The Design of Bone Scaffolds for Implantation into Femoral Defects for a Male Adolescent

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Abstract:

Tissue engineering is a growing application in the medical field, in which human tissue can be regrown instead of implanted. Bone tissue scaffolds simply are formed by seeding Mesenchymal Stem Cells (MSCs) on a three-dimensional template, and be able to function normally in the implanted body. These features need to be tested in-silico.

The objective of this research was to design a feasible scaffold that could be implanted into human femoral defects through the process of modeling and in silico testing. The scaffold cross-sections were designed using Draftsight, a 2D CAD software and FlexPDE, a finite element analysis system. For each design, a load was set as xstress and placed in the u (vertical) direction. These scaffolds were placed, through software, at the base of the femur. The femoral dimensions and properties tested were those of an adolescent male.

Two designs were made, one similar to that in literature, and another original scaffold, placed on the dorsal end of a full femur bone. Poly L Lactic acid was the material used for the scaffold in testing, and both the Young's Modulus and Poisson's Ratio were found for it. The first design was a rectangular cross-section and proved energy consuming when placed in the FlexPDE. The second cross-section and bone were ultimately drifted slightly, displacing the entire system. The main reasons for such drifting were due to issues with boundary conditions to eliminate drift. The loading that was placed on the edges of the cross-section along with adjustment of some of the boundaries of bone created newer problems.

Through this experiment, the various loads and stresses a human bone can withstand were understood. This information will be helpful in future designs in understanding what locations and environments can affect scaffold integrity. Future steps would be to create a consistent scaffold that can be successfully implanted in real human femoral defects.

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Introduction:

Tissue engineering is a field of research that focuses on the regrowth of hard or soft tissue. Instead of the surgical replacement of a tissue using alternative grafts, scientists attempt to form completely new tissue that can be grown from one's own cells. By placing a scaffold laced with the patient's cells into the body, part of a tissue structure can be regrown without risk of rejection from the immune system. To understand the scaffolding process for tissue engineering, the tissue being regrown must be understood (O'Brien, 2011). For example, we take a look at the structure of the bone.

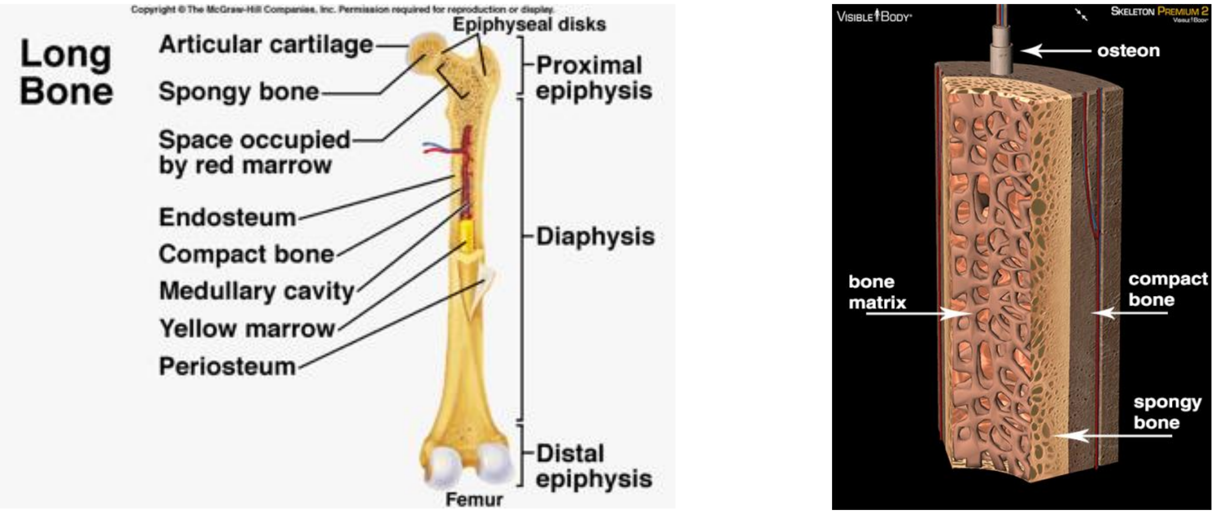

Figure 1: *These are the main components of a long bone, along with an interior view of the long bone as well.* https://www.google.com/search?q=bone&source

The function of bone is to support the body, protect the vital organs, transform muscular motion to movement, store minerals & growth factor and produce blood cells (Liao, 2008). Bone changes throughout life. Children and adolescents growth taller and stronger due to changes in their bones and the elderly can lose bone strength over time. Bone is the structure that holds the body together, so it is essential that it remain formidable and structurally intact. It is important to understand the bone itself, to finally attempt to design a feasible scaffold design (Leong, 2003).

