UBSJ
Fall 2008
Vol. I No. 1

UIC Bioengineering Student Journal

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PREFACE

In his excellent book on technical writing (The Craft of Scientific Writing, Springer 1996), Prof. Michael Alley inspires and instructs. The instruction we all need as we work to become better writers, while the inspiration is essential as we strive to overcome the lethargy and inertia that make getting started on a writing exercise (e.g., term paper, proposal, presentation) so difficult. One reason for starting the UIC Bioengineering Student Journal (UBSJ) is to provide more opportunities for students to get started with serious technical writing earlier in their undergraduate and graduate careers. Unfortunately, most college writing ends soon after the freshman year and until a senior or graduate thesis rolls around few opportunities arise in or out of engineering classes. Another justification for the UBSJ is to introduce students to the professional process of submission, review and revision that all technical publications adhere to. In creating each issue of the UBSJ Bioengineering students serve multiple roles: authors, reviewers and editors. Thus, by submitting a paper and by participating in the review of the work of their peers, students gain experience in all aspects of professional journal publication. Finally, as with all skills, practice leads to improvement. Writing for UBSJ provides an opportunity to develop writing skills in a student-friendly environment. In the UBSJ the focus is on structure, format and context – not deadlines, exams or grades. When writing a single author article for UBSJ each student can experiment with different stylistic tools; the basic structure of each article is fixed, not to inhibit creativity, but to stimulate – as in a haiku poem or sonnet – an economy of words and thoughts. Prof. Alley teaches that all technical writing should be precise, clear and forthright, as well as fluid, familiar and concise, but engineers look for patterns and instruction in rhetoric does not follow a schematic, an algorithm or a process flow diagram; writing well is as Prof. Alley says a “craft” or “skill” to be acquired by observation, analysis and practice. I hope that the opportunity to publish in the UBSJ will inspire bioengineering students to perfect their writing skills. These skills complement technical and scientific proficiency and in many cases are the key to communicating the new ideas that are the currency of a successful career.

Richard L. Magin, PhD
Professor and Department Head
Bioengineering
EDITORIAL

This journal is designed to be different, a clarion call for students to look beyond engineering solutions to see the human side of the problem. Often we lose focus on the real issues that concern us and get wrapped in details that are not part of the solution. Hopefully down the line this journal will help be the catalyst that makes that possible. We decided to give this journal some trace of a soul, a reason to exist beyond the confines of technical writing, one where we tell you that it is ok to ‘dare to dream’, to be different and to see bioengineering from a more holistic aspect. This approach is illustrated by some articles and also in the two cover designs. (see Behind the Scenes)

There are a lot of people to thank but who cannot be thanked enough. A special thank you to Dr. Magin who envisioned this journal, to Luke who kept things on track, to the editorial board, the authors, the various professors who helped review the articles and all of you, our readers – thank you for being a part of this dream and helping to make it a reality.

The publication of this first issue will hopefully herald a new chapter where we truly begin to push our ‘definition’ of what bioengineering is and what it can be - a journey with endless possibilities; that is the vision and the dream. Join us as we change the world, one dream at a time.

Sylvester Rozario
Editor
BIOMECHANICAL EVALUATION OF PCL ONLAY GRAFT FIXATION: A COMPARATIVE STUDY
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Abstract
Although a tibial onlay technique for posterior cruciate ligament (PCL) reconstruction is advantageous, metallic screw fixation of the bone block is required. This may pose problems for future surgery (e.g., osteotomies, total knee replacement). In the present study, fourteen human cadaveric knees were randomized to receive either a stainless steel (SS) or bioabsorbable (Bio) fixation of the Bone-Patellar Tendon-Bone (BPTB) graft. Cyclic testing of each construct was performed followed by a load to failure test. Cyclic creep deformation showed no significant difference between the two groups (p= 0.8). The failure load (SS = 461± 231 N; Bio = 638± 492 N; p= 0.7) and linear stiffness (SS =116± 22 N/mm, Bio =106 ± 44 N/mm; p= 0.6) also showed no significant difference between the two groups. The results indicate that from a biomechanical perspective, bioabsorbable screws are a viable alternative to metal in the context of tibial onlay reconstruction.

Key Words: Knee, Posterior cruciate ligament, Onlay, Inlay, Reconstruction, Tendon graft

1. Introduction
Injuries to the PCL are significant injuries to the knee. They are often suffered in combination with posterolateral corner injuries or knee dislocations and may require reconstructive surgery. While arthroscopic techniques are attractive for their low morbidity, difficulties in graft passage and concerns about the proximity of the popliteal vessels have led some surgeons to use arthroscopically assisted open techniques. A direct PCL onlay or inlay technique is advantageous since the approach protects the vessels and enables the surgeon to directly visualize the entire tibial attachment site of the PCL, thus allowing for anatomic placement of the graft. One disadvantage of this technique has been that metallic screw fixation of the bone block is required. The use of permanent screws may potentially pose problems for future surgery (e.g., revision PCL reconstruction osteotomies, total knee replacement) that may be necessary. The purpose of this study was to evaluate whether fixation with a bioabsorbable screw provides similar initial fixation strength of the tibial bone block as stainless steel screw fixation.

2. Methods
2.1 Sample Preparation
14 human cadaveric knees (5 males, 9 females) were used in this study (mean age 54 yrs); these included five contralateral pairs. Dega scans were performed to determine bone mineral density (BMD). The tibia and central third of the patellar tendon, forming a bone-patellar tendon-bone (BPTB) complex, were harvested from each cadaver. The proximal BPTB and distal tibia were each potted in acrylic cement. Prior to fixation, geometric measurements of each graft were recorded.

2.2 Graft Fixation
Bone-patellar tendon-bone (BPTB) grafts were randomly assigned to either bioabsorbable PLA (SmartScrew®, Conmed/ Linvatec) or stainless steel screw (Synthes) fixation; contralateral knees did not receive the same screw type.

The BPTB graft was secured to the tibia using two 4.5mm screws. After fixation, spherical markers were glued on the BPTB complex and the two screws at 1cm increments for optical determination of regional BPTB deformation.

2.3 Biomechanical Testing
Testing was performed using a servohydraulic materials testing system (Instron 8874) along with a custom digital video digitizing system to optically determine soft tissue surface deformation. Digital Motion Analysis Software (Spica Technology Corporation, Maui, HI, USA) was used to compute relative displacements of the tissue markers. All tests were performed at room temperature and the tissues were kept moist during preparation and testing. For each specimen, the longitudinal axis of the PT graft was carefully aligned with the vertical axis of the actuator. A preload of 5N was applied to each specimen before testing and preloaded gauge length of the tendon measured. Cyclic tensile loading was
then performed at a displacement rate of 0.75 mm/sec between 20N and 100N for 500 cycles. After cyclic loading, the specimens were loaded to failure at 1 mm/sec. Nonparametric statistical analyses were computed using the Mann-Whitney test to compare screw types and a Kruskal-Wallis test followed by Dunn’s post-hoc test to determine differences among anatomic regions (distal, proximal and midsubstance). Statistical significance was assumed for p<0.05.

3. Results

Bone mineral density was similar between the stainless steel and bioabsorbable groups (p=0.7). No significant differences between the two groups were detected with regard to cyclic creep deformation (p= 0.8) and secant stiffness (first cycle, p= 0.5; last cycle, p= 0.16) (Table 1). Structural properties also showed no significant differences between the two groups (i.e., failure load, p= 0.7; deformation to failure, p= 0.07; work to failure, p=0.5; and linear stiffness, p= 0.6). While significant differences between the two groups were not observed for the distal (p=0.7) and midsubstance (p=0.8) deformation at failure, proximal deformation was significantly higher for bioabsorbable fixation (p=0.02). Considering the two screw types together, surface deformation at failure varied significantly with anatomic region (p=0.003). Midsubstance deformation was significantly lower than both distal and proximal deformation. All samples failed at the tibial insertion site with the tibial bone block fracturing at the screws. Optical analyses showed that no slippage or screw displacement occurred during the failure test.

4. Discussion

To our knowledge, the current study is the first to examine biomechanical properties of PCL onlay fixation using bioabsorbable screws. Our results suggest that bioabsorbable fixation using a tibial onlay technique does not compromise the strength and stiffness characteristics afforded by metallic fixation. Hence, from a biomechanical perspective, bioabsorbable screws are a viable alternative to metal in the context of tibial onlay reconstruction.

Although PCL reconstructions can be successful, restoring native knee stability is challenging, and patients may progress to an arthritic state. In the latter case, further operative intervention such as osteotomies or arthroplasty may be necessary. Unfortunately, such major reconstructive surgeries typically require removal of metallic hardware. Use of bioabsorbable fixation can potentially eliminate future hardware problems after PCL reconstruction. The present study compared initial fixation strengths of two screw types in a cadaveric model. Clinical outcome studies are needed in order to more rigorously compare the performance and healing characteristics associated with these hardware.

5. References


Detecting Remote Homologues using Scoring Matrices Calculated from the Estimation of Amino Acid Substitution Rates of Beta-Barrel Membrane Proteins

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Abstract
Beta-barrel membrane proteins (MP) are found in Gram-negative bacteria, mitochondria and chloroplasts. They play important roles in metabolism of bacteria, where they are involved in transport of solutes in and out of the cell. Beta-barrel proteins may also act as proteases, lipases and may be important for cell-cell adhesion. Currently, there are about 30 solved structures of beta-barrels. Although the number of beta-barrel folds is fairly small, it is possible to expand the amount of available structural information by homology modeling using existing structures as templates. The scope of structure prediction may be widened by finding remote homologues of the existing structures. To improve the sensitivity of the database searches and the quality of sequence alignments, I study evolutionary history of transmembrane segments of beta-barrel membrane proteins. This can be achieved by estimating substitution rates with a Bayesian Monte Carlo approach, which can be used to detect remote homologues. Transmembrane, extracellular and periplasmic regions of beta-barrel proteins experience different evolutionary pressure, which results in different substitution rates. In the present work, I will focus on transmembrane regions of beta-barrel MP. Results on amino acid substitution rates, scoring matrices, and database searches for remote homologues will be discussed.

Keywords: Substitution rate, Scoring matrices, Beta barrel membrane proteins, Bioinformatics

1. Introduction
The development of molecular biology sequencing tools meant the appearance of publicly available databases. For example, GenBank [1] is the database for DNA sequences and SwissProt or PIR [2, 3] are examples of protein databases. The Protein Data Bank (PDB) [4] is the one for structure of biomolecules (especially proteins). All these databases have experienced an exponential growth in the last decade. For the research of this vast amount of biological information, it was crucial to develop local sequence alignment tools, such as FASTA [5] and BLAST (Basic Local Alignment Search Tool) [6], cornerstones of bioinformatics. The principle behind these tools is simple: given two sequences, an efficient dynamic programming algorithm is used to find the best possible alignment between them. For instance, given a query protein sequence and a protein database, a list of the most significant alignments is retrieved. These resulting homologous sequences may give decisive clues to infer protein function, structure or both.

Sequence analysis of homologous proteins showed that certain amino acid substitutions occur more frequently than others, due to physical, chemical or structural reasons, which prompted the use of scoring matrices as a punctuation system. The classic PAM (Percentage of Acceptable point Mutations) matrices [7] were based on robustly accurate alignments of closely related proteins, from which target frequencies for any desired evolutionary distance were extrapolated using a time-reversible Markov model [8, 9]. BLOSUM (BLOcks of Amino Acid Substitution Matrix) matrices [10] avoid such extrapolation by estimating target frequencies directly from different evolutionary distances by using the ungapped segments of multiple sequence alignments. BLOSUM62 is the default matrix for the popular database search BLAST, while FASTA is usually used with BLOSUM50 matrix. An update of PAM matrices based on the same counting approach that PAM and BLOSUM, using a much larger database is the Jones-Taylor-Thornton (JTT) amino acid substitution matrix [11], widely used in phylogenetic analysis [11-13].

