Different approaches to the Biomechanical Characteristics of Biomaterials for Tissue Engineering Scaffolds

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Cell Material Interaction

Scaffolds for tissue engineering

Successful tissue engineering:

development of extracellular conditions which induce the cell response: adhesion, proliferation, ECM molecules.

Cell –Material interaction take place at the *cell-biomaterial* interface

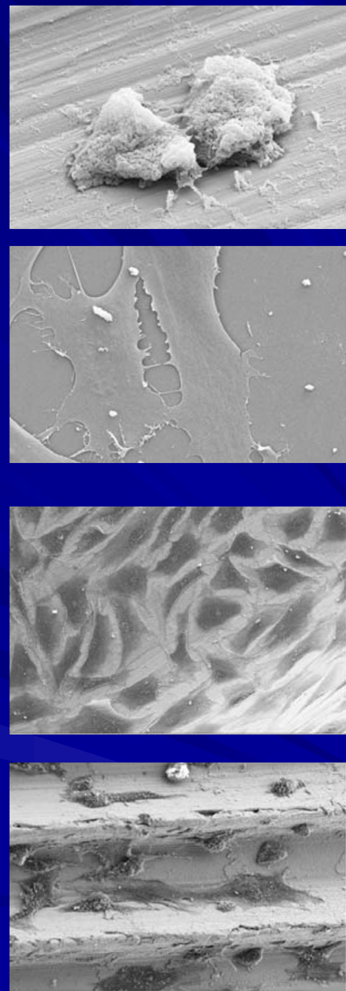
→

***Cells covering the scaffold* surface is the crucial event determining the cell responses at the biomaterial surface.**

Cell functions

- The initial attachment phase of cells to the scaffold surface is pivotal in ensuring successful tissue engineering.

Anselme K, 2000



The **physicochemically driven attachment** of cells on substrates has profound effects on the subsequent:

- cell adhesion
- spreading
- attachment strength
- proliferation
- migration
- phenotypic differentiation.

U. Meyer et al, 2005

Tissue engineered scaffolds

Explosion of tissue engineering research and the associated need for new materials with specific, controllable biological responses and biodegradability

Polymeric scaffolds with Natural biomacromolecules
coatings (Chitosan)
Biomechanical properties of surfaces

Natural scaffolds with Acellular xenogeneic tissues
Biomechanical characteristics of bulk biomaterials for STE

Inorganic scaffolds and coatings
Biomechanical properties of surfaces (CNTs, TiO_2 ,HAP)

1. Surface Coatings & Modification

Much research attention has focused on enhancing cell attachment for improving tissue engineering of scaffolds **through surface modification and coatings.**

The surface properties of a material will influence the initial cellular events at the cell – material interface.

Natural biomacromolecules
polysaccharide biomaterials
Chitosan

- promising biopolymer for tissue engineering in orthopaedic, nerve or cardiovascular applications
  - ✓ **biocompatibility**
  - ✓ **biodegradability**
  - ✓ **non-toxicity**
  - ✓ **adsorption properties**
  - ✓ **enhance osteogenesis and nerve regeneration**
  - ✓ **cell adhesion**

It has been proposed to modify the surface properties of prosthetic materials for enhancing the attachment of fibroblasts, osteoblasts, Schwann cells