Review of Literature:

The human body contains two types of bone. The compact or cortical bone composes 80% of the human body's bone and is dense and stiff. The spongy or cancellous bone is porous and less dense, composing the other 20% of the human body's bone (Leong, 2003). Long bones contain three sections, the epiphysis, metaphysis and diaphysis. The epiphysis is located at the ends of the long bones; the metaphysis is in the middle of the long bone. The diaphysis is the midsection of the bone where primary ossification occurs (O'Brien, 2011). In adolescents the growth plate exists within the metaphysis of long bones. It is important to understand these facts designing a useful and scaffold.

The reason for this research is to evaluate the mechanical integrity of the scaffold, and the different pressures and stresses to which it will be subjected. The goal is to create a realistic scaffold design and record the different loads that it undertakes. The bone scaffold's purpose is to serve as a temporary template for the incorporation into the subjects' skeleton, so it must be structurally stable upon taking load.

The techniques that are currently implemented for repairing and replacing bone tissue are autografting and allografting. Autografts are portions of bone from one's own body that are fused into a defected area. An advantage of autografting is that there is minimal risk of pathogen transfer or disease transmission (Drosse, 2008). However, autographing is problematic because there can be substantial morbidity and chronic pain for the patient (Arafat 2011). The allograft is the second option. An allograft is a portion of bone from a cadaver that is incorporated in the defective area in the patient. The incision site is not large, but there are risks of disease transmission, immune rejection, pathogen transfer and the potential use of dangerous immunosuppressant's (O'Brien, 2011) (Phillips, 2013).Bone salvage surgery is another method. The surgery a preventive measure that is, primarily performed to remove bone and soft-tissue cancers occurring in limbs in order to avoid amputation ta a later date (O'Brien, 2011).

Bone tissue engineering can minimize the risks of each of these methods (O'Brien, 2011). For a scaffold to operate properly in the body that it is implanted it must be osteoinductive, osteocondive and osteointegrative (Hutmacher, 2000). Osteoinductive means that the scaffold allows cellular activities to occur properly. Osteoconductive allows the scaffold to grow upon a surface. Finally, osteointegrative is the connecting and functioning of the body and the scaffold together. The scaffold is useless and serves no purpose in the body without these abilities (Leong, 2003).

The scaffold has requirements. It must be biocompatible, i.e. cells must adhere to the scaffold and the materials used should not be harmful to the host tissue (Hutmacher, 2000). The scaffolds must be biodegradable, but they must also degrade slowly to ensure proper bone growth (O'Brien, 2011). Because the scaffold is not permanently implanted into the body, cells should proliferate through the scaffold and be able to produce their own extracellular matrix (Liu, 2007). High mechanical integrity is needed for the scaffold to accommodate to the area of implantation (Wang, 2007). High mechanical integrity is needed from the time of implantation to the completion of the remodeling process. The porosity levels depend on the bone type that is being mimicked. The higher porosity rates also ensure deep interconnecting pores, which allow oxygen and nutrients to be delivered to the cells of the scaffold (Liu, 2007).

Tissue engineering uses three categories of biomaterials for creating bone scaffolds: ceramics, synthetic polymers and natural polymers. Ceramics include tricalcium phosphate (TCP) and hydroxyapatite (HA), known for high mechanical integrity, low elasticity and brittleness (Liao, 2008). They have high biocompatibility rates. Professor Jeffrey Hollinger used HA coated and uncoated scaffolds to test biocompatibility. There proved to be a higher biocompatibility in the HA coated scaffolds (Hollinger, 2012). This shows that material is very important for scaffold functions. Synthetic polymers are also extensively used in scaffolding techniques. They offer a great degree of design freedom but risk rejection due to reduced bioactivity. Unlike synthetic polymer-based scaffolds, natural polymers are biologically active. Natural polymers are biodegradable and allow host cells to produce their own extracellular matrix (Armentano, 2010). However, natural polymers have low mechanical strengths so load bearing applications are a concern (O’Brien, 2011).

