BIOMECHANICAL TESTING OF PORCINE EPIDERMIS+DERMIS USING BIAXIAL STRAIN

ADVISOR: DR. HUANG
BY: COLIN FRAZIER
NCSU: MAE DEPARTMENT

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Introduction and Background with Literature Review

In the article, “Mechanobiology of Force Transduction in Dermal Tissue,” Silver, et al explain the forces and biochemical processes present in the dermis and epidermis under varying states from relaxation to extreme stress and the resultant macro and micro reactions. The article begins by mentioning the overwhelming theme of biological adaptation to stimuli, describing the response of mechanical stress on a cellular level: “…cells that sense mechanical stress via their substrate, respond by altering patterns of protein expression, thus remodeling their ECM to meet changing mechanical requirements” (3). This premise is prominent throughout the article, which is understandable given the intricacy of the biological evolution of organs, namely the skin, on the functional cell level.

Silver, et al portrays the dermis as an immensely important component partner in respect to the epidermis. There is an exchange of forces between the two layers. This relationship creates equilibrium for the biomechanical and biochemical processes to occur as a natural condition. He states, “External forces are transmitted through the epidermis to the dermis and the underlying subcutaneous tissues, while internal forces are transmitted through the dermis to the epidermis” (4). A sub-relationship of this force trade is the keratinocyte-keratinocyte cell junction. If external forces are applied to the air-epidermis interface, this cell junction will increase in tension. This actuation impressively mimics a biodefense mechanism to protect biological bonds under unwanted external stresses. Also, it is concluded that cyclic strain results in amplified protein production by keratinocytes (17). This could be one of the more essential underlying reactions in understanding the mechanical properties of the dermis.

While discussing the epidermis composition, Silver, et al declares the interface between the epidermis and the dermis contains the highest stress (5). An inference may be made that the most resistive biological components of the top two layers of the skin reside in close proximity to this interface. If the epidermis and dermis were chemically separated, how would the mechanical properties change in each? How would the mechanical properties be affected if the basement membrane were removed from the two layers? He later notes: “Cells grown in matrices that are restrained to eliminate contraction develop isometric tension; whereas cells grown in matrices floating freely in cell culture medium remain mechanically unloaded” (14). Silver mentions the affliction epidermolysis bullosa where the epidermis separates from the dermis upon any external stress. The two layers are connected through the hemidesmosome attachment plaques. Beneath the hemidesmosomes, the anchoring filaments composed of type VII collagen are “usually increased in density” (6). Without these anchoring filaments composed of type VII collagen, it may be possible the epidermis could be separated from the dermis during the first formation of the skin in utero.
The dermis is comprised of an upper layer, the papillary dermis, and a lower, reticular layer. The papillary dermis is twice the thickness of the epidermis and is composed of loose, small diameter collagen fibers and immature collagen fibers, while the reticular layer contains large interwoven collagen fibers and mature elastic fibers (6). A conclusion may be derived that the reticular may be physically stronger in mechanical principle, but the papillary layer may be more easily adaptable to external stresses. The dermis utilizes collagen fibrils consisting of type I and V collagens and coated with type III collagen. This is an important structural stability affiliation.

Silver, et al briefly mention tenascin-C in respect to the mechanical stability of the extracellular matrix though its interaction with collagen fibrils. Tenascin-C is not normally expressed in adults, but appears during joint development (7). An important prospective: Could tenascin-C be isolated for use in treating joint deteriorating conditions such as arthritis?

The spaces between collagen bundles decrease with age due to the increase in collagen fiber density. The fibers appear to unravel with increasing age. Collagen concentration increases up to a certain age, and then decreases thereafter. Silver, et al describes the collagen concentration of rats increasing up to six months and then decreasing until death (7-8). An interesting note here would be defining the exact cause of the tipping point in collagen concentration. Also, the GAG content decreases with respect to the amount of protein with increased age (8). Can the GAG content be measured, and does this cause collagen concentration to decrease?

It is imperative to recognize the material properties of skin rely heavily on the collagen and elastic networks. Silver, et al deliver a concise statement of mechanical behavior: “Elastic fibers are believed to contribute to the initial lo modulus portion of the behavior providing recovery to the collagen networks in skin, while the collagen fibers prevent premature mechanical failure of skin” (9). The collagen fibers align with the load direction during deformation. The collagen fiber preferred orientation will result in great stresses while a 90 degrees rotation will result in great strains as the fibers rotate under the external loading. Silver found that the max “stress in skin occurs after a biaxial strain of about 20%,” which will decrease with age (9).

Silver, et al concluded that the internal forces in the dermis were greater than the epidermis, which is significantly caused by the internal tension in the collagen fiber network (11). With this conclusion, it is possible to rationalize that the material properties of the skin may be mostly attributed to the dermis and not the epidermis. Silver found that living cells wield internal tensile stresses on their adjoining ECM, which raises the query of differentiation in material properties of \textit{in vivo} versus \textit{ex vivo} samples. Assumptions must be made and are a necessity in biological material property testing in order to expand and develop new research ideas.

In the article, “Viscoelastic Properties of Young and Old Human Dermis: A Proposed Molecular Mechanism for Elastic Energy Storage in Collagen and Elastin,” Silver, et al. examine variations in the mechanical properties of the dermis and the elastic fibers and collagen dependencies on age. With the epidermis only 0.1mm thick and the dermis between 1mm and 4mm thick, the ratio at its minimum, 10 times, and at its maximum, 40 times thicker in the dermis (1978). This supports the extensive research that has been collected on the dermis because it possesses the major mechanical properties of the skin. Also, they declare the dermis determines the mechanical properties “because the removal of the epidermis does not change the viscoelastic properties of the skin” (1979). The collagen and elastic fiber orientations, elastic spring constants, and other properties establish the dermis macro-mechanical properties during tensile and load testing.

Silver, et al. describes the collagen fibers becoming more compact as the age increases and appearing to unravel. Also, they mention studies done with retractive forces as a function of temperature made on elastic fibers showing elastic fibers behaved similar to rubber in respect to the random coil network (1978). One may speculate the correlation between the aging of elastic fibers and the aging of rubber as well. This knowledge demonstrates the adaptability of both the elastic and the collagen fibers to extensive strains. The strain rate, or aggression of the strain, is where the behavior of the dermis network components display their true micro-mechanical properties. In the results of their experiment, they found both elastic and viscous stress-strain curves are shifted by increasing the strain rate, but the last portion of the curve does not change with strain rate, instead, it decreases with increased age (1980).

Silver, et al. makes an observation on the density of elastic fibers in the papillary dermis in respect to age. The density decreases from about 2.5% to about 2% after ten years of age. In addition, “Elastic fibers in skin from older individuals appear to fray and contain holes” (1979). As the fibers fray, they become weaker mechanically, and the holes create stress concentrations in the material. Both of these changes show the enhanced weakness in the elastic fibers with increased age.

There is a great correlation between the dermis network and energy transfer in a system. Silver et al. states: “The mechanical response of skin to applied loads involves both a viscous component associated with energy dissipation and an elastic component associated with energy storage. Dissipation of leads applied to skin occurs by molecular and viscous sliding of collagen fibrils during alignment with the force direction, whereas elastic energy is stored as a result of stretching of flexible regions in the collagen triple helix” (1979). This is the most significant statement in the article. The arrangement of energy transformation may be modeled analogously
to a spring and damper system, which supports the importance of defining the elastic spring constant.

The stages of strain behavior in the dermis, from equilibrium to failure, are described by Silver et al. Up to 30% strain, the collagen network offers little resistance to deformation and the elastic fibers dominate, then from 30% to 60% the collagen fibrils begin to offer resistance and the elastic component dominates the deformation, and finally from 60% to failure, involves fibril defibrillation (1979). This viscoelastic behavior is only complicated by the difficulty of applying the correct strain rate during testing to allow the collagen fibers to realign properly. It is interesting to note Silver et al. used the average fibril diameter for collagen of 80nm and the elastic fiber diameter of 2.0µm during experimental calculations (1980). This size difference generates questions of material property dominance and demonstrates the importance of alignment and density inside the dermis.

Silver, et al. describe a particular part of the experimental setup before material testing: “…treated with solutions to remove epidermis and other cellular materials…” (1979). Even though the material properties are based from the dermis and the epidermis is negligible, the internal stresses present in the epidermis-dermis boundary in the skin do contribute the molecular properties near the area. By removing the epidermis, the stresses are released and the fibers may form an unnatural alignment orientation.

