Published in final edited form as:

J Biomech. 2011 May 17; 44(8): 1491–1498. doi:10.1016/j.jbiomech.2011.03.007.

Designing PolyHEMA Substrates that Mimic the Viscoelastic Response of Soft Tissue

Brian Holt^{1,2}, Anubhav Tripathi^{2,3}, and Jeffrey R. Morgan^{1,2}

¹ Department of Molecular Pharmacology, Physiology, and Biotechnology, Brown University, Providence, RI, USA

² Center for Biomedical Engineering, Brown University, Providence, RI, USA

³ Division of Engineering, Brown University, Providence, RI, USA

Abstract

Matching the mechanical properties of a biomaterial to soft tissue is often overlooked despite the fact that it's well known that cells respond to and are capable of changing their mechanical environment. In this paper, we used NaCl and alginate beads as porogens to make a series of micro- and macro-porous pHEMA substrates [poly(2-hydroxyethyl methacrylate)] and quantified their mechanical behavior under low-magnitude shear loads over physiologically relevant frequencies. Using a stress-controlled rheometer, we performed isothermal (37°C) frequency response experiments between 0.628 and 75.4 rad/s [0.01–12Hz] at 0.1% strain. Both micro- and macro-porous pHEMA substrates were predominately elastic in nature with a narrow range of G' and G'' values that mimicked the response of human skin. The magnitude of the G' and G'' values of the macro-porous substrates were designed to closely match human skin. To determine how cell growth might alter their mechanical properties, pHEMA substrates were functionalized and human skin fibroblasts grown on them for fourteen days. As a result of cell growth, the magnitude of G' and G'' increased at low frequencies while also altering the degree of high frequency dependence, indicating that cellular interactions with the micro-pore infrastructure has a profound effect on the viscoelastic behavior of the substrates. These data could be fit to a mathematical model describing a soft solid. A quantitative understanding of the mechanical behavior of biomaterials in regimes that are physiologically relevant and how these mechanics may change after implantation may aid in the design of new materials.

Keywords

shear; rheology; viscoelastic; poly (2 hydroxyethyl methacrylate); soft tissue

© 2011 Elsevier Ltd. All rights reserved.

Address correspondence to: Jeffrey R. Morgan, Associate Professor of Medical Science, Department of Molecular Pharmacology, Physiology & Biotechnology, Box GB-393, Brown University, Providence, RI 02912-G.

Conflict of interest statement

The authors have no conflicts of interest to disclose.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

The development and success of biomaterials can be distilled to three primary design criteria: physical/mechanical properties; mass transport properties; and biocompatibility/biomimetic capacity [Gheduzzi et al., 2006; Kohane et al., 2008; Tibbit et al., 2009; Williams, 2009]. The majority of biomaterial development focuses on mass transport profiles and biocompatibility/bio-mimetic capacity. These parameters are often prioritized because they represent the preliminary hurdles to restoring and/or supplementing biological functionality [Stevens et al., 2005]. Essential to all biomaterials, however, is the cell-biomaterial or cell-substrate interactions which represent a primary obstacle to integration and clinical success [Stevens et al., 2005]. Few investigators recognize the importance of the mechanical behavior of substrates beyond a cursory comparative characterization and many biological processes involve mechanics. Specifically, cutaneous wound healing relies heavily on the ability of cells to detect changes in the mechanics of their environment and the ability of cells to sense and respond to their environment is well established [Wang et al., 2003; Hoffman et al., 2009], as is the role mechanical stimuli plays in affecting cellular morphology and phenotype [Cowin et al., 2000; Hoffman et al., 2009]. Unfortunately, little effort is made to synthesize biomaterials with mechanical properties that match the tissue with which it will interact and even less is understood about how the mechanics of these biomaterials might change when cells and tissues interact with these biomaterials. Furthermore, even fewer studies have attempted to garner insights from mathematical models of the viscoelastic response of biomaterials with cells.

In this study, we made a series of micro- and macro-porous poly (2-hydroxyethyl methacrylate) [pHEMA] substrates with the intent of matching their mechanical properties to that of human skin. Despite differences in pore sizes, micro- and macro-porous substrates had similar swelling kinetics indicating that interstitial fluid flow was comparable. The elastic (G') and viscous (G'') moduli of the pHEMA substrates were measured by subjecting them to low-magnitude shear loads over a range of physiologically relevant frequencies. The introduction of macro pores caused the greatest reduction in G' and G'' and the closest approximation to human skin. However, when cells were grown on modified pHEMA substrates for 14 days, G' increased in the low frequency regime and G'' increased across the entire frequency regime when compared to unmodified substrates without cells. Additionally, cellular activity on modified substrates altered the degree of frequency dependence in the high frequency rheological response of the substrates. Lastly, we were able to fit these data to a soft-solid viscoelastic model that facilitates more quantitative comparisons between various modifications of pHEMA substrates with and without cells as well as comparisons to human skin. A quantitative understanding of the mechanical behavior of synthetic substrates before and after implantation may help to improve their performance.