Scaffolds intended for the use in leg and spinal bone should also be load bearing (O’Brien, 2011). Load is the amount that can be or usually is carried, a measure of weight or quantity varying with the type of movement. Sitting, standing and jumping all implement a different type of load. Loads are either static or dynamic and differ significantly; dynamic loads are usually larger. Other load types are, but not limited to, torsional and shear. A bone must be able to support different loads simultaneously and over a long period of time. When considering load, the material fatigue should also be regarded. Ramay and Zhang fabricated porous scaffolds that accommodate to the bone tissues load. The scaffold was composed of b-tricalcium phosphate (b-TCP) and hydroxyapatite. The HA's role in the scaffold was to maintain the mechanical integrity. To measure the strength and the load bearing capabilities of the scaffold, compression tests were performed. The results of these tests demonstrate that including HA into the scaffolds increased the mechanical integrity. The major concern for fabricated load-bearing scaffolds is the balance between the material porosity and the mechanical strength. However, tougher scaffolds had more success when implanted, hence tackling a question of fatigue (Zhang, 2004).

Solid Freefrom Fabrication (SFF) is an effective method to create templates for the scaffold and the scaffold itself. The three-dimensional shape and microarchitecture of the scaffold can be controlled using SFF. SFF uses a layer-by-layer system that allows complex designs to be made using computer aided drawing (CAD). Before a scaffold can be implanted, the testing of the design must first be conducted via CAD (Leong, 2003).

C. Liu created spherical scaffold designs and attached multiple spheres to create a solid cubical cross-section. The design and testing was done through CAD initially, and then the design was created for implantation into rabbit femoral defects in the hind legs. The designs were stable and were able to withstand the load, proving the effectiveness and accuracy of in silico design and testing (Liu, 2007).

Zhou Xiong used SFF with the low temperature deposition method to fabricate porous scaffolds. The scaffold was a poly (l-lactic acid) (PLLA) tricalcium-phosphate (TCP) scaffold that was implanted into 20 skeletally mature beagles. The scaffold was created using SFF to ensure specifics. Over a period of 24 weeks, the new bone had regenerated. The beagles were caged for 24 weeks, so dynamic load was kept to a minimum. The implanted scaffold had high biocompatibility rates, but the biodegradability rates weren't as high. The usage of both PLLA and TCP proved to benefit the overall effectiveness of the scaffold, in which bone tissue did grow in just over 6 months.

The purpose of the research was to design a useful scaffold cross-section that would be able to support a human load. Through the use of Draftsight, a 2D CAD program and FlexPDE, a computational finite element analysis system, two scaffold cross-sections were made and tested to see if their qualities, when placed in a human femur bone, would be able to withstand load and ultimately stay intact. Mathematical analysis was done to investigate load bearing capabilities of scaffold being considered for implantation. Ultimately, scaffold designs were made to test the load bearing abilities of these cross-sections when implanted in the femur of a human male adolescent.

Methods:

Bone structures and their properties were examined to understand the biomechanics of bone tissue. The motivation for creating such scaffolds was gained by trying to remove infected, defected or cancerous bone tissue by implanted a bone tissue scaffold. The bone that was heavily focused on was the femur. Scaffold designs that accommodate the load that the femur supports must be able to sustain stress that the femur will take in order for the design to be useful. The scaffold must tolerate shearing and distortion as well (Leong, 2003). To create the scaffold designs, the 2D CAD modeling program Draftsight, was used. Draftsight allows the creation of complex 2D and layered 3D designs. Layering is important for the scaffold to distinguish between the substrate and the true bone tissue. Multiple layers can also be drawn to ensure porosities or complex architectures. Draftsight enables the programmer to use polylines that allow for easy construction of complex boundaries. The first scaffold was rectangular scaffold taken from literature and tested in a different environment. The second scaffold was a detailed drawing of the femur with a scaffold placed at the bottom left-hand portion of the bone where the femoral condyle is located. Both scaffolds were tested in silico and used a Young's Modulus of .3 GPA and a Poisson's Ratio of 100 x 10^9 for the femur bone. The PLA scaffold used the same Young's Modulus but a Poisson's Ratio of 30 x 10^9 (Table 1).

The dimensions of the PLLA/TCP scaffold fabricated by Zhou Xiong in 2002 were used for the design (Figure 2). The material that was simulated to create the scaffold was poly lactic acid (PLA). The Young's Modulus and Poisson's ratio were derived for implementation into the partial differential equation using FlexPDE. FlexPDE is a computational program that solves partial differentiation equations and plots results. It can perform finite element analyses and visually represent the tension, stress, or pressure that is placed on the scaffold.