The results of the dermis property dependence on age are interesting. At high strains, the elastic slope increases for young dermis and decreases for old dermis, but for the viscous slope, both increases. Silver, et al. calculated the volume fractions for collagen in old and young skin at 0.096 and 0.166 (1980). This is an astounding 70% difference in collagen in respect to age. Also, they found that as the strain rate increased, the collagen fibril lengths decreased from 179µm to 19.6µm for young and 103µm to 10.4µm for old skin (1982). Then, they continue to mention a study indicating there is a bell curve in respect to age for tensile strength, ultimate load, and ultimate modulus of elasticity, but skin relaxation decreased from inception (1982). This may indicate that there is an age that everyone reaches which the peak performance of the dermis reaches a tipping point. In addition, an important conclusion is reached: “Increased values of the elastic spring constant at higher strain rates suggest that rapid stretching may prevent conformational changes in elastic molecules that may occur at lower strain rates” (1982). The α-helical segments in the elastin molecules supply the opposition to deformation at low strains, but as they unravel, the spring constant of elastic fibers decreases. This unraveling is more abundant as age increases causing the elastic spring constant to decrease (1982). In accordance to these observations, there may be a great need for more research to be done on the α-helical segments in elastin since these coils are able to store elastic energy during strain.

In chapter 3, “Linear shear response of the upper skin layers,” Geerligs, et al. describe the mechanical and rheological properties of the epidermis respective of the stratum corneum and dermis involvement. Early in the introduction, it is quickly stated the mechanical properties of each individual skin layer are important and must be appropriately resolved to force credible results while studying a particular layer in the skin due to the anisotropic, inhomogeneous, non-linear, and viscoelastic behavior. The internal stresses between the layers should also be involved in the mechanical property determination and modeling. Some of the internal stresses may be present in the boundary layer between the stratum corneum and the viable epidermis. Due to the thin nature of the stratum corneum of 10-20 microns, it cannot easily be separated from the epidermis. The viable epidermis ranges from 30-100 microns and may be separated from the dermis, but there are no methods available to separate the stratum corneum from the epidermis. Also, the attachment of the epidermis to the dermis has a nonuniform shape resulting in large cones of epidermal tissue penetrating the dermis, which could be interpreted as stress concentrations in this boundary layer and separating the epidermis from the dermis may cause tissue damage (34).

During the material testing of the epidermal samples, the preparation step is of utmost importance. Geerligs, et al. use a dermatome set at 300 microns to obtain the stratum corneum, 200 microns for split-thickness composed of epidermis and papillar dermis, and 400 microns for split-thickness composed of the former plus the reticular dermis. The split-thickness samples were stored in Hanks HEPES Balanced Salt Solution (HHBSS) for a maximum of 72 hours in an incubator. The viability was shown to not change after a storage period of 72 hours, but the material properties may change after the period of 72 hours (36).

The testing was performed on a rotational rheometer to determine the loss and storage moduli of stratum corneum and viable epidermis as a function of frequency, temperature and relative humidity. Stacking is a method widely used in mechanical testing for thin materials. The stratum corneum is subject to stacking in this experiment (41). An issue with this testing method is the coefficient of friction between the samples as they are exposed to external forces and any consequential sliding. The linear viscoelastic strain regime defined as the strain range in which the material properties are independent of the strain amplitude, must be identified in order to make the testing viable (40). The evaluation of the strain regime as well as the conditioning time is extremely important and useful knowledge for skin grafts and skin expansion. Geerligs, et al. found the linear viscoelastic strain regime is similar for stratum corneum, epidermis, dermis, and the split-thickness skin samples (42). This result shows the viability of the skin layers working in tandem during external stresses, normal and shear, to minimize the damaging effects of the
stimuli. Also, the results for stratum corneum showed a decrease in modulus with increasing humidity during the determination of the relative humidity effects on the storage and loss moduli (44). This observation is intuitive in the respect moisture is allowed to enter the material through surface crevices causing the increase in malleability.

The discussion of the behavior of the epidermis is insightful. Geerligs, et al. suggest the dermis has a lower shear resistance than the highly organized epidermis (47). This material property surely was created during the evolution of the skin organ to reduce the effects of external stimuli and shear stress damage. They also found the "current values for shear moduli are one order of magnitude lower than those in dry conditions and up to two orders of magnitude when fully hydrated." As the relative humidity increased, the stiffness of the stratum corneum decreased. They could not establish a clear relationship between the mechanical properties of the epidermis and the relative humidity (47). Real time imaging techniques such as the BioTester equipped with a CCD camera can monitor any real-time surface tissue damage during the mechanical testing.


In chapter 5, "Linear viscoelastic behavior of subcutaneous adipose tissue," Geerligs, et al. examine the mechanical properties for the subcutaneous adipose tissue widely accepted as negligible to the material properties of the skin. The belief is adipose tissue plays an important role in the load transfer between different structures in the body during breathing, body movements, or exercise (62). It seems the inconsistency of the mechanical properties creates the notion of extreme difficulty when attempting to model the behavior. Young's modulus is defined as 3.21 kPa in 70 samples of breast fat tissue (63). Geerligs, et al. use similar methods in testing as they did during the rheological stress testing of the epidermis. One difference this time is the frequency sweep was repeated three times to avoid tissue conditioning phenomena (66). They discovered an obvious dependency on temperature during the testing, but did not have an explanation of the causes. They also found there was no statistical difference in the properties of snap frozen and fresh samples (71). They did, however, find at 40 degrees Celsius and higher, the protein solidification process begins and phase transitions start occurring (72). An interesting note is that "Besides blood vessels and the collagen fiber network, no other significant composites are present in the adipose tissue" (72). There is immense reason to believe the collagen fiber network behaves similar to the dermis and may cause material property differences in respect to the direction of stretch and the fiber alignment.

In the article, “Biorheological Characteristics of Skin after Expansion,” Zeng, et al. examine the biorheological characteristics of skin after expansion by utilizing expanders implanted into dogs. They study the stress relaxation, stress and strain relationship, tensile strength, and observe the epidermis and dermis histology between the controls and expanded samples. This article is particularly interesting in respect to the conclusion they find at the end of the study.

Zeng, et al. explain the advantages of the expanders: “…the high quality of the tissue match, avoidance of new scars and reduction in the number of procedures necessary for reconstruction” (367). Zeng and his team concentrate on investigating the short term versus the long term changes in the skin after expansion and compare it to the short term and long term changes in the control (non-expanded) during the mechanical testing.

They used eight adult dogs and placed four expanders in each dog, but only expanded two. They removed them from the dogs after an 8-week expansion. The expanded tissue and the control tissue specimens were taken at 3, 6, 12, and 24 weeks. The stress relaxation was an remarkable test because they stretched the samples 80% and held them there for an impressive 20 minutes. They found that the expanded specimens relaxed more completely than the controls, but with increasing recovery time, the expanded skin and the controls began to exhibit similar behavior. After 24 weeks, the expanded skin curve mostly coincided with its control skin curve (368). Zeng, et al. does make a note: “Skin is a soft tissue, so its hysteresis loop is almost independent of the strain rate” (369). This is a fascinating observation because if the strain rate increases a great deal, it may be assumed the elastin and collagen fibers may not be able to stretch and rotate to adapt to the strain as fast as they do in lower strain rates, and therefore surpass hysteresis and go quickly into the strain.

Next, they stretched at 20mm/min until 25 Newtons was achieved during the stress and strain testing (370). Zeng, et al. preconditioned three times before its final curve was obtained. The result showed with increasing recovery time, the deviation became gradually smaller between the expanded samples and the controls (372).

Zeng, et al. then stretched at a constant speed of 20mm/min until rupture to examine the maximum load and calculate its tensile strength. The results showed the tensile strength of the expanded specimens was markedly reduced immediately after expansion (373). It is interesting to note that, once again, with the passage of time, at 24 weeks the expanded specimens showed little difference from the controls.

On the histological side, the “basal cells became more undulated and increased in number after skin expansion,” but, again, these changes disappeared 24 weeks after transfer in the epidermis. The dermis became much thinner after expansion as expected, but thickened close to the control thickness by 24 weeks. Even the dermal blood vessels displayed proliferation right after skin
expansion and became normal after 24 weeks (375). Lastly, “after expansion, dense fibrous capsules formed, which contained large amounts of collagen fibers. But these fibrous capsules were gradually absorbed and thinned, and no residual capsule remained 24 weeks after transfer” (377). It is extremely fascinating that the biomechanical and physiological recovery had such a similar time frame. The bottom line is that 24 weeks seems to be the ideal time for skin expansion recovery.


