Internal Scaffold Architecture Designs using Lindenmayer Systems

Binil Starly[1] and Wei Sun[2]

[1]University of Oklahoma, starlyb@ou.edu
[2]Drexel University, sunwei@drexel.edu

ABSTRACT

Scaffolds with designed interior pore architecture, predefined porosity and a well interconnected predetermined channel network has been the favored design approach for tissue engineering applications. Solid freeform fabrication (SFF) technologies have provided the capability to fabricate scaffolds with desired design characteristics due to its integration with CAD enabled tools. However, currently the interior macro pore design of scaffolds have been limited to simple regular shapes of either squares or circles due to inadequate CAD capability. In this paper we seek to enhance the design of the scaffold architecture by the use of rewriting schemas such as Lindenmayer systems (L-systems) which provide a powerful method to create complex branched networks. We have presented several exploratory case examples to show the applicability of using such rewriting systems to model nutrient delivery networks within hydrogel scaffolds without an overwhelming need for expensive computer hardware. Feasibility studies applying these systems to the fabrication of samples using an extrusion based SFF system is presented. The paper concludes by future research prospects in extending the concept of L-system design theory to newer applications.

Keywords: L-systems, Tissue scaffolds, Vasculature network, Space filling curves.

1. INTRODUCTION

Scaffold guided tissue engineering necessitates the need for biologically inspired artificial matrices to recreate the natural three dimensional microenvironment for conducive cell and tissue growth. These scaffolds provide the framework upon which cells can attach, migrate, proliferate and differentiate into the desired tissue. A measure of success within this multidisciplinary technological field is how well cells are able to survive and organize themselves to form the desired tissue intended for a specific application. Such organizational processes are largely influenced by cell interaction with the surrounding material, surface topography, scaffold interior architecture [1-3] and nutritional network that feed the cells.

The tissue scaffold micro-architecture is believed to influence the behavior of cells and the biological function of tissues by providing a nutritional pathway as well as a spatial distribution for cell growth and proliferation [4]. Several studies have indicated that cells can be coaxed to migrate and grow along the direction of designed micro-channels/struts, through which the required cell spatial distribution and tissue function can be realized [5-8]. In certain applications, certain kinds of cells such as chondrocytes need to be restricted within void spaces [9], and therefore the architecture must support such design requirements. The performance of current generation freeform fabrication (SFF) based scaffolds can certainly be improved if newer interior architectures can be realized. Pore shapes in the form of rectangular, circular and honeycomb architectures have been fabricated by different research groups [10-13]. However, the design has always been limited to such regular primitive shapes in the form of patterned straight lines, square or circular holes. This is primarily due to shortcomings of current CAD technologies either in representation of such micro-structures or the inability to transfer detailed microstructure information to SFF machines [14-16]. There is a need to expand the pore architecture variety to provide for better functionality and adequate nutrient supply into the interior of the scaffold.

In vertebrate animals, circulatory systems distribute essential nutrients and carry waste products to and from cells. They are composed of arteries, capillaries and veins on the order of one to tens of microns in dimension that form a closed system. Any engineered scaffold or matrix must have a nutrient distribution system that is essential to the success of tissue engineered products particularly for bone, liver, kidney. Very limited work has been reported on the

fabrication of a vasculature system within scaffolds or hydrogel matrices resulting in scaffolds that have limited thickness (less than 5mm). Larger and thicker scaffolds (10mm or larger) to treat critical size defects have been reported with limited success primarily due to the inability of cells to survive deep within the scaffold [17]. It has been hypothesized that thick scaffolds must have an inbuilt nutrient distribution network to allow for the uniform growth of cells within their matrices. This would allow sufficient time before the cells themselves can recreate their own capillary network (a process known as angiogenesis). Some of challenges faced by the scaffold and hydrogel fabrication research community on the creation of such a branched system are listed below:

a) Inability of current fabrication processes (either RP or chemical methods) to create an interconnected micro capillary network within the scaffold.
b) Chemical based methods of fabrication lack the capability of producing a well controlled interconnected network of channels within a scaffold or hydrogel. On the other hand, the growing acceptance of Solid freeform fabrication systems for the fabrication of scaffolds requires the input of a CAD model. The design of an intricate network of channels within a CAD model is memory intensive and cumbersome using existing commercially available CAD systems.
c) Even if a well distributed network of channels (such as scaffolds produced using FDM, PED) with a 0°/90° pattern is obtained, the pores are gradually filled up with the growth of cells, preventing the flow of nutrients deep into the scaffold. Hence, there does not seem to be an effective way of keeping the channels open for continuous delivery of nutrients.