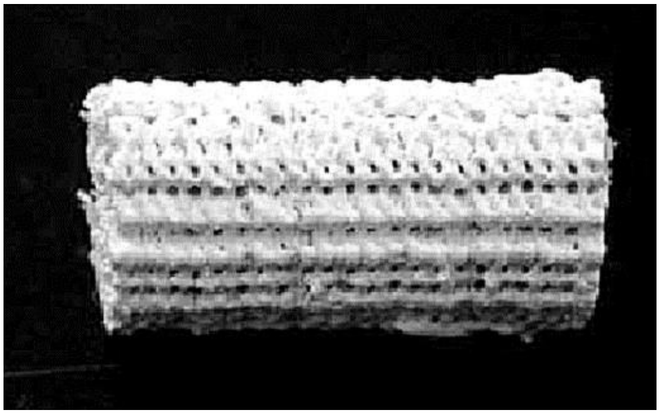

Figure 2*: The PLLA/TCP final scaffold design by Zhou Xiong (Xiong, 2002). This was the design that was remade through Draftsight to test if similar structure can withstand different loads in different environments.*

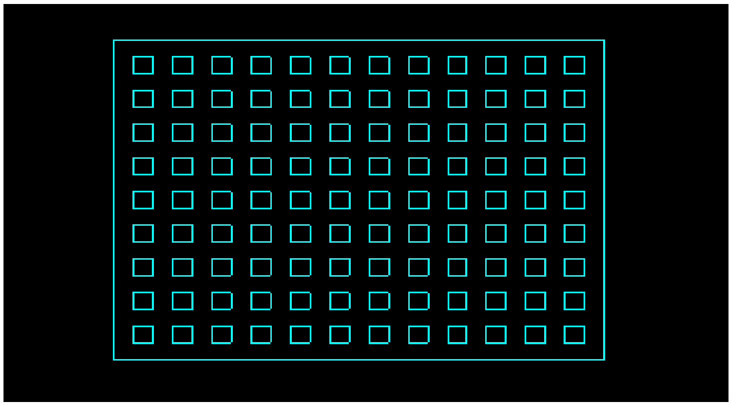

Figure 3a: *First Scaffold Design. The dimensions of this scaffold were similar to those of the scaffold made by Zhou Xiong(2002). The design is a rectangle with squares, which signify the pores in the scaffold. The material used for the design and imported into FlexPDE was poly lactic acid (PLA). This design was simplified significantly from the original (figure 3b).*

Certain aspects of stress can be tested, such as if an object shears when a load is added to it. The 2D drawings that were created on Draftsight were imported into FlexPDE, and specific dimensions and properties were added. Load was also added on the top horizontal region of the scaffold similar to a load on the femur bone. Two designs were created to test for possible implantation into femoral defects. The first design (figure 3a) was based off a successful substrate in literature that was fabricated to be implanted in Beagle femoral defects. This was a test design to see if the scaffold would still work in a different environment and maintain structural integrity with that of the of the PLLA/ TCP scaffold made by Xiong (2002). The design was made similar to that of the PLLA/TCP scaffold. The pores were kept at 400 µm, as stated in the article (Xiong, 2002). The first scaffold designs dimensions were 0.00076m by 0.001m (figure 3a), which were significantly smaller than the dimensions of the scaffold before modification, which were 0.0156m by 0.0204m (figure 3b).

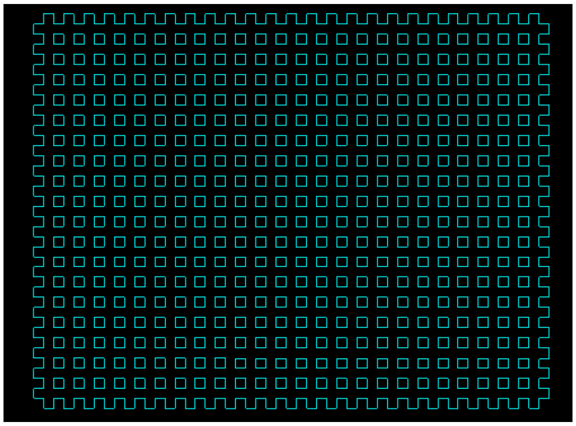

Figure 3b: *This is the original first scaffold cross-section. It was too large and its complex exterior pattern did not let it process in the FlexPDE program. The modifications that were made as shown in figure 3a made the model process faster, but not fast enough. Overall both designs were too process heavy and energy intensive.*

The 2D scaffold cross-section was made with the intentions withstanding loads consistent to a stationary male human adolescent standing on both feet with even weight distribution. The scaffold cross-section was rectangular. Following the pore morphology, squares holes were added in the scaffold design to specify the pores in the structure. The design was then imported into FlexPDE and the Young's Modulus and Poisson's ratio were added for the material PLA. The second design (figure 5) was a simplified drawing of the femur bone fixed with the medullary cavity to evince the hollow section where the marrow is situated.

