Unit-Cell Based Design and Modeling in Tissue Engineering Applications

Connie Gomez[1], Ali Shokoufandeh[2] and Wei Sun[3]

[1]Drexel University, cg24@drexel.edu
[2] Drexel University, as79@drexel.edu
[3] Drexel University, sunwei@drexel.edu

ABSTRACT

This work makes a three-fold contribution for a unit-cell based methodology for designing tissue scaffolds. We present a study on unit-cell informatics; we also propose computational methods to characterize unit-cells, and finally we develop criteria for connectivity between unit-cells. We will define a set of unit-cell parameters relevant for the geometrical, structural, mechanical, transport, and biological properties in tissue engineering applications. We will also present computation and engineering based approaches to evaluate unit-cell properties. We will also develop a combinatorial framework to study the unit-cell topological connectivity and scaffold properties. Using this information, we will be able to design an interconnected 3D porous scaffold to meet application requirements.

Keywords: Topological connectivity, tissue scaffolding, multi-scale modeling

1. INTRODUCTION

Current medical procedures seek to restore tissue function to patients with damaged tissues and failing organs through organ and tissue transplantation and implants. While transplant procedures have saved many patients, transplantation alone can not meet society's need for replacement tissue and organs. During 2004, there were 22,554 transplant procedures performed in the US [1]. However, more than 87,000 people are on waiting lists, 6,000 died while waiting, and costs our society more than $600 million each year [1] . Patients who do receive a transplant or an implant risk either additional morbidity sites when their own tissue is harvested or immune rejection when foreign tissue or material is placed inside their body.

Tissue engineering(TE) is seeking to restore tissue function by developing procedures and materials for tissue applications [2]. Among the major research areas in tissue engineering is developing procedures for three-dimensional cell growth which requires structural integrity during tissue regeneration, fluid movement throughout to all cells, and the ability to support multiple types of cells and tissue structures. Using scaffolds to facilitate 3D cell growth is seen as a crucial element in guiding and sustaining 3D tissue formation [3].

Scaffold development has been concerned with scaffold material, architecture, and fabrication processes. While this work focuses on developing a methodology for designing heterogeneous scaffolds, the current work in scaffold development establishes the technologies and the physical limitations which will impact our methodology. Currently, researchers are seeking scaffold materials that are biodegradable as well as biocompatible [3]. Another area of scaffold development is controlling scaffold architecture via the underlying fabrication process used. Fabrication methods include Solid Free Form (SFF) fabrication methods which can produce repeatable structures with interconnected pores [4]. Despite the research and development in these areas, the problem of creating an efficient method for generating a patient-specific heterogeneous scaffold remains unanswered.

While research is still being conducted into cell behavior within a scaffold, the ability to design based on cell behavior will be critical in scaffold design [5]. Furthermore, the inclusion of heterogeneity may cause one cell type to differentiate into different cells, forming a heterogeneous tissue or may allow different cell types to differentiate on the same scaffold forming a heterogeneous tissue. Heterogeneity can be designed into a scaffold by using different materials or different architectures. Heterogeneous scaffold designs based on unit cell assembly allow for gradients as well as specifying the scaffold structure in areas of shape changes in the mechanical properties. In conjunction with the mechanical properties, the unit cell methodology allows for a more detailed view of transport properties within a scaffold with the goal of creating specific flow conditions, unlike previous approaches. Fluid flow modeling has focused on single parameter evaluations in constructed models, such as permeability [6]. Other fluid flow work has focused on

flow through heterogeneous or random architecture [7]. We attempt to ensure connectivity between unit cells and force the connections to have a minimum threshold value.

Our characterization and unit cell based assembly process addresses the need for multi-scale scaffold design with interconnected pores using both the structure and the mechanical properties of the unit cells. The work presented in this article has two goals. Firstly, we define a set of critical unit cell parameters for representing the geometrical, structural, mechanical, transport, and biological properties for a tissue engineering application. Secondly, we describe a detailed characterization of the unit cell based on more than its pore size, porosity and mechanical properties, which have been the standards of scaffold evaluation prior to cell culturing.

The application of multi-scale modeling to tissue scaffolds is discussed in the section 2. The design criterion as well as the database system we have established for the unit cells is given in Section 3. The methods used during characterization are described in Section 4. Discussion and conclusions are given in the last section.