These challenges prompt the need to develop new design methodologies that address the use of creating nutrient delivery networks and guided struts within scaffolds without placing a memory overload on either graphic hardware or data transfer capabilities. In this paper, our objective is to develop new internal pore architecture designs by making use of rewriting systems that extends the general use of layer road patterns (0°/90°, 0°/60°) for extrusion and printing based SFF systems. The use of the recursive rewriting rules dramatically expands the pore architecture design space fabricated by such systems. The paper is organized as follows: The second section of this paper explain the basics of Lindenmayer systems, followed by sections on how such systems can be used to create interesting patterns without the overwhelming need for powerful CAD hardware/software systems. The fourth section details on how space filling curves generated using L-systems can be used to expand the pore structural library available for extrusion based SFF systems. In the last section, the paper concludes by a brief discussion on the results and future research prospects.

2. LINDENMAYER SYSTEMS

Lindenmayer systems (L-systems) were developed by a Hungarian theoretical botanist Aristid Lindenmayer in 1968 and most commonly used in the graphical modeling of growth and development of plants, bacteria and algae. It essentially consists of a set of axioms and rules used to generate recursive systems. The first L-system was used to model the growth of algae and is represented in Figure 1(a). The axiom is stated to be "AB" and the rules are set to be such that in successive rewriting steps, all A's are replaced by 'AB' and all B's are replaced by 'A'. Repeating the rewriting process iteratively for a finite number of steps, we generate a sequence of strings that are replaced simultaneously at each step of the process. Due to this parallel rewriting mechanism, L-systems have the capability to model biological phenomena such as cellular growth by simulating cell division and death.

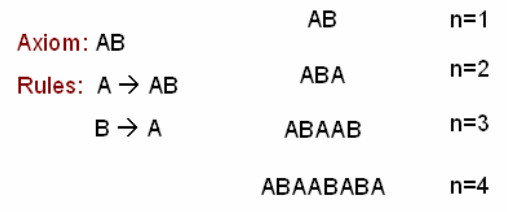


Fig. 1(a): Rewriting Process.

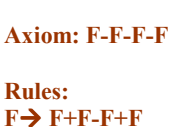


Fig. 1(b): Turtle Representation.

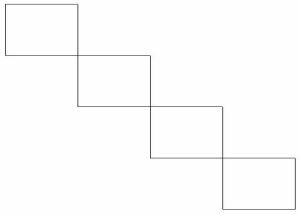


Fig. 1(c): 2D Pattern created using the rules presented in (b).

These L-systems can be extended to geometric interpretation using Turtle graphics, to generate fractal curves, space filling curves and plant like structures [18]. The notation of turtle graphics can be interpreted as follows. The state of the turtle is given by its geometric Cartesian coordinates (x,y) and the orientation of the turtle (θ). The turtle can be

instructed to move forward (F) and instructed to draw a line by a specified step distance (d), move forward without drawing a line (f), rotate counterclockwise (+) or clockwise (-) by a defined angle (δ). For example, consider the set of axiom and rules in Figure 1 (b). The axiom F-F-F-F at n=1 defines a rectangle. If all F's are replaced by F+F-F+F with δ=90° and repeated n=4 times, we obtain the pattern shown in Figure 1(c). The turtle representation can also be extended into the third dimension as shown in Figure 2. The axiom F is recursively rewritten n=3 times by the rule F&F-F&F, where '&' stands for pitch down by angle δ=90°. Other symbols such as '^'and '\' exist for the remaining axes of rotation. Complex geometric patterns both in 2D and 3D can be obtained using the recursive nature of L-systems. Extensions of the system representation include color code inclusions, geometric surface patch association, leaf-apex-branch notation etc. The authors refer readers to the excellent free online book resource by Prusinkiewicz that explain in detail how L-systems have been used to model plant growth and biological phenomena [19].

Axiom: F

Rule:
F→ F&F-F&F

Fig. 2(a): 3D Turtle Representation.

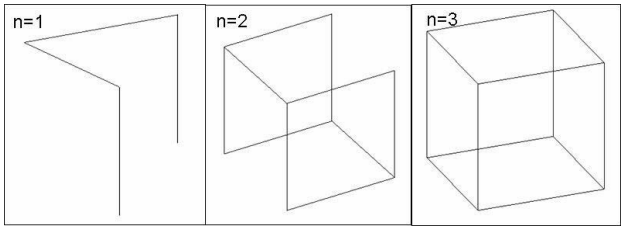


Fig. 2(b): Three dimensional representation of the L-system defined in 2(a)

The focus of our work is to explore the use the L-system concept in two specific tissue engineering application areas: First, to design nutrition networks within the scaffold structure using the L-system theory. Secondly, to define the interior pore architecture of a scaffold using space filling curves. Current CAD systems are incapable of generating complex interior patterns and most often rely on boolean operations and/or tool path manipulation to create interior pore structures. We have attempted to explore the use of L-systems as an alternate means of designing the interior architecture of scaffolds through a set of axioms and rule rewriting recursive systems. To the author's knowledge, no such previous application scenario has been reported.

3. VASCULATURE DESIGN USING L-SYSTEMS

Fig. 3: Vasculature network within a rat portal vein [22].

A number of techniques are currently available to model the vasculature system present in vertebrates. Most commonly, image and fractal based techniques model the geometric and flow characteristics to help address various circulatory diseases [20-22]. Karch, et al. used a modified computational method of constrained constructive optimization to create vascular tree patterns for simulated regions of tissue by taking into account arterial flow characteristics such as perfusion pressure, viscosity of blood, volume of perfusion etc [20]. Jom et al have analyzed the geometrical and fractal properties of the rat portal vein networks using micro MRI image based techniques as shown in Figure 3 [22]. The reconstructed model was used to study the effects of branching geometry on flow characteristics. These key papers suggest that in order to reproduce such complex networks within CAD systems and eventually in manufacturing, additional work is required that follows a Design for manufacturing (DFM) approach. To enable this, there is a need for design algorithms to include manufacturing constraints while developing the artificial nutrient delivery network tree.

The use of L-system is particularly attractive for vascular network design scenario for the following reasons:
1) The axiom and rule based rewriting scheme presents a simple yet powerful method to create fractal based networks.
2) Ease of inclusion of parameters and constraints within the rewriting system to meet specific design criteria through the use of conditional constraints.
3) Ability to associate 'features' or geometric entities within the rewriting system.
4) Ease of extensibility since L-systems were originally developed for plant modeling where branches, roots and nodes are common design features.

The following sub-sections describe how L-systems can be used in the creation of vascular patterns with sample scaffold shapes. For illustrative purposes, we have hypothesized a square shaped scaffold. The figures were created using a 30 day trial L-system software [23]. The generated vascular patterns are examples of circulation networks that can be used within cell encapsulated hydrogel scaffolds. The fourth section of this paper deals with the creation of space filling curves that is particularly useful for scaffolds fabricated using extrusion based SFF systems.

3.1 Layered Patterning of Branching Networks

Layered Manufacturing (LM) systems obtain layer by layer contour information from CAD models. Therefore, it is conceivable that 2D vasculature patterns can be laid out on specific layers and then transferred to the LM systems for fabrication. To create branch patterns, parametric L-systems can be used of the form shown in Figure 4(a). Successive generation of patterns yield branches of reduced length set forth by the parameter 's/R' as shown in Figures 4(b-c). The amount of branching steps will depend on several factors namely, resolution of LM system, perfusion necessity, material characteristics etc. Each of the patterns can be arranged within specific layers of the scaffold as needed and processed to obtain the necessary tool path instruction set as depicted in Figure 5.

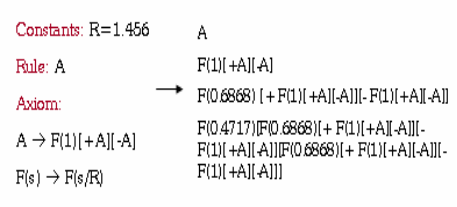


Fig 4(a): Parametric L-system.

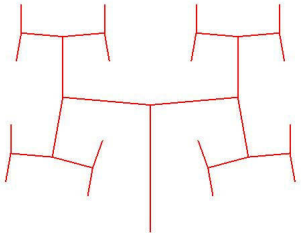


Fig 4(b): Branching pattern at n=5.

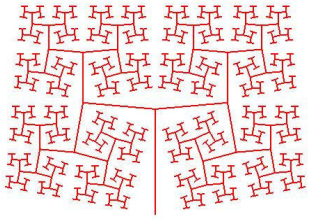


Fig 4(c): Branching pattern at n=10.

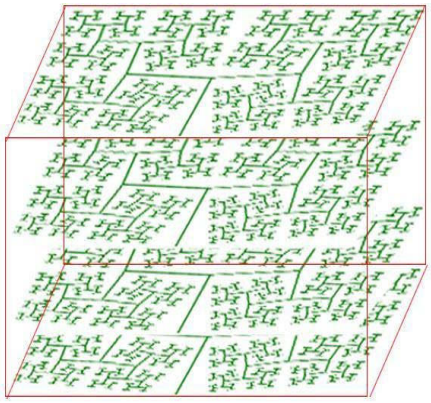


Fig 5: Final assembly of branching patterns on specific layers within the scaffold.

3.2 3D Branched Network

Branched patterns to form a three dimensional network can be formed within the interior of the scaffold through parametric L-system representation that branches out in three dimensions. An example of a branched network rewriting system is shown below in Figure 6 and graphically displayed in Figure 7. The length of the sub-branches is

controlled by the factor 'R'. The symbols '\' and '&' instruct the system to branch out in adjacent planes. The model can be further sliced to obtain the layer information and processed to instruct the specific RP system used. It should be noted that not all RP systems will be capable to generate a branched network. A nutritional flow channel network of the nature shown in Figure 8, known as the 3D Hilbert path is constructed and can be incorporated into a hydrogel scaffold. If cells are encapsulated in the scaffold, medium flowing through the channels can diffuse into the surrounding structure and thereby maintain cellular needs. It is therefore seen that complex pathways can be generated by recursive rewriting systems that do not require expensive computing systems.

```
#define R 1.45
Angle =80
N=4
Axiom: A
Rules:
A --> F(1)[+A][-A]\[+A]&[-A]
F(s) --> F(s*R)
```

Fig 6: 3D Branching L-System

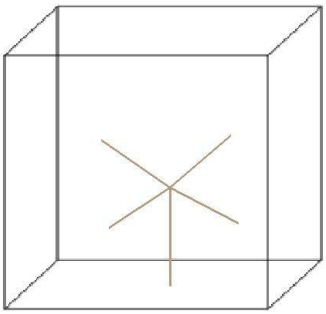


Fig 7(a): N=1 branch.

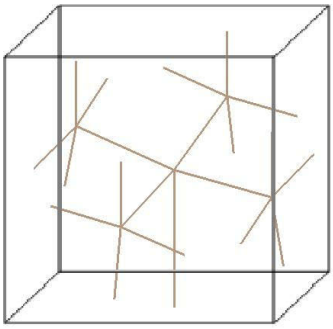


Fig 7(b): N=2 Branches.

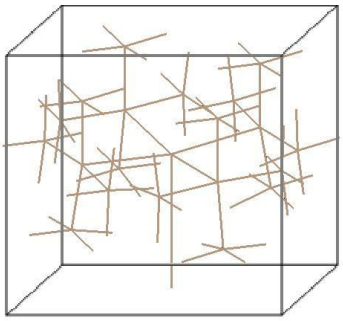


Fig 7(c): N=4 branched network.

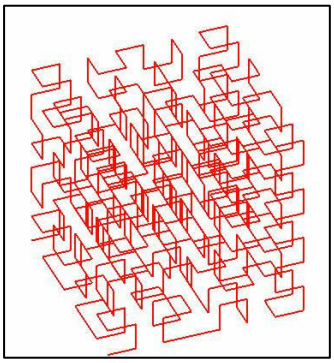


Fig 8: 3D Hilbert Flow Network.

4. SPACE FILLING CURVES

Space filling curves are a special class of L-systems and are defined as curves that fill up the given space in a particular order by either continuously changing directions or passing through every given point in the defined space [24]. The continuous functions that define these curves lie in the domain with a unit interval [0, 1], whose range lies in either the Euclidean 2-dimensional space (plane curves) or in the 3-dimensional space (volume curves). They have been extensively studied over the past century in fields of mathematics, information processing, database query, image compression. Examples of plane space curves are the Hilbert curves, Sierpinski curves and Peano curves. We will limit our discussion to only plane space filling curves that satisfy the following constraints:

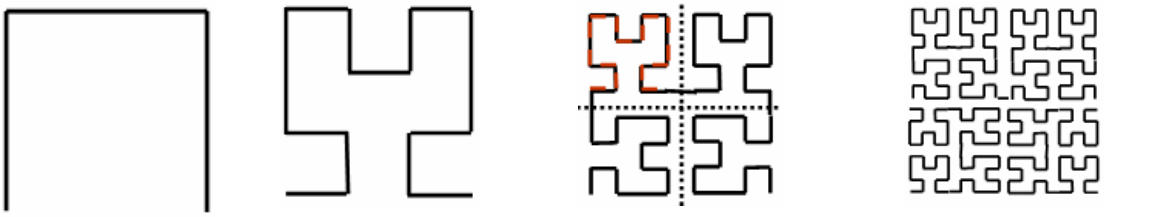
a) Curves in a single layer do not intersect.

b) Each point in the curve is at a unique constant distance from each other.

c) There is only one start and stop point of the curve in a single layer.

These three characteristics are particularly attractive for extruding polymer materials out of nozzles. The non intersecting feature allows patterns to be laid out in a layer by layer fashion. The constant distance between points helps in easy characterization in terms of being able to define a unit cell that makes up the pattern. The single start and stop feature of the curves helps in preventing multiple closures of the valve in a single layer without the agglomeration of material due to delayed response times. The Hilbert and the Sierpinski family of space filling curves satisfy all of the above criteria and its design formulation is described below. In addition, each of these curves can be generated using specific L-system rewriting rules.

4.1 Hilbert Curve Patterns



(a) Level 1 – Recursive Procedure begins at this level.

b) Level 2 – 2D Pattern that repeats to fill up a unit square.

c) Level 3 – Pattern repeated by changing the rotation of Level 2 in a sequence to form 4 quadrants

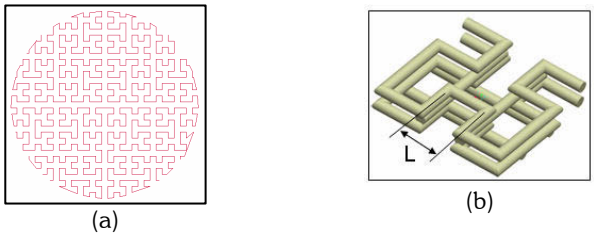
d) Level 4 – Process repeats with the distance between each vertex getting progressively lower.

Fig. 9: Hilbert curve recursive generation process.

The Hilbert curve was invented in the late 1890's by German mathematician David Hilbert and is perhaps the most popular of the space filling curves. The Hilbert curve is constructed using a recursive procedure described by Sagan and graphically shown in Figure 9 [24]. These curves can be used to construct the unit cell patterns required for scaffold design. Figure 10(a) represents the basic structure of the Hilbert curve pattern, while Figure 10(b) depicts the entire tool path pattern used to create the scaffold. The surface area of these structures is similar to any ordinary 0-90° pattern, but the spatial distribution and orientation of the struts differs considerably. The porosity (P) of the unit cell structure can be calculated using the following relation:

$$P = 1 - \frac{VolumeofUnitCell}{TotalVolume}$$

$$P = 1 - \frac{\pi d^2 L * 15}{4 * 9L^2 d} \qquad P = 1 - \frac{\pi d}{2.4L} \tag{1}$$



(a)

Circular Hilbert Curve Pattern clipped by a circle

(b)

3D Unit Hilbert Cell with characteristic length "L" and strut diameter "d"

Fig. 10: Scaffold structure with the Hilbert pattern.

