Mechanobiological Models for Intervertebral Disc Tissue Engineering

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Abstract: - Low back pain, which is often caused by disc degeneration, is a major health problem. Due to the limitations of the current treatments for degenerative disc disease, tissue engineering methods have been proposed. These methods present the opportunity to restore the functionality of the intervertebral disc by repairing or replacing the degenerated tissue. One of the major challenges in intervertebral disc tissue engineering is to recreate, in vitro, the physiological environment for optimal culturing of cells seeded in scaffolds constructs. So it is necessary to understand the link between the forces applied to a cell and its biological response. After a brief review of the fundamentals of the mechanical factors in tissue engineering, this plenary paper presents the mechanobiological models which are capable of optimizing the design parameters of the porous scaffolds and prediction of the stress distribution in different stages of the tissue engineering. On the basis of these infrastructure models and experimental results, we can gain a complete understanding of mechanobiology and the procedure of the tissue differentiation to finalize the setups of the instrumentations in intervertebral disc tissue engineering.

Key-Words: - Mechanobiological modeling, Intervertebral disc, Tissue engineering, Finite element methods

1 Introduction
Intervertebral disc tissue engineering presents the opportunity to restore the functionality of the intervertebral disc by repairing or replacing the degenerated tissue. In repairing the Intervertebral disc, the aim is to induce regeneration of the tissue in situ via biological manipulation, whereas replacing the intervertebral disc requires developing a functional tissue unit in vitro and implanting it into the body. The physiological properties of the disc are linked to the composition of its ECM\textsuperscript{1}. Thus, tissue-engineering methods tend to focus on techniques directed toward replenishing the ECM components of the disc to restore disc function. Tissue engineering is founded on three principal components (Cells, Scaffolds, Growth Factor and Mechanical Stress), which may be used independently or incorporated in combinatorial form

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{Principle components of the IVD tissue engineering}
\end{figure}

\textsuperscript{1} Extracellular Matrix
In general, the disc task in the spine is to maintain flexibility and motion. Along with the facet joints, it is responsible for carrying all the compressive loading to which the trunk is subjected. This includes different types of load and stresses, like dynamic loads, static loads, tensile stresses, torsion loads, shear stresses and a combination of tensile, compressive and shear stresses.

The nucleus carries the compressive loads and the annulus the tensile stresses. These changes with degeneration when the hydration of the disc is less and the tensile stresses in the collagen fibers of the inner annulus become compressive stresses.

Mechanical factors not only have a negative effect on the disc, evidence suggests that substantial biologic remodeling occurs in the intervertebral disc in response to mechanical stimuli. According to the important influence of the mechanical stress on the residing cells in the intervertebral disc, it is vital to propose suitable mechanobiological models as infrastructures for a better understanding of the mechanical behavior of this soft tissue and optimizing the tissue engineering parameters.

This plenary paper presents the mechanobiological models which are capable of optimizing the design parameters of the porous scaffolds and prediction of the stress distribution in different stages of the tissue engineering. First it discusses how we designed the algorithm for extracting the morphological parameters of natural tissues. Then it mainly presents two different types of mechanobiological modelling for studying the effect of parameters in mechanical response of tissue engineered intervertebral disc.

2 Extracting the Morphological Parameters of Tissue Scaffolds

As it mentioned, the first step of our researches in biomechanics of tissue engineering was focused on designing a suitable image processing algorithm which can extract the morphological parameters of tissue scaffolds [1]. In general, tissue engineering scaffolds supply 3D structures to facilitate cell proliferation, differentiation and migration and cause formation of new tissue in the defect site. Scaffold transfers mechanical loadings to the adjacent tissues and causes mechanical competence improvement of the defect site. Generally tissue engineering scaffolds must provide the following characteristics: (1) A suitable 3D structure with appropriate external geometry to integrate with the defect site and adjacent tissues. (2) A highly porous and interconnected network to supports cell attachment, proliferation and extra-cellular matrix formation as well as facilitates diffusion of nutrient and oxygen in and waste out of the scaffold. (3) A biocompatible and biodegradable structure with suitable surface chemistry. (4) Suitable mechanical properties to support tissue loadings and stimulate and direct cell differentiation and tissue regeneration.

One of the most important properties of the scaffolds that affect the other characteristics, are morphological properties. The purpose of this study was to determine the morphological properties of the scaffold using analysis of its electron-microscope image. Porosity and specific surface area are two main morphological parameters of a scaffold. Porosity is the most important morphological parameter of the scaffold which must meet some specific requirements. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients. On the other hand it causes very low stiffness and strength which can lead to failure in compare with natural biological tissue. The specific surface area of the scaffold is the total pore surface per unit apparent volume. This parameter implies how much surface per unit volume is available in the scaffold for cell attachment. The specific surface area of the scaffold should be as large as possible.

The mentioned parameters of a porous medium can be extracted mathematically using correlation functions and analysis of electron-microscope image of the material. Furthermore correlation functions may be used to estimate physical properties of porous medium such as electrical conductivity, elastic constants and permeability. Two point correlation function may be calculated using digital image processing methods. We used this method to extract morphological properties of type I collagen scaffold using its electron-microscope image. Figure 2 shows a SEM picture of a sample scaffold.