2. UNIT-CELL BASED MULTI-SCALE MODELING

The considerations and obstacles impeding successful tissue regeneration could come from a number of sources. Since tissue regeneration is dependent on variables such as the type of seeded cells and the mechanical signals delivered via applied loading, tissue engineering must have the capability to model cellular behavior at the micro-scale(lower scale) and tissue behavior at the macro-scale(higher scale). Additionally, tissue engineering must form a bridge between these two scales through the introduction of an intermediate scale, a meso-scale. The meso-scale will span both the micro- and macro- scales and will utilize information from both scales. It is on this scale that we introduce our unit-cell and our unit-cell design methodology.

2.1 Micro-scale and Macro-scale Tissue Information

This paper will focus on the methods and tools use to close the gap between the micro-scale and the macro-scale for tissue-engineered unit based scaffold design [8, 9]. The goal of any scaffold design is to provide cells with an environment that will provide the heterogeneous conditions needed for cell survival, cell proliferation and functioning tissue development [10, 11]. For this, the scaffold design should mimic the natural tissue's structure at the micro-scale and its response to conditions at the macro-scale. As in Fig. 1, the first step is to identify the damaged portion of the bone. This tissue has a heterogeneous micro-scale architecture with effective mechanical, structural and transport properties. The assembled scaffold will experience loading conditions and design parameters at the macro-scale. An integral part of this step is a fundamental understanding of the tissue, which in our case is bone. All this information is then incorporated into unit-cell design at the meso-scale, which lies between the micro and macro-scales.

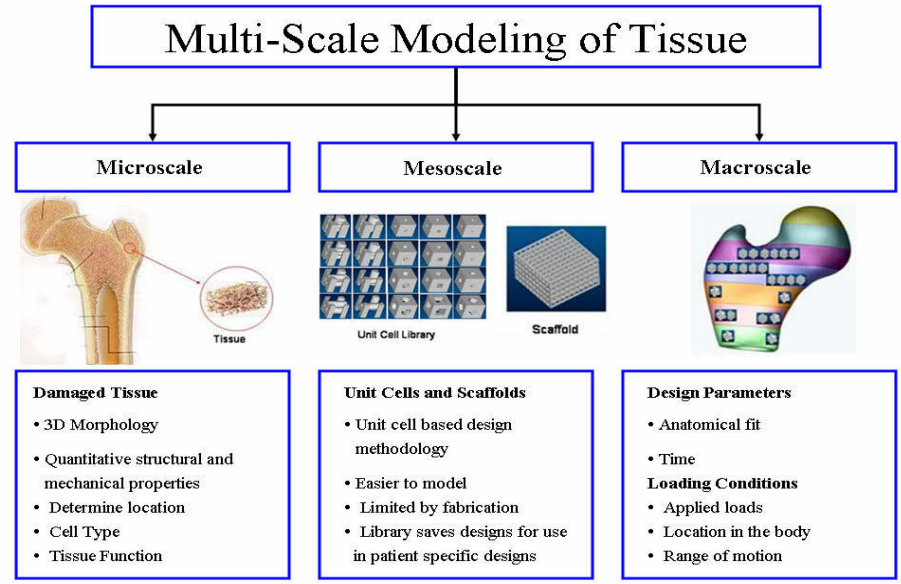

Fig. 1: Multi-scale Modeling: The left column identifying the damaged bone that requires replacement. The bone can be studied at the micro-scale and the macro-scale. At the micro-scale, we can determine the bone's morphology and

quantify its properties. On the far right at the macro-scale, we determine the loading conditions on the bone. We introduce a meso-scale that creates a continuum between the micro- and macro-scales. At this level we introduce a unit-cell methodology for bone scaffold designs.

2.1.1 Micro-scale Characteristics of Bone

The micro-architecture of bone and even the bone surface can be seen using current Scanning Electron Microscopy (SEM) technology. The architecture appears random, but in each bone type there are several types of substructures or features which are repeated. For example, trabecular bone contains calcified struts, which allow for open spaces and give the tissue its spongy quality [12]. While current technologies can capture images of the bone, the computational cost of capturing enough information and doing a reconstruction exceeds current technological limits. However, these images can provide pore size and porosity data. In addition to the tissue structure, it is at this level where we can identify the cell types that produce and remodel the bone, namely osteoblasts and osteocyctes [13]. However, at this scale, the connection between an applied load to the bone and the mechanical signal a cell receives is not completely understood.

