Some Problems in Computational Mechanobiology

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Mechanobiology?

• Consequences of mechanical forces on tissues
  – Mechanical forces injure tissues
  – Mechanical forces may act within tissues at the cellular level to regulate biological processes
    • Bone remodelling (Wolff’s law)
    • Tissue differentiation (More general problem)

  » Experimental mechanobiology
  » Computational mechanobiology
Cells

• Over 300 distinct cell types in the body.

  – Cells have been created by **differentiation** from a parent cell, called a stem cell.

• In the adult body there is a sub-population called **“mesenchymal stem cells”**
Mesengenesis

Mesenchymal Stem Cell (MSC)

Proliferation

Commitment

Lineage Progression

Differentiation and Maturation

Osteoblast
Chondrocyte
Myoblast Fusion
Stromal Fibroblast
Tenoblast
Preadipocyte

Osteocyte
Chondrocyte
Myocyte
Stromal Cells
Tenocyte
Adipocyte

Bone
Cartilage
Cardiac
Stroma
Tendon
Adipose
Cells have an amount of ‘independent life’
Stem cells

- Commitment
- Differentiation
- Mechano-regulated processes

Try to discover mechano-regulation rules for tissue differentiation
Cartoon description of the process of formation of a organ consisting of different tissue types.
1) Consider an arbitrary domain loaded over part of the surface

2) Stem cells resident outside the domain

3) Stem cells disperse into the domain
4) Stem cells divide (mitosis) and proliferation occurs

5) … and stem cells simultaneously migrate within the domain
6) Stem cells commitment

7) Stem cell differentiation
8) Differentiated cells express new tissues

- Osteoblasts → Bone
- Chondrocytes → Cartilage
- Fibroblasts → Fibrous connective tissue

Cartilage → Fibrous Connective Tissue → Bone
Computational mechanobiology

(i) Boundary value problem to determine local mechanical stimuli within the domain

(ii) Relate local mechanical stimuli to cell expression (tissue formation)

Historical review
Pauwel’s hypothesis of tissue differentiation (1940)

Hydrostatic pressure

Pauwels, 1957
Perren’s *interfragmentary strain theory* (1979)

“a tissue which ruptures or fails at a certain strain level cannot be formed in a region of precursor tissue which is experiencing strains greater than this level”

Interfragmentary strain (%)

- Bone
- Cartilage
- Granulation tissue

Carter and Beaupre. Tensile strain / Hydrostatic stress (2001)

Prendergast et al. (1997), *Mechano-regulation in a fluid/solid mixture*
• Conclusion: several mechano-regulation theories proposed for prediction of tissue differentiation.

Tissue differentiation: mechano-regulation in a fluid/solid mixture

• **AIMS:**
  - Create a *testable* hypothesis
  - Test whether or not tissue differentiation and bone regeneration can be simulated during processes for which experimental observations are available.
1) Define a vector of relevant cells, e.g.

\[ n = \begin{align*}
& n_{\text{stem\_cell}} \\
& n_{\text{fibrous\_tissue\_cell}} \\
& n_{\text{cartilage\_cell}} \\
& n_{\text{bone\_cell}}
\end{align*} \]

2) Model cell migration, proliferation and death

\[ \frac{dn^i}{dt} = D^i \nabla^2 n^i + P^i(S)n^i - K^i(S)n^i \]

where

- \( n^i \) is the number of cells,
- \( P(S) \) and \( K(S) \) is a proliferation rate that is dependant on The mechanical stimulus
3) At any site there may be a mixture of tissue types. If \( n_t \) is the number of tissue types

\[
\sum_{j=1}^{n_t} \phi_j = 1
\]

