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Investigation of Surface Mechanical Environment as an Optimization Criterion for Improved Tissue Engineering Scaffolds

by

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ABSTRACT

INVESTIGATION OF SURFACE MECHANICAL ENVIRONMENT AS AN OPTIMIZATION CRITERION FOR IMPROVED TISSUE ENGINEERING SCAFFOLDS

by

Brandon Bucklen

Trabecular bone, the porous bone found predominately in the spine and ends of long bones, is a mechanically regulated tissue. The hierarchy of bone consists of several levels of structure such as raw collagen and calcium phosphate on the microscale to trabecular packets, which are constantly being remodeled by bone cells on the tissue level. The remodeling of bone is believed to be explained through the concept of functional adaptation—where bone is a maximum strength yet minimum weight material. In functional adaptation, phenomenological models are able to predict the density distributions and bone shapes that are witnessed in vivo to a certain degree. Functional adaptation assumes there is an equilibrium state in which no changes in bone mass or structure will occur at the bony surface. Topological and mass changes are incurred on a local level when equilibrium is not achieved. The combination of these local changes produces a self-organized structure – meaning that the global bone shape is explained by simple local rules. Unfortunately, neither tissue engineering nor medical device design has incorporated the knowledge base of functional adaptation of bone into their orthopedic designs.
The objective of this dissertation work was to examine how the concept of functional adaptation could be applied to tissue engineering of bone in so much as it leads to the development of a computer-aided tissue engineering (CATE) framework. The idea was to increase the specificity in which implant/scaffold architectural shape can be matched to tissue mechanical properties of the spine (or other locations), as well as matched to an individual patient who has experienced fracture. Because a variety of mechanical stimuli have been proposed in the functional adaptation literature, the first step of this work was to categorize the most probable variables that explain mechanical loading of trabecular bone in the spine. This was accomplished through reverse engineering cadaver specimens into μ-finite element models. Two algorithms were developed for scaffold design, which makes use of the mechano-transductive principles specifically designed for the pre-determined mechanical variables. Finally, a framework for assembling scaffolds from local building blocks, which are derived from bone was proposed.
I am grateful for my advisor, Michael Liebschner, and his contribution to my work. I appreciated his insights and ideas throughout the many developments and changes in the scope of this project. I would also like to thank my committee members for their participation in this work, Kyriacos A. Athanasiou, K. Jane Grande-Allen, Gemunu H. Gunaratne, and Matthias Heinkenschloss. I especially valued the extra-departmental feedback from Dr. Heinkenschloss.

My fellow members of the computational and experimental biomechanics laboratory (CEBL), Matthew Wettergreen, Jeremy Lemoine, Wafa Tawackoli, Kay Sun, Alistair Templeton, and the several undergraduate/high school contributors to my work, have enriched the time and quality of my graduate school experience. I am especially grateful for Matthew Wettergreen who contributed to the Bone-Derived CAD Library and whose research provided an excellent complement to my own. I would like to thank Jeremy Lemoine for his training on the μ-CT.

Lastly, I would like to thank Rice University, the National Science Foundation as a funding source, Dr. Wei Sun and the members of the Computer-aided Tissue Engineering Laboratory at Drexel University for their generosity in collaborations, materials, and even housing. I would like to acknowledge Dr. Tony Keaveny for supplying several image data sets used in various stages of this research.

Special thanks to Robert Chang and Lauren Shor of CATE Lab for enhancing my experience in Philadelphia, PA. Thank you to my family, and especially my nieces, who remind me of home.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>3DP</td>
<td>3-d printing</td>
</tr>
<tr>
<td>abs.max.pr.e</td>
<td>Absolute maximum principal strain</td>
</tr>
<tr>
<td>abs.max.pr.s</td>
<td>Absolute maximum principal stress</td>
</tr>
<tr>
<td>abs.min.pr.e</td>
<td>Absolute minimum principal strain</td>
</tr>
<tr>
<td>abs.min.pr.s</td>
<td>Absolute minimum principal stress</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AW</td>
<td>Adapted window</td>
</tr>
<tr>
<td>BMU</td>
<td>Basic multicellular unit</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density</td>
</tr>
<tr>
<td>B-rep</td>
<td>Boundary representation</td>
</tr>
<tr>
<td>CA</td>
<td>Cellular automaton</td>
</tr>
<tr>
<td>CAD</td>
<td>Computer-aided design</td>
</tr>
<tr>
<td>CATE</td>
<td>Computer-aided tissue engineering</td>
</tr>
<tr>
<td>CPsrft</td>
<td>Closed poly surface</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>DW</td>
<td>Disuse window</td>
</tr>
<tr>
<td>ESO</td>
<td>Evolutionary structural optimization</td>
</tr>
<tr>
<td>evol</td>
<td>Volumetric strain</td>
</tr>
<tr>
<td>FEA</td>
<td>Finite element analysis</td>
</tr>
<tr>
<td>FEM</td>
<td>Finite element modeling/method</td>
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<tr>
<td>FDM</td>
<td>Fused deposition modeling</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>HA</td>
<td>Hydroxyapatite</td>
</tr>
<tr>
<td>HCA</td>
<td>Hybrid cellular automaton</td>
</tr>
<tr>
<td>ICC</td>
<td>Intelligent cavity creation</td>
</tr>
<tr>
<td>l/d</td>
<td>length-to-diameter</td>
</tr>
<tr>
<td>max.pr.e</td>
<td>Maximum principal strain</td>
</tr>
<tr>
<td>max.pr.s</td>
<td>Maximum principal stress</td>
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<tr>
<td>min.pr.e</td>
<td>Minimum principal strain</td>
</tr>
<tr>
<td>min.pr.s</td>
<td>Minimum principal stress</td>
</tr>
<tr>
<td>MOW</td>
<td>Mild overload window</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NURBS</td>
<td>Non-uniform rational β-splines</td>
</tr>
<tr>
<td>PCL</td>
<td>Polycaprolactone</td>
</tr>
<tr>
<td>PID</td>
<td>Proportional-integral-derivative</td>
</tr>
<tr>
<td>PLA</td>
<td>Poly lactic acid</td>
</tr>
<tr>
<td>POW</td>
<td>Pathological overload window</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>RCO</td>
<td>Rhombitruncated cuboctahedron</td>
</tr>
<tr>
<td>Rhino</td>
<td>Rhinoceros3d</td>
</tr>
<tr>
<td>RP</td>
<td>Rapid prototyping</td>
</tr>
<tr>
<td>RTO</td>
<td>Restricted topology optimization</td>
</tr>
<tr>
<td>sed</td>
<td>Strain energy density</td>
</tr>
<tr>
<td>SFF</td>
<td>Solid freeform fabrication</td>
</tr>
<tr>
<td>SI</td>
<td>Superior-inferior</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>SIMP</td>
<td>Solid isotropic material with penalization</td>
</tr>
<tr>
<td>SL</td>
<td>Stereolithography</td>
</tr>
<tr>
<td>SLS</td>
<td>Selective laser sintering</td>
</tr>
<tr>
<td>STL</td>
<td>Stereolithography file format</td>
</tr>
<tr>
<td>S/V</td>
<td>Surface-to-volume</td>
</tr>
<tr>
<td>T-9</td>
<td>Thoracic level 9</td>
</tr>
<tr>
<td>UD</td>
<td>Uniformity diagnostic</td>
</tr>
<tr>
<td><em>vms</em></td>
<td>Von Mises stress</td>
</tr>
<tr>
<td><em>μ</em>-(root word)</td>
<td>Micro-(root word)</td>
</tr>
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</table>
**Chapter 1: Introduction and Objectives**

1.1. Introduction

Currently, stand-alone tissue substitutes for the regeneration of bone in the case of osteoporotic lesion or fracture repair are incomplete in their role to restore joint function. Improved methods must be applied to provide more patient-specific, mechanically optimal, and biologically capable replacements, either to existing grafts or tissue-engineered substitutes. In order to design such replacements detailed information is required about the material properties, architectural organization, graft/biomaterial surface-interface chemistry, mechanical environment, and longevity of the replacements. Computer-aided Tissue Engineering (CATE) incorporates computer-based methods such as computer-aided design (CAD), topology optimization, rapid prototyping (RP), and bio-informatics databasing in order to better design and manufacture tissue replacements to match patient and biological site properties. In order for CATE to be relevant to the design process of an implant or scaffold (structural support element for cells and other bio-factors), information about the nature of trabecular bone and how it functions must be applied to the design—much of which is currently unknown.

1.2. Overall Objective

The goal of this dissertation work was to investigate the mechanical properties of the trabecular bone surface, the location of metabolic activity, and implement this information into several enabling technologies which will add to the existing, but undeveloped field of CATE.

1.3. Specific Aims

1.3.1. Specific Aim #1 (Chapter 3)
To determine the trends in mechanical properties on the surface of a representative sample of trabecular bone, in order to confirm which engineering measures are a likely functional adaptation stimulus (under direct deformation of the tissue).

1.3.2. Specific Aim #2 (Chapter 4)

To apply the results of the tissue level functional adaptation stimuli of trabecular bone into the virtual design of scaffolds or implants which will accentuate the qualities discovered in SAI.

1.3.3. Specific Aim #3 (Chapter 5)

To develop a general CAD/CATE framework for trabecular bone substitutes which utilize other non-surface properties such as permeability, porosity, stiffness, etc. in a manner specific to the patient.
Chapter 2: Background

One goal of this dissertation was to determine the role of architectural organization of trabecular bone as relates to its mechanical surface properties by investigating a random sampling of trabecular bone. The designers of scaffolds for tissue engineering have long recognized the importance of material selection, biocompatibility, and more generally an open-pore architecture for nutrient transfer; however, have neglected the role of material organization on the final tissue produced. This research aims to discover the importance of selected optimization variables in order to determine if a dominant surface mechanical environment may be introduced in scaffold design, leading to an increase in the quality of mineralized tissue produced, and likely a better integration between tissue and scaffold interface. The motivation for improvements in tissue engineering strategies and implant design is well-justified, as the current technologies and medical solutions suffer from drawbacks, some of which could be alleviated by CATE. A brief background of related information is presented here.

2.1. Bone Mechanics

Each tissue in the body has a unique function, and therefore a different set of mechanical requirements. Bone has an exceptional list which includes the capability to provide structural rigidity, act as a reservoir for ions and calcium regulation, and provide a framework for the transfer of muscle forces [1]. The composition of bone is 65% mineral, 35% organic matrix, cells, and water [2]. The mineral phase is unrefined hydroxyapatitite, with various trace amounts of magnesium, fluoride, and carbonate. The organic portion is primarily type I collagen, and about 10% noncollagenous proteins,
such as osteocalcin, osteopontin, and osteonectin [2]. The mechanical properties of bone, therefore, are determined by the composite interaction between mineral and organic phases, as well as the orientation (woven, lamellar) in each scale size.

In load bearing applications we are largely interested in osteoporotic or weak regions of trabecular bone, since fracture typically occurs in this area. Additionally, the major load bearing portion of bone in the spine, a common site of fracture among the elderly, is trabecular bone [3]. Trabecular bone grows and adapts through changes in tissue quality on its exposed surface. Trabecular packets, or parallel sheets of lamella organized in preferential angles within a trabeculae, are directly remodeled at their surface by osteoblasts and osteoclasts, unlike remodeling that occurs in cortical bone away from the surface. Therefore, trabecular bone represents a more metabolically active tissue form due to the large surface area/volume ratio.

The inability of bony defects to be adequately and quickly replaced has led to a conglomeration of research seeking to provide cell seeded scaffolds for both mechanical support and biological functionality. This need arises in a number of clinical situations including tissue degeneration due to osteoporosis [5, 6], voids generated by tumor resection [7], damage due to trauma [8], and a variety of other genetic diseases affecting the formation of mature bone [9].
2.1.1. Mechanical Usage

Because bone is a living tissue, the manner in which local mechanical signals are transduced is salient. If a tissue’s mechanical usage may be understood, then tissue engineering solutions may be formulated accordingly. Wolff’s Law, theorized in 1892, was the first to credit bone mechanics for bone shape [10]. His trajectorial theory stated that trabecular bone struts intersected at right angles that were aligned with the principal stress axes, which has since been proven incorrect and updated [11, 12]. A mechanical usage window was introduced by Harold Frost in order to explain the metabolic adaptation of bone to mechanical signals (see Figure 2, adapted from [13]). This terminology as well as his “mechanostat” theory helped to explain the apparent level biological machinery of bone. The “usage” of bone is defined as the voluntary mechanical loads on a skeleton during a typical week [14], and is delineated by units of strain. Studies have shown that strain levels and strain rates in all matter of humans and animals are predominately constant [15], which makes strain a robust metric of consideration.

Frost’s “mechanostat” was one of the first theories to adequately explain many facets of mechanically induced bone formation through a lumped-parameter model. Without considering all the cellular detail, his model describes a control process of bone mass/strength changes. The term “mechanostat” comes from the analog to thermostat; where deviations from a set point trigger the mechanism to turn “on”, while with no deviation the mechanism remains “off” [16]. Frost’s model was able to predict thirty-two verifiably occurring phenomena related to bone, such as the existence of a safety factor in load bearing bones [13].
The usage is described in four quadrants as seen in Figure 2. In the disuse window (DW), strains below 50µε cause an osteopenia-type loss of mass where material near marrow is evenly resorbed such as occurs in disuse and senile osteoporosis. In the adapted window (AW) that spans the remodeling and modeling thresholds, strains between 100 – 1000µε, trigger conservation remodeling – where architecture and strength are maintained yet “old” tissue is replaced by “new” tissue. In the mild overload window (MOW), bone mass increases not because of a higher remodeling metabolism, but because of lamellar modeling drifts which seek to restore the lower strain levels of the AW. At strains larger than 3000µε, microdamage accumulation is proportionally larger than the reparation speed by remodeling drifts, resulting in decreased strength in the pathological overload window (POW) [14]. Additionally, the intermediate set points, or thresholds, may be subject dependent or altered by a state of disease to explain bone drifts [17].
Figure 2. Mechanical Usage Window of bone. MESr, MESm, and MESp are the remodeling, modeling, and pathological mechanical thresholds, respectively. Below the MESr, biochemical stimuli is required to drive adaptation. Within MESr – MESm, mechanical loading is the dominate force. Below the MESp mechanical loading is reversible, while above the MESp, irreversible damage may occur.

There are some limitations to Wolff's Law and the “mechanostat” theory made evident in recent years. Dynamic loading is not elegantly addressed, but is critical in the mechanotransduction of at least bone [18] and cartilage tissue [19]. In bone, a short bout of dynamic loading increased bone formation by recruitment of surface cells [20], while longer loading regimes at similar magnitudes appeared to be detrimental to a loaded implant within a rat tibia, when compared to static controls [18]. Alternative cell types
also have differing sensitivities to magnitude of loading. Cartilage cells prefer smaller dynamic loading [21], while it is unclear if there is a preference in bone. Thus the effect of the magnitude and type of loading is not addressed by Wolff's Law or the "mechanostat"; neither does each qualify completely why the architecture of bone appears the way it does morphologically.

2.1.2. Structural Considerations

Mechanical properties are not constant in each structural reference frame. The structural hierarchy from largest to smallest is the whole bone, architectural, tissue, lamellar, and ultrastructural levels (see Table 1). The whole bone level (3 – 750mm) describes the most macroscopic look at bone, including muscles and tendon attachment sites. The two distinct architectural regions (75 – 300μm) are trabecular bone composed of rods and plates found in the ends of long bones, and cortical bone, comprising the concentric shaft of long bones. The tissue level (20 – 100μm) consists of individual trabeculae or osteons which have properties determined from the constituent material, a combination of organic matrix and mineral, as opposed to apparent properties that include void space [22]. The lamellar level (1 – 20μm) is one step smaller, and is composed of sheets of collagen and minerals deposited by osteoblasts [23]. The ultrastructural level (.06 - .4μm) is the smallest organizational level which consists of chemical interactions and quantum level relations, and is less often considered due to difficulty in its characterization [22].

The ability to determine mechanical properties at each individual level is somewhat limited due to mechanical testing protocols, yet there is much information on
the tissue and architectural levels. For an in-depth review see Liebschner et al [24]. As per Table 1, the architectural modulus values for trabecular [25] and cortical bone [26] are less than that of the respective tissue properties [26, 27]. This occurs because the former is estimated from a continuum composite of bone and its included void space, while the latter captures the mineral phase and as result is an order of magnitude larger. Naturally, the whole bone level [24] yields a wider spectrum of modulus values – as the structure of the whole is composed of its individual hierarchies, each of which has an associated range.

Table 1. Structural Hierarchy and Mechanical Properties of Bone

<table>
<thead>
<tr>
<th>Level</th>
<th>Dimensions</th>
<th>Modulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-bone level</td>
<td>3–750 mm</td>
<td>3.9–71 GPa</td>
</tr>
<tr>
<td>Architectural level</td>
<td>75–200 µm (T)</td>
<td>0.0001–2 GPa</td>
</tr>
<tr>
<td></td>
<td>100–300 µm (C)</td>
<td>2.90–57.7 GPa</td>
</tr>
<tr>
<td>Tissue level</td>
<td>20–75 µm (T)</td>
<td>0.76–20 GPa</td>
</tr>
<tr>
<td></td>
<td>20–100 µm (C)</td>
<td>13.8–21.1 GPa</td>
</tr>
<tr>
<td>Lamellar level</td>
<td>1–20 µm (T)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3–20 µm (C)</td>
<td></td>
</tr>
<tr>
<td>Ultrastructural level</td>
<td>0.06–0.4 µm (T)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.06–0.6 µm (C)</td>
<td></td>
</tr>
</tbody>
</table>

T, trabecular bone; C, cortical bone.

The architecture has a terrific impact on the structural properties of bone, and in turn, on its mechanobiology. Native tissue "sees" mechanical stresses coincident with the shape and stress patterns on the local level, which are not adequately capture by a single "modulus" value. The structural hierarchy of bone is one of the most compelling arguments for a biological machinery, and ranges from the radial configuration of growing osteons, to the rods and plates of trabecular architecture, to the symmetric interface which occurs during endochondral ossification [28]. One simple example illustrating the importance of architecture may be seen in Figure 3. Consider a composite bone/cell or scaffold/tissue arrangement where the composite experiences a uniform level of deformation, or
isostrain. In the case of the bone/tissue arrangement, tissue will deform consistent with the scaffold but will experience much lower internal stresses (assuming tissue has a lower modulus). Isostress, where each constituent receives an equal stress, results in proportionately larger deformation of the softer material, in this case the tissue. All bone architectures may be viewed as receiving some combination of isostress and isostrain. Therefore, a given architecture contains a complex milieu of stress and strain profiles which are a function of the geometry.

Bone architecture is not only patient-specific, but site-specific. The ratio of trabecular bone to cortical bone, bone mineral density, and boundary geometry of a femur will be different than that of a vertebral body. Figure 4 illustrates some of these key architectural differences for a trabecular portion of a human vertebral body and iliac crest. The former, has a combination of mainly rods and some plates, while the latter is composed of thick plate sections, devoid of a truss-like structure. Aside from morphological

**Figure 3. Isostrain and Isostress of a composite material.** In isostrain each component has a uniform deformation, while in isostress each material has a uniform stress. The stress and strain, for isostrain and isostress, respectively, are in general “additive” but depend on the moduli and volume fraction of each component.
differences, there are clear structural anisotropy distinctions. The vertebral sample is more aligned in the superior-inferior direction in accordance with the direction of load due to weight in the spine. The iliac crest is less organized in its fabric directions. Additionally, the two samples (though unmeasured) probably have similar yield and ultimate strains on the apparent level, but the stresses they experience are drastically dissimilar [29]. Disuse osteoporosis, which is sensitive to mechanics, will affect the vertebral body, causing perforations and reductions in trabeculae, though the iliac crest will not be vulnerable due to its lower S/V. The implication for tissue engineering is that constructs must be designed with a priori information on the site, its boundary geometry, and the loading it experiences.

Figure 4. Site-specific architectures of the trabecular portion of a human, lumbar vertebral body (left) and iliac crest (right).

2.2. Bone Metabolism

The three types of bone cells that are responsible for bone turnover are the osteoblast, osteoclast, and osteocyte. The functional unit describing some combination of osteoblasts/osteoclasts responsible for a local change in mass is known as a bone metabolic unit.
Osteoclasts derive from pre-osteoclasts which derive from the bone-marrow, hematopoietic, monocyte lineage. Osteoblasts derive from precursor blood cells of the mesenchyme, and are single-nucleated, cuboidal cells with a well-developed rough endoplasmic reticulum, responsible for the synthesis of bone matrix proteins. Once the mature skeletal system is established following the process of endochondral ossification in long bones, the bone cycle consists of remodeling and maintenance of the pre-existing structure.

In a quiescent state the surface of trabecular bone is composed of bone lining cells, which are morphologically flattened osteoblasts.

Figure 5. Remodeling of the Adult Skeleton [30].

Connected to these surface cells are osteocytes which are former osteoblasts that have become embedded in the lacunae (pore spaces created from previous osteoclast activity). Additionally, embedded osteocytes are connected to each other through cellular processes traversing tiny canalicular channels (< 20 nm), encompassing a complete loop of networking (osteocyte-osteocyte, osteocyte-osteoblast). On the non-mineralized side,
there is the marrow space containing precursor cells, and adipose cells (which are among the same lineage as osteoblasts).

Signaling begins when either a mechanical disruption is caused (either through micro-crack formation or a change in loading) or a biochemical factor causes osteocyte apoptosis or a stimulatory release of factors such as nitric oxide, prostaglandins, etc. which begins the cascade. There are many local factors as well as hormones that have shown to be influential in this cycle. Here, we outline briefly one possible communication path (see Figure 5).

Once the osteocyte has responded, either by halting the production of Sclerostin, an antagonist to bone formation, or by release of local factors, the stromal cells respond by differentiating into pre-osteoblasts and secrete factors such as macrophage colony stimulating factor, which signals the differentiation of marrow cells into pre-osteoclasts. The pre-osteoblasts express ligands (RANK-L) that are complementary to receptors of the pre-osteoclast (RANK). When this pathway is activated, several pre-osteoclasts will merge and form a multinucleated osteoclast. The osteoclast proceeds to resorb bone at a localized, but presumably random, spot for approximately two weeks, via the creation of sealing zone along its ruffled border where an acidic environment is created, and material decomposed through cathepsin and hydrolitic enzymes. Subsequently, the osteoclast will undergo apoptosis, the time scale of which is determined by factors such as estrogen. It has been shown that in post-menopausal women, the resorption depth of the osteoclast exceeds the average of 50 μm. Moreover, bone density treatments involving market biphosphonates serve to disrupt the cytoskeletal proteins responsible for creating this sealing zone, thereby reducing the effectiveness of the osteoclasts.
Mature osteoblasts secrete osteoprotegerin which binds with free-floating RANK-L. When enough mature osteoblasts are present, the effect of bone resorption will be reduced. Osteoblasts are believed to be signaled through a number of pathways. However, once they line the cavity they will deposit osteoid, osteopontin, osteocalcin, and other factors. When the osteoid is approximately 6 μm deep, it will undergo mineralization and continue to increase in density for up to four months. During the process, some of the osteoblasts will die, others become osteocytes in the newly formed bone, and some will transform into bone lining cells of the completely remodeled surface, until a new, localized, quiescence is achieved.

2.3. Bone Maladies

2.3.1. Osteoporosis

Bone remodeling involves the interplay of various cellular, hormonal, and metabolite contributors. Most significantly, specific bone cells called osteoblasts deposit osteoid that will later be calcified, while osteoclasts create an acidic environment that helps break down calcified matrix. The balance of the osteoblastic and osteoclastic action determines the amount of calcified tissue present in a local, biological environment. It is known that bone mass in men and women increases steadily until the approximate age of 30, reaches a plateau, and then declines as a result of age, or post-menopausal induced changes [31]. The decline that occurs after middle age is thought to decrease bone mass at about 0.6 % per year in both sexes before the onset of osteoporosis [32]. Because men as a general population reach a higher percentile of gross bone mass, they are naturally less likely to develop characteristic bone-mass “diseases”. The
discrepancy between bone fragility between men and women when first observed, offered explanation that fracture risk was in part governed by a predisposition to genetic factors. Studies including those focusing on bone mineral density (BMD) changes under weightlessness or bed rest, concluded that indeed a baseline of mechanical stimulation is required in order to maintain bone, implying that mechanical disuse may have an analogous effect to age-related or post-menopausal changes in bone density.