2.1.2 Macro-scale Characteristics of Bone

At the macro-scale, bones serve as structural support to the body during daily movements. Those movements apply forces on the bone which serve as signals to the body during bone remodeling. Where greater forces are applied, there is a greater distribution of compact bone. One example of this occurs in the femural head. The head experiences large forces as it supports the upper body and the impact incurred during walking. The head is composed mostly of compact bone, which has a higher elastic modulus than trabecular bone. The elastic properties and the anatomical geometry can be generated using imaging technologies, such as Magnetic Resonance Imaging (MRI), and micro-Computed Tomography (micro-CT). These images can be use to quantify the structural and mechanical properties of the tissue through a Quantitative Computed Tomography (QCT) [14] and homogenization approaches [11]. This distribution of compact and trabecular bone make bones heterogeneous structures.

2.1.3 Meso-scale

To bridge these two scales and to produce a heterogeneous scaffold, we introduce a meso-scale and a unit-cell-based methodology. The meso-scale is the platform on which the tissue's morphological, structural, and mechanical properties, and the loading conditions for the tissue location will be used to design a heterogeneous scaffold. The meso-scale model will incorporate this information into a unit-cell design that will bridge the micro-scale and the macro-scale. The advantage of introducing this scale is that changes to the micro-scale can be linked to changes in the meso-scale, which can subsequently be linked to changes in the macro-scale, thereby forming a continuum between the scales.

2.2 Unit-cell Based Scaffold Framework

The framework for this approach consists of designing unit cell structures, characterizing the unit cell structures, and saving the unit cell informatics into a database. These unit-cells will then be assembled into a larger heterogeneous structure that has the outer anatomical geometry required by a specific patient. The scaffold structure will be designed to meet the particular needs of the patient by assembling the unit-cells into a structure that has the mechanical, structural and transport properties required by the application.

2.2.1 Micro-scale: Unit-cell Internal Architecture

Individual unit-cell design focuses on generating architecture which meets the preference ranges of a particular cell and meeting the global loading conditions it must withstand. This includes designing for porosity, pore size, features such as rods and struts, and even the angles between these features. By including these in the design, the biological environment is being mimicked except for inherit randomness found in the natural tissue.

2.2.2 Meso-scale: 3D Unit-cell and Unit-cell Library

A unit-cell is the smallest non repeating volumetric representation which will be used within the heterogeneous scaffold [15]. Unit-cells must incorporate all the mechanical, structural and transport properties gathered from the micro-scale and the macro-scale as well as adhering to fabrication limitations and biocompatibility requirements [16]. Due to the number of cells that could be used in the implant and the range of loading conditions possible in the human body, each scaffold must be designed for a particular application. Creating a heterogeneous scaffold using this approach will require using different unit-cell designs. Therefore a unit-cell library will be compiled and will contain unit-cell designs

and their information for scaffold assembly at the meso-scale level. Multi-scale modeling will allow a more accurate understanding of tissue requirements at different scales to better utilize this library to produce a tissue scaffold that will deliver an equivalent environment.

2.2.3 Macro-scale: Unit-cell as a Macro-anatomical Tissue Scaffold

The unit cell design will fill the anatomical geometry, which is obtained from the macro-scale. Scaffolds created using this unit-cell based approach will require a fabrication process that uses biocompatible materials. The process must be able to create the complex architecture with the appropriate mechanical properties. Fabrication will utilize 3D printing technologies that can create complex heterogeneous structures at the meso-scale. Namely, this approach will use fused deposition modeling (FDM) [17] and precision extrusion deposition (PED) [18].

3. UNIT-CELL INFORMATICS

Much like the field of gene sequencing, tissue engineering faces an exponentially growing amount of information from biological, design, and manufacturing advancements. Utilizing this flood of information will be crucial to regenerating functional tissue, especially to the areas of tissue scaffold design, cell co-culturing, and modifying cell behavior. As our work focuses on unit-cell based scaffolds, we are interested in the information that affects the initial unit-cell design and the subsequent unit-cell placement in a tissue scaffold. Similar to the bioinformatics gathered to maintain the biological information of genes, unit-cell design and assembly must be based on biological, structural, and transport information. Gathering and maintaining all this information for unit-cell based scaffolds results in the new field of Unit-Cell Informatics. Unit-cell informatics will provide the information used for unit-cell selection and placement in a tissue scaffold. The unit-cell based scaffolds can be assembled using algorithms that insure multiple parameters, such as connectivity, porosity, and elastic modulus are satisfied. The algorithm is created based on the geometry, structure, and transport properties requirements for given cell type. Those requirements are based on the environment it needs to thrive.

Designing environments that promote cell growth under normal loading conditions require insight into the environment we are trying to mimic, namely a naturally occurring heterogeneous tissue. Therefore it is essential that the target tissue environment be analyzed and its properties used in subsequent unit-cell scaffold designs. The information gathered from the tissue includes geometry, function, cells present, cell configuration, fluid properties and loading conditions. This work will define a set of critical unit-cell parameters for the aforementioned properties.

3.1 Unit-cell Design Considerations

Tissue scaffolds and subsequently unit-cells are designed to meet various considerations, listed in Table 1. Also listed in Table 1 are possible design selections, which can alter a unit-cell's properties. Many of the parameters describing the possible design selections are important to several areas. This creates interdependence between possible design selections and unit-cell properties. This aspect of tissue scaffold design means that the design approach will be required to evaluate unit-cells based on multiple factors.

3.1.1 Mechanical Design Considerations

From the Table 1, we know that mechanical properties include structural integrity, architectural stability, strength and stiffness. These mechanical requirements are determined using mechanical testing or the QCT approach for a given location within the natural tissue. To meet those requirements, the unit-cell will need to be constructed such that its effective Young's Modulus (E_{Eff}) in each direction is equivalent to the tissue under consideration. The effective Young's Modulus in turn is dependent on the Young's Modulus (E) of the bulk material and the geometry of the given unit-cell [19]. Likewise, the other mechanical properties of effective shear stress (G_{Eff}) and Poisson's Ratio (υ_{Eff}) will depend on the properties of the scaffold material and the geometry [19]. However, it should be noted that the list of materials available for tissue scaffold applications is limited by the need for biocompatiblitiy and biodegradability of materials. The biological needs of the seeded cell will also dictate the geometry a unit-cell in terms of porosity (ϕ), pore size (d_{pore}), and pore area (A_{pore}) [11]. The tissue under consideration is able to function within a limited space under variable temperature conditions; as a result we need to gather information concerning the density (ρ) and the thermal expansion coefficient (α). While these parameters have been related to mechanical properties due to global loading, they are also important to other areas due to cellular needs [20].

3.1.2 Biological Design Considerations

The biological requirements, which are dictated by the desired tissue and are cell specific, include cell loading, distribution, attachment, proliferation, and tissue formation [16]. The biological requirements start with identifying the cell which will seed the scaffold and the medium which will support cell growth. The selection of the cell and medium will also eliminate some of the possible materials. Scaffold material selection is based on the cell's ability to attach to the material and the medium's reaction to the material.

Design Considerations	***Possible Selections Affecting Property***
Mechanical • Scaffold structural integrity • Internal architectural stability • Scaffold strength and stiffness Biological • Cell loading, distribution and nutrition • Cell attachment and growth • Cell-tissue aggregation and formation	 • Biomaterial selection • Internal architecture • Porosity and pore distribution • Fabrication method • Layout • Pore size and interconnectivity • Vasculature
Geometric • Anatomical fitting	• Scaffold external geometry
Transport • Nutrient and oxygen delivery • Waste removal • Growth factor and drug delivery	• Interconnectivity • Permeability selection
Fabrication • Temperature ranges during process • Control • Resolution	• Process parameters • Materials

Tab. 1: Design Considerations: On the left, the design considerations for tissue scaffolds are divided into five groups. Each group has particular needs it must address. In the right column, the selection options that directly impact the needs being addressed in one or more groups.

The cell selection will also determine the interior architecture in terms of porosity (ϕ), pore size (d_{pore}), pore area (A_{pore}), and pore angles (θ_{pore}). These parameters are directly correlated to cellular behavior [21]. For a given cell, its biological requirements also extend to the flow patterns and conditions that must exist for a cell to attach to the unit-cell surface and to receive adequate amounts of nutrients and growth factors. Currently, cell preferences are not completely known, but they must be identified as completely as possible with the prospect that they will yield information crucial to successful tissue growth [22].

3.1.3 Geometrical Design Considerations

Geometry must be considered both at the macro-scale and at the meso-scale when examining the tissue. At the macro-scale, the overall anatomical geometry of the patient must be gathered and retained for later scaffold design. This information can be gathered from the patient via Computed Tomography (CT) or magnetic resonance imaging (MRI). These images undergo a reverse engineering process to reconstruct the outer geometry of the scaffold [16]. For the purposes of scaffold implants, the boundaries of the volume (regions of the scaffold) are constructed from the anatomical information gathered during the reverse engineering reconstruction.

At the meso-scale, cell type and cell behavior will dictate the limitations of architectural features present. The tissue will also have a structural form which could also be mimicked in the unit-cell design. For example, bone tends to have either rod or plate like structures, depending on their location in the body. The architectural information can be gathered in the form of parameters, such as length. As discussed in Section 3.1.4, geometrical relationships also directly affect transport properties.

3.1.4 Transport Design Considerations

Since mass and fluid transport are essential for providing the cells with the materials needed to differentiate, the natural tissue can provide information on the fluid and the flow conditions for a given cell type. Firstly, the fluid reaching the tissue has a density (ρ_{fluid}) and viscosity (μ_{fluid}). We also maintain the interior geometrical information $\left(A_{pore}\right), \left(d_{pore}\right)$ and (ϕ). Coupling this information with the geometric information already gathered and velocity (V), pressure (P), and temperature (T), the local flow conditions can be used to determine the Reynold's number (Re), as in Eqn. 3.1, and therefore the type of flow at the inlet and the outlet of the unit-cell [23]. The unit-cells assembly will be determined in part by inlet and outlet flows. At the same time, fluid and mass transport through the tissue apply stresses on the tissue which affects cell behavior. This architecture insures that the tissue cells have the necessary nutrients for cell growth but also underlines the need to have interconnect fluid pathways [13].

$$\mathrm{Re} = \frac{\rho_{fluid} V d_{pore}}{\mu_{fluid}} \tag{3.1}$$

3.2 Fabrication Design Considerations

While the natural tissue can yield information needed to mimic an environment for cell growth, it is important to realize that current technologies can not reproduce the local geometries of natural tissues. For this reason, the limitations and the processes for scaffold fabrication need to be considered during scaffold design. Fabrication processes have limitations, such as feature size limits, material selection and the accuracy of its control system. These limitations must be considered when designing the unit-cell architecture and aligning unit-cells to form the overall scaffold. It is therefore important that assembly processes be able to take such matters into consideration.

3.3 Unit-cell Informatics

Heterogeneous tissue scaffold designs need to meet the design requirements of a particular application for biological requirements, material properties, structural properties, and transport parameters. The parameters for unit cell design and characterization constitute our set of unit cell informatics and are in Table 2. The unit-cell-assembly methodology is in turn based on these parameters. The desired characteristics for a scaffold are dependent on the particular cell or cells to be cultured on the scaffold. While the optimal environments for culturing and co-culturing specific cells is still under investigation, unit-cell characterization will provide scaffold designers information for unit-cell selection once preferred environmental conditions are determined [24].

4. UNIT-CELL CHARACTERIZATION

With the informatics of natural tissue categorized by design consideration, we developed a set of computational and engineering based approaches to evaluate the unit-cell properties. By applying these approaches to any unit-cell design, we can then use the properties of the unit-cells for comparison with the properties we want to mimic in the natural environment.

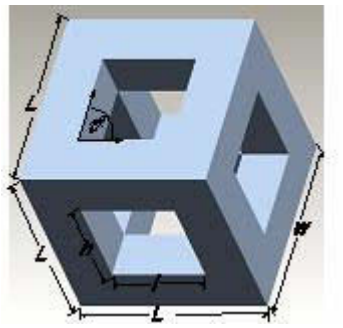

Fig. 2: Two-Phase Unit-Cell: Sample Two-Phase Unit-Cell with the structural dimensions labeled. These dimensions depicted in the figure form the parameters that describe the geometry of the unit-cell features.

4.1 Morphology Characterization

The first task when characterizing a unit-cell design is to determine the morphology of the unit-cell. Due to the heterogeneous nature of unit-cell design based on either multiple materials, heterogeneous structure or both, the number of phases present in a unit-cell system need to be determined and labeled. For example, in Fig.2, we have a two phase system consisting of the scaffold material and the pore/fluid space. The space occupied by each state is also recorded as a function of the spatial points which constitute the phase. With each phase, we also create a corresponding skeletal representation. This representation will greatly reduce the work for aligning unit-cells. With each additional unit-cell type into a scaffold, the number of alignment operations increases exponentially. For this reason, we have sought a unit-cell representation that can handle thousands of operations efficiently.