4) The diffusion coefficient for cells of type \( i \) thorough a volume can be give as

\[
D^i = \sum_{j=1}^{n_t} D_{ij} \phi_j
\]

where

\( D_{ij} \) is the diffusion coefficient for cell \( i \) in tissue \( j \).
5) The proliferation rate may be independent of the stimulus, or more generally, an optimum stimulation for proliferation may exist so that:

\[ P^i(S) = a_i + b_i S + c_i S^2 \]
• Cyclic strain increases proliferation of osteoblasts, but not magnitude dependant - Kasper et al, 1998
• Large strains (10,000 µstrain) increased proliferation of fibroblasts compared to lower strains (3,000 µstrain) - Jones et al, 1991
• Cartilage explant studies show chondrocyte death increases with applied stress in a dose dependant manner - Loening et al, 2000

\[
\begin{pmatrix}
P_{\text{bone}} \\
P_{\text{cartilage}} \\
P_{\text{fibrous}} \\
P_{\text{stemcell}}
\end{pmatrix}
= 
\begin{pmatrix}
a_{\text{bone}} & 0 & 0 \\
a_{\text{cartilage}} & 0 & 0 \\
0 & b_{\text{fibrous}} & c_{\text{fibrous}} \\
a_{\text{stemcell}} & 0 & 0
\end{pmatrix}
\begin{pmatrix}
1 \\
S \\
S^2
\end{pmatrix}
\]
6) Stem cell differentiation regulated by mechanical stimuli:

\[
0 \leq S \leq n \quad \text{...bone\_resorption}
\]

\[
n \leq S \leq 1 \quad \text{...bone}
\]

\[
1 \leq S \leq m \quad \text{...cartilage}
\]

\[
m \leq S \quad \text{...fibrous\_connective\_tissue}
\]

7) Introduction of an ad hoc hypothesis that the mechanical stimulus is a function of substrate strain and fluid flow:

\[
S = \frac{\gamma}{a} + \frac{v}{b}
\]

Experimental evidence from cell culture experiments
8) Once stem cell differentiation has been provoked the stimulus needs to be related to the rate of tissue formation in the form of an evolution equation:

$$\frac{d\rho_i}{dt} = f(\Delta S, n_i)$$

9) Evolution equations have only been worked out for bone

$$\frac{d\rho}{dt} = \begin{cases} 
  f_1(S) & S \leq S_{\text{min}} \\
  0 & S_{\text{min}} \leq S \leq S_{\text{max}} \\
  f_2(S) & S_{\text{max}} \leq S 
\end{cases}$$
10) Graphically for bone we have

\[ \frac{d\rho}{dt} \]
Tissue differentiation: Introduction to fracture healing.

1. Granulation tissue formation
2. Callus ossification
3. Modelling
4. Remodelling
Tissue differentiation during fracture healing

MU = Muscle
NB = New Bone
FC = FibroCartilage
CC = Cartilage
OB = Old Bone
MC = Med. Cavity
Calculation of fluid flow and strain stimuli

• Biphasic poroelastic constitutive model

In biphasic poroelasticity material, the solid stress, \( (\sigma_s) \) and fluid stresses \( (\sigma_f) \) are given by:

\[
\sigma_s = \phi^s p I + \lambda e^s I + 2 \mu \varepsilon^s,
\]

\[
\sigma_f = -\phi^f p I
\]

where \( e \) and \( \varepsilon \) denote the dilatational strain and the total strain in the solid phase,

\( p \) is the apparent pressure in the fluid,

with \( \phi \) denoting the volume fraction,

and \( \lambda \) and \( \mu \) being the Lamé constants.
Methods: Theoretical model of cellular migration

- Diffusion coefficient will depend on the tissue phenotype:
  - $D_{\text{granulation}} = 0.6$
  - $D_{\text{fibrous}} = 0.1$
  - $D_{\text{cartilage}} = 0.05$
  - $D_{\text{bone}} = 0.01$

\[
D = \frac{n_{\text{max}} - n}{n_{\text{max}}} D_{\text{granulation}} + \frac{n}{n_{\text{max}}} D_{\text{tissue mixture}}
\]

\[
E = \frac{n_{\text{max}} - n}{n_{\text{max}}} E_{\text{granulation}} + \frac{n}{n_{\text{max}}} E_{\text{tissue mixture}}
\]
Methods - Mechanical Model

- Axisymmetric Finite Element Model

Axial Loading
500 / 700 N

Time (sec)