2. Materials and methods

2.1. Preparation of micro- and macro-porous pHEMA substrates

To make micro-porous substrates, 1.0mL of stock solution containing 40mL of ophthalmic grade 2-hydroxyethyl methacrylate [HEMA: Molecular weight: 130kDa] (Polysciences, Warrington, PA), 160mL of 0.8M NaCl, 0.450mL of ethyleneglycol dimethacrylate [EGDMA] (Polysciences, Warrington, PA), and 0.135g of azobisisobutyronitrile [AIBN] (Aldrich Chemical, Allentown, PA) were added to a 12 well polystyrene cell culture plate (Figure 1). Once mixed, the solution was cross-linked with ultraviolet light for 60 minutes (South New England Ultra Violet Company, Branford, CT). For macro-porous substrates, dried alginate beads (as described in the Supplemental Section) were added prior to

polymerization. Following polymerization, substrates were removed, cooled to room temperature and ~10mL of double de-ionized water (dH₂O) added to hydrate the sample.

To improve cell adhesion, pHEMA substrates were treated with 1, 1' carbonyldiimidazole [CDI] (Sigma Aldrich) [Fukano et al, 2006]. Dried pHEMA substrates were incubated in 20mL glass vials with 30mM CDI in 1,4-dioxane at 37°C for ~2.5hrs [Fukano et al, 2006]. Following incubation, samples were rinsed several times with 1,4-dioxane and transferred to sterile phosphate buffered saline [PBS] (Mediatech, Manassas, VA) with 0.75% (w/v) gelatin type B (Sigma Aldrich), derived from bovine skin. Substrates were incubated at 37°C overnight before storage in PBS.

To measure swelling kinetics, dried (~22°C) substrates were weighed and then added to separate, 2L volumes of de-ionized water equilibrated at 37°C. Three micro- and macro-porous substrates were removed at various times over 180 minutes and weighed.

2.2. Cell culture

Normal human fibroblasts (NHFs) from human foreskins were cultured in NHF medium containing Dulbecco's Modified Eagle's Medium [DMEM] (Invitrogen, Grand Island, NY), 10% fetal bovine serum [FBS] (Hyclone Laboratories, Logan, UT), 1% penicillin/streptomycin [P/S] (MP Biomedicals, Irvine, CA) at 37°C in 5% CO₂. After reaching ~70% confluence, cells were trypsinized (0.05% trypsin)(Hyclone Laboratories, Logan, UT), counted and seeded (20,000 cells/well, ~5,300 cells/cm²) onto pHEMA substrates in 12 well dishes. Cell attachment and proliferation on pHEMA substrates were determined using the WST-1 cell proliferation assay. Briefly, substrates were equilibrated in NHF medium overnight and 72 hours after seeding with cells, 125μL of WST-1 reagent (Roche Diagnostics, Indianapolis, IN) was added to each well (1:10). The plate was incubated for 2hrs at 37°C in 5% CO₂ and then agitated for ~1min. The supernatant was removed and absorbance measured at 420nm with a reference of 600nm. The student t-test was applied to determine statistically differences significant ($p \ll 0.001$).

2.3. Rheological characterization

Small amplitude oscillatory measurements were performed using a controlled-stress rheometer (AR-2000N, TA Instruments, Newcastle, DE) with parallel plate geometry (8mm diameter) and specimen-dependent gaps [Holt et al. 2008] (Figure 1). Circular punch sections (8mm) of the substrates were placed, top side up, on waterproof sandpaper (~20 by 10mm) (Norton 320 Grit, Saint-Gobain Inc, Worcester, MA) held to the bottom plate by double sided adhesive tape (Scotch 666, 3M, St. Paul, MN) for no-slip shear [Tsubouchi, et al. 2006]. The 8mm parallel plate geometry was lowered to a gap of 1600 to 2200 μm, depending on the thickness of the sample and the maximum value of the normal force [Holt et al. 2008]. Critical to this loading protocol was the maintenance of a consistent compressive normal force. Samples were uninhibited by boundary constraints ensuring strain transmission throughout the material. The maintenance of the no slip boundary condition was determined by monitoring the magnitude of shear stress and its repeatability [Holt, et al. 2008]. Samples were hydrated during testing with 1mL of PBS and all measurements were performed at 37°C. Oscillatory tests were conducted at strains of 0.1% over a frequency range of 0.628 to 75.398rad/s, divided into two isothermal sweeps to ease data collection; a higher range (6.283 to 75.398rad/s) and a lower range (0.628 to 6.283rad/s). Samples were loaded from high to low frequencies to maintain the optimal oscillatory torque, collecting ten data points per decade.

2.4. Soft-solid viscoelastic model

A soft-solid viscoelastic model was applied to the rheological frequency sweep data. The following model describes solids with an inherent weak internal structure that liquefies at high frequency:

$$\frac{G'(\omega)}{G_e} = (\omega\tau)^k \{ \cos(k\pi/2) + \sin(k\pi/2)k(k+1)/(2\omega\tau) \} \Gamma(k+1) \quad (1)$$

$$\frac{G''(\omega)}{G_e} = (\omega\tau)^k \{ \sin(k\pi/2) - \cos(k\pi/2)k(k+1)/(2\omega\tau) \} \Gamma(k+1) \quad (2)$$

Given G' and G'' data of a given material, the fit parameters are k , G_e , and τ . Note, that as $k \rightarrow 0$, $G'(\omega) = G_e$ and $G''(\omega) = G_e \omega\tau$ [Larson 1999]. An abridged derivation can be found in the Supplemental Information.