The quality of the results obtained performing BLAST searches against protein databases depends strongly upon the choice of the proper scoring matrix and these commonly used matrices are not exempt from problems. For example, the counting methods behind their calculations present two main problems: the systematic underestimation of substitution in certain branches of a phylogeny, and the inefficiency in using all the information contained in the amino acid residue sequences [14].
Assuming the counting methods would be sufficient, BLOSUM and PAM have been derived from globular proteins that have a particular “standard” amino acid composition. The compositional adjustment of amino acid scoring matrices has been proposed from different approaches for other globular proteins with a non-conventional amino acid composition [15-18]. The same adjustment is required for membrane proteins based on their different structural features, different amino acid composition, and residue exchangeabilities [19], as a consequence of a different environment in which they are found (the lipid bilayer). Alpha helical membrane proteins account for 20-25% of all proteins encoded in a typical genome [20]. Their central importance in many cellular processes makes it of great importance to increase the ability to detect related membrane proteins. Several different approaches have been followed trying to achieve this goal, as the inclusion of information from topology prediction [21], the development of non-symmetric scoring matrices [22], the construction of substitution matrices based on the prediction of transmembrane segments [23] and the construction of scoring matrices for alpha-helical membrane proteins following the BLOSUM method [24].

However in this paper, I am interested in the beta-barrel-fold of membrane proteins. Beta-barrel membrane proteins (MP) [25] are found in the outer membrane of Gram-negative bacteria, mitochondria, and chloroplasts. They can adopt that particular 3D topology probably based on the composition of the outer membrane: the lipopolysaccharide. There are only a few structures of beta-barrels currently solved to date. Finding remote homologues of the existing structures may widen the scope of structure prediction. Substitution rates have been extensively studied in soluble proteins as I explained above and for alpha helical membrane proteins, but not in beta-barrel MP, due to the scarcity of available data. In this paper I studied evolutionary history of transmembrane segments of beta-barrel membrane proteins by estimating substitution rates with a Bayesian Markov Monte Carlo approach. A series of scoring matrices that I named **beta-barrel Transmembrane Matrices** (bbTM) specific for transmembrane region of beta-barrels were calculated and tested for detection of remote homologues of beta-barrel MP. I compare my results with the popular BLOSUM62 matrix.

2. Methods

I selected a dataset [26] of 7 non-homologous β-barrel membrane proteins with available X-ray structure (1A0S, 1BXW, 1FEP, 1I78, 1KMO, 1NQE, 1QJ8, 2OMF). For each protein sequence, I performed a BLAST search against NCBI NR database and selected homologous sequences with 20-90% sequence identity (e-value <10^-10). Two are the main criteria for this selection: (i) the identity is based on the sequence of transmembrane fragments, and (ii) a maximum of two gaps are allowed for every individual transmembrane fragment.

From these finally selected sequences, a phylogenetic tree is calculated using a maximum likelihood method [12]. With the information provided by this phylogenetic tree and with the part of the sequences that belongs to the transmembrane segments of these membrane proteins, a Bayesian Markov Monte Carlo simulation was carried out to estimate the substitution rates, using the method developed by Tseng and Liang [27]. A brief summary of this method: given the sequence divergence (branch lengths) of a calculated phylogenetic tree using a Maximum Likelihood method, and given a set of homologous sequences, the probability of observing all residues in the given sequence is:

\[ P(S \mid T, Q) = P(x_1, \ldots, x_n \mid T, Q) = \prod_{n=1}^{n-1} p(x_n \mid T, Q) \]  

For the estimation of the \( Q \) matrix, a continuous time Markov model for residue substitutions is implemented using a Bayesian approach, where the prior distribution \( \pi(Q) \) is employed to encode the past knowledge of amino acid substitution rates for proteins. The instantaneous substitution rate \( Q = \{q_{ij}\} \) is described by a posterior distribution \( \pi(Q \mid S, T) \), which summarizes the information contained in the given sequences \( S \) and in the optimal tree topology \( T \). After integrating the prior information and the likelihood function, the posterior distribution \( \pi(Q \mid S, T) \) can be estimated up to a constant. Therefore, our goal is to estimate the posterior means of rates in \( Q \) as summarizing indices (2):

\[ E_{\mu}(Q) = \int Q \cdot \pi(Q \mid S, T) \, dQ \]  

For this study I used both uniform uninformative priors and the priors obtained from BLOSUM62, which gave similar results. Then, a Markov chain is run to generate samples drawn from the target distribution \( \pi(Q \mid S, T) \). Starting from a rate sample \( Q_0 \) at time \( t \), I generate a new rate matrix \( Q_{t+1} \) using the proposal function \( T(Q_0, Q_{t+1}) \). The proposed new matrix \( Q_{t+1} \) will be either accepted or rejected, depending on the outcome of an acceptance rule \( r(Q_0, Q_{t+1}) \). This is achieved by using the Metropolis-Hastings acceptance ratio \( r(Q_0, Q_{t+1}) \) to either accept
Detecting Remote Homologues using Scoring Matrices Calculated from the Estimation of Amino Acid Substitution Rates of Beta-Barrier Membrane Proteins – D. Jiménez-Morales

or reject $Q_{t+1}$ depending on whether the following inequalities hold:

$$u \leq r(Q_t, Q_{t+1}) = \min \left\{ 1, \frac{\pi(S, T) T(Q_t, Q_{t+1})}{\pi(Q_t, S, T) T(Q_{t+1}, Q_t)} \right\}$$

(3)

Where $u$ is a random number drawn from the uniform distribution $U[0,1]$. With the assumption that the underlying Markov process is ergodic, irreducible, and aperiodic [28], a Markov chain will reach the stationary state.

3. Results

The substitution rates obtained in this study for every beta-barrel MP are very different from those derived from BLOSUM62, as shown in Figure 1. In these plot representations can be observed the different pattern of substitutions estimated between the twenty amino acids: the larger the number of substitution between two amino acids in the analyzed sequences, the bigger the bubble.

With the beta-barrel Transmembrane Matrices (bbTM) that I derived from the estimated substitution rates, I performed BLAST searches using each of the transmembrane fragments as a query sequence. The number of hits obtained by bbTM and BLOSUM62 matrices are summarized and shown for different ranges of e-values (Figure 2). bbTM matrices produce both larger number of hits (45 vs 41) and smaller e-values than BLOSUM62.

Fig 1. (a) Substitution rate of TMSs of beta-barrel MP
(b) Substitution rates derived from BLOSUM62

This method was used to estimate the substitution rate for each of the 7 protein sequences from the dataset described above. From each of this substitution rates, I derived the residue similarity scoring matrices beta-barrel Transmembrane Matrices (bbTM), and these are the ones that will be used for database searches. This is done by calculating the residue similarity score $b_{ij}(t)$, from the substitution rates, between residues $i$ and $j$ at a different evolutionary time, $t_e$ (e=1, 2… 300), from the rate matrix $Q$. But in this study I only used the scoring matrix at time $t=1$.

The target database for all the blast searches was the predicting bacterial membrane protein database PROFtmb [29].

Fig 2. Number of hits obtained from BLAST using both bbTM and BLOSUM matrices grouped by range of e-values

bbTM matrices were calculated for seven beta-barrel MP of known structure. For each of these sequences I performed a BLAST search using its own derived scoring-matrix (table 1: highlighted in grey) and the matrices calculated from other beta-barrel-membrane proteins, against the same database. The number of hits and e-values obtained from these BLAST searches were very similar for all bbTM matrices tested (Table 1), obtaining even better results for
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some of them, indicating that bbTM matrices can capture the substitution pattern characteristic of transmembrane beta-strands independently from the original sequence from which they were derived.

4. Conclusions

Substitution rates have been estimated for beta-barrel membrane proteins and are very different from BLOSUM62 (figure 1). The scoring matrices derived from these rates can be used for detection of remote homologues of known structures through BLAST searches: more homologous sequences can be detected and with better e-values (figure 2). All the transmembrane sequences studies share a poor sequence identity. Despite this fact, the BLAST results obtained are similar when any transmembrane sequence is used as a query with any of the matrices derived from other transmembrane sequence, indicating that the substitution rates estimated from very different beta-barrel membrane proteins share a strong common pattern of substitution.

5. Acknowledgement

Support from NIH GM079804, GM081682 and NSF DBI-0646035 is gratefully acknowledged.

6. References


Abstract
Measuring tissue mechanical properties, elastography, has a significant importance in medical diagnosis. Early detection of cancer tissue and characterization of carotid plaques are examples of where information regarding the tissue elastic properties are significantly important. This paper is a review of the literature of Magnetic Resonance Elastography. This work describes the elastography method in both mechanical and magnetic resonance imaging point of views. The basic theory of magnetic resonance imaging that is related to the elastography and soft tissue acoustics is covered. The reconstruction of material properties from an image is also discussed. Recent developments in the area are given with the examples of previous studies.

Keywords: Magnetic resonance elastography, Phase contrast imaging, Material property reconstruction.

1. Introduction
Tissue elasticity is an emerging parameter in medical diagnosis. The values of bulk modulus, shear modulus and loss modulus may provide significant information where other imaging modalities are inadequate to create contrast. Over the last twenty years a lot of progress has recorded in visualizing the elastic properties of soft tissues. One method that has developed and gained significant acceleration during the last ten years is Magnetic Resonance Elastography (MRE) [1].

MRE is a phase contrast technique, which is developed to image the propagating transverse acoustic strain waves in soft tissues or tissue like media [2]. In MRE the medium is subjected to mechanical excitation, which creates an oscillatory motion in the tissue. These oscillations are perpendicular to the direction in which they propagate, thus they are also called shear waves. Additionally the mechanical excitation is synchronized with the imaging gradients of the MR system. This synchronization facilitates the imaging of the transverse motion of acoustic waves.

Although the shear wave images are related to the material properties through parameters such as wave speed, wavelength, attenuation or propagation direction, they do not provide information about the elastic properties until they are reconstructed. There are different approaches for reconstruction of elastic properties. Some of them are local-frequency-estimator, algebraic inversion of the differential equation, variational method, and finite element based reconstruction [3].

2. Theory
MRE can be clearly understood if soft tissue elasticity and acoustics are well understood, together with the MR basics and phase contrast imaging.

