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Some Problems in Computational Mechanobiology

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Mechanobiology ?

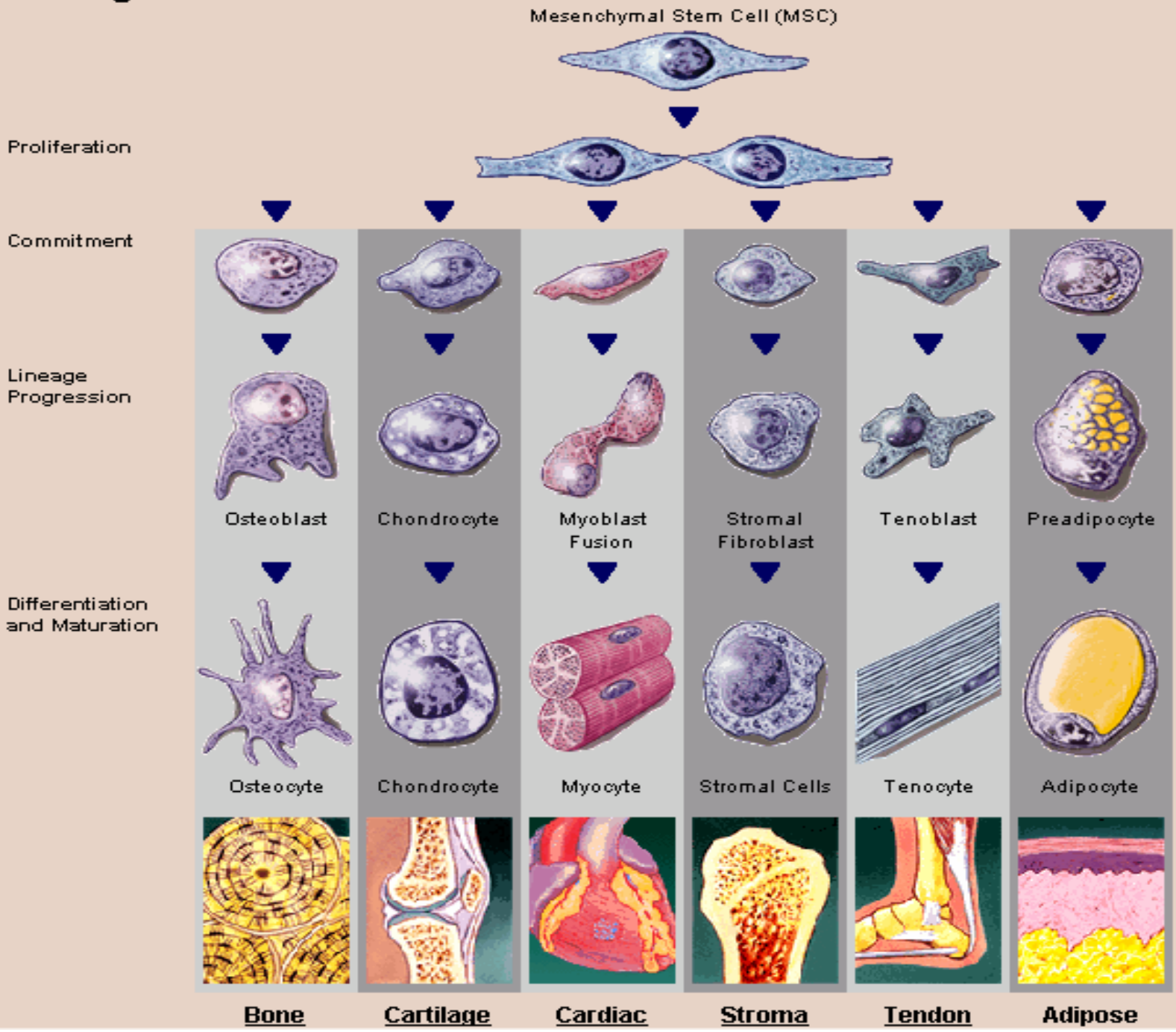
- Consequences of mechanical forces on tissues
  - Mechanical forces injure tissues
  - Mechanical forces may act within tissues at the cellular level to regulate biological processes
    - Bone remodelling (Wolff's law)
    - Tissue differentiation (More general problem)

    - van der Meulen and Huiskes (2002), *Why Mechanobiology?* J. Biomech. 35, 401-414.
      - » Experimental mechanobiology
      - » Computational mechanobiology

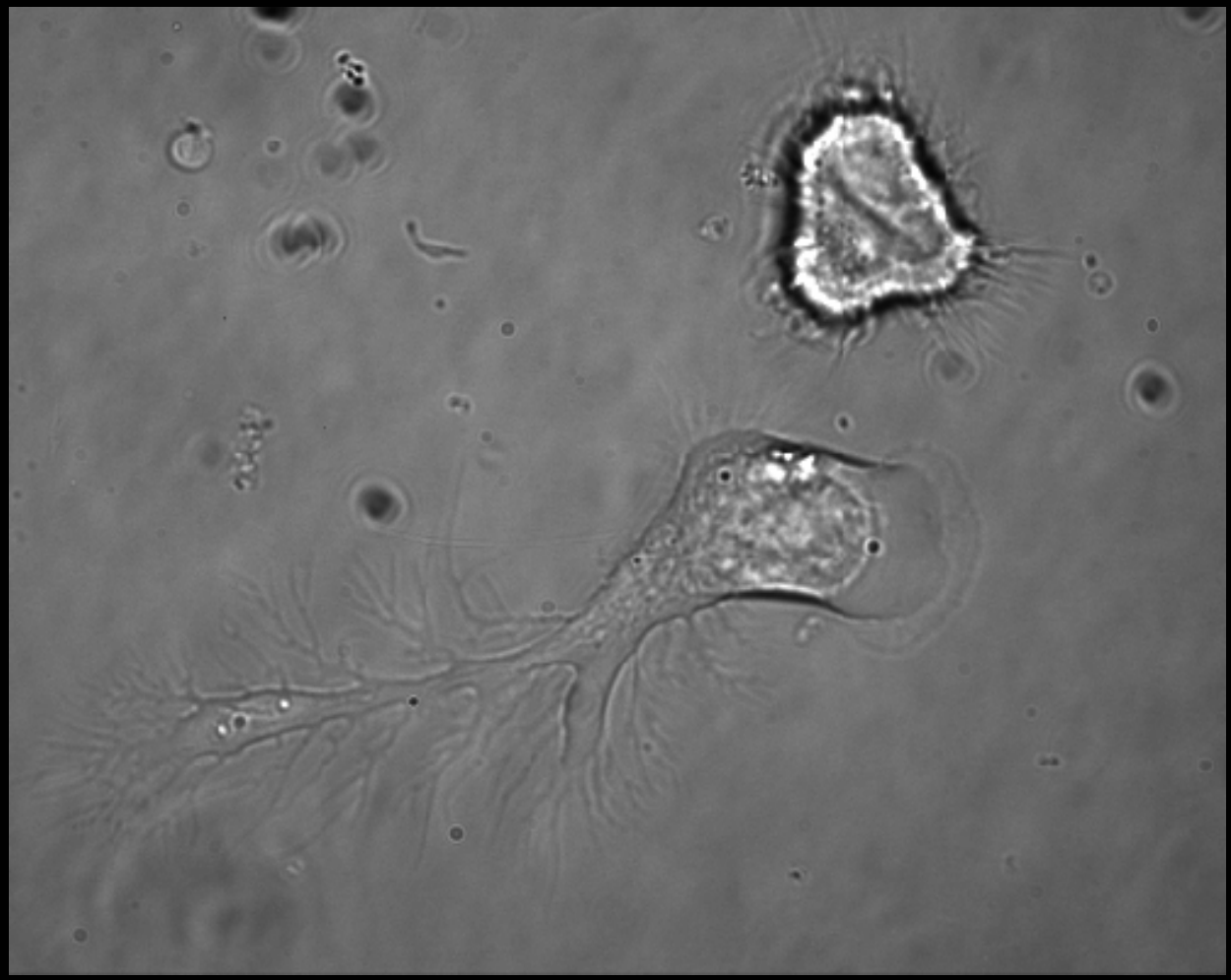
Cells

- 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



Cells have an amount of ‘independent life’



Stem cells

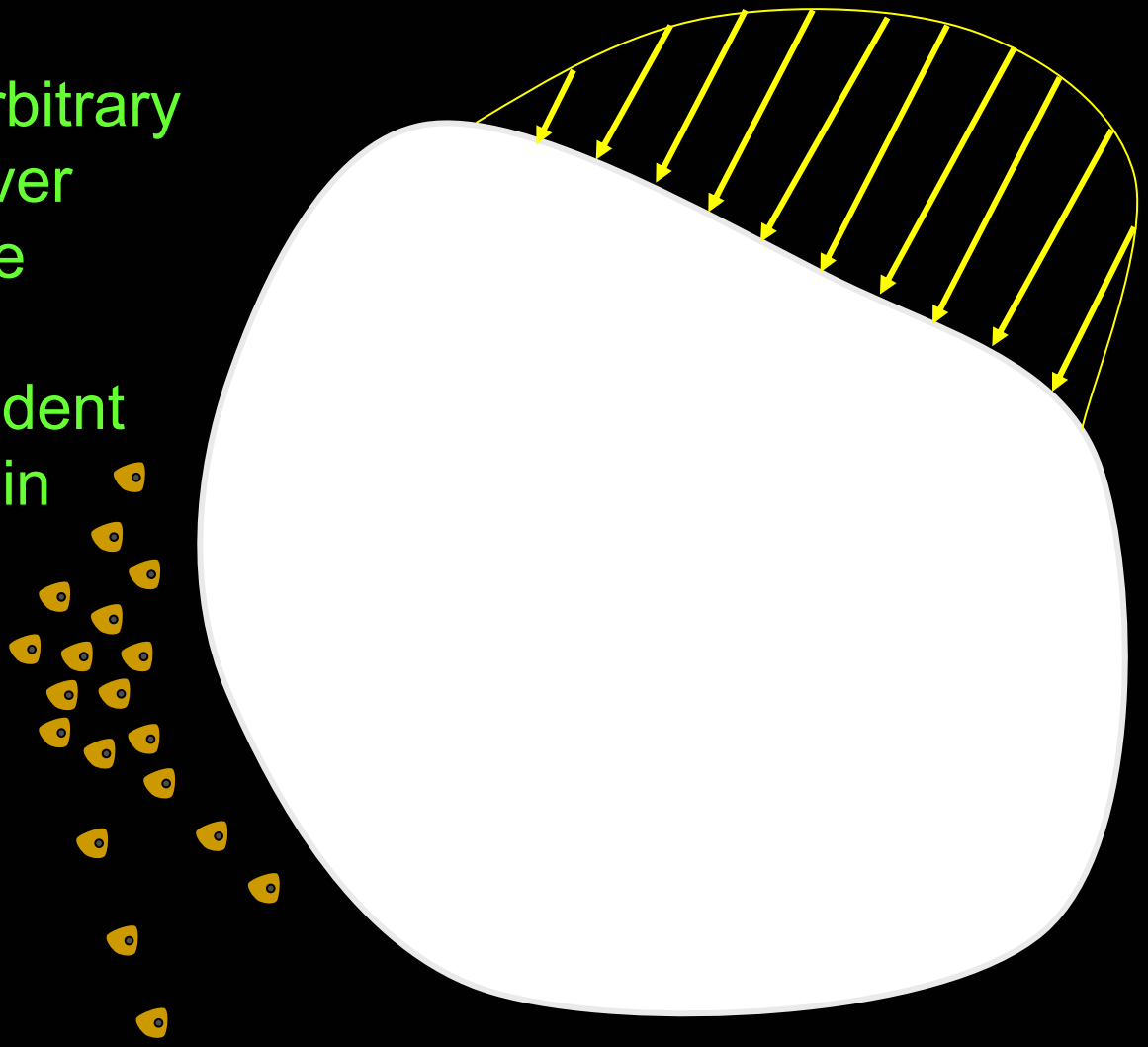
- Commitment
- Differentiation
- Mechano-regulated processes

Try to discover mechano-regulation rules for tissue differentiation

Cartoon description of the process of formation of a organ consisting of different tissue types.

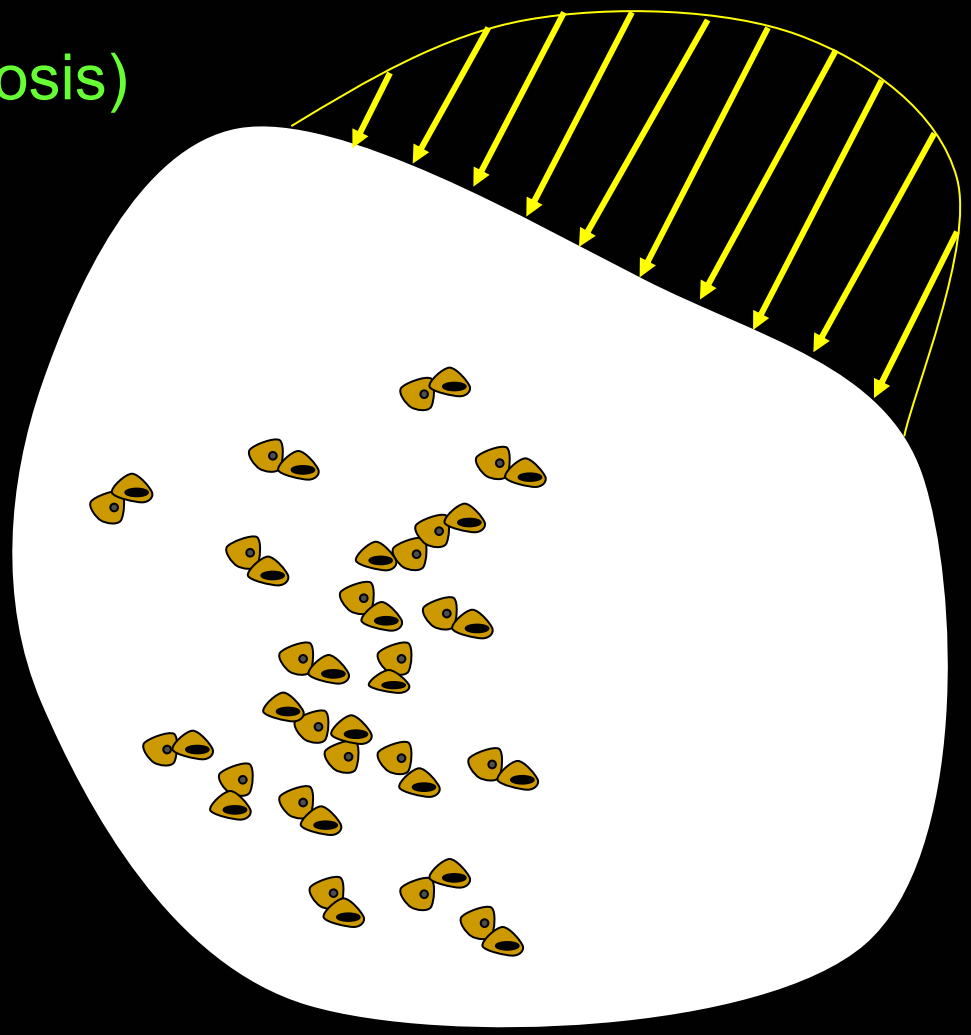
1) Consider an arbitrary domain loaded over part of the surface

2) Stem cells resident outside the domain



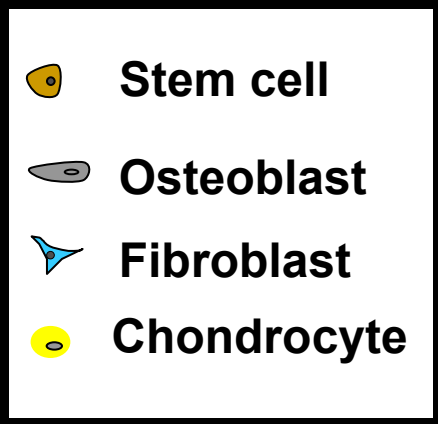
3) Stem cells disperse into the domain

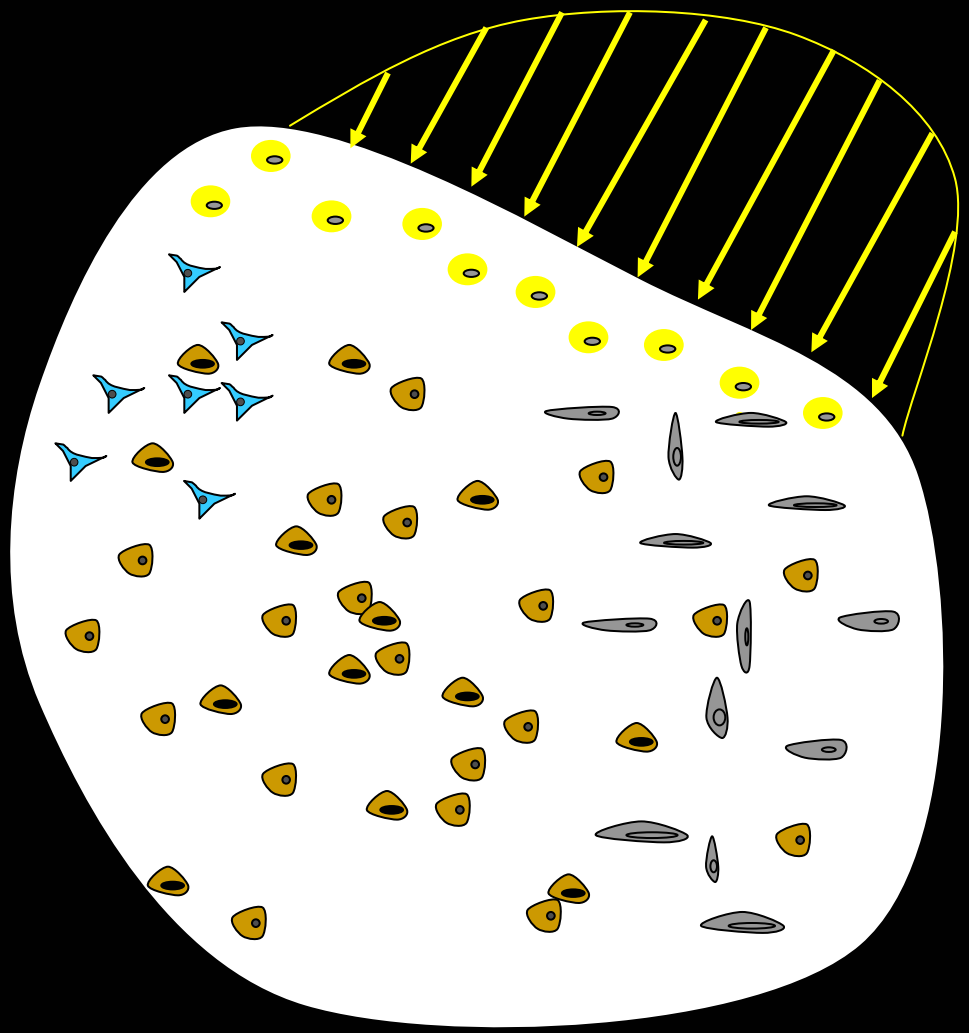
4) Stem cells divide (mitosis) and proliferation occurs