3. Results and discussion

3.1 Micro-pores dominate swelling kinetics and substrate super-structure of pHEMA substrates

Swelling experiments showed that micro- and macro-porous pHEMA substrates possessed very similar swelling kinetics (Figure 2). There was a marked degree of swelling from time $t = 0$ to 60mins during which approximately 60% of the swelling capacity of both micro- and macro-porous substrates was attained. This was supported by a similar trend in the equilibrium water content. The micro-porous substrates swelled more rapidly and possessed higher equilibrium water content when compared to the macro-porous substrates. A linear fit to the log – log of the swelling factor versus time data up to 60% of equilibrium showed a similarity in the swelling exponent for the micro- and macro-porous substrates, 0.804 ± 0.254 and 0.698 ± 0.211 , respectively. The similarity in the swelling exponents suggests that fluid movement is similar for both substrates and occurs predominately via the small pores created by NaCl and phase separation which is present in both micro- and macro-porous substrates. Micro and macro- porous substrate superstructures were confirmed through scanning electron microscopy [SEM] and supported by swelling kinetics findings. SEM analysis of micro and macro-porous substrates showed the substrates types possessed pore sizes ranging between 20 and 50 μm and 250 and 500 μm , respectively [Figure 3]. The micro- and macro-pore size ranges are intended to replicate the porosity critical to percutaneous interfaces that exist in nature and cellular survival in vitro [Pendegrass et al., 2006]. The ability of porous scaffolds and surfaces to promote endothelial cellular growth and integration is well established [Grosse-Siestrup et al., 1984; Fukano et al., 2006; Narayan et al., 2008; Tibbit et al., 2009]. The micro- and macro-pore designations were defined as less than or greater than 100 μm , respectively; a previously reported paradigm [Le Huec et al., 1995]. Furthermore, juxtaposition of the observed swelling kinetics with those reported in the literature indicates the micro- and macro-porous substrates are similar to “super absorbent” materials. These materials are known to possess high degrees of pore density and interconnectivity. However, further characterization, including bi- or tri-phasic dynamic mechanical analysis and porosimetry, are required to complete the characterization.

3.2. Large pores decrease viscoelasticity of pHEMA substrates

Constant strain (0.1%) was applied to micro- and macro-porous pHEMA substrates over frequencies from 0.628 to 75.40 rad/s, a physiologically relevant range (Figure 4). The

addition of NaCl during polymerization of the pHEMA substrates and increasing its concentration up to 0.9M had little to no effect on either the elastic (G') or viscous (G'') moduli of these micro-porous substrates. However, the G' and G'' values of the macro-porous substrates (alginate beads as porogen) were significantly reduced by nearly a full order of magnitude and more closely matched the viscoelastic behavior of human skin (Table 1). Similar to human skin, the viscoelastic response of all the pHEMA substrates increased gradually over the frequencies tested, with the greatest increase occurring in the high frequency range ($\omega > 10$ rad/s). Moreover, the G' and G'' curves did not intersect indicating that the viscoelastic behavior of all pHEMA substrates is primarily elastic in nature as is the case for human skin.

3.3 Surface functionalization improves cellular attachment on pHEMA substrates

To enhance cell attachment, pHEMA substrates were modified with CDI and gelatin attached (Figure 5). Normal human fibroblasts (NHF) were seeded on the substrates, incubated for 72hrs and cell growth quantified using WST-1. When compared to controls, substrates with covalently linked gelatin supported increased cell growth, indicating well adhered/attached cells on the substrate surface.

3.4. Cells alter the viscoelasticity of pHEMA substrates

To determine if cell growth influenced viscoelasticity, rheological measurements were made on micro- and macro-porous substrates on which cells had been growing for 14 days (Figure 6). In various controls where cell attachment and growth were not optimized, the pHEMA substrates had nearly identical viscoelastic behavior. For pHEMA substrates optimized for cell growth (gelatin attached via CDI), the viscoelastic behavior changed in response to cell growth. In the low frequency regime ($0.628 \leq \omega \leq 10$ rad/s), these substrates with cells showed a significant increase in the elastic and viscous components of the rheological response. In the high frequency regime ($10 < \omega \leq 72.4$ rad/s), the micro-porous substrates with cells showed a decrease in elastic response, indicating a decrease in frequency dependence. In contrast, the high frequency regime for the macro-porous substrates with cells showed no changes from the controls. These observations indicate that the presence and activity of cells on the substrates affected the rheological response, particularly at higher frequencies.

In human skin at low frequencies (≤ 10 rad/s), the rate of increase for G' and G'' was gradual ($G' \sim G'' \sim \omega^{0.05}$). Micro- and macro-porous substrates with cells showed similar trends in G' and G'' ($G' \sim \omega^{0.23}$, $\omega^{0.42}$ and $G'' \sim \omega^{0.27}$, $\omega^{0.27}$, respectively). Despite an increase in magnitude of the elastic and viscous moduli, micro- and macro-porous substrates with cells at $\omega \leq 10$ rad/s, showed slower rates of increase of the elastic response while maintaining the viscous trend ($G' \sim \omega^{0.04}$, $\omega^{0.12}$ and $G'' \sim \omega^{0.24}$, $\omega^{0.25}$, respectively). At higher frequencies ($\omega > 10$ rad/s), the rate of increase in G' in control, micro- and macro-porous substrates with cells were very similar ($G' \sim \omega^{1.75}$, $\omega^{1.89}$, respectively), but trends in the modified micro- and macro-porous substrates with cells were dissimilar ($G' \sim \omega^{0.1}$, $\omega^{1.23}$, respectively). Micro-porous substrates with cells showed a significant decrease in elastic frequency dependence ($G' \sim \omega^{0.1}$), while macro-porous substrates with cells showed a rate of increase of G' similar to control macro-porous substrates without cells.

These data suggest that cellular activity reinforces the elastic components while reducing frequency dependence. Cell growth suppresses the global fluid flow within the micro-porous infrastructure, evidenced in the high frequency regime ($\omega > 10$ rad/s). The low frequency behaviors of substrates with cells were more similar to responses seen in human skin [Holt et al., 2008], other cells/soft tissues [Alcaraz et al., 2003; Nicolle et al., 2005], and F-actin gels and keratin/intermediate filament networks [Gardel et al., 2004; Ma et al., 1999, 2001];

Shin et al., 2004]. Cellular activity may block the micro-pore network increasing the magnitude of the elastic and viscous response of the substrate while also disrupting the interstitial fluid flow in the substrate which causes frequency dependence. The frequency dependence in macro-porous substrates may be caused by free fluid motion/evacuation from the macro-scale pores. The degree of viscous frequency dependency in protein modified, micro- and macro-porous substrates ($G'' \sim \omega^{0.5}$ and $G'' \sim \omega^{0.45}$, respectively) are similar to the untreated types ($G'' \sim \omega^{0.58}$ and $G'' \sim \omega^{0.61}$, respectively), indicating the effect of changes in fluid flow behavior has little impact on the viscous component of the substrates' viscoelastic response.

3.5. Soft-solid model for PHEMA with cells

The rheological data for the pHEMA substrates were fit to a mathematical model describing a soft-solid. In the high frequency regime, the response was non-linear, but over the low frequency range (≤ 10 rad/s) the fit was excellent (Figure 7). A parameter reduction analysis showed that the model could be reduced from three parameters to two, fixing the retardation time, τ and κ at 1.15 and 0.09, respectively. The model reduced to a one parameter fit for equilibrium relaxation modulus, G_e . For micro-porous substrates with and without cells, G_e values were 2,500 and 8000, respectively. Likewise, for macro-porous substrates with and without cells, the G_e values were 3,000 and 15,000, respectively. These parameter value fits represented a 3 and 5 fold increase in equilibrium relaxation modulus due to the effects of cells on the substrates. Model parameter sensitivity showed the elastic response was most responsive to changes in the equilibrium modulus, G_e , while the viscous response was most sensitive to the equilibrium modulus and scalar exponent, G_e and k , respectively (Figure 7). Although not a traditional comparative tool, parameter sensitivity further highlighted the complexity of depicting the viscous component of the viscoelastic response.

When considering the complete frequency range (0.6283 – 75.4 rad/s), the soft-solid model fails to grasp the high frequency regime response of the NHF-pHEMA co-culture composites (data not shown). However, when focusing on the low frequency regime (≤ 10 rad/s) containing physiologically relevant frequencies, the model replicates the elastic behavior of the substrates, with and without cells, well (Figure 6) but fails to reproduce the viscous response. This was supported by the mean percent difference of experimental data to model prediction for G' and G'' (Table 2) and lesser so by the correlation coefficient. The correlation coefficient, a trend comparison index between -1 (reciprocally correlated) and 1 (perfectly correlated), showed that the mean correlation coefficients for G' and G'' for the micro- and macro-porous substrates were 0.921 and 0.917, respectively (Table 2). Together, the mean percent difference and correlation coefficient highlights the capacity of the model to replicate the trends of the rheological behavior but fails to capture the complexity of the viscous response.

4. Conclusions

Understanding the mechanics of cell-substrate interactions is critical to the success of biomaterials for many applications, especially in soft tissue. The viscoelasticity of both micro- and macro-porous substrates were predominately elastic in nature and the viscoelasticity of macro-porous substrates were the most similar to skin. At low frequencies ($\omega \leq 10$ rad/s), micro- and macro-porous substrates with and without cells showed very similar magnitudes and trends in G' and G'' ($G' \sim G'' \sim \omega^{0.25}$ and $G' \sim G'' \sim \omega^{0.35}$, respectively). The trends were similar to those seen in skin ($G' \sim G'' \sim \omega^{0.5-1.0}$) despite differences in magnitude. At high frequencies ($10 < \omega \leq 72.4$ rad/s), micro- and macro-porous substrates with cells had very distinct and dissimilar behaviors. Micro-porous substrates with cells were largely frequency independent ($G' \sim \omega^{0.7}$). Macro-porous substrates with cells maintained their frequency dependence at $\omega > 10$ rad/s, mimicking the

rate of increase of their untreated counterparts ($G' \sim \omega^{1.23}$ and $G' \sim \omega^{1.89}$, respectively). Micro- and macro-porous substrates with cells showed similar viscous component responses ($G'' \sim \omega^{0.5}$ and $G'' \sim \omega^{0.45}$, respectively). These data suggest that cellular activity affects interstitial fluid flow and reinforces the elastic components of the substrates while reducing frequency dependence. Lastly, we adapted a soft-solid model and found it useful to model the viscoelastic behavior of these substrates.

The frequency dependent rheological behavior of cells cultured on soft substrates is an area of increasing interest. At the cellular level, several studies have used tensegrity and soft glassy rheology to describe the cytoskeletal remodeling-mediated viscoelastic rheological behavior [Fabry et al. 2001, Mandadapu et al. 2008, Stamenović 2008, Kollmannsberger et al. 2009] with differing conclusions. The soft-solid viscoelastic model of this paper is capable of reproducing and replicating dynamic shear behavior and trends in the frequency response of skin as well as porous synthetic substrates and may be useful for understanding cellular responses as well as the design of new soft tissue analogs. With regards to whole skin, this model may form the basis for a more advanced finite element model capable of describing the load conditions around percutaneous devices. Future models will need to account for the more pronounced biphasic and frequency dependent nature of the porous substrates with cells in order to achieve an improved understanding of the differences between these synthetic substrates and normal skin. Future models will need to include additional, frequency regime specific, time constants capable of accurately describing the physiological relevant frequency range.