Chitosan

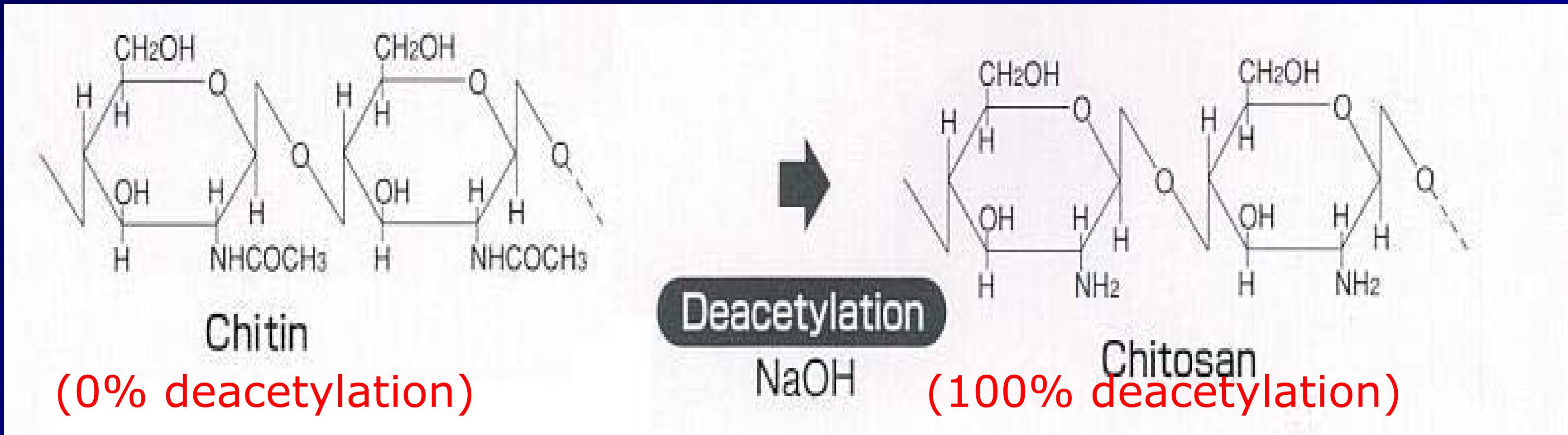


***Figure 1**: Molecular structure of the polysaccharides' repeating units.*

- When the fraction of acetylated amine groups is lower than 0,35-0,40 then the copolymer is referred to as chitosan.

Osteoblast → chitosan

Approach to the biomechanical characteristics

The study of the initial osteoblast response to the biomechanical surface characteristics of chitosan coated glass:

a. the evaluation of cell attachment

b. the development of the attachment – detachment strength of osteoblasts on chitosan substrate and

c. the osteoblast cell spreading.

Materials and methods

B. Surface characterization

X-Ray photoelectron spectroscopy (XPS): chemistry of the surface / confirmation of immobilization

Atomic Force Microscopy (AFM): nanotopology/roughness of the chitosan film compared to the non-coated glass.

Wettability: Static contact angles were measured with a Goniometer using the sessile drop technique. (room temperature with deionized water).

Materials and methods

C. Evaluation of cell attachment

Human bone marrow derived osteoblasts of 2nd-4th passage were allowed to attach undisturbed in a humidified incubator to chitosan-coated glass or non-coated glass surfaces (control) for 15, 30, 45 and 60 minutes.

1. Cell morphology
2. Attachment (number of cells attached)
3. Spreading (average cell area)

onto the biopolymer surface were determined using IMAGE PRO analysis on scanning electron microscopy (SEM) images.

Materials and methods

C. Evaluation of cell attachment

4. Cell Detachment Strength:

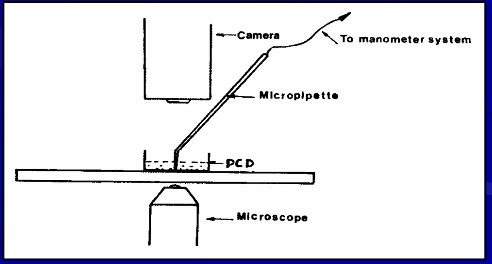
Using a micropipette technique, osteoblasts were aspirated into the micropipette until total detachment. The tip of the micropipette was bent at an 130^{o} angle to its corpus so as to apply forces vertically to the surface. The separation process and the suction pressure as well were recorded on videotape.

For evaluating the attachment strength, we introduced

The "separation impulse"

$$I = \int F(t)\, dt$$

where *F* is the product of the aspiration pressure and the cross-sectional area of the micropipette, and *t* is the application time.



Athanassiou G and Deligianni D (2001) J Mater Sci Mater Med

Results

A. Surface characterization

1. Chemical characterization

XPS showed a nitrogen component on the chitosan modified glass surface , based on the amino (-NH2) groups and amine bond (-CONH) in chitosan.

