction data were based on the average of n = 20 thickness measurements per histological image per graft using the ImageJ software (NIH freeware). The dermal stress σₑ(t) was replaced by Sₑ(t), the experimentally measured strength of engineered dermis at these time points. Thus, Eq. (2) furnishes σₑ(t).

These estimates of σₑ(t) are viewed as lower bounds to the epidermal stress Sₑ(t) for two reasons. First, the epidermis may not be at peak strength when the UTS of engineered tissue is reached. Second, the substitution σₑ(t) = Sₑ(t) supplies an upper bound to σₑ(t), so that Eq. (2) furnishes a lower bound to σₑ(t).

The resulting five values of σₑ(t) were used to calibrate a four parameter sigmoidal function (Tichopad et al., 2002):

Sₑ(t) = A + \frac{2A}{1 + e^{-\frac{t - \tau}{\Delta}}}

(3)

This function is commonly employed to describe cell activity, such as keratinocyte differentiation versus [Ca²⁺] (Walker et al., 2006), as well as intracellular bonding strength (Baumgartner et al., 2000). Specifically, A, Δ, τ, and are chosen to minimize the squared error between the σₑ(t) values obtained from Eq. (2) and the predicted Sₑ(t) values from Eq. (3). The nature of the sigmoidal function is that Sₑ(t) monotonically increases with time from Sₑ(t = τ) = A to Sₑ(t) = A + Δ to Sₑ(t > τ) = A+2Δ, where t is the time scale for these transitions.

3. Results

3.1. Cellular organization, viability, and tissue morphogenesis

Histological evaluation of the ES at t = 4, 7, 10, 14, and 21 days showed changes in epidermal organization with time (Fig. 1A–D). Stratum corneum grew thicker and more apparent with time. As the epidermis showed changes in epidermal organization with time (Fig. 1A–D), so did the basal keratinocytes (Fig. 1A–D, arrows). At all time points, ES was comprised of a densely populated epidermis with a continuous epidermal basal cell layer. The dermis contained a relatively sparse population of...
fibroblasts in the porous collagen matrix which became thinner with time (Fig. 1A–D).

Engineered skin barrier formation, as measured indirectly by surface hydration, showed a distinct drop in surface electrical capacitance with increasing days in culture (Fig. 2A). At day 4, the surface of the ES was fully hydrated (no barrier formation) with SEC values significantly greater than at all other time points (5515.9 ± 279.2 pF). By day 14, the surface of the ES became drier with ES hydration reaching normal levels of human skin hydration (Fig. 2A). Additional culture time (21 days) did not significantly alter surface hydration (Fig. 2A).

Analysis of cellular metabolism via MTT revealed an increase in metabolism with culture time until day 14 (Fig. 2B). At culture day 21, metabolism was reduced by ~14% compared to day 14 but it was still significantly higher compared to days 4 and 7 (Fig. 2B). Quantification of cell number per time point also indicated an increase in total number of cells per field of view with days in culture reaching a maximum at days 10 and 14 (179 ± 17 and 161 ± 21 cells/20 × field) with a slight decrease in cell number observed at later time points (145 ± 21 cells/20 × field at day 21).

Immunostaining for laminin-5, a major protein constituent of the basement membrane, indicated that basement membrane formed by day 7 and was maintained throughout the culture period (Fig. 3). Positive staining for involucrin, a cytoplasmic protein precursor of the epidermal cornified envelope, was present at days 14 and 21 but was not observed at day 7 (Fig. 3). Involucrin was present in the day 10 samples; however, staining was diffuse and not as intense compared to later time points (Fig. 3B).

3.2. Mechanical properties

The tensile tests revealed that ES substantially strengthened and stiffened over the culture period, with more modest
variations in the relaxation time exponent and strain to failure. In particular, the average 21-day strength (632 ± 222 kPa) was ~10 times that for day 7 (Table 1, Fig. 4A) and the average 21-day stiffness (0.077 ± 0.002 N/m) was ~7 times that for day 7 (Table 1, Fig. 4B). The average relaxation time exponent decayed modestly from ~0.22 to ~0.15 over this period, and the average strain to failure first increased and then decreased, with the average 21-day elongation ~0.8 times that for day 7 (Table 1). The associated regression analyses revealed a significant linear correlation with time for average strength and stiffness, but not for other mechanical properties (Fig. 4).