This design used two drawing layers, a red 'bone' layer and a blue 'scaffold' layer (figure4). The polyline in Draftsight was used to create this design. The scaffold was implanted on the lateral side of the design at the distal end of the femur, at the femoral condyle. The design was precisely measured and calculated to relate to the femur of a young adult who had a bone tumor removed in that location. The scaffold is

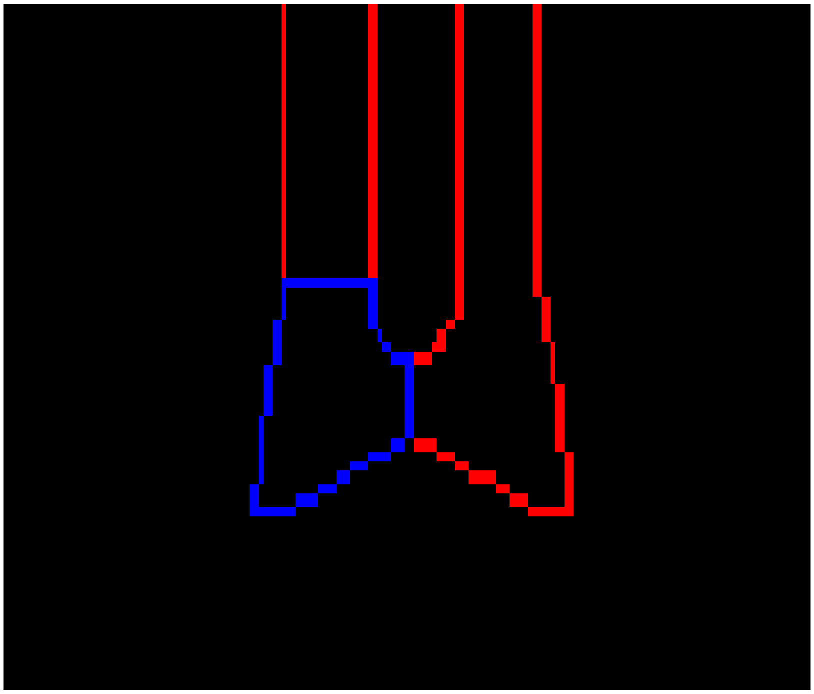

Figure 4: *The 'scaffold' section in blue, with 'bone' section in red. The design was made using polylines to make rounded edges to all more realism to the cross-section. The 'scaffold' region was made of PLA.*

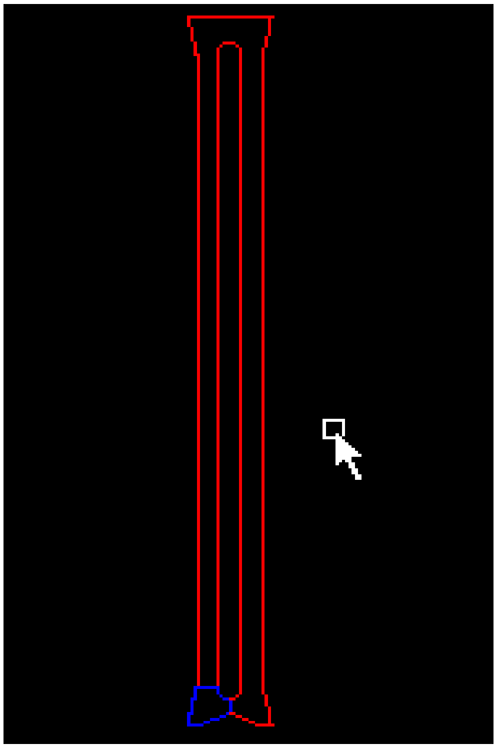

Figure 5: *Full second scaffold design. The dimensions of a male adolescent femur bone were used for the cross-section. The loads were consistent with the dimensions of the scaffold. Detailed architecture was made for more realism and practicality.*

intended to repair the limb. The final measurements of the femur bone with scaffold were 18 inches in length and 2 inches in diameter. These dimensions were added to the design by the aid of the smart dimension tool, which allowed precise measurements to make the scaffold dimensions as realistic as possible. The Poisson's ratio and Young's Modulus of the material were defined in the program to ensure the material consistency in FlexPDE. Also in FlexPDE tension and vibrate templates were initially used as the backbone to write the script for the cross-section.

Material	Bone	PLA Scaffold
Young's Modulus	100e+9	30e+9
Poisson's Ratio (GPA)	.3	.3

Material Properties Table (1)

Results:

For each scaffold design, a variable was set as xstress. This means that load xstress was placed in the u direction (vertical load). The response to the stress applied is strain. The strain is the displacement, which is shown in the graphs.