Osteoporosis is a disease causing an increase in the porosity of bone. The major symptoms are a reduced thickness of trabeculae in cancellous bone, radial narrowing of compact bone, and a deterioration of bone micro-architecture (loss of trabeculae). As the micro-architecture is altered, load transfer within the bone changes creating an imbalance in the normal intensities of force and improving the likelihood of fracture. Thus, fracture often occurs due to a loss of the strut connectivity with a minimum of observable trauma [33]. Studies have shown that below a threshold of approximately 1.0 g/cm², an increased risk of fracture exists for the femur and vertebra [32]. In its technical definition, osteoporosis and its less severe counterpart, osteopenia, are statistical constructs based on the standard deviation from mean bone density samples of normal, young adults. Standard deviations of 1-2.5, and > 2.5 constitute osteopenia and osteoporosis, respectively.

The cause of the imbalance in the operation of BMUs, osteoclast absorption of bone followed by osteoblast deposition of bone, is not completely known. Many sources are responsible for affecting the remodeling including local polypeptides, steroid hormone, and thyroid hormone activity [34]. Because osteoblastic and osteoclastic actions are not governed independently (in remodeling) but rather osteoclast signals or
signals released through the resorbed matrix directly stimulate osteoblasts it is important to understand that several possibilities exist for the way in which bone mass declines. For example, a lack of estrogen after menopause causes osteoclast activity to change by increases in local tumor necrosis factor and increased interleukin levels, which may both inhibit the death of osteoclasts as well as improve their proliferation [34]. Primarily, trabecular bone is known to be affected in this form of post-menopausal osteoporosis (Type I). In age-related osteoporosis (Type II), it is estimated that both cortical and trabecular bone is vulnerable because osteoblast differentiation is compromised more than the osteoclast turnover rate (exemplary of Type I), even though both types could result in the same net bone loss. It is postulated that the progression of Type II is slower as a result of the reduced metabolic activity and reduced surface area to volume ratio of cortical bone when contrasted with trabecular bone. It has also been noted that the number of bone cells may be more influential than their activation levels in determining bone mass balance [35].

An individual’s bone mineral density (BMD) is a consequence of factors such as nutritional intake [36], predispositions of heredity [37], predispositions of gender [32, 37, 38], predispositions of race [38, 39], and external lifestyle decisions [40, 41]. Gender is undeniably linked to bone mass. Riggs et al sustained that one in three women will suffer from vertebral fracture by age 65, and that one in three women, and one in six men will develop a hip fracture by “extreme” old age

Type I

Type I osteoporosis is the accelerated bone loss associated with the hormonal deficiency of estrogen or the male equivalent. Riggs et al. was the first to identify Type I
(post-menopausal) osteoporosis as distinct from Type II (senile, age-related) [37, 42]. However, it was noted that many people do not exhibit Type I or Type II exclusively, but rather the type is used to describe the way in which bone turnover occurs. Type I characteristically appears in women 15-20 years after menopause. The etiologies are a comparatively (with respect to Type II) sudden loss of bone mass, mainly affecting regions of high turnover in trabecular bone such as vertebral bodies of the spine, and extremities of long bones. The loss of estrogen begins a cascade of events. First, parathyroid hormone (PTH) in the absence of estrogen is free to mobilize calcium stores [32]. Secondly, PTH is reduced in response to the serum calcium levels, which incites a secondary response hindering the renal absorption of calcium through lowered hydroxylation of Vitamin D [32, 37]. Patients identified with Type I symptoms have expressed lower levels of PTH hormone [32, 42]. Though estrogen is implicated as the primary determinant for Type I, as Rubin concluded, it stands to reason that other factors must be responsible since not all post-menopausal women with similar estrogen levels develop the disease.

**Type II**

Type II osteoporosis is the bone loss associated with advanced age, arbitrarily deemed to be the age of 75. This version of the disease is much more subtle, such that those developing fracture may have bone density measurements just below their normal, age-matched counterparts [37, 43]. Type II affects men and women and is characterized in the vertebra by trabecular thinning, with no immediate changes in the architecture or constituency of bone tissue itself. Both cortical and trabecular bone loss seems to occur proportionately within this type [37]. Because the turnover of trabecular bone is on the
same order as cortical bone, yet cortical bone exhibits higher strength, the incidences of fracture tend to increase with age. Thus, the manifestation of bone deterioration is often seen much later than in Type I, while the frequency of fracture incidence increases with the same (or greater) rate with age.

Another possibility why certain individuals may experience more rapid age-related loss is due to the nature of cellular mechanisms. Based on observation, Parfitt described a fast and a slow type of osteoporosis [44]. In the fast occurring case, osteoclast resorption dominates causing deeper cavities, promoting the formation of perforations in trabecular bone. In cortical bone, the perforations lining the endosteal surface create the effect of a thin layer of trabecular bone, which has two consequences. First, the surface area is larger providing a more ample environment for additional osteoclasts. Secondly, the architectural properties are reduced in greater proportion to the simple reduction in mass (perforations may sever trabecular-like struts). In the slow type, the amount of infilling of an osteoclast cavity by osteoblasts takes place at a slower rate. Results are milder, indicative of trabecular thinning, where the architectural loss of strength is proportional to the amount of material lost, but there is no morphological change in connectivity. Despite this observation, the most important piece of information remains unknown—the impetus responsible for causing fast or slow age-related loss.

Furthermore, there is no consensus among the literature that the rate of bone loss accelerates in extreme old age, though fracture risk does. In fact, this was a major point of contention for Frost, who claimed that osteoporosis was not necessarily a failure of the skeleton to adapt to its mechanical environment. Instead, a change took place in the mechanical environment shifting from non-traumatic to traumatic. Fracture risk,
therefore, may be a function of initial bone mass, or external factors affecting the metabolic rate at which natural, age-related bone deterioration.

The morphological changes ensuing with age can be dramatic [31]. In vertebrae, there is a 70-80% decline in bone strength, though only a 35-45% reduction in density, signifying the non-linear relationship. The remodeling process itself is responsible for 1-3 μm of thinning, mainly on the horizontal, non-loading bearing struts. One resorption cavity can penetrate a depth of 50 μm, implying that only two layers of resorption could easily sever a thin trabeculae (typical trabecular thickness 100-200 μm). It is easy to see how the load bearing capability of even a dense network of trabeculae may be quickly disrupted. Furthermore, once a strut is severed, there is a minimal chance that it will be reconnected, unless the interfaces remain in good contact. Thus, resorption could develop because of a lack of load transfer. Even if a bone stimulating agent like sodium fluoride is administered [45], formation may only serve to thicken the two severed ends, unable to improve the mechanical properties and increased tissue quality. This irreversibility has inspired the rationale of preventative prophylaxes toward osteoporosis, and encouraged more accurate imaging measures and bone density estimation before fracture.

Disuse Osteoporosis

A localized or generalized bone loss resulting from diminution of mechanical stress has been termed disuse osteoporosis [46]. Disuse osteoporosis is considered a subset of secondary osteoporosis, where Type I and Type II make up primary osteoporosis. Amelioration of secondary disuse effects on bone are important for topics
such as long term space-flight, recovery from bed-stricken diseases, and spinal cord injury.

Disuse osteoporosis has some distinct differences from its primary counterparts. First, bone tissue loss is localized to the specific regions which are immobilized or inactive. However, as a repercussion of homeostasis, in some instances non weight bearing portions of the skeleton such as the skull showed in increase in BMD, while weight bearing bones experienced an expected decline under bed rest [46, 47]. Some morphological changes caused by immobilization also deviate from primary osteoporosis. Yonezu found that periosteal bone resorption was present in the femur in rats after immobilization through sciatic neurectomy [46, 48], which reveals a marked difference between Type II osteoporosis signified through endosteal resorption [49]. Further corroborations by additional studies [50-52] of immobilized spinal cord injury patients showed an initial bone loss and a subsequent stabilization toward a constant value. In fact, human bed rest studies [53] and animal studies [54] revealed a heightened resorption early on followed by stabilization. The specific time scale during which resorption is carried out also depends on the disuse model. For example, the largest decline in BMD was not reached after 6 months in space [50, 55-57], and was reported as 6 months and between 1-2 years in separate studies after SCI injury [50, 54, 58, 59]. The location of loss is also critical. In the majority, bone tissue in the lower back/hip and femoral head area experience heavy losses of upwards of 50 - 60 % [50, 60, 61]. These bones, therefore, exhibit the most pronounced feedback to the changing mechanical environment because they are responsible for bearing much of the body weight during normal circumstances.
2.3.2. Fracture

Ultimately, the risk of osteoporosis is that it inevitably leads to fracture. Annually, 1.5 million people will suffer a bone disease related fracture [63]. It is estimated that because of the aging population the incidence of osteoporosis, and likewise fracture, will increase. By 2020, it is expected that one in two Americans over age 50 will be at risk of developing osteoporosis of the hip [64]. The most common fracture sites are the spine, wrist, and hip. In particular for trabecular bone of the spine, it is suggested that 20-25 percent of postmenopausal White women have at least one moderately deformed vertebra [65]. Falls account for about one-fourth of the spine fractures that come to clinical attention [66].

There are several different types of spinal fractures. The most common is a wedge fracture which results in the compression of the anterior portion of the vertebral body, typically caused through flexion (the act of bending forward) of the spine (Figure 6).

6A). Another type of fracture is the burst fracture which results from the bursting of intervertebral disc or endplate due to direct compression. The result is a stable but typically painful fracture with fragments contained within the fractured body (Figure 6B). Lastly, torsional influences can cause dislocations or fracture in the middle of the vertebral body in a diagonal direction (Figure 6C) [67].

2.4. Mechanical Adaptation

Adaptation of trabecular bone toward an applied external load is an accepted fact. Nevertheless, despite this much generalized understanding of a perceptible adaptation, the governing cellular mechanism and stimulatory mechanical distributions are unidentified. Most often, the best investigations only pinpoint that certain morphological properties (trabecular thickness, number, connectivity, volume fraction) are affected dynamically by induced mechanical loading [68, 69] with no basic formulations of how the biomechanical surface environment contributes to the cell response. From a practical standpoint, the biomechanical fields on the surface are the direct presentation of the stimuli in which bone cells must respond if, in fact, they are locally and mechanically regulated as has been implicated [70, 71].

2.4.1. Generalized Mechanical Adaptation

Mechanical adaptation has been observed through osteoporosis, changes in bone volume during periods of disuse and contralaterally space flight, and in response to foreign materials such as implants. Guldberg et al. discovered that implant shape was a factor responsible for the level of osteo-integration achieved [69]. In this study, hydraulic
bone chambers were used to provide uniaxial mechanical load to metaphyseal portions of the canine femur in vivo. Porous, bone-like coatings of sintered titanium spheres better

Figure 7. Porous coated implant study. Radiographs reveal the effects of implant shape on osteointegration.

resembling trabecular architecture were fused onto several implant shapes (see Figure 7).

All implants resulted in decidedly inferior bone-implant integration and bone resorption in regions just below the surgical site. This outcome was attributed to resorption of lateral trabeculae due to a realignment of the applied loading direction and due to an insufficient loading magnitude. Nevertheless, conical implant designs (right) had the least resorption of those tested, partially explained by its stress-state which contains gradients at the interface site under simplified unconfined compression – coincidentally corresponding with the axis of maximum shear stress in an ideal material.

2.4.2. Specific Mechanical Loading Types

Three types of mechanical loading types have been considered: 1). fluid shear stress, 2) direct deformation, and 3). changes in stored energy. Fluid shear stress is
believed to be a major mode of cell transduction. Small-diameter canals surrounding osteocytic cell processes cause large pressure gradients even when small mechanical loads are applied to the exterior. These loads are thought to induce a fluid gradient, which then acts as a shear stress on the osteocytes [72]. One way mechanical signals influence tissue formation is through differentiation of cells. Figure 8 portrays the relationship between interstitial fluid velocity, strain, and the differentiation pathway [73, 74]. It is clear that bone tissue prefers a balance of small strain and small fluid velocities, and an overexposure (to either) promotes fibrous tissue. You et al found that human and rat osteoblastic cells are less sensitive to substrate deformation than oscillatory fluid flow, at least in regard to expression of messenger RNA levels of osteopontin and intercellular calcium concentrations, two factors thought to be responsible for bone cell recruitment and proliferation [75]. Despite these findings, there certainly evidence of strain-mediated remodeling at the cellular level. Cowin and Weinbaum described how strain may be amplified in the lacuna-canalicular network. Their argument was that flow generated through the canaliculi (small connecting tubules) passes over the fiber glycocalyx and causes a hydraulic resistance. This resistance then distends the cell, producing a hoop stress [76] and a straining of the cell in the direction of flow. You et al further deduced that the effect of the drag force on the pericellular matrix is an order of magnitude higher than the shear stress, which can lead to hoop strains 10 to 100 times larger than the applied strain [77]. Direct experimental measures of stored energy have been much sparser because there is no good experimental protocol for its measurement. Kunnel et al suggested that hysteresis energy may be a valid indicator of anabolic growth in bones upon mechanically applying a cyclical load to neonatal mouse tibias [78]. Inferences to
stored energy, however, are most often accomplished through model techniques in which the mechanical properties of the tested material are known so that the stresses may be ascertained [79]. The model of Ruimerman et al on three-dimensional representations of trabecular micro-architecture concludes that strain energy density leads to the closest approximation in trabecular bone morphology [80]. This conclusion was based on comparing the results of their simulations with reasonable values of volume fraction, trabecular spacing, and net full turnover with the values observed experimentally.

There is evidence to suggest that bone adapts in a dynamic way based on the frequency and magnitude of loading it experiences. Nevertheless, studies in this arena may be conflicting as application of dynamic loading is almost always associated with an extra load pattern, such as the addition of interstitial fluid flow in an animal model at all but the lowest magnitudes. Most studies corroborate that increased frequency has anabolic responses in bone up to a certain traumatic level [81], and at strain magnitudes as small as $10 \mu \varepsilon$ [82]. Very recently, it has been proposed that

![Figure 8. Relationship between interstitial fluid velocity, strain, and tissue response.](image)
frequency is more important than mechanical strain magnitude [68], as ovariectomized rats subjected to whole-body vibrations at a high frequency of 90 Hz produced ~200 % more bone volume (as measured in the tibia), then age-matched controls and those specimens which were vibrated at 45 Hz.

2.4.3. Mechanical Engineering Variables

Suppose trabecular bone or an implant biomaterial occupies the domain \( \Omega \in \mathbb{R}^3 \) and is contained in a given reference domain, say the unit cube. Let \( \Gamma \) denote the scaffold boundary, i.e., the boundary of \( \Omega \). The bone or implant is displaced by a force on the boundary causing a displacement \( \mathbf{u} \) of the bone or implant due to applied forces. 

**Equation 1:**

\[
\mathbf{u} = \begin{pmatrix} u \\ v \\ w \end{pmatrix}
\]

The displacement is governed by the constitutive equations of linear elasticity relating stress to strain. The mechanical response of the bone is measured in terms of mechanical engineering variables which are described in this section. These variables are a function of the strain, \( \mathbf{\varepsilon}(\mathbf{u}) = \frac{1}{2} \left( \nabla \mathbf{u} + \nabla \mathbf{u}^T \right) \), and the stress, \( \mathbf{\sigma}(\mathbf{u}) = \mathbf{C} \mathbf{\varepsilon}(\mathbf{u}) \), where \( \mathbf{C} \) is positive definite fourth order tensor describing the elasticity. The infinitesimal state of stress or strain for an arbitrary point can be expressed in component form as the matrices,

**Equation 2:**

\[
[\mathbf{\sigma}] = \begin{bmatrix}
\sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\
\sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\
\sigma_{zx} & \sigma_{zy} & \sigma_{zz}
\end{bmatrix},
\quad
[\mathbf{\varepsilon}] = \begin{bmatrix}
\varepsilon_{xx} & \varepsilon_{xy} & \varepsilon_{xz} \\
\varepsilon_{yx} & \varepsilon_{yy} & \varepsilon_{yz} \\
\varepsilon_{zx} & \varepsilon_{zy} & \varepsilon_{zz}
\end{bmatrix}
\]

respectively.
**Von Mises Stress**

Von Mises stress has typically been used in failure analysis to predict the onset of plastic deformation during ductile failure. The requirement is that the deviatoric strain energy as computed by the second deviatoric invariant, $J_2$, within the material not exceed the estimated material shear stress determined through uniaxial tensile tests. As ductile materials fail during shear, this dimensionless stress value has been shown to accurately represent failure in these cases.

Let the stress be decomposed into its mean stress values, $\sigma_m = \frac{1}{3}(\sigma_{xx} + \sigma_{yy} + \sigma_{zz})$, and its deviatoric components (suggesting shape not volume changes) such that,

\begin{align*}
\begin{bmatrix} [\sigma] = [\sigma_m] + [\sigma_d] \\
\end{bmatrix} = \begin{bmatrix} \sigma_m & 0 & 0 \\
0 & \sigma_m & 0 \\
0 & 0 & \sigma_m \\
\end{bmatrix} + \begin{bmatrix} \sigma_{xx} - \sigma_m & \sigma_{xy} & \sigma_{xz} \\
\sigma_{yx} & \sigma_{yy} - \sigma_m & \sigma_{yz} \\
\sigma_{zx} & \sigma_{zy} & \sigma_{zz} - \sigma_m \\
\end{bmatrix}
\end{align*}

The second deviatoric invariant can be expressed as,

\begin{align*}
\text{Equation 4:} \quad J_2 = \frac{1}{2} ([\sigma_d] \cdot [\sigma_d]) = \frac{1}{2} \left( 2 \left[ \frac{2}{3} \sigma_{xx} + \frac{1}{3} \sigma_{yy} + \frac{1}{3} \sigma_{zz} \right] + \sigma_{xy}^2 + \sigma_{xz}^2 + \ldots \right)
\end{align*}

and may be simplified to the following form in terms of the stress components,

\begin{align*}
\text{Equation 5:} \quad J_2 = \frac{1}{3} \left( \sigma_{xx}^2 + \sigma_{yy}^2 + \sigma_{zz}^2 \right) - \frac{1}{3} \left( \sigma_{xx} \sigma_{yy} + \sigma_{xx} \sigma_{zz} + \sigma_{yy} \sigma_{zz} \right) + \left( \sigma_{xy}^2 + \sigma_{xz}^2 + \sigma_{yz}^2 \right)
\end{align*}

Let the yield stress of a physical material be $\sigma_y$. During a monotonic, uniaxial tensile test to yield there will be only one applied stress component,

\begin{align*}
\text{Equation 6:} \quad [\sigma] = \begin{bmatrix} \sigma_y & 0 & 0 \\
0 & 0 & 0 \\
0 & 0 & 0 \\
\end{bmatrix}
\end{align*}
Therefore the mean and deviatoric form of applied stress can be written as,

\[
[a_{m}] + [a_{d}] = \begin{bmatrix} \sigma_x / 3 & 0 & 0 \\ 0 & \sigma_y / 3 & 0 \\ 0 & 0 & \sigma_z / 3 \end{bmatrix} + \begin{bmatrix} 2\sigma_x / 3 & 0 & 0 \\ 0 & -\sigma_y / 3 & 0 \\ 0 & 0 & -\sigma_z / 3 \end{bmatrix}
\]

The \( J_2 \) value will serve as the upper limit for elasticity or the lower limit for plasticity which defines our failure criteria and can be computed for the material from Equation 4,

\[
J_{2,\text{material}} = \frac{1}{3} \sigma_y^2
\]

The von Mises stress is derived by ensuring the mathematical definition of \( J_2 \) as defined by Equation 5 not exceed the material value of the applied stress as in Equation 8,

\[
J_{2,\text{experienced}} < J_{2,\text{material}}
\]

\[
J_2 < \frac{1}{3} \sigma_y^2
\]

Rearranging terms to isolate the yield stress produces the following inequality,

\[
\sqrt{3J_2} < \sigma_y
\]

\[
\sqrt{\left(\sigma_{xx}^2 + \sigma_{yy}^2 + \sigma_{zz}^2\right) - \left(\sigma_{xx}\sigma_{yy} + \sigma_{xx}\sigma_{zz} + \sigma_{yy}\sigma_{zz}\right) + 3\left(\sigma_{xy}^2 + \sigma_{yz}^2 + \sigma_{zx}^2\right)} < \sigma_y
\]

\[
\frac{1}{\sqrt{2}} \sqrt{\left(\sigma_{xx} - \sigma_{yy}\right)^2 + \left(\sigma_{yy} - \sigma_{zz}\right)^2 + \left(\sigma_{xx} - \sigma_{zz}\right)^2 + 6\left(\sigma_{xy}^2 + \sigma_{yz}^2 + \sigma_{zx}^2\right)} < \sigma_y
\]

The left hand side of the equation is known as the von Mises stress,

\[
\sigma_{vm} = \frac{1}{\sqrt{2}} \sqrt{\left(\sigma_{xx} - \sigma_{yy}\right)^2 + \left(\sigma_{yy} - \sigma_{zz}\right)^2 + \left(\sigma_{xx} - \sigma_{zz}\right)^2 + 6\left(\sigma_{xy}^2 + \sigma_{yz}^2 + \sigma_{zx}^2\right)}
\]

Note that in this derivation we made no assumptions about linear behavior or anisotropy of the material.
**Strain Energy Density**

Strain energy represents the elastic energy stored in a material undergoing deformation. The total strain energy in a material is the compliance which is inversely proportional to the stiffness. For an individual material element the strain energy density (\(sed\)) (local work involved in deformation) can be expressed in a form similar to von Mises stress, as a dot product of the stress and strain which has units of energy per unit volume.

Equation 12:

\[
\text{\(sed = \frac{1}{2} (\langle \sigma \cdot \varepsilon \rangle) = \frac{1}{2} \left( \sigma_{xx} \varepsilon_{xx} + \sigma_{xy} \varepsilon_{xy} + \sigma_{xz} \varepsilon_{xz} + \ldots \right) \)}
\]

With the assumption of isotropy and linear behavior we may apply the definition of Hooke’s Law for three-dimensional solids and rewrite the equation as,

Equation 13:

\[
\text{\(sed = \frac{1}{2} (\langle \sigma \cdot \varepsilon \rangle) = \frac{1}{2} \left( \sigma_{xx} \left( \frac{\varepsilon_{xx}}{E} - \nu \left( \sigma_{yy} + \sigma_{zz} \right) \right) + \sigma_{xy} \left( \frac{1 + \nu}{E} \sigma_{xy} \right) + \ldots \right) \)}
\]

which may be simplified into,

Equation 14:

\[
\text{\(sed = \frac{1}{2E} \left( \sigma_{xx}^2 + \sigma_{yy}^2 + \sigma_{zz}^2 \right) - \frac{\nu}{E} \left( \sigma_{xx} \sigma_{xy} + \sigma_{xx} \sigma_{xz} + \sigma_{yy} \sigma_{xy} \sigma_{yz} \right) + \frac{1 + \nu}{E} \left( \sigma_{xy}^2 + \sigma_{yz}^2 + \sigma_{xz}^2 \right) \)}
\]

where \(E\) is the isotropic Young’s Modulus and \(\nu\) is the Poisson’s ratio. The form of Equation 14 strongly resembles that of Equation 10 with three similar terms scaled in different ways according to the material properties. In the case of fully plastic loading, or an incompressible criteria (\(\nu = 1/2\)), Equation 14 may be simplified into,
Equation 15:

\[ \text{sed} = \frac{1}{2E} \left( \left( \sigma_{xx}^2 + \sigma_{yy}^2 + \sigma_{zz}^2 \right) - \left( \sigma_{xx}\sigma_{yy} + \sigma_{xx}\sigma_{zz} + \sigma_{yy}\sigma_{zz} \right) + 3\left( \sigma_{xx}^2 + \sigma_{yz}^2 + \sigma_{xz}^2 \right) \right) \]

\[ \text{sed} = \frac{1}{2E} (3J_2) = \frac{1}{2E} (\sigma_{vm}^2) \]

Therefore, plastically, the distributions of von Mises and strain energy will provide roughly similar trends, though one can expect differences under, at least, partially elastic loading.