In the article, “Biomechanical Characteristics Investigation on Long-Term Free Graft with Expanded Porcine Skin,” Zhang, et al. discuss the significance of an expanded skin free graft biomechanical properties compared to normal free grafts, normal skin, and expanded skin. The method of skin expansion is done by increasing the volume of an expander to produce pressure to the skin tissue for extending skin and obtaining extra skin (864). They chose to study this particular method due to the fact that local expansion is often not an option, such as a large area skin default or lacking normal skin surrounding the skin default (865). This is a novel study because Zhang states “the biomechanical modeling of the skin flaps during long-term free graft with expanded skin have not been studied yet” (865).

Zhang, et al. use young pig’s skin because the skin is “mechanically and histologically similarly to human skin” (865). They used four pigs for the experiment. The layout of the research is best explained by Table 1 which shows the experimental setup of the subjects (865):

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Note: N(n = 8) normal skin; E(n = 8) expanded skin without graft; NG(n = 8) normal skin free graft after three months; EG(n = 8) expanded skin free graft after three months.

The method in which they fill the expanders is explained: “20-30 mL saline solution was injected into the expander every two days until the solution was totally given 360 mL. The saline solution injection should be finished during one month.” He goes on to note: “Clinic studies indicated that the expanders should be kept for about one month after the expansion was finished. That can efficiently reduce the skin shrinkage and increase the expansion area” (865). During the experimental setup clarification, Zhang, et al. make an important statement about porcine skin biomechanical properties: “The skins should be cut in a consistent orientation for eliminating the
error caused by the anisotropic biomechanical properties of the skin” (866). If the direction is unknown of the porcine skin, mechanical testing with incremental rotations may be done to ensure maximum stresses are discovered in each porcine skin sample regardless of mount orientation. Another important part of the setup is removing the adipose tissue to expose the dermis (866).

Zhang, et al. composes some thoughts about preconditioning the samples. “This is necessary because data on preconditioned specimens are less variable than unpreconditioned specimens. The number of precondition cycle is dependent on the tissue and the method of preparation. Preconditioning was continued until a reproducible, identical curve was obtained over several cycles” (866). It seems preconditioning samples is respective of the experiment, but is necessary to obtain viable results. They go on to describe the stress-strain testing and give details about their preconditioning: “In stress–strain test, the specimens were loaded and unloaded under a constant rate of 10 mm/min for four cycles. It was seen that the hysteresis loop decreased between successive cycles and eventually disappears. After three cycles, the specimens were regarded as preconditioned and the fourth time data were used for calculation and analysis” (866).

The results of the stress-strain relationship are interesting. The stiffness of the samples was the greatest in the normal group, second greatest in the expanded skin without the graft, third stiffest group was the expanded skin free graft after three months, and surprisingly the least stiff group was the normal skin free graft after three months. The expanded skin free graft group is more similar to that of the normal skin group than the normal skin graft group (867). This result is promising to the clinical patients which may need large areas of skin replacement.

Zhang, et al. put together an important statement about the method of stress relaxation testing: “It is indicated that about 35-45% of the initial stress was relaxed at 600s” (867). The results they found show “that the expanded skin specimens are more difficult to get relaxed than that of the unexpanded skin specimens after grafting for three months” (867). This may be due to the internal stress already induced on the specimens causing the elastin to be maximally stretched and the collagen fibers to be at the peak rotation orientation position for stretching.

Zhang, et al. discuss previous assumptions and why their study was important: “Previous experiment studies indicate that expanded skin occur recessive changes, such as increasing epidermis thickness, thinning hypoderma, rupture of elastic fibers, formation of tiny thrombus, fibrinolysis, the viscoelasticity of skin decreased and so on” (868). They declare a promising method for skin grafts is on the horizon, “Recently some experiments indicated that the biomechanical properties of the expanded skin would recover after 3–6 months free from expansion and would be close to that of the normal skin. The biomechanical characteristics of the free graft with expanded skin will recover with the increased recover period” (868). This is an
important discovery because patients will be able to use a small portion of skin to graft and expand to cover large areas without any biomechanical difference from the surrounding skin as long as the recovery period during expansion is acknowledged properly.


In the article, "A Review of Tissue-Engineered Skin Bioconstructs Available for Skin Reconstruction," Shevchenko, et al. discuss skin substitutes and the need for these tissue-engineered bioconstructs in patients where normal autographs are not applicable. The article begins discussing the necessity for skin replacement, such as a wound, and classifying them into epidermal, superficial, partial-thickness, deep partial-thickness, and full thickness. An interesting note about epithelial regeneration in wounds is that "the hair follicles of human skin contain a reserve of stem cells, located in the bulge region of the follicle, which are capable of self-renewal" (229). This describes the basis for natural superficial partial-thickness healing and is utilized as a factor in the creation of tissue-engineered constructs.

Shevchenko, et al. describe the clinical gold standard treatment in full-thickness injuries: "...split-thickness autologous skin grafting. Epidermis with a superficial part of the dermis is harvested with a dermatome from an undamaged skin donor site and applied to the full-thickness wound. Being applied to the wound, capillaries of the split skin graft form anastomoses or 'plug in' into the existing capillary network to provide nutrients for graft survival..." (230). It seems the autologous skin graft has much less of a rejection rate and, in the majority of skin substitutes, leads to a permanent skin bioconstruct, hence why this method is the gold standard. In cases where this gold standard cannot be applied and the donor sites are not available, the "use of cultured autologous keratinocytes and/or bioengineered skin substitutes" are utilized (230). The most important factors are the anatomical and functional purposes of the skin substitutes in respect to their success. They must be "safe for the patient, be clinically effective, and be convenient in handling and application" (231). The actual biomaterials used must not "be toxic, immunogenic or cause excessive inflammation, and should also have no or low level of transmissible disease risk" (231). Functionally, the biomaterial also must "provide pain relief, prevent fluid and heat loss from the wound surface and protect the wound from infection." Also, "is cost-effective, readily available, user-friendly and possesses a long shelf life" (231).

Shevchenko, et al. explain the central classifications of currently available skin-substitute products: "Anatomical structure: dermo-epidermal (composite), epidermal, dermal; Duration of the cover: permanent, semi-permanent, temporary; Type of the biomaterial: biological (autologous, allogeneic, xenogeneic), synthetic (biodegradable, non-biodegradable); Skin substitute composition regarding cellular component: cellular, acellular; Primary biomaterial loading with cellular component occurs: in vitro, in vivo" (231).

The dermo-epidermal composite skin is the most advanced and most expensive skin substitute due to the complexity. These types of bioconstructs are "based on allogeneic skin cells, incorporated into a dermal scaffold" (231). An extremely important detail to note: "in order to produce permanent dermo-epidermal skin substitutes, it appears that either allogeneic or
autologous fibroblasts can be used but only autologous keratinocytes can be used to achieve permanent closure of the skin defect" (236). In respect to this review by Shevchenko, et al., this particular detail seems to be a large factor for all the bioconstructs discussed.

The first bioconstruct discussed in the review is the allograft, Karoskin. The allograft is defined: "Human viable split-thickness cadaveric allograft is used as a temporary measure to cover the wound until it is possible to close it with a permanent skin graft" (236). A few interesting details about the allograft, Karoskin, is that it becomes vascularized in three to four weeks and "the dermal part of the graft becomes partly incorporated into the wound and serves as a dermal bed for further autologous skin graft applications" (236). When discussing the next bioconstruct, Shevchenko, et al. state, "allogeneic cells of the construct [Apligraf] do not survive after one to two months in vivo," and later, "autologous cells are not rejected by the host" (239). This is a good example of the need for a wide variety of skin substitutes purposed for different treatments. There is no one size fits all approach to these bioconstructs. There is, however, only one "three-dimensional reconstructed skin substitute which has achieved clinical use and has been found to be very promising...PermaDerm...is based on the collagen sponge, seeded with autologous fibroblasts and keratinocytes. It therefore delivers permanent wound closure and can be viewed as a true skin substitute" (240). This is a significant breakthrough for tissue-engineered bioconstructs. The largest issue of a true skin substitute is the "lack of immune cells, sweat glands and hair follicles" (240).

Shevchenko, et al. discuss epidermal substitutes and the difficulty of development in respect to the attachment to the dermis: "This can be partly attributed to the fact that CEAs (Cell Cultured Epithelial Autographs) contain terminally differentiated keratinocytes in which integrin expression responsible for attachment to the underlaying matrix is altered" (241). The strength and importance of the basement membrane to the epidermis is described: "Subconfluent keratinocyte suspensions contributed to an earlier basement membrane formation with a mature dermal-epidermal junction region when compared with CEA sheets" (242). Epicel, EPIBASE, and EpiDex are epidermal substitutes and use a "patient's own keratinocytes which are grown to confluency within 15 days to form CEA sheets" (242). This seems an efficient method for an autologous creation. Many of the skin bioconstructs discussed in the review use a silicone material for support layers such as MySkin and Integra Dermal Regeneration Template (242-244).