2.1. Tissue elasticity and acoustics
Elasticity information of a medium can be acquired either statically or dynamically. Static material property estimation can be done by monitoring the deformation of tissue under a static load. The results obtained in this way can provide information for isotropic materials. However most of the biological soft tissue is anisotropic. Dynamic material property estimation can be done by visualizing the wave motion in the medium. Among two different acoustic wave types, compression waves and shear waves, that can propagate inside the tissue, shear waves are more feasible to visualize, because of their wave speed.

\[ c_s = \sqrt{\frac{\lambda}{\rho}}, \quad c_v = \sqrt{\frac{\mu}{\rho}} \]

The compression wave speed is dominated by the bulk modulus (\(\lambda\)) where the shear wave speed is dominated by the shear elasticity (\(\mu\)). This basic formulation for wave speeds yields a compression wavelength of meters in the interested frequency band. However for shear waves the wavelength is on the order of centimeters. It is not practical to fit a standing compression wave in a medium of interest due to its length, but multiple shear waves, with the given wavelength, can fit in a test specimen. Therefore MRE uses transverse acoustic (shear) waves.
2.2. Magnetic Resonance Elastrography

MRE is a dynamic elasticity assessment technique that is based on phase accumulation in precessing atomic nuclei (spins) undergoing harmonic motion. This harmonic motion of the spins is due to a mechanical actuation. Magnetic Resonance phase encoding is possible if this mechanical motion and motion sensitizing imaging gradients are synchronized. The derivation of phase shift for moving spins occupying a certain voxel in the presence of a magnetic field gradient is given by [2,3,4]:

\[ \phi(t) = \gamma \int_{0}^{\tau} G_r(t) \cdot \vec{r}(t) dt \]  

(2)

\( G_r(t) \) is the magnetic field gradient in synchronization with the mechanical excitation, \( \vec{r}(t) \) is the position vector for the considered set of spins, \( \gamma \) is the gyromagnetic ratio. In the case of harmonic mechanical oscillation these spins undergo simple harmonic motion

\[ \vec{r}(t) = \vec{r}_0 + \vec{\xi}(r,t) \]  

(3)

Here \( \vec{r}_0 \) represents the mean position of the spins and \( \vec{\xi}(r,t) \) represents the cyclic displacement. The harmonic component of displacement can be rewritten as

\[ \vec{\xi}(r,\theta) = \vec{\xi}_0 \cos(kr - \omega t + \theta) \]  

(4)

where \( k \) is the wave number. Substituting this expression into (2)

\[ \phi(r,\theta) = \gamma \int_{0}^{\tau} G_r(t) \vec{\xi}_0 \cos(kr - \omega t + \theta) dt \]

\[ \phi(r,\theta) = \frac{2\gamma NT(G_r,\vec{\xi})}{\pi} \sin(kr + \theta) \]  

(5)

Where \( N \) is the number of gradient cycles, \( T \) is the period of mechanical excitation [1]. This shows that the phase shift is proportional to the dot product of the displacement and the gradient vectors as well as the number of gradient cycles. This is a useful property when imaging small displacement oscillations, as more phase is accumulated through higher number of gradient cycles [5, 6].

As a conclusion; oscillatory motion of the spins produces a phase shift when coupled with oscillatory imaging gradients at the same frequency. This phase shift phenomenon can be used to visualize the shear wave motion in the medium.

3. Mechanical Actuation Methods

Shear waves in tissue are transverse acoustic waves, the direction these waves propagate is perpendicular to the direction of the oscillation. The appropriate mechanical actuation method to create such waves is harmonically forcing the tissue in the transverse direction while this transverse motion is transferred in the longitudinal direction by wave motion. An example might be a contact plate on the tissue surface moving parallel to the surface [4-7]. In this example the friction force between the plate and the tissue should be enough to create motion without slipping, otherwise the plate can be replaced with a sharp object sticking in the tissue. Images acquired by employing this method will show waves parallel to the excitation direction. An alternative contact method can be a needle penetrating deep inside the tissue. The axial motion of a cylindrical needle can also create shear waves propagating in the radial direction.

![Figure 1. Examples of waves at different directions](image_url)
attached rod. This torque can be transferred to the medium by means of a mechanism. In addition to that an actuator mechanism can also be driven via an electric motor, located outside the magnet, and the motion can be translated by a linking element such as a belt. Alternatively piezoelectric elements, which can be placed in any orientation in the magnetic field, can be used to create mechanical motion [5, 8]. Piezoelectric actuators have a higher force output however they work in smaller range of displacements, this makes these kind of actuators suitable for micro imaging applications.

Another method to induce motion inside the tissue is Focused Ultrasound (FUS) [9]. FUS uses the radiation pressure of a high frequency acoustic source, the spherical cap shaped design of the transducer focuses the ultrasonic beam to the focal point of transducer. Modulation of this radiation field creates a harmonic force inside the tissue. Displacements created by FUS are relatively low compared to the other direct techniques. However it is advantageous because it creates motion inside the tissue rather than at surface, this eliminates the attenuation problem to a certain level.

4. Inversion Methods

In order to obtain material properties, the displacement information given by the phase image should be further processed. The equation of harmonic motion for an isotropic, linearly elastic medium is given by [3, 10 - 12]:

\[ \mu \nabla^2 u + (\lambda + \mu) \nabla (\nabla \cdot u) = -\rho \omega^2 u \]  

(7)

Where \( u \) is the vector of displacements, \( \lambda \) is the bulk modulus, \( \mu \) is the shear modulus, \( \rho \) is medium density and \( \omega \) is the excitation frequency. There are various solution techniques for different boundary conditions, when the material properties are known and the above equation is going to be solved for unknown displacements. Solving this equation for material properties, in the presence of known displacements, needs an inverse method.

4.1. Local Frequency Estimation (LFE)

The shear wave speed (or phase speed) is given in (1) this initial definition of the wave speed does not describe the complex nature of this quantity. Assuming Voigt model [13], it can be expressed more precisely as:

\[ c_s = \frac{2}{\rho} \frac{\mu_1^2 + \omega^2 \mu_2^2}{\mu_1 + \sqrt{\mu_1^2 + \omega^2 \mu_2^2}} \]  

(8)

Also the logarithmic decrement between at least two successive points is:

\[ \delta = \ln \frac{W(x)}{W(x + T)} = \frac{2\pi \mu_2}{\mu_1 + \sqrt{\mu_1^2 + \omega^2 \mu_2^2}} \]  

(9)

Both the log decrement and the wavelength can be measured from a line profile of the shear wave image. These measured values can be used to solve for the shear elasticity (\( \mu_1 \)) and shear viscosity (\( \mu_2 \)). The fundamental shortcoming of this method is being not able to provide close estimates where at least half a wavelength cannot be fitted, and material or geometry discontinuities, both are due to low resolution of the method. The main weakness of this method is the assumption of plane waves, which states waves are propagating in a single direction, neglecting the wave motion in other direction and reflections.

4.2. Algebraic Inversion of the Differential Equation (AIDE)

In this inversion method, the partial differential equations (7) are inverted at different spatial positions, assuming, local homogeneity and isotropic material properties [14]. This solution is for the strong form (derivative form) of the dynamic differential equation. Equation of motion (7) can be rewritten as:

\[ \mathbf{A} \begin{bmatrix} \lambda + \mu \\ \mu \end{bmatrix} = -\rho \omega^2 \begin{bmatrix} u_1 \\ u_2 \\ u_3 \end{bmatrix} \]  

(10)

\[ \mathbf{A} = \begin{bmatrix} A_{11} & A_{12} \\ A_{21} & A_{22} \\ A_{31} & A_{32} \end{bmatrix} = \begin{bmatrix} u_{i,ii} & u_{i,ii} \\ u_{i,ii} & u_{i,ii} \\ u_{i,ii} & u_{i,ii} \end{bmatrix} \]  

(11)

Then the solution is

\[ \begin{bmatrix} \lambda + \mu \\ \mu \end{bmatrix} = -\rho \omega^2 (\mathbf{A^* A})^{-1} \mathbf{A^*} \begin{bmatrix} u_1 \\ u_2 \\ u_3 \end{bmatrix} \]  

(12)

\( \mathbf{A^*} \) is the conjugate transpose of the matrix \( \mathbf{A} \)
Displacements from neighboring pixels are also used for calculation of the second derivatives in (11) and (12). AIDE techniques depend on the presence of motion, rather than planar shear wave propagation, in the region of interest. This condition is satisfied with MRE data.

### 4.3. Variational Method (VM)

Alternatively, the weak form (integral form, variational form) of the differential equation, with appropriately chosen, smooth test functions, can be used to estimate the material properties [15, 16]. The product of the differential function and the test function is integrated over the domain of interest. Similar to AIDE, VM also assumes material homogeneity and isotropy in the region of interest. However calculating the derivatives of the displacements is not necessary in VM, analytical derivatives of the test functions are used instead. This brings the advantage of selecting appropriate test functions at the boundaries so that the method will not lose resolution, as in the case of AIDE, in which calculated derivatives cause huge discontinuities at the boundaries.

### 4.4. Finite Element Based Inversion

Finite Element based inversion method iteratively solves the forward problem over a sub-zone to find the solution for the inverse problem [10, 17-19]. An error function is defined such that

\[
F(\mathbf{E}) = \sum_{i=1}^{N} \sum_{l=1}^{3} (u_{il}^c - u_{il}^m)^2
\]

where superscript ‘c’ denotes ‘calculated’ and ‘m’ denotes ‘measured’ for displacements and ‘l’ stands for a sub-zone. The search algorithm iterates to minimize this error function on a given sub-zone. The error function plays an important role together with the search algorithm, such as Newton’s method, in the determination of the parameters for next iteration. When the error is minimized for a sub-zone, method suggests persisting with another sub-zone until the whole domain is covered.

The method is relatively slow especially for 3D reconstruction; therefore a parallel computing scheme is developed. Since each sub-zone is independent from other sub-zones, parallelization is effective.

### 5. Examples and Discussion

MRE has been applied on various specimens on different systems at different scales. Initial experiments were conducted on agar/agarose gel samples as well as some animal soft tissue, such as porcine kidney, bovine liver, bovine fat and muscle. The first human MRE experiment is conducted on breast to study some breast cancer lesions [4]. Some studies compared their elasticity results with other mechanical test results [7], those presented good correlation between two experiments. For better resolution MRE was carried to higher magnetic fields, a voxel size of 35μm×35μm with a slice thickness of 500μm was obtained by an 11.7 T microimaging system [5]. The lower limit for the static magnetic field was also studied; a 0.1 T system was reported to work well with know MRE techniques [20]. Most of the MRE research in the literature is focused in frequencies lower than 1000 Hz. This is mainly due to the rapid attenuation of shear waves at higher frequencies and gradient limitations both in amplitude and frequency. One study in the literature goes up to 5 kHz using an electromagnetic actuator, these experiments are conducted on silicone gels specimens and hyaline cartilage [6].

More specific applications of MRE were reported recently. Human skeletal muscle was imaged using different directions of mechanical excitation and motion encoding. Due to the fibrous structure of muscles, motion in these tissue highly depends on the actuator orientation [21, 22]. One other study is particularly concentrated on human vessel wall elasticity imaging [23]. Motion is induced in vessels in a direction transverse to the vessel axis. These oscillations are propagated on the vessel and in the surrounding media using the vessel as a wave guide. Elastic properties of the vessel can be extracted using a similar method discussed in the previous section.

Elasticity reconstruction from shear wave images, is build upon Voigt material model; at some studies it is shown that this model doesn’t represent the actual behavior of the tissue. Therefore fractional order material models are utilized [24, 25].

### 6. References


Implantable cardiac pacemakers are imitations of the heart our body has. Every second our heart beats is thanks to a group of cells that generate electrical activity. These specialized cardiac muscle cells then separate charged particles and leak some into the cells of the heart. This then produces an electrical impulse and causes the heart to contract. The cells that do this are part of the sinoatrial node, the natural pacemaker. This system in our body causes about 72 beats per minute. However, since for the heart to beat properly, the generated impulse must go through a specific path to the ventricles, the lower half of the heart, sometimes problems such as, irregular heart beat, hypertrophic cardiomyopathy, and congestive heart failure may arise. Hence, to improve this natural system the use of implantable cardiac pacemakers came into need.

The size, functionality, and longevity of the pacemaker have increased with the development of technology in microelectronics. Sources show that nearly 600,000 pacemakers are implanted worldwide, each year. And since 1960 nearly 3 million have been implanted. [5] A pacemaker has two functions and both are equally important. First, its main function is cardiac stimulation, which is done by a pulse generator. This generator depends highly on specialized circuitry and sends the pulse via output circuits at a certain time interval. This is done via time control and control circuits that senses pulse events via sensing circuits. Secondly, the pacemaker has to log and monitor its own workings.