Tensile tests reveal that ED did not strengthen or stiffen over time, in contrast to ES. At day 7, ED and ES had relatively similar average strength and stiffness but ED values actually

![Image](image1)

Fig. 3. Immunostaining of engineered skin at days: 7 (A), 10 (B), 14 (C), and 21 (D) for cell nuclei (blue), laminin-5 (green), and involucrin (red). At day 7 (A), the basement membrane has formed (green) while little staining for involucrin, a marker of epidermal differentiation is observed. Involucrin staining, and thus epidermal differentiation, becomes more evident as the engineered is incubated for longer periods of time. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Mechanical properties of engineered skin and engineered dermis determined by uniaxial tensile testing.

<table>
<thead>
<tr>
<th>Engineered skin Days in culture</th>
<th>Strength S (kPa)</th>
<th>% Elongation at failure</th>
<th>Linear stiffness (N/mm)</th>
<th>Engineered dermis Strength Sd (kPa)</th>
<th>% Elongation at failure</th>
<th>Linear stiffness (N/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>23.8 ± 9.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.6 ± 17.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.009 ± 0.003&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.3 ± 7.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>108.2 ± 3.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.005 ± 0.001</td>
</tr>
<tr>
<td>7</td>
<td>61.4 ± 43.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.2 ± 33.7</td>
<td>0.011 ± 0.004&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63.8 ± 10.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>62.5 ± 7.2</td>
<td>0.005 ± 0.001</td>
</tr>
<tr>
<td>10</td>
<td>178.1 ± 56.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107.1 ± 41.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.027 ± 0.009&lt;sup&gt;h&lt;/sup&gt;</td>
<td>59.3 ± 3.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>59.9 ± 11.7</td>
<td>0.004 ± 0.002</td>
</tr>
<tr>
<td>14</td>
<td>425.1 ± 157.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.1 ± 20.7</td>
<td>0.044 ± 0.009&lt;sup&gt;h&lt;/sup&gt;</td>
<td>48.8 ± 7.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>51.8 ± 4.2</td>
<td>0.003 ± 0.002</td>
</tr>
<tr>
<td>21</td>
<td>632.3 ± 221.7</td>
<td>69.0 ± 23.8</td>
<td>0.077 ± 0.023</td>
<td>138.5 ± 41.3</td>
<td>50.9 ± 13.3</td>
<td>0.006 ± 0.003</td>
</tr>
</tbody>
</table>

<sup>a</sup> p < 0.001 vs. day 10, 14, and 21.<n> <sup>b</sup> p < 0.05 vs. day 7, 10, and 14.<n> <sup>c</sup> p < 0.01 vs. day 10, 14, and 21.<n> <sup>d</sup> p < 0.05 vs. day 21.<n> <sup>e</sup> p < 0.001 vs. All.<n> <sup>f</sup> p < 0.01 vs. day 14 and 21.<n> <sup>g</sup> p < 0.05 vs. day 21.<n> <sup>h</sup> p < 0.01 vs. 14&21<sup>h</sup> <sup>i</sup> p < 0.01 vs. 21<sup>h</sup>
decreased from day 7 to day 14 and more than doubled from day 14 to day 21. The net result was that ED values are only \( \frac{1}{C^{24}} \% \) to \( \frac{2}{C^{24}} \% \) of ES values at days 14 and 21 (Table 1, Fig. 4). Average ED elongation to failure ranged from 0.3 to 0.7 times that for ES between day 7 and day 21. Overall, average elongation to failure and stiffness of ED did not improve over the 21-day culture period (\( p > 0.05 \), Table 1). The ED relaxation time exponent could not be determined since the ED samples generated insufficient load at 10% strain (Fig. 4B).

### 3.3. Correlation of tissue morphogenesis and viability with mechanical properties

Ultimate tensile strength of ES was plotted against surface electrical capacitance and absorbance (Fig. 5) to determine if mechanical properties correlate with measures of proliferation and differentiation (Fig. 5). Strength correlated more significantly with lower surface electrical capacitance (Fig. 5A) compared to increased cellular metabolism (Fig. 5B).

### 3.4. Composite strength model

Application of the composite strength model (Eq. (2)) showed that the average epidermal contribution, \( \sigma_{\text{e}}(t)fe \), increased to a 21-day value that is \( \sim 70 \) times the 7-day value (Table 2). The results clearly showed that at day 10 and beyond, ES strength derives primarily from the epidermis. Even though the epidermal/dermal thickness ratio was \( \sim 0.2 \) at day 21 (Table 2), the ES strength in Eq. (2) had a contribution \( \sigma_{\text{e}}(t)fe = 515 \text{kPa} \) from the epidermis compared to \( \sigma_{\text{d}}(t)fd = 111 \text{kPa} \) from the dermis (Table 2).