Shevchenko, et al. also discuss the importance of dermal substitutes: "Wound be preparation and the resultant recipient surface are very important for an effective graft take" (243). The quality of the dermal substitute is directly correlated to the success of the construct with the patient. The Hyalograft 3D is an interesting product because "it lays down ECM components conditioning the wound for split skin grafting. This material is reported to improve in vitro epithelial organization and dermal-epidermal junction maturation in organotypic skin bioconstructs" (245). The biomaterials used for these bioconstructs discussed in this review are mostly based on collagen because of its "biocompatibility and bioconductivity" (246).

The importance of the biomechanical characteristics of skin substitutes is noted by Shevchenko, et al. in detailed explanation. They state six major issues which need to be addressed during the formation of skin substitutes: mimicry of natural skin properties such as resistance to tearing, cell
invasion properties, mechanical properties such as elasticity, stiffness, viscosity, viscoelasticity, failure, and brittleness, modes of measurement for these mechanical properties, comparative to natural skin - should it be measured in vivo or ex vivo, and the dependence of location on the body (246). All of these are valid observations and must be taken into account during the development of tissue-engineered constructs. In respect to the human skin, "the epidermis appeared to contribute rigidity and elasticity, while the dermis was far more viscoelastic in nature, suggesting that the skin is mechanically a two-phase system" (248).

Future endeavors of tissue-engineered skin bioconstructs must take into account all the functions of natural skin: “protective barrier, touch and temperature sensation, excretion, perspiration, thermoregulation, protection from ultraviolet rays, synthetic function, and aesthetic function” (249). Meeting all of these requirements is a timely pursuit that must encompass a vast array of research areas from adult stem cells to natural regeneration.


In the article, "Effect of Dermal Thickness, Tissue Composition, and Body Site on Skin Biomechanical Properties," Smalls, et al. discuss correlations of biomechanical properties of skin using two biomechanical devices and skin thickness using a third ultrasound device. They completed the study with 30 females between the ages of 21-66 with a range of BMI from 18 to 41 kg/m² (44).

The first device, the BTC-2000™ utilizes a circular aperture to measure skin deformation response under a predetermined negative pressure. In this case, they use a vacuum of 10mmHg/s for 15 seconds with a 5 second relaxation time (44). The areas of the body that are tested are the thigh, calf, and shoulder. The device measures elasticity using the elastic recovery, elastic deformation, elasticity percentage, laxity using acute elastic deformation after negative pressure displacement, stiffness using the stress/strain slope, and energy absorption using the area under the stress/strain curve (44). These biomechanical properties together develop good insight into correlations of age and body location. This will create a relationship of biomechanical property ranges depending on site location for clinical human skin sample testing.

The second device Smalls, et al. utilize is the Cutometer® SEM 575 Skin Elasticity Meter® which measures the vertical deformation of the skin using an optical system. The device measured $U_r$, the elastic recovery and the $U_f$, the elastic deformation. This information determined the biological elasticity: $U_r/U_f$ (44). This relationship is a common measurement obtained during shear stress skin experiments and skin stretching experiments, such as this study.

The third device is the Dermascan C® version 3 which measures the thickness of the skin using an ultrasound (44). This device is impressive because the user may define the layers of the skin
in respect to the depths. The epidermal boundary and the dermal/subcutaneous fat boundary are defined for this experiment (45).

The results Smalls, et al. found in this study are interesting. Some of the correlations are predictable, yet some may pose second thought to the full validity of some skin expansion studies. The “skin thickness was significantly correlated with stiffness, energy absorption, and $U_r/U_f$ for the shoulder sites” (45). BMI was significantly correlated negatively with stiffness and skin thickness and positively correlated with energy absorption in the shoulder (45). In respect to the calf, this location had significantly lower values for laxity, laxity percentage, elastic deformation, energy absorption, elasticity, elasticity percentage, $U_r$, $U_f$, and $U_r/U_f$ compared with the thigh and shoulder body sites. Also, the calf site was much stiffer than the thigh and shoulder (45). This result may be mostly due to the muscle tone prominent in the calf. “The shoulder and calf were significantly different for all measurements, clearly highlighting tissue variation” (47). This is an important point to mention because biomechanical properties are extremely dependent on site location and should be in the front position of the experimental setup of all skin property or skin expansion studies.

The most interesting result is shown with the correlation of side dominance to biomechanical properties. “Significant differences were noted in the left vs. the right side of the body for elastic deformation, elastic recovery, energy absorption, stiffness, $U_r$, and $U_r/U_f$” (45-46). This result is understandable for the shoulder site, but may not normally be assumed for the thigh and calf sites. This may draw the conclusion that the entire dominant side of humans has greater stiffness and different biomechanical properties than the non-dominant side.

Smalls, et al. determined that the “increase in age correlated to a decrease in the biomechanical properties of the skin, likely the result of the degeneration of the elastic and collagen network of the dermis and subcutaneous tissue” (47). “For the shoulders…No correlations were observed for age and skin thickness” (46). A fascinating result from the study that they found was “Energy absorption at the shoulder could be predicted from a linear combination of dermal thickness and age” (46). Rarely do the biomechanical properties of the skin show a linear correlation to age. Smalls, et al. show the importance of site location for all skin biomechanical properties studies.


In the article, “Preparation and Characterization of a Novel Skin Substitute,” Castagnoli, et al. discuss novel methods of preparation of skin substitutes with a concentration on a successful dermis using chemical and non-chemical techniques. A central reason this study is performed is: “Dermal skin substitutes may be used to handle the problem of donor site shortage when dealing with major skin loss” (1). The aim is: “An in vitro construction of a skin substitute made up of an alloplastic acellular glycerolized dermis (AAGD) scaffold directly seeded with low-density keratinocytes” (2). A successful treatment with dermal skin substitutes “requires low antigenicity, the capacity for rapid vascularization and a stable dermal template” (1). The current
technology for permanent wound coverage is using human alloplastic grafts to support cultivated autologous keratinocytes (CEA), but is not always reproducible (1).

Castagnoli, et al. first began the study by isolating the dermis by stripping the epidermal layer using two chemical processes and then a manual stripping. They obtained human split-thickness skin grafts from healthy individuals to culture keratinocytes. These primary cultures of human keratinocytes were grown to approximately 80% confluence after about fourteen days (2). After staining, they performed a quantitative analysis of the dermis infiltrating cells with “separate consideration given to the subpapillary and reticular dermis” (3).

The results of this study generally describe the differences between the three methods of preparation in respect to removing the epidermis from the dermis: 1) using dispase II which cleaves the anchoring system between the dermis and epidermis, 2) using trypsin, which breakdown the single cells, producing a cellular suspension, and 3) manually separating without the use of an enzyme (3). The first, and extremely important, result discussed in this article is the basement membrane presence using the three methods of separation. The dispase II completely removed the basement membrane, the trypsin had left only a few incomplete membranes, and the manual method left the membrane completely intact in the dermis (3-4).

Next, Castagnoli, et al. analyze the keratinocytes adhesion in the three methods. Only a few cells attached to the dermal sections in the dispase II treatment due to the lack of basal membrane. The trypsin method displayed inconsistent cell attachment. The manual method showed one or two continuous cell layers at 7 days, pluristratified epithelium at 14 days, and differentiated epithelium with a corneous layer at 21 days (4). “The presence of immunocompetent cells in the AAGD tissue was determined by immunochemistry on the tissue sections” (4). The manual method was the only technique which left the basement membrane intact, so it was selected and used for the preparation of the biosubstitute scaffold (5).

Castagnoli, et al. assert: “the choice of alloplastic dermis as a scaffold is determined by the fact that it retains almost all healthy mechanical skin properties, being compact and elastic, able to take into the bed wound, providing a barrier against invading organisms: it is considered, to date, the best skin substitute available” (6). They discuss the importance of the basement membrane preservation as “fundamental for both adhesion and cell growth” and “promotes the growth and differentiation of the keratinocytes seeded on the dermis” (7-8). Castagnoli, et al. confers the biosubstitute viability: “Taken together our immunohistochemical results suggest that the newly formed epithelium of the biosubstitute was comparable to an epidermis in active regeneration as is observed during the process of wound healing, where the keratinocytes are in an activated state” (10). These are tremendous breakthroughs that assist in the search for the perfect permanent skin biosubstitute for clinical applications.