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

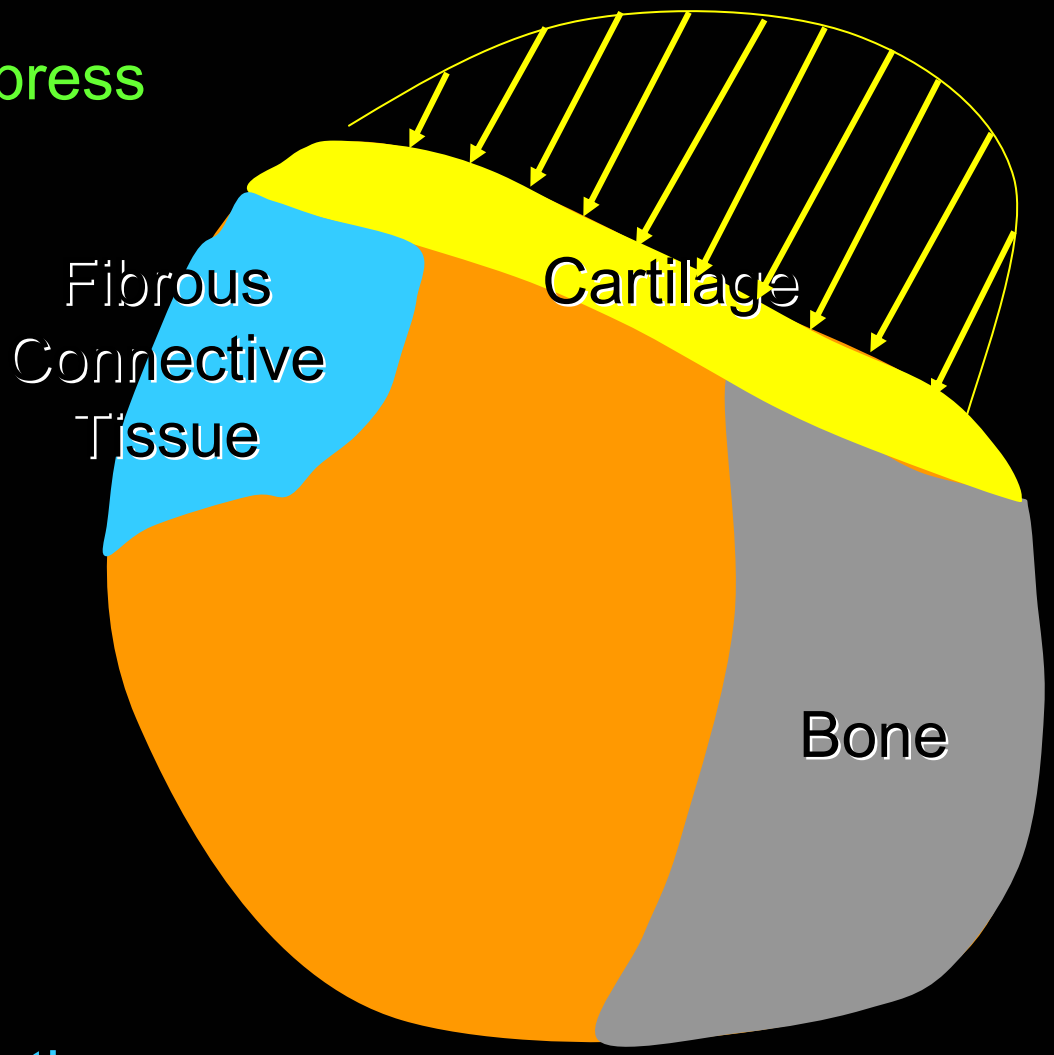
↓

cartilage

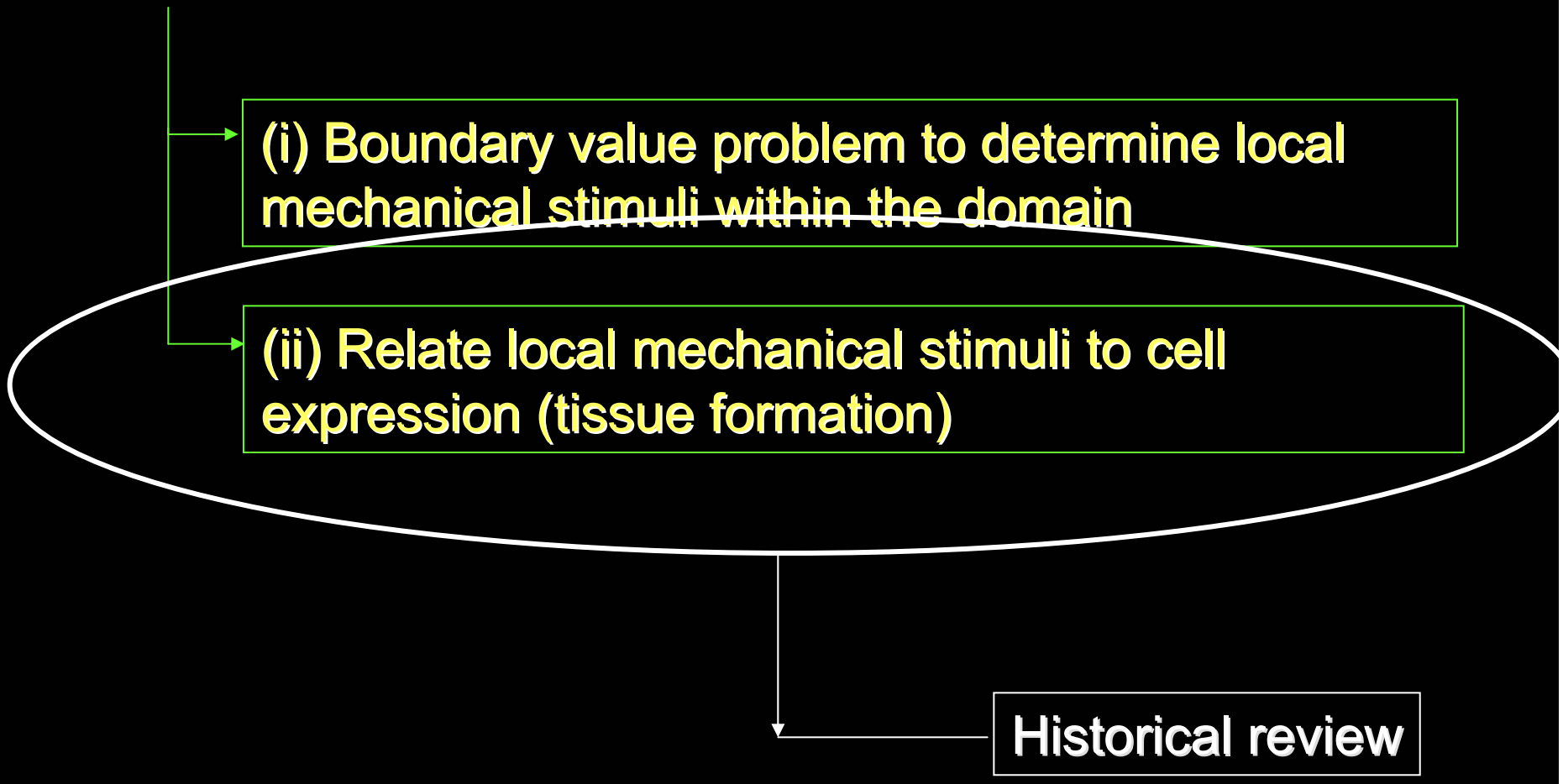
fibroblasts

↓

fibrous connective tissue



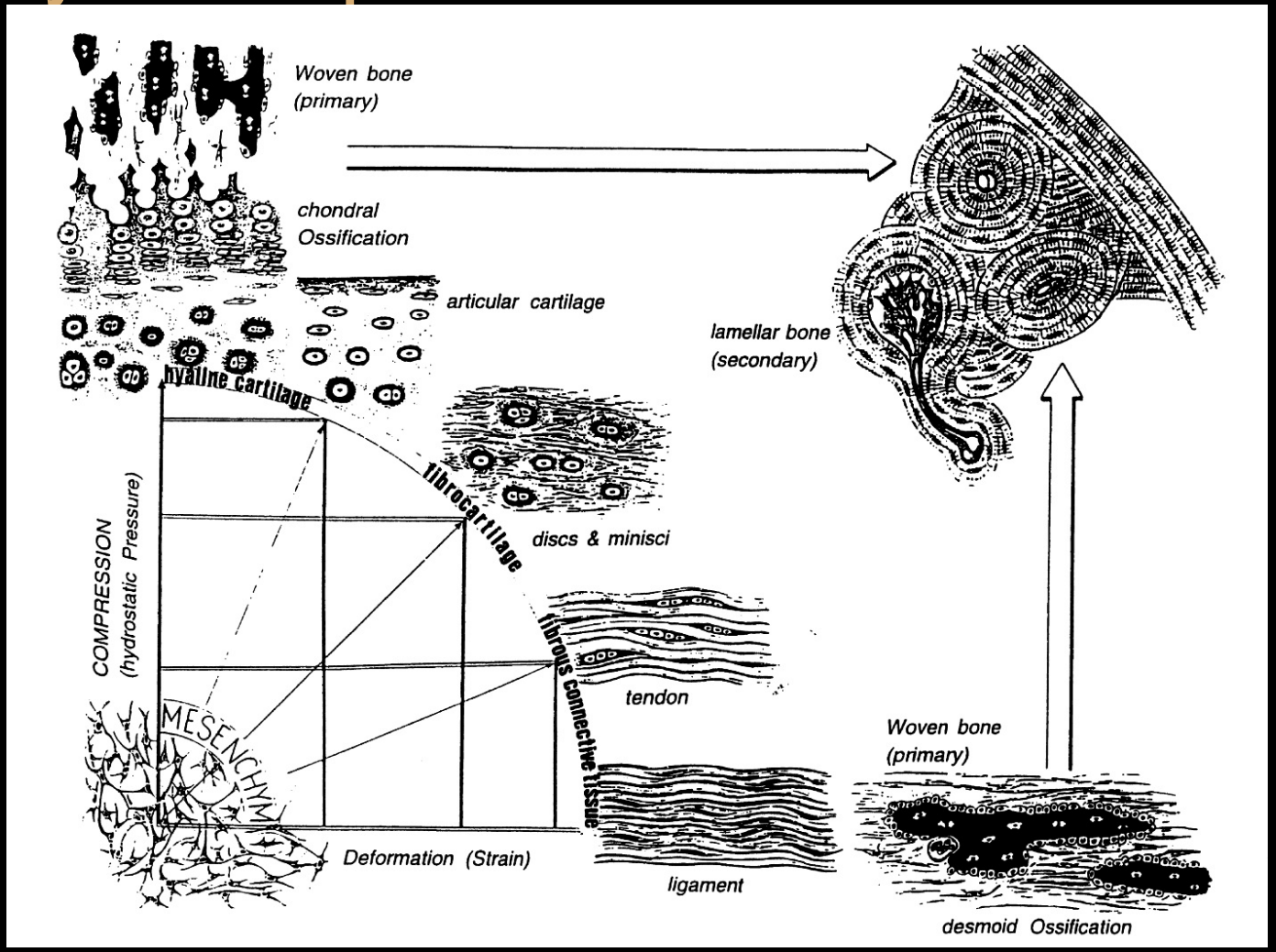
Computational mechanobiology



Pauwel's hypothesis of tissue differentiation (1940)

Pauwels, 1957

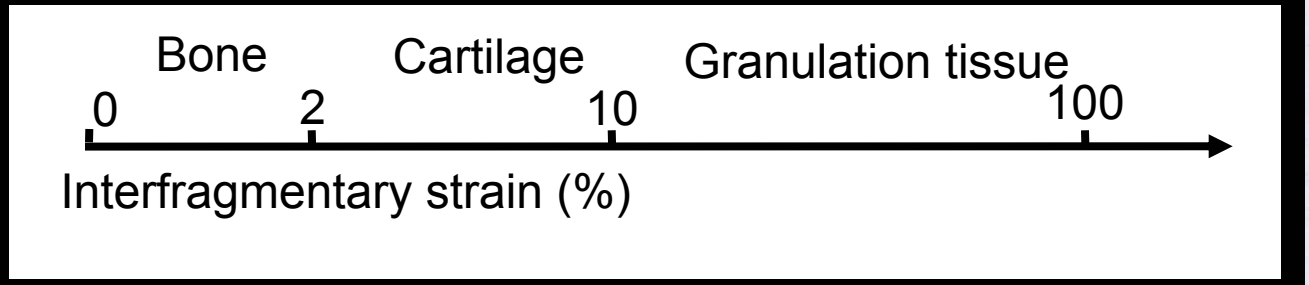
Hydrostatic pressure

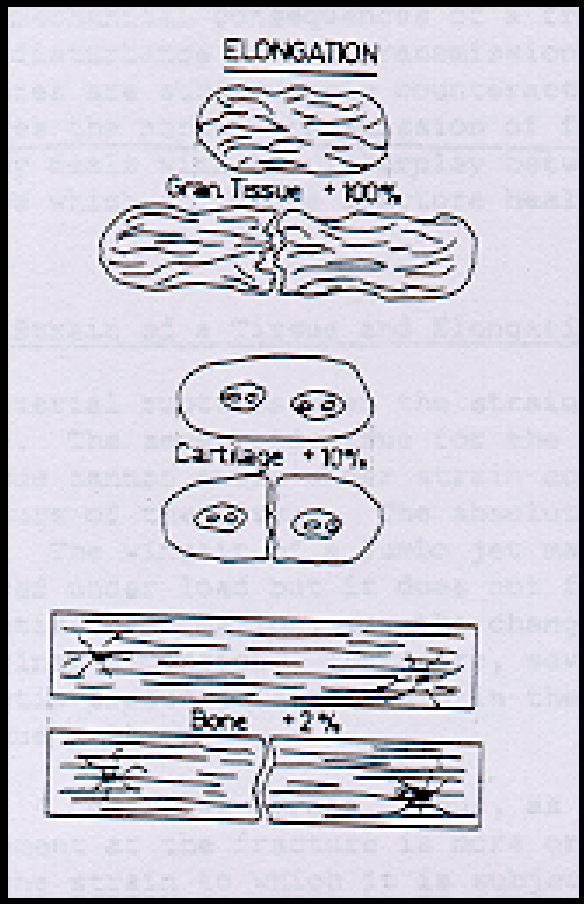


Strain

Perren's *interfragmentary strain theory* (1979)

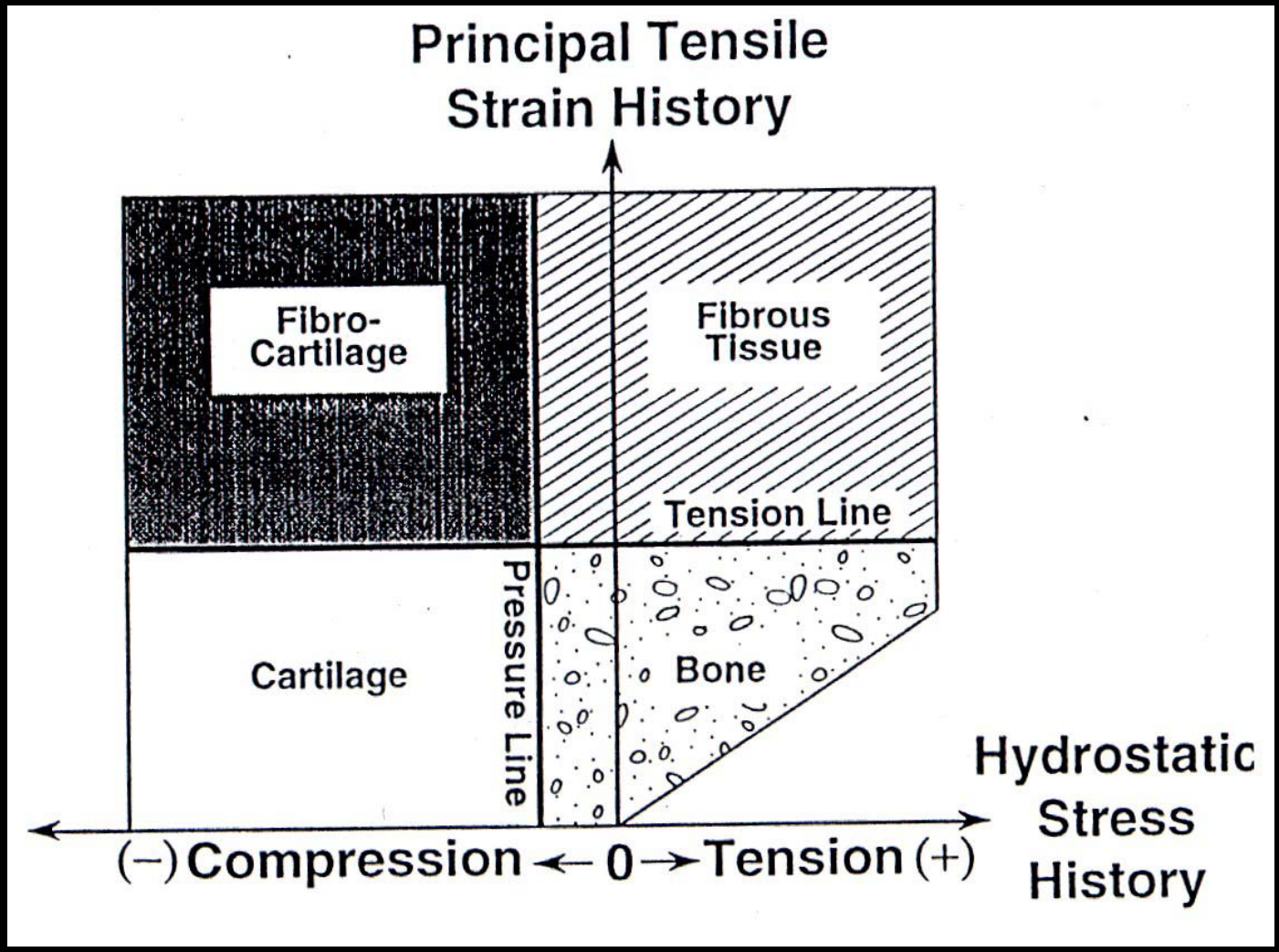
"a tissue which ruptures or fails at a certain strain level can not be formed in a region of precursor tissue which is experiencing strains greater than this level"





