The Role of Mechanical Loading in Bone Remodeling:
A Literature Review

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by

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Abstract

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This thesis investigates the factors influencing bone remodeling within the human skeleton with a focus on developing methods for constructing prosthetic bone scaffolds containing cells to progenerate into living bone upon implantation in the body. These porous scaffolds would ideally regulate events such as cellular proliferation and intracellular signaling after surgical implantation. The overarching goal is to identify materials, geometries, and other properties of the scaffold design in order to generate replacement tissue that replicates the original bone structure and geometry.

Bone remodeling is the process of simultaneous removal of old bone and replacement with new bone powered by the coupled actions of osteoclasts and osteoblasts, cells that resorb bone and produce bone, respectively. While bone remodeling occurs more intensely during skeletal development, it continues throughout a human's lifetime, repairing microscopic damage resulting from stress and fatigue on the body. There are many different models that describe how remodeling may occur as well as what initiates the remodeling response to damaged bone.

Historically, biologists explored tissue development primarily in terms of chemical and electrical signal pathways controlled by genes. However, recently published studies have implied that bone cells may be able to sense and react to mechanical forces. These forces likely have a crucial role in stimulating remodeling to occur based on the existing three-dimensional geometry of the bone and how well suited it is to handle the forces. Many independently published studies have investigated singular mechanical factors in the stimulation of bone remodeling as well as the resulting implications for the design of implanted skeletal scaffolds. However there remains a lack of publications that analyze multiple studies in order to examine their similarities and discrepancies. Reviews linking multiple studies are valuable tools for moving bone engineering theories into practical realities.

Based on the hypothesis that stresses that develop among bone cells are control mechanisms in regulating bone remodeling, a literature search was conducted. Two critical factors were examined in detail: the effects of pore geometry and the stiffness of the substrate on the rate and concentration of cell proliferation. Experimental results of conducted studies as well as theoretical results using finite element analysis and analytical equations were compared and contrasted. Finally, conclusions were synthesized from each study into general observations that are important to the future creation of bone scaffolds.
The Role of Mechanical Loading in Bone Remodeling: A Literature Review

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Introduction

The aim of this thesis is to investigate the factors influencing bone remodeling within the human skeleton with a focus on developing methods for constructing prosthetic bone scaffolds containing cells to regenerate into living bone upon implantation in the body. Much like a hip or knee replacement, porous scaffolds would replace bone removed after an injury or cancer ablation and act as a physical support structure (1). These scaffolds would be cultured with bone cells and ideally would regulate events such as cellular proliferation and intracellular signaling after surgical implantation. The porous structures would have channels that allow for the migration and proliferation of cells throughout the scaffold. The overarching goal is to identify materials, geometries, and other properties of the scaffold design that would dictate where and at what concentration bone cell growth and division would occur in order to ultimately generate replacement tissue that replicates the original bone structure and geometry. In order to do this, the subsequent bone modeling and remodeling that would occur as cells proliferate and form bone must be quantitatively predictive. Bone engineering is an important and relatively new field of biomedical engineering. Prosthetic bone implantation could improve the lives of those affected by skeletal injuries, bone cancers, and degenerative diseases such as spinal muscular atrophy and osteoporosis.

This thesis is focused on how the concepts of bone remodeling might best be replicated to generate this prosthetic bone. Research was centered on the role of
mechanical loading in bone remodeling. For many years, biologists explored tissue development primarily in terms of chemical and electrical signal pathways controlled by genes (2). However, recently published studies have implied that bone cells may be able to sense and react to mechanical forces. These forces likely have a crucial role in stimulating remodeling to occur based on the existing three-dimensional geometry of the bone and how well suited it is to handle the forces. In essence, bones are exhibiting survival techniques through a feedback mechanism – forces are generating microdamage but are also instigating bone remodeling to repair the microdamage (3). Many independently published studies have investigated singular mechanical factors in the stimulation of bone remodeling as well as the resulting implications for the design of implanted skeletal scaffolds. However there remains a lack of publications that analyze multiple studies in order to examine their similarities and discrepancies. Reviews linking multiple studies are valuable tools for moving bone engineering theories into practical realities.

The hypothesis that bone loading and stresses are control mechanisms in regulating remodeling was a driving force in the selection of articles reviewed in this paper. Studies suggest that bone remodeling is controlled by a multitude of factors including the inhibition or expression of various proteins and the location of adhesion sites attached to the cytoskeleton (4, 5). For this purpose, two critical factors are examined in detail: the effects of pore geometry and the stiffness of the substrate on the rate and concentration of cell proliferation (6-9). After a preliminary review of approximately twenty articles, four primary articles were chosen for further focus in this
paper. These articles served as the base for conclusions synthesized from information gathered from all of the sources.

Future analysis of the conclusions generated through these studies would focus on how bone remodeling could be quantitatively predictive and how it might best be replicated to generate prosthetic bone that would be accepted by the skeletal system and progenerate into living bone. Ideally scaffolds would have a specifically designed microstructure that would create certain levels and types of mechanical loading, including shear, tensile or compressive loading. This predictive mechanical loading would stimulate remodeling and optimize the growth of the new bone cells.

**Organization of the Report**

This report is organized topically, as shown in the schematic in Fig. 1. It begins with a general background on bone and bone cells and their relevance to the project. Then the two primary factors under investigation are expanded. The first section on the role of pore geometry begins with a summary of two independent published experiments, and the section concludes with the implications of the experimental results. The second section on the role of substrate stiffness also begins with a summary of two experiments and finishes with conclusions drawn from the results. Both sections include experimental results and as well as theoretical comparisons using finite element analysis and analytical equations. The final section of the paper synthesizes the conclusions from each study into general observations that are important to the future creation of bone scaffolds. This section also discusses future work to be conducted if this project is carried forward.
Figure 1. Schematic of research process
(Source: http://www.nlm.nih.gov/medlineplus/ency/imagepages/17156.htm)

**Thesis:** Stresses between bone cells are control mechanisms in regulating bone remodeling

**Sources of Information**

- Literature Search
- Finite Element Analysis
- Analytical Solutions (Equations)

**Factors Influencing Remodeling:**

1) *Pore Geometry*
2) *Substrate Stiffness*
3) Inhibition & Expression of Proteins
4) Location of Adhesion Sites

**Conclusions:**

- Quantify critical factors in order to create stresses to regulate remodeling activity
- Stimulate remodeling activity in porous scaffolds to generate replacement tissue with the same geometry and structure as the original bone
Background: Bone & Bone Cells

Human bones are composed of three primary substances: water, minerals, and a collagen matrix (10). Collagen is a structural protein that gives a bone its tensile strength as well as flexibility, while minerals give the bone rigidity and compressive strength. A particular bone's strength is dependent on the relative amounts of these three substances. The two primary types of bones are trabecular (or cancellous) bone and compact (or cortical) bone. Trabecular bone has a porosity of approximately 75-95% and is found in vertebrae, flat bones, and at the ends of long bones. Nonmineralized porous spaces within the bone matrix contain bone marrow, which is a tissue primarily made up of blood vessels and nerves. The pores throughout trabecular bone are interconnected and filled with marrow. Compact bone is denser (with a porosity of approximately 5-10%) and is found in the shafts of long bones.

There are two primary categories of bone cells: those that resorb bone and those that form bone. Resorption bone cells are closely related to macrophages, cells that migrate throughout other body tissues to remove debris and foreign materials. Formation bone cells are closely related to fibroblasts, cells that produce structural molecules in other body tissues. Between these categories, there are four types of bone cells: osteoclasts, osteoblasts, osteocytes, and bone lining cells.

Osteoclasts are multinuclear cells formed in bone marrow. These cells resorb bone by first demineralizing it with acids and then dissolving the remaining collagen with enzymes manufactured by the cell. Osteoclasts are able to erode through bone at a rate of
tens of micrometers per day. Osteoblasts are mononuclear cells that produce osteoid, the organic portion of the bone matrix containing collagen, non-collagenous proteins, and water. As the osteoid calcifies, water is replaced by minerals and eventually the osteoid is accepted into the bone, typically replacing the bone resorbed by the osteoclasts. Osteocytes are osteoblasts that essentially embed themselves in a bone by producing osteoid into their immediate surroundings which calcifies into bone before the osteoblast can migrate to another location. Osteocytes are surrounded by cavities called lacunae which are connected through tunnels called canaliculi. It is thought that the canaliculi allow osteocytes to communicate with each other and sense mechanical stress between neighboring cells, a concept pivotal to the theory that mechanical stress is a control mechanism in bone remodeling. Finally, bone lining cells are osteoblasts that remain on the surface once bone formation or reformation is completed. Bone lining cells also communicate with osteocytes and are thought to initiate bone remodeling as well as contribute to the osteocytes' ability to sense mechanical stress.