**Volumetric Strain**

The volumetric strain is the summation of the normal direction strain components or the change in volume (ΔV) with respect to original volume (V₀), such that,

Equation 16:

\[ \varepsilon_{\text{vol}} = \varepsilon_{xx} + \varepsilon_{yy} + \varepsilon_{zz} = \frac{\Delta V}{V_0} \]

For an isotropic, linearly elastic material, volumetric strain may also be expressed through the summation of the normal stresses which act independently of shear components as,

Equation 17:

\[ \varepsilon_{\text{vol}} = \frac{\sigma_{xx}}{E} - \nu(\sigma_{yy} + \sigma_{zz}) + \frac{\sigma_{yy}}{E} - \nu(\sigma_{xx} + \sigma_{zz}) + \frac{\sigma_{zz}}{E} - \nu(\sigma_{xx} + \sigma_{yy}) \]

\[ \varepsilon_{\text{vol}} = \frac{1 - 2\nu}{E} \left( \sigma_{xx} + \sigma_{yy} + \sigma_{zz} \right) \]

This metric will help determine if swelling or contraction of the material of bone is significant.
**Principal Values**

An arbitrary state of stress or strain can be represented in terms of its orthogonal unit normals, \( n \). For example, in two dimensions, the traction stress \( S \) (or force vector passing through the surface, \( F \)) may be decomposed (Figure 9) from the internal stresses according to Equation 18.

\[
S = \begin{bmatrix} S_x \\ S_y \end{bmatrix} = \begin{bmatrix} \sigma_{xx} & \sigma_{xy} \\ \sigma_{yx} & \sigma_{yy} \end{bmatrix} \begin{bmatrix} n_x \\ n_y \end{bmatrix}
\]

Equation 18:

A similar argument is applicable in three dimensions. Principal directions differentiate the orientation of the coordinate system which maintains the surface traction yet only consists of normal stress components.

This transformation of the coordinate system may be expressed as,

\[
S = \begin{bmatrix} S_x \\ S_y \end{bmatrix} = \begin{bmatrix} \sigma_{xx} & \sigma_{xy} \\ \sigma_{yx} & \sigma_{yy} \end{bmatrix} \begin{bmatrix} p_x \\ p_y \end{bmatrix} = \begin{bmatrix} \sigma_p & 0 \\ 0 & \sigma_p \end{bmatrix} \begin{bmatrix} p_x \\ p_y \end{bmatrix}
\]

Equation 19:
From Equation 19, it can be seen that this problem is equivalent to the classic eigenvalue formulation,

\[
\begin{bmatrix}
\sigma_{xx} & \sigma_{xy} \\
\sigma_{yx} & \sigma_{yy}
\end{bmatrix}
\begin{bmatrix}
p_x \\
p_y
\end{bmatrix}
-
\begin{bmatrix}
\sigma_p \\
0
\end{bmatrix}
\begin{bmatrix}
p_x \\
p_y
\end{bmatrix} = 0 \Rightarrow ([\sigma] - [\sigma_p]) \mathbf{p} = 0
\]

The characteristic equation is found by solving for the determinant of the parenthetical term of Equation 20. Its roots, are therefore the principal stresses, \( \sigma_p \), and the coordinate system or principal directions are defined though the unit normal vector, \( \mathbf{p} \).

\[
\sigma_p = \left( \frac{\sigma_{xx} + \sigma_{yy}}{2} \right) \pm \sqrt{ \left( \frac{\sigma_{xx} - \sigma_{yy}}{2} \right)^2 + \sigma_{xy}^2 }
\]

**Tresca Stress**

Tresca failure criteria is similar to that of von Mises stress and is concerned with ductile failure which occurs during maximum shear stress. However, instead of considering the distortion energy, the maximum shear stress in a material element is used which can be expressed in terms of the principal values accordingly,

\[
\sigma_{\text{Tresca}} = \max \left( \frac{\sigma_{p,1} - \sigma_{p,2}}{2}, \frac{\sigma_{p,2} - \sigma_{p,3}}{2}, \frac{\sigma_{p,1} - \sigma_{p,3}}{2} \right)
\]

It can be noted that the Tresca criteria is slightly more conservative than von Mises stress.
2.5. Computer-aided Tissue Engineering

The emergence of interactive computing environments has led to computer-aided tissue engineering (CATE) – which according to Sun et al. “encompasses computer-aided design (CAD), image processing, manufacturing and solid free-form fabrication (SFF) for modeling, designing, simulation and manufacturing of biological tissue and organ substitutes [83]. This virtual environment, deemed CAD, is critical for joining components, checking part validity, exporting parts to a proper format for FEA, or preparing a part for rapid-prototyping.

The concept behind CATE for biological replacements of bone is that the complex architecture of bone is too difficult to replace with a scaffold/implant replica which takes into account the small feature sizes, mechanical properties of the hierarchical scale in which they exist, fluid flow properties (permeability) and pore connectivity, and matching biomaterial properties. Therefore, as an alternative, the replacement of a bone defect could be depicted as the assembly of smaller sub-volumes of simplified unit cells which have characteristics that are discretely known and able to be manufactured. In this way, the creation of an implant consists of the optimization problem of selecting and matching primitive shapes which match the local properties within the sub-volume they are meant to replace yet “fit” together. This is a complicated problem which brings into play several engineering tools such as 1). CAD, for the design of unit-cells, 2). FEA, for the analysis of unit cell mechanical properties, 3). Optimization, for the fluid flow and topological mechanical properties, 4). Direct fabrication using RP techniques, for the production of biomaterials, amongst others such as image-analysis, databasing, etc.
The process of CATE for a vertebral replacement can be visualized in Figure 10. Following a non-invasive CT or MRI, the contour of the vertebral replacement in reconstructed using CAD and surface-contouring methods. The peripheral boundary of the vertebral shell is isolated and the interior is replaced by smaller sub-volumes of known properties. The part is then created through the combination of RP and casting methods from a suitable biomaterial. The scaffold can then be seeded with cells or directly implanted at the surgical site.

2.5.1. Computer-Aided Design

Creating an anatomic model of a tissue is a complicated task that can be completed in several ways. In general, the process involves translating the image data from an imaging modality (which produces a stacked, series of 2-D pixel intensities
representing a volume), into a CAD model which consists of a vector-based, boundary representation (B-rep) of a solid.

The image data is converted into a solid volume in one of two ways. Two-dimensional contouring involves segmenting (typically manually) each CT slice individually by tracing the border considered to be part of the volume. CT or post-processing software will identify the surface voxels chosen to represent the border and use a marching cubes algorithm to produce a tiled surface of individual surface triangles, connecting the layers. Unfortunately, this operation does not ensure a topologically connected shape, and is not directly suitable for a vector-based CAD program. Most often user-intervention is required to check that morphing of surfaces between layers is done successfully, as typically the marching cubes algorithm will produce topological inconsistencies and missing triangles [84]. Even if the surface triangles are created successfully, the format is not directly importable into a vector-based CAD software, and each polygon will have to be manually converted into a B-rep representation.

The second method involves retaining the volumetric information of the scan. If the data is thresholded to produce the tissue area of interest, then a volumetric connectivity operation can be implemented in order to ensure which tissue portions are directly “touching” each other. This operation ensures the topology of the structure which then aids in the creation of surface-triangles. The major drawback is the extra information required to represent the whole volume of the structure, rather than 2-D contours of the surface [85].
2.5.2. Finite Element Modeling

Finite element modeling is a numerical tool developed to allow calculation of parameters within a complicated geometry. It originated out of a natural extension to be able to expand mechanical calculations from simple idealizations to actual geometries. In this way engineering components may be broken up into pieces (elements) which exhibit certain identifying characteristics and solved under global boundary conditions. Mechanical, heat transfer, fluid, convectional, and electro-magnetic systems have been solved using this method. For solid mechanics problems, properties such as elastic modulus, Poisson's ratio, and any anisotropic elasticity constants may be assigned to individual elements, upon which the problem may be solved to compute displacements, stresses, and strains. The equations that make this solution possible are derived from global solution of Newton's equilibrium equations, moment equilibrium, and the constitutive relation of elasticity.

Finite Element Derivation

Suppose trabecular bone or an implant biomaterial occupies the domain \( \Omega \in \mathbb{R}^3 \) and is contained in a given reference domain, say the unit cube. Let \( \Gamma \) denote the scaffold boundary, i.e., the boundary of \( \Omega \). The bone or implant is displaced by a force on the boundary causing a displacement \( \mathbf{u} \) of the bone or implant due to applied forces. The governing equations for elasticity (in the absence of boundary conditions), where \( b \) is a body force per unit volume acting on \( \Omega \), and \( \rho \) is the density are described by Cauchy's first law of linear momentum,

---

Equation 23:
\[ \nabla \cdot [\sigma] + \rho b = 0 \]
Equation 23 expresses how the stresses are related to the body forces assuming a linear, Taylor series expansion of the stresses in equilibrium. The constitutive equation is given in a consolidated form where redundant entries are removed, where \( \nu \) is the Poisson’s ratio, \( E \) is the elastic modulus, and the strain is defined in terms of the derivatives of displacement.

\[
\begin{bmatrix}
\sigma_{xx} \\
\sigma_{yy} \\
\sigma_{zz} \\
\sigma_{xy} \\
\sigma_{xz} \\
\sigma_{yz}
\end{bmatrix} = \frac{E}{(1+\nu)(1-2\nu)} \begin{bmatrix}
1-\nu & \nu & \nu & 0 & 0 & 0 \\
\nu & 1-\nu & \nu & 0 & 0 & 0 \\
\nu & \nu & 1-\nu & 0 & 0 & 0 \\
0 & 0 & 0 & \frac{1-2\nu}{2} & 0 & 0 \\
0 & 0 & 0 & 0 & \frac{1-2\nu}{2} & 0 \\
0 & 0 & 0 & 0 & 0 & \frac{1-2\nu}{2}
\end{bmatrix}
\begin{bmatrix}
\frac{\partial u}{\partial x} \\
\frac{\partial v}{\partial y} \\
\frac{\partial w}{\partial z} \\
\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \\
\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y} \\
\frac{\partial u}{\partial z} + \frac{\partial w}{\partial y} + \frac{\partial v}{\partial x}
\end{bmatrix}
\]

In order to solve this differential equation with the finite element method it will be necessary to estimate the displacement with basis/shape functions. By multiplying the residual of a weight function, \( w \), and integrating over the domain, it is possible to solve for the coefficients of the assumed displacement function. The weighting function allows the integral to vanish exactly, while the approximated residual itself is not zero. In the Galerkin case, the weight function is chosen as the derivative of the approximated displacement. Equation 25 expresses the method of weighted residuals.
Use of the Green-Gauss theorem allows us to decouple the volume integral in a similar way to integration by parts, which has the added advantage of reducing the divergence of stress into a term which does not require a derivative (Equation 26). This permits the use of a displacement approximation that is only first order differentiable, instead of second order differentiable.

\[ \int w (\nabla \cdot [\sigma] + \rho b) dV = \int w [\sigma]^T n dS - \int (\nabla w)^T [\sigma] dV + \int w \rho b dV = 0 \]

Rewriting Equation 26 as

\[ \int (\nabla w)^T [\sigma] dV = \int w \rho b dV + \int w [\sigma]^T n dS \]

where the left hand side will later be discretized into the more well-known “Kx”, and the right side indicates the sum of the forces (body + traction).

Now in a position to be able to discretize the domain into distinct nodes for computational purposes, it is time to select the element type. For the 8-noded linear brick element used in this case, one must assume that the displacement, \( \mathbf{u} \), within each element can be expressed as,

\[ \mathbf{u} = a_1 + a_2 x + a_3 y + a_4 z + a_5 xy + a_6 yz + a_7 xz + a_8 xyz \]

\[ \mathbf{u} = [a_1, a_2, a_3, a_4, a_5, a_6, a_7, a_8]^T [1 x y z xy yz xz xyz] \]

which makes the displacement linear in any one direction. For each node, the x and y coordinates may be substituted into Equation 28, and the coefficient matrix \([\mathbf{a}]\), may be solved in terms of \( u_1...u_8 \). Substituting the solved matrix \([\mathbf{a}]\) into Equation 28 allows one to express the displacement in terms of basis functions which relate the coordinates at any
point within the element to the coordinates of the nodes. In this way Equation 28 may be expressed as,

\begin{equation}
\mathbf{u} = \begin{bmatrix}
N_1 & N_2 & N_3 & N_4 & N_5 & N_6 & N_7 & N_8 \\
N_1 & N_2 & N_3 & N_4 & N_5 & N_6 & N_7 & N_8 \\
N_1 & N_2 & N_3 & N_4 & N_5 & N_6 & N_7 & N_8 \\
\end{bmatrix}
\begin{bmatrix}
u_1 \\ v_1 \\ w_1 \\
v_2 \\ v_2 \\ w_2 \\
v_3 \\ v_3 \\ w_3 \\
\vdots \\
\vdots \\
\vdots \\
\end{bmatrix}
\end{equation}

where \( N_i \) is the shape function determined from the columns of the solution of \( \mathbf{a} \) substituted into Equation 28, and \( \begin{bmatrix} u_1 & v_1 & w_1 \end{bmatrix} \) is the nodal displacement of the corresponding finite element. The advantage of this approach is that the displacements at the nodes are no longer a function of \( x, y, \) and \( z \), but rather depend on the shape functions.

Furthermore, the derivative of the displacements can be used to create the matrix representation of the strain (see Equation 24), which when multiplied by \( \mathbf{D} \) provides the stress. Substituting into the left hand side of Equation 27 the matrix representation of \( \text{grad}(w) \) and the discrete definition of stress, and applying the Galerkin criterion that \( w \) is equal to the test function \( N_i \), results in,

\begin{equation}
K'\mathbf{u} = \int_V [B]^T \mathbf{D}[B] dV [u_1 \quad v_1 \quad w_1 \quad u_2 \quad v_2 \quad w_2 \quad u_3 \quad v_3 \quad w_3 \quad \ldots \quad u_8 \quad v_8 \quad w_8]^T
\end{equation}

which represents the left hand side of Equation 27 for a single finite element. \([B]\) is a matrix of the shape function derivatives replacing \( \text{grad}(w) \), and \( \mathbf{D} \) was previously defined. The integral is completed numerically through Gaussian quadrature, and the element stiffness matrices are assembled into a global system matrix.

The right hand side of Equation 27 is completed by a similar method. First, assume that there are no body forces such that the first right hand side term drops out. The second term is simply the integral of the traction forces applied at the boundary. The
known forces are multiplied by the weight function, $N$, and assembled into the global degrees of freedom. There is no need to integrate each element because of the surface integral.

**Voxel Models**

Voxel models are introduced into finite element applications essentially for convenience. Voxels, or hexahedral brick elements, are a natural element choice for creating bone models from clinical or μ-CT data. Additionally, large scale models may be applied to complicated geometries in an effort to ease common meshing problems which occur at interfaces, such as the facet joints in a human vertebral body. Most often however, the purpose of voxel models is to introduce stress computations at a smaller level, such as the microstructural or tissue level. The primary difficulty regarding the use of voxel models are the large problem sizes necessary to represent elements on the size order of tens of microns.

Another common application is to solve for the elastic stiffness or compliance tensors using these methods. It has been shown, high resolution isotropic materials converge as well as non-linear, continuum models [86]. Homogenization theory is used to estimate the continuum properties of a repeated microstructure and formulates how microstructural strains are related to apparent strains [87]. In essence, it states the tissue strain is the superposition of the apparent strain and a fluctuating strain caused by a locally periodic microstructure. While the theory can estimate continuum properties and tissue level properties of cancellous bone, the accuracy is much higher for apparent properties ($\sim 73\%$). The assumption of local periodicity assumes that the microstructure
is either an orthogonal beam structure in repetition or contains a circular void, which is obviously not the physiologic case.

2.5.3. Topology Optimization

Topology optimization, the study of optimal material configurations, has received significant attention in the field of tissue engineering and implant design. For orthopedic applications the scaffold generally has inferior mechanical properties with respect to native bone, so the problem is often a formulation of a maximum stiffness material that maintains a minimum porosity for osteo-induction. Additionally, if the scaffold is designed as part of a system to release biofactors, the problem definition changes. Hollister et al solved a topology optimization problem which was able to match effective stiffness and porosity values for trabecular bone of the mandibular minipig using ceramic or polymer biomaterial properties [88]. This method was known as ‘restricted topology optimization’ (RTO) because the base material was assumed to have a 3-d pore structure where only the diameters of the pore could be adjusted. Hollister used homogenization theory to related how the topology of a single unit cell relates to a scaffold theoretically composed of that unit cell repeated infinitely (Figure 11a).

Because RTO was not able to match all nine orthotropic elastic properties of bone, a more generalized approach was used.

Figure 11. RTO (a) and generalized optimization techniques (b) to produce unit cells that compose a scaffold [89].
that was able to produced unit cells of arbitrary geometry that could match all homogenized properties [89] (Figure 11b).

Aside from matching material properties and porosities, specific scaffold/implant design needs to incorporate tissue-scaffold interactions and permeability requirements, which currently have not been developed. Moreover, the use of homogenization method where the design is composed of one, repeated unit cell, does not offer a design solution that is regionally heterogeneous as occurs naturally in trabecular.

Recently, a simulation has been proposed which described material degradation and tissue regeneration – the first of its kind [90]. Degradation is important as the concept for tissue engineering with biodegradable polymers entails a load sharing throughout the lifecycle of the implant site as synthetic material degrades and new tissue forms as visualized in Figure 12.

![Figure 12. Tissue regeneration with a degradable implant. As de novo tissue forms the mechanical characteristics of the implant change.](image)

The numerical simulation by Adachi and colleagues was able to mimic the degradation of a scaffold over 150 days, where the initial scaffold was devoid of tissue ingrowth, and the
final implantation site consists only of de novo tissue. Unfortunately, many of the assumptions are as yet unproven \textit{in vitro}. For example the degradation was assumed to occur randomly or occur through hydrolysis which is simplified through the basic diffusion equation. Additionally, models for tumor-induced angiogenesis near blood vessels as well as mechanobiological models are used to model tissue growth.

2.5.4. Rapid Prototyping

Stereolithography (SL) consists of a bath of photosensitive resin which is cured in the presence of an ultraviolet laser beam. A part is first designed in CAD and converted into a stereolithography file (STL). The file is used to build a part in a slice-by-slice fashion. A mirror guides the curing path of the laser, and after an entire layer is produced, the build platform reduces its position to make room for the next sequential layer which is bonded together through the curing process. In certain part designs, superior layers require support structures so that 'overhangs' do not occur if there is no non-bonded material beneath. In this case, the support structures need to be removed manually [91]. Additionally, a patterned mask can be used in the absence of a focused laser, so that only light passing through the mask will irradiate the photopolymer [92], leading to improved resolution such as \(\mu\)-SL. Of the RP techniques, SL is generally considered to have the highest resolution which can approach \(\sim 50 \mu m\) [91]. Unfortunately, a major drawback involves the limitation on manufacturing parts which are photopolymerizable. Some groups have mixed hydroxyapatite (HA) into the polymer in order to create composite shapes. The photopolymer was melted away and the solid
HA sintered to make a solid part [92]. Negative molding techniques can be used, but typically incur a loss in feature resolution.

The process of Selective Laser Sintering (SLS) is similar to SL in that parts are created in a layer-by-layer fashion by a laser. Instead of a liquid resin, powder particulates are used and stored in a powder bath. The powder is heated by an infrared laser to a temperature just beyond its melting temperature along focal points outlining the shape. A wider variety of biomaterials can be prepared in powder particulate form like polystyrenes, polycarbonates, and nyons, such as poly lactic acid (PLA) and polycaprolactone (PCL) which offers a major advantage [93, 94]. PCL has a compressive modulus in the lower end of trabecular bone (~60 MPa). After a layer is built, the build platform descends and a wiper spreads an evenly distributed layer of powder for the subsequent layer. Additionally, the unaffected powder offers a support structure unlike the liquid resin in SL. The resolution of SLS is determined by the powder particulate size and the laser focal size.

Three-dimensional printing (3DP) is another additive technique. The concept makes use of a powder bath, like SLS, but instead the particles are bound through a polymer binder which is printed by traditional inkjet print heads. The binder is a mix of colored resin and binder which allows parts printed in color which can be useful in conjunction with finite element methods. The major disadvantage is again materials, as the commercialized machines use starch or gypsum which makes the final parts relatively fragile. The non-bonded powder offers a support system for the powder while the solid portion is printing. Blowing away unused powder in the final stages can also be difficult if it becomes embedded in small cavities [94].
Fused Deposition Modeling (FDM) is an extrusion based system where thermoplastic filaments are melted by heating and extruded into a pattern of a part. Commercially based systems often offer a build material and a support material. The support material is automatically added to contain and transmit forces through the structure as it is being manufactured. Upon completion, the support material is removed through a chemical process, leaving the desired part. These systems have been used with PCL, Poly propylene (PP), PCL-HA, etc [93] and reported as small as 250 μm filament diameters. In recent years RP techniques have gained recognition for the direct fabrication of parts instead of prototypes.

2.6. Biomaterials

Orthopedic operations comprised more than 20% of all operations in the U.S. In the 1980s, the number one orthopedic operation was open reduction and internal fixation of a fracture, which was the 11th most common overall type of surgery [95]. Biomaterials used for bone tissue engineering are relatively sparse because of the limited manufacturing techniques available to produce highly porous, mechanically robust scaffolds. Scaffolds can be created by either direct manufacturing processes (which additively create material) or indirect means (fabrication of a mould). The best bone replacement materials are thought to be autografts (bone harvested from the same individual), followed by allografts (bone harvested from donors). Autografts are free from immune-responses and possess the osteoconductive qualities which induce bone

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formation. In some cases allografts are preferred to avoid donor site morbidity or multiple surgeries, as is the case with bone tumor removal.

Metals are useful for implant fixators and bone tissue engineering because of their superior mechanical, bio-inert properties. Porous titanium, nickel-titanium alloy, or hydroxyapatite coated titanium are some common examples. Of the top ten orthopedic operations, four of ten make use of metals: open reduction of a fracture and internal fixation (one), placement or removal of a fixator without reduction (sixth), arthroplasty of the knee (seventh), and total hip replacement (eighth) [96]. The three most common metal implant types are stainless steel (316L), cobalt alloys, and titanium alloys. Low carbon stainless steel is composed of mostly iron, with equal parts chromium and nickel. Chromium is added for corrosion resistance, while nickel is used to counteract the effect of a crystallinity change caused by the chromium addition from FCC to BCC, which has inferior mechanical properties [96]. Cobalt-chromium (~ 60% Co – 40 % Cr) alloys are used for their superior corrosion resistance, but are difficult to machine. Typically they need to be cast in high melting temperature ceramic casings. Titanium alloys can be manufactured at various strengths and are used for bio-integration and corrosion resistance when TiO₂ forms. Addition of nitrogen, carbon, or oxygen can significantly increase the mechanical properties through solid solution strengthening acts as a barrier to moving dislocations. Titanium alloys, in general, have a smaller Young’s modulus yet higher yield and fatigue strength than Co-Cr or stainless steals making them suitable for orthopedic purposes [96].

Synthetic calcium phosphates are often used for bone replacement materials because of the commonality of many the elements of bone, such as calcium. Synthetic
hydroxyapatite (HA) has a Ca to P ratio of 1.67. The commercially available product differs from unrefined HA found in bone in that it has phosphate ions instead of carbonate and is without several other impurities. The effect is artificial HA is hardly soluble in water and osteoclast resorbability is poor. Nevertheless its application as a biomaterial seems to produce contact at the bone-biomaterial interface with very little granulation. Tricalcium phosphate has also been used which as a Ca to P ratio of 1.5, but its higher resorbability is a problem despite its faster degradation rate. As a result, a biphasic calcium phosphate was developed which is a combination of HA and β-Tricalcium phosphate. The use coral derived HA/calcium carbonate composites have been used for several reasons. The coral skeletons are composed of calcite which is dissolvable and forms HA in a chemical reaction and has pore sizes in the range of bone tissue (300 μm). Unfortunately its mechanical properties are unsuitable for most implant applications.

Biopolymers are often considered for bone tissue engineering in the hopes that the degradation rate will match the regenerated mechanical properties reducing the need for a second surgery. A class of aliphatic polymers such as polylactic acid, polyglycolic acid, polycaprolactone and copolymers are the most common. One problem with these polymers which degrade by hydrolytic cleavage of the polyesters is that they lose mechanical strength well before they lose their mass [97]. Moreover, when mass is loss, it tends to occur quickly resulting in inflammation due to the acid byproducts. Nevertheless, by varying the copolymer ratio, degradation times can be tailored from several months to years [98].
Chapter 3: Investigation of Functional Adaptation Stimuli under Direct Deformation using a Reverse Engineering Approach

This objective of this chapter is to investigate a sampling of trabecular bone in order to determine some "effective" biomechanical trends in its tissue properties relevant to its mechano-biology. Subsequently, these features will be used as design goals for CATE in later chapters. Trabecular bone from the spine, subjected to various types of loading requirements, is examined. We quantify the driving force, the same way functional adaptation models of bone have described the process, as the difference between a local value and an average value. This uniformity diagnostic (UD) is used to look at various engineering measures of stress and strain, particular those which represent the extrema (have the largest or smallest UD). The extrema are likely mechanical, functional adaptation stimuli.