A least squares regression line of the sigmoidal function in Eq. (3) was fit to the average \( \sigma_{\text{e}}(t) \) values at the five time points (\( t = 4, 7, 10, 14, \) and 21 days), with \( R^2 = 0.99 \) (Fig. 6). The resulting fitted parameters were \( A = -57 \text{kPa}, \lambda = 1316 \text{kPa}, t_0 = 10.4 \text{ days}, \) and \( \tau = 1.22 \text{ days} \) (Table 2). This corresponded to \( S_0(t = t_0) \)

![Image 1](image1.png)

**Fig. 4.** Scatter plot and linear regression lines for mechanical properties of engineered skin (ES) and engineered dermis (ED) as a function of time: (A) ultimate tensile strength, (B) linear stiffness, and (C) relaxation rate (exponent \( n \) from Eq. 1). The relaxation rates could not be determined for engineered skin at days 4 and 7 and the engineered dermis at all time points because the magnitude of force was too small.

![Image 2](image2.png)

**Fig. 5.** Engineered skin (ES) strength versus (A) surface electrical capacitance and (B) cellular metabolism. Note the strong correlation between surface capacitance and strength and relatively weak correlation between metabolism and strength.

### Table 2

<table>
<thead>
<tr>
<th>Time</th>
<th>( f_e ) (kPa)</th>
<th>( f_d ) (kPa)</th>
<th>( \sigma_{\text{e}}(t) ) (kPa)</th>
<th>( \sigma_{\text{d}}(t) ) (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.09±0.04</td>
<td>0.91±0.04</td>
<td>-43.8</td>
<td>-4</td>
</tr>
<tr>
<td>7</td>
<td>0.08±0.05</td>
<td>0.92±0.05</td>
<td>92</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>0.12±0.05</td>
<td>0.88±0.05</td>
<td>1029</td>
<td>123</td>
</tr>
<tr>
<td>14</td>
<td>0.16±0.08</td>
<td>0.84±0.08</td>
<td>2441</td>
<td>391</td>
</tr>
<tr>
<td>21</td>
<td>0.20±0.19</td>
<td>0.80±0.19</td>
<td>2575</td>
<td>515</td>
</tr>
</tbody>
</table>

* Derived from Eq. (2) using \( S \) and \( S_0 \) values from Table 1, and \( f_e \) and \( f_d \) values from Table 2.

* \( S_0 \) values from Table 1.
and ECM deposition (Sun et al., 2009).

chitosan scaffolds showed steady cell proliferation, IL-6 production, and ECM deposition (Sun et al., 2009).

containing fetal bovine serum and other growth factors/proteins without additional cells and in fibroblast growth medium often prior studies, dermal fibroblasts were cultured on scaffold constructs that promote proliferation and ECM production (Sun et al., 2009; George et al., 2008). In contrast, the UCMC 161 medium used to culture skin (co-culture of fibroblasts and keratinocytes) was designed to promote epidermal proliferation and differentiation while maintaining fibroblast viability. Prior studies showing BrdU staining of ES culture in this medium indicated that the epidermis was highly proliferative but very few fibroblasts in the dermis were actively proliferating (Kalyanaraman et al., 2008). As the culture medium is formulated to maintain fibroblast viability and promote epidermal proliferation and differentiation, it is not surprising that dermal strength in our model was lower than that of studies that utilize DME-based mediums with FBS supplements where fibroblasts are highly proliferative and deposit large amounts of ECM.

The present work showed that ES strength increased approximately linearly with time and epidermal strength fit well to a sigmoidal function (Fig. 6). The epidermal contribution to ES strength increased the most with culture time over a period from \( t = t_0 \) to \( t = 9.18 \) to saturation at \( t = 11.62 \) days (Fig. 6), whereas the dermal contribution was essentially constant. The saturation in epidermal strength coincided with the point where ES surface hydration reached normal human skin values (Fig. 2A) and proteins of the cornified envelope appeared in the epidermis, indicating that the epidermis was nearing terminal differentiation (Fig. 3). The observed plateau in epidermal strength was expected as the epidermis undergoes significant stratification and differentiation during the culture process, reaching a plateau in maturation after approximately 12–14 days in culture (Powell and Boyce, 2008; Powell et al., 2008, 2009). Additionally, the storage modulus of the epidermis is reported to decrease from the outermost stratum corneum to the most viable, inner most layer of the epidermis the stratum basale (Kendall et al., 2007) and would thus be significantly weaker.

4. Discussion

Epidermal differentiation increased with time in culture as evidenced by stratification of the epidermis (Fig. 1), decreased epidermal hydration (Fig. 2A), and positive involucrin staining (Fig. 3). These data correlated well with several models for ES that showed skin maturation with time at the air–liquid interface (Powell et al. 2009; Sun et al., 2005; Pouliot et al., 2002; Butler et al., 1999).