- Biphasic Material Properties

<table>
<thead>
<tr>
<th>Granulation Tissue</th>
<th>Fibrous Tissue</th>
<th>Fibro-cartilage</th>
<th>Marrow</th>
<th>Woven Bone</th>
<th>Cortical bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young’s modulus (MPa)</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>300</td>
</tr>
<tr>
<td>Permeability (m^4/Ns)</td>
<td>1E-14</td>
<td>1E-14</td>
<td>5E-15</td>
<td>1E-14</td>
<td>3.7E-13</td>
</tr>
<tr>
<td>Poisson’s ratio</td>
<td>0.47</td>
<td>0.47</td>
<td>0.47</td>
<td>0.47</td>
<td>0.30</td>
</tr>
</tbody>
</table>
Calculation of cell spreading through the callus

• Basic equation

\[
\frac{dn}{dt} = D \nabla^2 n + ns(c) - kn.
\]

\( n = \text{number of cells} \)

\( D = \text{diffusion co-efficient} \)

\( s(c) = \text{mitosis rate per cell} \)

\( c(x,t) = \text{concentration of a metisis inducing factor} \)

\( k = \text{apoptosis / cell removal rate}. \)
- Mesenchymal cells may originate from:
  1. Surrounding tissues
  2. Inner cambial layer of periosteum
  3. Medullary cavity
Tissue differentiation: Mathematical model

- **t = 0**
  - Beginning of regenerative phase

- **t + 1 day**
  - Loading conditions
  - Rule of mixtures applied to determine tissue properties in each element
  - New tissue phenotype

**Biophysical stimuli**

![Image showing interstitial fluid flow and collagenous tissue strain](image)

- Fibrous Connective Tissue
- Fibro-cartilage
- Bone

**Graph showing interstitial fluid flow (μl/s) vs. collagenous tissue strain (%):**

- Y-axis: Interstitial Fluid Flow (μl/s)
- X-axis: Collagenous Tissue Strain (%)
Tissue differentiation: simulation of resorption

Hydrostatic pressure

Interstitial Fluid Velocity (μm/sec)

Tissue Strain (%)

Strain

Fibrous connective tissue

Fibro-cartilage

Woven bone

Resorption

2.4 \times 10^{-4}

3 \times 10^{-4}

3.75

11.25
Axisymmetric FE model – 3 mm gap

Axial loading

Iteration 1

- 6000 MPa (Bone)
- 10 MPa (Cartilage)
- 2 MPa (Fibrous tissue)
Fracture gap influence – 1 mm gap

Axial loading

Iteration 1

6000 MPa
Bone
0.2 MPa
10 MPa
Cartilage
0.2 MPa
2 MPa
Fibrous tissue
0.2 MPa
Fracture gap influence – 6 mm gap

Axial loading

Iteration 1

- 6000 MPa
- Bone
- 0.2 MPa
- 10 MPa
- Cartilage
- 0.2 MPa
- 2 MPa
- Fibrous tissue
- 0.2 MPa
**Results** – Regulation of biophysical stimuli in the regenerating tissue

![Graph showing the relationship between interstitial fluid flow and tissue shear strain for different tissue types.](image)
Does the model predict any difference in the clinically measured variable?
Tissue differentiation: discussion

1) Simulations collaborate the hypothesis that tissue differentiation is mechanically regulated - pattern of tissue formation follows histological observation

2) Moreover, this result has a biomechanical explanation.
   - In the beginning there is displacement control. However load felt by external callus is not much affected by displacement – ossification begins there
   - Only when bridging occurs does the force transfer via the external callus leaving the interfragmentary callus unloaded; it then ossifies – this causes a transition to force control
   - The load transfer path changes again to be via the internal callus and the external callus resorbs
Prendergast et al. (1997), *Mechano-regulation in a fluid/solid mixture*
Osteochondral defects

• Articular cartilage defects, caused primarily by traumatic events will, if untreated, lead to large-scale degenerative changes and osteoarthritis - Buckwater & Mankin, 1998