The most common pacemakers are the “Demand” pacemakers. These are better than the initially used “Fixed” rate pacemakers because unlike the fixed pacemaker, which sends signals at a fixed rate, the Demand pacemakers sense when the heart is beating on its own and sends pulse only when necessary.

The telemetry system element that automatically measures and transmits data, for memory, programmability and testability has greatly helped doctors understand and diagnose patients. The data logged in the pacemaker helps the doctors know how the natural heart is working and if the patient needs to be prescribed extra medicine or may need other alternatives such as heart transplant to better his/her health. Initially, these kinds of pacemakers were less of an implant because they needed to be connected externally to AC power. The problem with this was mobility. A patient could move only as far as the cord length would let him/her…somewhat like a vacuum machine for the carpets. In 1957, the first battery-powered pacemaker was developed, and in 1960, the first implantable one was developed. However, the battery life was only 12-18 months. Hence, would require surgery about every year to replace the battery.

Then came US Patent 4,117,212 - Lithium Iodine Battery. James Moser and Alan Schneider developed the lithium-iodide Battery. Though the patent is under Moser and Schneider’s name, the work on this originated in 1967 at Jet Propulsion Laboratory. The theory behind this battery’s working is the “charge transfer complex” – an electron donor-electron acceptor complex characterized by electronic transition(s) to an excited state in which there is a partial transfer of electric charge from the donor to the acceptor. Soon after this development, Wilson Greatbatch, an inventor himself, recommended the lithium-iodide-polyvinylpyridine system to power pacemakers. And finally in 1972 it was first implanted in the human body.

The reasoning behind the use of a lithium-iodide battery in pacemakers is the battery life. These batteries can last for almost 10+ years and have had a great positive impact on the lives of patients needing pacemakers. For one, it has replaced the mercury zinc battery, which can cause serious problems due to its mercury content. Secondly, its long life has reduced the need to replace batteries often, hence reducing the need for repeated surgeries. Thirdly, the chemistry in the battery offers a high energy density and a long shelf life, and has a very low self discharging rate that results in the long shelf life. Another great feature of this battery is that it has a stable voltage through much of its life span. This attribute greatly helps in predicting a safe and easy replacement time.
The pacemaker battery – M. Hanif

Figure 2. Lithium-Iodide Cell

The chemistry behind this battery cell is a very simple yet very elegant one; it is a system of solid electrolyte in the cell that is made of the combination of two elements, lithium and iodine, resulting in lithium iodide. The complexity is the reaction between polyvinylpyridine (PVP) and iodine. This thermal reaction between the two forms the cathode material, an electric conductor. And presence of free, unpaired, electrons in the material has been confirmed by electron spin resonance studies.

The increase in thickness of the solid electrolyte and the electronic conductivity decrease causes the battery to display a high internal impedance which increases as the cell discharges. Hence, it is not capable of giving high power. This battery system does not perform well at 60 C, however, it does perform well at 37 C, the normal human body temperature. At the body temperature, is can distribute around 10 – 200 microamperes of current, and a volumetric density that is nearly perfect to operate the pacemaker.

The anode side of the battery is comprised of a development of a corrugated anode. These ridges increased the surface area of the anode and the macroscopic distortions of the anode cell discharge were reduced. At first, the anode was physically painted with a PVP solution in a volatile solvent. Then developments helped better control of the coating weights and helped make the production effective and efficient. One method used was a film of PVP, which was cast on the anode. Another development was to inseminate an inert substance with a PVP solution, then drying the solvent and cutting it into suitable shape to press onto the anode.

Finally a very major development came with the making of thinner cells. These cells introduced in 1984, had a thickness of 5 mm. Modern technology has helped make these cells even thinner, but the 1984 development help this progress.

Though the lithium iodide battery has helped greatly, it may not be in need anymore. Since it still requires a surgery about every 10 years, scientists are trying to develop a way to completely exclude these batteries. In 2004, BBC news reported studies at Biophan Technologies in New York, where a “biothermal battery” is being developed. This battery’s development is still in very early stages, but its mechanism would be to use the body’s own heat to provide electricity to the pacemakers.

Another technology being developed is a million British pounds project, a microgenerator. The researcher David Hatherall from Zarlink Semiconductor is developing this device. He says "The ability to fit and forget implantable devices in terms of their power supply will have significant clinical and quality of life benefits". [6] Not only will this development help in progressing the biomedical field, but will also reduce financial burdens for the patients, since the replacement surgeries can cost nearly 10,000 pounds, roughly, 25,000 dollars. Additionally, it will help reduce health risks for patients, since they need to be put under general anesthetic to undergo this surgery.

References


Evaluating the Growth of Tissue-engineered Cartilage Using MRI

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Abstract
Tissue-engineered cartilage is a promising approach for cartilage regeneration and repair. In this study, measurements of the development of tissue-engineered cartilage with quantitative magnetization transfer imaging (QMTI), MR relaxation times (T1 and T2) and apparent diffusion coefficient (ADC) were conducted over a 3-week growth period. These MRI results were correlated with subsequent biochemical analysis for glycosaminoglycan (GAG) contents. Bound proton fraction (BPF) and cross relaxation rate (k) show a statistical increase after one week in the tissue culture which was highly correlated with the increase of GAG. This study demonstrates that the QMTI can be used to assess the changes of the extracellular matrix (ECM) during tissue-engineered cartilage development, as reflected in BPF. Thus BPF can be used as a marker for evaluating the tissue-cartilage development process.

Keywords: Tissue-engineered cartilage, Quantitative magnetization transfer imaging, Bound proton fraction, Cross relaxation rate, Cartilage evaluation.

1. Introduction
Arthritis and degenerative diseases of cartilage affect millions of Americans. One in five (21%) adults in the United States report having doctor diagnosed arthritis, costing $128 billion annually [1]. Tissue-engineered cartilage is a promising approach for cartilage regeneration and repair [2]. Yet, noninvasive visualizing and evaluating the distribution of functionally important macromolecules (like GAG and collagen II) in the engineered cartilage still need further study. MRI techniques are amenable to the assessment of early stages of cartilage disease [3]. As a relatively new way of generating contrast in MRI, magnetization transfer (MT) is sensitive to the density of macromolecules in tissue structures such as ECM [4]. Magnetization transfer imaging (MTI) has been used for the evaluation of tissue-engineered cartilage [5,6]. However, the parameter most often used in these studies - magnetization transfer ratio (MTR) - is highly dependent on the imaging conditions. The Bound Proton Fraction (BPF), a parameter derived from quantitative magnetization transfer imaging (QMTI) [7], directly reflects the percentage of macromolecules in the total extracellular matrix (ECM), and hence is a more objective parameter than MTR. For example, BPF was used recently in the diagnosis of Multiple Sclerosis [8]. To our knowledge, however, there is no report of the use of BPF to monitor the development of the tissue-engineered cartilage, e.g., Glycosaminoglycans (GAGs); therefore, we performed a series of experiments on tissue-engineered cartilage to seek a correlation between BPF and cross-relaxation rate (k), and other conventional MRI parameters: T1, T2, and ADC, as well as direct biochemical methods.

2. Materials and Methods

2.1 Sample Preparation
Tissue-engineered constructs were generated by seeding gelatin cubes with mesenchymal stem cells (MSCs) [9]. The constructs then were divided into two groups: treatment and control. One group was cultured in chondrogenic differentiation medium, while the other group was cultured only in basic DMEM medium. The constructs were examined using MRI every seven days over a three-week period.

2.2 MRI Experiments
All MRI experiments were conducted using a 56-mm vertical bore 11.74 T Bruker Avance imaging spectrometer with a micro-imaging gradient insert with a maximum gradient strength of 200 G/cm and a 5-mm diameter saddle coil (Bruker Instruments). MTI was performed using the spoiled 3D MT-GRE pulse sequence with following parameters: TR/TE/α=36ms/1.9ms/10°; FOV = 8.0×8.0mm; slab thickness = 2.0mm; 3D matrix = 128×128×16; NEX = 1. A Gaussian RF pulse with peak power of 25 µT was used as the MT pulse. The MT-weighted images were acquired for offset frequencies Δ = 1, 1.5, 2, 3, 4, 6, 8, 12, 15, 20, 25, 31, 37, 43, 50 kHz. A water-cooling system was used to keep gradient temperature between 25 and 30 °C. The T1, T2, and ADC for the samples were also measured [10].
2.3 Biochemical Analysis

After MRI analysis, the GAG content of the matrices at each time period was assessed by a modification of the dimethylmethylene blue method [11]. The matrices were digested in 1 ml of protease (2.5 mg/ml; Type XIV Bacterial from Streptomyces Griseus, Sigma) in TBS solution in a 55 ºC water bath overnight. A 100 µl aliquot of the digest was assayed for total GAG content by addition of 2 ml of 1,9-dimethyl methylene blue dye solution (Polyscience Inc., Northampton, UK). Absorbance at 535 nm was determined with a spectrophotometer (LKB Biochrom Ultraspec).

2.4 Data Processing

A specific region of interest was localized in the treatment and control samples. T1, T2, and ADC were calculated using a least squares single exponential fitting model implemented in MATLAB 7.1. BPF and k were extracted with a program written using Henkelman’s two-pool model [7, 12]. The results of MRI data and biochemical data are reported as the mean ± std. A student t test was performed between data from the control and the treatment groups. Statistical significance was defined as p = 0.05.

3. Results

Fig.1. shows MT weighted MR images of the constructs cultured in the three-week period. The difference between the control and treatment construct at each stage is shown. The treatment group has a lower intensity in the images after the first week. Also, after one week in culture, there are significant differences (p<0.05, n=6) between control and treatment groups for the MRI parameters: T1, T2, ADC, BPF, and k, as well as the GAG content determined by biochemical analysis (Fig. 2. and Table 1).

The BPF and k of the constructs cultured in the chondrogenic differentiation medium (treatment) showed a significant difference (p<0.05, n=6) at all stages of development (Fig.2. B & C). The BPF and k of the constructs cultured in the chondrogenic differentiation medium (treatment) were highly correlated (0.98 and 0.93, respectively, shown in Fig. 3.) with the increase of GAG content in the constructs.

Fig.1. MT weighted images of the constructs in the three-week period. The offset frequency of MT pulse is 2000 Hz. A. MR Image of one construct after MSCs was seeded and before cultured in different medium (week 0). B. C. and D are MR images of constructs after one week, two weeks, and three weeks in culture, respectively.
Evaluating the growth of tissue-engineered cartilage using MRI – W. Li

Fig.2. Changes of GAG, BPF, k, T1, T2, and ADC during the three-week growth period. A. GAG content measured by biochemical analysis. B. bound proton fraction (BPF) from MTI; C. cross-relaxation rate (k) from MTI; D. longitudinal relaxation time (T1); E. transverse relaxation time (T2); F. apparent diffusion coefficient (ADC).

Fig.3. Graphs showing the relationship between GAG content in chondrogenic differentiation medium (treatment) and bound proton fraction (BPF) and cross-relaxation rate (k) in three-week’s growth stages. The correlation coefficient was shown in each graph.
Evaluating the growth of tissue-engineered cartilage using MRI – W. Li

Table 1. Changes of GAG, BPF, k, T1, T2, and ADC measured during the three-week growth period

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GAG (mg/ml)</strong></td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>8.9±2.06</td>
<td>15.8±1.54</td>
<td>10.3±0.86</td>
<td>33.6±4.99</td>
</tr>
<tr>
<td><strong>BPF (%)</strong></td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>0.14±0.02</td>
<td>0.14±0.03</td>
<td>0.18±0.03</td>
<td>0.52±0.06</td>
</tr>
<tr>
<td><strong>k (s⁻¹)</strong></td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>3.12±0.03</td>
<td>3.12±0.03</td>
<td>2.97±0.05</td>
<td>2.60±0.06</td>
</tr>
<tr>
<td><strong>T1 (s)</strong></td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>70.85±1.62</td>
<td>70.85±1.62</td>
<td>60.64±1.63</td>
<td>48.78±2.47</td>
</tr>
<tr>
<td><strong>T2 (ms)</strong></td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>70.85±1.62</td>
<td>70.85±1.62</td>
<td>59.27±1.23</td>
<td>50.76±1.39</td>
</tr>
<tr>
<td>*<em>ADC (<em>10⁻³ mm²/s)</em></em></td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>1.33±0.02</td>
<td>1.33±0.02</td>
<td>1.28±0.05</td>
<td>0.98±0.07</td>
</tr>
</tbody>
</table>

4. Discussion

In this study, tissue-engineered cartilage constructs were cultured and analyzed with 11.74 T micro-MRI system and biochemical methods every seven days over a 3-week period. Six groups of the constructs were examined for each week. The small changes observed in the MRI parameters measured during the growth of tissue-engineered cartilage (T1, T2, and ADC) indicate that these parameters will not be effective for monitoring engineered cartilage development. On the other hand, both bound proton fraction (BPF) and the cross-relaxation rate ($k$) increased substantially during the study (factors 9 and 4, respectively). In addition, the high correlation between BPF and construct GAG content suggests that extracellular matrix (ECM) development can be observed by quantitative magnetization transfer imaging. Since BPF reflects the fraction of macromolecules to free water in tissue, the increase of BPF mirrors the increase of ECM in the constructs. Cross-relaxation rate ($k$) donates the magnetization exchange between the macromolecules and the free water in tissue. Its value is sensitive to the MR experiment condition and MR imaging protocol [13]. Further study is needed on the relation of $k$ and ECM in the constructs.

5 Conclusions

Bound proton fraction (BPF) from quantitative magnetization transfer imaging (QMTI) can be used as a marker for evaluating the cartilage development process.

5. References

1. http://www.arthritis.org/resources/gettingstarted


**T1ρ MEASUREMENT IN MRI OF TISSUE ENGINEERED CARTILAGE**

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**Abstract**

Degenerative diseases of cartilage affect millions of people. Tissue engineered cartilage is important to repair and treat the osteoarthritis. MRI is a noninvasive way to monitor the growth of cartilage tissue. During the 12 days' growth period, spin-lattice relaxation time in the rotating frame (T1ρ) decreases continuously in the stimulated group while there is no apparent decrease of spin-spin relaxation time (T2) in simulated in our experiments. Our preliminary study shows that T1ρ may be more sensitive to the glycosaminoglycans growth of the tissue-engineered cartilage than T2.

**Keywords:** T1ρ, cartilage, Tissue engineering

**1. Introduction**

Osteoarthritis (OA) is the most common form of arthritis, currently affecting over 20 million people in the U.S [1,2]. The early diagnosis of OA requires the ability to noninvasively and nondestructively detect degenerative changes in glycosaminoglycans (GAG) concentration and collagen network integrity. MRI has been used in detection of early degeneration of cartilage. Since tissue engineered cartilage is a promising way to repair and treat human cartilage [3]. It is important to find an efficient parameter to depict the GAG growth of tissue-engineered cartilage. It has been proved that T1ρ is more sensitive to the biochemical change than T2 [4].

The spin-lock method makes it possible to study relaxation at very low magnetic fields [5,6]. In Spin-lock experiment, spins are locked to the xy plane by applying a continuous RF pulse without spin-dephase. In the rotating frame, the net magnetization decays with a constant relaxation time T1ρ because of the existence of B1 generated by the continuous RF pulse. Since the relaxation in the rotating frame is similar to the spin-lattice relaxation, T1ρ is called spin-lattice relaxation in the rotating frame. In biological tissues, frequency dependence on relaxation rates, relaxation-dispersion, may arise from (i) rotational motion of a fraction of water bound to proteins, (ii) exchange of protons on macromolecules with bulk water and (iii) the nonaveraged residual dipolar interaction (RDI) of spin associated with oriented macromolecules in the tissue [4]. T1ρ experiment can be performed at high fields with high SNR while it can provide the potential information about the low frequency motions (~few kHz).

**2. Material and Method**

MRI experiments were conducted at 11.74 T (500 MHz for protons) using a 56 mm vertical bore magnet (Oxford Instruments, Oxford, UK) and a Bruker DRX Avance Spectrometer (Bruker Instruments, Billerica, MA, USA). MR images were acquired using a Bruker Micro 5 imaging probe with triple axis gradients (maximum strength 200 G/cm) and a 5 mm diameter RF saddle coil was used to transmit/receive the nuclear magnetic resonance signals. The scanner was controlled by ParaVision imaging software.

**2.1 Phantom Study**

Firstly, 2%, 4%, 8% agarose gels and 0.03 mMol MnCl₂ are prepared in the 5 mm diameter tube. Then we put the tube in the probe and select the MR parameters as TE/TR = 8 ms/2000 ms, Slice thickness = 1 mm, B₁ =100 µT, FOV = 8.0 mm × 8.0 mm, Matrix size = 128×128, NEX = 2, TSL = 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 ms.

T1ρ relaxation rate obeys the following expression:

\[
\frac{1}{T_{1\rho}} = K \left( \frac{1.5\tau_c}{1 + 4\omega_1^2\tau_c^2} + \frac{2.5\tau_c}{1 + \omega_2^2\tau_c^2} + \frac{\tau_c}{1 + 4\omega_p^2\tau_c^2} \right)
\]

where \( K = \frac{3}{\mu^2 \gamma^4 / 160 \pi^2} \) is a constant including Planck’s constant \( h \) and the distance \( r \) between dipoles, \( \tau_c \) is the rotational correlation time, and \( \omega_1 \) and \( \omega_2 \) are the frequencies of the spin-lock and main magnetic fields, respectively [7].

The intensity of the measured magnetization follows:

\[
M_r = M_0 e^{-TSL/T_{1\rho}}
\]

where TSL is the duration of the spin-lock pulse.
2%, 4%, 8% agarose gels and 0.03 mMol MnCl₂ are measured with the MR methods shown in the previous passage. The $T_{1ρ}$ values are fitted by the equation (2) in Matlab 7.0 and the respective values are listed below:

<table>
<thead>
<tr>
<th></th>
<th>2% agarose gel</th>
<th>4% agarose gel</th>
<th>8% agarose gel</th>
<th>0.03 mMol MnCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1ρ}$ (ms)</td>
<td>92.6±7.1</td>
<td>620±32</td>
<td>368±1.5</td>
<td>212±23</td>
</tr>
</tbody>
</table>

Table 1 $T_{1ρ}$ values of phantom study

And the $T_{1ρ}$ map of the phantoms is obtained by fitting pixel by pixel in the 10 slices, which is shown in the Fig. 1.

2.2 Engineered Cartilage Study

Tissue-engineered constructs were generated by seeding gelatin cubes with mesenchymal stem cells (MSCs) [8]. The constructs then were divided into two groups: stimulated and control. One was cultured in chondrogenic differentiation medium, while the other was cultured only in basic DMEM medium. TE/TR = 8 ms/2000 ms, Slice thickness = 1 mm, $B_1$ =100 µT, FOV = 8.0 mm × 8.0 mm, Matrix size = 128×128, NEX = 2, TSL = 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 ms.

In Fig. 2 we show $T_{1ρ}$ weighted MR images (day 6 and day 12) of control and chondrocyte growth stimulated tissue samples (gelatin cubes implanted with MSCs on day 0).

Fig. 2. $T_{1ρ}$ weighted images at 11.7 T of cartilage constructs at 6 days (A) and 12 days (B) of growth in cultured in chondrogenic differentiation medium. In these images, a SL-SE pulse sequence was used with the following parameters: TR/TE = 2000 ms/8 ms; FOV = 8.0 mm × 8.0 mm; Slice thickness = 1.0 mm; 2D matrix = 128×128; NEX = 2; $B_1$ = 40 µT; TSL = 90 ms.

3. Results and Discussion

Our hypothesis is that developing tissue will display differences in the relative amount of the “so-called” free and bound water fractions. Our preliminary data of $T_2$ and $T_{1ρ}$ are shown in the bar charts of Fig. 3 and Fig. 4. The $T_{1ρ}$ of the stimulated tissue begins to decrease on day 3 and continues to fall through day 12, while the control tissue values are unchanged. However, we can’t see $T_2$ decreasing tendency of stimulated tissue.

Fig. 3. $T_2$ values derived from $T_2$ weighted images for control and growth stimulated constructs. The data are the mean ± one standard deviation for 4 measurements.
Fig. 4. $T_1\rho$ values derived from $T_1\rho$ weighted images such as those in Fig. 2 for control and growth stimulated constructs. The data are the mean ± one standard deviation for 4 measurements.

In the early stage of cartilage tissue regeneration, the free water content doesn’t change a lot. This is the reason why $T_2$ doesn’t show significant change. However, the water bound to macromolecules begins to change and we can see the significant decrease in the $T_1\rho$ figure. Therefore, we can conclude that $T_1\rho$ is more sensitive to monitor the early regeneration of tissue-engineered cartilage.

4. References


THREE-DIMENSIONAL IMAGING OF OXYGEN TENSION IN A RAT RETINA
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Abstract
Optical three dimensional imaging method to measure oxygen tension in the retina is described in this paper. Oxygen tension maps were generated from phosphorescence lifetime images using a frequency domain technique in which the phase relation between the modulated excitation laser light and sensitivity of the imaging camera was varied. A series of two-dimensional phosphorescence section images were acquired and used to reconstruct depth-displaced en-face images of the retina. These images were then used to create three dimensional oxygen tension maps of the retina. This phosphorescence image technique is non-invasive, and information for all pixels is obtained at the same time, so no pixel to pixel scanning is required. The method was tested in four rats while those rats breathe 10 %, 21 %, and 50 % oxygen. A noticeable variation (27 ± 4 mmHg for 10 %, 53 ± 7 mmHg for 21 %, and 139 ± 34 mmHg for 50 %) in oxygen tension in retinal artery was observed under different breathing conditions. This technique may potentially [1, 5] aid in the understanding of retinal oxygen dynamics under different physiologic states and in health and disease.

Keywords: Phosphorescence lifetime, Phosphorescence Imaging, Oxygen Tension, Three Dimensional Imaging, Retina.

1. Introduction
Oxygen and nutrients are the most important factors for retinal cell survival. Insufficient oxygen delivery and retinal hypoxia is considered one of the leading causes of eye diseases such as diabetic retinopathy, glaucoma, and age-related macular degeneration [1-5]. While hypoxia is known to occur at the end stage of disease, the relationships between blood flow insufficiency, vessel patency and tissue hypoxia in the early stages of retinal disease remains unknown. For example, in diabetic retinopathy it is not clear whether tissue hypoxia precedes or is a consequence of the capillary closure and non-perfusion in the retina. Currently, the role of oxygen in many retinal diseases is not well understood and technologies that allow better diagnosis and treatment of retinal diseases are greatly needed.

Several methods are now available for studying retinal oxygenation. The oxygen sensitive micro-electrode technique is accurate but invasive and disturbs the retinal micro-environment [6]. Other methods such as magnetic resonance spectroscopy and magnetic resonance imaging are non-invasive but limited in resolution as compared to optical imaging. Oxygen tension has been measured using phosphorescence imaging technique [9,10] but is limited in depth discrimination. Although a probe injection necessary, phosphorescence imaging is a non-invasive method that does not alter the retinal micro-environment and allows high precision measurements of the oxygen content of the retina.