The aim of this study was to fabricate synthetic porous substrates that approximate the *superstructure and* mechanical behavior of soft tissue such as skin and to determine the mechanical changes that occur when cells are grown on these substrates. Our rheological measurements show that pHEMA substrates are predominately elastic in nature with a narrow range of G' and G'' values that mimic the response of human skin. Substrates with micro-pores more closely matched human skin and cell growth on all substrates preferentially enhanced the elastic response. It is important to note that the effect of cell culture on the pore diameter within the substrates is critical to the understanding of the complex mechanical behavior observed in this study. More accurately, the mechanism by which biological activity on the mechanically matched substrates can alter the mechanical behavior of the substrate hinges on this interaction. It can be argued that the observed mechanical adaptation in response to cellular activity originated from the disruption of interstitial fluid flow within the given substrates. Although a plausible hypothesis, a detailed study on bi- or tri-phasic dynamic mechanical tests and imaging analyses are required to quantitatively validate the mechanism and the connection to cellular alteration of pore size. A quantitative understanding of the mechanics of synthetic materials prior to implantation, as well as how these mechanics change after implantation due to cell growth may aid in the design of new materials that retain their mechanical match with soft tissue after implantation. Such materials would be expected to dissipate stress discontinuities between materials and tissues, minimizing adverse cell responses and aid in soft tissue integration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The project described was supported by Grant Number F31AR054202 from the National Institute Of Arthritis And Musculoskeletal And Skin Diseases. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Arthritis and Musculoskeletal and Skin Diseases or the National Institutes of Health.

We would also like to thank Dr. Bruce Caswell, Professor Emeritus of Engineering from Brown University for suggesting the soft-solid viscoelastic model utilized during the course of this study.

References

- Alcaraz J, Buscemi L, Grabulosa M, Trepas X, Fabry B, Farre R, Navajas D. Microrheology of human lung epithelial cells measured by atomic force microscopy. *Biophysical Journal*. 2003; 84(3):2071–2079. [PubMed: 12609908]
- Bajpai SK, Shubhra S. Investigation of swelling/degradation behavior of alginate beads crosslinked with Ca^{2+} and Ba^{2+} ions. *Reactive & Functional Polymers*. 2004; 59:129–140.
- Bajpai AK, Kankane S. Evaluation of water sorption property and in vitro blood compatibility of poly(2-hydroxyethyl methacrylate) (PHEMA) based semi interpenetrating polymer networks (IPNs). *Journal of Material Science: Materials in Medicine*. 2008; 19(5):1921–1933.
- Bavaresco VP, Zavaglia CAC, Reis MC, Gomes JR. Swelling and Tribological Properties of Poly (HEMA) Hydrogels. *Ma*. 2004
- Cabodi M, Cross VL, Qu Z, Havenstrite KL, Schwartz S, Stroock AD. An Active Wound Dressing for Controlled Convective Mass Transfer With the Wound Bed. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*. 2007; 82B(1):210–222.
- Cacou C, Muir IF. Effects of plane mechanical forces in wound healing in humans. *Journal of the Royal College of Surgeons of Edinburgh*. 1995; 40(1):38–41. [PubMed: 7738896]
- Carone TW, Hasenwinkel JM. Mechanical and Morphological Characterization of Homogeneous and Bilayered Poly(2-hydroxyethyl methacrylate) Scaffolds for Use in CNS Nerve Regeneration. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*. 2006; 78B(2):274–282.
- Chehroudi B, Brunette DM. Subcutaneous microfabricated surfaces inhibit epithelial recession and promote long-term survival of percutaneous implants. *Biomaterials*. 2002; 23(1):229–237. [PubMed: 11762842]
- Clark RAF, Ghosh K, Tonnesen MG. Tissue Engineering for Cutaneous Wounds. *Journal of Investigative Dermatology*. 2007; 127(5):1018–1029. [PubMed: 17435787]
- Cowin SC. How Is a Tissue Built. *Journal of Biomechanical Engineering*. 2000; 122(6):553–569. [PubMed: 11192375]
- Danion F, Varraine E, Bonnard M, Pailhous J. Stride variability in human gait: the effect of stride frequency and stride length. *Gait Posture*. 2003; 18(1):69–77. [PubMed: 12855302]
- Discher DE, Janmey P, Wang Y. Tissue Cells Feel and Respond to the Stiffness of Their Substrate. *Science*. 2005; 6:214–221.
- Fabry B, Maksym GN, Butler JP, Glogauer M, Navajas D, Fredberg JJ. Scaling the Microrheology of Living Cells. *Physical Review Letters*. 2001; 87(14):148102. [PubMed: 11580676]
- Farley CT, Gonzalez O. Leg stiffness and stride frequency in human running. *Journal of Biomechanics*. 1996; 29(2):181–186. [PubMed: 8849811]
- Fukano Y, Knowles NG, Usui ML, Underwood RA, Hauch KD, Marshall AJ, Ratner BD, Giachelli C, Carter WG, Fleckman P, Olerud JE. Characterization of an in vitro model for evaluating the interface between skin and percutaneous biomaterials. *Wound Repair and Regeneration*. 2006; 14(4):484–491. [PubMed: 16939578]
- Gardel ML, Shin JH, MacKintosh FC, Mahadevan L, Matsudaira PA, Weitz DA. Scaling of F-actin network rheology to probe single filament elasticity and dynamics. *Physical Review Letters*. 2004; 93(18)
- Gheduzzi S, Webb JJC, Miles AW. Mechanical characterisation of three percutaneous vertebroplasty biomaterials. *Journal of Material Science: Materials in Medicine*. 2006; 17(5):421–426.
- Grosse-Siestrup C, Affeld K. Design criteria for percutaneous devices. *Journal of Biomedical Materials Research*. 1984; 18:357–382. [PubMed: 6736073]
- Gutmann AK, Jacobi B, Butcher MT, Bertram JE. Constrained optimization in human running. *Journal of Experimental Biology*. 2006; 209(Pt 4):622–632. [PubMed: 16449557]
- Hadgraft J. Skin deep. *European Journal of Pharmacology and Biopharmacology*. 2004; 58(2):291–299.

- Hoffman BD, Crocker JC. Cell Mechanics: Dissecting the Physical Responses of Cells to Force. *Annual Review of Biomedical Engineering*. 2009; 11(1):259–288.
- Holt B, Tripathi A, Morgan JR. Viscoelastic Response of Human Skin to Low Magnitude Physiologically Relevant Shear. *Journal of Biomechanics*. 2008; 41(12):2689–2695. [PubMed: 18672246]
- Jacobsson, M.; Tjellstrom, A. *Clinical Applications of Percutaneous Implants*. Technomic Publishing Co; Lancaster: 1991.
- Janmey PA, Euteneuer U, Traub P, Schliwa M. Viscoelastic Properties of Vimentin Compared with Other Filamentous Biopolymer Networks. *Journal of Cell Biology*. 1991; 113(1):155–160. [PubMed: 2007620]
- Knabe C, Grosse-Siestrup C, Gross U. Histologic evaluation of a natural permanent percutaneous structure and clinical percutaneous devices. *Biomaterials*. 1999; 20(6):503–510. [PubMed: 10213352]
- Kohane DS, Langer R. Polymeric Biomaterials in Tissue Engineering. *Pediatric Research*. 2008; 63(5): 487–491. [PubMed: 18427292]
- Kokshenev VB. Dynamics of human walking at steady speeds. *Physical Review Letters*. 2004; 93(20): 208101. [PubMed: 15600973]
- Kollmannsberger P, Fabry B. Active soft glassy rheology of adherent cells. *Soft Matter*. 2009; 5(9): 1771–1774.
- Larson, RG. *The Structure and Rheology of Complex Fluids*. Oxford University Press; New York: 1999.
- Le Huec JC, Schaefferbeke T, Clement D, Faber J, Le Rebeller A. Influence of porosity on the mechanical resistance of hydroxyapatite ceramics under compressive stress. *Biomaterials*. 1995; 16(2):113–118. [PubMed: 7734643]
- Lévesque SG, Lim RM, Shoichet MS. Macroporous interconnected dextran scaffolds of controlled porosity for tissue-engineering applications. *Biomaterials*. 2005; 26:7436–7446. [PubMed: 16023718]
- Liu Q, Hedberg EL, Liu Z, Bahulekar R, Meszlenyi RK, Mikos AG. Preparation of macroporous poly(2-hydroxyethyl methacrylate) hydrogels by enhanced phase separation. *Biomaterials*. 2000; 21(21):2163–2169. [PubMed: 10985489]
- Lou X, Vijayasekaran S, Chirila TV, Maley MAL, Hicks CR, Constable IJ. Synthesis, physical characterization, and biological performance of sequential homointerpenetrating polymer network sponges based on poly(2-hydroxyethyl methacrylate). *Journal of Biomedical Material Research*. 1999; 47(3):404–411.
- Ma L, Xu J, Coulombe PA, Wirtz D. Keratin filament suspensions show unique micromechanical properties. *Journal of Biological Chemistry*. 1999; 274(27):19145–19151. [PubMed: 10383419]
- Ma L, Yamada S, Wirtz D, Coulombe PA. A ‘hot-spot’ mutation alters the mechanical properties of keratin filament networks. *Nature Cell Biology*. 2001; 3(5):503–506.
- Mabilleau G, Stancu IC, Honoré T, Legeay G, Cincu C, Baslé MF, Chappard D. Effects of the length of crosslink chain on poly(2-hydroxyethyl methacrylate) (pHEMA) swelling and biomechanical properties. *Journal of Biomedical Materials Research Part A*. 2006; 77A:35–42. [PubMed: 16345096]
- Malkin, AY.; Isayev, AI. *Rheology: Concepts, Methods, and Applications*. Vol. 1. ChemTec Publishing; 2005.
- Mandadapu KK, Govindjee S, Mofrad MRK. On the cytoskeleton and soft glassy rheology. *Journal of Biomechanics*. 2008; 41:1467–1478. [PubMed: 18402964]
- McClarence, E. *Close to the Edge: Branemark and the development of Osseointegration*. Quintessence Publishing Co; Chicago, Illinois: 1993.
- Narayan D, Venkatraman SS. Effect of pore size and interpose distance on endothelial cell growth on polymers. *Journal of Biomedical Materials Research Part A*. 2008; 87A:710–718. [PubMed: 18200559]
- Nicollé S, Lounis M, Willinger R, Palière JF. Shear linear behavior of brain tissue over a large frequency range. *Biorheology*. 2005; 42(3):209–223. [PubMed: 15894820]