![Fig 2: Scanning electron micrographs of type I collagen matrix; Bar indicates 100 μm.](image-url)
At the first step, thresholding process with an appropriate threshold value should be applied on the image. After thresholding process, the grayscale image will convert to binary image. This means that every pixel has one of two values (0 or 1) depending on its grey intensity. On the basis of this data we can calculate two point correlation functions and extract the value of desired morphological parameters. For example two point correlation function curve of this scaffold is shown in Figure 3.

According to this curve, the value of porosity and specific surface area of the scaffold is calculated \( \phi = 0.62 \) and \( s = 0.0118 \, \mu m^{-1} \) respectively [1].

On the basis of this algorithm, we developed a program to extract the morphological parameters of desired scaffolds for the purpose of IVD tissue engineering. So in next step, it is necessary to develop complex mechanobiological models to consider the effect of different parameters in mechanical response of tissue engineered intervertebral disc.

3 Triphasic Poroelastic Model of Tissue Engineered Intervertebral Disc

This mathematical model considers a charged hydrated tissue engineered intervertebral disc as a mixture consisting of: (1) a porous, permeable, charged solid phase; (2) an incompressible fluid phase; and (3) ion phase with two ion species, i.e., anion and cation (Figure 4).

According to the Biot theory, derivation of the governing equation of the fluid was based on empirical evidence that the fluid flow in porous media obeys Darcy’s law. By the way, this model is strictly based on the laws of continuum mechanics [2].

As it was shown [3, 4], agreement of the results of this mathematical model with analytical results and experimental data validate our model for application in studying the biomechanics of the intervertebral disc as a hydrated soft tissue.

On the basis of this infrastructure model we can gain the capability of optimizing the design parameters of porous scaffolds and prediction of the stress distribution in different stages of the tissue engineering. To have a short review of three main exemplary problems, we can mention to the investigation of the role of porosity in scaffold manufacturing, effect of fixed charge density and water content on mechanical response and studying the nutrition criterion in IVD tissue engineering procedure.

3.1 Investigation of the role of porosity

We considered a homogenous two dimensional version of our mathematical model, to analyze a simple sagittal slice of the disc [4]. In the model, we assumed that the bottom surface of the sagittal slice is impermeable, frictionless, insulated, and rigid so that there is no vertical flow. The top edge was loaded with pressure P. In order to compare the
difference between different porosity, a long duration (200s) was applied, and the value of P was chosen as a ramp from zero to $2 \times 10^6$ Pa in test duration (0–200s).

Figure 5 shows the displacement at top with different porosity in mentioned 2-D model. With increasing time, the differences in the displacement at top between different cases increase. Figure 5 also indicates that the displacement at top increases with increasing the porosity.

Fig.5: Displacement at top with different porosity

On the basis of this exemplary application of our mathematical model and extracting other morphological parameters [1], we gain different range of porosity which is suitable for adjustments of the instrumentation setups for various types of scaffold manufacturing.

3.2 Effect of FCD and water content on mechanical response of engineered tissue

As we predicted, our infrastructure model is capable for this study. A homogenous two dimensional version of this mathematical model [4], can analyze the effect of fixed charge density in a simple sagittal slice of the disc. For the compression creep problem, $\sigma_0=10$ KPa was applied to the 2-D intervertebral disc model. The effect of fixed charge density on creep deformation was investigated with three different amounts ($FCD=0.1, 0.2$ and $0.3$) and plotted in Figure 6. In next step, the effect of water content on creep deformation was investigated with three different amounts ($\zeta_w=0.7, 0.8$ and $0.9$) and plotted in Figure 7.

Fig.6: Effect of FCD on the creep behavior

Fig.7: Effect of water content on the creep behavior

3.3 Investigation of the nutrition criterion in IVD tissue engineering procedure

In this case first we derived two fluid exchange factors [4]. The first one is the volume of fluid exchange per cycle, which represents the volume of disc included in the fluid exchange region. So we calculated this factor by dividing the final total volume of fluid exchange by the total number of cycle which occurred during the dynamic loading simulation. The second one is the volume of fluid exchange between the disc and the surrounding media per unit time. So we calculated this factor by dividing the final total volume of fluid exchange by the total duration of the dynamic loading simulation. After definition of these two factors, we calculated the results of our mathematical mode by changing cycle length from zero to 12 hours in dynamic loading. To derive a criterion in fluid transport study, we maximized these two mentioned fluid content do not affect the equilibrium strain significantly.

Fig.6: Effect of FCD on the creep behavior

Fig.7: Effect of water content on the creep behavior
factors and normalized it to define a nutrition index. Figure 8 shows how this criterion changes regarding to different cycle lengths.

![Figure 8: Variations of the nutrition criterion with cycle length](image)

By the way, different results of this infrastructure model can lead in logical algorithms in intervertebral disc tissue engineering in order to reduce trial and errors.