Skeletonization is a process that has been utilized by digital imaging applications for the past few decades [25]. Intuitively, the creation process of a skeleton for a 2D object is to find the centers of the largest circles which fit inside the object and touch two or more points of the object's boundary. The set of centers constitutes the skeleton. Each point belonging to the skeleton contains the radius of the circle used to create the skeleton. As shown in Fig. 3, four single-phase objects have been drawn along with their skeletal representations. The skeletal representation for either phase can be generated and used for alignment. Skeletonization can be done by decomposing a unit-cell into either a set of 2D layers and creating skeletal representations for each layer of the unit-cell or by creating a 3D skeleton of the unit-cell, depicted in Fig. 4. The skeleton representations will be used to create topological connectivity between assembled unit-cells.

Design Consideration	***Characterizing Parameter***
Mechanical	E_{xx} , E_{yy} , E_{zz} , G_{xy} , G_{yz} , G_{xz} υ_{xy} , υ_{yz} , υ_{xz} , E_{xx}^{Eff} , E_{yy}^{Eff} , E_{zz}^{Eff} , G_{xy}^{Eff} , G_{yz}^{Eff} , G_{xz}^{Eff} υ_{xy}^{Eff} , υ_{yz}^{Eff} , υ_{xz}^{Eff} , d_{pore} , A_{pore} , θ_{pore}^{x} , θ_{pore}^{y} , θ_{pore}^{z} , ρ , α , ϕ
Biological	ϕ_{solid} , ϕ_{fluid} , d_{pore} , A_{pore} , θ_{pore}^{x} , θ_{pore}^{y} , θ_{pore}^{z}
Geometric	ϕ_{solid} , ϕ_{fluid} , d_{pore} , A_{pore} , θ_{pore}^{x} , θ_{pore}^{y} , θ_{pore}^{z} , l,h,w,r
Transport	ρ_{fluid} , μ_{fluid} , d_{pore} , A_{pore} , ϕ_{solid} , ϕ_{fluid} , T , P , V_x , V_y , V_z , k
Fabrication	T,l,h,w,r

Tab. 2: Unit-Cell Informatics: In the left column, design considerations are divided into five groups, mechanical, biological, geometric, transport, and fabrication. In the right column, the parameters that describe the material properties (Young's Modulus, Shear Modulus, and Poisson's Ratio), fluid properties, geometry, and scaffold conditions are listed. The material properties are for the *xx, yy, zz, xy, xz, yz* directions, both for the bulk material and effective material. Note, some of the parameters are in more than one location.

4.2 Geometry/structural Characterization

The architecture of each unit-cell is dependent on the tissue it is mimicking. While tissues can be similar, their location in the body affects their internal architecture in order to handle the repeating loading conditions experienced at that location. For this reason, a unit-cell can be designed to mimic the natural material. Designs include the use of rods and plates to mimic bone. This would allow unit-cells to be classified by the architectural elements present in the unit-cell.

The specific geometry of each unit-cell design will affect the mechanical properties and is critical for the fabrication process. Geometry determines the stress distributions within a structure and the maximum loading conditions a structure can withstand [26]. The geometry also determines the resolution requirements for the fabrication process. For our work, the geometry of a unit-cell denotes the size and dimensions of the architectural features as in Fig. 2.

Structurally, the goal of scaffold designs is to mimic the mechanical and transport properties and the overall anatomical fit for future placement into the body. Prior work has used porosity and pore size as the basis for scaffold evaluation [27]. Pore size $\left(d_{pore}\right)$ has been a parameter of most scaffold systems, and a large amount of research has been undertaken to create desired pore sizes repeatedly while using different fabrication processes [11, 28]. It is known that cells will only cross a specific spatial distances and obstacles in an environment. Cell movement limitations due to architecture are not completely understood but current evidence demonstrates cell behavior in the presence of different

architecture can vary greatly [11]. The pore size is related to the pore shape and is denoted by both the area of the pore and the angles of the pore. Efforts to mimic nature can lead to irregular pore shapes that make these parameters important to the unit-cell structure characterization.

Porosity, (ϕ_f) is a critical parameter for characterization of scaffolds and is specific to the type of cell that will grow on the scaffold. Porosity is the fluid or contour-space volume-fraction of the unit-cell. The porosity in a two-phase unit-cell structure is the total volume of one phase over the total volume of the entire system. While this will yield the geometric porosity of the unit-cell structure, the unit-cell operating with fluid flowing through it will have an effective porosity. The effective porosity denotes how much of the available flow capacity is being used for flow through the unit-cell. The ideal unit-cell structure has an effective porosity equal to its geometric porosity, so that all areas within the unit-cell structure that are in contact with the flow pathways. Areas where fluid does not flow are known as closed or dead porosity. Dead porosity spaces are where cells have the least chance for survival or growth due to lack of available nutrients.