In the article, “Effects of Subcutaneous Expansion on the Mechanical Properties of Porcine Skin,” Belkoff, et al. discuss the biomechanical, histological, and biochemical effects of conventional expansion of porcine skin to rapid expansion, called intraoperative expansion. The conventional expansion may take anywhere from eight to twelve weeks using multiple appointment during the time to fill the expander below the skin. This study is examining alternative methods because there are some issues with this method such as cosmetic deformity, infection, and ischemia (117).

Belkoff, et al. describe the biomechanical properties of the skin in elegant detail: “…the gradual stiffening of skin to extension…is reportedly due to uncrimping and alignment of collagen fibers in the dermis. Young immature skin exhibits a more highly crimped and disorganized network of collagen, so relatively more tensile deformation is needed to align these fibers. The slope of the linear range is considered to be a measure of the tensile modulus of collagen fibers. This mechanical parameter correlates with the concentration of more mature (crosslinked) collagen. The tensile properties (modulus and strength) of skin also depends on body orientation. Collagen fibers have a preferential orientation in skin that affects the tensile properties and may be related to the state of in vivo tension. When skin is deformed beyond its elastic limit, collagen fibers are damaged. This ‘plastic’ deformation results in a permanent set (an inability to retain its original dimensions) and a corresponding decrease in the tangent modulus of the skin” (117-118).

This study was done with six female pigs because “the young pig has skin which is mechanically and histologically most similar to human” (118). The conventional method utilized 200 mL expanders which were placed under the skin and expanded four times in 50 mL intervals at 0, 7, 14, and 21 days. Specific elliptical shapes where drawn on the porcine skin to measure skin expansion. The skin was excised for testing. A control was done on the opposite side of the pig to test normal skin (118). During the surgery anesthesia was used. Since skin is adaptable to stress under normal conditions, a thought arises if specific mechanical or biological effects could be associated with anesthesia and wound healing, such as the excision of the skin here under anesthesia. Also, important to note that there are differences in skin on the dominant side of the body versus the non-dominant.

The rapid, or intraoperative, expansion was done with 200 mL expanders also, but expanded the entire volume in an hour to aggressively force expansion. A second biopsy was taken from each of the pigs after eight weeks to test the recovery of the tissue (119). Belkoff, et al. examined the histological “slides for the following: (1) alignment, thickness, and fragmentation or degeneration of collagen fibers; (2) alignment, thickness, fragmentation, and quantity of elastin fibers; and (3) number of fibroblasts” (119).

The results showed during the surgery: “The acutely (intraoperatively) expanded skin…was difficult to manipulate. It appeared grossly to be much thinner than the control sin; however, this impression was not supported by actual thickness measurements” (120). This may have been perceived in this manner due to blood pooling out of the location of expansion to release the tension on the area. Belkoff, et al. state: “There was no significant difference, however, in skin thickness between skin from either expansion site and their respective controls” (120). These results show a significant recovery, especially in the intraoperative samples. They also found that the stiffness and tangent modulus were the same, but the time zero showed “transversely oriented
skin specimens were typically as stiff or slightly stiffer than their longitudinal counterparts” (120). During the second biopsy, “however, the longitudinal specimens were stiffer than their transversely oriented counterparts” (120). They note that “this direction-dependent difference was significant only for the intraoperatively expanded skin specimens” (120). This may be due to the collagen fibers having time to convert back to the original orientation during the conventional method, but the intraoperative expansion was too aggressive.

The most interesting mechanical measurement to note is the testing of the control delivered 8.14 MPa as the ultimate stress, but conventional method conveyed 5.50 MPa (120). The overall mean modulus of the porcine samples was 29.8 MPa (122). Also, the ultimate stresses for all the methods of expansion showed significant differences in longitudinal and transversely oriented specimens (121). This shows the importance of collagen fiber orientation in the material testing of the skin. The biochemical testing showed the “total collagen content increased significantly from 70.72 ± 4.29 to 87.26 ± 4.29 mg/g over the period from the initial biopsy to final biopsy” (121). This four week change in collagen content shows the biochemical adaptation of the dermis during recovery.

Belkoff, et al. discuss the results: “Skin subjected to 4 weeks of conventional expansion exhibited a drop in tangent modulus (although not statistically significant) and a significant decrease in ultimate stress (strength). These changes indicate a weakening of the dermal collagen and may suggest a remodeling process initiated by the altered state of stress on the skin during expansion. These changes may also be associated with the formation of fibrous tissue around the implant” (121). They also note: “Examination of light micrographs did not indicate evidence of a disruption of the collagen network which would be expected if plastic deformation were to occur” (121). An interesting discussion point made by Belkoff, et al. is that “dermal collagen was recruited from surrounding tissue” of the expanded samples (121). However, after four weeks of healing time, all the biomechanical and histological tests showed no significant difference between the expanded tissue and normal tissue (122). This, once again, shows the adaptability of the collagen fibers in the dermis.


In the article, “Evaluation of Biomechanical Properties of Human Skin,” Edwards, et al. discuss the biomechanical properties and property testing methods of the skin in vitro versus in vivo. They state some of the basic functions of the 1.7 m², 4 kilogram organ: protection against friction, impact, pressure, cutting, and shear, dissipation or conservation of heat, and grip (375). The functions of the skin listed are exactly the properties researchers are interested in testing. In vivo testing may show how the skin reacts to external forces of stretch, shear, torsion, compression, and indentation while still on the body and is able to be repeated. “The serial testing of one site possible with nondestructive in vivo tests may provide information on the function and the kinetics change in mechanical properties. The numerical values given by in vitro tests usually exhibit a smaller spread than those from in vivo tests” (375). Edwards, et al.
make a valid point that, overall, in vivo testing tests function and in vitro testing tests properties (375).

In vitro testing methods involve the excision of skin before testing begins. “The site and orientation of the specimen are very important, as anisotropic preexisting or ‘resting’ tension of skin exists as a result of structural strain, normal habitual body movements, and underlying joints or musculature. Such resting tension gives rise to the characteristic gaping of wounds, where a linear cut across the lines of tension produces an elliptical wound. The lines of maximum tension were originally mapped by and are named for Langer in the late 19th century” (376). This supports the importance of the properties in respect to the collagen fiber alignment and the existing tension between the epidermis and dermis layers of skin. Edwards, et al. explain: “…skin exhibits a rate-dependent resistance to applied stress, and if load is applied too fast it may in fact rupture at stress levels much lower than the ultimate stress levels determined by other tests” (376). The strain rate of mechanical testing is extremely important, especially in viscoelastic materials.

In vitro testing is a valid comparison to in vivo because “in experiments where both in vitro and in vivo tests have been done on the same (animal) subjects, the two agree fairly well” (376). There are several important biomechanical properties of human skin discussed in this article. One interesting property is creep: “…for a given load the specimen will continue extending after application, a phenomenon called creep. If skin samples are stretched at a steady rate then unloaded with the same velocity, it is found that the stress-strain curves for the extension do not coincide with the curves for the unloading cycle, forming what are termed hysteresis loops. The area under the stress-strain curves represents the energy put into the system, and the area between the loading and unloading curves represents the energy used or lost in the system (376). They state the importance of collagen: “…the strength and elastic properties of skin are determined by the collagen content (specifically the ‘insoluble collagen’ content), and not the elastin or ground substance components” (377). Unfortunately, these biomechanical property tests cannot be fully reproduced in an in vivo environment.

In vivo testing may show the adaptability of the skin to overcome external stresses. “The force required to stretch the skin and maintain the new tab separation is recorded...The separation results in an initial peak force which immediately drops due to the adaptation of the collagen meshwork of the dermis (relaxation)” (377). Edwards, et al. make an interesting observation: “When [tabs] are stuck to the skin and moved apart, the skin is prevented from ‘necking’ in the region of strain application and, therefore, experiences an effective stress in a direction orthogonal to the extension axis” (378). This is a valid point. Is there extra tension in the skin during in vitro testing due to necking that should be occurring in both directions during biaxial testing? In vivo testing seems to centrally show the functions of the skin as an organ of protection and adaptability, while in vitro testing displays the properties of the skin as a biomaterial.

In the article, “The Effect of Composition and Microstructure on the Viscoelastic Properties of Dermis,” Ventre, et al. discuss the extracellular matrix microstructure and the mechanical behavior of the upper and lower layers of the dermis, papillary and reticular, respectively, by performing enzymatic digestion and mechanical shear frequency analysis. They introduce the skin as a heterogeneous material, but more importantly, the dermis due to the mechanical complexity, and differences, of the two main layers in the dermis: “…dermis itself is a heterogeneous tissue: the spatial assembly and the relative amount of the extracellular matrix (ECM) microconstituents is not uniform throughout its thickness (Sorrell and Caplan, 2004) and it should be expected that different dermal strata will contribute to the overall mechanical response differently” (430). Ventre, et al. deliver a brief description of the two main layers: “As a first approximation, dermis can be divided into two layers: a papillary layer, closer to the epidermis, where collagen fibres are packed in thin bundles of less than 10 μm in diameter and the deep reticular dermis, formed by an entangled mass of collagen bundles (more than 50 μm), forming a looser network. Moreover, the transition between the papillary and reticular layers is not abrupt: collagen fibres change in dimension and architecture in a gradual manner” (430). This description shows the heterogeneity that the dermis possesses microstructurally.