3.1. Introduction

Trabecular bone, the porous bone found in the spine, is a mechanically sensitive, metabolic tissue. Despite knowledge of its micro-architectural adaptability with mechanical loading, little is known of the specific mechanical impetus for the control system of cell recruitment governing this activity. This research has basic science and many practical implications such as the study of osteoporosis, specimen-specific scaffold design for guided tissue regeneration, and computer-aided tissue engineering (CATE). In computational adaptation models, several mechanical parameters produce believable bone shapes. Here, we attempt to the answer the question, "What mechanical

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3 Information in this chapter in preparation to Journal of Biomechanics
environment prevails on the surface of trabecular bone, the location of bone formation/resorption to incite its mechano-sensitive nature?" A reverse engineering approach is applied which utilizes finite element analyses conducted on virtual bone cubes of cadaveric human vertebral bodies to determine biomechanical trends. Trends were quantified with a metric representing the level of tissue uniformity and comparisons were made between types of mechanical variables. The results demonstrate that there is no difference between surface uniformity and whole volume uniformity, and that strain energy density (sed) profile is likely the most similar at any point within the tissue, corroborating the concept of high stiffness. Surprisingly, we found that von Mises stress acts as a conflicting design goal of relative non-uniformity. Of the parameters evaluated, sed uniformity was the over-riding isolatable variable. However, its fluctuation between bone samples was the largest of all parameters corroborating its sensitivity as biomechanical indicator of architecture, yet limited usefulness as a universal descriptor of bone shape.

Bone is frequently identified as a mechanically optimal material, yet the individual design goals of which it satisfies remain dubious. Understanding its biomechanics has implications for clinical research such as osteoporosis [5, 99], cancer [7], trauma [8], genetic diseases [9], and engineering research of structural and topological optimization [100].

Bone is an ideal investigatory tissue due to its high stiffness (conducive for stress based, μ-finite element analysis (FEA)) and well-known propensity for mechano-transduction [79]. Disregarding unavoidable genetic predispositions to architectural form, trabecular bone responds to physical stimuli by mechanically regulating its micro-
architecture as a result of a cellular control process [11, 13, 71, 101-108]. It is most commonly believed that osteoblasts and osteoclasts prescribe the deposition and absorption of bone, respectively, as sensed through the osteocytic syncytium—a fluid-like network connecting osteocytes embedded in a mineralized matrix. The literature is contradictory as to the mechano-sensitivity of each cell type, its relationship in the control process, and the method of mechanical stimulation (fluid shear or direct mechanical deformation) applied to the lining cells, despite a large number of studies investigating bone-cell regulation.

Functional adaptation of bone, the idea that the architecture is a result of conflicting design goals of mechanical integrity and minimum weight, has received much interest in the biomechanics community, especially after the introduction of FEA. Many design formulations have been developed. Frost proposed the mechanostat theory based on the idea that there is specified set point or strain level in which larger strains would stimulate growth, and disuses would trigger remodeling in bone [109]. Cowin developed a theory called adaptive elasticity which formulated bone as a continuum mechanics problem of a reacting chemical medium subject to thermodynamic constraints. The change in mass was governed by the difference between the strain matrix and a reference strain matrix of set points [110]. In order to simplify the unidentified reference values of adaptive elasticity, Huiskes et al. and Carter et al. used the scalar value of strain energy density (sed) as the driving force for bone modulus changes when compared to a reference sed [106, 111, 112]. Weinans et al. used an augmented formulation where the density was updated in time through an effective, apparent (whole bone) sed representing the average loading state of bone [113]. Beaupre et al. developed a model where the driving stimulus
was the difference between a daily stress stimulus and an equilibrium attractor state. This stress stimulus was defined as the average between the effective stress experienced over the different loading types which occur during a day. The apparent stress was related to the effective stress through empirically fit equations [62, 114]. Mullender and Huiskes proposed a more phenomenological approach which considered osteocytes as the origins of the mechanical excitation whose signal is dissipated through an exponentially decreasing distance function to the surface. The signal is based on the difference of the *sed* rate and an equilibrium rate. In this way, an individual surface cell receives the summation of local, osteocyte signals [79, 115-117]. These simulations have been able to predict many facets of the remodeling cycle such as the emergence of new trabecular orientations based on loading patterns, trabecular thickness, connectivity and mass as is seen in disuse osteoporosis, and the effects of estrogen deficiency [116]. In all these literature sources, the overarching trend is that bone mass (derived in terms of density or modulus) is augmented as a function of the proportionality between a mechanical signal and a reference value.
Regardless of the exact mechanism involved in the adaptation cycle, it can be reasonably assumed that the mechanical environment of the trabecular bone surface is a contributing factor or driving force of any resultant architectural change for several reasons. First, the surface area to volume ratio of cancellous bone is high (\( \sim 20 \)). Secondly, even with the commonly assumed hypothesis that osteocytes are the enablers of mechanical adaptation, the mechanical signal is first 'sensed' on the surface, most notably through the existence of microcracks which initiate on free surfaces. The concept that a mechanical driving force is directly related to the level of uniformity on the surface is also well justified. Recently, a study using a simulation in which the surface layer of trabecular bone attempted to reach a constant level of strain energy density by either

![Figure 13. Schematic of image-based finite element modeling. Trabecular bone cubes (a) were isolated virtually from the native vertebra, translated into finite element models (b, shaded), and subjected to various loading conditions. Mechanical parameters were isolated on the surface elements of the bone cubes (b, solid). Histograms were calculated which describe the uniformity of the surface (c) and a uniformity diagnostic was calculated (d) which is function of the mechanical parameter, its mean, and mode.](image-url)
adding or removing material, found that the resultant architecture was synonymous with the outcome of the structural optimization formulation of maximum stiffness [118]. This compelling result reveals that a bone adaptation model concerned with only local surface uniformity changes can produce a globally optimized structure. In fact, the relationship between local strain energy density uniformity and global maximum stiffness has been established and applied to the hierarchy of trabecular bone [119, 120] to produce reasonable architectures. The idea of nature attempting to encourage structures with uniform surface energies and stresses has been in existence for some time. Mattheck identified certain curved boundaries seen in nature as boundaries of zero notch stresses, ideal for fatigue resistant engineering applications and the avoidance of micro-cracks [121]. Others have directly applied these theories of uniformity on the bone surface to simulations using stress instead of strain energy density as the objective [117].

Though the existing computer models are successful in predicting expected trends and changes in fabric direction, they nevertheless lack the specificity to detect differences between independent mechanical parameters [80]. Many studies have taken the approach of developing a numerical model of functional adaptation and comparing that to the architecture of bone. The goal of our study was to provide a direct measure of the surface environment of excised trabecular bone samples, rather than relying on fitting model parameters to match experimental data as a validation. To investigate possible mechanical driving forces of trabecular bone (Figure 13), we applied the following reverse engineering methodology outlined below.
3.2. Methods

**Image-based Finite Element Modeling**

Sixteen human, thoracic level number 9 (T-9), vertebral bodies were scanned (70 kVp, 114\(\mu\)A) at 30\(\mu\)m resolution using Micro Computed Tomography (\(\mu\)-CT) (\(\mu\)CT 80, Scanco Medical AG, Bassersdorf, Switzerland). Twenty virtual bone cubes (5mm edge length) were extracted from random locations within vertebral bodies using an image editing software (ANALYZE, Analyze Direct, Inc., Lenexa, KS) and resampled into 50\(\mu\)m voxel dimensions. The binary data was translated into tetrahedral finite element meshes (in order to overcome the surface artifacts associated with sharp-angled voxels noticed in a preliminary study) using a custom written program. The elements consisted of standard 4-noded tetrahedral elements. FEAs were conducted using ABAQUS 6.7-1 (Abaqus, Inc., Pawtucket, RI), with linear, scalable material properties, using an isotropic tissue modulus [122], under a variety of simulated loading conditions including

- unconfined compression in the dominant superior-inferior (SI) spinal axis
- unconfined compression SI with a 10 degree, anterior-posterior offset (toward the posterior)
- unconfined compression SI with a 25 degree, anterior-posterior offset (toward posterior)
- scaled triaxial compression (scaled triaxial compression involves applying displacements in all three orthogonal planes according to the measured relative stiffness. For example, the stiffest direction will receive the largest strain, which has the effect of producing a relatively uniform stress state, internally)
- unconfined compression in conjunction with medial-lateral (twisting) shear strain
unconfined compression in conjunction with bending (flexion) shear strain

**Statistical Data Analysis**

Twelve mechanical parameters \( m \) [maximum principal strain \((\text{max.pr.e})\), minimum principal strain \((\text{min.pr.e})\), absolute maximum principal strain \((\text{abs.max.pr.e})\), absolute minimum principal strain \((\text{abs.min.pr.e})\), volumetric strain \((\text{evol})\), von Mises stress \((\text{vms})\), maximum principal stress \((\text{max.pr.s})\), minimum principal stress \((\text{min.pr.s})\), absolute maximum principal stress \((\text{abs.max.pr.s})\), absolute minimum principal stress \((\text{abs.min.pr.s})\), strain energy density \((\text{sed})\), and tresca stress] were recorded and isolated for the centroid of each element of the trabecular structure. An index was tabulated in order to define the uniformity of the mechanical field variable according to a histogram. A single large peak in the histogram indicates a majority of the elements have a common value, thus supporting uniformity, while a flat histogram with multiple, smaller peaks indicate a wide distribution in the data. The uniformity diagnostic (UD) metric was tabulated as,

\[
\text{Uniformity Diagnostic (UD)} = \sum_{i=1}^{n} \left( \frac{1}{2} \frac{v_i}{v_{\text{tot}}} \left( \hat{m}_i - \frac{v_i}{v_{\text{tot}}} \sum_{i=1}^{n} \hat{m}_i \right)^2 + \frac{1}{2} \frac{v_i}{v_{\text{tot}}} \left( \hat{m}_i - L_{\text{mode}}(\hat{m}_i) \right)^2 \right)
\]

where \( \hat{m}_i \) is the scaled, normalized \((w.r.t.\ its\ maximum\ value)\) mechanical parameter (i.e. stress, strain, etc.) which is a function of the displacement field, \( n \) is the number of elements (either surface or whole volume), and \( L_{\text{mode}} \) is an operator calculating the most frequently occurring value of \( \hat{m} \). The uniformity diagnostic included two parts: 1) the contribution due to differences between the distribution and its mean, and 2) the contribution due to differences between the distribution and its mode. Each element is
weighted to so that its contribution to the diagnostic depends on its volume fraction, $v$. Smaller magnitudes of the diagnostic concluded that the tissue is more uniform, while larger values signaled the existence of mechanical gradients of $m$.

A single analysis (1-way-ANOVA) using solely data from unconfined compression (the major mode of physiological loading in the spinal column) contributed to knowledge regarding significant differences between the mechanical parameter type. Two-way-ANOVA provided the tool to distinguish the interaction effects of loading type and mechanical parameter. Three-way-analysis-of-variance aided in deciphering between the main effects of trabecular samples, mechanical parameter type, and loading scenario. It was assumed that all three categories would contribute to the variation in the numerical model. In all cases, the Tukey-Kramer method was applied for multiple comparison testing, with probability of type I error, $\alpha = .05$. 

Figure 14. Conversion of image data to tetrahedral element meshes. Binary data sets (A) were converted into tetrahedral elements (B) through a combinatory method of surface fitting and volume meshing.
Figure 15. Process of isolating surface tetrahedrals. Surface voxels (B) are easily isolated from the original volume set (A). The tetrahedral mesh (C) is superimposed onto the surface voxels to produce surface tetrahedrals (D).

**Trabecular Bone Surfaces and Volumes**

Based on our hypothesis that the uniformity levels of mechanical distributions on the surface of trabecular bone would be an indicator of the driving force, we conducted an introductory comparison of the bone surfaces versus whole volumes. Tetrahedral element meshes were created through binary voxel datasets of a bone cube (Figure 14). First, individual elements of the bone cube which did not have connected neighborhoods (6 - element connectivity) were removed to ensure a single volume. Next, an iso-surface was fit to the data in MATLAB 6.5 (Mathworks, Inc., Natick, MA) using the
"isosurface" command and the vertices and connectivity were written to an ABAQUS input file for a 3-noded triangular element. In ABAQUS the surface mesh was converted into a volume mesh through a built-in command. The volume coordinate data were re-exported. Surface tetrahedrons were identified by a superposition of the tetrahedral mesh onto the surface layer of the voxel mesh as visualized for a simplified 2-D case in Figure 15. Surface voxels (B) were identified from the volume dataset (A). The tetrahedral mesh (C) was mapped onto (B) resulting in those tetrahedral elements which have one or more overlapping nodes in the vicinity of a surface voxel (D). Ten bone cubes were subjected to uni-axial unconfined compression and the uniformity diagnostic was calculated for all parameters according to Equation 31 for two cases of \( n \) representing surface elements only and all elements, respectively. Statistical comparisons were made using a two-tailed paired \( t \)-test, and it was determined that there was no statistical difference between whole-volume uniformity and surface-uniformity for any mechanical group. For all subsequent analysis whole-volume uniformity was conducted.

3.3. Results

Unconfined compression is the load case predominately seen in the spinal column during non-traumatic motion. Analysis of this loading case provides the most accurate picture of trabecular bone’s static constraints. One-way-ANOVA comparing the means of the twelve mechanical parameters within each of the modeled bone cores suggested a statistical significant difference \( (p<.05) \) between them. After post-hoc analysis of the same parameters (Figure 16), it was found the non-uniformity of mechanical parameters could be separated into at least two groups with increasing UD: \([sed, max.pr.s] <\)
Von Mises stress was the most non-uniform of the mechanical parameters and was statistically more non-uniform than five other mechanical parameters. Additionally, the normalized standard deviations (w.r.t. mean values) which represent fluctuation due to bone sample architecture were similar for most parameters except for sed (Figure 16, Figure 17) during unconfined compression. Strain energy density had the largest normalized standard deviation, or variability in the uniformity metric across bone samples, of all the mechanical parameters investigated. Overall, sed and vms profiles represented the two extrema in the mechanical uniformity. The behavior of the sed profile was such that, with respect to other parameters, it maintains the most uniform distribution of values and is likely the most similar at any point within the tissue. Conversely vms acts in the opposite manner and is the most apt to be disjoint or variable.

![Figure 16. Summary statistics for unconfined compression. The means and standard deviations are reported for various mechanical parameters (left), and the significance comparisons of a one-way-anova (right) reveal which groups are statistically different. Overlapping markers signify no statistical difference between groups.](image-url)
To verify that the uniformity of \textit{sed} was not due to insensitivity of this parameter to the random micro-architecture of trabecular bone tissue, we repeated the analysis on symmetric polyhedra that had previously been indicated as building blocks for tissue engineered constructs [123, 124]. The results (Figure 18) showed that other architectural configurations in fact don’t exhibit the same ranking in mechanical profiles as exists in human vertebral trabecular bone. However, \textit{sed} was the most uniform for two of the three architectures tested.

The interaction effect between the mechanical parameter choice and loading type was statistically significant \((p < .05)\), which confounds the interpretation of each factor individually. Figure 19 depicts the means of each mechanical parameter over the various loading scenarios. There were minor interactions between several of the parameters. It can be seen that a large number of major interactions occur in the unconfined compression with bending load case, indicated by intersecting lines. Both, the relative rankings and magnitudes of the means changed, making the predictability of
the main effects suspect. In order to further investigate the role of bending, it was removed from the model and the interaction effect tabulated. In this case, the p-value \((p = 0.888)\) was negligible, eliminating the interaction effect. To further verify bending as the culprit, another load case was removed from the model (unconfined compression w/ twisting) which had no effect \((p < .01)\), and both twisting and bending removed from the model which had a significant effect \((p = 0.960)\). Therefore, bending is responsible for the interaction effect between loading type and mechanical parameter.

In an effort to keep bending, a common source of spinal loading, in the model and still draw conclusions about a subset of trabecular bone, samples were isolated which had no significant main effects of specimen variation. Samples S4, S5, S12 were removed and considered as outliers (Figure 20). The remaining samples were separately analyzed, again, using 2-way-ANOVA. The results show the interaction effect was removed \((p = 0.30)\), thus, main effects could be isolated (Figure 21, Figure 22). The mechanical profile ranking was very similar to the single factor analysis.

Figure 18. Comparison of mechanical parameters for non-bone structures. Three geometric structures, hollow pore (top), rhombitruncated cuboctahedron (middle), and truncated hexadron (bottom) all at a similar porosity (86%) under unconfined compression.
(Figure 16), but more of the groups were distinctly different from one another at the lower end of the spectrum ($sed < [max.pr.s, evol] < [others]$). Though $vms$ was the most non-uniform, statistically it could not be separated from $tresca$ and several other principal stresses/strains.

Also within this subset it can be seen that the loading case significantly affects the uniformity (Figure 22). More specifically, unconfined compression and its off-axis, SI counterparts were not significantly different from each other. Unconfined compression w/ twisting was not statistically different from triaxial compression, but all were statistically different than unconfined compression w/ bending.

![Diagram of interaction effects between loading types](image)

Figure 19. Interaction effects between loading types. The means of the uniformity diagnostic change with loading type. Unconfined compression and its off-axis derivatives remain relatively constant. There are only minor interaction effects with triaxial compression and unconfined compression + shear (twisting), while most of the interaction occurs during unconfined compression + shear (bending), indicating a difficulty in predicting the mechanical surface state of trabecular bone when subjected to bending.
3.4. Discussion

It is not altogether surprising that sed exhibits uniform mechanical profiles because trabecular bone has been implicated as a stiffness-optimized cellular solid. With a high stiffness material which has minimum total strain energy, it would be expected that a large percentage of the elements have a relatively constant, yet minimum value. Visually, this is depicted through histograms (Figure 23) of the bone tissue. Strain energy density has the most uniform distribution and lowest UD. Additionally, Nowak and others found that a uniform sed objective can correspond to a global, maximum stiffness criterion [118], which is confirmed through this study in the case of trabecular bone.

In the human, vertebral, trabecular samples tested, 100 % had strain energy as its most uniform distribution, 80% had max.pr.s as its 2nd most uniform distribution, and only 30 % had either tresca or vms as its most non-uniform distribution in unconfined compression. The sed outcome supports trabecular bone as at least a partly, stiffness-optimized material and all previous literature which has assumed bone was mechanically robust. Additionally, the high degree of uniformity of max.pr.s is consistent with the idea Wolff’s trajectorial theory—that bone architecture aligns toward
the principal stresses. A surprising finding, however, is that the standard deviation of the \textit{sed UD} is decidedly larger than that of all other mechanical parameters tested, and in some cases nearly twice as large (Figure 17). The subsequent interpretation must be that in comparing two randomly selected trabecular bone samples, one would expect both to have \textit{sed} as its most uniform parameter within the tissue, yet would also expect the relative level of \textit{sed} uniformity between them to be the most likely of all parameters to be different. Therefore, \textit{sed} uniformity between bone samples is capable of a great deal of fluctuation. The results of uniform \textit{sed} support its use as a functional adaptation stimulus for bone adaptation models. The high degree of deviation between specimens corroborates that it is sensitive to variations in the trabecular architecture, which we interpret as a supporting argument for its continued use in computational modeling. Unfortunately, it does not offer a complete description of bone shape as evidenced by similar rankings in non-bone architectures (Figure 18). Several mechanical or non-mechanical variables may be required in addition to provide a more complete tissue-level description.
A noteworthy finding was that \textit{vms} had the largest UD, or non-uniformity within the bone tissue. Unfortunately, \textit{vms} non-uniformity was not statistically separable from several other mechanical parameters such as \textit{tresca} and several principal stresses/strains which share its non-uniformity (Figure 21). Uniform \textit{sed} alone cannot completely account for the architecture, or trabecular bone would be composed of very basic architectural units. For example, in unconfined loading the optimal resulting architecture would be only vertical struts with no horizontal supports to meet solely the \textit{sed} requirement. Therefore, either non-mechanical design objectives must be at work, or the architecture is a result of the superposition of different load cases experienced as Beaupre suggested [62, 114]. Here, we entertain the idea that the cellular control process of bone could seek to actively maintain a prescribed non-equilibrium of \textit{vms}, as a conflicting design goal as our data suggest. It is hypothesized that remodeling is stimulated under this mechanical condition through a mechanism, not unlike other biological machinery, such as cellular transporters which use work to create ionic gradients as a driving force, and neurons which use electric potential as a driving force. Trabecular bone, being mechanical in nature,
however, may make use of stress non-uniformity as a driving force to maintain a mechanical gradient, while at the same time achieving a high stiffness (uniform sed).

Analysis of non-bone architectures gives us several important pieces of information (Figure 18). First, two of the three architectures have sed as the lowest UD. This verifies that sed does not uniquely describe bone architectures, but several load-bearing configurations. Therefore, a more complete description involving the uniformity of several mechanical variables may be relevant. Applying the statistical ranking of all mechanical bone variables seen in Figure 21 toward non-bone architectures results in discarding the hollow pore architecture (Figure 18, top) and truncated hexahedral (Figure 18, bottom) as being similar to trabecular bone. Trabeculae are commonly depicted in a simplified sense as an interconnected orthogonal rod lattice, similar to the truncated hexahedral, but that is not consistent with our data. The Rhombitruncated Cuboctahedron (middle) is the most similar to trabecular bone rankings.

Strain energy density and vms are related mechanical parameters. They are not universally coupled in uniformity as perceived through their rankings in non-bone structures (Figure 18). Though the sed profiles were not always the most uniform independent of bone, they were distinctly low. The

Figure 22. Multiple comparison plot of loading types which exhibited no interaction effect between loading type and mechanical parameter. Letters indicate statistically distinct groups (p<.05)
sensitivity of strain energy to predicting changes in architecture was observed by analyzing its comparison to $vms$ through a cross-sectional slice of trabecular bone. Recalling that $sed$ corresponds to the superposition of the stress tensors which cause a volumetric change, and those which cause distortional shape changes, $vms$ will be proportional to just the distortional part of $sed$. When we compared the mechanical profiles of a particular line of elements in an axial slice of trabecular bone (Figure 24), we found that $vms$ fluctuated in a greater way, picking up more of the changes in the mechanical environment and architecture, while $sed$ followed the same pattern of “jumps” in data but with buffered magnitude. This effect is mostly likely due to the addition of the volumetric term. Despite, this relative insensitivity in $sed$ to spatial location, the normalized UD remains the most sensitive to changes in the bone architecture.
Figure 23. Surface mechanical profiles for a single bone sample, S7. Histograms are shown plotting the percentage of the surface at various normalized values of the mechanical parameter. The values of the uniformity diagnostic are also included. *Sed* is very uniform across this sample, as well as *max.pr.s* which has a distinct peak, and *evol* while *abs.min.pr.e, vms, tresca* share the largest non-uniformity.

It was shown that the loading type significantly accounted for the averaged surface distribution (Figure 22). For example, in physiological unconfined compression, mechanical profiles were largely dissimilar across bone tissue, suggesting an incomplete use of the architecture mechanically, and the existence of definitive mechanical gradients on the surface. Also, there was very little change in uniformity for slightly off-axis loading of 10 and 25 degrees, meaning that the architecture of bone has a built-in leniency to changes in loading, and will thus only be susceptible to atypical daily loading regimes. Bending (flexion) and triaxial loading proved to emphasize uniformity of the surface, though bending is responsible for coupling the mechanical parameter with the loading type, confounding the interpretation of the individual influences.

We propose that future studies further investigate the importance of *vms* and other non-uniform engineering variables and incorporate them into computational adaptation models instead of solely a *sed* constraint.
3.5. Summary

There are several important findings presented in this study. The goal of the study was to ascertain the "effective" trends of an average piece of trabecular bone in the thoracic spine subjected to various loading scenarios that could easily occur during normal daily activities. The results were quantified according to the UD, by which low values show tissue homogeneity and large values heterogeneity. The most important conclusions were that:

- Strain energy density, maximum principal stress consistently have the lowest UD
  - Implications: Bone is partly stiffness optimized and aligned in the principal stress directions
- Strain energy density cannot fully account for the trabecular architecture because
  - Other load bearing architectures show similar features.
  - Variation in sed UD is the largest of all mechanical variables
  - Implications: Entire mechanical ranking of bone must be included for a complete description and/or non-mechanical factors included
Compression from 0 – 25 degrees off-axis is indistinguishable in UD

Implications: bone has a built in leniency to off-axis loading.

Unconfined compression + bending produces an interaction effect between mechanical variables and loading type.

Implications: bending may be the most severe load case mechanically and has the largest energy-backbone similar to a fully-stressed environment, which is corroborated through wedge fractures.
Chapter 4: Scaffold Optimization Based on Bio-mimetic Principles

In the previous chapter, we have identified certain trends regarding the mechanical environment of an "average" piece of trabecular bone from the spine using statistics. As a subset of the results, it was shown that strain energy density was the most uniform of mechanical parameters and had the lowest UD over an average of loading conditions. Several principal stresses/strains, tresca, and von Mises stress were statistically, and equivalently the most non-uniform.

The goal of this study is to design the architectural shape of scaffolds in a way that promotes the design goals of Chapter 3. Because we cannot separate a single mechanical parameter of non-uniformity it will not be practical to design for this factor. Therefore, sed uniformity which was statistically separable from other parameters will be the design goal here.

This chapter is divided into two parts which are two different methods that can be used to promote the sed distribution. In the first section we discuss a method that uses a bio-mimetic design approach similar to the concept of Frost's mechanostat theory to adjust the shape of an initial scaffold configuration. The shape is composed of equivalently sized pieces (voxels) so that the pieces may be removed and added easily to change the topology. Only voxels on the surface may be changed which mimics the process of bone metabolism. At the end of that section sed is discussed in particular. In the second method, we use a topology optimization method called "hybrid cellular automaton" to produce a maximum stiffness material. A maximum stiffness material will have a uniform sed distribution.
4.1. Voxel-Based, Surface Scaffold Design

Despite recent need-based advances in orthopedic scaffold design, current implants are unsuitable as "total" scaffold replacements. Both mechanical requirements of stiffness/strength and biological stipulations dictating cellular behavior (attachment, differentiation) should be included. The amount of mechanical stimulation in the form of stresses, strains, and energies most suitable toward implant design is presently unknown. Additionally unknown is if whole-bone optimization goals such as uniform and non-uniform driving forces are applicable to a scaffold-bone interface. At the very least, scaffolds ready for implantation should exhibit mechanical distributions (dependent on loading type) on the surface within the typical mechanical usage window of bone. Scaffold micro-architectures can be strategically shifted into that window.

The overall goal of this study was to produce micro-architectures tailored to a more uniform mechanical distribution, while maintaining the morphological properties necessary to sustain its mechanical integrity. The mechanical adjustment stimuli investigated were von Mises stress, strain energy density, maximum principal strain, and volumetric strain. Scaffold models of a similar volume fraction were generated of three initial architectures (Rhombitruncated Cuboctahedron (RCO), hollow sphere, and trabecular-like bone cube) using high resolution voxel mapping. The resulting voxels were translated into finite element meshes and solved, with a specially written iterative solver created in Fortran90, under confined displacement boundary conditions.

The result was verified against a commercial software. Once the mechanical distributions were identified one of two methods was chosen to alter the configuration of

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material in Cartesian space. The success of the alteration was judged through a diagnostic based on the histogram of mechanical values present on the surface of the micro-architecture. The first method used a compliant approach and, for the case of stress, reinforced locations on the surface with large stresses with extra material (strategically taken from the least stressed portions). The second method used a simulated annealing approach to randomly mutate the initial state in a “temperature” dependent manner.

Results indicate that the mechanical distributions of the initial scaffold designs vary significantly. Additionally, the end state of the adjustment demonstrated anisotropy shifts toward the direction of loading. Moreover, the adjustment methods were found to be sensitive both to the mechanical parameter used for adjustment and the portion of the surface adjusted at each increment. In conclusion, scaffolds may be adjusted using a mechanical surface-based objective, as the surface of the scaffold is crucial toward its in vivo acceptance. This technique provides some mathematical specificity toward the whole of computer-aided tissue engineering.

Scaffolds intended for orthopedic purposes are not carefully designed to fit the mechano-biological requirements of the implant site. This chapter presents a formulation of an optimization problem which will provide some mathematical specificity toward the whole of computer-aided scaffold engineering. The particular problem is formulated to achieve a uniform distribution of a mechanical parameter on the scaffold surface, which is hypothesized to improve the tissue-scaffold integration under load bearing applications. The choice of the optimization function is based in the bone literature, though different formulations of a “uniformity” requirement do exist. Two methods were
introduced as a first attempt to solve the optimization problem, and the preliminary results are discussed.

Bone has been identified, both in experiment and simulation, as a mechanically regulated, self-organized material [108]. The cellular components of bone respond to mechanical stimuli at the surface, though it is unknown which form of stimuli are responsible for this regulation. Models using gradients in mechanical parameters (such as strain energy density [125], and stress [117]) as well as differential models of osteoclast and osteoblast activity [79] have been able to produce “bone-like” architectures, further supporting the idea of mechanical regulation in three dimensions. Despite difficulties in localizing the effect of one particular mechanical loading type in experiments, evidence does exist for bone-shape regulation from 1). direct cellular deformation [76], 2). force intensities (stresses) through fluid flow or direct deformation [76, 126], and 3). changes in stored energy [79]. Because many of these loadings are not independent of each other, it is easy to see why specific information about their contribution to bone metabolism has remained elusive.

Nevertheless, there are definitive statements about the regulation of whole-bone one can apply to the optimization of a scaffold. Evidence exists in nature for both the uniform and non-uniform surface hypothesis. In one example, it was shown that bone responds through minimizing the stresses at the surface which is believed to reduce the probability of fatigue fracture or crack propagation [127]. Similar evidence exits for tiger claws, tree branches, tree roots, nuts, and shells [121]. We hypothesize that whole-bone optimization goals will be relevant to the tissue-scaffold interface.
Topology optimization has only recently been applied to the study of bone, as a natural extension of the theories intended for structural mechanics. These problems, however, have predominately been formulated to minimize the compliance of the structure (a scalar measure of its stiffness), which is known to have a unique solution. With respect to scaffold design, Hollister et al. has contributed to the field by applying theories of topology optimization [88, 89] to the design of a spinal fusion cage [128] while accounting for large porosities (which are necessary for fluid perfusion in vivo).

Until recently, the formulation of our scaffold design problem was not pragmatic. In other words, technology did not exist to manufacture (or even model) scaffolds in a specifically designed manner. Different types of rapid prototyping technologies such as stereolithography and fused deposition modeling now are available which are approaching the resolution of the tissue level (10 – 100 μm) [84, 129]. Some initial work in rapid prototyping systems have shown the capability of delivering cells, gels, and cell aggregates into the scaffold matrix [130]. Nevertheless, many improvements must still be made to the types of materials able to be processed, and the resolution of the feature sizes in these systems.

4.1.1. Optimization Problem

Suppose the scaffold occupies the domain Ω and is contained in a given reference domain, say the unit cube. Let Γ denote the scaffold boundary, i.e., the boundary of Ω. The displacement u of the scaffold due to applied forces is governed by the equations of linear elasticity. We are interested in finding a scaffold Ω such that a given mechanical quantity m, which depends on the displacement and which is defined
on the scaffold boundary $\Gamma$ is as uniform as possible. We use the square of the deviation of $m$ from the average,

\begin{equation}
\text{Equation 32:}
\left(\sqrt{\int_{\Gamma} \left( \frac{m(u(x)) - \frac{1}{\int_{\Gamma} dx} \int_{\Gamma} m(u(x)) dx}{\frac{1}{\int_{\Gamma} dx} \int_{\Gamma} m(u(x)) dx} \right)^2 dx}\right),
\end{equation}

as a measure of non-uniformity. In addition to achieving uniformity of $m$, we also want to maintain a certain porosity of the tissue. As a first attempt, we model this by requiring a constant volume fraction, $\int_{\Omega} dx = \text{vol}$.

Our scaffold design problem is given as follows.

\begin{equation}
\text{Equation 33:}
\begin{align*}
\text{Minimize } & \left(\sqrt{\int_{\Gamma} \left( \frac{m(u(x)) - \frac{1}{\int_{\Gamma} dx} \int_{\Gamma} m(u(x)) dx}{\frac{1}{\int_{\Gamma} dx} \int_{\Gamma} m(u(x)) dx} \right)^2 dx}\right), \\
\text{subject to } & -\text{div}\sigma(u)(x) = f(x) \quad \text{in } \Omega, \\
& u(x) = g(x) \quad \text{in } \Gamma_1, \\
& \sigma(u)(x)n(x) = t(x) \quad \text{in } \Gamma_2, \\
& \int_{\Omega} dx = \text{vol}
\end{align*}
\end{equation}

where the stress $\sigma$ and the strain $\varepsilon$ are given by $\sigma = \sigma(u) = C\varepsilon(u)$ and

\[\varepsilon(u) = \frac{1}{2} (\nabla u + \nabla u^T),\]

respectively, where $C$ is a symmetric positive definite fourth order tensor specifying the material properties of the scaffold, and where $\Gamma_1$, $\Gamma_2$ are a partition of the scaffold boundary. The unknowns in our optimization problem are the domain $\Omega$ and the displacement $u$. 
In our initial computations, we consider one of the following mechanical quantities, volumetric strain, maximum principal strain, von Mises stress, and strain energy density,

\[
m(u) = \begin{align*}
\varepsilon_1 + \varepsilon_2 \\
\max \left( \frac{\varepsilon_1 + \varepsilon_2}{2} \pm \sqrt{\left( \frac{\varepsilon_1 - \varepsilon_2}{2} \right)^2 + \left( \frac{\varepsilon_{12}}{2} \right)^2} \right) \\
\sqrt{\sigma_1^2 - \sigma_1 \sigma_2 + \sigma_2^2} + 3\sigma_{12}^2 \\
\frac{1}{2} \left[ \sigma_1 \varepsilon_1 + \sigma_2 \varepsilon_2 + \sigma_{12} \varepsilon_{12} \right]
\end{align*}
\]

where the value of \( m \) is written for the two dimensional case for brevity. When applicable the three-dimensional extensions are used. Note that all of these quantities are volumetric quantities. Hence, in the definition of the objective function we replace integration over the boundary \( \Gamma \) by integration over all the voxels that share a face with the boundary \( \Gamma \).

The scaffold design problem stated above is the initial version. The choice of the mechanical quantity, the measure of uniformity, the modeling of porosity, and components of the mathematical problem formulation are still under investigation and will likely be modified. The scaffold design problem is a particular type of topology optimization problem [131]. These optimization problems are difficult to solve. For the efficient solution of the scaffold design problem, one has to find a representation of the shape that can capture the fine scales of tissues and that allows the application of mathematical optimization. Among the approaches used are density functions [131, 132], level-set methods [133], and other implicit function based methods [134].

Another challenge arises from the size of the problem. Many partitions are required to describe the domain. These partitions reflect the method used to solve the
problem such as if the reference domain is included with the partitioning. Voxels models of trabecular bone attempting to calculate tissue level stresses and strains in a whole bone have used up to 7.6 million elements [135]. A discretization of just a portion of a whole bone still requires hundreds of thousands of elements to ensure each trabecular thickness is partitioned by several elements, thus adequately approximating changes in the displacement field.

4.1.2. Heuristic Solution Procedure

Implementation of the above optimization was done through a heuristic update procedure. The heuristic rule is that uniformity can be achieved by the addition of material in areas with a large mechanical parameter, $m$, and the removal of material with a small mechanical parameter, $m$. The heuristic procedure works by applying the heuristic to a fixed amount of material (i.e. fixed number of surface elements per iteration), thus satisfying the porosity constraint, which describes the domain $\Omega$. The procedure for the scaffold minimization problem is then (see Figure 25):

- Select in initial scaffold geometry, satisfying the porosity constraint.
- Discretize the domain into hexahedral voxel elements.
- Apply the loading conditions and compute the displacements and mechanical parameter, $m$.
- Determine which elements are in contact with the boundary, $\Gamma$.
- Coincidently remove $n$ surface elements in the lower $p$ percentile of the mechanical parameter and identically add $n$ surface elements to a location in
contact with each of the elements in the upper \( p \) percentile of the mechanical parameter (where \( p \) has the effect of changing the number of surface elements moved in each step).

- Evaluate the minimization integral, go to step 3, repeat, or exit.

![Diagram of material transfer](image)

**Figure 25.** Example of strain energy density as an objective function. Material within a vulnerable trabecular-like strut is then reinforced by transfer of material from lower energy locations. The increase in material locally acts to reduce the surface energy gradients resulting in a more uniform surface profile as the higher energies (red) tend to propagate outside gradient boundaries.

### 4.1.3. Examples

**Unconfined compression, \( m \Rightarrow \text{von Mises stress} \)**

An example problem is presented to highlight the concept of the heuristic method and its behavior. An initial architecture known geometrically as a Rhombitruncated Cuboctahedron (of 86% porosity) was chosen to represent a possible bone scaffold. The heuristic approach was applied to an unconfined loading case, where the mechanical
objective, $m$, was set equal to the von Mises stress description. Elemental material properties were described as isotropic ($E = 1000$ MPa, and $v = 0.3$). A specialized code adapted from Hughes et al. [136] and Kwon et al. [137] was written in order to more efficiently solve the finite element problem over larger domains as each repeating unit cell contained approximately 48,000 solid elements. This iterative solver was written in Fortran90 and used a preconditioned (Jacobi) conjugate gradient search path to update the degrees of freedom. A post processor was written in order to compute the desired mechanical parameters from the displacement degrees of freedom. Evolution of the shape under the applied heuristic method is shown in Figure 26.

Figure 26. Heuristic method for unconfined compression with von Mises stress as the mechanical parameter.

The success of the heuristic update scheme on the minimization of the non-uniformity may be evaluated by a histogram of the form seen in Figure 27. The normalized von
Mises stress with respect to the maximum value is plotted for a single repeating unit cell, where 3% of the surface is evolved at each step. The outcome is that over the procedure, the surface profile is contracted to the left and expanded in the ordinate. Large stress values are reduced and a greater percent of the surface is located around a single value, as per our definition of uniformity. Iterations greater than 20 do not significantly improve the profile. Figure 28 shows the effect of the heuristic on a slice of the unit cell. Stress concentrations characteristic of sharp corners are ameliorated by an induced curvature and cross member thicknesses are increased in the loading direction and reduced in the non-loading directions (loaded in the + x-dir).

**Confined compression, \( m \Rightarrow \text{von Mises stress} \)**

The same example was analyzed for the case of confined compression, which signifies that the non-loaded faces of the unit cube are prohibited from displacing in the direction parallel to their respective normal vector (no Poisson expansion). The histogram results are shown in Figure 29. In this more complex loading case, the heuristic method improved the initial state, creating a shape with a superior surface profile. In subsequent
steps (>20), the surface profile is not significantly improved, thus implying a local minimum of non-uniformity. The resulting shape's fabric direction is oriented with the loading (not shown), though a larger percentage of struts in the off-axis direction are preserved. Confined compression provides some reaction force in the non-load bearing direction.

Effect of surface front, $m \Rightarrow$ von Mises stress

The rate at which the heuristic procedure progresses, as well as the equilibrium state, depends on the rate at which the shape evolves. The effect of this surface front was investigated qualitatively by comparing the results of a 10% surface front at each step with a 3% surface front. The front is indicative of what portion of the surface is forced to change at each step. The results are presented in Figure 30 for an unconfined loading description. The larger surface front (10%) does not capture the equilibrium peak which can be found in the smaller surface front (3%). In fact, the larger surface front diverges into a shape with an inferior surface profile in later steps.

Effect of mechanical parameter, $m_1 \Rightarrow$ von Mises stress, $m_2 \Rightarrow$ strain energy

The selection of the mechanical parameter to be optimized also has an effect on the success of the heuristic. The influence of the mechanical parameter is demonstrated in an
example showing the histogram results of von Mises stress and strain energy density for
the same initial architecture, under
confined compression (see Figure 31). The strain energy histogram is uniquely
different from that of the von Mises stress despite the fact stress is linearly related to strain. The result of the sed optimization is inherently more uniform, such that, improvement from the heuristic is seemingly less significant than that of the von Mises stress case. However, the mechanical sensitivity of bone cells to these stimuli is unknown. Hence, even small improvements may be perceptible.

**Alternative Method**

The minimization of Equation 33 may be solved in additional ways. Simulated annealing is an example of a random search technique that can be used [138, 139]. For an overview see Kirkpatrick et al. [140]. Briefly, like all metaheuristic techniques, this process uses an intelligent search method to compensate for a lack of information on the derivative of the objective function. An algorithmic analogy to the metallurgical process of annealing is made. In annealing, the minimum energy state of the metal depends on the rate of
cooling. Rapid cooling will result in a non-equilibrium state. Algorithmically, a global solution will be found if local minima are avoided. To this end, a “temperature” variable (sensitivity) is included so that trial solutions resulting in a worsened value of the objective function can be accepted with some probability. Thus, a more robust search is done which is less likely to stagnate in a local minimum.

In the scaffold design problem, trial solutions representing possible scaffold configurations are constructed and the integral of Equation 33 is evaluated. An augmented, discrete version of the integral was used as a diagnostic,

\[
\sum_{i=1}^{N} \left( \frac{\hat{m}(u(x))}{N} - \frac{1}{N} \sum_{i=1}^{N} \hat{m}(u(x)) \right)^2,
\]

where \( \hat{m} \) is the normalized mechanical parameter with respect to the maximum value, and \( N \) is the number of boundary elements. An example is illustrated on a small, cross-beams architecture (Figure 32). Confined compression was used with \( \hat{m} \) as the normalized von Mises stress. New trial solutions were created by mutating a defined percentage of the surface, which is a function of the temperature.
sensitivity. At the initial sensitivity, 20% of the surface was adjusted, and there was ~ 80% probability of accepting a larger value of the diagnostic. As the sensitivity decreases, the scaffold shape is less likely to change. The approach is successful as can be seen by the reduction of the diagnostic, but requires a significant number of iterations. Each evaluation necessitates the solution of the finite element problem, making it only suitable for 2-D or small 3-D problems.

4.1.4. Discussion

A mathematical optimization problem was presented for bone scaffolds, based on the literature, using a particular formulation of minimizing the “non-uniformity” for various mechanical parameters. Investigation of the relative contribution of the mechanical parameters in vitro is pending. In order to implement the optimization on a large scale, a heuristic method was chosen. The heuristic demonstrated that the uniformity on the surface was improved under multiple loading cases. Nevertheless, because of the implied nature of a heuristic, there is no guarantee of improvement at each step. The method appeared to find local minima.

Figure 32. Initial cross-beams architecture (top) and simulated annealing result of uniformity diagnostic (bottom).
(see Figure 29) as little improvement was witnessed after ~ 20 iterations, and depended on the size of the surface front at each step (see Figure 30). The heuristic was not equally successful on all forms of the mechanical parameter, even though all are derived from the displacement. Additionally, all possible solution spaces will not be encountered using this method. For example, in Figure 26 we find that vertical beams are formed in the loading direction. However, the number of beams is identical to that of the initial architecture. In this respect, the method seems to have a finite capacity to merge beams and fill/create holes to produce any shape. The method, then, is very dependent on the initial architecture selected which has both negative and positive attributes. First, the scaffold may be initially designed toward other optimization goals (i.e. compliance). Conversely, it is more time consuming and manual for the designer. Other types of methods exist to solve Equation 33. Simulated annealing was presented as one option which uses a random mutation of the trial solution. This method is not suitable for large problems, and uses no information on the derivative of the minimized variable. Consequently, its behavior is not partial toward a particular choice of $m$. Ideally, gradient methods would be used; however, it is unclear how to represent the shape with such methods. A density representation of the shape would require the solution of the finite element problem over the entire reference domain, and does not clearly define a surface. Implicit surfaces or level-set methods would also require additional computations in order to identify the border of the boundary, $\Gamma$.

It is important that scaffolds intended to replace bone resemble, in structure and function, some aspects of the native site. While an exact replication is not required, it is important that certain "bone-like" features be preserved. For example, the transformation
in shape seen in Figure 26 is highly divergent from trabecular bone. One way this problem could be eliminated is through more stringent porosity constraints. Porosity constraints over the entire domain are insufficient to regulate many morphological features important toward describing trabecular architecture such as pore size, trabecular thickness, etc. Future work will involve applying the porosity constraint over sub-domains. Unfortunately, the heuristic method is not well suited to more complicated constraints because it is unclear how to change the heuristic rule for partitioned sub-

Figure 33. Evolution of RCO unit cell after A). 0 Iterations, B). 2 Iterations, C). 5 Iterations, and D). 9 Iterations, in unconfined compression.
Additionally, the uniform surface hypothesis does not require the scaffold to be geometrically homogeneous. There are potentially a number of solutions that will have an acceptable "uniformity" on the surface. Using an initial scaffold starting point that resembles trabecular bone can account for some of this heterogeneity. Some initial solutions will satisfy Equation 33 more than others, so it is important to determine at the start if the purpose of the adjustment method is to provide topology changes (independent of the initial guess) or merely shape improvements to existing topology. Topology changes could represent extrema of the mechanical state which is important for basic science research, while shape changes have more relevance to the practical implementation of implantable scaffolds, where some information about the scaffold morphology is already known.

4.1.5. Discussion – Strain Energy Density

Strain Energy Density, in particular, was the parameter isolated from Chapter 3.

Figure 34. Strain Energy Density plot of RCO unit cell at Iteration 0 and Iteration 9.
We looked at the evolution of the RCO unit cell using the voxel-based heuristic method and a 10% surface front in Figure 33. Characteristically, under this loading condition vertical struts are formed. A display of the strain energy contours for the initial and final shape is depicted in Figure 34. One can see the resulting shape is not necessarily relieved of peak stresses. In fact, the peak stresses increase, particularly near the cell interfaces whose skeleton topology is not permitted to evolve for loading condition purposes. The overall distribution of strain energy is toward the average of the initial configuration (i.e. near the yellow-green-aqua color scheme) which increases some of the initially lower values and decreases some of the initially higher values.

The effect on the uniformity diagnostic itself is that throughout the iteration history, most of the parameters achieve smaller UD or increased uniformity (Figure 35). Strain Energy Density, in particular, achieves the lowest value which makes the proposed heuristic method applicable for our purposes. Unfortunately in this process, we have no
control of the non-sed parameters. For example, minimum principal strain and von Mises are non-uniform in bone, but not in our scaffold optimization solution.

The voxel-based surface method does not perform as well in other types of loading conditions. For example, in unconfined compression + bending the heuristic solution procedure was able to produced scaffolds with material anisotropy (Figure 36).

However, the results of the UD reduction show only a moderate improvement in strain energy density in early iterations followed by an increase in the UD of most mechanical variables (Figure 37). The reduction of max.pr.e was an unexpected result of the optimization.

Figure 36. Evolution (in profile view) of RCO unit cell after A). 0 Iterations, B). 2 Iterations, C). 5 Iterations, and D). 9 Iterations, in compression + bending. The bending vector is in the -x direction.

Figure 37. Adjusted RCO unit cell in compression+bending (left), and UD reduction (right).
While in both unconfined compression and unconfined compression with bending, the magnitude of the *sed* UD is significantly reduced, the rankings of the mechanical variables changes only in a minor way. For example in both load cases *sed* of the unoptimized and optimized shapes remain the lowest. Moreover, *tresca* and *vms* remain in the non-uniform spectra of the rankings consistent with trabecular bone. The most notable change in ranking was *max.pr.e* which moved positions in ranking toward a more non-uniform state. Most other variables moved one or two positions at the most as seen in Figure 38.

![Figure 38. Rankings of mechanical variables before and after voxel-based surface adjustment in two different load cases.](image-url)
4.2. *Implicit Hybrid Cellular Automaton Method for Scaffold Design*\(^5\)

A hybrid cellular automaton (HCA) method was adopted which attempted to solve a global objective function by reformulating the problem as a local application of rules to individual neighborhoods of elements. It was discovered that bone adaptation models based on sensing and receptor cells of local regions could produce geometries consistent with the global outcomes from the field of topology optimization, which led to the development of HCA. Here, we incorporated the design variable as the distance from the iso-surface representing the boundary of the topology. By this, the shape of the structure is known at every point during the optimization process unlike density-based methods which suffer from intermediate densities. The implicit field is expressed in terms of nodal-shape functions so different types of problems may be solved such as those with interfaces not-coincident with mesh boundaries, and surface-objective functions. An extended finite element method is used on a structured mesh, which makes use of regular cellular automaton lattices possible. The method does not require computations of numerical gradients and may be useful for large-scale, 3-D problems.

The hybrid cellular automaton (HCA) model for topology optimization is a method that was recently developed and inspired by the phenomenological activity of bone-cell metabolism [141]. In particular in trabecular (or spongy) bone, a local driving force encourages material in regions which are ‘over-stressed’, and removes material in regions which are ‘under-stressed’. The mechanism of material addition and resorption is through its cellular components such osteoblasts and osteoclasts, respectively, which interpret the mechanical signal. The exact mechanical signaling pathway and stimulant is

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\(^5\) Information in this chapter in process of submission to *Computer Methods in Applied Mechanics and Engineering*
under investigation, but strain energy density \((sed)\) appears to work well in computational models.

It was discovered that ordinary differential equation models mimicking the process of bone cell metabolism produced architectures that resembled shapes seen in the field of topology optimization, specifically those of minimum total strain energy or compliance [118]. Though we now understand that the mechanical objectives of bone are more complex than simply maximum stiffness, these early models inadvertently bridged a gap between local phenomenological rules and the global objective of maximum stiffness.

Cellular Automaton (CA) is a process of applying local rules to a partition of a larger domain in order to produce a "self-organized" global design [142]. In CA, the domain is discretized into square, equally sized lattice cells and the design variables (e.g. defining the topology) are given finite values. For every time period, the same local rule involving a limited neighborhood of surrounding cells is applied to each lattice cell in a simultaneous fashion. The result is used to update the design, and the process is repeated until a convergence is reached.

CA was originally formulated by von Neumann in the 1940s who was investigating the idea self-replication in biology, and the idea of robotic self-assemblies [143]. However, interest in the subject was nurtured when a thought experiment was proposed by Conway by which simple rules were used to determine a two-state, black or white, condition of a 2-D automaton [144]. This simulation known as the "Game of Life" produced a complex picture of a matter oscillating between order and disorder, producing intricate patterns despite the fundamental simplicity of the local rules. Consequently, this spawned interest in the concept that much of nature's complexity is the result of
locally discrete rules which gives rise to global self-organization. In practice, CA is implemented when the governing equations are unknown, but certain inferences may be made about individually confined interactions. CA has been used to study traffic patterns, earthquake and forest fire propagation, proliferation of cells on Petri dishes, liquid and fluid diffusion, crack propagation, etc. [145-151].

There are several examples of the application of CA toward structural design [141, 142, 152-154]. Inou et al. used CA by comparing an effective stress with a reference stress in a local region of elements. The result was used to adapt the Young's modulus. If the modulus became excessively low it was removed, thus changing the topology of the structure. The theory of evolutionary structural optimization (ESO) shares many similarities with CA, though is not explicitly linked. In this process an unchanging reference stress is compared with each element, and if the current stress is less than the referenced stress it is removed from the model [154]. Later, bi-directional ESO methods were used to add material in highly stressed regions [152]. While ESO is not implemented on a neighborhood of elements, it does contain a local update rule and reference value like CA.

Most recently, Tovar et al. introduced a method which used the volume fraction of a cell (density) as the CA variable of interest, and the finite element method (FEM) to evaluate the field states, which they termed hybrid cellular automaton (HCA). The prefix 'hybrid' was coined to denote a process which utilized the FEM solution of the entire structure in order to update the local variables, where typical CA does not. The method produced optimal structures, but made use of density penalizations in order to drive each element's volume fraction to the empty-filled (0-1) state. In their method, the average
sed is evaluated in a local neighborhood for every lattice point. The size of the local neighborhood can vary, and will be discussed later. The local driving stimulus is the difference between the averaged neighborhood value and a reference sed which is predetermined. The cellular automaton activates the remodeling process according to a function of the computed driving stimulus. Different stimulus functions have been shown to affect the computational tendencies of the process. Tovar et al. proposed a Proportional-Integral-Derivative (PID) control system in order to magnify and/or dampen the signal. In this study, we only consider proportional control.

Topology optimization is the process of determining the optimal layout of material in a specified design space. Typically it is mathematically rigorous and necessitates expressing the quantity to be optimized, and deciding on an update procedure which is a function of the gradient of the constraints and the objective function. Many types of methods have been proposed, but most prevalent are density methods where the topological characteristic is the volume fraction of a cell. In the SIMP (Solid Isotropic Material with Penalization) approach, intermediate densities are penalized, and in the homogenization approach, a microscopic void structure is imposed on a cell and related to the macroscopic volume fraction, so that the final topology is the superposition of cells with different void sizes [132].

Two methods of interest have been employed which have not used volume fraction (density) as the topological characteristic, but have used an implicit function definition of the boundary. The level set method updates the boundary of the topology by solving for the velocity of the moving boundary front according to the optimization criteria [133]. The regularization method, numerically relaxes the Heaviside step (impulse) function so
that it may be differentiated, and standard topology updates schemes may be applied [134].

In this study, we combine the extended finite element method (the solution of the finite element problem which incorporates the Heaviside of the implicit function into the weak form of the equilibrium equations), and the HCA approach. The result is a design tool which appears to be robust, is computationally efficient, conducive to parallel computing, and allows for the solution of a sub-class of problems that would not otherwise be possible with density-based methods. The method avoids most mesh dependent artifacts and checkerboarding, most likely because it makes use of an averaged local neighborhood of elements, effectively acting as image filtering technique. The only negative consequence is that because a global objective function is never formed, a relationship between the problem definition and local rules needs to be established \textit{a priori}.

4.2.1. \textit{Problem Definition}

In this section the optimization problem is described. The optimization goal is to find the structural design $\Omega$ with boundary $\Gamma$ that provides an optimal material. We assume the material in $\Omega$ is homogeneous, linearly elastic, and subject to small deformation plane stress. The design is restricted to the design space of $\Omega_{\text{des}}$. We define the topology (and cellular automaton) quantity as the implicit, distance function from the boundary isosurface so that the structural design $\Omega$ is defined by the implicit function, $\phi(x)$,
Equation 35:
\[
\phi(x) = 0 \quad \text{on } \Gamma \\
\phi(x) > 0 \quad \text{inside } \Omega \\
\phi(x) < 0 \quad \text{outside } \Omega
\]

In a restricted space, the implicit function can be considered a surface as visualized in Figure 39. In this way, as the contours of the surface morph, so do the boundary and solid portion of the structure. Additionally, we impose the constraint on the upper and lower bound of the implicit function,

Equation 36:
\[
\text{if } |\phi(x)| > \alpha, \text{ then } \phi(x) = \alpha \text{sign}(\phi(x))
\]

to contain the range. The Heaviside function \( H \) is introduced to integrate a physical quantity \( f \) over only the solid portion of the structure \( \Omega \),

Equation 37:
\[
\int_{\Omega} f \, d\Omega = \int_{\Omega_{\text{sol}}} H(\phi(x)) \, f \, d\Omega
\]
\[
H(\phi(x)) = \begin{cases} 
0 & \text{if } \phi < 0 \\
1 & \text{if } \phi > 0
\end{cases}
\]

To define the problem we reference the relationship observed between global-local problem definitions [118, 120], and draw the analogy of minimizing the total strain energy Equation 38a, with the expression of minimizing the non-uniformity (maximizing the uniformity) of strain energy density across the design domain at each infinitesimal point \( i \) (Equation 38b),
Equation 38:

\[
\begin{align*}
\text{Minimize} & \quad \int_{\Omega_{\text{ext}}} H(\phi(x)) e(x)^T D e(x) d\Omega \\
\text{Minimize} & \quad \int_{\Omega_{\text{ext}}} \left( \lim_{A \to 0} \frac{1}{A} \int_{A} H(\phi(x)) e(x)^T D e(x) d\Omega - \frac{\int_{\Omega_{\text{ext}}} H(\phi(x)) e(x)^T D e(x) d\Omega}{\int_{\Omega_{\text{ext}}} H(\phi(x)) d\Omega} \right) d\Omega
\end{align*}
\]

where \( e \) is the consolidated strain matrix, and \( D \) is the elasticity matrix for the plane stress condition. The expression of Equation 38a is the structural optimization problem of maximum stiffness usually subjected to a restriction on the volume fraction. Equation 38b suggests adding up the difference between the infinitesimal strain energy density at point \( i \), and the total strain energy per unit volume, across the design space.

The second term of Equation 38b represents the \textit{sed} of the design domain, which is known to be constant for an optimal material, and is the result used for the heuristic update scheme in topology optimization of Equation 38a. If it were not constant, it would indicate that one point of the structure stores energy in more or less efficient
manner than an adjacent point, but this is not the case for a maximum stiffness material, that is homogeneous and isotropic at every point. We can choose any value for the constant \( s_{ed} \), but intuitively it makes sense to use the initial configuration for entire design space for an artificial time, \( t = t_0 \).

\[ U_{ref} = \frac{\int_{\Omega_{ref}} H(\phi(x)) e(x)^T D e(x) d\Omega}{\int_{\Omega_{ref}} H(\phi(x)) d\Omega} \bigg|_{t = t_0} = \text{constant} \]

In order to solve the integral of Equation 38b, the integrand must be minimized for every point which produces a local minimization problem – a correspondence established by Tovar et al [120],

\[ \text{Minimize } |e_i|, \]

\[ e_i = \left( \frac{1}{A_n} \int_n U dn \right) - U_{ref} \]

The local error/driving function to be minimized, \( e_i \), is the difference between the average, strain energy \( U \) of a local area neighborhood \( n \) with area \( A_n \) and a reference strain energy, \( U_{ref} \), taken to be the average strain energy of the initial configuration.

Smaller local neighborhoods should more accurately approximate Equation 38b as limit of the area is reduced. Reformulating Equation 40 in terms of \( \phi(x) \), the definition of strain energy, and the Heaviside function yields,
Equation 41:
Minimize \( |e_i| \),  
\[ \text{s.t. } -\alpha \leq \phi_i \leq \alpha, \quad \alpha > 0 \]
\[ e_i = \frac{1}{A_n} \int \int_{\Omega} H(\phi(x)) \varepsilon^T D \varepsilon d\Omega - U_{\text{ref}} \]

The solution of Equation 41 involves determining the strains within the structure. The governing equilibrium equations for the elastostatics problem with the absence of body forces may be converted to the weak-form equations using the principle of virtual work (For details see [134, 142]),

Equation 42:
\[ \nabla \cdot [\sigma] = 0 \quad \Rightarrow \int_{\Omega_{\text{des}}} H(\phi(x)) \delta \varepsilon^T \sigma d\Omega = \int_{\Gamma_t} \delta u \cdot \tilde{t} d\Gamma \]

where \( \sigma \), \( \Omega_{\text{des}} \) have been defined previously, \( \Gamma_u \) and \( \Gamma_t \) denote the displacement and traction-prescribed boundary conditions of the whole boundary \( \Gamma \), \( [\sigma] = [\sigma_y] \), \( \sigma \) is the consolidated stress vector of non-redundant entries, \( u \) is vector of nodal displacements, \( \delta \) is the variational test function, and \( \tilde{t} \) is the applied traction vector of components \( t_i = \sigma_y n_j \), where \( n_j \) is the outward normal of the \( x_j \)-th component.

4.2.2. Implicit HCA Discretization/Solution

To solve the posed local design problem of Equation 41, \( \Omega_{\text{des}} \) must be discretized for the calculation of strain energy, and an update procedure for \( \phi(x) \) must be chosen which reliably minimizes the driving force, \( e_i \). The implicit function is defined in terms of the nodal quantities of its corresponding finite element,
Equation 43:
\[ \phi(x) = \sum_{l=1}^{n_{\text{nodes}}} N_l(x) \phi_l \]

where \( N \) are the standard shape functions, and \( \phi_l = \phi(x_l) \) where \( x_l \) are the nodes.

Structured finite elements are used because the standard cellular automata neighborhood consists of a regular neighborhood of equally sized regions. Rewriting Equation 41,

Equation 44:

Minimize \(|e|_l|\),

s.t. \(-\alpha \leq \phi_l \leq \alpha, \quad \alpha > 0\)

\[ e_l = \left( \frac{1}{A_n} \sum_{n} \int_{A_n} H(\phi(x)) \mathbf{e}^T \mathbf{D} \mathbf{d} \right) - U_{\text{ref}} \]

where \( \Omega_e \) is the element domain, gives the discrete problem definition. We define the update points to be the nodes, not the elements, because of the nature of the implicit function definition Equation 43. The weak form of Equation 42 reduces into a linear system of equations,

Equation 45:

\[ \sum_{e=1}^{N_e} K_e u_e = \sum_{l=1}^{N_l} f_{\text{app}} \]

which is the summation over \( \Omega_{\text{des}} \) consisting of \( N_e \) finite elements and \( N_l \) boundary elements with discretized applied traction forces \( f_{\text{app}} \). The elemental stiffness matrix is described as,

Equation 46:

\[ K_e = \int_{\Omega_e} H(\phi(x)) \mathbf{B}^T \mathbf{D} \mathbf{B} d\Omega \]

where \( \mathbf{B} \) is the standard strain-displacement matrix consisting of shape function derivatives. The finite element solution of the nodal displacements in Equation 46 is
done through the extended finite element method (for details see Belytschko et al. [155]).

The derivation of the element stiffness matrix depends on whether or not an isosurface boundary is passing through the element as shown for the 2-D case in Figure 40.

If the element is completely inside $\Omega$ then the integration is straightforward with $H(\phi(x)) = 1$. For example, in a bilinear displacement field with 4-noded quadrilateral elements, the element is integrated using a 2x2 Gaussian quadrature.

If the element is entirely outside $\Omega$, the integration is also straightforward but to avoid numerical singularities, we use $H(\phi(x)) = \Delta$, where $\Delta$ is a small positive number. In the case where at least one node is inside $\Omega$ and an isosurface passes through the element (Figure 40, middle), we use the following methodology of embedding the Heaviside function into stiffness matrix of the structured mesh.
o Divide the element into \( n \) divisions \((n \geq 100)\)

o Use the shape function definitions to approximate the iso-surface

o Fit a linear least-squares regression and determine the boundary intersections

o Compute Delaunay triangulation inside \( \Omega \)

o Numerically integrate stiffness matrix over each triangular domain

In this way, the partition of unity is achieved through numerical quadrature of the triangular (2-D) or tetrahedral (3-D) domains, and the volume fraction of each cell may still be computed exactly. Moreover, because the Heaviside function is embedded within the structured mesh and no remeshing is done, the CA method which requires a regular lattice, becomes feasible. In fact, the method of embedding the topology in the Heaviside function represents a major advantage by utilizing a cellular automata description of a structured mesh, which may still capture a continuous and smooth topology.

In order to update the topological characteristic we note that the update is most appropriately a simple function of the current, static state of the computed error function, \( e_t \).

\textbf{Equation 47:} \\
\[ \phi_t(t+1) = f(\phi_t(t)) \]

We consider the update of the topological characteristic at iteration \( t \) in the iteration history using the proportional control method of Tovar et al. [141] as

\textbf{Equation 48:} \\
\[ \phi_t(t+1) = \phi_t(t) + \Delta \phi(t) \]

where \( \Delta \phi(t) = c_p \times e_t(t) \)

so that \( c_p \) is a problem dependent scalar affecting the rate of convergence.
The neighborhood of influential elements can vary. We investigated several neighborhood configurations as seen in Figure 41, square-4 (S4), square-16 (S16), square-36 (S36), radial-12 (R12), and radial-24 (R24). The square configurations are similar to the Moore neighborhood, but the Moore neighborhood assumes the element (not the node) is the attribute to be updated. We used the fixed boundary type so that CA lattice points outside the grid contain a value of ‘0’ or no contribution, which comes into play when evaluating nodes near the border of $\Omega_{des}$.

4.2.3. Implementation

The overview of the algorithm implementation is presented Figure 42. Convergence of the HCA algorithm occurs when the change in total volume between iterations becomes acceptably small. According to Tovar et al. we use a two time point history average in case of oscillating behavior so that,

Equation 49:

$$\frac{|\Delta V(t)| + |\Delta V(t-1)|}{2} \leq \varepsilon_{vol}$$
Recasting the Implicit Function

In certain cases, as the nodal quantities evolve over the HCA procedure, mesh artifacts will develop which appear as wavy lines like those seen in Figure 43a. These artifacts are a consequence of
the artificially imposed upper
and lower bounds of the
implicit function in Equation
36. Experience shows that
removing the bounds on $\phi(x)$
will lead to a smooth
evolution of the shape over
time. Indeed, this is an
expected result, as neither the
density-based HCA method
[141] or topology
optimizations using the
implicit method [134] have shown mesh-dependencies or checkerboarding, with the
exception of a neighborhood of zero cells. Nevertheless, in order to keep the imposed
range in $\phi(x)$ we employ a recasting procedure of the implicit function.

![Flowchart of Implicit HCA Method](image)

Figure 42. Flowchart of Implicit HCA Method.
Figure 43. Implicit Function Recast. When wavy artifacts (a) appear on the topological border, \( \phi(x) \) is recast to a more uniform distribution which fits specified point values (b), producing the new implicit function (c), which maintains the same domain, yet is artifact-free (d).

The recasting procedure redistributes the implicit function in a more continuous manner, essentially creating a linear buffering distance between \( \alpha \) and \(- \alpha\), without affecting \( \Omega \). First, points along the iso-surface are selected as the boundary intersections of each element (Figure 43b). Next, an erosion of \( \Omega \) is performed to produce a skeletonized internal structure which are given a value of \( \alpha \). Conversely a dilatation of \( \Omega \) is used to create the external plateau \(- \alpha \). The nodal values of the implicit function are computed (Figure 43c) by solving a system of linear equations which expresses \( \phi(x) \) according to a set of radial basis functions (For details see [155]). The resulting topology
is free from the wavy line artifacts (Figure 43d). Recasting is done intermittently throughout the optimization procedure when,

Equation 50:

\[ c_r(t) \geq \varepsilon_{\text{cast}} \]

where \( c_r(t) \) is the percentage of elements in \( \Omega_{\text{des}} \) which contain nodal values of both \( \alpha \) and \(-\alpha\) within the same element, and \( \varepsilon_{\text{cast}} \) is the casting threshold.

**Algorithm**

The overall flow of operations is defined according to the subroutine,

- \( \phi(x) \) is determined for the initial configuration at iteration \( t = 0 \)
- Implicit FEM is used to determine the nodal displacements, \( u_t \)
- Convergence, based on volumetric changes, is checked.
- Mesh artifacts, based on the distribution of \( \phi(x) \), are checked
  - If mesh artifacts exist, \( \phi(x) \) is recast
  - If convergence has not been reach, \( \phi(x) \) is updated by Equation 47.
  - If convergence has been reached, terminate.

Practically, the algorithm consists of two parts, the FEM problem, and the cellular automaton update. In the FEM, elements are sorted between interior elements not containing an iso-surface, exterior elements not containing iso-surface, and active boundary elements, those containing an iso-surface. The most expensive operation appears to be the linear-regression step used to subdivide the active boundary elements into solid triangles/tetrahedrals. Here, we used the direct solution method of the linear system of equations, but for 3-D problems, solutions involving iterative methods should
be used. As far as parallel applications are concerned, while the update of the implicit function (Equation 48) is a local quantity (i.e. readily divisible), the solution to the finite element problem (Equation 45) is not as directly divisible though element-by-element methods do exist.

![Common Topological Examples](image)

**Figure 44. Common Topological Examples.** Implicit HCA was able to reproduce optimal configurations for several classical problems.

4.2.4. Application and Performance

Several examples of the performance of the implicit HCA method were conducted on some common topological problems (Figure 44). A design space of 30x30 (each cell 1mm x 1mm) was applied to a cantilever loaded in the bottom right with a load, $P = 1 \text{ N}$, isotropic Young’s modulus, $E = 1 \text{ N/mm}^2$, Poisson’s ratio, $\nu = 0.3$, and a unitary thickness under the plane stress condition (Figure 44, left). Additionally, a cantilevered beam of 39x24 cells was loaded along it’s center plane (Figure 44, middle), and a center-loaded beam of 50x20 cells with end-supports (Figure 44, right) were examined with similar mechanical properties. The neighborhood was chosen as S4 (see Figure 41). In
all cases the values of \( c_p, \varepsilon_{cast}, \varepsilon_{vol} \) were adjusted in a problem-dependent manner, and we are not concerned with magnitudes at this time, as they will be discussed later. In all cases the initially solid configuration of \( \Omega = \Omega_{des}, \phi(x) = \alpha \), where \( \alpha = 0.2 \) was used, along with reference strain energy, \( U_{ref} \) equal to the average strain energy of the initial configuration. The algorithm was able to reproduce the optimal topologies for all three classical examples (Figure 44, bottom).

\[ \text{Figure 45. Shape Evolution of Middle-loaded Cantilever, } c_p = 0.05/U_{ref}. \text{ The time course history (d) of } \Omega \text{ in } \Omega_{des} (30x30 \text{ grid}) \text{ with parameters, } \varepsilon_{vol} = 5, \varepsilon_{cast} = \infty \text{ is shown at (a) iteration 6, (b) iteration 11, and (c) iteration 18.} \]
Figure 46. Shape Evolution of Middle-loaded Cantilever, $c_p = 0.02/U_{ref}$. The time course history (d) of $\Omega$ in $\Omega_{des}$ (30x30 grid) with parameters, $\varepsilon_{vol} = 3$, $\varepsilon_{cast} = \infty$ is shown at (a) iteration 14, (b) iteration 25, and (c) iteration 41.

The effect of the proportional control parameter, $c_p$, on the behavior of convergence was examined. A 30x30 grid was applied to the cantilever loaded along its central axis with similar neighborhood, initial conditions, and mechanical properties as above. Proportional control was set to $c_p = 0.05/U_{ref}$, recasting removed from the model $\varepsilon_{cast} = \infty$, and the convergence parameter set to $\varepsilon_{vol} = 5$. The evolution of the shape (Figure 45) shows the final total strain energy (compliance) approaches $\approx 6$, which is in agreement with Tovar et al [141], after 18 iterations. The topology does not achieve a cavity until iteration 6, but then gradually reduces its volume fraction until convergence. The same example with a different proportional parameter ($c_p = 0.02/U_{ref}, \varepsilon_{vol} = 3$) is shown in Figure 46, and exhibits virtually the same behavior, except that $>40$ iterations
are required to reach convergence, illustrating the importance of fine tuning the proportional control for each problem. In contrast, choosing a larger value of $c_p$ or increasing the rate of change (not shown), will cause the convergence to become unstable, but is generally very obvious from the evolution of the shape over time, and can thus be easily avoided.

The spatial neighborhood of influence will also affect the final topology of the structure. Figure 47 shows the finishing topologies for the respective neighborhoods of Figure 41. The simulation was run with $c_p = 0.05/U_{ref}$, $\varepsilon_{vol} = 2$, $\varepsilon_{cast} = 0.02$, (indicating that if > 2% of the elements had corresponding nodes equal to both $\alpha$ and -$\alpha$, the function is recast). All but the S36 neighborhood is able to maintain the correct topology, yet the R24 neighborhood has only very small peripheral cavities. The most optimal looking solutions are the S4, S16, and R12 neighborhoods. The S16 and R12

![Figure 47. Effect of Local Neighborhood. The neighborhoods of Figure 41, respectively, result in the finishing topologies for a). square-4 (S4), b). square-16 (S16), c). square-36 (S36), d). radial-12 (R12), and radial-24 (R24) with parameters $c_p = 0.05/U_{ref}$, $\varepsilon_{vol} = 2$, $\varepsilon_{cast} = 0.02$.](image)
solutions are very similar and produce a more curvilinear result when compared to the sharp angles incurred by S4. The trend is that the larger the neighborhood, the more "smeared" is the final topology. In light of the problem definition Equation 38b this is an expected result as the local strain energy density is only accurately reflected as the neighborhood area approaches zero. Nevertheless, the neighborhood could be used as a design tool to adjust the coarseness or fineness of the result. The S4 neighborhood appears to be sufficient for the simulations we have run.

The role of the initial configuration on the resulting topology was inspected. Two initial configurations were tested; a cantilever (Figure 48, left) and a frame structure
(Figure 48, right). The algorithm parameters were $c_p = .07/U_{ref}$, $\varepsilon_{vol} = 1$, and $\varepsilon_{car} = .03$, and the reference strain energy, $U_{ref} = 4.8 \times 10^{-3}$ N/mm$^2$, corresponding to the initially solid configuration $\Omega = \Omega_{des}$, $\phi(x) = \alpha$, where $\alpha = 0.2$. The evolution of total strain energy in time is shown.

In both cases the final topologies reached a stable, converged value similar to compliance of Figure 45. However, the resulting topologies do not match the optimal configuration. According to Belytschko et al. [134], the gradient of the compliance in the vicinity of the optimum is very small. Therefore, substantial changes in the topology will be incurred with relatively negligible improvement in the compliance, near the optimum. It appears that implicit HCA is not able to overcome this barrier for any arbitrary initial condition, but in all loadings we examined, the initially solid configuration was able to produce an optimal topology. Most likely because of neighborhood averages, very small differences in compliance will not be sufficiently accentuated. Nevertheless, nearly optimal solutions are achieved with any initial configuration. Moreover, there appears to be a relationship between the initial configuration and the final topology. The frame structure maintains the initial internal cavity into its final structure, while the cantilever does not.

4.2.5. Conclusion

The implicit HCA method makes use of local control rules (CA) in combination with structural finite element methods (FEM). The problem is formulated as local difference between a neighborhood of values and a reference strain energy. This CA approach has been used to model complex biological systems, through discrete,
simultaneously applied, local rules. The method is simple in that it requires no gradient, is extremely fast in the number of iterations it takes to solves, and is conducive to be transferred into the 3-D problem utilizing parallel processing of sub-domains. The design domain is formulated in terms of an implicit function so that the exact topology is known at every point during its optimization making it conducive to solving a class of problems involving crack propagations and/or local surface objective functions that could not otherwise be examined.

Tovar et al. created a hybrid cellular automata method using density as an update rule [120]. This method differs from theirs in that we use the implicit function definition of the topology, which has several subsequent effects such as changing the nature in how the FEM is completed, as well as the convergence characteristics. Additionally, this algorithm has several intended uses which their method cannot adequately address. For example, we have proposed a design problem for implant architecture based on the mechano-biological properties of bone [156], which is posed as an optimization problem on the scaffold surface. Surface properties cannot easily be incorporated into density-based problem. In our formulation, surfaces can be defined through a surface layer as the shape of the structure is known as it evolves. Penninger et al. has used the HCA method as a control system of cell behavior to describe the anisotropic properties of bone in a hierarchical approach [119]. In their formulation, checkerboarding occurs in unit cells as a consequence of trying to apply surface rules to elements with intermediate densities. Additionally, this method can handle the appearance of cracks or holes inside an element without any remeshing which is an advantage when micro-cracks develop such as is the case with trabecular bone and reduces the degrees-of-freedom for solution. The
technique shares many similarities with [134], but differs because the problem is not formulated as a global objective function and therefore does not require regularization of the Heaviside function nor the computation of any derivatives.

The convergence behavior is controlled though a problem dependent proportionality constant which has positive and negative consequences. Selecting an appropriate value can cause convergence in relatively small number of iterations (~20), while other methods can requires substantially more (~100) [134]. Unfortunately the process is less automated as care should be taken to fine tune the convergence characteristics.

The application of a regular grid of CA lattices is made possible through the extended-finite element method which embeds the Heaviside function into the structured design domain directly. Implicit HCA is inherently free from any numerical instability, except artifacts which arise through a hard-limit on the upper and lower bounds of the implicit function. Use of a large neighborhood or custom initial configurations can lead to sub-optimal results which are easily avoided, but might serve as purposeful design tools.

4.2.6. Discussion – Strain Energy Density

The mathematical development of implicit HCA involves a local error driven function which minimizes the total strain energy of the scaffold. In order to validate that minimization of the total sed is synonymous with our previously defined tissue uniformity diagnostic (see Equation 31), a ranking of the mechanical variables was conducted as before. The middle-loaded cantilever was converted into a 3-D model by extruding the surface by a unit dimension. It was discretized into a linear, tetrahedral
mesh, with similar material properties as Chapter 3 (Figure 49, left). The resulting von Mises plot reveals the symmetry in stress distribution of the optimized architecture (Figure 49, right). The normalized UD confirms that \textit{sed} is significantly more uniform than any other mechanical parameter which agrees the previous conclusions of trabecular bone. Much like the voxel method of scaffold design, there is little control of the remaining non-\textit{sed} parameters. Nevertheless \textit{min.pr.e}, \textit{tresca}, and \textit{vms} are near the higher end of the UD range, which is in agreement with spinal trabecular bone.

4.3. Summary

In this chapter, two methods for the development of scaffold/implant topologies are proposed which are material independent and promote \textit{sed} uniformity. One method utilizes a local process of reinforcing high energy regions with more material from lower energy regions. It requires the use of voxels to geometrically assemble and change the topology. The second method utilizes an implicit function which also locally updates the topology through the adjustment of a moving iso-surface. Each method has advantages and disadvantages which are worth discussing (Table 2). Both methods have the
advantage of not requiring any complicated numerical gradients, which most topology
topology
topology
methods will require. Calculation of gradients involves numerical derivatives over the
design space and is computationally expensive. Both methods are suitable for 3-D design
spaces, although implementation of the Implicit HCA is more difficult for several
reasons. First, voxels with an iso-surface have to be partitioned into tetrahedral elements
for integration. Next, the recasting procedure of fitting an implicit function must be
solved for the 3-D case.

Table 2. Comparison of Scaffold Adjustment Methods.

<table>
<thead>
<tr>
<th>Advantages:</th>
<th>Disadvantages:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heuristic, Voxel-Based Method</td>
<td>No global problem formulation</td>
</tr>
<tr>
<td>• No complicated gradients</td>
<td>• Voxel surface numerical errors</td>
</tr>
<tr>
<td>• Easy, 3-D manipulation</td>
<td>• Unpredictable convergence</td>
</tr>
<tr>
<td>• Successful for Strain Energy</td>
<td>• Contingent on initial configuration</td>
</tr>
<tr>
<td>• Volume fraction is defined</td>
<td>• Requires connectivity for FEM</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Implicit HCA Method</td>
<td>Disadvantages:</td>
</tr>
<tr>
<td>• No complicated gradients</td>
<td>No global problem formulation</td>
</tr>
<tr>
<td>• Possible for 3-D</td>
<td>• Has problem-dependent variables</td>
</tr>
<tr>
<td>• Successful for Strain Energy</td>
<td>• FEM solution more complex</td>
</tr>
<tr>
<td>• Predictable convergence</td>
<td>• More difficult for 3-D.</td>
</tr>
<tr>
<td>• Requires no connectivity constraint</td>
<td>• Volume fraction adjusted through</td>
</tr>
<tr>
<td></td>
<td>the reference stress</td>
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</tbody>
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Also the finite element solution has more degrees of freedom than the voxel method
which only considers the solid portion of the structure. Both methods are capable of
producing structures of uniform sed but the convergence characteristics of the voxel-
method is much more unpredictable (see Figure 35). Additionally, voxel-methods suffer
from numerical artifacts on their surface which will accentuate the error of the UD when
calculated on the surface. The Implicit HCA method produces continuous and smooth evolutions in the shape and is capable of merging and adding holes throughout the process regardless of structural connectivity. The voxel method inherently requires the structure be connected or the forces will not realistically transfer throughout the structure. Therefore, the voxel method is dependent on the choice of the initial architecture and has a limited ability of adding new features. An advantage, however, is that the volume fraction of the scaffold may be built into the structure through the initial configuration as material is never added nor removed only relocated, positionally, during the process. The implicit HCA method can account for different porosities, but it requires adjusting the reference strain energy density of Equation 39.
Chapter 5: Deriving Computer-aided Tissue Engineering Constructs for Bone Tissue

In Chapter 3, strain energy density was isolated as an applicable mechanical-architectural property of spinal, trabecular bone. In Chapter 4, two methods were proposed to design scaffolds according to that distribution, which both have certain advantages and disadvantages. It was also noted, that strain energy density alone, is not sufficiently descriptive to account for all the architectural features trabecular bone exhibits.

In this chapter, I propose a methodology for CATE which will create a construct that is locally specific to a patient’s mechanical or non-mechanical properties. In simple terms, if we cannot provide a full description of the architecture, the next best option is to copy the prominent features, such as localized elasticity, permeability, porosity, etc. I describe the process of developing libraries of architectures derived from trabecular bone itself which will inherently have many of the intangible design requirements of bone, built-in. This CAD process is described in terms of matching patient-specific elastic moduli.

In the second part of the chapter, I describe a methodology for importing large-scale datasets, such as trabecular bone datasets obtained through CT scans, into a CAD framework. Computer-aided design is the necessary glue in which product development processes much pass through in order to get to pre-production and production validation. Without having a passable method of importing tissue structures into a CAD format, bio-modeling and analysis of tissue-implant constructs is not possible. I describe a novel methodology which greatly reduces the data storage requirements.
5.1. Bone-Derived CAD Library for CATE

To aid in the development of scaffolds for orthopedic tissue engineering purposes, we propose a library of easily-assembled architectural sub-units, deemed tissue primitives, that may be strategically merged according to various characteristics. In particular, for bone, mechanical characteristics such as the regional stiffness in a continuum sense, micro-architectural levels of mechanical surface strain, void fraction amount and orientation, as well as permeability will be critical both individually and in concert. As the influences of these factors are elucidated, the potential to successfully engineer scaffolds improves. Here we expound upon previous research of creating assembled scaffolds from derived analytical shapes, and extend it to encompass the native architecture of human trabecular bone, prepared from repeated patterns witnessed in the interior portion of various T-9 vertebral bodies. With the use of building blocks designed from the physical characteristics of bone itself, we streamline the bio-compatibility of these implants, until the role of architecture and form within bone has been adequately determined from basic science. We report several results; namely, the description of a number of tissue primitives and interfaces with commentary on their morphological characteristics and the integration of unit blocks into a global assembly using a regional bone density map.

The idea of designing synthetic scaffolds for guided tissue regeneration [157, 158] is attractive for several reasons. First, by supplying a base material, the locations upon which tissue will integrate with the surrounding environment are known a priori. As a consequence, several schemes may be developed based on the degradation of the scaffold

or changes in morphology over time [159-162] which can influence how subsequent
tissue may form. Secondly, by controlling the shape, or at least the surface contour, one
can manipulate the mechanical environment in which tissue exists [89, 121, 163]. This
was shown to be influential on cellular metabolism [108, 164, 165]. Additionally, the
morphology indicates, based on a superposition of isostress/isostrain measurements,
which areas of the scaffold would be overloaded once implanted at an osteoporotic site
and could thus lead to tissue necrosis [166-168]. Finally, with control over the scaffold
domain comes control over the scaffold interfaces, or transitions from one type of
architecture to another, which if joined incongruously can lead to disjoint stress profiles
as well as cellular sparsity.

The goal of this study is to present a library of tissue primitives (unit building blocks
and interfaces) to be implemented in computer aided tissue engineering (CATE) [169,
170]. These unit blocks may be merged according to various qualities, some of which are
illustrated in this manuscript. The characteristics are open to the designer, but we wanted
demonstrate the ability to match tissue primitives according to a density map of the
subregions within a human vertebral body. Density or volume fraction is a suitable
metric determined from image intensity, though the authors expect that more significant
metrics related to tissue regeneration will need to be identified. Nevertheless, the
correlation between bone mechanical properties (stiffness and strength) to apparent
density is irrefutable [171, 172]. Using principles of an assembled library, scaffolds may
be created in a site-specific manner with conformations that resemble bone. This process
may be preferred over current scaffold generation techniques that make use of random
processes of solvent casting or gas leaching to create the necessary void spaces [173, 174].

To date, many of the biomaterials used as scaffolding have mechanical behavior inferior to the constituency of bone [171, 175, 176]. Therefore their design cannot always be biomimetic (or at least derived biomimetically) as proposed here. Given a case where the architecture of the scaffold needs to deviate from natural tissue, as in fulfilling the demand of exceptional stiffness, we recommend an assembly of derived analytical shapes [123, 177]. In the absence of this necessity, a biomimetic approach using a composite of bone-derived architectures may be beneficial for the many of the non-mechanical tangibles (e.g. volume fraction) and qualitative intangibles (e.g. fluid perfusion, metabolic waste removal, and those listed previously).

5.1.1. Unit Cubes and Interfaces

There are two types of design approaches to consider when creating scaffold building blocks. The first is to create a variety of scaffold shapes which are characterized throughout the spectrum of the independent design variable. This is particularly useful if a specific shape or repeated pattern is recognized from the outset. For example if one preferred a design favoring the volume fraction of a given shape, experience indicates that scaffolds need at least 60% porosity to account for nutrient delivery and below approximately 90%, the mechanical integrity will be suspect [123, 178, 179]. On the other hand, if the shape is the unknown, but the interfaces and contact angles of adjacent unit blocks are known, then the inverse problem, usually a shape/topology problem, may
be solved as long as a design rule or objective is provided. Figure 50 illustrates the two conceptual differences.

Though the focus of this paper will be on shape recognition of tissue primitives, i.e. a known shape, we provide a brief example to illustrate usefulness the interface method. The solution of the inverse problem is a topology optimization problem. These types of problems are difficult to solve. One has to find a representation of the shape that can capture the fine scales of tissue and that allows the application of mathematical optimization. Among the available approaches are density functions [132, 180], level-set methods [133], evolutionary structural optimizations (ESO) [181-183], and other implicit function based methods [134].

For demonstrative purposes and ease of implementation, a heuristic method was chosen known as the modified method of intelligent cavity creation (ICC) which is a subclass of ESO. This method has the advantage of dictating the number of cavities (voids) created during the process, and is relatively easy to implement. The major disadvantages are that the optimization goal is not well formulated and the computational order is of the scaffold dimension cubed. Briefly, a completely solid material is used as

Figure 50. Unit cube and interface methods of primitive design. The unit cube architecture is known (A), or the interface (B) and loading conditions are known.
the starting geometry and reduced to a final shape through a combination of surface erosion (nibbling ESO) and cavity creation. The reduction serves to minimize the addition of peak stresses caused by material reduction, by removing only "unneeded" material. A metric is used to determine "unneeded" material and when the internal stress state may tolerate the addition of a cavity (for a complete description see [181]). The fixed number of cavities regularizes the problem and the numerical instabilities often

![Figure 51. Interface method. The boundary conditions (A) determine the stress state (B) which dictates the final shape (C) of any optimization method (in this case ESO).](image)

associated with the inverse description.

The interfaces and contact angles of the bone cube in Figure 50 were estimated to create boundary conditions (Figure 51A). Assumed displacement boundary conditions of equal magnitude were applied along the trabecular axes. The elastic modulus of the solid material was $1 \times 10^6$ times stronger than void material and had a Poisson's ratio of 0.3. The created routine used an interactive routine to retrieve the von Mises stress (AB AQUS, Inc., Pawtucket, RI) (Figure 51B) with optimization rejection ratios of 0.01 and a maximum of two internal cavity initiations. The final internal architecture had a volume fraction of 36% (Figure 51C). The advantage of this technique is that interfaces
to the adjacent cube structures are already known and each cube can be separately analyzed/optimized.

5.1.2. Identification of Primitives

The immediate objective of this study was to identify and implement patterns of trabecular bone into a descriptive stiffness library composed of unit cubes and interfaces. Each library unit is referred to as a primitive, and is the combination of one unit cube and six interfaces.
Figure 52. Tissue primitive library. The unit blocks (A) are attached with interfaces on all six sides (B) resulting in a tissue primitive. Unit blocks are limned at their “native” volume fraction (see Figure 55).

The feasibility of joining two primitives is handled by a library subset of seven dissimilar interfaces, which share a common surface area (Figure 52B). The stiffness of unit cubes was calculated (Page 132), and was used, along with its morphology (Figure 52A) to present an example of the application of the library to a partial scaffold assembly (Page 134).

In an effort to isolate repeating patterns within trabecular architectures, 20 sections from 10 T-9 human vertebral bodies were scanned (μCT Scanco80, Basserdorf,
Switzerland) at 30 μm isotropic resolution. We reconstructed the image sets into segmented, binary, trabecular bone datasets of voxel slice dimensions 2048 x 2048. Architectures were loaded into an image processing suite (Analyze Direct, Inc., Lenexa, KS) and viewed in the three physiological planes in > 2 mm thick sections. In most cases, trabecular subsections were translated into stereolithography files for 3-D viewing (Figure 53A). Because each individual bone contains many complex repeating patterns, often joined with other repeating patterns, the number of each unit shape could not be documented. Nevertheless, there was enough evidence to construct computer models of derived tissue interfaces (Figure 52B) and unit cubes (Figure 52A).

The architecture of bone was examined on three length scales. The first and simplest length scale representing a junction of two single rods or a junction of rod with a plate was not considered significant. The second length scale was unit blocks witnessed on at least two trabecular lengths. The final scale, lengths of more than four trabecular lengths were scarcely found with any consistency, possibly due to difficulties in manual identification, but most likely because trabecular bone is seemingly a random conglomeration of simpler repeating units. Therefore, this length was not included at any point in the library. Repeated patterns, such as the highlighted regions in Figure 53A, were translated into tissue primitives (Figure 53B) for the library. Emergent patterns could be found in numerous locations illustrated by the abridged version of the Sunglasses shape portrayed in Figure 53C.
Figure 53. Identification of tissue primitives. Tissue primitives were identified on several length scales in several physiological directions from μCT scans. They were identified (A), derived into CAD representations (B), from emergent, repetitious patterns (C) within the samples.

The primitive library consists of five Archimedean, five tissue-derived, two Johnson, and two Catalan solids. The solids are respectively: (Truncated Icosahedron, Truncated Dodecahedron, Truncated Octahedron, Truncated Tetrahedron, Cuboctahedron), (Armchairs, Archway, Folding Chairs, Ball and Stick, Sunglasses), (Bi-Augmented Triangular Prism, Augmented Hexagonal Prism), and (Deltoidal Icositetrahedron, Triakistetrahedron). Incidentally, the Catalan solids are duals of the Small Rhombicuboctahedron, and Truncated Tetrahedron, respectively. Morphological features of the non-wireframe versions of these polyhedra have been well documented [184]. In all cases, architectural features were noted repeatedly within bone samples. In most cases, closed 3-D versions did not exist, but were included in closed form for space-
filling and regularity purposes. For example, in Figure 53A a partial, wireframed version of a combined face of the Deltoidal Icositetrahedron (highlighted, bottom-left) can be clearly seen, with the appropriate angles (~98° face edge, ~21° protrusion), but does not exist in closed form as pictured in Figure 52.

Tissue-derived shapes which bore no resemblance to previously recorded polyhedra were also documented. The Armchairs unit cube consists of 4 curvilinear plates joined at a central junction. The Archway represents a distorted “X” interface extruded in a curvilinear fashion along the height. The Folding Chairs is a spring-like shape which when projected in two of three directions forms a commonly occurring hexagonal pattern. The Ball and Stick model is a basic orthogonal rod model, where the created rhombi exist at 60° angles, also corresponding to a regularly observed trend. The final Sunglasses shape was a 3-D version of a curvilinear figure-eight pattern (Figure 53C). The locations of the primitives within the bone was location dependent, but not exclusive. For example, the hexagonal projections tended to exist in axially cut sections while in coronal views, the orientation of the struts was in-line with loading, resulting in more rectangular interfaces and rectangular unit cubes. Often the transition from pentagonal shape to a rectangular shape could be observed by a diminutive or unused strut, within the coronal view.
5.1.3. Interfaces

For effective load transfer, tissue primitives must connect to other tissue primitives through designed interfaces (Figure 52B). Figure 54 portrays interface matching and the common surface area between adjoining interfaces. The diagonal entries represent a perfect joining with a normalized surface area of one. The off-diagonal elements reflect the common area and symmetrically the relative percent of cross-section filling, calculated as the fraction of the common portion over the superposition of both areas (intersection/union). Each interface has a basis of values which describe how well a given interface relates to another. For example, the circular interface matches only with itself or the hexagonal interface with any mechanical integrity, reflecting the limitations of the library, and the necessity of multiple interfaces. Ideally, each primitive’s interface would provide a perfect matching. However, because unit cube architectures were derived, and not designed, a universal interface is not possible.

<table>
<thead>
<tr>
<th>Interface</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
<th>Value 4</th>
<th>Value 5</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.04</td>
<td>0</td>
<td>0.07</td>
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<tr>
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<tr>
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<td></td>
</tr>
<tr>
<td>Pentagon</td>
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<td>0.34</td>
<td>0.39</td>
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<tr>
<td>Circle</td>
<td>0.47</td>
<td>0.35</td>
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<td>0.25</td>
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</tbody>
</table>

Figure 54. Interface matching. Seven interfaces can be matched with each other according the relative fraction of intersecting areas. Each interface forms a mathematical basis which has a spectrum of values.
Moreover, simple solid plate attachments or similar constructs would hinder the permeability and porosity of the scaffold. For that reason, native bone interfaces were derived as well. Architectures with drastically dissimilar or similar stiffness values are readily attachable in order to avoid further stress concentrations.

5.1.4. Characterization of Primitives

Morphological analysis

Morphological analysis was conducted on each unit cube in order to assist in characterizing the properties of the library. A “native” volume fraction for each unit cube
was determined by calculating the mean of the range of possible volumes (Figure 55). The lower bound was calculated in each unit cube with similar strut diameters, signifying that it is a function of the number of struts and spatial arrangement. We determined the upper bound by expanding each diameter until a unique feature of the shape was obscured (such as a hole closing in on itself). The only exception was the Arm Chair which was given an average plate thickness commensurate to the strut diameter. The Armchair and Truncated Icosahedron were the most porous while the Ball and Stick was the least. Similarly the range in material volumes tended to increase with respect to the mean value. In other words the least porous material also existed over the widest range (Ball and Stick).

Table 3 portrays a more robust analysis of each architecture. Volume fractions range from 0.09 - 0.35, though most of the shapes are within 80 – 90 % porosity, which is suitable for scaffold engineering.

In general, a high surface to volume ratio (S/V) is advantageous as the available area for fluid flow over attached cells will be higher. Of the identified shapes, the Armchair had the largest surface to volume ratio, and Archway the smallest. The average Connectivity Index, or number of struts connecting to a vertex, and length to diameter ratio was also reported (Table 3).
Finite element analysis

A finite element analysis was conducted on each unit cube in order to characterize the spectrum of modulus values. The unit block modulus could be later used as a parameter to select shapes for the assembly of a site-specific scaffold. The analysis was completed in ABAQUS. A seeding density of 0.75 was used in most cases providing on average 37,000, linear tetrahedral elements. Each architecture was assigned isotropic material properties of $E = 2$ GPa and $v = 0.3$. The stiffness and elastic modulus in the loading direction were calculated in the standard manner through the summation of the reaction forces. The normalized values (with respect to the isotropic modulus) and the orientation in which they were tested are reported in Figure 55.

5.1.5. Assembly of the Scaffold

Generation of a density map

A density map was constructed which represents the local volume fraction of material within 3 mm sub-volumes, chosen to be the bounding dimension of the each primitive unit. Each section of this three-dimensional matrix maps an averaged volume fraction to that region. Through this procedure, apparent properties may be used to identify the placement of primitives which were designed at a length scale of several trabeculae based on manufacturability and available materials.

The modulus (stiffness) of each primitive was assumed to correlate well with apparent density. Only the relative distribution of values were important for this proof of principle, though any number of correlation relationships, linear or power law, have been
Table 4. Interface matching to tissue primitives. The seven interfaces can be matched to the primitive matching of an interface onto a unit cube is depicted through Table 4. The fraction reported is the contact volume of the internal side of the interface and unit cube, divided by the volume of the interface.

<table>
<thead>
<tr>
<th>Interfaces</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>Truncated Dodecahedron</td>
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<td>Deltoidal Icosa - tetrahedron</td>
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</table>

Table 4. Interface matching to tissue primitives. The seven interfaces can be matched to the primitive unit blocks based on the percentage of common contact volume.

<table>
<thead>
<tr>
<th>Interfaces</th>
<th>1</th>
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<th>4</th>
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</table>

Scaffold assembly

The modulus values were ranked according to their magnitude, and the density map subdivided into 14 ranges, representing each of the 14 shapes. The available matching of an interface onto a unit cube is depicted through Table 4. The fraction reported is the contact volume of the internal side of the interface and unit cube, divided by the volume of the interface.
In practice, Boolean operations in Rhinoceros 3D (McNeel Associates, Seattle, WA) provided this functionality. Each row of the table corresponds to one of six faces. Values below a certain threshold provide decidedly poor contact volume for load transfer through the interface and into the unit cube, and therefore, should be avoided. We chose a threshold of 0.1; however the exact number may be modified by the user depending upon the input criteria. The scaffold assembly, then, was produced in the following fashion:

- Unit cubes were placed according to their ranking (modulus) in Cartesian space.
- Interfaces were selected between each face by applying the threshold value for Table 4, below which an interface is not considered suitable to transfer the load from its designated unit cube. A perfect match is always chosen between adjacent unit cells when available, under the assumption no perfect match has preference over another (i.e. each has an adequate area for load transfer).
- When no perfect match is available, select between the available interfaces (Table 4), those which provide the largest relative match (Figure 54).