• Defects that penetrate the subchondral bone (osteochondral defects) are invaded by mesenchymal cells from the underlying bone marrow which form a repair tissue usually characterised as fibrous, fibrocartilage or hylaine-like cartilage - Wakitani et al, 1994

• Extensive degeneration occurs in approximately half of osteochondral defects after 6 months – Furukawa et al, 1980
Introduction

• Day 3: Mesenchymal cells present in depths of defect and invading periphery of clot.
• Day 7: Fibrous clot infiltrated by mesenchymal cells throughout defect.
• Day 14: Superficial fibrous layer seen; Deeper layer of chondroid cells forming; intramembranous woven bone forming in adjacent marrow.

* Shapiro et al., 1993
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- Week 3-8: Endrochonral bone formation in depths of defect; Chondrocyte layer well developed; Repair cartilage closely apposed to residual cartilage in some defects but separated in others.
- Long term: Superficial cartilage fibrillation, decreased staining of matrix with time.

* Shapiro et al., 1993
Objectives

• Use the theory to test the following hypothesis:

  – The local biomechanical environment is a major influence on tissue differentiation in the repair of osteochondral defects.

  – Degradation of the repair tissue is due to fibrous tissue formation, which is mechanically inferior to articular cartilage, and subsequently leads to cell death at the articular surface.

  – Tissue engineered cartilage or scaffolds will improve the repair of osteochondral defects.
Methods: Finite element model of osteochondral defect

- Femoral chondyle
- Subchondral bone
- Articular cartilage
- Meniscus
- Defect
Methods: Finite element model of osteochondral defect
Methods: Boundary conditions in the finite element model

- Partially loaded: 250N, \( P = 0 \)
- Fully loaded: 700N, \( P \neq 0 \)

Unknown pressure boundary condition
Methods: Integration of repair tissue

\[ \tau = Ae^{bn} \]

\( \tau \): shear stiffness

\( n \): cell number at interface element
Methods: The Algorithm

- Create FE model and set initial cell concentration at $t = 0$
- Run diffusion analysis $D \nabla^2 n$
- Calculate cell mitosis and apoptosis
- Run structural analysis
- Calculate max strain and fluid flow
- New cell phenotype
Methods: The Algorithm
Methods: The Algorithm

\[ t = 0 \]

Create FE model and set initial cell concentration

Run thermal analysis \( \nabla^2 n \)

Calculate cell mitosis and apoptosis

Run structural analysis

New material properties determined from rule of mixtures

Smoothing procedure applied

New cell phenotype

Calculate max strain and fluid flow

\[ t = t + 1 \]
Results: 10mm Defect

Iteration 1

- 6000 MPa
- Bone
- 0.2 MPa
- 10 MPa
- Cartilage
- 2 MPa
- Fibrous tissue
- 0.2 MPa

Bone
Subchondral bone
Cartilage
Results: 10mm Defect
Results: 10mm Defect

Graphs showing the relationship between iteration number and cell number, as well as the change in Ochedral Shear Strain (%). The graphs illustrate the strain as a function of iteration number and cell number.
Results: 14mm Defect
Results: 14mm Defect
Future work:
Future work:

• Determine the influence of tissue engineered cartilage and scaffolds on the repair process in osteochondral defects based on their mechanical properties
On the importance of mechanics in mechanobiology

- Falsifiability & testability of theories [Karl Popper].
- Progress high if theories with many potentially falsifying hypotheses have been developed, severely tested, and found to be upheld.
- Approach I (Analyse skeletal elements ‘as they are’) versus Approach II (experiment with mechanoregulation algorithms) theories
- Optimization vs. Mechanoregulation
- Mechanics should serve to make hypotheses more precise and therefore more testable.

Laws Governing Biological Construction of Skeletal Forms
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Graduate Research Students, particularly Damien Lacroix PhD and Danny Kelly MSc

Rogramme for Research in Third Level Institutions

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Tissue differentiation: mechano-regulation in a fluid/solid mixture

Fluid flow

Strain

Interstitial Fluid Velocity (μm/s)

Fibrous Connective Tissue

Fibro-cartilage

Bone