Ventre, et al. used two-year-old bovine samples shaved and defleshed for the testing. They cut 15 millimeter diameter disks, and then removed the top 1 millimeter of to remove the influence of hair follicles and stiff keratins during the tests. They then used a confocal microscope with a 10x lens to measure both thicknesses of the papillary and reticular dermis (431). The enzymatic treatments were completed by the following procedure: “A total of 15 upper dermis and 5 lower dermis specimens were used for dynamical mechanical-enzymatic characterization in order to assess the effects of selective digestion of ECM components on the viscoelastic response of each dermal layer. Each sample was subjected to small deformations so that the test could be run twice on the same specimen, i.e. before and after the enzymatic treatment. The testing protocol consisted of four sequential steps, namely, (1) equilibrating the specimen in the same buffer solution of the intended enzymatic treatment but without the enzyme; (2) testing of the specimen to obtain the control; (3) selective digestion of specific ECM component; (4) testing of the same treated sample” (431). The experimental setup to find the viscoelastic response was completed using dynamic mechanical testing through oscillatory shear in a parallel plate with the 15 millimeter diameter specimens (431). The testing is used to find the storage and loss moduli separately. “The dynamic moduli G’ and G” depend only on the frequency and are named ‘storage modulus’ and ‘loss modulus,’ respectively. Their exact physical meaning is reported in any standard textbook of viscoelasticity; here suffice it to say that G’ represents the specific elastic energy stored in one cycle, while G” is proportional to the viscous dissipation of energy per cycle” (432).
The results of Ventre, et al. are interesting due to the disparity between the upper and lower dermal layers. “For both strata $G'$ is significantly higher than $G''$ over the whole frequency range, moreover the moduli of upper dermis are higher by about an order of magnitude than the corresponding ones of lower dermis” (432). The results of the enzymatic treatments are specific to the treatment. When the trypsin treatment is utilized, “The effect of the trypsin treatment on the dynamic moduli spectra of both upper and lower dermis is reported...The average value of $G'$ of both strata is lower in the case of treated samples than the control. In particular, the upper dermis samples undergo a more prominent decrease than the lower dermis ones...” (432). In respect to the elastase treatment, “Treatment with elastase produces a significant decrease in both $G'$ and $G''$ over the whole frequency range” (432).

Ventre, et al. discuss the papillary and reticular dermis results by thoroughly describing the adaptation of the microstructure. “In the natural state, upper dermis shows higher dynamic moduli than the corresponding ones of lower dermis. This can be interpreted on the basis of structural and compositional features: upper dermis comprises mainly papillary dermis, which is composed of densely packed thin collagen fibres entrapped within a three-dimensional network of elastic fibres. When a macroscopic stress is applied, collagen fibres cannot glide and relax the stress, hence a considerable fraction of the provided energy is stored as elastic energy in the elastic fibres...On the other hand, lower dermis, being composed entirely of reticular dermis, is constituted by an entangled mass of wavy collagen bundles that are not constrained by any elastic network. The small stresses involved during the oscillating deformation do not allow the bundles to straighten enough to be able to withstand the applied stress and this results in a decreased value of $G'$. These data are also confirmed by the dissipation factor: in the low-frequency regime (i.e. below 0.1 Hz) the larger collagen bundles of lower dermis have time to uncrimp and reorient, thus damping more energy than the thin and highly packed collagen whiskers of upper dermis” (433). Ventre, et al. then discuss the effect of the presence of elastin: “According to Meyer et al. (2000), elastic fibres in bovine papillary dermis form a three-dimensional network, which is interconnected with the thin collagen fibres. In reticular dermis, on the other hand, elastin is not functionally present; therefore, we expect that elastase treatment would have an effect only on upper dermis. Our results show that elastin digestion produces a significant decrease of both $G'$ and $G''$ despite elastin comprises less than 3% of the skin wet weight” (434). There is no doubt that this research displays the importance of acknowledging there are two specific layers to the dermis and they both deliver properties important to the structure. The difficulty is the separation, whether physically or visually, using microscopy, because the boundary of the two layers is not subtle, but a more of a blended conversion.


In the article, “Reinforced Bioartificial Dermis Constructed with Collagen Threads,” Seo et al. discuss the novel method of creating a composite scaffold utilizing a collagen sponge reinforced with a collagen mesh to develop a bioartificial dermis with elevated biocompatibility and strong mechanical properties. They clarify the basic necessary factors of wound healing is a synergistic effect of a combination of cells, growth factors, and scaffolds (745). Next, they discuss the importance of scaffolds: “Scaffolds provide the substrates to which the cells can attach during the initial phase, and degrade after the completion of wound healing” (745). They explain that using PGA in a collagen sponge keeps the sponge from shrinking and not inhibiting the movement of transferred cells, but the hindrance of using PGA is that the biocompatibility is not as qualified as collagen (746). Unfortunately, there are caveats to using synthetic materials versus natural grafts, and vice versa. Seo, et al. explain: “Synthetic materials may afford better plasticity and controlled biodegradation than grafts prepared from natural ECM materials, but they are also less biocompatible. However, natural ECM materials may be less suitable in many instances because of their weaker mechanical properties. So, many researchers have designed composite or reinforced scaffolds so as to overcome these shortcomings” (749).

To prepare the collagen threads in the mesh, Seo, et al. utilized type I calf skin collagen and circulated a coagulation solution. They layered the threading in a 9x9 (threads) cross-linked collagen thread mesh, five layers deep using 254 nm UV for 12 hours. The collagen sponge was formed next using a collagen-chondroitin-6-sulfate solution (collagen-CS). They then immersed the collagen mesh into the collagen-CS solution to form the reinforced collagen sponge (746). Next, Seo, et al. obtained normal human skin and aseptically isolated fibroblasts, and mechanically stripped the epidermal layer (747). The next step was extremely important to the success of the bioartificial dermis: “Human skin dermal fibroblasts, suspended in DMEM supplemented with 10% FBS, were seeded onto the unreinforced and reinforced collagen sponges (diameter 15 mm, thickness 2.0 mm). To obtain a very high cell seeding density, the cells were seeded onto the sponges in a dried condition” (747). For the final test of the quality of the bioartificial dermis, they performed the ultimate tensile strength and compression testing after seven days of culturing. Both tests were completed using a 10% strain rate.

The results of Seo, et al. are impressive and fascinating. The collagen mesh they formed of collagen threads showed an average diameter of 55 µm (748). The histology testing observed the collagen threads and cells. “The reinforced bioartificial dermis was constructed of a porous collagen sponge containing collagen mesh and fibroblasts. It showed homogenous distribution of cells into the pores and on the surface of the collagen sponge and mesh” (748). This result shows the high-quality cell transferability that is usually an obstacle. Another good result: “The fibroblasts adhered rapidly to the reinforced collagen sponge and attached completely in less than 24 h. These cells were cultured in the reinforced collagen sponge for 7 days, during which
time the pores in the sponge became filled with fibroblasts that secreted ECM to form a bioartificial dermis” (748). A cell-friendly environment is demonstrated: “This resulted in the virtual disappearance of the pores. Unlike monolayer cultures, where contact inhibition limits cell growth, this porous structure provides an environment for cell growth and ECM deposition to occur in a three-dimensional configuration” (748).

The mechanical testing results are even more impressive. The sponges with the collagen mesh reinforcement, versus just the sponge alone, showed a ten-fold increase in ultimate tensile strength, and then displayed a twelve-fold increase during the compression test (748). Seo, et al. explain the implications and the applications this research may develop: “In addition to the high degree of biocompatibility, it will be possible to control the degree of strength of these scaffolds through changes in the diameter of the threads, the quantity of the threads, the thickness of the mesh, and different thread weaves used to form the mesh. Using these procedures, it will be possible to made stronger or weaker materials for use in different applications” (748). They mention using this technique for various other applications such as synthetic cartilage, bone, and blood vessel formation (749). The mechanical properties are always demonstrated from a microstructure level in any material which is why the basic building blocks such as fibroblasts and collagen are extremely important to obtain the results Seo, et al. were fortunate to achieve.