There are relatively few investigations of ES and ED mechanical properties (Powell et al., 2009; LaFrance et al., 1995) with no studies as a function of culture time. The present work demonstrated that UTS and stiffness increased significantly with time in ES but they increased more modestly or even degraded with time in ED (Fig. 4A, Table 1). Structural proteins, including involucrin, loricin, and trichohyalin, are produced as ES matures, imparting biomechanical strength to the epidermis (Candi et al., 2005; Nemes and Steinert, 1999). The absence of these proteins in a less differentiated epidermis, characteristic of early culture times (Fig. 3), has been shown to alter murine skin biomechanics and could reduce strength and stiffness in ES. For example, loricin-deficient mice are more susceptible to epidermal damage by mechanical stress (Koch et al., 2000; Ishida-Yamamoto and Iizuka, 1998).

This study shows that ES stiffness and ductility did not change significantly with time (Fig. 4A, Table 1). ED strength decreased from day 7 to day 14 and then approximately tripled in value at day 21. This increase was likely a result of dermal thinning (Fig. 6A) and a constant maximum load at failure. These data contrast with prior studies showing increased dermal thickness, extracellular matrix production, and cellular proliferation with culture time.

Culture of human dermal fibroblasts on porous collagen scaffolds showed significant increase in DNA content and a ~2-fold increase in procollagen type-I production over a 2 week culture period (George et al., 2008). Rat dermal fibroblasts cultured on collagen–chitosan scaffolds showed steady cell proliferation, IL-6 production, and ECM deposition (Sun et al., 2009).

However, the present study had different culture conditions. In prior studies, dermal fibroblasts were cultured on scaffold constructs without additional cells and in fibroblast growth medium often containing fetal bovine serum and other growth factors/proteins that promote proliferation and ECM production (Sun et al., 2009; George et al., 2008). In contrast, the UCMC 161 medium used to culture skin (co-culture of fibroblasts and keratinocytes) was designed to promote epidermal proliferation and differentiation while maintaining fibroblast viability. Prior studies showing BrdU staining of ES culture in this medium indicated that the epidermis was highly proliferative but very few fibroblasts in the dermis were actively proliferating (Kalyanaraman et al., 2008). As the culture medium is formulated to maintain fibroblast viability and promote epidermal proliferation and differentiation, it is not surprising that dermal strength in our model was lower than that of studies that utilize DME-based mediums with FBS supplements where fibroblasts are highly proliferative and deposit large amounts of ECM.

These data also provide insight into optimizing profiles for mechanical stimulation. Engineered tissues are often stimulated following the principles of functional tissue engineering (FTE) where engineered organs are subjected to the patterns of activity and the magnitude of stressors encounters in normal use (Butler et al., 2000). While this concept has been widely utilized for engineered tendon (Butler et al., 2000, 2009), our current data suggest that modifications to this theory are warranted in engineered organs that undergo significant differentiation, as

\[
\sigma_0 (t) = \frac{G.C. Ebersole et al. / Journal of Biomechanics 43 (2010) 3183–3190}{2^{\frac{t}{t_0}}} C_0
\]

\[
R = 0.99
\]

\[
S_d (t) = 2575 \text{ kPa}
\]

\[
S_e (t) = 1259 \text{ kPa}
\]

\[
S_{e+\delta} (t) = 1259 \text{ kPa}
\]

\[
S_{e+\delta} (t) = 2575 \text{ kPa}
\]

\[
S_d (t) = 1259 \text{ kPa}
\]

\[
S_{e+\delta} (t) = 2575 \text{ kPa}
\]

\[
S_d (t) = 1259 \text{ kPa}
\]
the “in use” stimulation profiles will likely damage the maturing tissue.

5. Conclusions

The present study suggests that the mechanical properties of engineered skin (ES) are highly dependent on culture time and morphogenesis. At shorter culture times (e.g., 4 and 7 days), the dermis is largely responsible for the strength of ES but at larger culture times (e.g., 14 and 21 days), the epidermis is largely responsible. It is postulated that differences in medium formulation between the present and prior studies affect fibroblast senescence, so that strategies to improve fibroblast activity and ECM production could lead to the most significant improvements to ES biomechanics. Overall, knowledge of how engineered tissue biomechanics evolve with time will help foster new strategies to improve these properties and set limits to mechanical stimulation profiles to prevent tissue damage during culture.

Conflict of interest statement

The authors have no financial or personal conflicts of interest associated with the presented work.

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