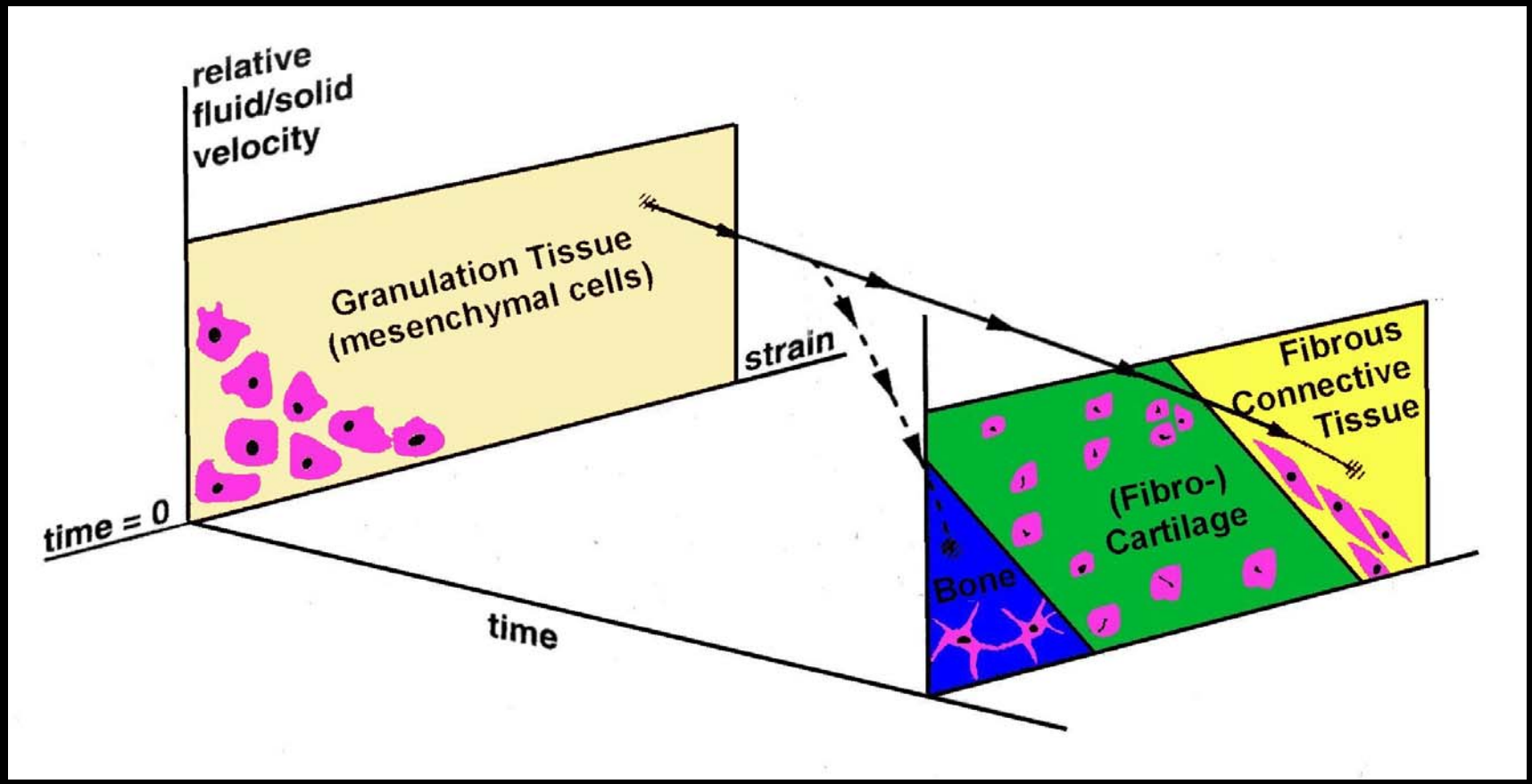
Perren S.M. ***Clin. Orthop. Rel. Res.*** **138:175-196, 1979**

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



Carter D.R., Beaupré G.S, *Skeletal Function and Form.* Cambridge University Press, 2001

Prendergast *et al.* (1997), *Mechano-regulation in a fluid/solid mixture*



Prendergast P.J., Huiskes R. and Søballe K. *J. Biomechanics* 30:539-548, 1997

- Conclusion : several mechano-regulation theories proposed for prediction of tissue differentiation.

- see *Mechanics of Bone Regeneration*, P.J. Prendergast, M.C.H. van der Meulen, In Bone Mechanics Handbook, (S.C. Cowin, Ed.), CRC Press, Boca Raton, 2001

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.**

Theoretical development - 1

1) Define a vector of relevant cells, e.g.

$$n = \begin{Bmatrix} n_{stem\_cell} \\ n_{fibrous\_tissue\_cell} \\ n_{cartilage\_cell} \\ n_{bone\_cell} \end{Bmatrix}$$

2) Model cell migration, proliferation and death

$$\frac{\mathrm{d}n^{\mathrm{i}}}{\mathrm{d}t} = D^{\mathrm{i}}\nabla^2 n^{\mathrm{i}} + P^{\mathrm{i}}(S)n^{\mathrm{i}} - K^{\mathrm{i}}(S)n^{\mathrm{i}}$$

where

n^{i} is the number of cells,

$P(S)$ and $K(S)$ is a proliferation rate that is dependant on The mechanical stimulus

Theoretical development - 2

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

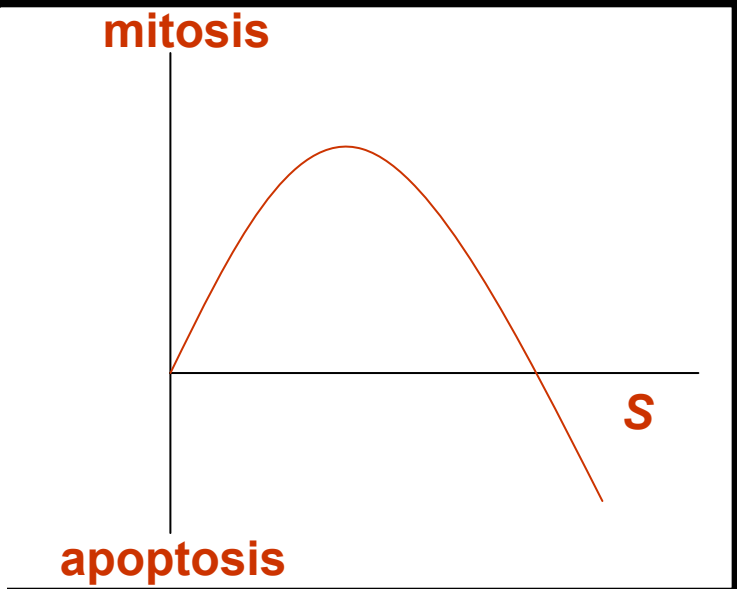
$$D^i = \sum_{j=1}^{n_t} D_{ij}\phi_j$$

where
D_{ij} is the diffusion coefficient for cell *i* in tissue *j*.

Theoretical development - 3

5) The proliferation rate may be independent of the stimulus, or more generally, an optimum stimulation for proliferation may exist so that:

$$P^{i}(S) = a_i + b_i S + c_i S^2$$



Theoretical development - 4

- 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

$$\begin{Bmatrix} P_{bone} \\ P_{cartilage} \\ P_{fibrous} \\ P_{stemcell} \end{Bmatrix} = \begin{bmatrix} a_{bone} & 0 & 0 \\ a_{cartilage} & 0 & 0 \\ 0 & b_{fibrous} & c_{fibrous} \\ a_{stemcell} & 0 & 0 \end{bmatrix} \begin{Bmatrix} 1 \\ S \\ S^2 \end{Bmatrix}$$

Theoretical development - 5

6) Stem cell differentiation regulated by mechanical stimuli

$$0 \le S \le n.....bone\_resorption$$

$$n \le S \le 1.....bone$$

$$1 \le S \le m.....cartilage$$

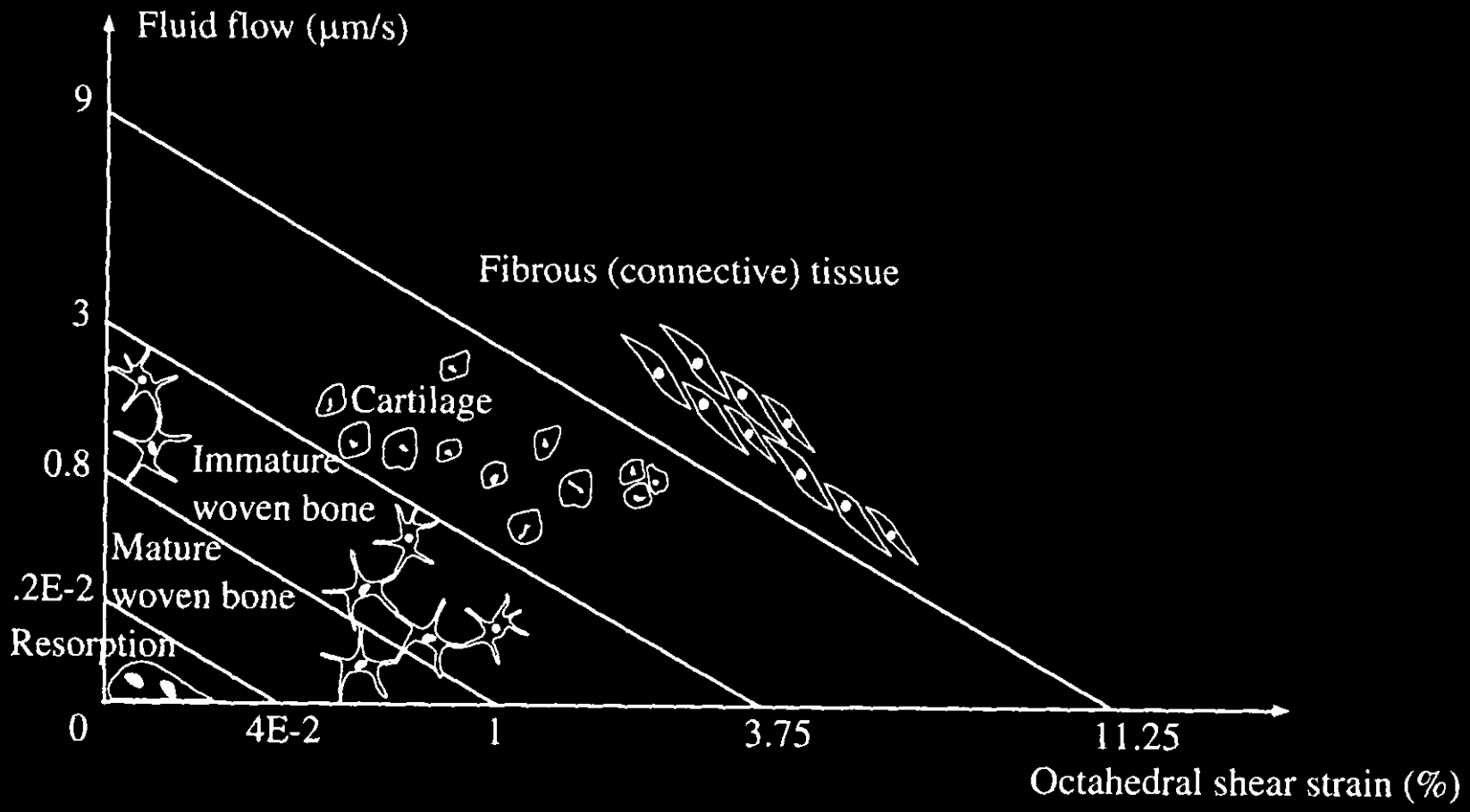
$$m \le S.....fibrous\_connective\_tissue$$

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

Theoretical development - 6



Theoretical development - 7

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:

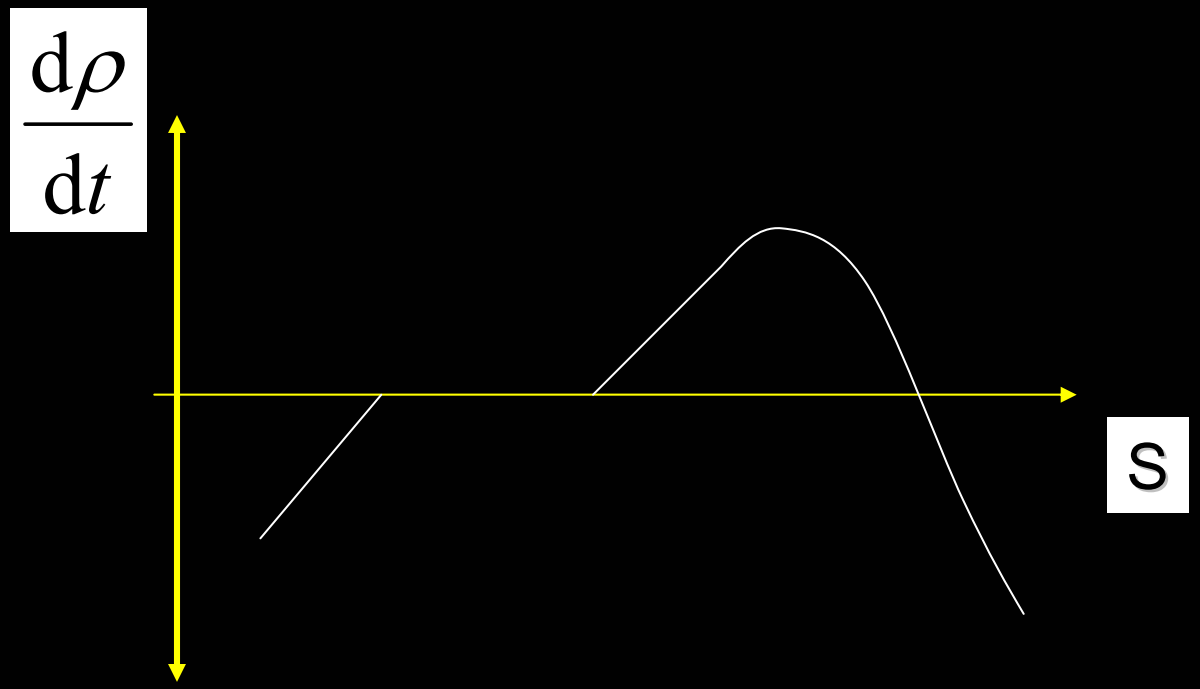
$$\frac{\mathrm{d}\rho_i}{\mathrm{d}t} = f\left(\Delta S, n_{\mathrm{i}}\right)$$

9) Evolution equations have only been worked out for bone

$$\frac{d\rho}{dt} = \begin{cases} f_1(S) & S \le S_{\min} \\ 0 & S_{\min} \le S \le S_{\max} \\ f_2(S) & S_{\max} \le S \end{cases}$$

Theoretical development - 8

10) Graphically for bone we have



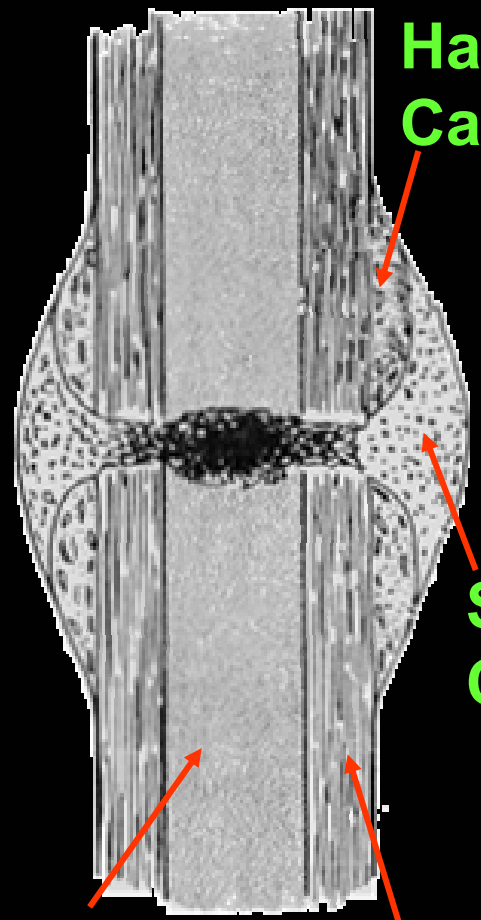
Tissue differentiation: Introduction to fracture healing.