As animals develop and mature, their bones must also not only grow, but be shaped appropriately. Therefore bone must be removed in some places and added in others through osteoclastic and osteoblastic activity. Growth occurs through bone modeling and remodeling. Bone modeling involves the independent actions of osteoblasts and osteoclasts, and through modeling, a bone's size and shape is altered. Once a skeleton reaches maturity, the rate of bone modeling is greatly diminished. On the other hand, bone remodeling involves the coupled actions of osteoblasts and osteoclasts in sequence. Remodeling is episodic rather than continuous, and though reduced after skeletal
maturity, continues through an individual's lifetime. The purpose of remodeling is to repair microscopic damage resulting from stress and fatigue on the body. There are currently a number of models that describe how remodeling may occur as well as what initiates the remodeling response to damaged bone.
Pore Geometry

Patterns of individual cell behavior guide architectural changes in tissue, through several of the mechanisms such as changes in local cell adhesion, cell shape, and the rate of cell proliferation (6). It is apparent as a result of many studies that specific geometric patterns in cellular growth drive tissue formation, but what initiates these patterns? Experimentally, it appears that cells regulate patterns of growth in order to favor and sustain certain geometrical structures while eliminating others. The goal then, in regards to the development of bone scaffolds, is to intentionally organize a population of cells in a way that initiates the desired patterns of cell proliferation. This will allow tissue engineers to dictate where cell proliferation should occur and at what concentration.

The work of Nelson et al. (6) hypothesizes that tissue form dictates its function – that is, the shape of the tissue regulates its own patterns of cell proliferation. The authors believe that cell growth patterns are influenced by the variations in internal mechanical stresses throughout the architectural framework of the tissue. To test their hypothesis, Nelson et al. (6) constrained the form of endothelial cell proliferation by culturing the cells on ECM islands of various shapes. In another study, Rumpler et al. (7) looked beyond the individual cell to investigate the effect of macroscopic tissue geometry on cell proliferation. They hypothesized that cells can probe their environment and sense forces over long distances relative to their size. The results of the experimentation by Rumpler et al. (7) showed that the growth behavior of osteoblast-like cells was influenced by the geometry of channels within their artificial three-dimensional bone matrix. Both articles'
results suggest that a quantitative understanding of macroscopic curvature-driven growth could dictate the design of pores in scaffolds for tissue engineering.

*Nelson et al.* (6) cultured bovine pulmonary artery endothelial cells on substrates created on cover slips using elastomeric stamps. The stamps contained a relief that dictated islands of cell growth in desired micropatterns and any unstamped regions on the cover slips were accordingly rendered nonadhesive in order to restrict cell growth to specified regions. The cells were spread on the substrata and once a monolayer of cells formed, the cells were exposed to a growth media for 48 hours. Over a period of several days, the cell proliferation rate decreased until it was nearly undetectable in the centers of the islands. However, around the outer edges of the islands, cell proliferation continued for several weeks. Final samples were stained and visualized using an Orca charge-coupled device camera attached to an inverted Nikon TE200 microscope. Images of 50 samples were directly stacked to show the general distribution of the rate of proliferation.

The stacked images revealed that the cells on the edges of islands proliferated more than cells in the centers. Additionally, in noncircular islands, cells at the corners of the islands proliferated more than the edges or the centers. Increasing the area of these islands increased the magnitude of proliferation at both the edges and the corners, and *Nelson et al.* (6) concluded that the proliferation scales with the size of the island. On rectangular islands, cells on the short edges were found to proliferate more than cells on long edges. An example of fluorescently imaged cell proliferation on a rectangular island can be seen in Fig. 2.
The experimental findings suggest that the pattern and concentration of proliferation depends on the geometry of the substrate. Higher rates of proliferation at the edges of the cell layer might have been stimulated by a mechanism such as contractile tension that could have propagated cell proliferation from the bulk tissue outward towards the edge. Alternately, increased cell proliferation could be induced directly at the edges, as a result of decreased cell-cell adhesion around the exterior of the cell layer. However, results of the experiment revealed that significantly lower proliferation occurred at the long edges of rectangular islands than the edges of square islands with the same length. This suggests that proliferation is based on the overall geometry of the island, supporting the hypothesis that cell proliferation is propagating outwards from the bulk tissue rather than being stimulated directly at edges of islands. In order to explore this idea further, Nelson et al. (6) next developed an experiment to determine whether the
hypothesized contractile tension in the cell layer was generating a predictable pattern of mechanical stress.

A finite element computational model was developed to simulate the previously conducted experiment. A sheet of cells was modeled as contracting against substrata by connecting the contractile cell sheet onto a passive base with a fixed bottom surface. Contraction of the cells was simulated by decreasing the length of the contractile layer and then computing the resulting maximum principal stresses produced.

For both circular and noncircular cell layers, the simulated cell sheet produced patterns that corresponding with the observed patterns of proliferation. For the square and rectangular layers, increased stress was observed at the exterior edges and concentrated at the corners. A detailed image of the FEM results for the stress concentration in the square cell layer can be found in Fig. 3B. Figure 3D shows the FEM results of the stress concentration in the circular cell layer while Fig. 3E shows the imaged results of cell proliferation during the first experiment.