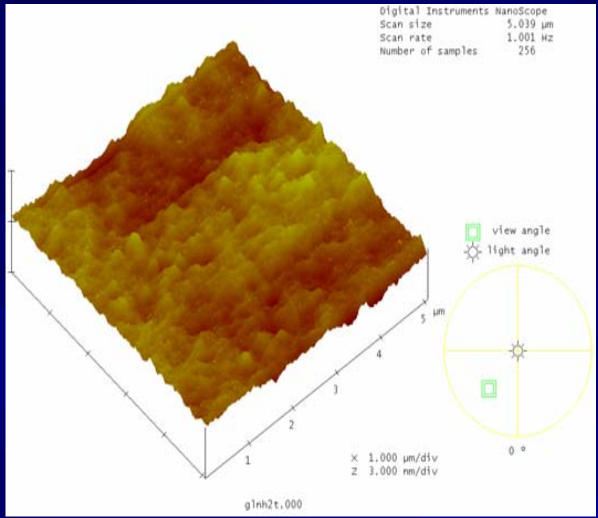
	N	Si	O	C
glass	0	26.7	64.4	8.9
chitosan	1.5	24	53.2	21.2

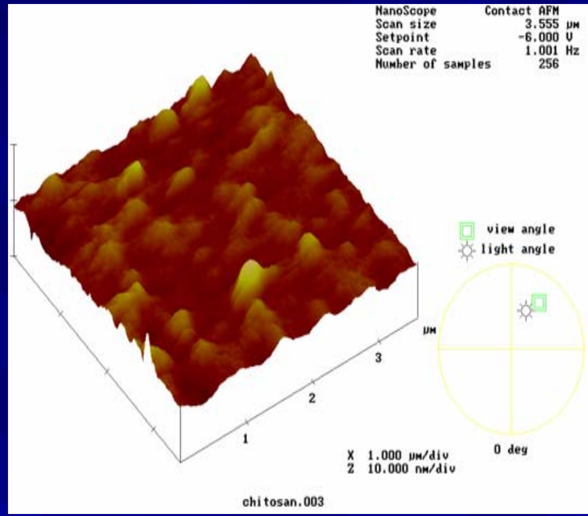
Table 1. Atomic composition (%) of the surface layer of glass and chitosan film on glass substrates, obtained from XPS analysis.

Results

A. Surface characterization

AFM characterized nanotopography





substrate	Ra (nm)
control	0.190±0.070
chitosan	0.779±0.207

310% increased Ra
($p<0,05$)

Table 2. Roughness values (mean ± SD) of non-coated glass and chitosan-coated glass, as obtained from AFM.

Results

A. Surface characterization

Surface wettability

non-coated glass	chitosan
$18 \pm 2\ ^{o}$	$45 \pm 4\ ^{o}$

Table 3. Water contact angles of the substrates ($p<0,002$)

Results

SEM images

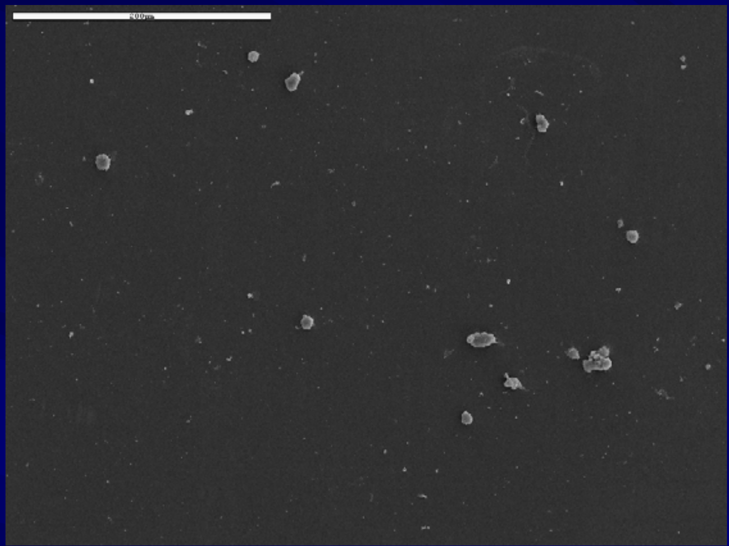


Fig 3a.15 min control

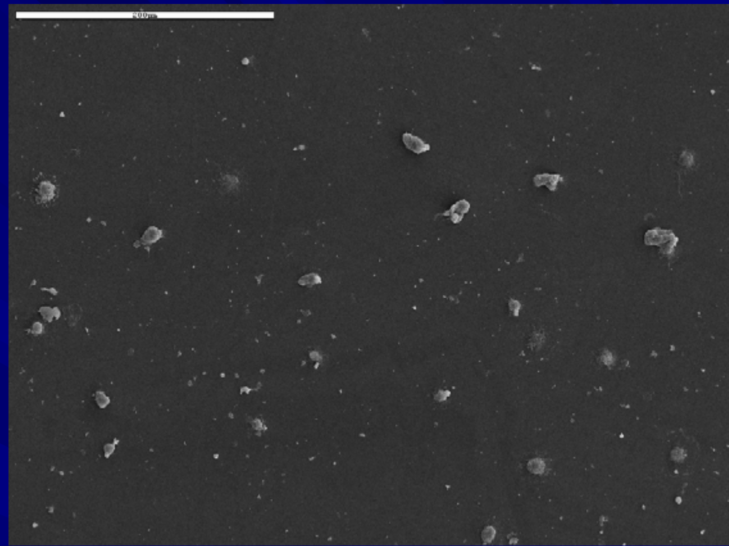


Fig 3b. 15 min chitosan

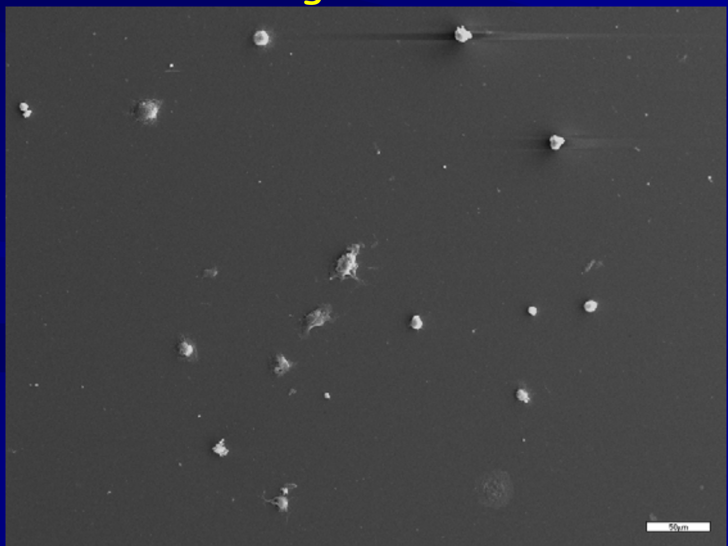


Fig 3c. 30 min control

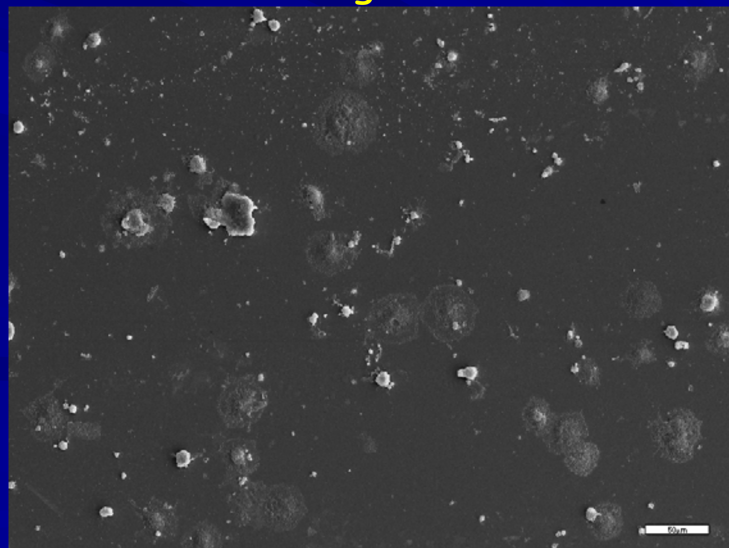


Fig 3d. 30 min chitosan

Results

B. 1. SEM images

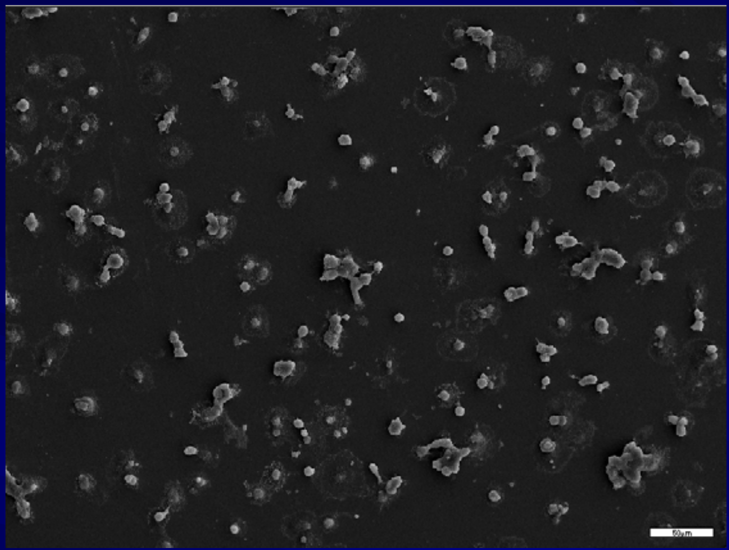


Fig 3e. 45 min control

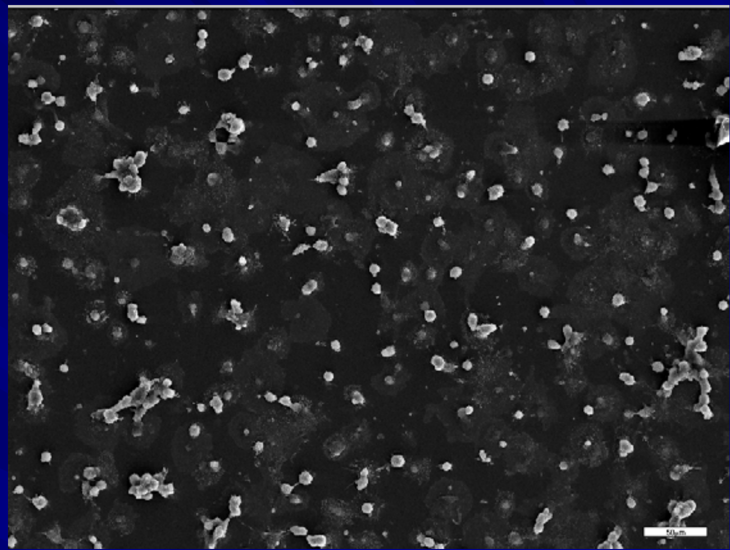


Fig 3f. 45 min chitosan

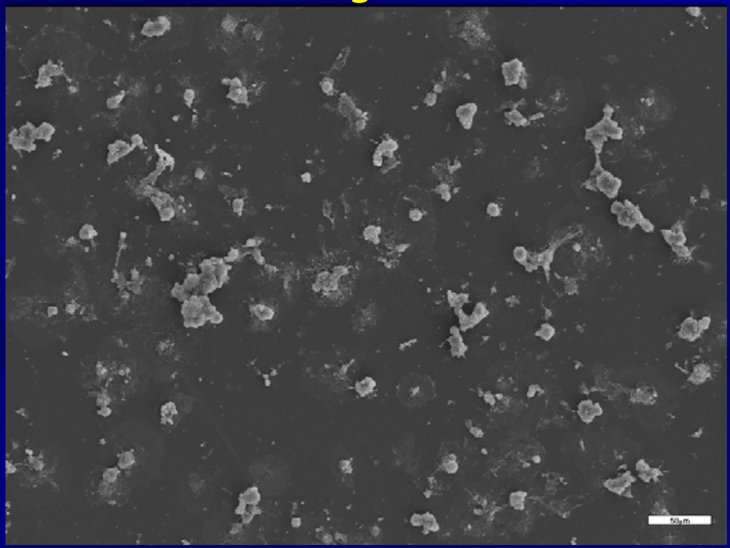


Fig 3g. 60 min control

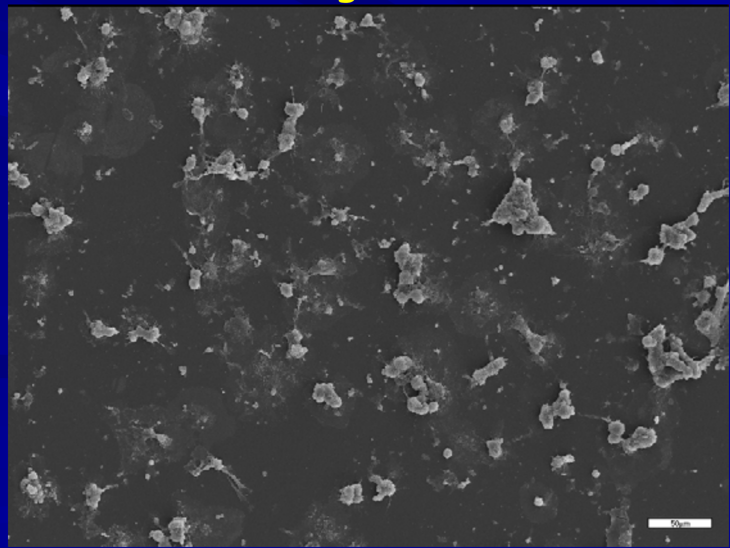


Fig 3h. 60 min chitosan

Results

B. 2. Cell attachment

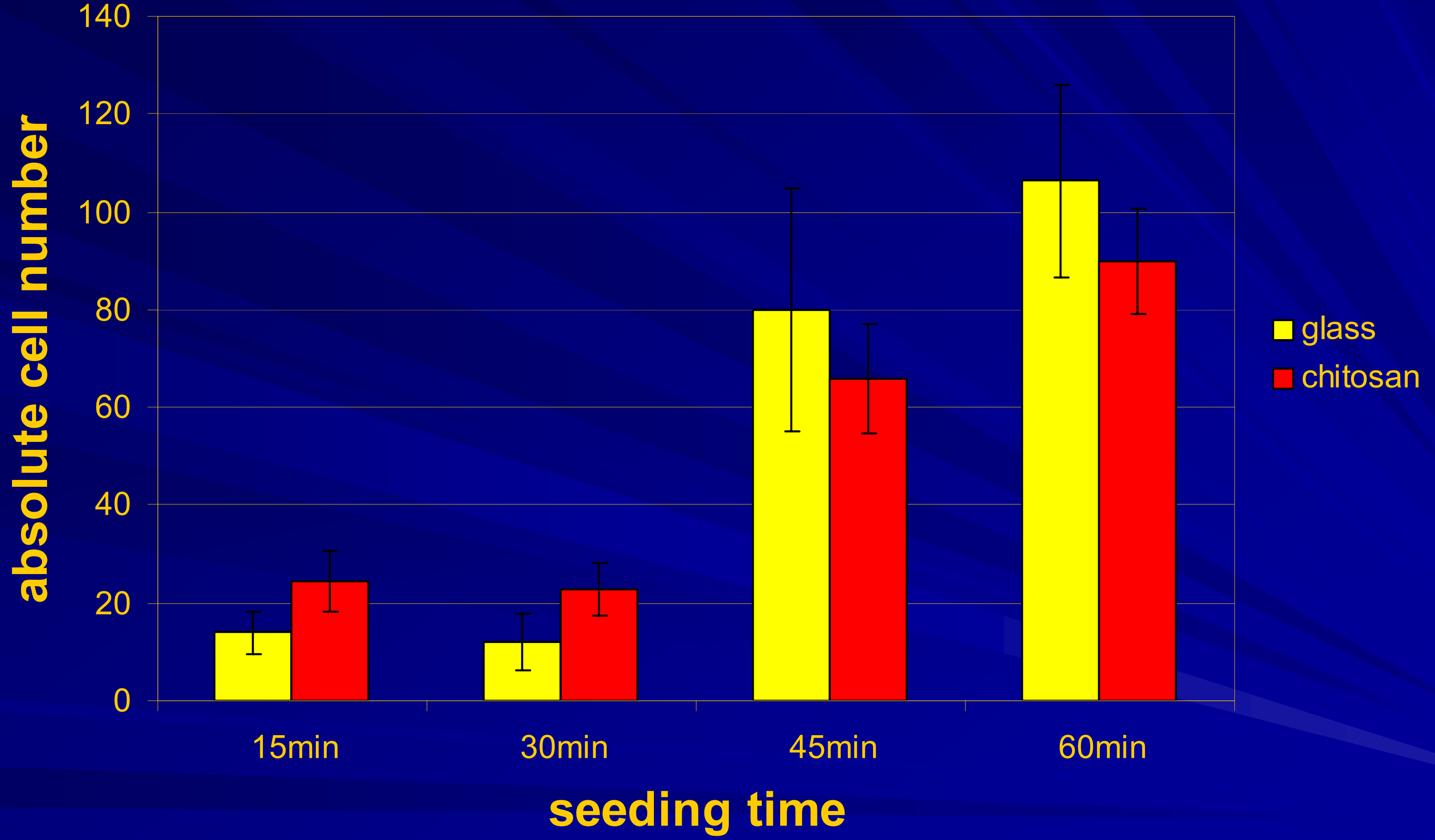


Figure 4. Mean ± SD values of absolute number of cells attached on control and chitosan surfaces. Values are calculated using IMAGE PRO for equal random SEM images of the samples.