4 Macroscopic Finite Element Model of Intervertebral Disc

The stress distributions inside healthy and degenerated discs are investigated [5] and have been shown to change with disc degeneration. Because the functional properties are affected it significantly affects load-bearing and kinematical patterns on the lumbar spine [5]. The range of pressures in the disc during daily activities is investigated and shown in different researches. These researches show that abnormal mechanical loads may provide a pathway to disc degeneration, and give an idea of the height of these loads. Simple mechanical stimulations cannot cause disc degeneration, for this a complex mechanical stimulation combining forward and lateral bending of the spine followed by violent compression is needed [5]. Experimental measurements of in vivo intradiscal stresses are difficult. Therefore, different finite element approaches have been made in recent years to gain a better understanding of the load distribution in the spine and especially in the disc. So in tissue engineering procedure, it is necessary to study the biomechanics of intervertebral disc by means of a macroscopic model.

4.1 Geometrical Model

We developed a reconstruction model of the spine based on the CT and MRI data-based anatomical structure of the spine by using the software Mimics. A young man with no history of present and past disc disease was selected as normal subject. Initially L3–L4 motion segment data were taken in the axial direction. The CT slice images had a slice thickness of 1 mm. First, we adopted grey threshold segmentation algorithms for 2D image slices. As we know, the bone grey value is bigger than other tissue’s. We can separate the bone tissue from the other tissue by setting grey value. But the automated grey threshold segmentation may result in artificial isolated section or discontinuous boundary as the grey value of cancellous bone is lower than cortical bone and similar to other tissue such as soft tissue. That’s why we took the conventional manual segmentation to modify the contour and boundary of 2D slice image of spine. The 3D L3–L4 segmentation modeling was fundamentally performed based on a set of axial slices in Mimics software. The 3D model of the L3–L4 segment is shown in Figure 9.

![Figure 9: 3D model of the L3-L4 segment](image)

We translated the data from the Mimics file format in order to import the data to the finite element method software Ansys11. We finally created the geometric model of the L3–L4 segment in Ansys11.

4.2 Finite Element Model

Material properties of the different tissues were extracted from the literature [5]. The fluid like behaviour of the annulus ground substance was simulated by using an incompressible, hyper-elastic, two parameter Mooney–Rivlin formulation. The stress–strain behaviour of the collagen fibers were described with a non-linear function based on previous measurements [5]. Figure 10 shows the finite element model in Ansys11.
For considering the loads and boundary conditions, the inferior endplate of the lower vertebral body (L4) was rigidly fixed, by anchoring it. In simulations, pure unconstraint bending moments of 2.5, 5, 7.5 and 10 Nm in flexion, extension, lateral bending and axial rotation were applied to the superior endplate of the upper vertebral body (L3). Nonlinear large deformations were used for calculation. To ensure the convergence, four to six substeps were included, whereas each substep was iteratively determined using the "Newton–Raphson" approach.

Values of flexion and extension of this simulation are compared with experimental data in Table 1 and 2, to show the validity of the model.

Table 1: Comparison of the values of flexion

<table>
<thead>
<tr>
<th>Bending Moment (N.m)</th>
<th>2.5</th>
<th>5</th>
<th>7.5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE Model</td>
<td>6.22</td>
<td>7.98</td>
<td>9.63</td>
<td>11.95</td>
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<tr>
<td>ROF (Deg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental Data</td>
<td>6.64</td>
<td>8.38</td>
<td>10.11</td>
<td>13.35</td>
</tr>
</tbody>
</table>

Table 2: Comparison of the values of extension

<table>
<thead>
<tr>
<th>Bending Moment (N.m)</th>
<th>-2.5</th>
<th>-5</th>
<th>-7.5</th>
<th>-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE Model</td>
<td>5.38</td>
<td>8.12</td>
<td>8.95</td>
<td>12.02</td>
</tr>
<tr>
<td>ROF (Deg)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Experimental Data</td>
<td>5.15</td>
<td>7.92</td>
<td>10.28</td>
<td>12.86</td>
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</tbody>
</table>

5 Conclusion

One of the major challenges in the intervertebral disc tissue engineering is to recreate, in vitro, the physiological environment for optimal culturing of cells seeded in scaffolds constructs. So it is necessary to understand the link between the forces applied to this soft tissue and its biological response.

Also in tissue engineering procedure, it is necessary to study the biomechanics of intervertebral disc by means of a macroscopic model to gain a better understanding of the load distribution. After a brief review of the fundamentals of the mechanical factors in tissue engineering, this plenary paper presents the mechanobiological models and algorithms which are capable of optimizing the design parameters of the porous scaffolds and prediction of the stress distribution in different stages of the tissue engineering. So first we discussed how we can extract the morphological parameters of the desired tissue. Then a novel triphase poroelastic model of intervertebral disc was presented that can simulate the effect of different parameters of tissue engineering. At the end a macroscopic finite element model was discussed which can help us to gain a better understanding of the load distribution in the spine and especially in the disc. On the basis of these infrastructure models and our predicted experimental results, we can gain a complete understanding of mechanobiology and the procedure of the tissue differentiation to finalize the setups of the instrumentations in intervertebral disc tissue engineering.

References: