CHARACTERIZATION OF EFFECTIVE MECHANICAL STRENGTH OF CHITOSAN POROUS TISSUE SCAFFOLDS USING COMPUTER AIDED TISSUE ENGINEERING

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ABSTRACT

Tissue engineering can be understand as the development of functional substitute to replace missing or malfunctioning human tissue and organs by using biodegradable or non-biodegradable biomaterials such as scaffolds to direct specific cell types to organize into three dimensional structures and perform differentiated function of targeted tissue. The important factors to be considered in designing of microstructure and there structure material were type of bio-material porosity, pore size, and pore structure with respect to nutrient supply for transplanted and regenerated cells. Performance of various functions of the tissue structure depends on porous scaffold microstructures with dimensions of specific porosity, pore size, characteristics that influence the behaviorand development of the incorporated cells. Finite element Methods (FEM) and Computer Aided Design (CAD) combines with manufacturing technologies such as Solid Freeform Fabrication (SFF) helpful to allow virtual design and fabrication, characterization and production of porous scaffold optimized for tissue replacement with appropriate pore size and proper dimension. In this paper we found that with the increase in the porosity of tissue scaffolds(PCL, HAP, PGAL & Chitosan) their effective mechanical strength decreases by performing the modeling & simulation with CAD & FEM package (Pro/E & ANSYS respectively) and validating the results with in vitro fabrication of Chitosan scaffold by performing in vivo mechanical testing.

Keywords:

Tissue Engineering, Chitosan, Tissue Scaffolds, CATE

1. INTRODUCTION

Tissue engineering with computer aided designing has emerged as an excellent approach for the repair/regeneration of damaged tissue, with the excellent potential to overcome all the limitations of autologous and allogenic tissue repair. Tissue engineering is a an excellent and latest approach to resolve the damaged tissue and organ problems.

Biodegradable biomaterials plays a significant role in tissue engineering by serving as 3Dimensional synthetic frameworks commonly referred to as biodegradable scaffolds, matrices,

or constructs for cellular attachment, proliferation, and in growth ultimately leading to new tissue fabrication and development. Both synthetic polymers and naturally derived polymers have been extensively investigated as biodegradable and biocompatible biomaterials.

The biodegradable scaffolds with high surface area to volume ratio favors living cell adhesion, proliferation, migration, and differentiation with ingestion of nutrients, all of which are highly desired properties for tissue engineering applications. Therefore, current research in this area is driven towards the fabrication and characterization of scaffolds for tissue engineering applications

1.1. Tissue Scaffoldsfor organ

Tissue scaffold are the synthetic bioresorbable or biodegradable bio-polymers that are functional substitutes to replace missing tissues and organ of humans, to provide a temporary substrate to which the transplanted cells can stick or adhere is the primary role of a biodegradable scaffold.

The important factors which is to be considered with respect to nutrient which are important for the growth of cells, supply to transplanted and regenerated cells are porosity, pore size and pore structure for porous scaffolds with a large surface-area-to volume ratio and a large void volume are desirable for attachment, growth, maximal cell seeding, ECM production, and vascularization. Pores in biodegradable scaffolds of same diameter in an identical scaffold are preferable to yield high surface area per volume provided the pore size is greater then the diameter of a cell in suspension.[1]

1.2. Computer Aided Tissue engineering

The utilization of computer-aided technologies in tissue engineering has evolved in the development of a new emerging field of Computer-Aided Tissue engineering (CATE). CATE comprises of computer imaging technology like CT scan, MRI, CAD/CAM and modern design and manufacturing technology like solid free form fabrication.Through the use of CATE, the design of intricate three dimensional architecture of biodegradable scaffold can be realized and these scaffolds can be fabricated or manufactured with reproducible identical to assist biologists in studying complex tissue engineering problems. The classification of CATE is done in three major categories (1) computer-aided tissue anatomical modeling; (2) computer-aided tissue classification; and (3) computer-aided tissue implantation [2, 15]. CATE enables many novel approaches in modeling, design, and accurate fabrication of complex tissue substituteswith enhanced functionality for research in patient specific implant analysis and simulation. On the other hand if the mechanical properties of material of biodegradable tissue scaffolds are known then with the help of Finite Element Modeling (FEM) with the help of tools like ANSYS we are able to predict the behavior of complex structures, such as multilayer system [3-9]. When tissue scaffold is in vivo condition then there will be some microscopic loads(compressive loads and fluid flow) as the tissue differentiation proceeds, it is difficult to determine the local mechanical stimuli sensed by the cells at a microscopic level,for the study of stress strain relationship at microscopic level Finite Element Analysis is used. Finite element analysis (FEA) and Computer Aided Design (CAD) combines with manufacturing technologies such as Solid Freeform Fabrication (SFF) helpful to allow virtual design with accuracy, characterization and production of biodegradable scaffold optimized for tissue and body part replacement, make possible to