1 Granulation tissue formation

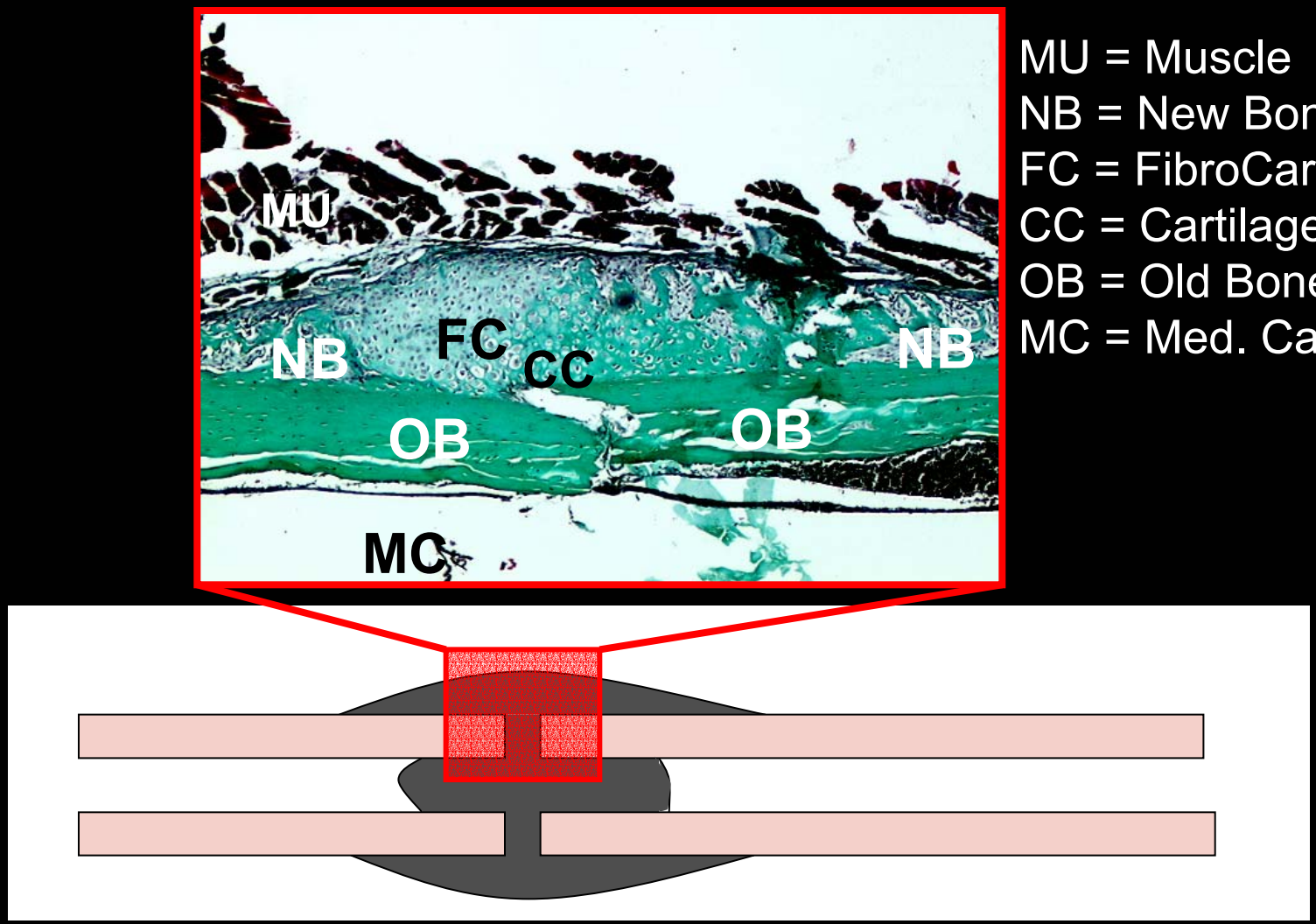
2 Callus ossification

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 poroelastity material, the solid stress, (σ_s) and fluid stresses (σ_f) are given by:

$$\sigma_s = \phi^s pI + \lambda e^s I + 2\mu\varepsilon^s ,$$

$$\sigma_f = -\phi^f pI$$

where e and ε denote the dilatational strain and the total strain in the solid phase,

p is the apparent pressure in the fluid,

with ϕ denoting the volume fraction,

and λ and μ being the Lamé constants

Methods: Theoretical model of cellular migration

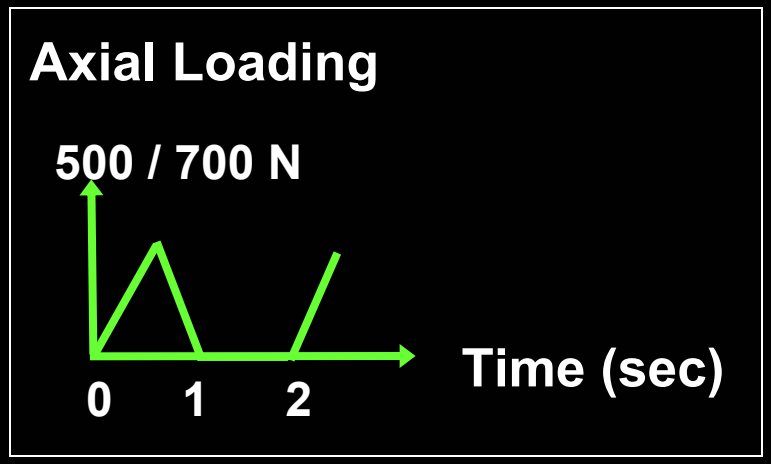
- Diffusion coefficient will depend on the tissue phenotype:
  - $D_{granulation} = 0.6$
  - $D_{fibrous} = 0.1$
  - $D_{cartilage} = 0.05$
  - $D_{bone} = 0.01$

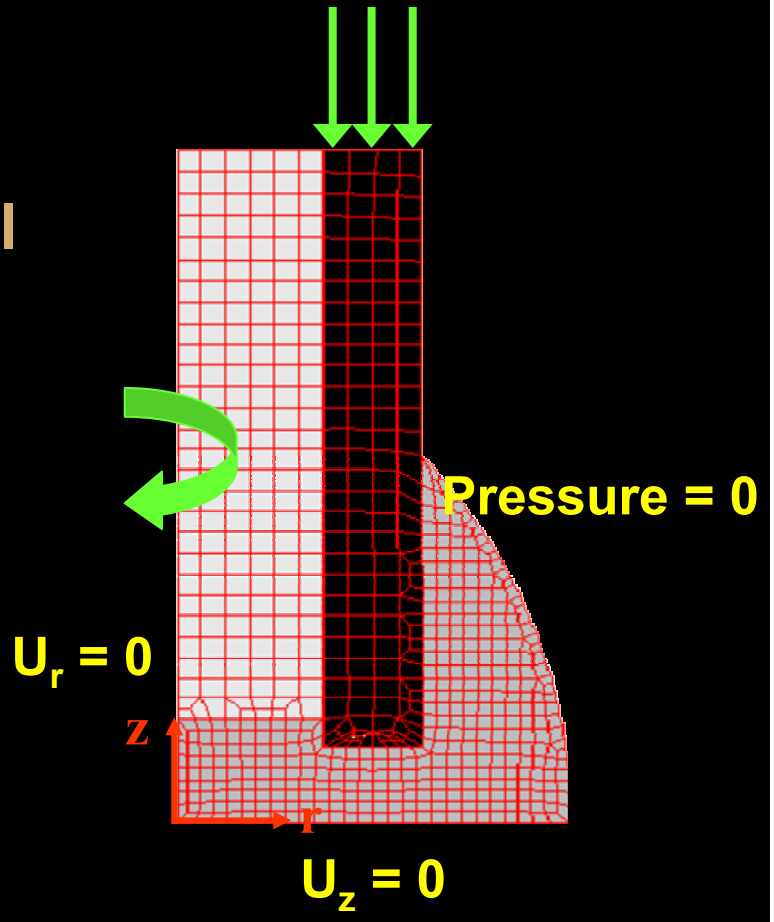
$$D = \frac{n^{\max} - n}{n^{\max}} D_{granulation} + \frac{n}{n^{\max}} D_{tissue\_mixture}$$

$$E = \frac{n^{\max} - n}{n^{\max}} E_{granulation} + \frac{n}{n^{\max}} E_{tissue\_mixture}$$

Methods - Mechanical Model

- Axisymmetric Finite Element Model





- Biphasic Material Properties

	Granulation Tissue	Fibrous Tissue	Fibro-cartilage	Marrow	Woven Bone	Cortical bone
Young's modulus (MPa)	1	2	10	2	300	4590
Permeability (m^4/Ns)	1E-14	1E-14	5E-15	1E-14	3.7E-13	3.7E-13
Poisson's ratio	0.47	0.47	0.47	0.47	0.30	0.30

Calculation of cell spreading through the callus

- **Basic equation**

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

n = number of cells

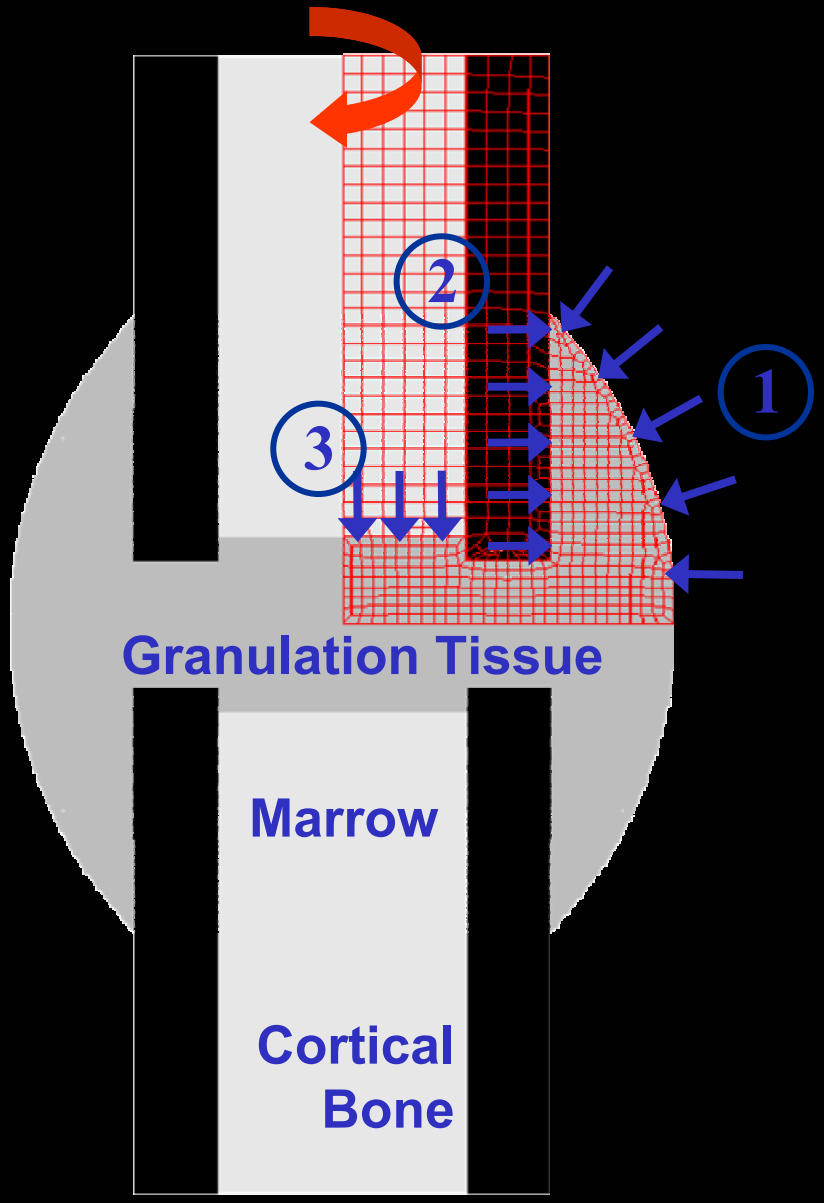
D = diffusion co-efficient

s(c) = mitosis rate per cell

*c(**x**,t)* = concentration of a metisosis inducing factor

k = apoptosis / cell removal rate.

Methods – Cell spreading



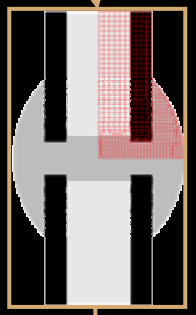
- Mesenchymal cells may originate from:
  1. Surrounding tissues
  2. Inner cambial layer of periosteum
  3. Medullary cavity

Tissue differentiation: Mathematical model

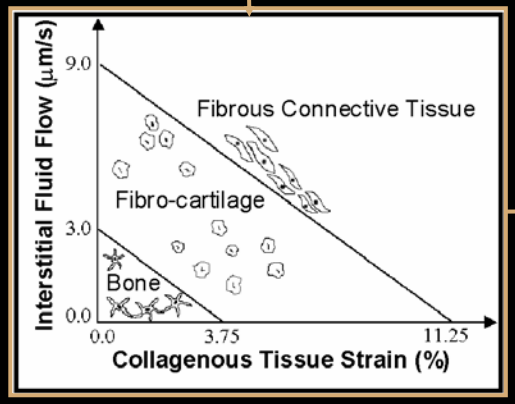
t =0

Beginning of regenerative phase

Loading conditions



Biophysical stimuli



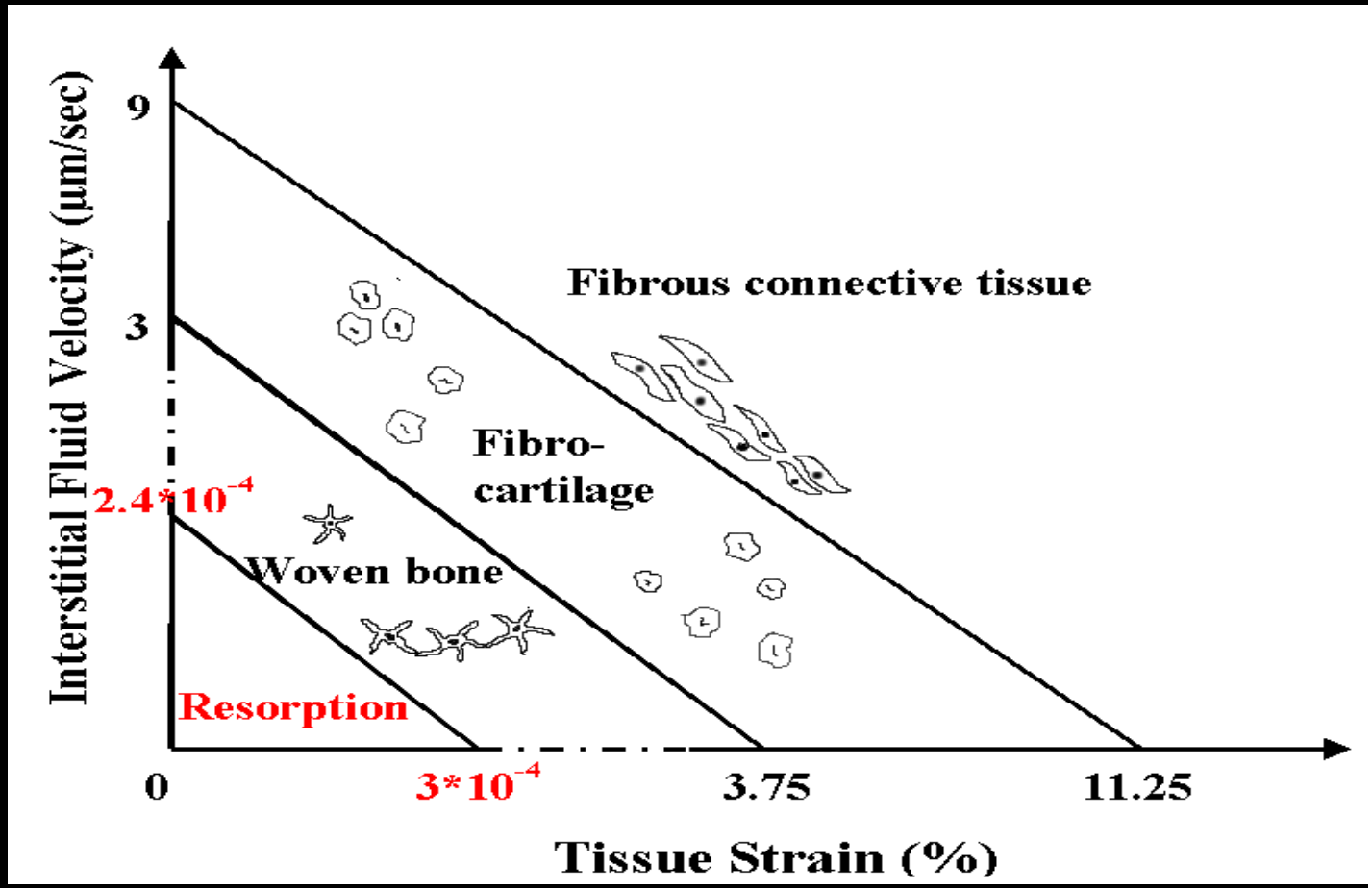
New tissue phenotype

Rule of mixtures applied to determine tissue properties in each element

t + 1 day

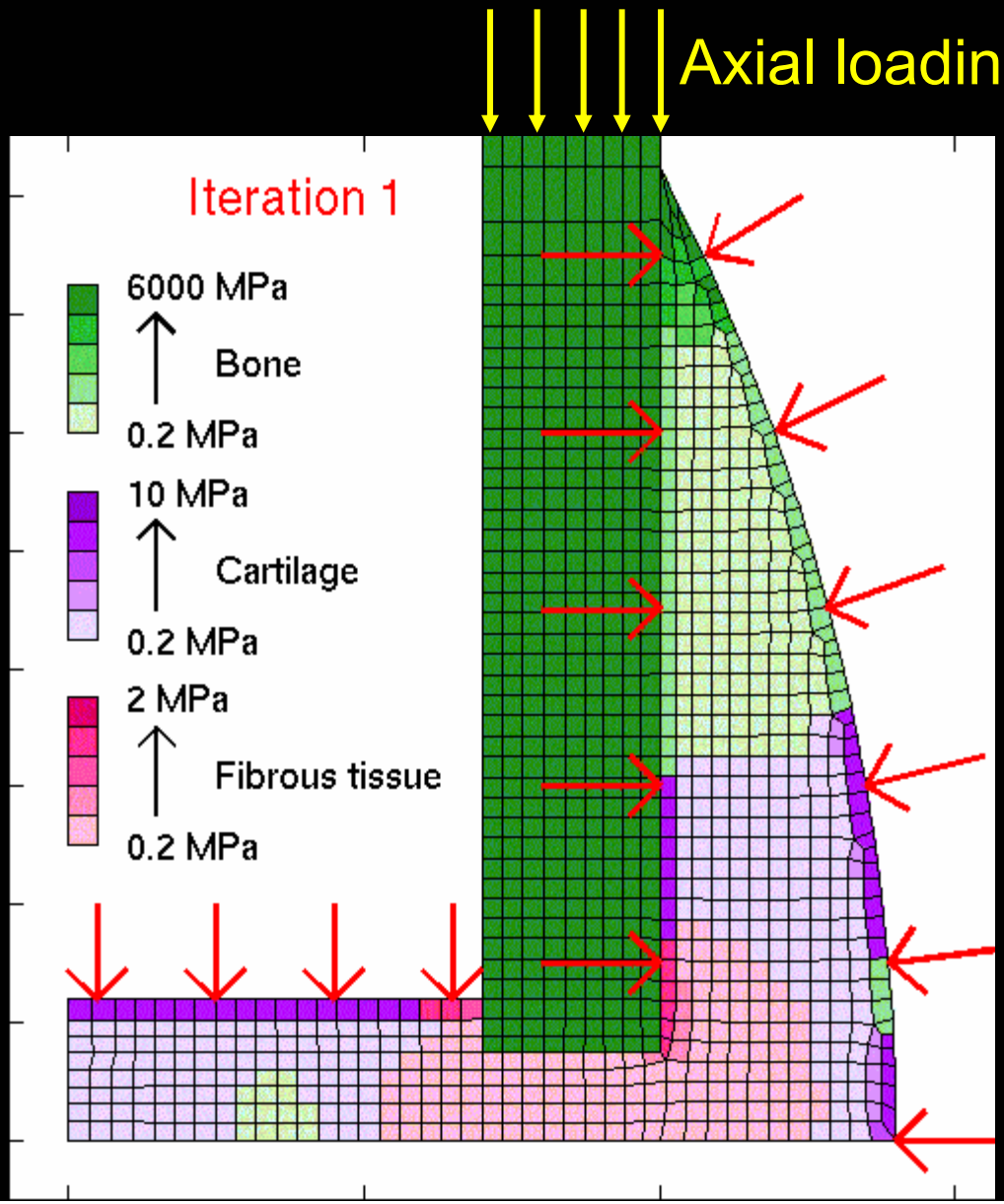
Tissue differentiation: simulation of resorption