Each of the structural features has geometric dimensions, which are requirements during the fabrication process. A feature's fabrication feasibility is limited only by the fabrication resolution. As in Fig. 2, a unit-cell will have features that have lengths, widths, heights, and angles (l, L, w, h, and θ). During characterization, that geometrical information will be recorded. The geometrical information will then be compared to the fabrication resolution. It will also be important to fluid flow through the unit-cell, which is discussed in Sec. 3.1.4.

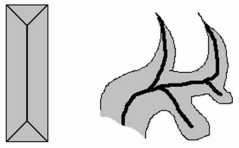

Fig. 3: Sample Skeleton Representations: Examples of 2D figures and their skeleton representations. The skeleton representations capture a unit-cell's morphology and topology. Skeletons are created by fitting circles that touch two or more boundaries inside the figure. The circle centers create the skeleton.

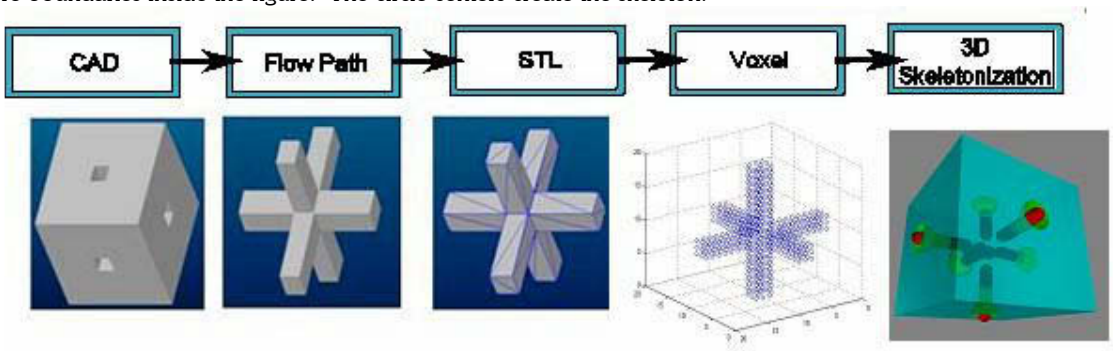

Fig. 4: 3D Unit-Cell Skeletonization Method: An STL file is created for either the pore space or the scaffold material. The STL is then voxelized. A skeleton representation will be created for the entire unit-cell.

4.3 Mechanical Characterization

The mechanical properties rely both on the material properties of the phase and the phase geometry. The properties relating to the material need to consist of the standard 6 different directions, *xx, yy, zz, xy, xz, and yz*. The material properties needed for characterization include Young's modulus (E_{ij}), shear modulus (G_{ij}), Poisson's ratio (υ_{ij}) and the coefficient of thermal expansion (α) [19], where *i* and *j* range over *x, y, and z*. Different material choices have different bulk Young's modulus (E_{ij}) values. Therefore, material choice directly affects a scaffold's ability to mimic the biological environment and for a given unit-cell structure, the material will have a bulk Young's modulus and an effective Young's modulus. This does not fully characterize the unit-cell in terms of scaffold material. The scaffold material's surface affects cell attachment and fluid flow due to its surface roughness (e). Rougher surfaces decrease the velocity threshold that will produce turbulent flow [29]. The structural material also needs to be characterized by its effective Young's modulus (E_{ij}^{Eff}), effective shear modulus (G_{ij}^{Eff}), and its Poisson's ratio (υ_{ij}^{Eff}). These material

values are necessary to understand the structural material's behavior when loaded under shear stresses and the effect that tensile loading will have on material along its length and across its width.