design and manufacture very complex tissue scaffold structure with functional components that are difficult to fabricate.

2. MATERIALS & METHODS

2.1 Chitosan

Chitosan, a linear polysaccharide consisting of (1, 4)-linked 2-amino-deoxy-β-D-glucan, is a deacetylated derivative of chitin biopolymer. It has been found to be good candidate for synthesizing scaffolds because of its biocompatibility, biodegradability, non-toxicity, antimicrobial and high affinity towards proteins etc. Chitosan can also be easily fabricated into films, fibers, foams etc. Chitosan scaffolds are used for tissue engineering purposes and also find its use in drug delivery. Chitosan (CS) is widely used as scaffolds for the regeneration of bone tissue, Skin Tissue, nervous tissue, etc.Porous chitosan scaffolds were prepared by the controlled lyophization of chitosan solution or gels, and by the elecrospinning technique. Pure chitosan scaffolds show poor mechanical properties

Porous Chitosan scaffolds were prepared by the controlled lyophization of Chitosan solution or gels, and by the elecrospinning technique. Pure chitosan scaffolds show poor mechanical properties. Some researchers have prepared hybrid Chitosan scaffolds to improve its load bearing property. S. Phongying et al.[10] reported that Chitosan scaffolds could be directly prepared from the chitin whiskers. Zhang et al.[11] reported about the fabrication of porous Chitosan scaffolds by thermally induced phase separation. Zhang et al.[12] also reported about the fabrication of porous chitosan tubular scaffolds by a novel mold casting/lyophilizing method. Zheng et al[13] prepared a novel Gelatin/Montmorillonite-Chitosan (Gel/MMT-CS) nanocompositebioscaffoldvia the intercalation process and the freeze-drying technique, using the important ice particulates as the porogen materials. The Gel/MMT-CS scaffold has good mechanical properties and controllable degradation rate. Hydroxyapatite and alginate hybridized Chitosan scaffolds were also reported

2.2. Modeling of Unit Cell of Scaffold

The designing of the model unit cell is done with the help of Pro/Engineering CAD package which is having the different porosity with three different biomaterials: Hydroxyapatite (HA, E =14,000 MPa, ν = 0.25), Polycaprolactone (PCL, E =400 MPa, ν= 0.33), and copolymer of polylactic acid and polyglycolic acid (PLGA, E = 1200 MPa, ν = 0.33) are considered in this study. If the edge length of the unit cell for the scafold and the edge length of the square channel for the scaffold are L and A, respectively, the porosity of the scaffold can be determined by:-

$$p = \frac{(3LA^2 - 2A^3)}{L^3}$$ A<L, for square channel (Fig.1)

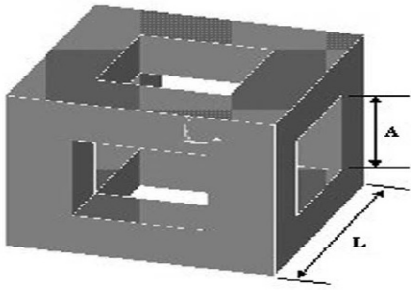

Figure 1 Unit cell scaffold with open square pore

After the designing of the scaffold of six different pore size (Fig.2) in Pro/Engineering then the models are imported in the ANSYS software for the stress analysis with different mechanical properties for all different pore size models. .The models are discretized in to tetrahedral segments (Fig. 3) of size .5mm through meshing. After discretization the displacement of .1mm is given to every pore size model in the positive direction of X-axis.A total of 18 simulations is done with different porosity range between 0% to 95%. Then the results are calculated for effective Young Modulus which will describe the mechanical behavior of scaffold with the increase in its porosity

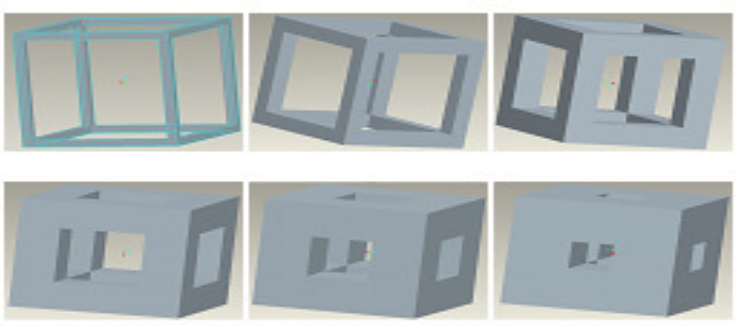

Figure 2. Scaffold unit cells of six different pore sizes

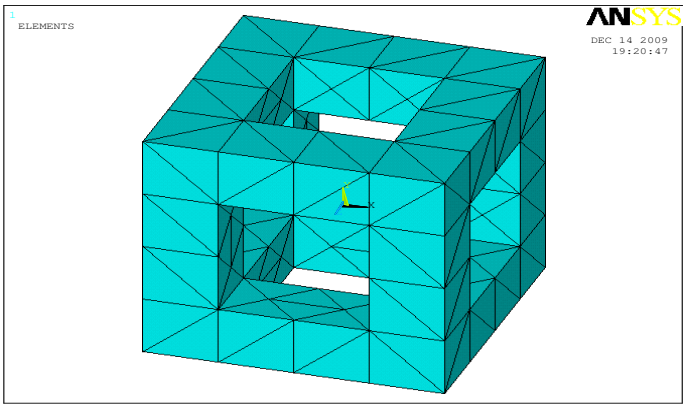

Figure 3 Discretized unit cell of scaffold

2.3. In vitro fabrication of Chitosan Scaffolds

Fabrication of Chitosan scaffold is by formation of composition of Chitosan with two other biopolymerAliginate and Pectin in different proportion as we know that Chitosan alone is not having a good biocompatible and biodegradable properties, with the help of lyophilization technique and freeze drying technique which will help in giving porous scaffolds.The three different pore size samples are fabricated then Scanning Microscopy is done for pore size deduction. Mechanical compression testing is done by following the ASTM F 2103 – 01 code on INSTRON 1195 TESTING MACHINE with cross head movement .5 mm/min with gage length 4.00

2.4. Dimension of samples

The sample is cut in the shape of a cylinder having diameter 12mm and height is 6mm. The force on the sample was applied perpendicular to the longitudinal axis of the conduits at a cross-head speed of 1 mm/min, and the loads were measured at variable strains of 20%, 40%, and 60%.

Cross Section area= 153.938 mm^2
Gage length = 6.00 mm
Full Scale = 0.5 kN

Scale Factor = 1.000

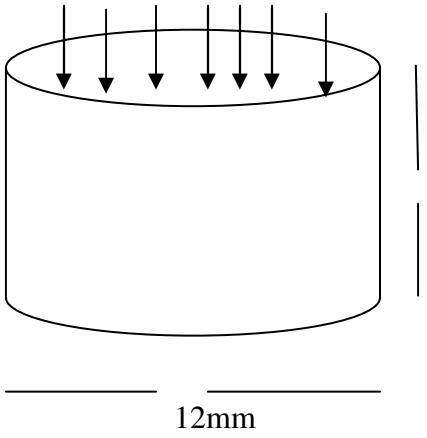

Figure 4 Fabricated porous Chitosan tissue scaffolds

3. RESULTS AND DISCUSSION

Results were obtained after the eighteen simulations run on ANSYS after modeling in Pro E software for six different pore size models and these results were compared with the results obtained by the Z. Fang B. Starlyetal(14). Then the deviation in their effective young's modulus were observed were near about same as obtained by Z. Fang B. Starly et.al., as shown in Fig. 5 the average stress in X direction with the help of which the Effective young's modulus is obtained which is having the porosity of 20%, the deviation of Effective Young's Modulus on increasing porosity of Hydroxyapetite(HA), Polyglycolactic Acid(PGAL) &Polycarpolactum(PCL) respectively, with the increase in the porosity of tissue scaffold there is a relative increment in the Effective Young's Modulus with in all three biomaterials(HA, PCL & PGAL), as we increase the porosity of the tissue scaffolds there is an increase in the Effective shear Modulus, the behavior of the Chitosan is predicted ie. how the chitosan tissue scaffold behave under the compression loading with different porosity and with the increase in the porosity there will be a decrease in the mechanical properties of Chitosan tissue scaffold too.

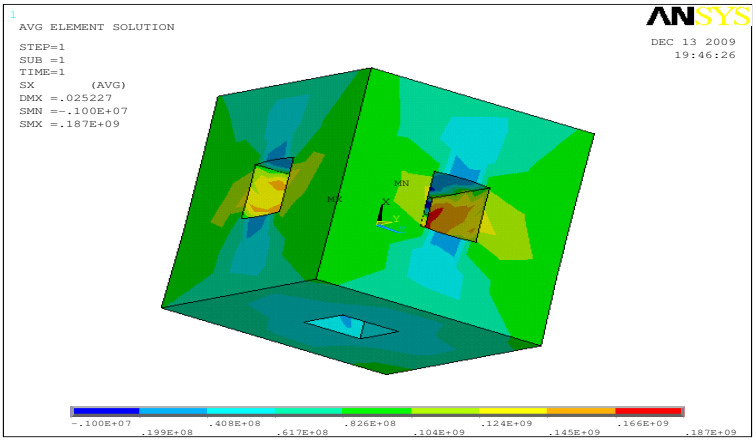

Figure 5. Load distribution on Chitosan model

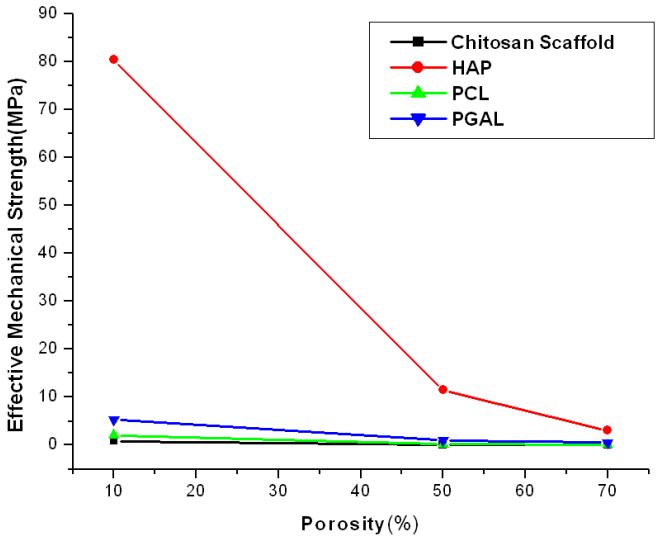

Figure 5Comparison of mechanical Strength of Chitosan with HA, PCL & PGCL

4. CONCLUSION

A computer-aided characterization approach for evaluation of mechanical properties and structural heterogeneity of porous tissue scaffolds was presented in this thesis. The central idea of the characterization approach is the use of Computer Aided Tissue Engineering this approach enables the design and fabrication of porous tissue scaffold of exact pore size and of appropriate mechanical strength where this porous scaffold is implemented.

Results of the characterization in the above experiment show that the effective mechanical properties of the composite Chitosan scaffold are functions of the scaffolding materials which are used for its fabrication, the orientation of deposition layout pattern, and the overall porosity of the scaffold structure. In general, the scaffold structures behave with anisotropic mechanical properties and the degree of the anisotropy is depending on the deposition layout pattern.

The effective mechanical strength/properties like stress and starin decrease with the increase of the porosity for all three scaffolding biomaterials (HA, PCL, PGAL), as shown in Figure 5. Chitosan scaffold shows same type deviation in there mechanical properties as with the increase in its porosity its mechanical properties decreases but the mechanical strength of the Chitosan is very low as shown in Fig. 5 so it is to be concluded that Chitosan is a potential biomaterial for nerve repair. Lyophilization&Freezdrying are the techniques are used to generate the porous chitosan tissue scaffold through which the size of porous tissue scaffold is controlled which will be helpful in fabrication of appropriate mechanical strength tissue scaffold.

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