These results suggest that the patterns of the cells may generate mechanical stresses that drive the observed rates of proliferation. To test this suggestion, Nelson et al. (6) explored whether geometries with edges contained decreased predicted stress would accordingly have lower proliferation rates. A useful geometry to consider is the annulus with an eccentric hole, as shown in Figs. 3F, 3G, and 3H. FEM results reveal that this shape has the lowest predicted contractile stress at the concave inner edge of the hole and the highest predicted contractile stress at the convex outer edge furthest from the hole. These predictions correspond to the experimental proliferation rates shown in Fig. 3H.
It is apparent that differences in edge curvature, such as corners, result in increased cell proliferation. Even in the absence of changes in the local edge geometry, based on results from circular cell layers, proliferation appears to occur more intensely in
areas of high stresses. Nelson et al. (6) next sought to directly measure the forces occurring in the experimentally cultured cell layers to verify the FEM results.

In order to directly measure forces, islands of cells were cultured on an elastomeric force sensor array. This type of sensor contains a high density of vertical microneedles which act as deflecting cantilevers. The deflection distribution of the microneedles is then used to report the forces experienced by the cells across the island.

The sensor results confirmed that at the outer edges significantly more force was exerted on cells than at the inner edges of the islands. Other observations were consistent, including the idea that stresses were higher along the short edges of rectangles and highest at the corners, directly corresponding with experimental cell proliferation rates.

As is apparent from the work of Nelson et al. (6), the geometric arrangement of the local environment is a critical factor in dictating cell behaviors such as proliferation. Additional studies have shown that on a nanoscale, cells respond to the geometry of adhesion sites as well as the stiffness of the substrate, and these factors can determine cell shape (5, 8-9). The influence of the geometry of the cellular environment has been studied, but data is not readily available for tissue-level cellular behavior based on the collective surface geometry (7). This information is important because the effect of macroscopic pore geometry on tissue formation will directly lead to a better understanding of ideal scaffold design which would feature pores to promote cell formation, migration, and growth.
Rumpler et al. (7) designed an experiment to model tissue growth that included three-dimensional hydroxylapatite (HA) plates containing channels in four different shapes: triangular, square, hexagonal, and circular. Each channel shape was reproduced in three different channel sizes (with perimeters of 3.14, 4.71, and 6.28 mm). These plates were placed in a culture of murine pro-osteoblastic cells. Once the cells proliferated for 21 days, the resulting stress fibers were imaged using a confocal laser scanning microscope. Results showed that tissue grew uniformly in the circular channels and thicker at the corners of the other geometries. As the average curvature (i.e. the average angle of the corners) increased, tissue thickness at the corners accordingly increased and tissue thickness across the flat channel faces decreased. Regardless of the initial shape, the behavior of the tissue led to a central round opening through the channel, as can be seen in Fig. 4. For each shape in a channel of the same size, the total amount of tissue was approximately the same, so it appears that on average, total cell proliferation is independent of shape. Other experiments suggest additional mechanisms such as protein expression that may control total cell proliferation rates.
Fluorescence microscopic imaging revealed the orientation of the actin stress fibers between the cells of the tissue, as shown in Fig. 5. Cells that grew outside of the channel on the flat plate showed stress fibers in a completely random orientation. On the other hand, cells neighboring the inner edge of the tissue in the channel showed a strong stress fiber alignment along the tissue-interface. Rumpler et al. (7) feel that these results suggest that mechanical forces are developing in regions of high curvature – the corners, where the strongest stress fiber orientation is exhibited. In turn, these higher mechanical forces are stimulating the increased cell proliferation rates at these regions. Though their experimental procedures are different, these results directly parallel with the results of Nelson et al. (6). Cells are experiencing higher mechanical forces at regions with high radii of curvature, and these forces are controlling the distribution of cell proliferation.

Rumpler et al. (7) observed that their results strongly mimic the mechanisms of
surface tension. Surface tension is driven by the minimization of energy through the minimization of surface area. Through a mechanism termed curvature-driven growth, surface tension tends to reduce the curvature of an environment in order to decrease the surface area. The curvature-driven growth model is based on the assumption that local growth rate is proportional to the local curvature. Applying this model to this experiment, it would be expected that the rate of change of the average tissue thickness would be proportional to the average curvature. The equation for the average curvature $K$ for a closed convex curve, as given by Rumpler et al. (7), is

$$K = \frac{2\pi}{P}, \quad [1]$$

where $P$ is the perimeter. Based on this relationship, the average curvature and, through applying the proportionality, the average tissue thickness should be the same for a fixed perimeter, as observed experimentally and discussed earlier. Additionally, channels with smaller perimeters and thus larger average curvatures should have a higher average tissue thickness, again a result that is observed experimentally.