Hydrostatic pressure

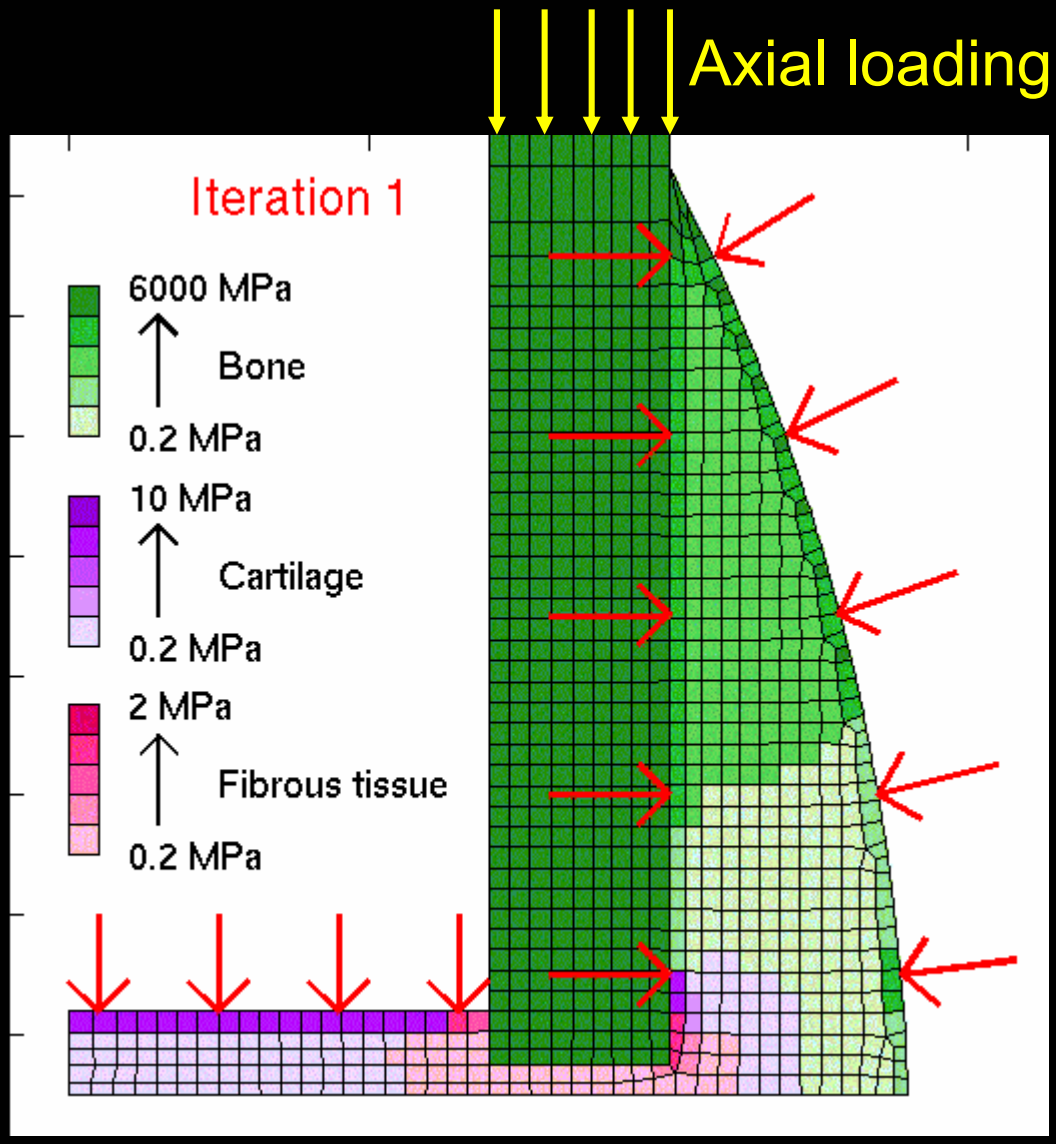


Strain

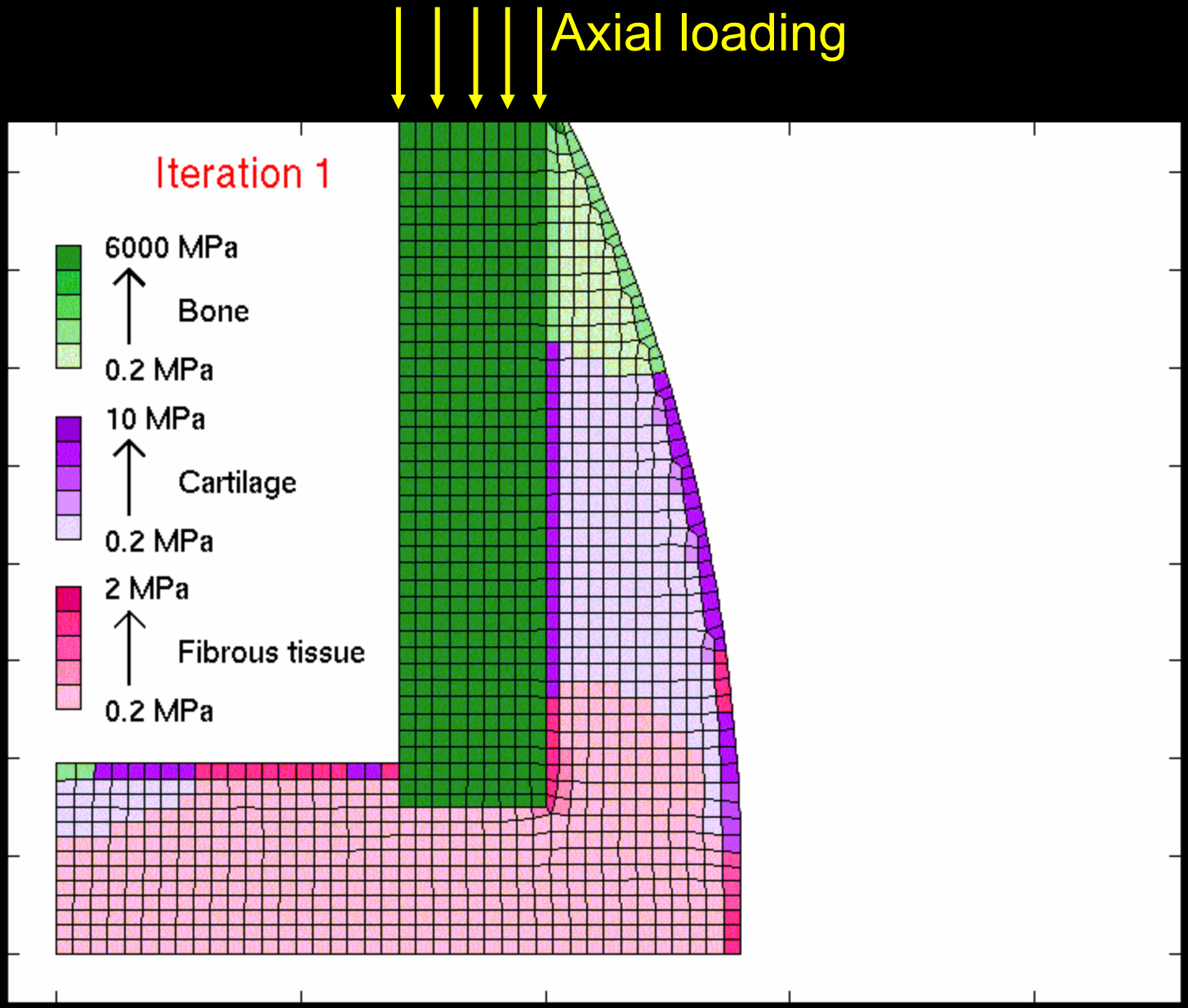
Axisymmetric FE model – 3 mm gap



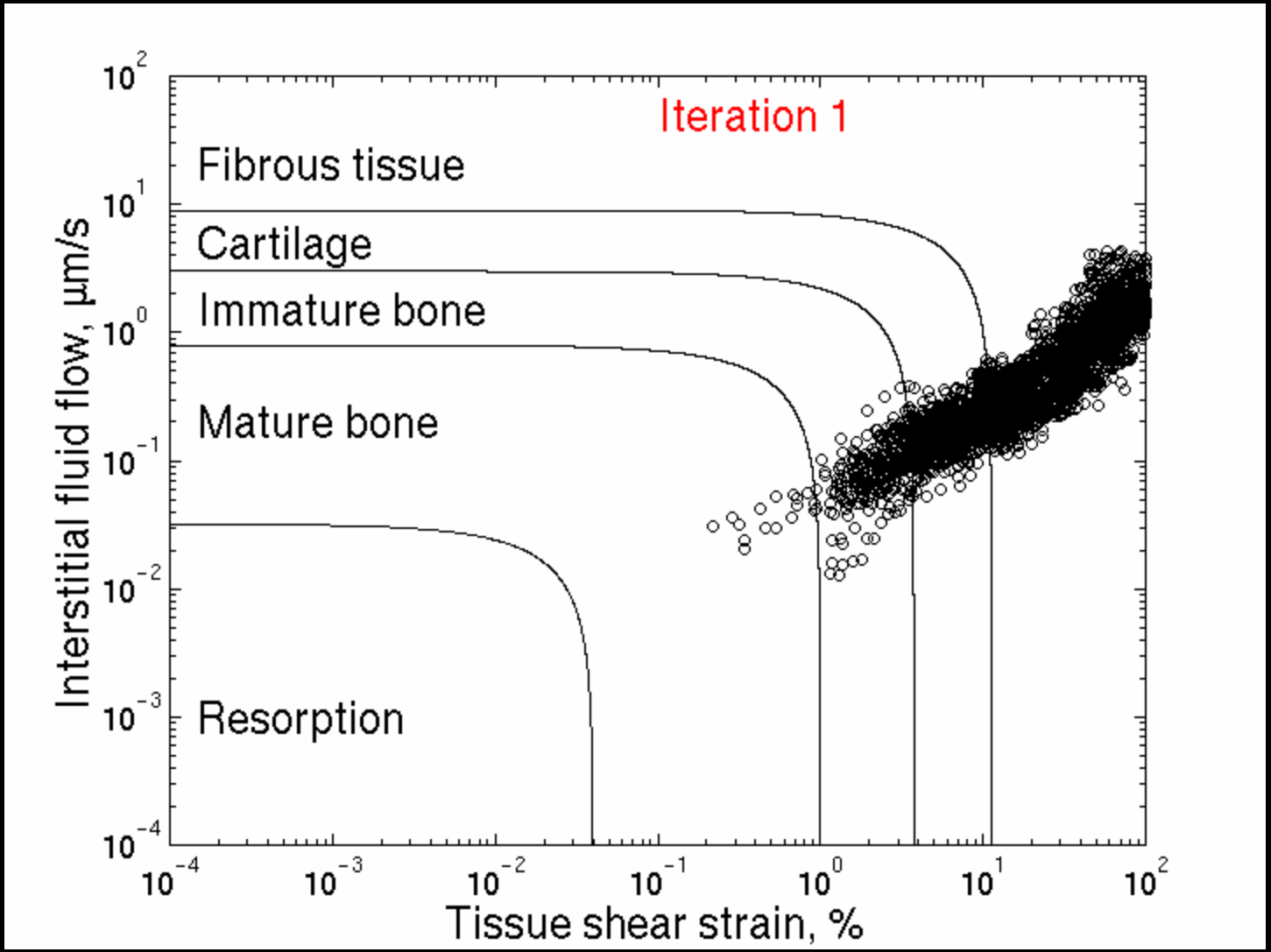
Fracture gap influence – 1 mm gap



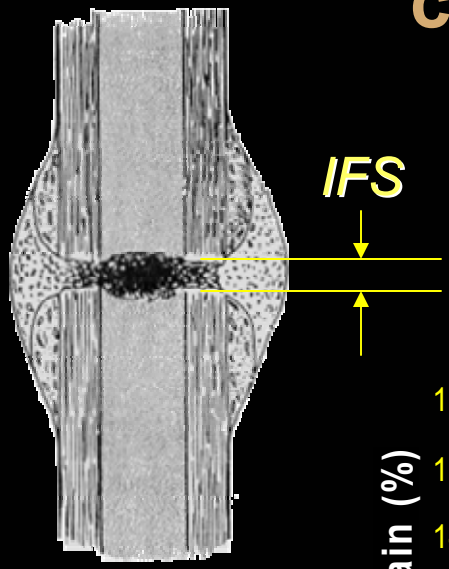
Fracture gap influence – 6 mm gap

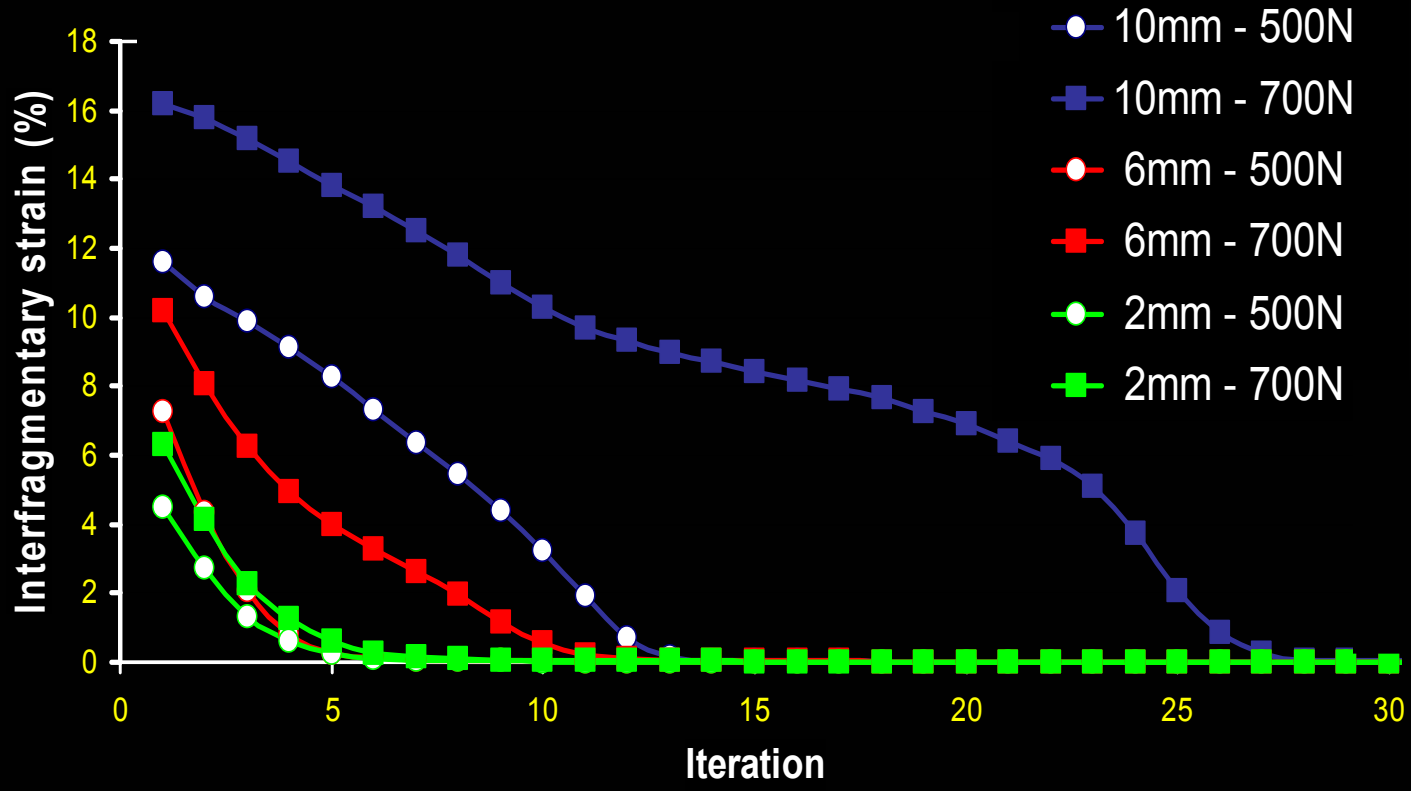


Results – Regulation of biophysical stimuli in the regenerating tissue



Does the model predict any difference in the clinically measured variable?