Frequency domain phosphorescent lifetime imaging will likely transform oxygen measurements in the retina because the oxygen levels across the entire retina can be accurately recorded and displayed in a two dimensional oxygen map through a single measurement. Although the current capabilities of frequency domain phosphorescence lifetime imaging technologies are functional and are currently being used for retinal diseases research, further development and refinement of these systems is required for the consistent generation of highly accurate retinal oxygen maps.

A phosphorescence imaging method has been developed for studying oxygen tension in retina and brain [9,8]. In this paper a novel optical three dimensional imaging system to measure the oxygen tension in retina was investigated.

2. Theory

2.1 Phosphorescence Lifetime Imaging

The origin of phosphorescence lifetime imaging is based on oxygen dependent quenching of
phosphorescence. A palladium porphyrin probe, Pd-meso-tetra [4-carboxylphenyl] porphine, and albumin are injected into the bloodstream to generate a phosphorescence signal [11]. The intensity of the phosphorescent signal of the probe is inversely related to oxygen level in the bloodstream. The phosphorescence emission can be characterized by an exponential decay function:

\[ I(t) = I_0 e^{-t/\tau} \]  

where \( I(t) \) is the phosphorescence intensity, \( I_0 \) is the maximum intensity at \( t=0 \), and \( \tau \) is the lifetime of the phosphorescent decay. A relationship between phosphorescent lifetime and oxygen is described by the Stern-Volmer equation:

\[ \frac{\tau_0}{\tau} = 1 + K_q \tau_0 P_O^2 \]  

where \( \tau_0 \) is the initial phosphorescence in the absence of oxygen, \( K_q \) is the bimolecular-quenching constant for the probe, and \( P_O^2 \) (mmHg) is the concentration of the oxygen quenching agent. From equation (2), the oxygen tension, \( P_O^2 \), of the tissue can be determined using the value of \( \tau \) which is experimentally derived.

2.2 Frequency Domain Lifetime Imaging of Oxygen Tension.

To determine the phosphorescence lifetime \( \tau \), a frequency domain technique is utilized. When a sinusoidal modulated light is used to excite the probe, a phosphorescence emission will result that is also sinusoidal but shifted by a phase angle \( \theta \) and modulated by \( m \), where \( A \) and \( a \) are the amplitude of the excitation and emission, respectively[10].

![Figure 1. The relationship between the phosphorescence excitation and emission signal where \( \theta \) is the phase delay of the emission signal.](image)

Figure 1. The relationship between the phosphorescence excitation and emission signal where \( \theta \) is the phase delay of the emission signal.

\[ m = \frac{a}{A} \]  

Where \( m \) is the modulation factor, Phosphorescence lifetime imaging uses a digital charged coupled device (CCD) - camera to acquire the intensity of the phosphorescence emission in an image format. The intensity recorded at each pixel of the CCD is represented by

\[ I(\theta_D) = k[pd](1 + \frac{1}{2} m_D m \cos(\theta - \theta_D)) \]  

where \( \theta_D \) is the phase shift of the CCD intensifier, \( m_D \) is the modulation profile of the CCD intensifier, \( k \) is a constant and \( [pd] \) is the concentration of the probe. By acquiring an image set across an intensifier phase shift from 0 to \( 2\pi \), \( \tau \) can be determined from both the phase shift and modulation of the phosphorescence signal. The lifetime of the phosphorescent decay is related to phase shift by [9]:

\[ \theta = \tan^{-1}(\omega \tau_\theta) \]  

where \( \omega \) represents the modulation frequency and \( \tau_\theta \) represents the apparent lifetime. The apparent lifetime of the phosphorescent signal is related to the modulation \( m \) by

\[ m = (1 + \omega^2 \tau_m^2)^{-1/2} \]  

where \( \tau_m \) is the apparent modulation lifetime[9]. To simplify the calculation, the intensity data recorded by the CCD is expressed in linear form as a function of the phase shift

\[ I(\theta_D) = a_0 + a_1 \cos(\theta_D) + b_1 \sin(\theta_D) \]  

where \( a_0 \), \( a_1 \) and \( b_1 \) are the fitting parameters [12]. After determining the fitting parameters through regression, the phase \( \theta \) can be determined by

\[ \theta = \tan^{-1}(b_1 / a_1) \]  

and modulation \( m \) with

\[ m = \sqrt{a_1^2 + b_1^2} / a_0 \]
3. Methods

A schematic diagram of the optical system used to capture images is shown in figure 2. The system consisted of a laser delivery path and phosphorescence imaging path. For laser delivery, a diode laser at wavelengths of 543 or 532 nm was expanded and projected at an oblique angle on the retina. A cylindrical lens was placed in the path of the incident laser beam to form a focused line on the retina. In the imaging path, the optics of the slit-lamp biomicroscope formed an image of the retina on a digital charged coupled device (CCD) camera. A filter with a transmission cutoff wavelength of 645 nm, matching that of the oxygen probe phosphorescence emission, was placed in the imaging path. Due to the angle between the incident laser and imaging path, an optical section phosphorescence image was acquired by a digital CCD camera. The laser line was scanned with a galvanometer to create a series of horizontally displayed phosphorescence section images.

A total of four rats were imaged. The rats were anesthetized using ketamine and xylazine (30-35 mg/kg). The pupils were dilated with 2.5% phenylephrine hydrochloride and 1% tropicamide. A flat glass cover slip with hydroxypropyl methylcellulose was placed in front of the cornea of the eye to overcome the cornea optical power and prevent dehydration of the cornea.

The rats were placed in front of slit-lamp biomicroscope and procedures were carried out according to American National Standard Institution [13]. Images were captured in left and right eye in each rat under the breathing conditions of normoxia (21% oxygen), hyperoxia (50% oxygen) and hypoxia (10% oxygen). The PO$_2$ in the retinal artery was measured by blood gas analysis and found to be 195 mm Hg (50% oxygen), 84 mm Hg (21% oxygen), and 55 mm Hg (10% oxygen).

The series of horizontally displayed phosphorescence section images are shown in figure 3. To create en-face images of the retina displaced in depth, an image reconstruction procedure was completed in Matlab (R14 MathWorks Inc, Natick, MA) the reconstructed images are staked together to create the depth. This reconstruction process is shown in Figure 3.
4. Results

$PO_2$ in retinal artery was found to vary with breathing conditions. Under 10%, 21% and 50% oxygen, the mean $PO_2$ in retinal artery for four rats was measured to be $27 \pm 4$ mm Hg (10% oxygen), $53 \pm 7$ mm Hg (21% oxygen) and $139 \pm 34$ mm Hg (50% oxygen). $PO_2$ measurements in retinal artery for different breathing conditions is shown in table 1.

Table 1 – Phosphorescence imaging measurements of (a) mean oxygen tension (mm Hg) for three different breathing conditions of oxygenation and (b) mean oxygen tension (mm Hg) for four healthy rats at different breathing conditions.

<table>
<thead>
<tr>
<th></th>
<th>Rat # 1</th>
<th>Rat # 2</th>
<th>Rat # 3</th>
<th>Rat # 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>10%Oxygen</strong></td>
<td>24 ± 3</td>
<td>29 ± 5</td>
<td>25 ± 4</td>
<td>30 ± 2</td>
</tr>
<tr>
<td><strong>21%Oxygen</strong></td>
<td>50 ± 4</td>
<td>55 ± 7</td>
<td>58 ± 9</td>
<td>52 ± 7</td>
</tr>
<tr>
<td><strong>50%Oxygen</strong></td>
<td>133±35</td>
<td>145±29</td>
<td>136±33</td>
<td>141±38</td>
</tr>
</tbody>
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<table>
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<tr>
<th></th>
<th>Total mean value (mm Hg)</th>
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<tr>
<td><strong>10%Oxygen</strong></td>
<td>27 ± 4</td>
</tr>
<tr>
<td><strong>21%Oxygen</strong></td>
<td>53 ± 7</td>
</tr>
<tr>
<td><strong>50%Oxygen</strong></td>
<td>139 ± 34</td>
</tr>
</tbody>
</table>

Table 2 – Blood gas analysis measurements of (a) mean oxygen tension (mm Hg) for three different breathing conditions of oxygenation and (b) mean oxygen tension (mm Hg) for four healthy rats at different breathing conditions.

<table>
<thead>
<tr>
<th></th>
<th>Rat # 1</th>
<th>Rat # 2</th>
<th>Rat # 3</th>
<th>Rat # 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>10%Oxygen</strong></td>
<td>49 ± 7</td>
<td>59 ± 3</td>
<td>53 ± 5</td>
<td>57 ± 4</td>
</tr>
<tr>
<td><strong>21%Oxygen</strong></td>
<td>80 ± 3</td>
<td>85± 7</td>
<td>83 ± 6</td>
<td>86± 4</td>
</tr>
<tr>
<td><strong>50%Oxygen</strong></td>
<td>187±35</td>
<td>190±30</td>
<td>200±20</td>
<td>205±15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Total mean value (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>10%Oxygen</strong></td>
<td>55 ± 5</td>
</tr>
<tr>
<td><strong>21%Oxygen</strong></td>
<td>84 ± 6</td>
</tr>
<tr>
<td><strong>50%Oxygen</strong></td>
<td>195 ± 25</td>
</tr>
</tbody>
</table>

Three dimensional intensity images are shown in figure 4. Oxygenation in retinal artery increased with increased inhaled percent oxygen. In order to validate our $PO_2$ measurements, $PO_2$ in retinal artery was measured using blood gas analysis and is shown in table 2. A comparison of $PO_2$ measurements using phosphorescence imaging and blood gas analysis is shown in figure 5.
Three-dimensional imaging of oxygen tension in a rat retina – Y. Z. Rawash

5. Discussion:

The frequency-domain approach for phosphorescence lifetime measurements can be used to generate two and three-dimensional PO2 maps for rat retina. Using this technology to measure oxygen tension in the rat’s eye has potentially advanced investigation of oxygen dynamics in transgenic and knock out rat models of retinal disease, such as diabetic retinopathy and age-related macular degeneration.

As shown in table 1 and 2 the PO2 measurements in four rats under different breathing conditions appear reasonable as it gives good noticeable variation under different breathing concentrations. For all PO2 maps produced in this study the modulation frequency was fixed at 1600 Hz. Since the relationship between phase and oxygen tension was described in Eqs. (2) and(5), the oxygen tension sensitivity is proportionally depend on this modulation frequency. A modulation frequency of 1600 Hz provides acceptable sensitivity across the physiologic range, higher modulation frequencies may give better oxygen tension maps. Further investigations for the relationship between oxygen tension maps and modulation frequencies can be done, because the optical chopper used in this study can be modulated as fast as 3000 Hz.

Two-dimensional maps of oxygen tension is shown in figure (4), three-dimensional oxygen tension maps of the retina is generated by staking the two-dimensional oxygen tension together –figure 4.C- . The staking two-dimensional oxygen tension maps does not give us accurate three-dimensional representation of oxygen tension maps of rat retina. However, this way gives us good idea of how the retinal three-dimensional map will be.

6. Conclusion

A new optical three dimensional imaging method to measure oxygen tension in rat retina is described. Two dimensional oxygen tension maps were generated using a frequency-domain approach for phosphorescence lifetime measurement. It was shown that oxygenation in retinal artery increased with increased inhaled percent of oxygen, although there was some variation between our method and blood gas analysis. This variation may be due to sampling location. Blood gas measurements were taken near the heart of the rat, while oxygen tension was measured from images captured in the rat eye. Second, oxygen is consumed during its movement through the blood from heart to eye, which may lower the oxygen levels measured in the eye.