The physics behind curvature-driven growth has been explained for various occurrences such as membrane mechanics, crystal growth, and phase transformations. The driving force behind these phenomena is that surface tension results in curvature-driven growth that seeks to reduce the surface area as much as possible. Rumpler et al. (7) feel that this concept can be related to the behavior they observed during cell proliferation on the HA plates. The alignment of the stress fibers parallel to the tissue interface suggested that this was where mechanical forces were developing. They
theorize that cell-cell tensile interaction could be stimulating a surface tension-like phenomenon which causes increased tissue growth at areas of large curvature. This idea corresponds to the findings of Nelson et al. (6) that cell proliferation is concentrated in areas of large mechanical force. This curvature-driven growth leads to increasing cell organization through a feedback mechanism (similar to the feedback theories discussed in the next section) in which the curvature transmits a message to the cells which proliferate and cause a change in the curvature, which in turn, feeds back again to the cells (7). Eventually, this feedback mechanism would cause a channel to be completely filled with tissue – an important idea in the design of porous scaffolds for bone implantation. Additionally, it may even explain how osteoblasts naturally in bone work to fill in the gaps left by the osteoclastic cell resorption.

Based on the work of Nelson et al. (6) and Rumpler et al. (7), it is apparent that pore geometry is a critical factor driving the design of porous bone scaffolds. When cell proliferation occurs within a geometrically-defined environment, stresses develop between neighboring cells through mechanical forces. These stresses are based on the local curvature, with higher mechanical forces resulting from increases in curvature, which was modeled through FEA and demonstrated experimentally. Regions of high mechanical forces correlate with regions of increased cell proliferation. Cell proliferation begins at the corners of channels and only occurs on the faces of the channels when cell growth out of the corners creates a radius of curvature along the once-flat faces.

A commentary article by Ingber (2) on the work of Nelson et al. (6) raises further theories on the reasons for the observed tissue behavior. He theorizes that cells are
seeking to minimize the local strain and stress they are subjected to (2). Through the tensile cell-cell interaction, they sense the radius of curvature on a macroscale based on mechanical forces and begin proliferating at regions of high stress. This pattern of cell proliferation leads to a decrease in the local curvature. Cell proliferation continues, only reaching the faces of the channels when they are integrated into the local curvature through cell growth out of the corners, and eventually results in a circular hole. A circle has the lowest average curvature and therefore can achieve the lowest levels of stress and strain. The idea that the tissue wishes to minimize stress and strain can also be seen in the influences of substrate stiffness on cell proliferation, which will now be explored in the following section.
Stiffness of the Substrate

In the article by Rumpler et al. (7) previously referenced in this paper, it is mentioned that the stiffness of the culture substrate has been experimentally shown to control the differentiation of cells. For example, neuronal cells are formed on softer substrates, muscle cells on stiff substrates, and osteonal cells on rigid substrates. As substrate stiffness increases, more stable focal adhesion sites are established leading to a more organized cell cytoskeleton. The stability and location of cell adhesions sites has also been explored as a control mechanism in bone remodeling (5).

This mechanical response to substrata is crucial to the arguments for the roles of mechanical loading in bone remodeling. Substrate stiffness is relevant to the design of scaffolds through material selection and the corresponding stiffness. The following reviewed articles by Discher et al. (8) and Bischofs and Schwarz (9) provide support for the importance of substrate stiffness. In particular, Discher et al. (8) discuss the idea of a feedback loop between cells and their extracellular microenvironment through transmitted forces. Bischofs and Schwarz (9) seek to numerically model how stress and strain propagate through substrata in situations of interest and use these models to predict how cell mechanosensing of this stress and strain drives cell growth and orientation.

Tissue cells need to adhere to a solid in order to remain viable (8). These adherent cells plus the substrata they adhere to creates a relatively elastic microenvironment that
allows pushing and pulling between neighboring cells and the external substrate. Through their anchors, tissue cells are able to probe their environment and pull on the surrounding substrata. Discher et al. (8) review studies that show that tissue cells not only apply force to substrates but also respond based on the sensed resistance.

Previously, research has been directed at the response of individual cells to external forces such as fluid flow and direct stretching or twisting. Now scientists are beginning to focus on cell-exerted forces which involve a feedback loop between the cell and the elasticity of its microenvironment. The contractile forces in these cells are generated by the interactions of actin and myosin filaments which are cross-bridged and allow the cell to respond to the resistance of the substrate. Based on this feedback, the cell adjusts its adhesions, cytoskeleton, and overall state. Various experiments have shown the presence of these feedback loops in epithelial cells, fibroblasts, muscle cells, neurons, as well as other tissue cells. The question Discher et al. (8) asked was whether there is an observable contrast between the behaviors of cells cultured on much more compliant tissues, gels, and cell sublayers versus those cultured on plastic, glass coverslips, and more rigid materials.