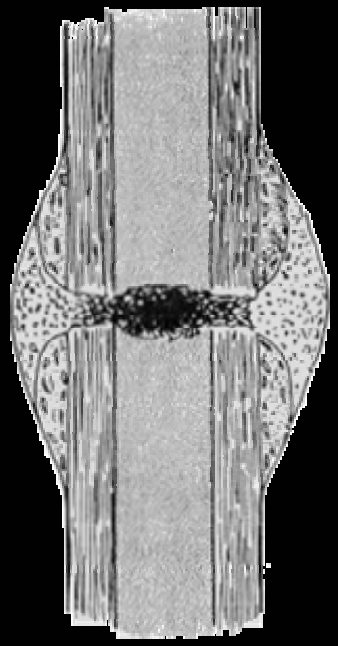




Tissue differentiation: discussion

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



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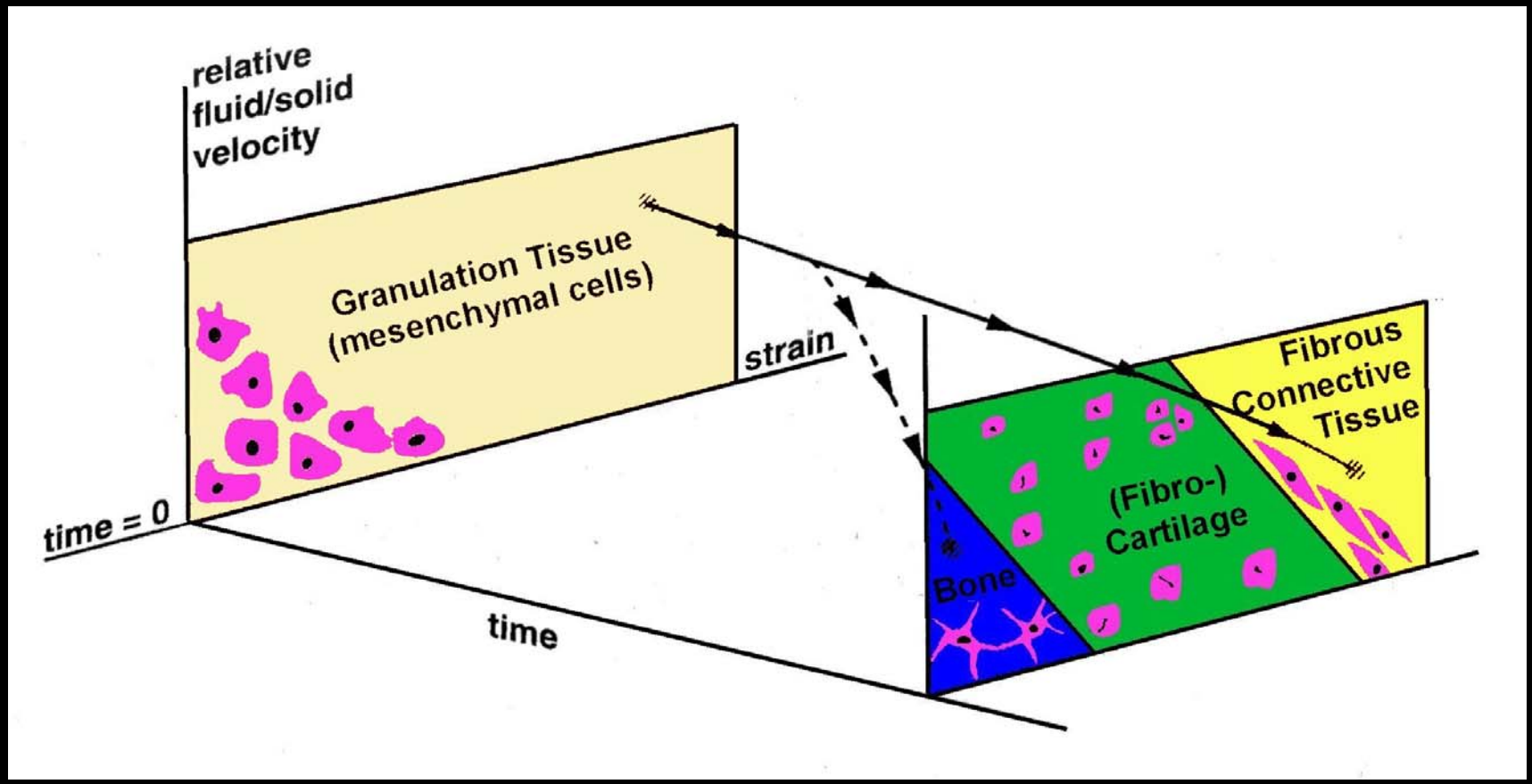


Prendergast P.J., Huiskes R. and Søballe K. *J. Biomechanics* 30:539-548, 1997

Osteochondral defects

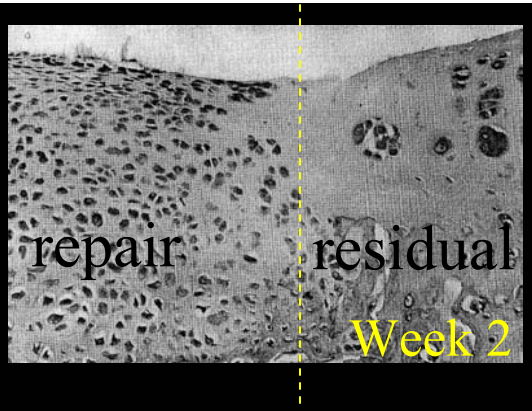
- Articular cartilage defects, caused primarly by traumatic events will, if untreated, lead to large-scale degenerative changes and osteoarthritis - Buckwater & Mankin, 1998

- 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 inflitrated by mesenchymal cells throughout defect.
- Day 14: Superfical fibrous layer seen; Deeper layer of chondroid cells forming; intramembranous woven bone forming in adjacent marrow.



* Shapiro et al., 1993

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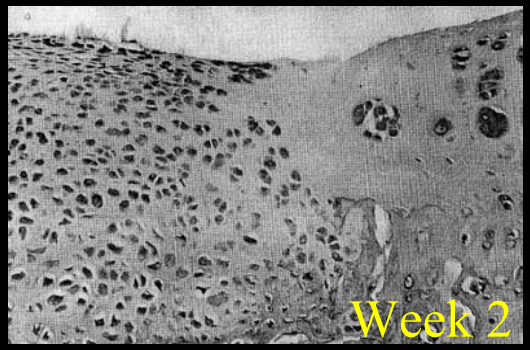
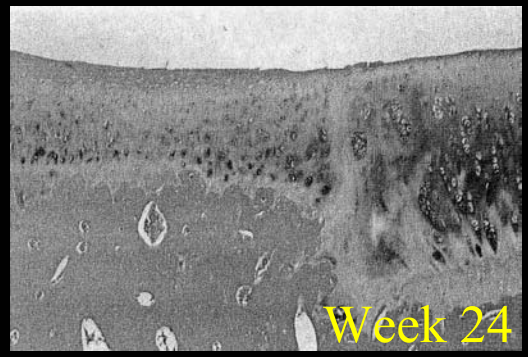
- Week 3-8: Endrochonral bone formation in depths of defect; Chondrocyte layer well developed; Repair cartilage closely apposed to residulal cartilage in some defects but seperated in others.

* Shapiro et al., 1993





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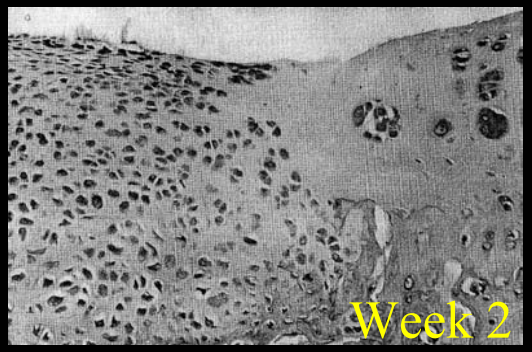
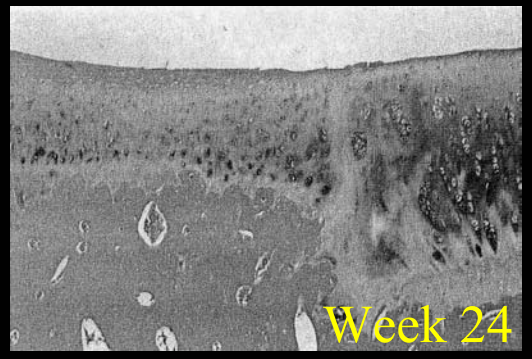
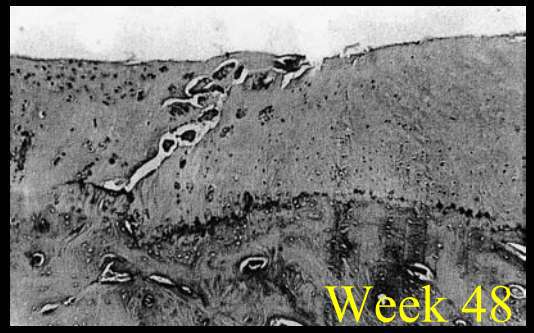
- Long term: Superficial cartilage fibrillation, decreased staining of matrix with time.

* Shapiro et al., 1993



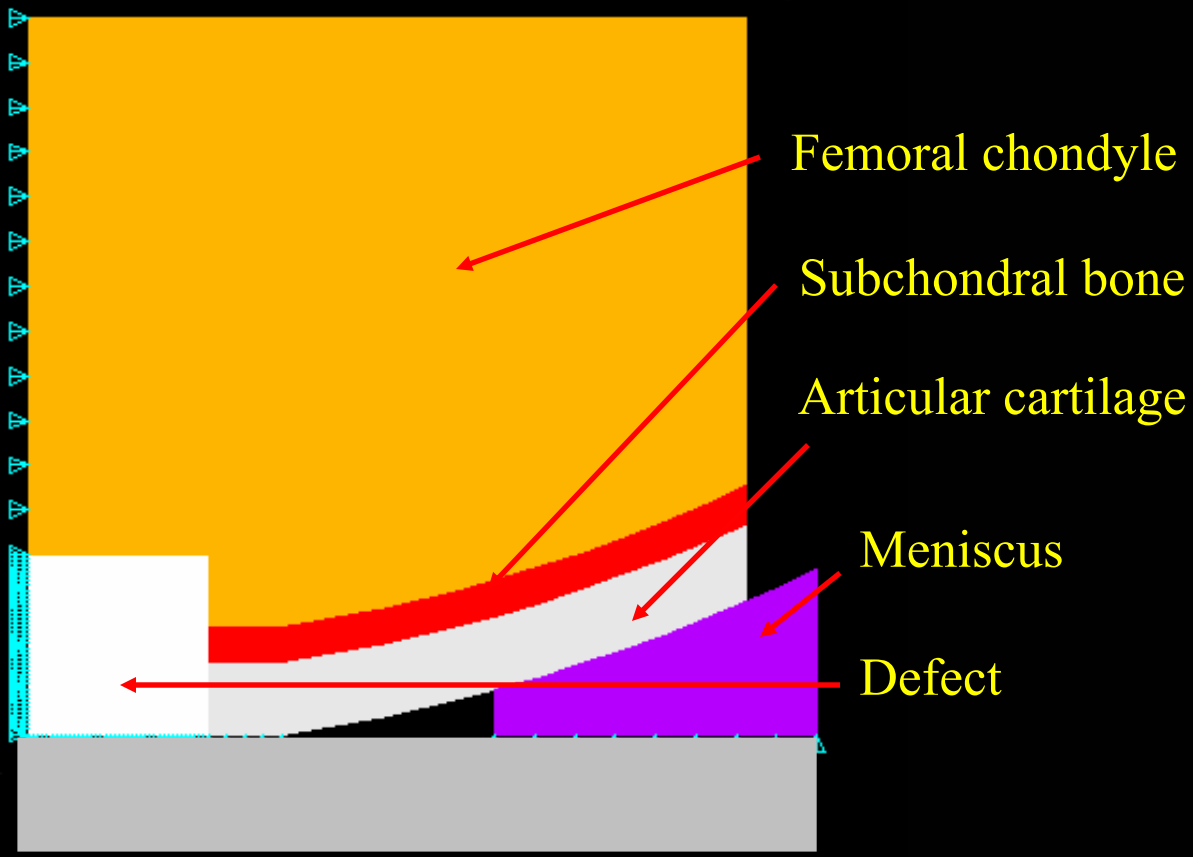




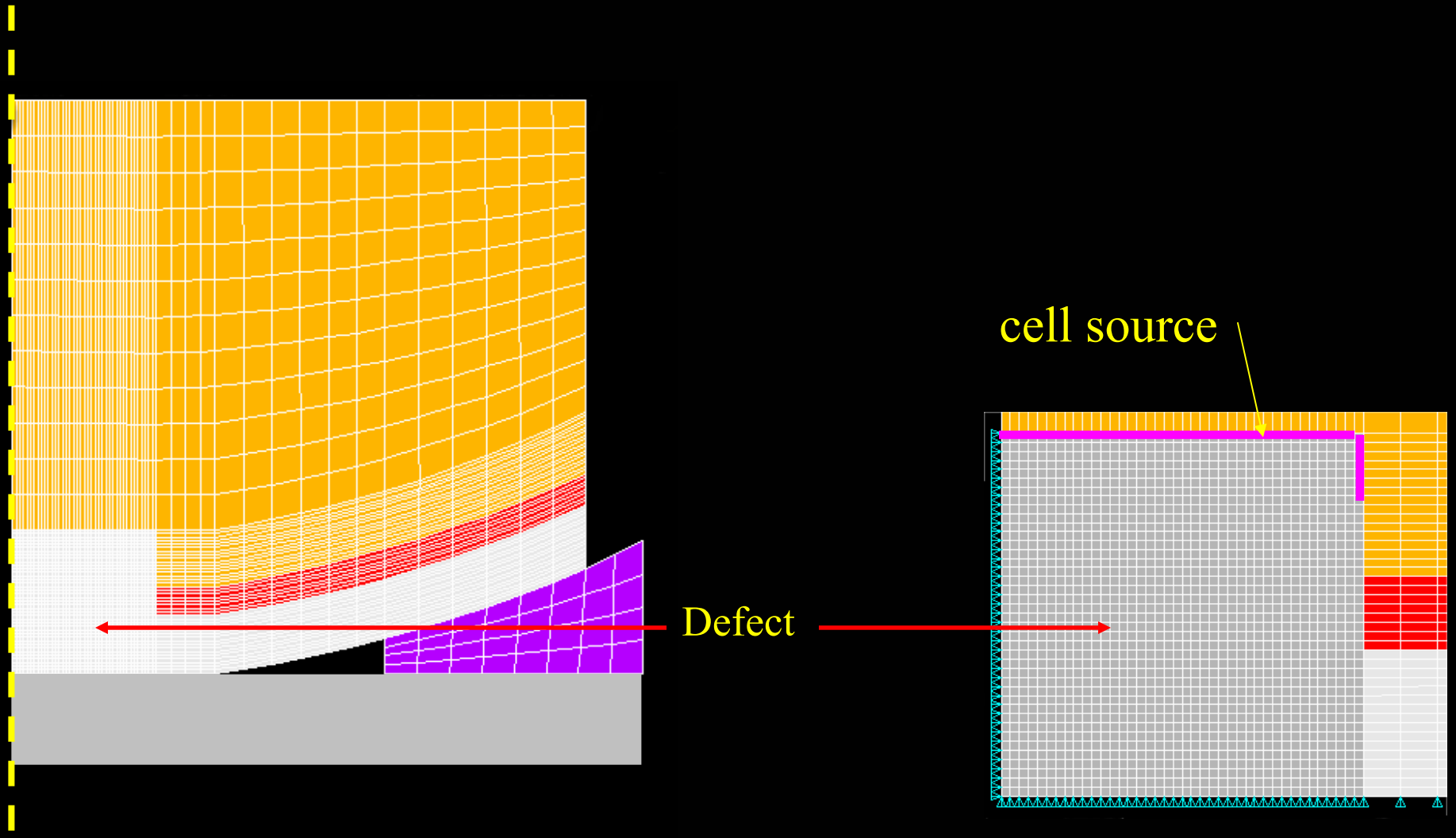
Objectives

- Use the theoryto 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.

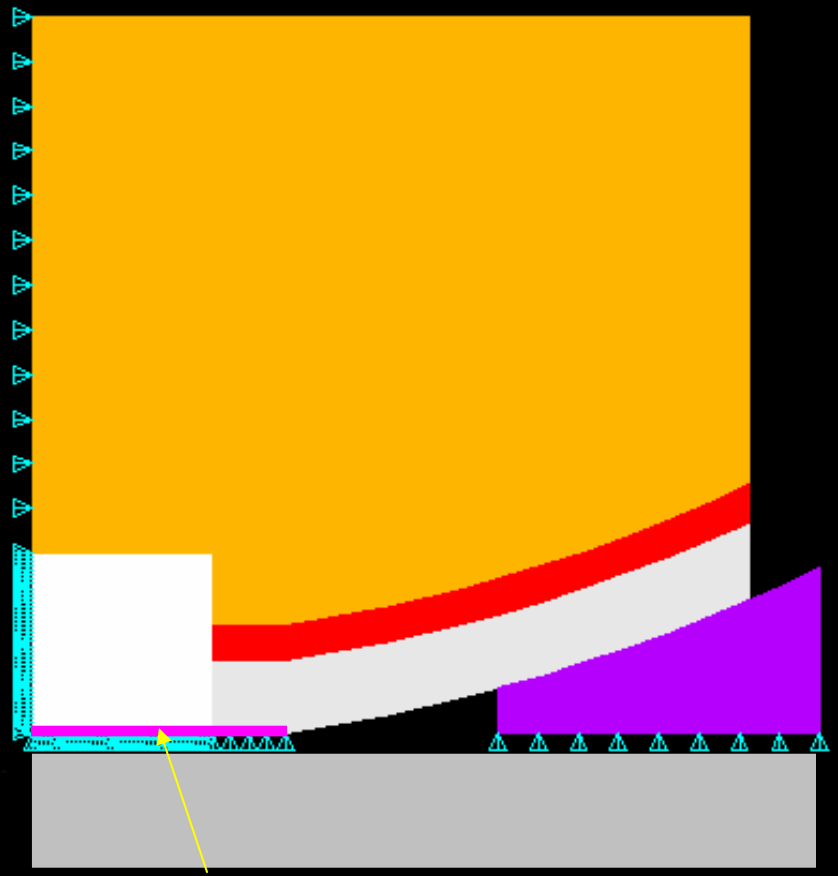
Methods: Finite element model of osteochondral defect



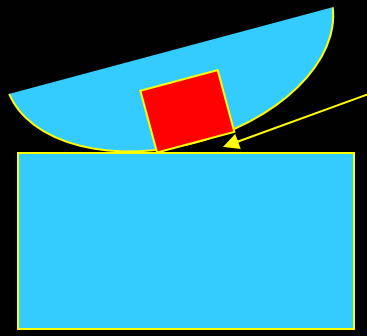
Methods: Finite element model of osteochondral defect