Our method consists of several stages. Data acquisition, image reconstruction, oxygen tension mapping, and three dimensional visualization of intensity images and oxygen tension maps. The disadvantage of this method is that the oxygen-sensitive probe is bound to albumin and remains in vasculature of the eye, thereby prohibiting oxygen tension measurement in the retinal tissue. The advantages of this method are in its potential to measure and to visualize oxygen tension in retinal artery directly. However, future improvement in this method to permit better depth discrimination and higher precision are required.
6. References


NOVEL MULTI-MODAL SYSTEM FACILITATES SIMULTANEOUS CHEMICAL AND ELECTROPHYSIOLOGICAL RECORDINGS IN ISCHEMIC STROKE MODEL

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Abstract
Integration of microdialysis as a means to obtain neurochemical data from the infarct core while attaining real-time electrophysiological recordings allows for a multi-faceted, more comprehensive approach to stroke research. Microdialysis is a technique capable of quantifying the concentrations of neurotransmitters such as glutamate, aspartate, and taurine, each of which is important in the stroke cascade. This study uses a novel multi-modal system with a cranial platform designed through a rapid-prototyping technique specifically for use in a photothrombosis-induced stroke. Once fabricated, the platform was attached to the skull above the location of the craniectomy and a microdialysis probe, recording electrode, and fiber-optic light probe were lowered into or to the surface of the brain. In this study, we report microdialysis recordings obtained before, during, and after photothrombosis. Findings indicate consistent increases from baseline concentrations to peak post-ischemic concentration in all three neurotransmitters assessed: glutamate, aspartate, and taurine. Glutamate and aspartate concentrations peaked within approximately 20 minutes of stroke at levels 3 and 1.25 higher than baseline levels, respectively. Taurine peaked within 30 minutes after stroke induction, at levels approximately 2.5 times higher than baseline concentrations.

Keywords: Microdialysis, Electrophysiology, Ischemic stroke

1. Introduction
Understanding the dynamics of the complex stroke cascade begins with the use of effective techniques and experimentation. The ability to understand its short and long-term neurotoxic events has the potential for saving millions of lives each year. Stroke is the third leading cause of death in the western world and the leading cause of disability in the United States [9]. Ischemic strokes comprise 80% of all stroke diagnoses, which indicates there is a complete or partial occlusion of a blood vessel in the brain [11]. This blockage results in a cutting off of oxygen, called hypoxia, which leads to an entire cascade of biochemical interactions. The lack of oxygen and glucose flowing to the neurons causes a vast decrease in ATP production as well as a breakdown of uptake carriers and gated membrane channels. This allows high levels of the amino acid glutamate out of the cell and toxic levels of calcium and other ions into the cell, causing necrosis in the infarct core. Mitochondrial failure results in release of damaging oxygen free radicals. Prolonged exposure to high levels of glutamate, free radicals, and excitatory amino acids threaten undergo apoptosis [11].

A common neuroscience application to observe and analyze neural tissue is microdialysis. Microdialysis is a simple way to sample the extracellular fluid for a specific area of the brain over a period of time to gain an understanding neurochemical dynamics. The procedure consists of pumping a fluid, typically artificial cerebral spinal fluid, into the brain by way of a concentric-tube probe with diameters commonly between 200 and 500 microns. The perfusion fluid is pumped in through the inner concentric tube and reaches the tip of the probe, where diffusion occurs. The membranous tip of the probe is selectively permeable with a known molecular weight cutoff, typically 20 or 100 kDa. After collecting molecules through diffusion in the tissue, the fluid is then pumped out through the outer concentric tube of the probe and into a collection vial for analysis.

For the purpose of this research, concentrations of several amino acids and neurotransmitters in post-ischemic tissue have revealed information
that contributes to greater understanding of the stroke itself. Microdialysis recordings of neurotransmitters is commonly done for glutamate[10], GABA[13], dopamine[5] and many others. Our research focused on the post-ischemic rise in glutamate, aspartate, and taurine levels in the extracellular space. These are classified as excitatory amino acids and each plays a role in the damage incurred by ischemic stroke. Increased levels of glutamate in the extracellular space can be attributed to the breakdown of neuronal activity in hypoxic conditions. The high levels of intracellular calcium due to depolarized ion pumps initiate release of the excitatory neurotransmitters glutamate and to a lesser degree aspartate and taurine into the extracellular space. These three EAAs have each been shown to have specific neurotoxic effects at high concentrations [8]. More precisely, the high EAA levels increase the stimulation of the previously blocked NMDA receptors, resulting in a further cascade of biochemical changes. Within the cell, an influx of calcium and other ions initiate many irreversible damages, such as proteolysis of the microfilaments, breakage of membrane phospholipids, free-radical formation, and ultimately cell death [1]. Glutamate is the major neurotransmitter present in the CNS and as a result has a more pronounced effect when released. The net increase in each of these amino acids and the specific neurotoxic consequences unfold in the minutes and hours following a stroke.

The component which distinguishes this study from past studies [2, 7] is its inclusion of the microdialysis procedure into a study with real-time electrophysiological recordings. The implementation was able to be performed by means of a multi-modal cranial platform tool developed through rapid prototyping technology in a protocol developed by Tek et al (2007) [12]. The rapid prototyping approach allowed for computed aided design (figure 1A) and fabrication of a cranial platform (figure 1B) for use in animal experiments involving photothrombosis and a linear array of probes for recording data. The multi-modal device is pictured in Figure 1. The design used in our study contained precisely fabricated locations for the insertion of the fiber-optic light, microdialysis probe, and one or more recording electrodes. The microdialysis probe and recording electrode in our study were lowered 3mm into the cortical tissue to record from extracellular space while the fiber optic light was lowered only lowered to the surface of the brain.

Figure 1. (A) Multi-Modal Cranial platform CAD Rendering (B) Implanted Cranial Platform from our study, with anterior and posterior denoted by inset (A—P)

2. Methods

2.1 Animal Procedure

All surgeries in the study were carried out in male Sprague-Dawley rats weighing 350-400 g (n=2). Procedures were conducted in compliance with the AAALAC accredited Animal Care Committee of the University of Illinois at Chicago. Anesthesia was induced using a gaseous 4% halothane mixture for 10 minutes followed by a KXA (0.1 ml/100g body weight mixture of Ketamine, 100 mg kg⁻¹, Xylazine, 5 mg kg⁻¹, and Acepromazine, 2.5 mg kg⁻¹) intramuscular injection. Additional 0.1 ml KXA doses were applied throughout the surgery as needed to maintain a steady anesthetized state. Pulse rates, oxygen saturation levels, and paw-pinch reflexes were used to assess anesthetic stability throughout the surgery. To obtain access to the cranial surface, a 2 cm incision along the midline cranial suture was made and subcutaneous tissue was separated and/or incised to obtain access to the skull surface. A 5 x 5 mm craniectomy was made using a 5 mm diameter hand-operated trephine bit to expose the dura. The craniectomy site was 5.0 mm post and 5.5 mm lateral to bregma.

2.2 Microdialysis

Following removal of dura, the multi-modal cranial platform was aligned in the orientation
shown in Figure 1B. Using the platform as a guide, two additional holes were drilled anterior and posterior to the craniectomy site for anchoring bone screws to be located to stabilize the cranial platform during the remainder of procedure. The guide cannula, (PE100 tubing (O.D. 1.52 mm, I.D. 0.86 mm, Intramedic, Sparks, MD)) was then lowered to the cortical surface through the slot in the cranial platform. A dummy probe (O.D. 0.65 mm) was lowered into the guide and 3 mm into the tissue. A 25 micron diameter tungsten recording electrode was also lowered 3 mm into the tissue through the cranial platform, 2 mm anterior to microdialysis probe, for design feasibility reasons but recording was not performed in this procedure. After 30 minutes, the dummy probe was removed and a concentric tube microdialysis probe (O.D. 0.5 mm, CMA, Stockholm, Sweden) was inserted. Perfusion with 0.9% NaCl was achieved with a syringe pump (Eicom, Kyoto, Japan) at 2 µL/min flow rate. Samples were collected for 10 minute intervals, then immediately placed into -20 °C storage until High Performance Liquid Chromatography (HPLC) analysis could be performed on the samples. Samples were collected for one hour prior to induction of stroke, for twenty minutes during photothrombosis, and for one hour after completion of stroke induction. The rat was sacrificed at approximately 40 minutes after completion of the photothrombosis procedure. The timing of this event was chosen due to expected concentration peaks for each amino acid occurring in the first 10-20 minutes follow induction of stroke.

2.3 HPLC Analysis

The following solvents, solvents, and buffers were prepared for HPLC procedure: Solvent A: 10 mM Na2HPO4, 10 mM Na2B4O7, 0.5 mM NaN3, pH 8.2, Solvent B: Acetonitrile:Methanol:water, 45:45:10, Injection dilu tant: 1500 uL conc H3PO4 in 100 mL buffer A, OPA reagent : 10mg of O-Phthaldehyde (OPA) in 1 mL methanol and add 16uL of 3-mercaptopropionic acid. This was diluted with 95 uL of injection diluent (1500 uL conc H3PO4 in 100 mL buffer A) before injection and injected immediately after derivatization. The reversed-phase Vydac 218TP54 C18 column (Hesperia, CA, USA) using a Biocad derivatized samples were analyzed on a SPRINT HPLC system (Applied Biosystem CA, USA). A flow rate of 1 mL/min using solvent A (10mM Na2HPO4, 10 mM Na2B4O7, 0.5 mM NaN3, pH 8.2 in Milli-Q water) and solvent B (Acetonitrile:Methanol:water, 45:45:10) was used. The column was equilibrated with 2% solvent B. After sample injection, the column was eluted with a linear gradient from 2% solvent B to 57% solvent B in 15 min. The derivatives were detected using Waters 474 Scanning fluorescence detector (Waters cooperation USA) with excitation at 230 nm and emission at 450 nm. The retention times of OPA derivative of Aspartate, Glutamate and Taurine are 4.1, 6.1 and 13.1 min respectively.

3. Results

Two acute surgeries were performed with successful photothrombosis-induced ischemic stroke occurring in each animal. Microdialysis data showed a consistent pattern of post-ischemic chemical dynamics (see Figure 2). Glutamate increased from baseline values of 1.48 ± 0.22 to a post-ischemic peak concentration of 6.40 pmol/µL, occurring 10 minutes after the completion of the cortical infarction. Aspartate showed a similar pattern, with a baseline concentration of 0.600 ± 0.43 pmol/µL, reaching a post-ischemic peak of 0.8 pmol/µL within 20 minutes of the completion of photothrombosis. Taurine concentrations were generally much higher, with baseline concentrations of 30.34 ± 17.29 pmol/µL followed by a post-ischemic peak concentration of 102.6 pmol/µL, measured 20 minutes post-photothrombosis. Similar trends were seen in studies by Feustel et al. (2004) and Zeng et al (2007) [6,15].

4. Discussion

The data findings were valuable verifications of the well-understood stroke cascade. Collected data must be able to relate the physiological event with the neurochemical result. In an ideal surgery, the only
physiological damage to the tissue would be in the infarct core and penumbra, occurring purely as a result of the photobleb thrombosis stroke technique. In practical application, especially when designing a multi-modal device, each probe or biosensor introduced into the neural tissue incurs at least moderate physical damage to the tissue and time must be allotted for the tissue to return to its equilibrium state. In this study, a dummy microdialysis probe was inserted for 30 minutes prior to insertion of actual probe, yet it remains uncertain as to whether the tissue has fully reached an equilibrium state prior to initiation of sample collection.