The resistance of a solid to stress is measured by its elastic modulus $E$, which is obtained through the application of force and measurement of the resulting relative change in length (i.e. the strain). Experimentally, most tissues and biomaterials exhibit a relatively linear stress-strain relationship up to approximately 10-20% strain at a constant slope of $E$. The different slopes for different materials create distinct elastic microenvironments for cells based on the stiffness of their substrate. Previously,
correlations have been made between increased cell adhesion and increased cell contractility, but it now seems that this sensing of the substrate stiffness is what is feeding back and influencing adhesion and cytoskeletal development.

Although Discher et al. (8) were not performing any primary experimentation, they cite examples where substrates have been adjusted from very soft to rigid. In soft gels, cell adhesion is dynamically diffused over the matrix. In stiff, highly cross-linked gels, cell adhesion is stable and static. Additionally, specific proteins identified as potentially signaling substrate stiffness to the cell are greatly enhanced on stiffer gel substrates. This indicates that indeed the cell is receiving information about the stiffness of its environment. A stiffer environment serves to reduce the tensile stress between neighboring cells through a higher elastic modulus and therefore a better ability to resist the stress.

Other experiments support the hypothesis that substrate stiffness is dictating cell proliferation. For example, on gels with an elasticity very close to the elasticity of relaxed muscle bundles, cultured cells exhibited distinct striations nearly identical to those found in actual muscle bundles. In another study, a monolayer of cells with an elastic modulus similar to that of the brain was first cultured on glass. Then further cells were cultured on this sublayer and the result was a framework of connective tissue that would be suitable for the neuronal branching found in the brain.

All experiments discussed by Discher et al. (8) supported the theory that as the elastic modulus of the substrate increases, a more organized and larger cytoskeleton is generated. The observation that substrate stiffness does influence cell growth forms an
important foundation for bioengineers designing bone scaffolds. Ideally, scientists would like to dictate the appropriate tissue formation through the development of fibrous scaffolds with appropriate fiber flexibility to promote desired cell growth.

The mechanical activity of neighboring cells has typically been attributed to their physiological function, that is to say, cells push and pull on each other in order to function properly. For example, fibroblasts are believed to initiate wound healing by pulling on their environment. Recent research has shown that there is an additional motivation for this mechanical interaction: by contracting against their environment, cells are able to sense the mechanical properties of the environment and react appropriately. Experimental results have shown that fibroblasts cultured on elastic substrates such as collagen gels will orient themselves in the direction of tensile strain and will actually migrate towards regions of higher rigidity and larger tensile strain. Uniaxial stretch will cause the cells to orient in the direction of the principal strain. Biaxial stretch will cause the cells to align themselves along the direction of pull between the fixed points, parallel to the free surfaces. If the substrate is cut perpendicular to the direction of the strain and a sufficient distribution of cells is present at the time, the cells will reorient themselves parallel to the new free surface that has been introduced at the cut.

The roles of substrate stiffness and focal adhesion sites combine to structurally reinforce the cell and signal further activity. As a result of mechanosensing at these adhesion sites, cells remodel their adhesion contacts and cytoskeleton, possibly changing position and orientation based on the mechanical properties of the substrate environment. The typical response of cells seems to be a preference for large effective substrate
stiffness and accordingly, as discussed in the section on the work of Discher et al. (8), a more organized cytoskeleton.

Through their work, Bischofs and Schwarz (9) aimed to calculate how the stress and strain are propagating through the substrate and cellular environment using a linear elasticity theory model. They then solved equations for different geometries and boundary conditions of interest in order to predict the position and orientation where cells would sense the maximum effective stiffness in their local environment. Accurate equations for a variety of situations are imperative to the creation of a quantitatively predictive model for predicting how bone remodeling might occur. It is important to note that the equations presented in this paper are not intended to precisely quantify any values; rather their purpose is to show general relationships among important variables.

Bischofs and Schwarz (9) suggest that cells position and orient themselves in order to sense maximal effective stiffness using input from the local elasticity of the substrate. Assuming that the extracellular environment can be described by the isotropic linear elasticity theory, they aim to define a quantity that utilizes the kind of information a cell can take from its environment through the use of contractile tension.