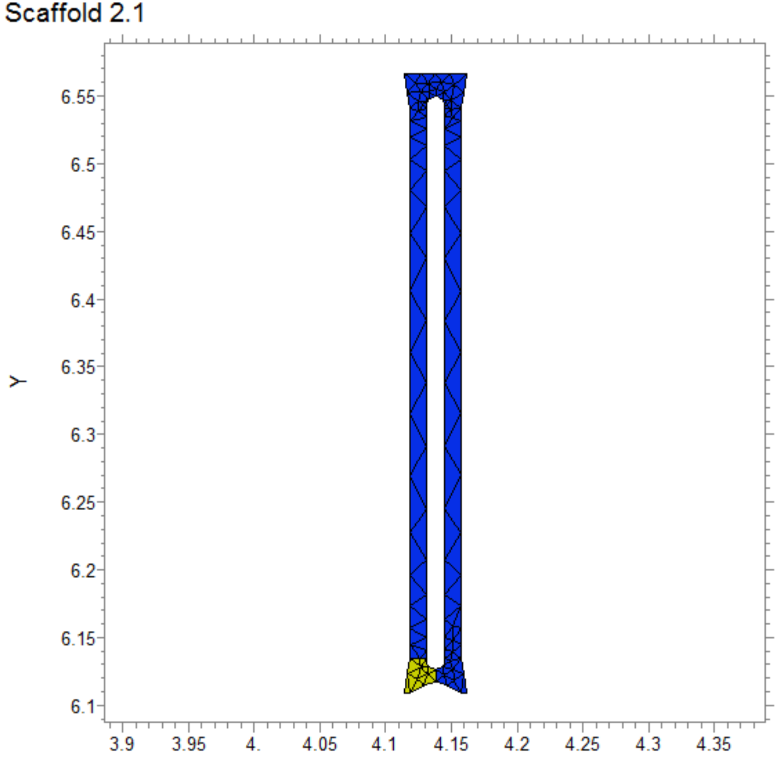

Figure 6: *This pre-displacement of the scaffold from FlexPDE before any stresses were applied. The graph is in meters.*

The first scaffold design (figure 3a) was energy intensive and process heavy, to the point when where the nodes exceeded the limits of the student version of the FlexPDE program.