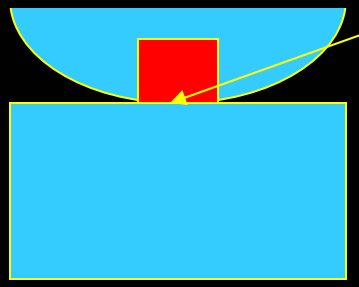
Methods: Boundary conditions in the finite element model



unknown pressure boundary condition

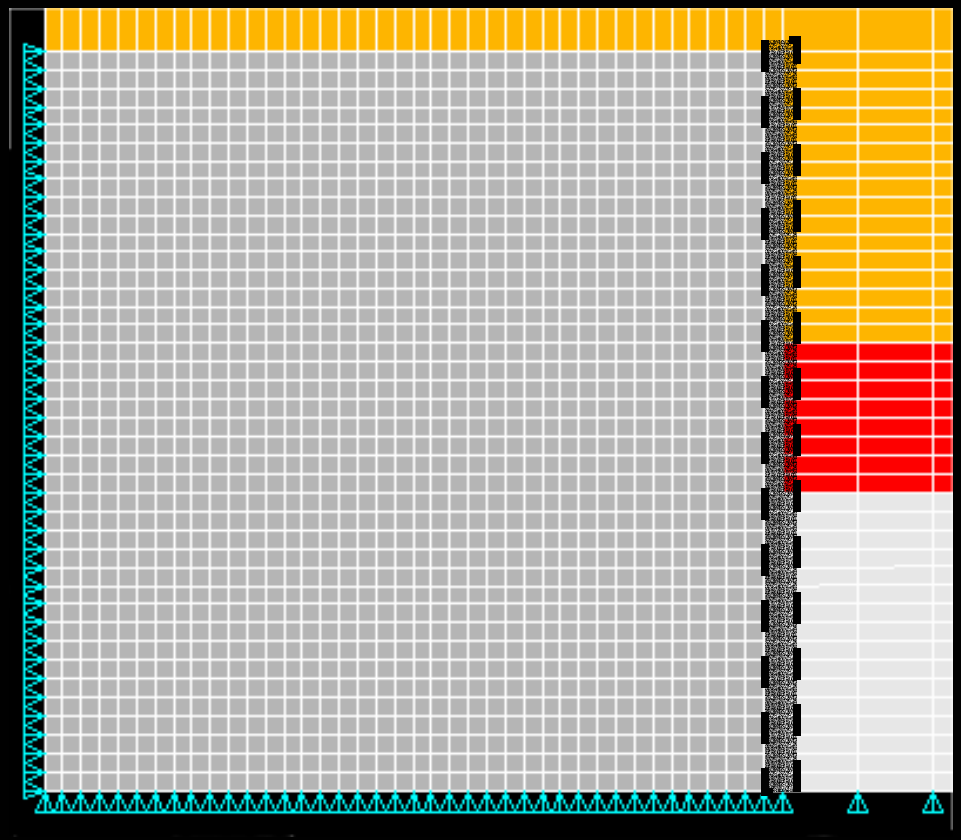


partially loaded: 250N



fully loaded: 700N

Methods: Integration of repair tissue



$$\tau = Ae^{bn}$$

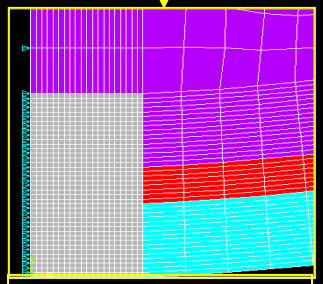
τ: shear stiffness

n: cell number at interface element

Methods: The Algorithm

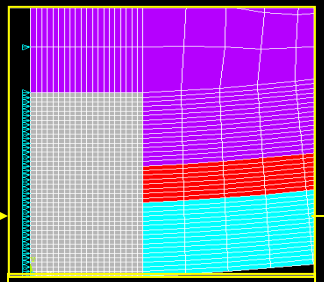
t = 0

Create FE model and set initial cell concentration



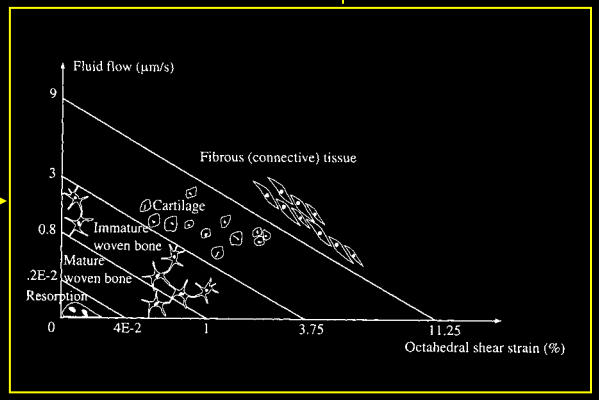
Run diffusion analysis $D\nabla^2 n$

Calculate cell mitosis and apoptosis



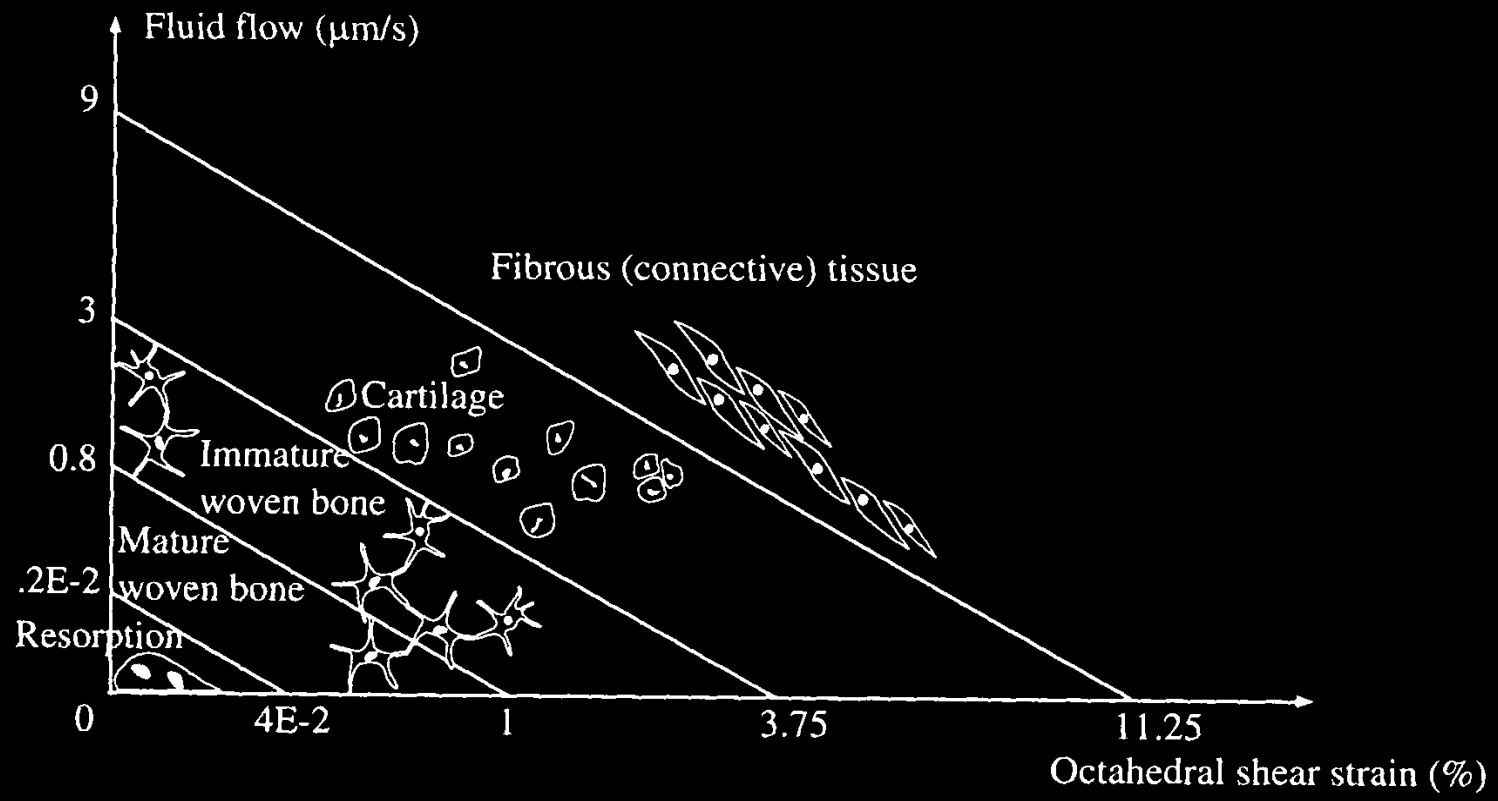
Run structural analysis

Calculate max strain and fluid flow



New cell phenotype

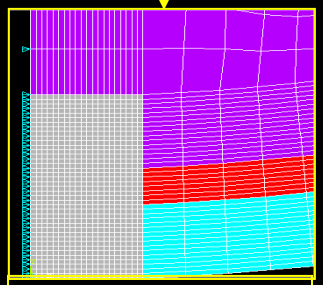
Methods: The Algorithm



Methods: The Algorithm

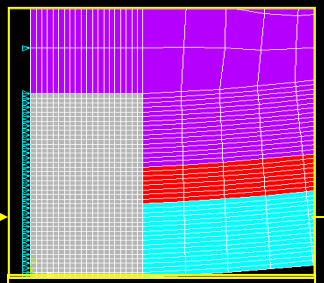
t = 0

Create FE model and set initial cell concentration



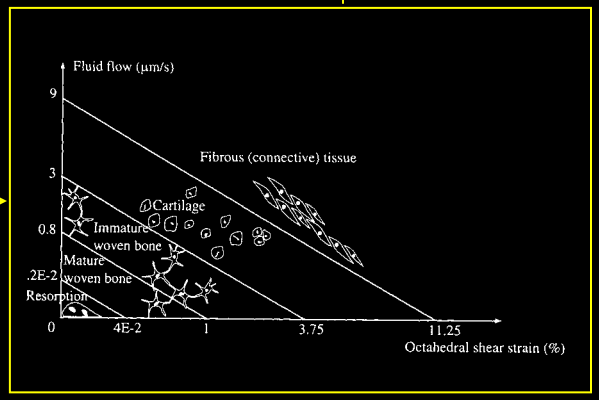
Run thermal analysis $D\nabla^2 n$

Calculate cell mitosis and apoptosis



Run structural analysis

Calculate max strain and fluid flow



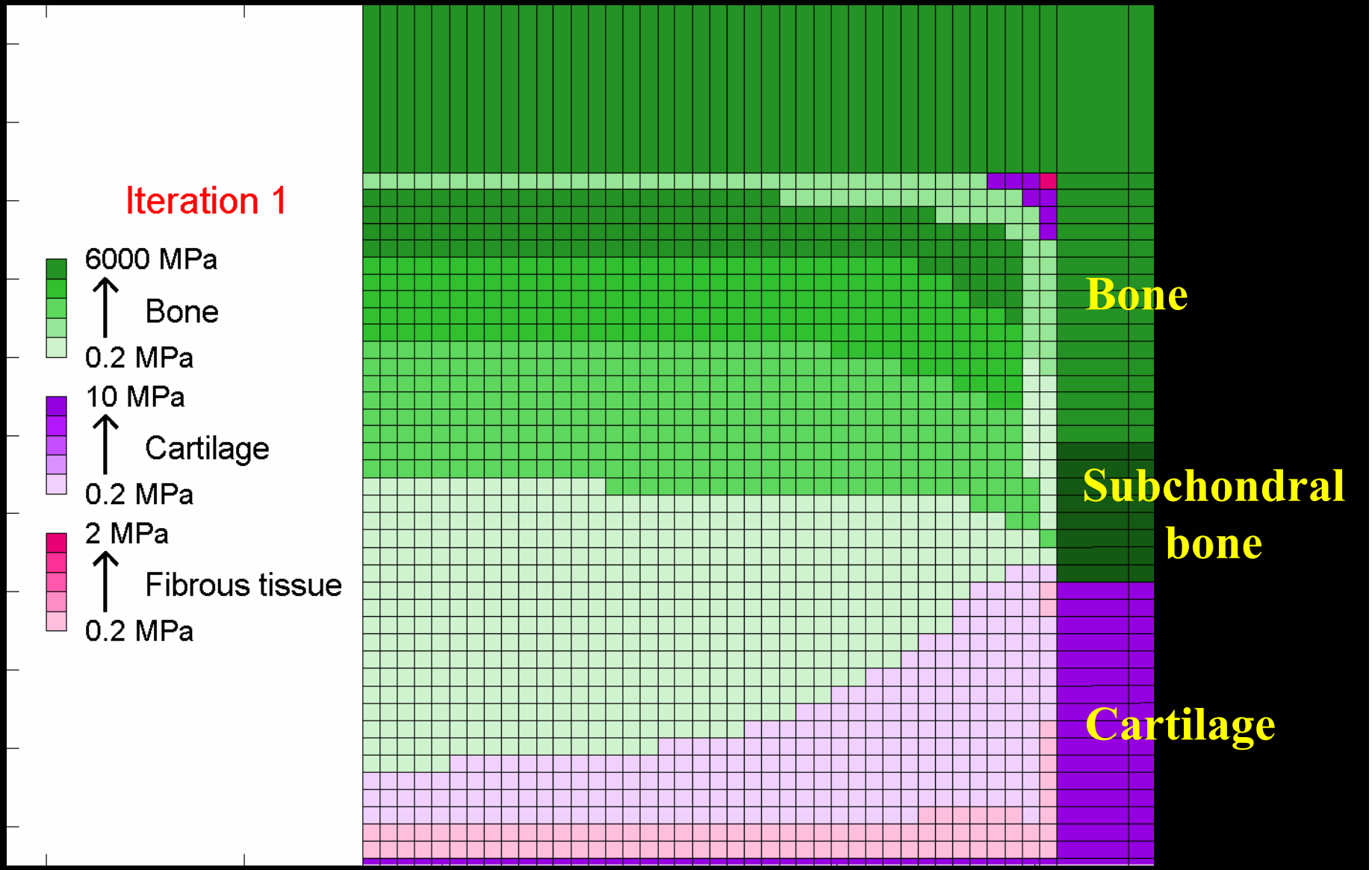
New cell phenotype

Smoothing procedure applied

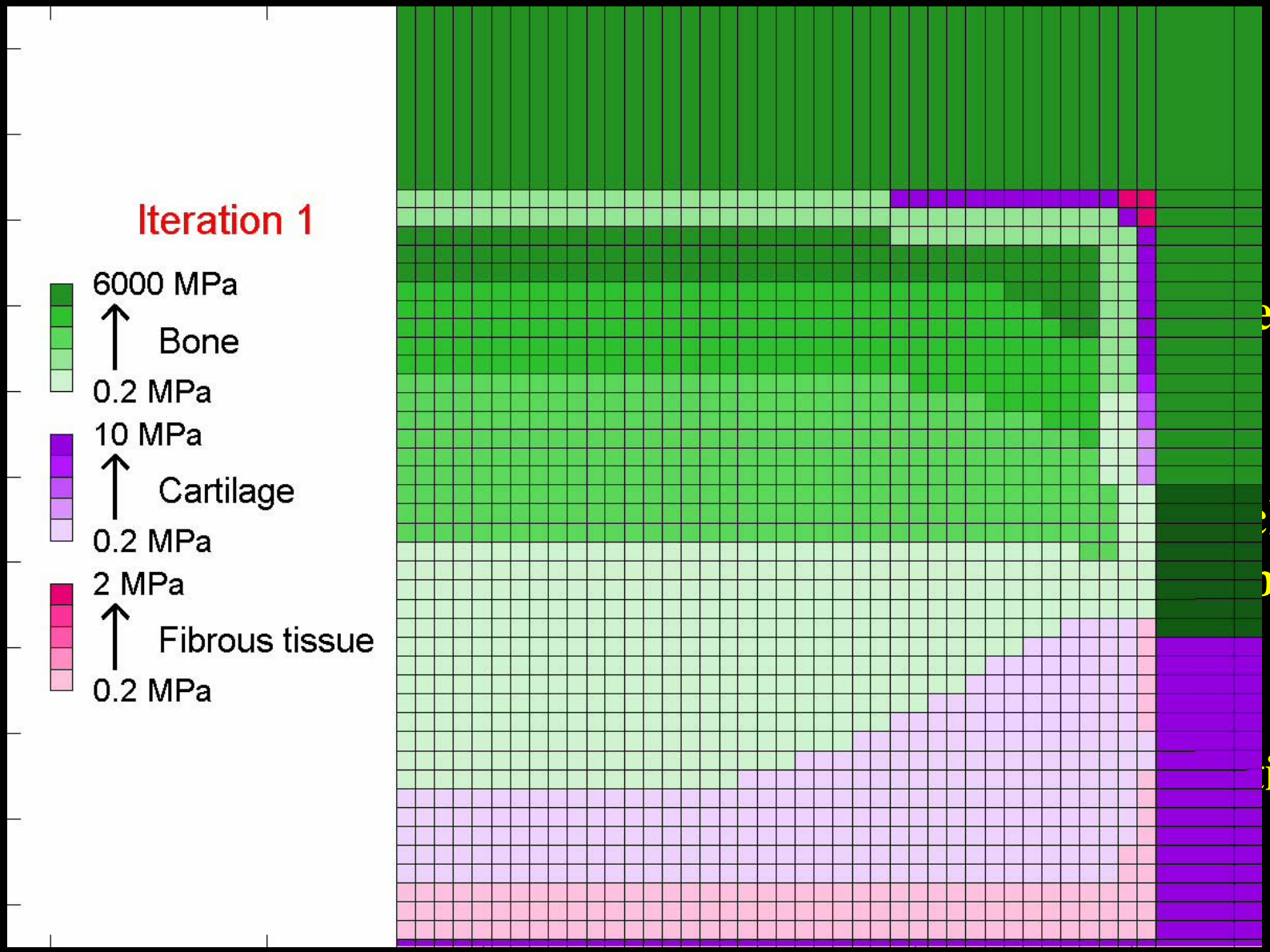
New material properties determined from rule of mixtures