In the absence of any prestrain, the work $W_0$ that a cell has to invest into its surroundings in order to build up a force $\vec{F}$ at a single contact position $\vec{r}_c$ is

$$W_0 = \frac{1}{2} \vec{F} \cdot \vec{\phi}_c(\vec{r}_c), \quad [2]$$

where $\vec{\phi}_c$ is the displacement caused by the cell (9). Work can be a measure of the effective stiffness of the elastic environment as it is probed through a single contact. The
displacement caused by the cell decreases as the rigidity of the environment increases.
Therefore based on the relationship in [2], the cell has to invest less work in order to
achieve a specific force at regions of high rigidity. That is to say, if $\tilde{\mathbf{u}}_e$ is decreased and
$W_0$ and $\bar{r}_e$ are held constant, then the force $\mathbf{F}$ achieved will increase. Thus it can be
concluded that one mechanism for sensing maximal stiffness could be that the cell can
sense when work is minimized at a contact.

In reality, the work investment necessary can vary in the presence of prestrain. The corresponding contribution of prestrain to work is

$$\Delta W = \mathbf{F} \cdot \tilde{\mathbf{u}}_e(\bar{r}_e)$$  \[3\]

where $\tilde{\mathbf{u}}_e$ is the displacement caused by the external strain (9). Therefore total cellular
work can be expressed as $W = W_0 + \Delta W$. Based on this relationship, a negative $\Delta W$
would reduce the amount of work that must be invested and thereby represent a larger
effective stiffening of the environment. Conversely, a positive $\Delta W$ increases the amount
of work that must be invested and represents a smaller effective environmental stiffening.
Thus, the quantity $W$ can characterize the elastic input from the environment that is
available to a mechanosensing cell; since the quantity considers both substrate rigidity
and prestrain, the value calculates for an effective stiffness independent of whether the
stiffness originates from substrate rigidity or prestrain. Bischofs and Schwarz (9)
conclude that calculating $W$ for situations of interest can measure the kind of information
a cell can garner from its elastic environment through active mechanosensing.
Different contacts throughout the extracellular environment are coupled so that overall force balance is ensured. This can be modeled by considering only pairs of opposing forces. In the elasticity theory used by Bischofs and Schwarz (9), this pinching force pattern is known as anisotropic force contraction dipoles, where a dipole strength is calculated based on the force magnitudes and separation. The aim, then, is to establish a model that will calculate the effect of external strain on the work required to build up a force dipole $P_{ij}$, which be written as

$$\Delta W = P_{ij}u_{ij}^{e}(\vec{r})$$  \ [4]$$

using the same notation as in the single-contact model. Like the case of the single-contact, the specific force pattern that minimizes $\Delta W$ is optimal. Because cell dipoles act in contraction, $P$ will always be less than zero and therefore $\Delta W$ will always be negative. Therefore a tensile strain, which corresponds to $u_{ij}^{e} > 0$, is always favorable because, in combination with $P$, it generates a negative value for $\Delta W$ which corresponds to an effective increase in stiffness. Alternately, compressive strain or $u_{ij}^{e} < 0$ would lead to a positive value for $\Delta W$, or an effective decrease in stiffness, which is undesirable.

Bischofs and Schwarz (9) begin their models with the principle that cells prefer maximal effective stiffness in their environment. This observation is supported experimentally and aligns perfectly with other factors discussed throughout this paper. As shown in the work of Nelson et al. (6) and Rumpler et al. (7), cell growth oriented along the sharp corners, i.e. the area with the highest rigidity, or effective stiffness, in its shape.
This phenomenon can also be reasoned through the analogy of a spring. The equation for a work in a spring is $W = F^2/2K$ (9). In order to produce a large force $F$ with minimal work $W$, the spring stiffness $K$ should be as large as possible in this relationship. A higher stiffness leads to a more efficient build-up of force, and the hypothesis is that this build-up of force leads to cell growth, a theory again supported by the work of Nelson et al. (6) who found that maximal cell growth corresponded to regions with the highest concentration of strain fibers. Thus the conclusion can be made that cells prefer maximal effective stiffness because it allows for force to be most efficiently built up.

When considering a cell interacting with external strain, the equations of isotropic elasticity result in

$$\Delta W = -\frac{Pp}{E}[(1 + \nu)\cos^2 \theta - \nu], \quad [5]$$

where $\theta$ is the orientation angle of the cell relative to the direction of the external tensile stress $p$ which is always less than zero (9). Based on the relationships in [5] and our previous conclusions, the optimal cell orientation, corresponding to a minimum value for $\Delta W$, occurs at $\theta = 0$. This suggests that the cell would orient with the direction of stress if at all possible, which as discussed before is experimentally observed. This explains why, for clamped boundary conditions, the cell senses maximal stiffness perpendicular to the edge, and for free boundary conditions, the cell senses maximal stiffness parallel to the edge, and orients accordingly.