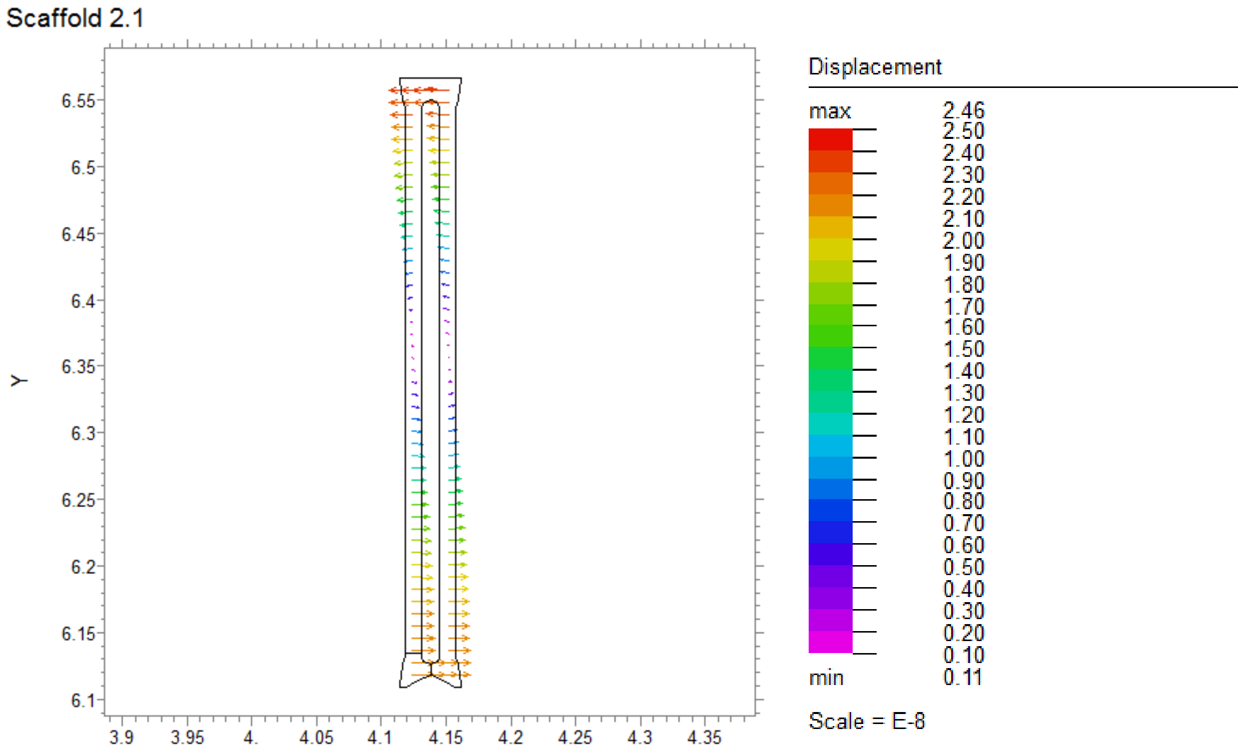

Figure 7: *The drifting of the scaffold, which occurred from the incorrect boundary conditions and from improper fixing of locations of nodes. The unit for the graph is 10^{-8}meters. This is the result of the vertical load that was placed.*

The dimensions were 0.00076m by 0.001m. This was a modification of the larger sized and more complex exterior design scaffold (3b). A dual core processor computer with the student version of FlexPDE was used to perform the FEA analysis the design in figure 3a. The drawing was complex, and took over an hour to process. When dealing with a 2D program, simplicity of the computation is preferred. The scaffold required many of the holes to be removed from the cross-section due its complexity.

For the second scaffold design the final measurements of the femur bone with scaffold were 18 inches in length (0.4572 meters) and 2 inches in diameter (0.0508 meters). There were eight distinct graphs describing the physics of the scaffold design. The first graph showed if the bone and scaffold were structurally intact (figure 6). The second graph displayed the displacement that occurred in the design through drifting (figure 7) (figure 8). The other graphs showed the x and y displacement along with the x and y stresses from different angles, but the first two graphs clearly showed the results of the test. The third graph did show the x displacement with a color chart, showing that there was maximum displacement at the bottom of the bone, where the scaffold was situated.