t = t +1

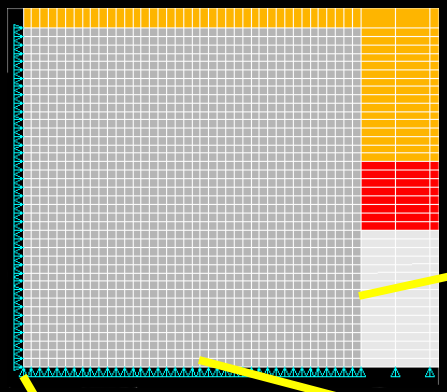
Results: 10mm Defect

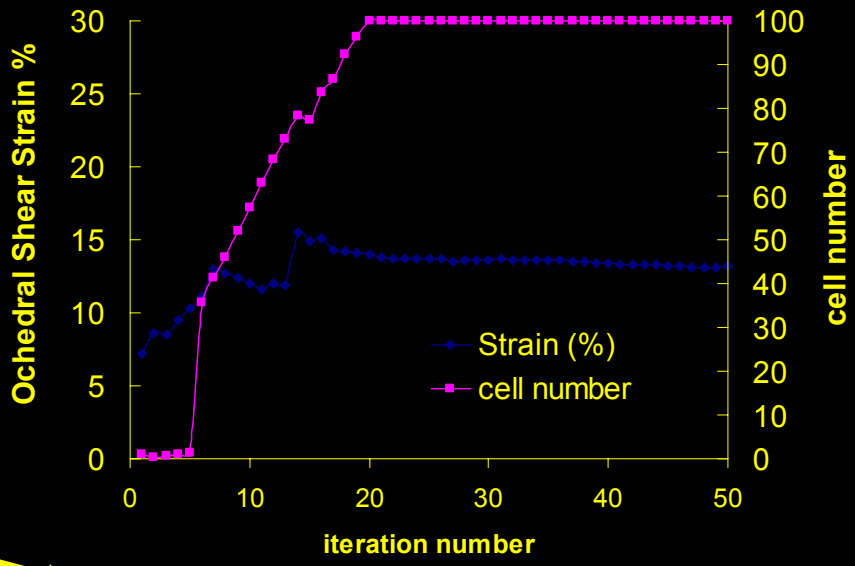


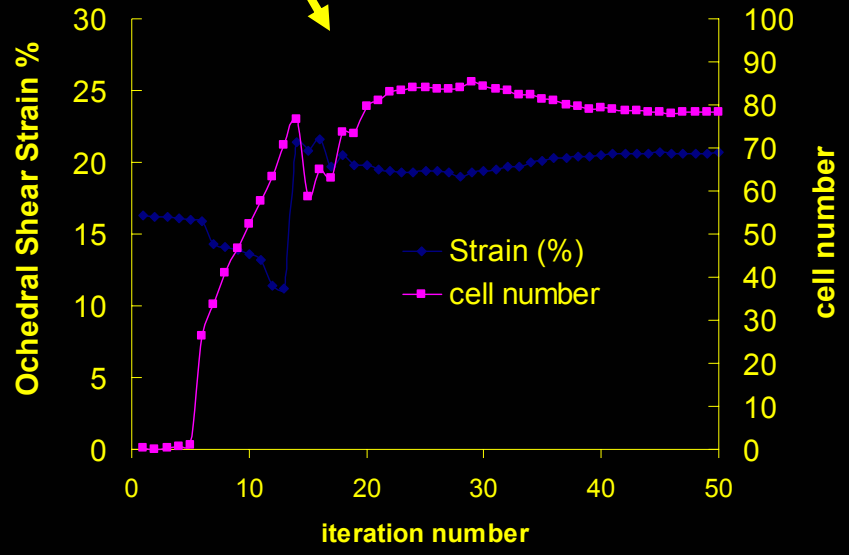
Results: 10mm Defect

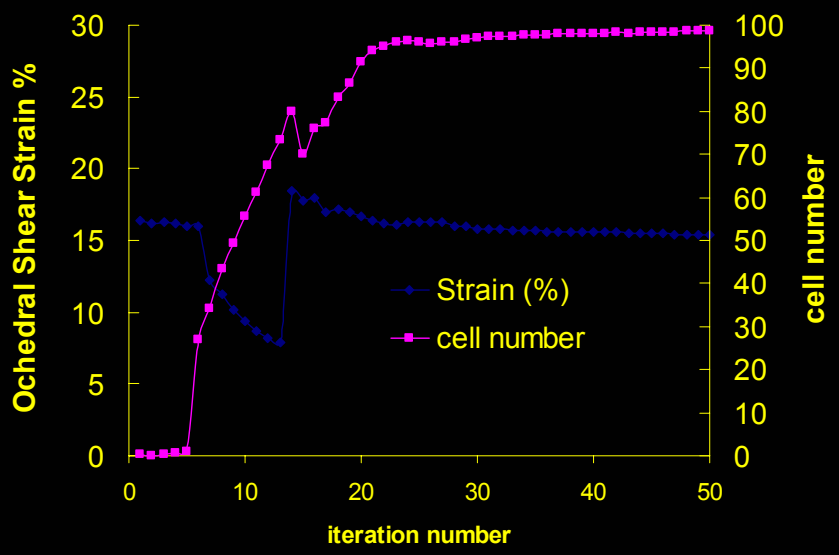


Results: 10mm Defect

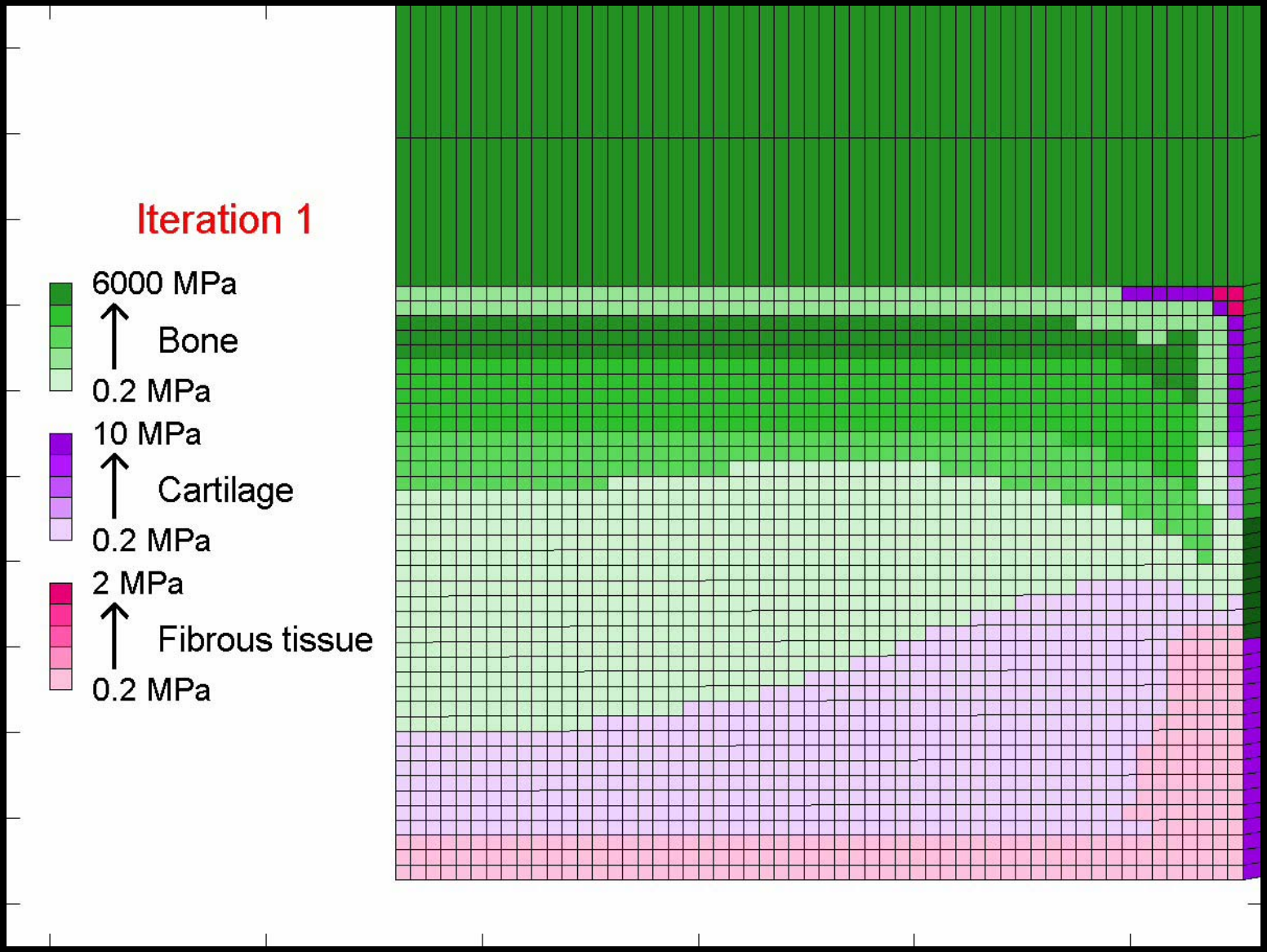




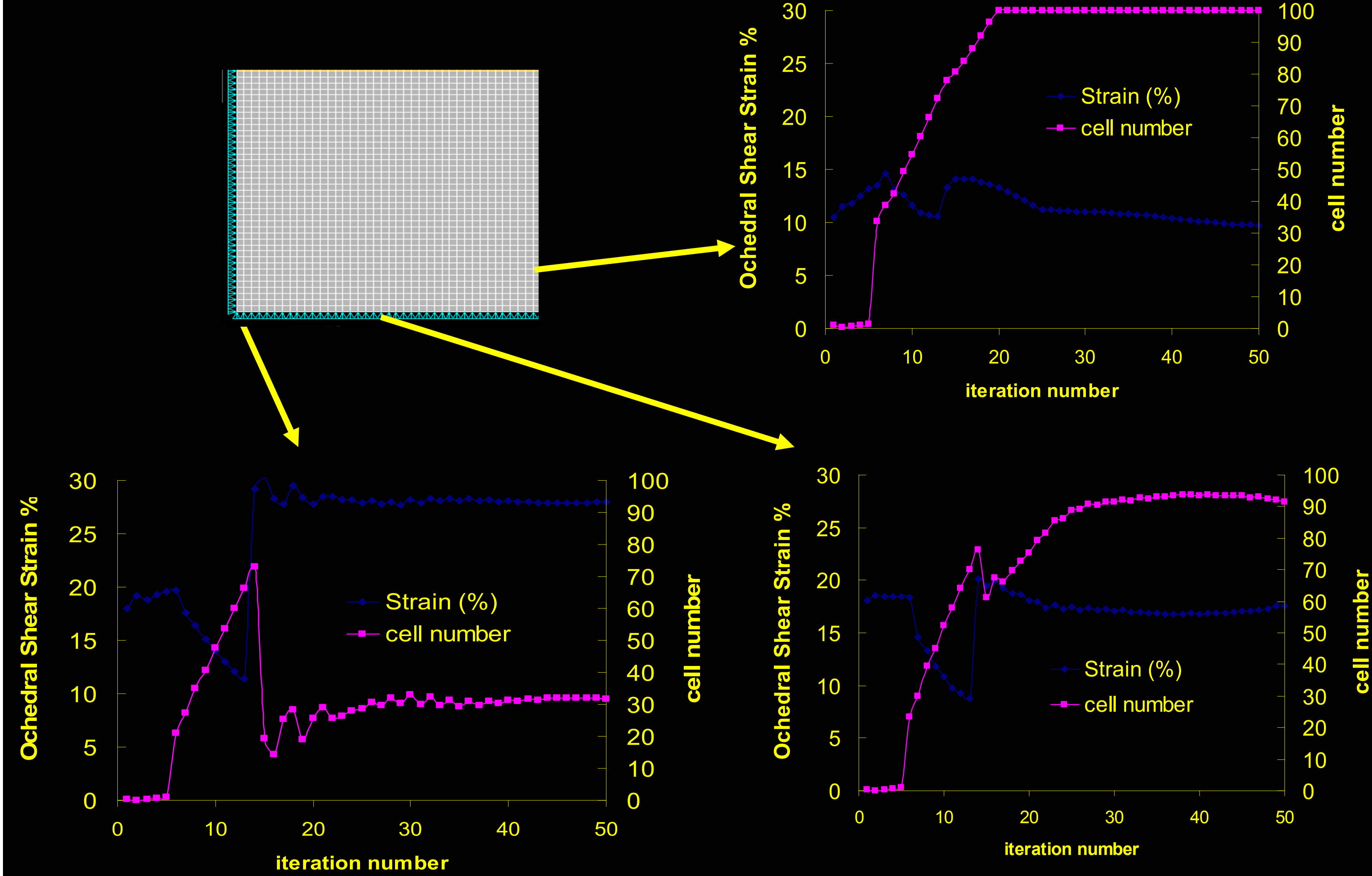




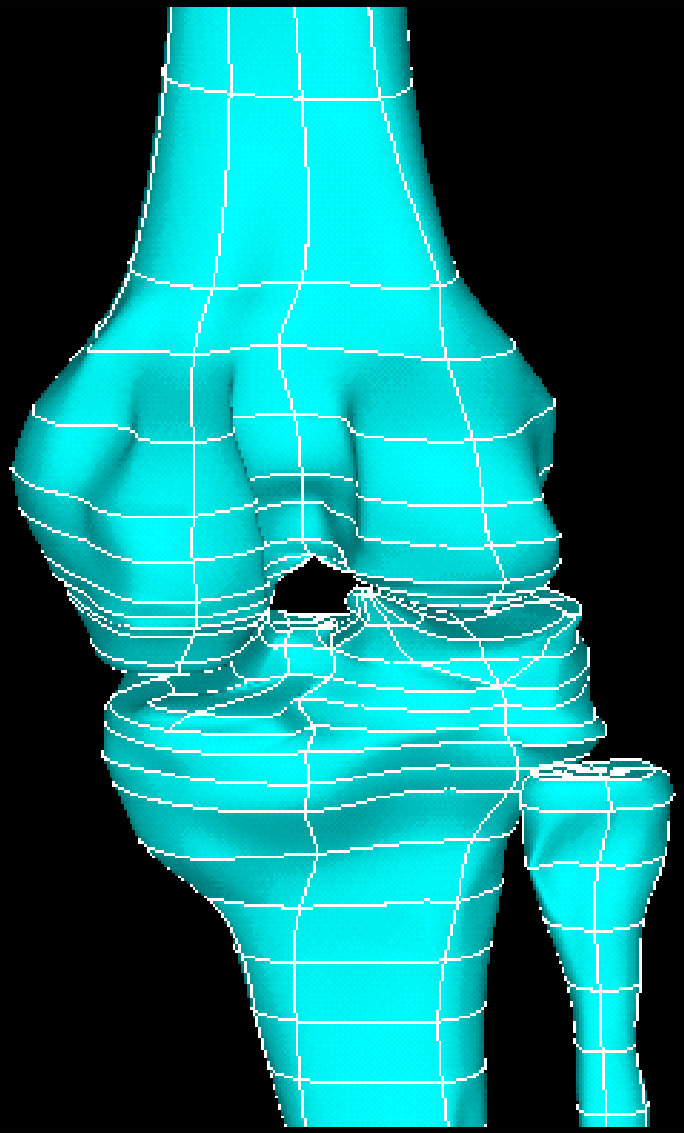
Results: 14mm Defect



Results: 14mm Defect



Future work:



Future work:

- Determine the infleunce 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

- Falsfiability & 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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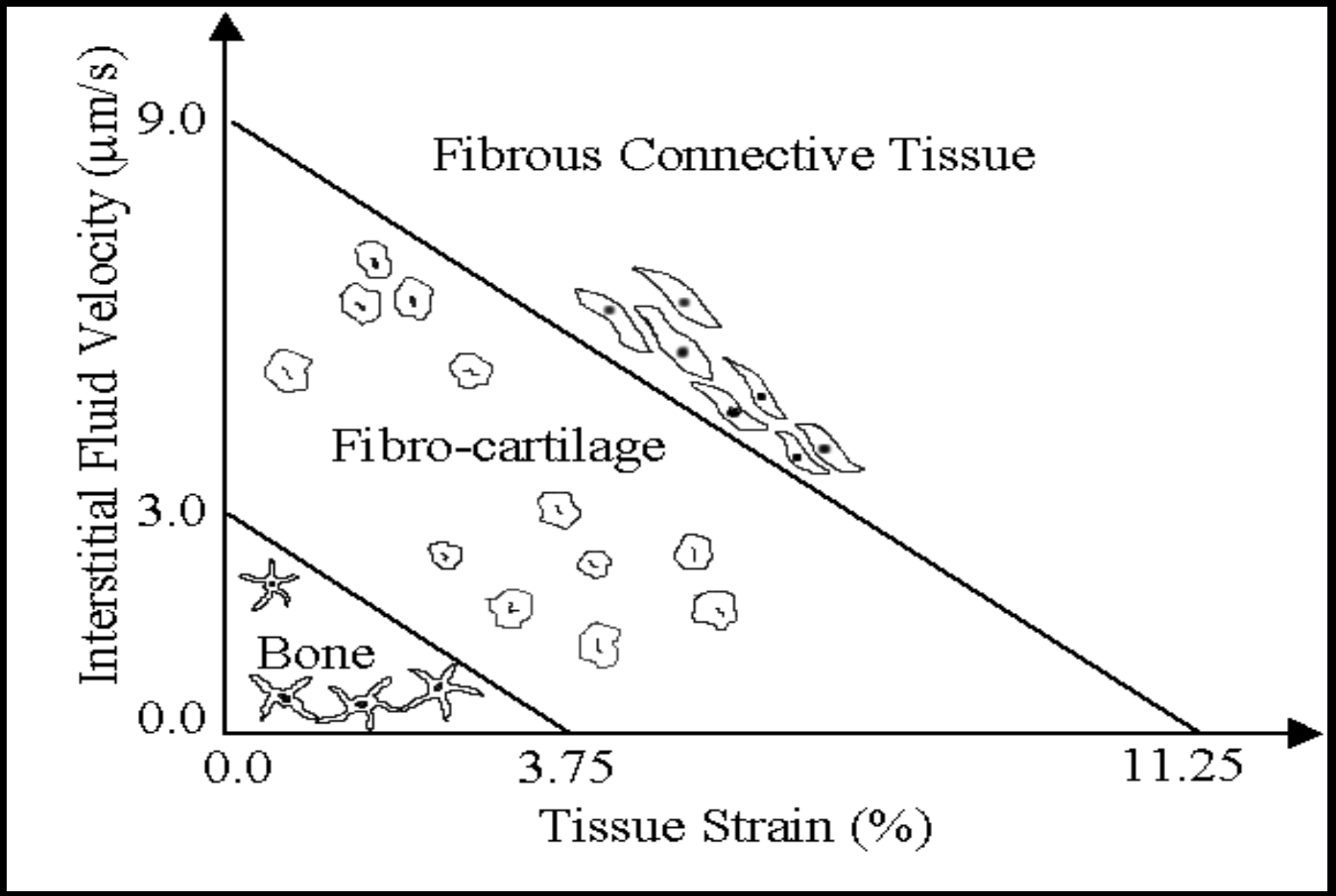
Trinity College, Dublin 2, Ireland

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

Fluid flow



Strain