The mechanical properties of a unit-cell can be determined by one of the four methods: rule-of-mixtures, mechanical testing, finite element analysis (FEA), or a homogenization process [15]. Rule-of-Mixtures averages the properties of the materials found in the sample based on the volume fractions of each phase [19]. In mechanical testing, a sample is fitted with strain gages that measure deformation under stress. Then has a compressive force applied. The experimental strain data is used for mechanical property calculation. This method will yield experimental information but is time consuming due to the need for physical samples [15]. The FEA method begins with the unit-cell that has been meshed. Then one surface of the unit-cell is held stationary while the opposite surface experiences an applied force. After the unit-cell undergoes deformation, the amount of strain is reported. Using the known stress via the applied force, the surface area on which it was applied, and the reported strain values, the mechanical properties of the unit-cell can be calculated using Hooke's Law [30]. Finally, a given unit-cell of a region can undergo a homogenization process to determine its effective mechanical properties [15]. The unit-cell will be treated as an anisotropic material, and therefore the Young's modulus, the shear modulus and the Poisson's ratio will be independent of each other. It begins by selecting unit-cell for homogenization. Next, during the preprocessing phase, a mesh is created. The process then goes on to solve six characterized cases for the homogenization equation with inputs from the stiffness matrix, the boundary conditions, and the force vector.

4.4 Transport Characterization

Similar to mechanical properties, fluid and mass transport rely on the fluid properties in the pore space and the geometry of the unit-cell. The transport properties also rely on existing fluid velocities or pressures. Current modeling systems allow any initial velocity or pressure to be applied such as in Fig.6. Being able to apply an initial forced velocity, a unit-cell is capable of experiencing different velocities, therefore different Reynold's numbers and in turn both laminar and turbulent flow. This means that initial conditions for a given environment also need to be recorded. The components of properties such as velocity will be recorded using a Cartesian coordinate system.

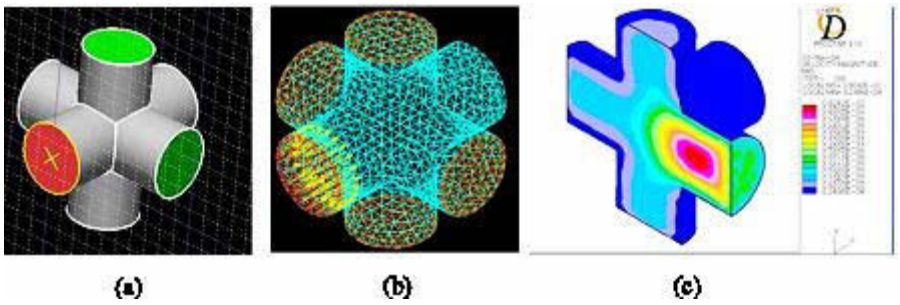

Fig. 6: Computational Fluid Dynamics model of a fluid space: The geometry of the fluid space is created or imported into STAR-CD, such as in (a), and initial fluid conditions are defined for the space, as in (b). In (c), we see the resulting interior velocity contours that are produced from the geometry and initial conditions [31].

Similar to using different materials for the unit-cell structure, different fluids inside the unit-cell will affect the transport properties and flow conditions [13]. Each fluid has a viscosity (μ), a density (ρ) and diffusion rate (κ) for a given temperature (T). These fluid properties are involved with determining the Reynold's number that affects the flow patterns present in the scaffold, the start of turbulence in a unit-cell structure and what stresses a cell would undergo [29]. The flow patterns will also determine where mass will move through a scaffold. In order to mimic the environment in which the cells will grow, both the fluid properties and the conditions found either in nature or in a bioreactor need to be part of the design process for a unit-cell structure and must be known for unit-cell structure characterization. The combination of geometry, fluid properties and an initial velocity can be seen in Fig. 6

5. CONCLUSION

This work introduces and makes a three-fold contribution to developing a unit-cell based methodology for designing tissue scaffolds. We have presented a study on unit-cell informatics, and proposed computational methods for unit-cell characterization. This approach is based on unit-cells that will fill regions within a scaffold and will meet all the design

considerations for a given application. Designing scaffolds based on unit-cell skeleton representations will reduce the representation complexity of the unit-cell and in turn the tissue scaffold. By using unit-cells, we can approximate both the micro-scale and the macro-scale properties of natural tissue, use current manufacturing methods, and reduce many hardships in computational tissue engineering.

Introducing unit-cells to tissue scaffold applications creates a meso-scale environment. This meso-scale serves to bridge the macro and the micro-scale requirements that stem from loading and from the biological needs of the seeded cells. Unit-cell design can incorporate biocompatible material, geometrical features, and fluid transport patterns a cell needs for attachment, proliferation, and growth. Similarly, unit-cell design can incorporate mechanical properties necessary to withstand loading conditions. Bridging these two scales and incorporating their requirements on one platform, the unit-cell, means that the scaffold environment created is closer to mimicking the natural tissue environment than if we based scaffold design on either micro or macro scale requirements.

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