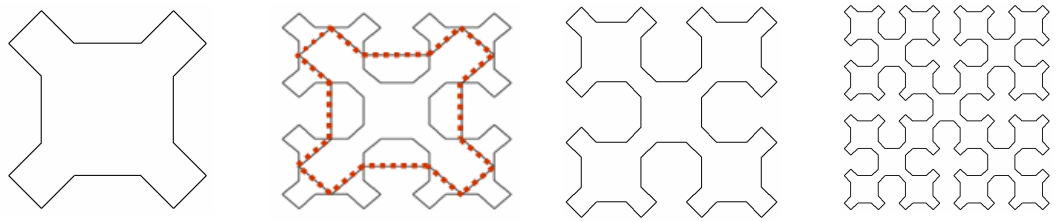
4.2 Sierpinski Curve Patterns

The Sierpinski curves are constructed together by four orientations of a three part curve as shown in Figure 11. The generated patterns are closed with both the start and end point being the same. Using these patterns, scaffolds with

interior structure consisting of alternating straight curves and Sierpinski curves can be produced as shown in Figure 12. The porosity of the unit cell structure can be calculated using the following relation:

$$P = \frac{\frac{\pi}{4} d^2 L * 20}{4.828^2 dL^2}$$

$$P = \frac{\pi d}{4.66 L} \qquad (2)$$



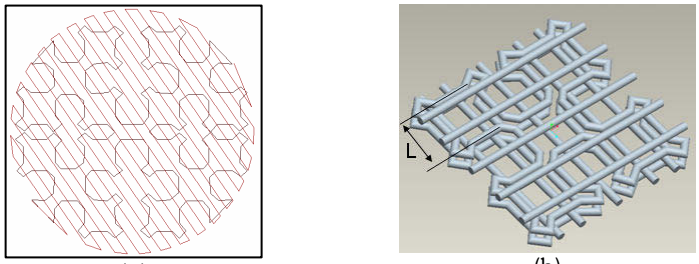
(a) Level 1 – Recursive Procedure begins at this level.

b) Level 2 – Four corners of the original curve are split to form the second level

c) Level 2 – The resulting pattern is a closed 2D pattern.

d) Level 3 – Higher levels can be produced by recursively orienting the original pattern.

Fig. 11: The Sierpinski curve at Levels 1, 2 and 3.



(a) Circular Sierpinski Curve Scaffold Structure

(b) 3D Unit Sierpinski Cells with characteristic length "L" and diameter "d"

Fig 12: Scaffold structure with the Sierpinski pattern.

4.3 Fabrication

We have implemented space filling curve designs using the Precision Extrusion Deposition system that has been developed at Drexel University. The basic set of operations works similar to the FDM concept, except here, no filaments are required and material can be added in the form of pellets or powder. This difference helps in avoiding filament preparation steps and having the flexibility of selecting a broader range of biomaterials for scaffold fabrication. We have used poly-e-caprolactone (PCL, Sigma Aldrich, Inc., Milwaukee, Wisconsin) to demonstrate the design of the space filling curves and heterogeneous patterns. The PED system consists of an XYZ position system, a material extruder system, a temperature control system, a data processing and system control software. The nozzle through which the material is extruded is controlled by the x-y positioning system which is in turn controlled by machine instructions generated from the models to be fabricated. The system control software monitors the deposition of material through the material extruder system according to the process tool path layer-by-layer to construct 3D porous scaffold. A detailed description of the machine setup can be found in Wang et al, 2004 [25]. Samples of the designed scaffold with space filling curves with feature sizes of around 250 μm scale level are shown in Figures 13.

Fig. 13: The Hilbert and Sierpinski Patterned Scaffold.