Protocol for Experimental Setup and Testing of Porcine Dermo-Epidermal Expansion

1) Turn the water bath to 37 degrees Celsius. Place the HBSS into the heated water bath.

2) Secure the porcine skin sample in HBSS and refrigerate if the sample is not already in HBSS. The porcine skin sample main contents are epidermis, dermis, and subcutaneous adipose tissue.

3) Remove the hair gently from the porcine epidermis with a straight razor blade.

4) Cut a 2mm x 2mm section from the porcine epidermis+dermis and place it into a 1.5mL centrifuge tube. Label the tube PED(x1)_(x2) where P=porcine, ED=Epidermis+Dermis, x1=pig number, and x2=section sample number from the pig. Freeze this sample tube.
5) Cut another 2mm x 2mm section from the porcine epidermis+dermis and place it into buffered 10% Formalin, pH 6.8-7.2. Label the container PH(x1)-(x2) where P=porcine, H=histology, x1=pig number, and x2=section sample number from the pig. Refrigerate this in buffered 10% Formalin.

6) Carefully cut five approximately 7mm x 7mm sections from the porcine skin sample.

7) Use a blade or scalpel to gently remove most of the subcutaneous adipose tissue.

8) Use the spring-loaded micrometer gauge to measure the thickness of each of the approximately 7mm x 7mm sections. Record the thicknesses for each sample under the label P(x1)-(x2) where P=porcine, x1=pig number, and x2=section sample number from the pig. For example, P42 is pig number 4, section sample 2.

   **Note:** The total number of samples to be tested is 48: two samples from 24 different pigs. A third sample from each pig will be tested to acquire data for a balloon skin expansion experiment. Five sections are cut to ensure the proper number of samples in case certain sections cut are not physically testable.

9) Place each of the five sections in separate 1.5mL centrifuge tubes containing HBSS with the labels P(x1)-(x2) and place them in a centrifuge tube holder.

10) Power on the BioTester and the heated bath. Fill the heated bath with the heated HBSS. Install the biorakes onto the actuators via the magnetic holders. Start the BioTester software. Calibrate the load cells if necessary with the spring. Reset the actuators using the software.

11) Next, use the dual or independent movement arrows in the software to move the biorakes into position, forming a 4mm x 4mm square boundary. The close proximity may be monitored by observing the live CCD camera image on the screen.

12) Obtain P(x1)-1 and place the section on the BioTester mounting crossbar stage. Raise the mounting crossbar stage by elevating the heated bath until the sample is at a level where it may be pierced by the biorake tines. Gently use the holder end of a set of tweezers to push down the tines and pierce P(x1)-1. Lower the heated bath to remove the mounting crossbar stage. Raise the heated bath until P(x1)-1 is submerged into the HBSS.

13) Create a regimen in the BioTester software. For this test, 35% stretch at 15 seconds is employed (15 second stretch and 15 second recovery with no hold time). Begin stretch #1 for Px1. After completion, save the file and sequence of images (.avi movie) as P(x1)-1-(x3)-(x4) where P=porcine, x=pig number, and 1=section sample number from the pig, x3=angle rotation number, x4=stretch number for this particular angle rotation number. For example, P4-2-3-2 is pig number 4, sample section 2, third angle of rotation, and the second stretch of this particular angle of rotation.
14) Each angle of rotation is stretched a total of five times. The first two stretches are saved, but are not used for final data analysis. The final three stretches are saved and used for an n=3 of the final data analysis. Wait 30 seconds between each of the five stretches.

15) The first angle P1-1-(1)-1 is considered the 0 degree angle of rotation, marked by a surgical pen. After the five stretches at 0 degrees, remove the sample from the biorakes by lowering the heating bath, placing the mounting crossbar stage onto the heating bath, and raising the stage to the sample height. Gently remove the sample with tweezers onto the stage. Lower the stage and rotate the specimen approximately 18 degrees clockwise (this is a rough degree estimate for five rotations within 90 degrees, i.e. 90/5=18). Repeat the five stretches for angle rotation two. Continue until all five rotations and 25 stretches are complete for the sample section.

16) Repeat steps 12-15 for a second section sample from the same pig. Only the data from two section samples from each pig need to be obtained for analysis.

17) Next, mount a third section sample from the same pig onto the biorakes. Using a similar BioTester software regimen, 35% stretch at 15 seconds, create a cyclic regimen to repeat this 35% stretch at 20 seconds 10 times with no lag time between stretches. Record this data as P(x1)C where x1=pig number and C=Cyclic. Thirty seconds after the tenth stretch in the regimen, stretch an eleventh time. Record this data as P(x1)P where x1=pig number and P=Post cyclic stretch.

18) Remove the sample from the biorakes. Shut down the software and then turn off the BioTester power and heated bath. Clean and sanitize the biorakes and place them into the proper case, empty the heated HBSS bath, and clean the surrounding area. Place the HBSS back into the refrigerator and shut off the water bath.
Results and Discussion of Biomechanical Property Testing of Porcine Dermo-Epidermis

1. Porcine epidermal plus dermal sample preparation

2. The samples are strained biaxially using 500mN load cells in the BioTester using tungsten biorakes with five tines each spaced 1mm apart.
3. The samples are biaxially strained using 500 mN load cells as feedback. Each sample is rotated to obtain the maximum stress and the orientation of the collagen fibers.

Figures 9-13: BioTester CCD camera shots, pre-strain, of samples mounted on biorake tines and submerged in heated HBSS

![Porcine Epidermis+Dermis Force vs Displacement](image)

Figure 14: Force vs. displacement of porcine epidermis+dermis sample. The data displayed is the 90 degree rotation orientation from the first placement (4th sample shown above from left to right).

Work Cited:
The BioTester regimen for the porcine skin expansion mechanical testing contains a 35% stretch: 15 second stretch and 15 second recovery time with no hold time.

Figure 15: Biotester initial screen with an input regimen (red), biorakes in place to support a sample (yellow), and load cell output (blue) with real-time graphical output (orange)
Figures 16-20: Porcine skin samples P1-1-(1-5). Each of these rotations was stretched two times initially and three additional times to obtain data. Note the coordinate system scribed by a surgical pen (L).
Figures 21-22: Actual data output from the two initial stretches of rotation 3: (P1-1-3-1 and P1-1-3-2)
The Figure on the left is the sample stretch at 0 seconds for the first rotation at 0 degrees. The Figure on the right is the sample at full stretch at 15 seconds.

The Figure on the left is the sample stretch at 0 seconds for the second rotation at approximately 22 to 25 degrees. The Figure on the right is the sample at full stretch at 15 seconds.
1. This section shows the data from the second sample of the second porcine specimen for each of the five rotations. Note each rotation is stretched five times. The second graph on each page displays the final three stretches at each particular rotation.
2. This figure shows the data from the second rotation. The graph displays the average of the final three stretches at that particular rotation with the standard deviation. This rotation was chosen due to the result of the highest forces being developed at the maximum displacement, and in this case, in the X direction.
The raw data results are a force versus displacement graph. This information is used to calculate the stress versus strain graph. Figures (23) through (27) and Equations (1) and (2) display the steps and equations utilized for this calculation:

\[
\text{Stress} = \frac{\text{Force}}{\text{Area}_C} = \frac{F_{xi}}{2.10(\text{mm})^4(\text{mm})} \quad \text{and} \quad \frac{F_{yi}}{2.10(\text{mm})^4(\text{mm})}
\] (1)

\[
\text{Strain} = \frac{L_f - L_0}{L_0} = \frac{L_{f(x)} - 4(\text{mm})}{4(\text{mm})} \quad \text{and} \quad \frac{L_{f(y)} - 4(\text{mm})}{4(\text{mm})} \quad \text{from 0 to 0.35 (35\%)}
\] (2)

where \(\text{Area}_C\) is the cross sectional area of the specimen, \(L_f\) is the final length of the sample at a specific time point during displacement, and \(L_0\) is the original length of the specimen.

![P2-1-2 Force vs Displacement](image)

Figure 23: Raw data: the force versus displacement graph for the five stretches including recovery for sample 1 from pig 2 during rotation 2
Figure 24: The sample displays a preliminary tension which must be released during the first two stretches, therefore the first two stretches are removed from the data: N=3

Figure 25: The three final stretches are averaged in the X and Y directions with standard deviations
Figure 26: Equations (1) and (2) are utilized to calculate the stress versus strain graph for both X and Y directions for the final three stretches of P2-1-2.