The models considered by Bischofs and Schwarz (9), up until this point, only considered single cells. The next step is to consider that external strain is caused by the
traction of neighboring cells through their elastic interaction. For the simplest case of two cells interacting, the equation presented is

$$\Delta W = \frac{P^2}{Er} g_v(\theta_1, \theta_2, \theta), \quad [6]$$

where \( r \) is the distance between the dipoles of the cells and \( g_v \) is a complicated function involving Poisson's ratio and the orientational degrees of freedom between the cells. Based on derivations external to the extent of this research paper, a minimum value for \( \Delta W \) occurs for completely aligned dipoles, independent of any other variables. This suggests that cells will seek to organize themselves by forming strings of cells in parallel to other external strain, an idea that is again observed experimentally.

Bischofs and Schwarz (9) showed that through numerical and finite-element methods, models can be developed to optimize the design of tissue implants based on geometry and boundary conditions. The purpose of presenting these equations is to show how general relationships that are observed experimentally can be confirmed through the relationships found in existing equations. This is relevant to the topic of bone remodeling for reasons discussed throughout this section and because models like these could be developed and applied when designing bone implant scaffolds in order to stimulate cell growth in preferred orientations based on the use of substrate materials with varying stiffness and boundary conditions.
Conclusions

_Nelson et al. (6) and Rumpler et al. (7) demonstrated that the shape of the tissue, based on its constraints, influences subsequent patterns of cell proliferation. It appears that cells are probing their environment and as a consequence, the concentration of cell proliferation is varying according to environmental feedback. On islands and within channels, cultured cells proliferated significantly more at the corners and short edges of their boundaries. Experimentation showed that cell proliferation was not simply stimulated at just the edges of their boundaries; rather it was based on overall geometry. Cells at both corners and short edges experienced increased tensile interaction which resulted in the higher concentration of proliferation. All cell proliferation eventually led to a round central opening, regardless of the initial shape. A circular opening has the lowest average curvature and consequently is the geometry that subjects cells to the least amount of tensile stress. Rumpler et al. (7) theorize that the tissue is seeking to reduce its overall surface area, similar to the mechanisms of surface tension, and Nelson et al.'s conclusions support this hypothesis. A minimal surface area minimizes tensile cell interaction as well as the overall stress and strain on the tissue._

_Discher et al. (8) and Bischofs and Schwarz (9) demonstrate that cells interact with their environment and feedback information that influences proliferation. Generally speaking, it appears that cells prefer maximal substrate stiffness and a more organized cytoskeleton. A stiffer environment reduces the amount of tensile stress due to its higher elastic modulus. In all studies examined, cell proliferation occurs in the presence of high_
rigidity, whether that rigidity is induced by a higher radius of curvature in the tissue geometry or by the substrate environment. This high rigidity allows a cell to produce the largest force with minimal work, leading to increased cell proliferation. These conclusions are important driving forces in the design of bone scaffolds. The goal in designing progenative bone scaffolds is to initiate desired patterns of cell proliferation in order to best model an appropriately shaped bone. The studies investigated above have made it apparent that the macroscopic geometry of the scaffold as well as the effective stiffness of the scaffold base material is critical to the success of potential designs.

The articles reviewed in this thesis only discuss a small fraction of the models that exist to describe remodeling. I believe that the complex nature of remodeling constitutes that bone engineers must consider a number of different influences when considering the design of bone scaffolds. Importantly though, I believe that these articles show how much overlap exists between models. All of these experiments were designed independently, yet their conclusions fit together. For example, Ingber (2) felt that within their channels, cells were trying to orient themselves so as to minimize stress and strain. Similarly, the models of Discher et al. (8) found that preferred cell orientation was along a stiffer substrate where minimal stress and strain was found. Based on its complexity, it may be that there will never be a perfect explanation for bone remodeling, so the focus should be on optimizing design rather than perfecting it. Bischofs and Schwarz (9) offer a wonderful example of how numerical models can be developed to help support trends that have been observed experimentally. I think that a future step in research could be to design experiments using the same channel mechanisms as Rumpler et al. (7). However,
in these experiments, there would be a desired goal for cell proliferation rates and concentrations, and the method would be adjusted to achieve those results. Similarly, the influence of substrate stiffness could be quantified through an experiment that cultured cells of substrates of varying material properties and stiffness. These results could help to find ideal materials for creating bone scaffolds. I believe that we would find that much like real bone, a successful scaffold would have variable material properties based on where it was to be implanted.

Although this thesis focuses on forces and stresses at the intercellular level, future work could also include studying the impact of macroscopic forces and stresses. Finite element analysis of bone models loaded with reasonable forces could be developed, based on reasonable and expected impact levels. In order to determine forces to use, further investigation into the loading of the skeleton during routine tasks would need to be conducted. I believe that we would find correlations between areas of high stress and areas of increased bone volume and density, similar to results at the cellular level.
References