5. DISCUSSION

Based on the preliminary results shown in sections 3.1 and 3.2, the L-systems used to generate the branched networks were selected with no prior definition of design criteria. The selected parameters are arbitrary and are shown primarily to prove the applicability of using the formulation of the L-system theory to the generation of complex vascular patterns. The benefits of using the L-system theory are:

a) Use of recursive systems to generate complex patterns without the excessive use of CAD graphic resources. The pattern trajectories can be generated in real time and can be implicitly associated with the outer architecture of the scaffold structure prior to manufacturing process plan generation.

b) Although not described in this paper, the L-system formulation can be modified to incorporate engineering design constraints into the axiom and rule definition through the use of conditional statements.

c) The L-system can be parameterized to generate branched networks for specific application depending on nutrition delivery needs. Thus a single L-system definition can be used to generate several topological networks while maintaining geometry.

d) STL files need not be generated and can be directly converted to process tool path instructions for fabrication, thus avoiding the bottlenecks of having to convert multi-scale models to the triangulated format.

e) The L-systems can be used to incorporate multiple material definitions within the formulation, hence providing the capability to generate heterogeneous models.

6. CONCLUSIONS AND SUMMARY

Modeling, design and fabrication of tissue scaffolds to meet multiple biological and biophysical requirements is always a challenge in tissue engineering. This is further amplified when designing load bearing scaffolds for bone and cartilage tissue application. In these cases, tissue scaffolds need to be designed with intricate architecture, porosity, pore size and shape, and interconnectivity in order to provide the needed structural strength, transport nutrients, and the micro-environment for cell and tissue in-growth. By selecting the appropriate unit cell interior structures, properties such as the effective mechanical properties, diffusion and permeability characteristics can be controlled. Depending on the fabrication method used, varying complex internal patterns can be fabricated. In this paper, we have presented the use of Lindenmayer systems in designing vasculature networks that could potentially be incorporated in hydrogel scaffolds. The use of a recursive based rewriting system provides a powerful method to create complex architecture patterns within the interior of scaffolds.

Future research efforts will be undertaken to explore the development of L-system axioms and rules that incorporate feature and manufacturing constraints. The goal would be to design scaffold interior architecture designs which take into account manufacturing process constraints and design specifications. Future cell culture studies need to be carried out to prove that if indeed such space filling curve patterns prove to be better in terms of achieving cell organization and distribution than the traditionally patterned scaffolds. Organs such as the liver have highly organized cellular structures. It is hoped that by providing complex internal patterns that mimic the cellular structure, cells can be guided to form the intended structure. Furthermore, the knowledge gained from such cell culture studies can also be used to optimize the applicable design and fabrication parameters for scaffolds in tissue engineering.

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7. REFERENCES

[1] Griffith, L. G.: Emerging design principles in biomaterials and scaffolds for tissue engineering, Ann N Y Acad Sci., 961: 2002, 83-95.

[2] Tran, K. T; Griffith, L.; Wells, A.: Extracellular matrix signaling through growth factor receptors during wound healing, Wound Repair Regen., 12(3), 2004, 262-8.

[3] Pirone, D. M.; Chen, C. S.: Strategies for engineering the adhesive microenvironment, Mammary Gland Biol Neoplasia., 9(4), 2004, 405-17.

[4] Langer, R.; Vacanti, J. P.: Tissue Engineering, Science, 260, 1993, 920-926.

[5] Prasad, C. K.; Krishnan, L. K.: Effect of passage number and matrix characteristics on differentiation of endothelial cells cultured for tissue engineering, Biomaterials, 26(28), 2005, 5658-67

[6] Hutmacher, D. W.; Schantz, T.; Zein, I.; Ng, K. W.; Teoh, S. H.; Tan, K. C.: Mechanical properties and cell cultural response of polycaprolactone scaffolds designed and fabricated via fused deposition modeling, Journal of Biomedical Materials Research, 55, 2001, 203-216.