The context of the data obtained through microdialysis must also be considered. The amount of molecules diffused across the membrane, even at this low flow rate, is typically only around 10% of the actual concentration in the tissue, but a study by Clough et al. quantified the loss due to diffusion in Formula (1).

\[
C_{\text{tissue}} = C_{\text{outlet}} / (1 - e^{-PS/F}) \quad (1)
\]

where PS is the permeability surface area product and F is the perfusion rate [4]. While reported data acknowledges this observation, it still serves as simply a sample of the actual concentration, one of the main disadvantages of the procedure. With respect to the stroke cascade, the relative concentrations (post-ischemic vs. baseline) remain more significant than the absolute concentrations.

The other main disadvantage of microdialysis is the low temporal resolution. Slow flow rates yield small volumes of sample unless run for periods of several minutes. Real-time data of the concentrations of chemicals can not be obtained as in electrophysiological recording. The upside, however, is the ability to follow overall trends in neurochemistry and their longer-term dynamics. While data from recording electrodes and other sensors of electrical activity provides a quantification and visualization of neuronal firing patterns, the role microdialysis must not be overlooked. Microdialysis advances the understanding by looking at a lower level, the amino acids in hypoxic tissue, to provide a more precise answer for why and when the steps in the stroke cascade occur.

Further work could pursue introduction an EAA inhibitor such as dihydrokinate (DHK) [14] or a competitive NMDA inhibitor such as EAA-090 [3]. Regardless of the mechanism, reduction of

Figure 2. (A-C) Concentrations of Glutamate, Aspartate, and Taurine in the following time scale: 0-30 minutes, prior to stroke. 30-50 minutes, during induction of stroke using photobleb thrombosis. 50-90 minutes, post-stroke (alive). 90-120 minutes post-stroke (sacrificed). Shown data was similar in each of two studies. Exact concentrations provided in Results.
the neurotoxic effects of EAA in cells is certainly one means to approach the solution. As seen in the data from this study, the peak concentrations for these EAA occur within the first 10-20 minutes of stroke induction in the rat model. This indicates a short window for meaningful intervention, which must be readily understood through future experimentation.

5. Acknowledgements

We would like to thank Dr. Bob Lee and Dr. Lasanthi Jayathilaka of the Protein Research Laboratory for their assistance as well as the technical assistance of the UIC Department of Bioengineering. The project was funded by the NSF (grant no. BES-0348145).

6. References


Neural Engineering is an increasingly popular concentration in Bioengineering. Producing some of the most innovative and exhilarating possibilities in contemporary medical science, this interdisciplinary field is progressing towards a comprehensive understanding of the human brain. The ability to gather information of neurological processes via neuroimaging is the foundation of this progress. This review calls for the awareness of the ethical questions raised by the ability to map, measure, monitor, intervene in and alter the brain. Developing new processes to manipulate neural tissue and efficacious interfaces for improved neuronal function extend beyond Bioengineering into fields as seemingly disparate as law, social science, public policy and philosophy. The ethical core at the intersection of these fields is ultimately the question of benefit versus harm in terms of how the mind is affected. Following an explanation of Glannon’s brain-body-self continuum, this review suggests considering his philosophical foundation in the study of neural engineering.

Walter Glannon, Canada Research Chair in Biomedical Ethics and Ethical Theory at the University of Calgary in Alberta, has written extensively on the topic of neuroethics. In Bioethics and the Brain (2006), Glannon defines neuroethics as “a branch of bioethics concerned with ethical issues arising from different ‘measures of’ and ‘interventions in’ the brain or central nervous system”. In the book, the author provides an integrative examination of the ethical questions raised by biotechnological advances, specifically in neurology, neurosurgery, and psychiatry. In six chapters, he addresses: brain, body, self; neuroimaging; pharmacological and psychological interventions; neurosurgery, psychosurgery, and neurostimulation; and brain death.

Some of the ethical questions addressed include the morality of using neuroimaging for diagnostic and predictive purposes; forced behavior control through pharmacological means; how much influence physiology and psychopharmacology have on cognitive function; to what degree are doctors or patients in control of the outcomes; do the benefits of neurosurgery, psychosurgery, and neurostimulation outweigh the risks; what is the applicability of standardized neuroimaging prior to any kind of neurological intervention; does the availability of these technologies mean that we should use them; and the debate of the withdrawal of life-support. Questions of benefit and harm ultimately rely on how the mind is affected. Benefits are consistent with satisfaction and interests, and are categorized as obligatory, prohibited, or permissible. Glannon assesses the ethical implications of the choice to use the techniques, procedures and drugs that are all generally permissible including artificial bioelectric and brain-computer interfaces. The complexities of the relationship between the brain and the mind complicate the process of making treatment decisions and are left for the doctor and the patient to determine for themselves.

Considering these questions, we can envision creating standardized neural imaging for all patients prior to the commitment to any kind of neurological procedure or treatment to serve as point of reference throughout the treatment. This standardization may prove helpful in various cases, specifically those in which doctors’ or family proxies are consenting for treatment for patients who are considered unable to make such decisions for themselves. These cases may indicate affected cognitive and conative, or volitional, abilities to consent to treatment as a result of the afflicting disorder. In this context, it is important to understand Glannon’s explanation of the integrative function of the brain, mind, and body. He points out that by affecting the brain via biotechnological mechanisms, we must be careful to attempt to fully understand the holistic affects of such treatments. Beyond the biomechanical interactions, he questions how we might compare and reconcile a case in which the neurobiological recommendations of the experts are incongruous with the intentions of the patient?

He demonstrates the interdependence of these distinct parts of the human being by providing a technical explanation of how the concrete brain interacts with the abstract mind and how the two comprise a part of the human organism. The brain is physically responsible for generating and sustaining cognitive, affective, and conative mental capacities in response to the body, itself, as well as the person’s external environment. The mind, however, “expresses itself through a chain of molecular events and processes. But…is more than just a function of molecules.” It is fair to consider the mind at the conceptual interstice of biochemistry and the philosophy of the mind which controls conscious
beliefs, desires, emotions and intentions and is to be considered a process or a set of properties.

He continues to explain how the brain and mind collaborate in an attempt to maintain homeostasis within what Glannon calls *the internal environment of the body* minimally including the central nervous system serving sensory and motor functions and the limbic system, generally accepted as the emotion control faculty. From the interplay of these minute factors the most interesting questions are raised.

Let us conclude with the consideration of and commentary on two accounts of one specific neurological disorder and its emerging ethics issues. Fictional and factual, I would claim that neither of the two temporal epilepsy patient models would deign to imagine the loss of their heightened mystical states. Their perceptions of preferred homeostasis would likely not agree with modern opinions influenced by the knowledge that untreated their lives will result in coma and brain death. One of the models is the non-fictional account of a woman, appropriately named Sister John who wished to maintain her intensified spiritual connection caused by an untreated brain tumor, a condition referred to as ‘the sacred disease’ dating back to Hippocrates. This woman’s desire to maintain having the option of this state is a function of molecules and the mind. The second example that considers whether Dostoevsky would have consented for any form of treatment for his epileptic seizures is one that shall decisively remain unanswered. Influenced by the literary phenomenon of ‘madness in Russian Literature’ I would claim that he would not: as he admitted, have used the periods immediately preceding his epileptic seizures to fuel his work.

Furthermore, consider whether he should have been required to consent to treatment; what if he had declined treatment? What moral code would substantiate his being forced to do so and under what auspices would have parties besides Dostoevsky consenting to treatment or improved neurological function have altered, if not erased, his works. How then would Dostoevsky define the benefits and risks of his situation in the modern context?

These neuroethical examples, considered in the context of contemporary society, clearly demonstrate the causality of affected cognitive and conative function due to a neurological disorder. In a final attempt to demonstrate the many layers of Glannon’s brain-body-self continuum in the context of the ethical questions related to treating temporal lobe epilepsy: Glannon approaches the mind as a series of molecular and theoretical processes that control the choice to accept or refuse treatment. The choice the mind produces is expressed via neurobiological cognitive thought. It is at this abstract intersection of the brain and the mind that some argue consciousness is produced in terms of the capacity for self-awareness. Second order psychopathologies created by impaired neurological function fundamentally differ from limited cognition due to psychiatric conditions like schizophrenia, depression, or bipolar disorder. Glannon describes mental illness as “disorders of the mind arising from dysfunction in the brain.” Dostoevsky and his fictional counterpart’s disorder, perhaps, might be called dysfunction of the mind due to disorders in the brain.

I recommend Glannon’s series of books that are progressively moving from meta-ethics to micro-ethics. Some of the issues this author continues to confront and redefine have piqued my interest in defining my study of bioengineering and interest in neural engineering in terms moral accountability. In recent decades this author has established a varied body of work with ethics at the epicenter. This book’s journey into neuroethics follows his inquiry into *Biomedical Ethics* and preceded his recent work published in the Spring of 2007 *Defining Right and Wrong in Brain Science*. I highly recommend getting hold of a copy.

-Lissette Hall
Behind The Scenes

Front Cover:

**UBSJ Logo and cover design: Gregory Kowalsky**

The design aesthetic in the cover eliminates the use of margins to show unconstrained thoughts; it represents a state where the mind is free to innovate. The flame in the logo represents the soul, spirit and fire that drives the bioengineer in the quest for perfection, the action potential apart from the literal significance represents that sudden stroke of inspiration; the singular moment of inspired clarity and the exhilarated rush obtained when the bioengineer suddenly sees past the data and identifies a problem and maybe even a solution and suddenly life begins to make sense again. The placement of the text in relation to the picture and the bold use of black and white represent a revision of the traditional designs of a journal.

**Picture by David Jiménez-Morales**

**Description:** The type of protein structure shown in the cover is almost-exclusively found in the outer membrane of gram-negative bacteria. Some of these bacteria have an enormous clinical impact, which points at these beta-barrel membrane proteins as good targets for the design of antimicrobial drugs. But given the scarcity of structural data available, the researchers have developed a method that use the evolutionary relationship between these proteins in order to more accurately detect them in the available protein-databases. This is represented as a few beta-barrel proteins surrounding their evolutionary history (a phylogenetic tree) looking for information about themselves.

Back Cover:

**New Laputa (detail) © 1999 Gregory Kowalsky**

The artwork is loosely based on discussions on nanochips. It was derived from a 3D analytical visualization, first used in a Web colloquium in 1998 (or so). Reproduced here with permission.

The idea of the picture was about a flying "ivory tower", as was the original island of Laputa, but without the oppression. In the "Voyage to Laputa" Swift wrote about Mars satellites, that were not known then. Also, he wrote a pretty funny piece there, describing a logic machine, that sound today like a very evil parody on a Semantic Web concept.
Call for Papers - Spring 2009

The deadline for submission is January 31, 2009
Details available on the BioeJournal Blackboard site

SUBMISSION PROCESS

We have setup a Blackboard site to streamline submissions. All submissions are to be made through this Blackboard site. Please adhere to the submission guidelines listed below:

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1. Only one author per paper/article.
2. The student must be a current UIC Bioengineering undergraduate/graduate student.
3. Only research and review articles may be submitted.
4. Permission must be taken from your advisor wherever applicable.
5. Research papers must not be more than 4-5 pages long and may be submitted in the prescribed format. (see under Article Template in the BioeJournal Blackboard site)
6. Review articles must be limited to a maximum of 4-5 pages in content and should be formatted as per guidelines in the article template.
7. The criteria of acceptance shall be based on volume of papers received, relevance, subject mastery, organization, appropriate documentation etc.
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9. All submissions will be peer reviewed and the author will be informed in advance if their article is selected for publication. Short listed papers will be further reviewed by members of the faculty.
10. Submissions to be made as MS Word files. Name files in the following format

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Questions?
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