An example of a portion of an assembled scaffold for human vertebral body is presented in Figure 56.
5.1.6. Conclusions

We have presented a library based on the assembly of regularly oriented tissue primitives in an attempt to improve the techniques of scaffold design. Though many of the factors and appropriate cues of what makes a good scaffold have yet to be elucidated, we believe that this technique can be effective even in the absence of this knowledge because its derivation utilizes the architecture of bone. An exact bone scaffold would be ideal, but the means to manufacture a biopolymer on the scale of bone for a substantial scaffold, is not currently attainable. Therefore, we have introduced a library in which the apparent properties, not tissue properties, may be matched in a patient/site specific manner, yet the architecture maintains much of the same tissue level shape (porosity, permeability) that are essential for its biological functionality. The shortcomings of this technique are that a single scalar relationship (density, modulus) is used as the scaffold assembly mapping, yet it is known that architecture as well as the density is necessary for a strength description. Moreover, the modulus value was obtained only in one loading direction, and a more thorough analysis would involve finding all the elasticity constants,

Figure 56. Assembly of scaffold using density map. Primitive unit blocks are assigned based on the density map and Figure 55. Interfaces are assigned based on Table 4 and Figure 54.
and, therefore, defining a very specific map. Nevertheless, even in this case, there would be the possibility of multiple architectural configurations that have similar elastic properties [89].

Of the designed unit cubes, the majority had porosities native to bone and within the window suitable for scaffold design. The S/V of each shape existed over a relatively small range (0.61 – 1.87). The Archway had the second largest normalized modulus value, but with a poor S/V, denoting that it is most useful as a support element, while the Arm Chair had the largest S/V, suitable for fluid perfusion, yet its \( l/d \) value of 11.50 could signify buckling, except for the strongest materials. Most \( l/d \) values were low, and safely under the buckling threshold. The Truncated Tetrahedron exhibited two distinct ratios within the same unit cube, signaling that bending or partial buckling is a possible failure mode. We did find that many of the sorted modulus values were in the same neighborhood, which introduced some error in the method of ranking the shapes in absolute terms. The modulus values corresponded with the volume fraction only weakly. The Arm Chair is a notable example which has the smallest material volume range (and material volume mean value), yet the third largest modulus value. At the other extreme, the Folding Chair and Cuboctahedron have a large volume fraction range, but behave poorly mechanically. Intuitively, the Ball and Stick fares well mechanically and has 65\% material volume.

The need for regularizing the domain into cubic volumes is necessary from an assembly and property matching perspective. A classical analog is splitting up a mechanics problem into finite elements that can be solved individually. Obviously, this engineered regularization does not occur naturally in bone. For example, a common
question when identifying primitives was: “Where does one border end and the next begin?” Additionally, many of the unit cubes did not exist in the three orthogonal planes, yet we plan to assemble them as such. These types of problems are not easily avoided with an architecture as complex as bone. Nevertheless, since scaffolds are intended as temporary structures nature may take care of this on its own.

Selecting the best interface for each face of a given unit cube is a problem that has multiple solutions. Load transfer from one primitive to the next requires that enough volume is present between the contact area of the unit cube and interior portion of the interface and that there is enough common surface area between interfaces, so that localized stress concentrations do not occur. Our currently methodology of selecting a tolerance to apply to Table 4, in order to decide which interfaces may be placed on a unit cube, is the factor responsible for allowing multiple solutions. Auxiliary methodologies, such as a mathematical optimization which maximizes the weighted average of the (unit cube – interface) and (interface – interface) contact values (Table 4, Figure 54) is plausible, and could induce a unique solution for any modulus map; Though negative consequences to any optimization objective function do exist, i.e. a maximum average connection area does not imply anything about individual unit cubes; Moreover, one stress concentration may be sufficient to trigger the scaffold’s failure.

Lastly, other alternatives such as interface methods were discussed and an example provided (Figure 51). The main shortcoming, here, is the computational order of such optimization strategies. Because an entire continuum is used, the order is of the dimension cubed, which is not yet conducive to large bone samples. Moreover, it is
generally not clear what internal loading vectors are present on the face of a unit cube at this time.
5.2. CAD Framework for High-Resolution Binary Datasets Like Trabecular Bone

Most structural engineering requires a virtual environment to prepare information for stress analysis. However, complicated bio-models have avoided using computer-aided design (CAD), by directly creating meshes. Complex analyses, such as joining several spinal levels together, adding an implant, cutting or splitting bone on an axis, or modeling an entire joint requires CAD. We highlight a reliably simple methodology of importing 3-D datasets (via μCT or high-res MRI) into a CAD environment that significantly reduces the stored information by taking advantage of their hexagonal nature. We compute the numerical solution of a bone-implant assembly under uni-axial compression as a validation.

The emergence of interactive computing environments has led to computer-aided tissue engineering (CATE) – which according to Sun et al. “encompasses computer-aided design (CAD), image processing, manufacturing and solid free-form fabrication for modeling, designing, simulation and manufacturing of biological tissue and organ substitutes [169]. This virtual environment, deemed CAD, is critical for joining components, checking part validity, exporting parts to a proper format for finite element analysis (FEA), or preparing a part for rapid-prototyping and direct printing.

The usefulness of CAD to the medical community is resounding. A number of possibilities are already realized clinically. Hieu et al. noted that three applications of CAD and RP in the medical community were in 1). the design of bio-models and surgical aid tools, 2). the design of surgical training models and medical devices and 3). the design of scaffolds for tissue engineering [185]. The primary and most currently used

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7 Work in this section is under revisions in *Journal of Biomedical Informatics*
function of CAD in the orthopedics industry is for the design of implant parts and
fixators. CAD along with RP technology reduces the time from conception to
implementation of an implant. A major role of CAD in the medical community is in the
reverse engineering problem of determining biological contour boundaries in which the
replacement scaffold should fill, either from imaging techniques or point mapping.
Reverse engineering has been applied to cranioplasty [186], in the case of skull injury or
tumor, as well as dentistry for creation of damaged teeth from a mold, and artificial knee
joint contours [187]. CAD also has utility in computer-assisted surgery and surgery prep
models and software used to plan surgeries and train practicing medical professionals.
For example, training tools for nurses working with intra-aortic balloon pumping [188],
and virtual reality software involving haptic feedback of the temporal skull have been
developed [189].

CATE in particular, holds the promise of being able to very specifically and
repeatably offer tissue substitutes and someday even whole organs with interacting cells
and scaffold housings. Spin-offs of this broad classification include organ printing of cell
slurries in defined micro-patterns [190, 191], scaffold design following
mechanotransduction principles [124], the use of bio-informatics to automatically
assemble scaffolds with different localized properties [170, 192], and the prediction and
interactions of cellular mass transport properties onto a scaffold environment [193, 194].
With all of these facets working together, the biomedical engineering community is
rapidly approach an era where emerging technologies may be combined. For example, a
bone defect can be digitally imaged, analyzed, virtually created, and its physical replica
built in an optimized and efficient manner using tools of FEA, topology optimization, design, and prototyping.

The major drawback of implementing the above procedures is that there is no integrated framework in which to unify the different engineering tools. For example, large-scale FEA algorithms have been written to solve the complex geometries of bone, yet they bypass a CAD environment because such datasets are too cumbersome for a personal computing environment in which most CAD software currently operates. CAD is sometimes used for the repair of stereolithography files which represent the complex architecture of image data through surface triangles (and are the common format for rapid prototyping). However, these meshes are not converted into NURBS (non-uniform rational β-splines) or vector-based boundary representation CAD formats, so only limited operations may be performed. Certainly useful CAD operations such as splitting, control-point shape morphing, smoothing, trimming, etc. cannot be fashioned on these native surface meshes. This prohibits joint modeling with multiple entities and the integration of tissue-implant assemblies into CAD. For this reason, most of the CAD usage associated with bio-modeling has to do with repeated and regular shapes which are less arduous to handle [169].

In addition to restricted computing resources, a limiting factor has to do with the nature of image data stacked in pixel form. High-resolution voxel finite element models, though aesthetically inaccurate on the surface due to right angles, are the standard for measuring biomechanical stresses and strains [195]. However, Boolean operations on many CAD platforms will fail when intersection planes are very small or coincident with each other, such as is the case with two voxels placed adjacently. For this reason we
have chosen to create our CAD model as three, planar models which, in the final step, are joined to create a single 3-D model. In this manner, our Boolean operations only consist of 2-D, planar surfaces which will never share a common border and are thus guaranteed to be without error, in contrast to a solid modeling approach.

To facilitate interest of incorporating native tissues in orthopedic tissue engineering and implant designs, we describe a simple solution which will function in the personal computing environment, and enable a CAD representation of image data.

5.2.1. Methods

Process Overview

A vertebral body was scanned (70 kVp, 114μA) at 30μm resolution using MicroComputed Tomography (μ-CT) (μCT 80, Scanco Medical AG, Bassersdorf, Switzerland). A cubic volume of bone was extracted (ANALYZE 7.1, Analyze Direct, Inc., Lenexa, KS) and thresholded to create a binary data set (representing bone ‘1’ and air ‘0’) of 50x50x50 voxels in size. The method is applicable to larger data sets as well.

The goal of this process was to be able to import information obtained from high-scale resolution CT or MRI data into a CAD framework. We used the CAD system, Rhinoceros 3D 3.0. (McNeel Associates, Seattle, WA), which is a NURBS-based surface modeler that also has the capability of running scripts so that the procedure may be automated. In order to ensure proper importing of our bone part into a commercial FEA package, it is imperative that a closed polysurface (CPsrf) is created, which means that the part does not have any gaps and is therefore considered a closed volume. Gaps
present in between surfaces will be interpreted as empty space between un-joined shells. Such files may require extensive repair.

To create a CPsrf of a highly porous tissue like trabecular bone, we employed these steps:

- Isolate the 3-D volumetric data set into three, 1-D planar shell data sets encompassing the entire volume (one for each Cartesian direction).
- Remove the troublesome planes which create Boolean problems during shell closing (diagonal spurs).
- Cluster planar surfaces by creating a clockwise contour around each connected island of faces.
- Write the coordinates of each contoured island in a sequential text file.
- Write the coordinates of each internal planar hole in a sequential text file.
- Execute Rhinoceros script which loads in all planar and hole data.
- Execute planar Boolean operations in each direction to create internal holes.
- Join all planes to create a CPsrf.

**Preparation of Contour Files**

The first step of the process is to obtain the surface faces of the 3-D bone volume which represents its bounding shell. We use a NURBS surface-modeling software which in-and-of itself is beneficial in data reduction compared to a solid modeler. The binary volume was loaded into MATLAB 6.5 (Mathworks, Inc., Natick, MA). The information is stored in a hexagonal, voxel format, which makes the isolation of surface planes simple. By looping through each bone voxel and keeping those faces which are not
duplicates to any other face, the bounding surfaces were stored in a 3-D matrix of 51x51x51, each of three directions corresponding to the 1, 2, and 3 of Cartesian space.

The equity of the proposed method lies in the reduction of information that a CAD system needs to store by clustering the faces which are adjacent to each other.

Figure 57 shows the difference between a NURBS representation of voxel planes (left), and clustered faces (right) of a 25x25x25 bone cube. By eliminating interior nodes which are not necessary to capture the shape of a hexagonal structure (such as one produced through imaging diagnostics), the use of commercial CAD software on high
resolution datasets becomes feasible. The reduction in data between the cases pictured in Figure 57 is highlighted in Table 5.

![Diagram](image)

**Figure 58. Coordinate ordering of clustered faces and diagonal spurs.** The connected planar islands of faces are clustered by tracing the perimeter coordinates. These coordinates (1-28) and the internal hole coordinates (a-d) are written in sequential text files that are later loaded into a CAD software. Additionally, diagonal spurs, such as the faces which meet at coordinate 27 must be removed in order to ensure proper closing of the bone cube.

Note that no information on the complexity of the micro-architecture has been lost. The number of surfaces is decreased by more than a third and the vertices by one half. Additionally, as the size of the bone cube increases, a larger economy of savings can be expected. The voxel planes model (Figure 57, left) took significantly longer to close (or to create a CPsurf) than the clustered faces model (Figure 57, right). On larger bone models experience dictates that because of memory requirements it will not be possible to close the Voxel Planes model. Clustered Face models may be closed in a matter of minutes on any standard computing platform.

After the creation of planar surfaces, the faces need to be clustered. For that purpose, we individually examined each planar layer and performed 1) a volume dilation in order to create the nodal skeleton representation (instead of an element or face skeleton representation), and 2) a perimeter tracing algorithm which lists in counter-clockwise fashion the exterior nodes of the connected islands (see Figure 58). The second operation
made use of the built-in function in MATLAB, "bwperim". Moreover, the nodal coordinates of each island of clustered faces were individually written to a text file in a sequential order (coordinates 1-28). By inverting the binary image, a similar procedure was done to create text files of the coordinates of the internal holes (coordinates a-d) which were used as part of a Boolean difference operation in CAD to create the planar holes inside the planar faces. This process is visually depicted in Figure 58. In this manner, six groups of text files were written: the 1-2-3 directions of filled surface plane coordinates and the 1-2-3 directions of internal holes.

Prior to the creation of these text files, certain planes must be removed which will create problems later in the joining phase. Those vertices which share two or more planar faces of connected islands, such as coordinate 27 of Figure 58, will create problems if not first removed from the model. As a result the two faces, which meet at a diagonal endpoint, referred to as a diagonal spur, must be removed from the model. The total number of these spurs is negligibly small, often less than 3 % of the volume of the bone cube, and can easily be removed without affecting the morphological characteristics in a significant way.

*Creating a Closed Polysurface*

A script was created for the Rhinoceros 3D, Visual Basic scripting environment, to automatically load in the text file information. The coordinate information was read into the script to automatically create planar NURBS surfaces. Six CAD layers were used corresponding to the filled surface planes and internal holes of each of the three directions. Three Boolean Difference operations were conducted on each of the three
directions of planar surfaces to create the internal cavities seen in Figure 58. The three directions when superimposed upon each other represented the bounding shell of the bone cube seen in Figure 59 for a smaller sub-volume of 25x25x25. All surfaces were selected and joined to create a CPsurf without error. Again, a CPsurf is suitable for exporting to any commercial FEA package.

Larger Data Sets

The limiting factor to the outlined methodology is the computer memory required to simultaneously join all the faces into a CPsurf. The process of joining or ‘gluing’ the surfaces together depends on the order of operations, so it is therefore not possible to join all the planes in the 1-2 directions, then join the result with direction-3. All planes must be joined together simultaneously in order to ensure a CPsurf. For larger datasets, it could become very time consuming to complete this step. In fact, through a trial-and-error approach, we have concluded that joining between 2000-3000 faces at once is the upper threshold to minimize the time of joining (determined based on physical memory 2.12 GB). In order to get larger datasets into a CAD environment, the problem must be allocated into sub-volumes. For example the original 50x50x50 voxel data set was
subdivided into eight, 25x25x25 data sets. These subdivisions may be incorporated in the automation process.

The theory with larger datasets is to apply the above methodology to multiple sub-volumes that may be joined in timely fashion, and then merge the resultant sub-volumes into a single CPsrf. Complications arise however, because there would be bounding cap interfaces between sub-volumes that would not allow the overall part to close. The methodology depicted in Figure 60 was used to circumvent that problem for larger data sets. First, sub-volume planes were imported as before, but with an extra voxel layer at interface boundaries. Secondly, the bounding cap faces of the extra layer were deleted creating a smooth transition across the interface boundary; However, redundant faces remained in the overlapping portions. The duplicate faces were easily selected and removed in the CAD platform. Each sub-volume layer was then joined separately. The resultant sub-volumes were joined to create a
single CPsrfs. The last two steps of this process are depicted in Figure 61 for eight sub-volumes (Panel A) and the CPsrfs (Panel B).

Figure 61. Joining Phase of Larger Data Sets. Eight sub-volumes (A) are used to create a CPsrfs (B) of a 50x50x50 cubic bone volume.

5.2.2. Applications: Stresses on an Assembly

In order to demonstrate the feasibility of our methodology, several common CAD operations were applied to the CPsrfs of the bone cube. The cube was 1) sectioned in half, 2) an orthogonal lattice tissue-engineered scaffold was inserted in the middle, 3) and the equivalent of the cylindrical Boolean operation was performed (using split and join commands) to create a bone-scaffold cylinder arrangement (Figure 62). Subsequently, the part was exported, then reimported into ABAQUS 6.7-1 (Abaqus, Inc., Pawtucket, RI) as a solid part with zero errors. The part was meshed with ~2,000,000 tetrahedral elements, given isotropic, linear material properties of 2000 MPa (within the range of trabecular bone and some biomaterials [89]) and subjected to uniaxial compression loading. The resulting von Mises distribution of the bone-implant assembly is shown in Figure 63. High stress concentrations existed predominately in the vertical beams of the
implant and dispersed into the upper and lower bone sample. Most likely the use of orthogonal angles created the high stress distribution within the implant, as well as some areas that are disjoint between the interfaces of the bone and implant contours. While the specifics of the analysis are inconsequential for an arbitrary implant design, we illustrate the framework which allowed the problem to be imported into the FEA environment.

Figure 62. Utilizing CAD Operations for an Implant-Assembly. The created bone cube was sectioned and Booleaned with an orthogonal implant to create a CPsrP ready for export.

5.2.3. Conclusions

There are many engineering advances that have affected biomedical applications. However, in so far as these advances may be applied to a practical area such as orthopedic implant design, CAD will always been the glue to the design stage. Importing large-scale bioinformatics requires a framework to reduce the amount of stored
information, which can be accomplished by utilizing the hexagonal nature of MRI and CT imaging modalities.

Difficulties with Boolean operations are alleviated by completing them two-dimensionally in planar a fashion. The amount of data is greatly reduced in this manner, and can be scaled for larger applications, as well as automated. The proposed methodology makes use of automation and CAD software that supports scripting to load in pre-formatted contour information as boundary representations that will allow full CAD functionality. The major drawback is the pre-processing effort required to create the slice file information using external software. Nevertheless; that portion is simplified by the ease of manipulating voxels. Though the methodology is useful for larger datasets, based on the concept of subset division, there is certainly an upper limit on the practical size of datasets that must be used, as a volumetric description is required as the first step. For micro-mechanical stress analysis of trabecular bone samples, this process appears to work well.
Chapter 6: Conclusions and Future Directions

The concept of functional adaptation – that bone architecture is maximum strength/minimum weight material which responds to gradients from an equilibrium mechanical state has existed for centuries. With the advent of computer methods, especially the finite element method, this concept has been corroborated through theories like adaptive elasticity [110], mechanostat [13, 16], and phenomenological models [79, 80, 116]. These computer models have utility in predicting phenomena associated with osteoporosis as well as the local bone response around prosthetics, such as hip stems, after implantation [106].

In the separate field of tissue engineering, focus has been placed on applying cells and growth factors to a scaffold base which provides structural support whilst cells gradually differentiate, proliferate, and produce extra cellular tissue matrix of the type desired. In general, the field of tissue engineering has not considered mechanical aspects as a valid contributor to its paradigm. However, in the case of orthopedic materials, especially bone, mechano-biological considerations highly corroborate the architectures seen in vivo, and may in fact be the most relevant factor to consider. Neglecting its role in either scaffold or implant design will undoubtedly be detrimental to the integration between native bone and the replacement material.

Previous studies have used topology optimization and CATE to design scaffolds which meet specific anisotropic scaffold design requirements, while achieving a high target porosity (which is important for nutrient transfer, removal of waste products, etc.) [89, 128]. However, no effort has been made to develop a design scheme for scaffolds which accentuates the functional adaptation characteristics of its host tissue. The driving
force as described through functional adaptation principles is simply ‘the difference between a reference value and the current value’ of a mechanical variable such as stress. By summing this tissue level quantity over the entire trabecular bone architecture, one is able to quantify an index which measures the global tissue level uniformity of bone. Low index values indicate the tissue is equally loaded, while high values indicate that there is heterogeneity in loading throughout the bone.

In Chapter 3, the uniformity index values (UD) were examined using a reverse engineering approach where trabecular bone data sets were converted into \( \mu \)-FEA models. Different mechanical loading variables (see 2.4.3. Mechanical Engineering Variables) were considered and it was found that strain energy density (sed) was the most uniform, and had the largest sensitivity to variations in trabecular architecture between samples, which supports its role as a functional adaptation stimulus. Unfortunately, sed is often the most uniform for several architectural shapes which bear load well, and was thus not able to fully account for the trabecular shape. Additionally, no other mechanical variable could be statistically isolated as a unique contributor. For further details see Section 3.4.

In Chapter 4, two methods were developed which promote sed uniformity as a functional adaptation stimuli. Both methods use mechano-biological principles to arrive at their “optimized” configurations. There are several advantages and disadvantages to each method which are summarized in Table 2. Comparison of Scaffold Adjustment Methods. Both methods are able to accentuate sed in a variety of loading conditions. In order to validate the adjusted architectures in a tissue engineering scheme the following numerical simulation was conducted.
6.1. Bone Growth on a Scaffold

The RCO unit cell was optimized in unconfined compression to produce an augmented shape with uniform $sed$ (see Figure 33). Cells which represent BMUs (dimension = 50 μm) were virtually seeded on the initial and optimized scaffolds (dimension = 3.2 mm), respectively. Twenty percent of all available scaffold surface locations were assigned to be BMUs using a random number generator, as the initial state. Tissue resorption and formation were simulated according to a mechanical set point, which was specified to be strain energy density according to Chapter 3 (Figure 64). A related tissue adaptation scheme has been used by Adachi et al [90].

Scaffold surface values above the upper threshold will trigger proliferation, while surface values below the lower threshold will trigger resorption. In a discrete sense, a bone tissue voxel will be added to the neighborhood when the local $sed$ value is above $\Gamma_u$ and removed from the neighborhood when below $\Gamma_L$. The scaffolds were exposed to 50 μm strain in unconfined compression and given material properties, $E_{\text{SCAFFOLD}} = 1000 \text{ MPa}$, $E_{\text{BMU}} = 200 \text{ MPa}$, $\nu = 0.3$. The threshold values were set to $\Gamma_u = 0.07$ and $\Gamma_L = 0.025$. The results of the evolution are shown in Figure 65. The
actual loading values are irrelevant for the study, but will need to be determined experimentally in the future.

The original scaffold loses bone tissue in areas which are unstressed which is intuitive in light of the finite element results (see Figure 34). Furthermore, tissue is added in areas of high sed. By iteration 5, there are clumps of tissue and the scaffold/tissue complex does not have a well distributed tissue pattern, despite a balance in the formation and resorption properties (only 3% of the initial tissue volume was resorbed). The augmented scaffold (Figure 65, bottom) has specific areas of peak energies which are caused by the fixed volume fraction of material as the topology of the scaffold was adjusted. Nevertheless, there is a larger percentage of the scaffold which is at a common value. The result is a narrowing of the scaffold’s mechanical distribution and a shifting of the mean value to a higher strain energy state. Consequently, fewer tissue elements are resorbed and more tissue elements are added to high strain energy density areas.

Figure 65. Evolution of original (top) and optimized (bottom) scaffold unit cell over 0, 1, 2, and 5 Iterations (left – right).
producing a more uniform tissue pattern on the scaffold surface. Two hundred percent more tissue is produced and the tissue pattern is superior. The effect of scaffold adjustment can be represented schematically as illustrated in Figure 66 as a shift to the right and narrowing of the functional adaptation window.

In Chapter 5, a Computer-aided Tissue Engineering (CATE) framework is discussed. While CATE can provide more specificity to scaffold design, another advantage is its ability to provide patient-specific or locally matched properties. With a simple CT scan and registration of that scan, a scaffold was constructed which matched the local Young’s modulus of a vertebral body. In Section 5.2, focus was placed on methods of image registration from high resolution CT data. In Section 5.1, the library of unit cells and their assembly into a scaffold is described (see Figure 56). In this way spinal fusion cages can be created in a manner which resembles many of trabecular bone’s repetitious patterns, yet can be produced from biomaterials or metals to match the mechanical properties of bone.
Future directions will focus on combining the techniques described in this thesis work into an automated and well-characterized database which could be realized clinically. By expanding the database to include mechanical (structural integrity, stress shielding avoidance), biological (cell attachment, ECM production), transport (fluid flow, permeability, porosity), and geometric (matching anatomic feature) properties, unit cells can be characterized and assembled through optimization processes as biological goals are elucidated. Further clarity in the knowledge of optimization goals required for bone tissue are necessary. Because of limitations in additive rapid prototyping, large spinal defects are the most appropriate site for the proposed library. Additionally, experimental models will be essential to validate the effectiveness of the proposed optimization schemes to the bone-communication network. Suggested studies will use \textit{in vivo} animal models with \textit{in vivo} CT scanning techniques that do not disrupt mature osteocyte-osteocyte and osteoblast-osteocyte communication networks.
References


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