- Palacci, P.; Ericsson, I.; Engstrand, P.; Rangert, B. Optimal Implant Positioning & Soft Tissue Management for the Branemark System. Quintessence Publishing Co; Chicago, Illinois: 1995.
- Pendegrass CJ, Goodship AE, Blunn GW. Development of a soft tissue seal around bone-anchored transcutaneous amputation prostheses. *Biomaterials*. 2006; 27:4183–4191. [PubMed: 16618500]
- Pipkin, AC. Applied Mathematical Sciences: Lectures on Viscoelasticity Theory. Springer-Verlag; New York: 1972.
- Presland RB, Jurevic RJ. Making sense of the epithelial barrier: what molecular biology and genetics tell us about the functions of oral mucosal and epidermal tissues. *Journal of Dental Education*. 2002; 66(4):564–574. [PubMed: 12014572]
- Schiraldi C, D'Agostino A, Oliva A, Flamma F, De Rosa A, Apicella A, Aversa R, De Rosa M. Development of hybrid materials based on hydroxyethylmethacrylate as supports for improving cell adhesion and proliferation. *Biomaterials*. 2004; 25(17):3645–3653. [PubMed: 15020139]
- Shin JH, Gardel ML, Mahadevan L, Matsudaira P, Weitz DA. Relating microstructure to rheology of a bundled and cross-linked F-actin network in vitro. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101(26):9636–9641. [PubMed: 15210969]
- Stamenović D. Rheological behavior of mammalian cells. *Cellular and Molecular Life Sciences*. 2008; 65(22):3592–3605. [PubMed: 18668200]
- Stevens MM, George JH. Exploring and Engineering the Cell Surface Interface. *Science*. 2005; 310(5751):1135–1138. [PubMed: 16293749]
- Tharmann R, Claessens MMAE, Bausch AR. Viscoelasticity of isotropically cross-linked actin networks. *Phys Rev Lett*. 2007; 98(8)
- Tibbitt MW, Anseth KS. Hydrogels as Extracellular Matrix Mimics for 3D Cell Culture. *Biotechnology and Bioengineering*. 2009; 103(4):655–663. [PubMed: 19472329]
- Tregear, RT. Physical functions of skin. Academic Press; London, New York: 1966.
- Tsubouchi K, Enosawa S, Harada K, Okamoto J, Fujie MG, Chiba T. Evaluation of the Relationship Between the Viscoelastic Stress and Strain of Fetal Rat Skin as a Guide for Designing the Structure and Dynamic Performance of a Manipulator for Fetal Surgery. *Surgery Today*. 2006; 36:701–706. [PubMed: 16865513]
- Vidaurre A, Castilla Cortázar I, Gómez Ribelles JL. Polymeric scaffolds with a double pore structure. *Journal of Non-Crystalline Solids*. 2007; 353(11–12):1095–1100.
- von Recum AF. Applications and failure modes of percutaneous devices: a review. *Journal of Biomedical Materials Research*. 1984; 18(4):323–336. [PubMed: 6234317]
- von Recum AF, Park JB. Permanent percutaneous devices. *Critical Review in Bioengineering*. 1981; 5(1):37–77.
- Wang YN, Sanders JE. How does skin adapt to repetitive mechanical stress to become load tolerant? *Medical Hypotheses*. 2003; 61(1):29–35. [PubMed: 12781637]
- Williams DF. On the nature of biomaterials. *Biomaterials*. 2009; 30(30):5897–5909. [PubMed: 19651435]

Fig 1.

Preparation and rheological measurements of porous pHEMA substrates. – (A) Micro- and macro- porous pHEMA substrates were polymerized by UV-cross linking. Micro pores and macro pores were created using NaCl and NaCl plus alginate beads, respectively. For cell growth, substrates were modified with CDI enabling gelatin attachment. (B) Micro- and macro-porous substrates, on which were cultured normal human fibroblasts, were subjected to an isothermal (37°C) oscillatory strain of 0.1% between 0.6283 and 75.398 rad/s, a physiologically relevant frequency range. The resulting G' and G'' values at various frequencies were measured.

Fig. 2.

Kinetics of swelling and water uptake of pHEMA substrates. Micro-porous (■) and macro-porous (●) substrates were dried, weighed and then added to de-ionized water equilibrated at 37°C over 180mins. At specific time intervals, substrates were removed and weighed. The resulting weights were used to calculate the swelling factor (closed symbols) and equilibrium water content (open symbols)(A). Linear curve fitting to a reduced ln-ln swelling factor v. time plot revealed information about the swelling kinetics of the micro- and macro-porous substrates (B).

Fig. 3. Scanning electron microscope [SEM] analysis of micro- and macro-porous poly(2-hydroxyethyl methacrylate) [pHEMA] substrates. Micro- and macro-porous substrates were prepared for SEM analysis and imaged to measure pore size and describe the porosity gradient within the substrates. Large pores ($\leq 500\mu\text{m}$) [Arrows] on the basal surface (imaged as the apical surface) of the macro-porous substrates were integrated into an interconnected small pore ($\sim 20 - 50\mu\text{m}$) network via an alginate leaching technique (A). Small pores were created using a phase separation technique that induced a high degree of interconnectivity (B). Micro-porous substrates were analyzed but images omitted due to redundancy.

Fig 4.

Rheological characterization of substrates mimicking the mechanical behavior of human skin. pHEMA substrates were subjected to an isothermal (37°C) oscillatory strain of 0.1% and the resulting G' (A) and G'' (B) values were measured. Micro-porous substrates had various concentrations of NaCl: No NaCl (■, □); 0.6M (●, ○); 0.7M (▲, △); 0.8M (▼, ▽); and 0.9M (◀, ▶). Macro porous substrates had 0.8M NaCl plus 1.5% (w/v) dried alginate beads (►, ►). Human skin is also shown for reference (◆, ◇).

Fig. 5.

Functionalization and cell growth on pHEMA substrates. pHEMA substrates were functionalized via 1,1' carbonyldiimidazole-mediated hydroxyl group activation and gelatin attached (A). Hydroxyl group activation and subsequent gelatin attachment was confirmed by FTIR spectrometry (B). Human fibroblasts were seeded on various control and modified pHEMA substrates and cell proliferation was measured using the WST-1 assay 72 hours after seeding cells (C). Cell growth was significantly increased when pHEMA were treated with both CDI and gelatin.

Fig. 6.

Viscoelastic response of micro- and macro- porous substrates with and without NHFs. Cells were grown on micro- (**A**, **B**) and macro-porous (**C**, **D**) substrates for 14days, after which substrates were subjected to an isothermal (37°C) oscillatory strain of 0.1% between 0.628 and 75.40 rad/s and G' (**A**, **C**) and G'' (**B**, **D**) were measured. Untreated substrates, with (●, ○) and without (■, □) cells and substrates treated CDI and gelatin with cells (▲, △) were measured and compared to human skin (◆, ◇). Note, cellular activity on modified substrates increased the magnitude of G' and G'' at low frequencies while also altering the degree of high frequency dependence, indicating that cellular interactions with the micro-pore infrastructure has a profound effect on the viscoelastic behavior of the substrate.

Fig. 7.

Adapted soft-solid model – A soft-solid viscoelastic model was applied to the rheological data on control, micro-porous and macro-porous substrates without cells (■, □) and modified micro-porous and macro-porous substrates on which normal human fibroblasts were cultured (▼, ▽ and ▲, △, respectively). [*open symbols denote G'' values*] The soft-solid model simulates viscoelastic solids with an internal structure that liquefies at higher frequencies. The resulting G' and G'' values predicted by the model (dashed lines) were compared qualitatively to experimental data.

Table 1

Quantitative comparison of untreated and gelatin modified, micro- and macro-porous substrates and human skin from isothermal (37°C), constant oscillatory strain (0.1%) experiments between 0.628 and 75.40 rad/s. Power law curves ($G' \sim G'' \sim \omega^x$) were fit to G' and G'' values within the low (≤ 10 rad/s) and high (> 10 rad/s) frequency regimes for comparison of the rate of increase of frequency dependent viscoelastic behavior. All pHEMA substrates are with normal human fibroblast cells.

	Micro-pore [untreated]		Micro-pore [protein modified]		Macro-pore [untreated]		Macro-pore [protein modified]		Human Skin	
	G'	G''	G'	G''	G'	G''	G'	G''	G'	G''
<i>Mean Values [10^3]</i>										
<i>Low Frequency (≤ 10 rad/s)</i>	2.88 – 5.90	0.171 – 0.356	8.47 – 9.40	0.558 – 1.10	1.73 – 6.07	0.129 – 0.281	15.70 – 22.80	1.69 – 3.27	0.361 – 0.435	0.092 – 0.098
<i>High Frequency (> 10 rad/s)</i>	7.57 – 18.20	0.419 – 1.270	9.47 – 11.40	1.20 – 3.06	8.57 – 271.0	0.274 – 0.823	25.50 – 286.0	3.54 – 8.23	0.448 – 1.27	0.097 – 0.190
<i>Power Law</i>										
<i>Low Frequency (≤ 10 rad/s)</i>	0.23	0.27	0.04	0.24	0.42	0.27	0.12	0.25	0.69	0.33
<i>High Frequency (> 10 rad/s)</i>	1.75	0.58	0.10	0.50	1.89	0.61	1.23	0.45	0.54	0.04

Qualitative comparison of experimental data from isothermal (37°C), constant oscillatory strain (0.1%) experiments between 0.628 and 10 rad/s on untreated, micro- and macro-porous substrates without cells and protein modified, micro- and macro-porous substrates with normal human fibroblasts to an adapted soft-solid model. Experimental data and model predictions were compared using percent difference (accuracy) and correlation coefficient (trend-matching). All substrates are with NHF cells.

Table 2

	Parameters	Micro-pore [untreated]	Micro-pore [protein modified]	Macro-pore [untreated]	Macro-pore [protein modified]
<i>Model Parameters</i>					
	Equilibrium Relaxation, G_e	2500	8000	3000	15000
	Retardation Time, τ	1.15	1.15	1.15	1.15
	Kappa, κ	0.09	0.09	0.09	0.09
<i>Average Percent Difference [96] (Experimental Data v. Model) [Model Accuracy]</i>	Elastic [Storage] Modulus, G'	16.5	3.94	14.2	3.94
	Viscous[Loss] Modulus, G''	61.3	44.3	26.6	10.1
<i>Average Percent Difference [96] (Experimental Data v. Model) [Trend Matching]</i>	Elastic [Storage] Modulus, G'	0.869	0.996	0.875	0.945
	Viscous[Loss] Modulus, G''	0.916	0.898	0.915	0.937