Figure 27: The average stresses are calculated for the X and Y directions and graphed as stress versus strain with standard deviations.
All five rotations must be compared in order to find the rotation with the greatest stress of all five rotations for sample 1 from pig 2: P2-1. Figures (28) through (31) display the average stresses calculated for the X and Y directions and graphed as stress versus strain with standard deviations for rotations 1, 3, 4, and 5. For P2-1, rotation 2 displayed the highest stress.

Figure 28: The average stresses of rotation 1 calculated for the X and Y directions and graphed as stress versus strain with standard deviations

Figure 29: The average stresses of rotation 3 calculated for the X and Y directions and graphed as stress versus strain with standard deviations
Figure 30: The average stresses of rotation 4 calculated for the X and Y directions and graphed as stress versus strain with standard deviations.

Figure 31: The average stresses of rotation 5 calculated for the X and Y directions and graphed as stress versus strain with standard deviations.
Figures (32) and (33) display the summarized results of the biomechanical testing of the dermo-epidermis porcine specimens (two samples) from pig number two.

**P2 Stress vs Strain for Stretch 3, 4, and 5**

![Graph of P2 Stress vs Strain for Stretch 3, 4, and 5](image)

**Figure 32:** Stress versus strain graph for both X and Y directions for the final three stretches of the two samples from P2

**P2 Average Stress vs Strain**

![Graph of P2 Average Stress vs Strain](image)

**Figure 33:** The average stresses are calculated for the X and Y directions and graphed as stress versus strain with standard deviations of P2
Figures (34) and (35) display the summarized results of the biomechanical testing of the dermo-epidermis porcine specimens (two samples) from pig number three.

**Figure 34:** Stress versus strain graph for both X and Y directions for the final three stretches of the two samples from P3

**Figure 35:** The average stresses are calculated for the X and Y directions and graphed as stress versus strain with standard deviations of P3.
Figures (36) and (37) display the summarized results of the biomechanical testing of the dermo-epidermis porcine specimens (two samples each) from pig number two and three.

**Figure 36**: Stress versus strain graph for both X and Y directions for the final three stretches of the two samples from P2 combined with P3

**Figure 37**: The average stresses are calculated for the X and Y directions and graphed as stress versus strain with standard deviations of P2 combined with P3.
Works Cited


Appendix: Final Presentation

**PURPOSE OF PORCINE EPIDERMIS+DERMIS BIAXIAL STRAIN TESTING**

**PURPOSE:** TO OBTAIN THE DIRECTIONAL BIOMECHANICAL PROPERTIES OF PORCINE DERMAL TISSUE
- PORCINE DERMAL TISSUE DISPLAYS MECHANICAL PROPERTIES SIMILAR TO HUMAN

**ULTIMATE GOAL:**
DEVELOP A BIOREACTOR THAT WILL MINIMIZE THE STRESS VARIATIONS IN THE DERMIS TO MAXIMIZE SKIN EXPANSION CAPABILITY

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**PORCINE EPIDERMAL AND DERMAL LAYERS**

Cross Section of Stresses in the epidermis and dermis and the Microstructural Components Driving the Results of Strain Testing[2]
Porcine Epidermis+Dermis
Biomechanical Experimental Setup

- Porcine skin from the belly area of a 12-month-old pig (upper left)
- Sectioned in approximately 7x7 mm² samples
- Subcutaneous fat tissue removed
- Thickness is measured by micrometer
- Samples stored in HBSS when idle

The BioTester: Specimen Setup for Biomechanical Testing

The BioTester developed by CellScale utilizes a biaxial stretching method to measure strain in both X and Y directions simultaneously using a 500mN load cell on each axis.
**BIOTester Software Setup for Biomechanical Testing**

![BioTester software setup diagram](image)

BioTester initial screen with an input regimen (red), biorakes in place to support a sample (yellow), and load cell output (blue) with real-time graphical output (orange).

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**The Importance of Biaxial vs. Uniaxial Stretch**

![Porcine Epidermis+Dermis Force vs. Displacement](image)

Porcine Epidermis+Dermis Force vs. Displacement

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[2]
MOUNTING THE PORCINE SAMPLE IN THE BIORAKES FOR EACH ROTATION (0-90 DEG)

• Each sample is mounted as 0 degrees (This is considered the first rotation (upper left))
• Each rotation is approximately 22-25 degrees clockwise from 0 to 90 degrees (5 rotations)
• Each rotation orientation is stretched 5 times

BIOMECHANICAL STRETCH SOFTWARE OUTPUT
**DIRECTIONAL BIOMECANICAL PROPERTIES OF THE DERMAL TISSUES**

The collagen fiber orientation creates varied directional stresses: Stiffer along the preferred collagen fiber direction.

The first two stretches of each rotation are removed from the data due to the pretension in the porcine skin remaining in the sample even after excision (n=3).

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**STRESS AND STRAIN CALCULATIONS**

- The output from the BioTester software is a force versus displacement data set and graph. This raw data is used to calculate the stress versus strain graph using the equations:

\[
\text{Stress} = \frac{\text{Force}}{\text{Area}_C} = \frac{F_{x_i}}{2.10(\text{mm}) \times 4(\text{mm})} \quad \text{and} \quad \frac{F_{y_i}}{2.10(\text{mm}) \times 4(\text{mm})}
\]

\[
\text{Strain} = \frac{L_f - L_0}{L_0} = \frac{L_f(x_i) - 4(\text{mm})}{4(\text{mm})} \quad \text{and} \quad \frac{L_f(y_i) - 4(\text{mm})}{4(\text{mm})} \quad \text{from 0 to 0.35 (35%)}
\]

where \(\text{Area}_C\) is the cross sectional area of the specimen, \(L_f\) is the final length of the sample at a specific time point during displacement, and \(L_0\) is the original length of the specimen.

*(Example data is from sample P2-1-2 biomechanical test)*
P2-1-2 STRESS AND STRAIN CALCULATION

- Note the Strain values shown on the graph displays values from 0.24 to 0.35 (24%-35%) to emphasize the nonlinear anisotropic behavior
- P2-1-2 represents the second pig, first sample from pig two, and the second rotation orientation

RESULTS FROM PORCINE SPECIMEN 2 AND 3: 2 SAMPLES EACH

- This displays the collagen fiber nonlinear anisotropic behavior
- The average stress and strains include all five rotations for the four samples. Since the biomechanical test utilizes biaxial strain, the X and Y orientations will often merge when averaging all rotations across the 90 degrees
PORCINE EPIDERMIS+DERMIS BIAXIAL STRAIN VISUAL AID:
P1-1-1-5 STRAIN TEST AT 35% STRAIN: 15 SECOND STRETCH, NO HOLD TIME, 15 SECOND RECOVERY

PORCINE EPIDERMIS+DERMIS BIAXIAL STRAIN VISUAL AID:
P1-1-1-5 STRAIN TEST WITH DISPLACEMENT POINT GRID
Other Methods of Biomechanical Testing of Skin

Techniques
- Biorheological: Shear
- Tension and Compression to Failure
- Viscoelastic, Stress Relaxation\(^4\) and Creep

Applications
- Balloon Expanders\(^3\)
- Artificial Skin
- Wound Healing
- Skin Substitutes

Skin Substitutes\(^1\) and the Future

Anatomical Structure
- Demio-Epidermal Composite
- Epidermal
- Dermal

Duration of the Cover
- Permanent
- Semi-permanent
- Temporary

Type of Biomaterial
- Biological
- Synthetic: Biodegradability

Cellular Component
- Cellular
- Acellular

Biomaterial Loading Occurs
- *In vitro*
- *In vivo*
REFERENCES


APPENDIX SECTION: P1-1-1-5 STATIC IMAGES

• The purpose of this Appendix is to display the static images associated with the videos in the presentation body.

• P1-1-1-5: Pig Specimen #1, Sample #1 from Pig #1, Rotation #1 (0 degrees), Stretch #5.

• The BioTester software package designed by CellScale is utilized to create the following images.
P1-1-1-5: RELAXED

P1-1-1-5: 35% STRETCH
P1-1-1-5: GRID RELAXED

P1-1-1-5: GRID AT 35% STRETCH
P1-1-1-5: DISPLACEMENT POINT RELAXED

P1-1-1-5: DISPLACEMENT POINT STRETCHED
P1-1-1-5: DISPLACEMENT POINT RELAXED

P1-1-1-5: DISPLACEMENT POINT STRETCHED
P1-1-1-5: STRAIN RELAXED

P1-1-1-5: E1 STRAIN AT 35% STRETCH
P1-1-1-5: E2 STRAIN AT 35% STRETCH

P1-1-1-5: EX STRAIN AT 35% STRETCH
P1-1-1-5: EY STRAIN AT 35% STRETCH

P1-1-1-5: PRINCIPLE ANGLE (R) AT 35% STRETCH