[7] Christopher, S. C.; Mrksich, M.; Huang, S.; Whitesides, G. M.; Ingber, D.: Geometric Control of Cell Life and Death, Science, 276, 1997, 1425-28

[8] Li, Y.; Ma, T.; Kniss, D. A.; Lasky, L. C.; Yang, S. T.: Effects of filtration seeding on cell density, spatial distribution, and proliferation in nonwoven fibrous matrices, Biotechnol Prog, 17(5): 2001, 935-44.

[9] Taguchi, T.; Xu, L.; Kobayashi, H.; Taniguchi, A.; Kataoka, K.; Tanaka, J.: Encapsulation of chondrocytes in injectable alkali-treated collagen gels prepared using poly(ethylene glycol)-based 4-armed star polymer, Biomaterials, 26(11) 2005, 1247-52.

[10] Rai, B.; Teoh, S. H.; Hutmacher, D. W.; Cao, T.; Ho, K. H.: Novel PCL-based honeycomb scaffolds as drug delivery systems for rhBMP-2, Biomaterials, 26(17). 2005, 3739-3748

[11] Darling, A.; Sun, W.: Freeform Fabrication and Micro-CT Characterization of Poly-ε-Caprolactone Tissue Scaffolds, IEEE Engineering in Medicine and Biology Magazine, 24, 2005, 78-83.

[12] Khalil, S.; Nam, J.; Sun, W.: Multi-nozzle Deposition for Construction of 3D Biopolymer Tissue Scaffolds, Rapid Prototyping Journal, 11(1), 2005, 9-17.

[13] Kalita, S. J.; Bose, S.; Hosick, H. L.; Bandyopadhyay, A.: Development of controlled porosity polymer-ceramic scaffolds via FDM, Materials Science and Engineering C, 23 [5], 2005, 611-620.

[14] Starly, B.; Lau, W.; Bradbury, T.; Sun, W.: Interior Architecture Design and Freeform Fabrication of Tissue Replacement Structures, Computer-Aided Design, 38(2), 2006, 115-124.

[15] Starly, B.; Fang, Z.; Sun, W.; Regli, W.: Three-Dimensional Reconstruction for Medical-CAD Modeling, J. of Computer-Aided Design and Application, 2(1-4), 2005, 431-438.

[16] Fang, Z.; Starly, B.; Sun, W.; Shokufandeh, A.; Regli, W.: A Computer Aided Multi-Scale Modeling for Direct Fabrication of Bone Tissue Structures, J. Computer-Aided Design and Applications, 2(5), 2005, 627-634.

[17] Sachlos, E.; Czernuszka, J. T.: Making tissue engineering scaffolds work. Review: the application of solid freeform fabrication technology to the production of tissue engineering scaffolds, Eur Cell Mater, 5, 2003, 29-39.

[18] Prusinkiewicz, P.: Modeling and Visualization of Biological Structures, In Proceedings of Graphics Interface, 1993: 128-137.

[19] Prusinkiewicz, P.; Lindenmayer, A.: The Algorithmic Beauty of Plants, Springer, ISBN0387972978 http://algorithmicbotany.org/papers/#abop

[20] Karch, R.; Neumann, F.; Neumann, M.; Schreiner, W.: Voronoi Polyhedra Analysis of Optimized Arterial Tree Models, Annals of Biomedical Engineering, 31(5), 2003, 548-563.

[21] Beard, D. A.; Bassingthwaighte, J. B.: The fractal nature of myocardial blood flow emerges from a whole-organ model of arterial network, J. Vasc. Res., 37, 2000, 282–296.

[22] Buijs, J. D.; Bajzer, Z.; Ritman, E. L.: Branching Morphology of the Rat Hepatic Portal Vein Tree: A Micro-CT Study, Annals of Biomedical Engineering, 34(9), 2006.

[23] http://algorithmicbotany.org/virtual_laboratory/

[24] Hans, S.: Space-Filling Curves, Springer-Verlag, New York, 1994. ISBN: 0-387-94265-3.

[25] Wang, F.; Shor, L.; Darling, A.; Khalil, S.; Sun, W.; Güçeri, S.; Lau, A.: Precision Extruding Deposition and Characterization of Cellular Poly-e-Caprolactone Tissue Scaffolds, Rapid Prototyping Journal, 10(1), 2004, 42-49.