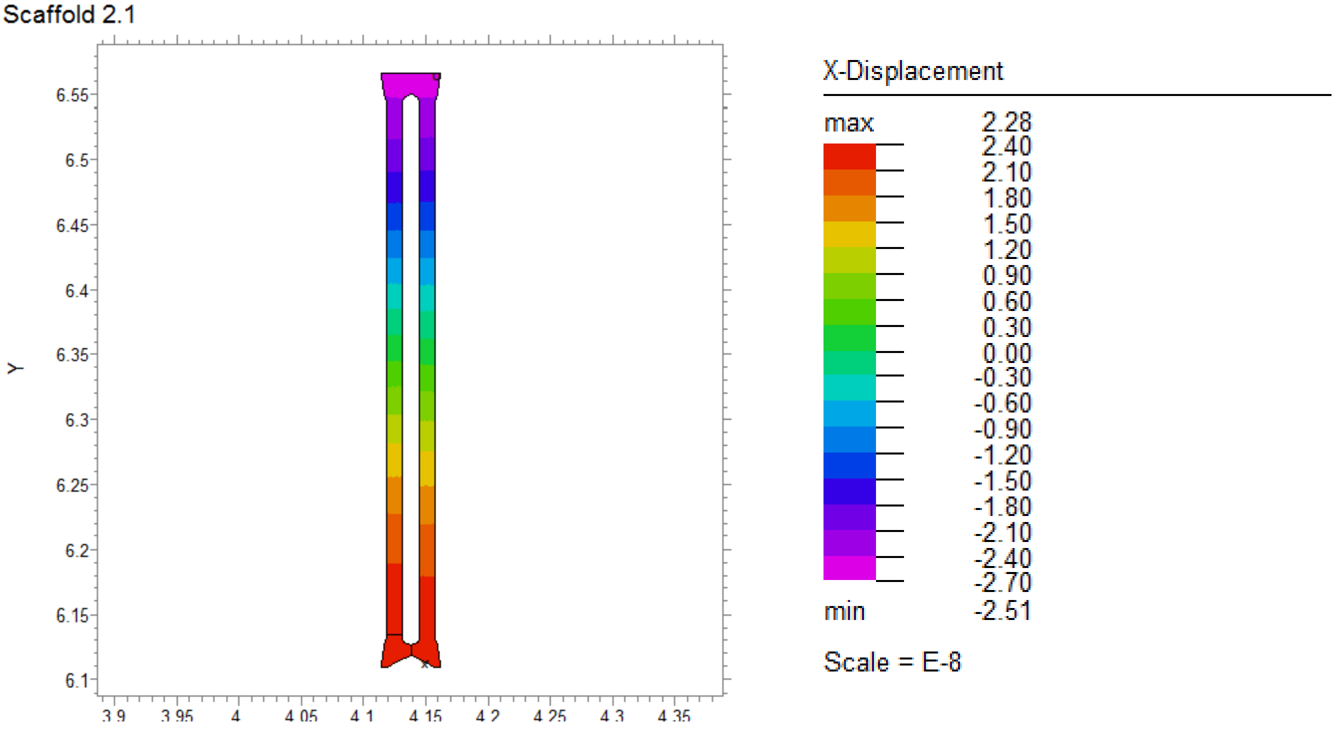

Figure 8: This shows the horizontal displacement from another view. The maximum displacement is shown at the bottom of the scaffold, where the scaffold was placed. This obviously shows that there is a flaw in the design. The red and purple have the same magnitudes, but different directions, and light blue is at 0 displacement. The graph is also in meters.

The scaffold and bone were ultimately drifting slightly during the calculations, giving the entire system a displacement. The intensities of the displacements were shown by color diagram, which red and purple at the highest and light blue at 0 displacement. The displacement was about 2.5 x 10^{-8} meters both directions. The drift took the form of a small rotation of the bone/scaffold system over time. The main reasons for such drifting were due to issues with boundary conditions to eliminate the drift. The loading that was placed on the edges of the cross-section along with the fixing of some of the boundaries of bone made newer issues arise. There were also 368 nodes used in this model, out of the 800 available nodes, making processing quicker but not as accurate.

Discussion:

The first scaffold design was created to compare to the structure that was made in the literature by Zhou Xiong to see if the design would scale up Xiong implanted the PLLA/TCP scaffolds into beagle femoral defects in the hind quarters (Xiong 2002), while the scaffold that was created to compare against it was meant for human femoral implantation. The first scaffold design resulted in an FEA model whose node count exceeded the number allowed by the student version of the software. The design had to be modified many times to the point that it would be better off creating a 3D design. The calculations took too much time, and therefore did not run. The removal of more holes from the original design (figure 3b) affected the initial purpose of the cross-section as well. This hampered the main objective of the scaffold analysis, and led to the decision to attempt a different method. Overall, the first scaffold design was not a useful cross-section. The second scaffold design that was created was an original design and portrayal of a true femur bone with vertical loads. Most of the literature deals with scaffolds that just go through compressive testing (Liu, 2007, Hutmacher, 2000, O'Brien, 2011). Other articles conducted compressive analysis as well as implantation into other organisms. The research that was conducted differs from other tests (Xiong, 2002, Hollinger, 2012) because there is not a simulation of a scaffold being placed in a human femoral defect. This is an application of tissue engineering that is heavily sought after. The material, PLA, which was used in the scaffold, was used in various tests, but the programs that were used were different from most professional research. Everything was done in silico, i.e. through software. This is similar to Leong's research in which a scaffold using CAD was made, and statistical testing was done on it to see if it is a plausible design (Leong 2003). The second scaffold design did have many problems though. There were constant complications with the boundary conditions. As more of problems arose, the more displacement the scaffold had. The loading on the edges of the scaffold and the fixing in place of nodes and components of the scaffold caused the design to not work properly. Because there was drift in the second scaffold, the actual displacement values were masked, preventing analysis of the scaffold. Several attempts to spatially fix the scaffold were not fruitful. Therefore, the second scaffold design was not an effective one. Both cross-sections overall did not prove to be effective designs.

Conclusion:

This study is a useful addition to the literature because it shows how various scaffold designs act upon pressure in realistic simulations. Properties of the elements were explicitly defined in the programs allowing for the most accurate depiction of tension and load stresses on scaffold material in silico. A simpler rectangular scaffold design and a realistic model of the femur bone were drawn using Draftsight, and a scaffold implant zone was also shown, and its properties were added. The main objective was not achieved, i.e. to design a feasible scaffold that could be implanted into femoral defects in human bone, but through the process of modeling and in silico testing, a greater understanding was achieved on how both biomaterials and human bone react to a specific load.

Future research for scaffold design is directed to biomaterials and how to find and utilize more effective ones in scaffolds. Other future research centers on the utilization of rapid prototyping (RP) in tissue engineering. This enables the production of three-dimensional scaffolds with complex geometries and very detailed structures. Adding micro- and nanometer details into the scaffolds could improve the mechanical properties of the scaffold as well. Finally, there should be CAD machines designed specifically for the fabrication of tissue engineering scaffolds.

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