control vs chitosan: $p<0.05$ for 15 and 30 minutes and $p<0.002$ for 45 and 60 min. (Student's t-test)

Results

B. 3. Cell spreading

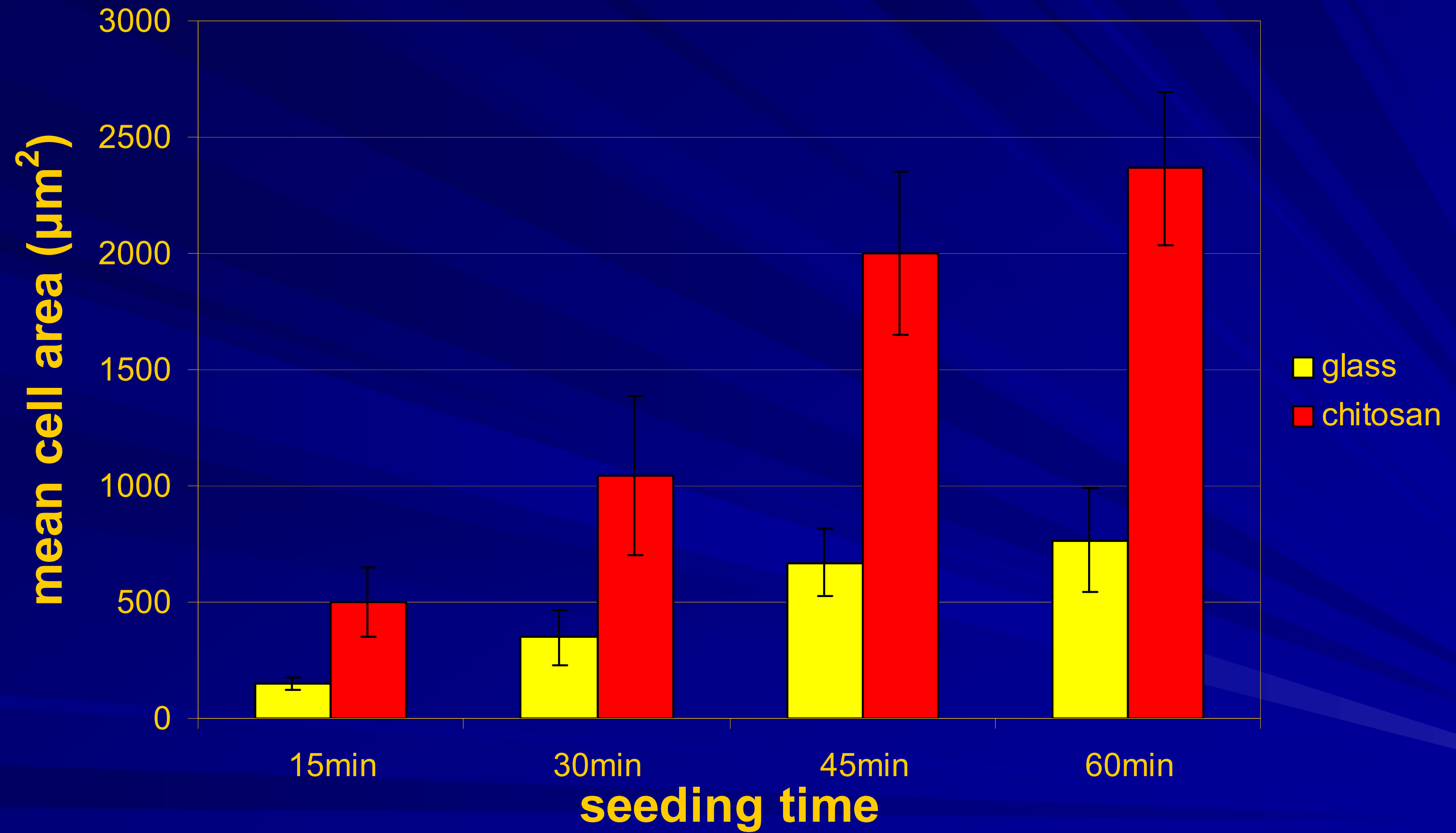
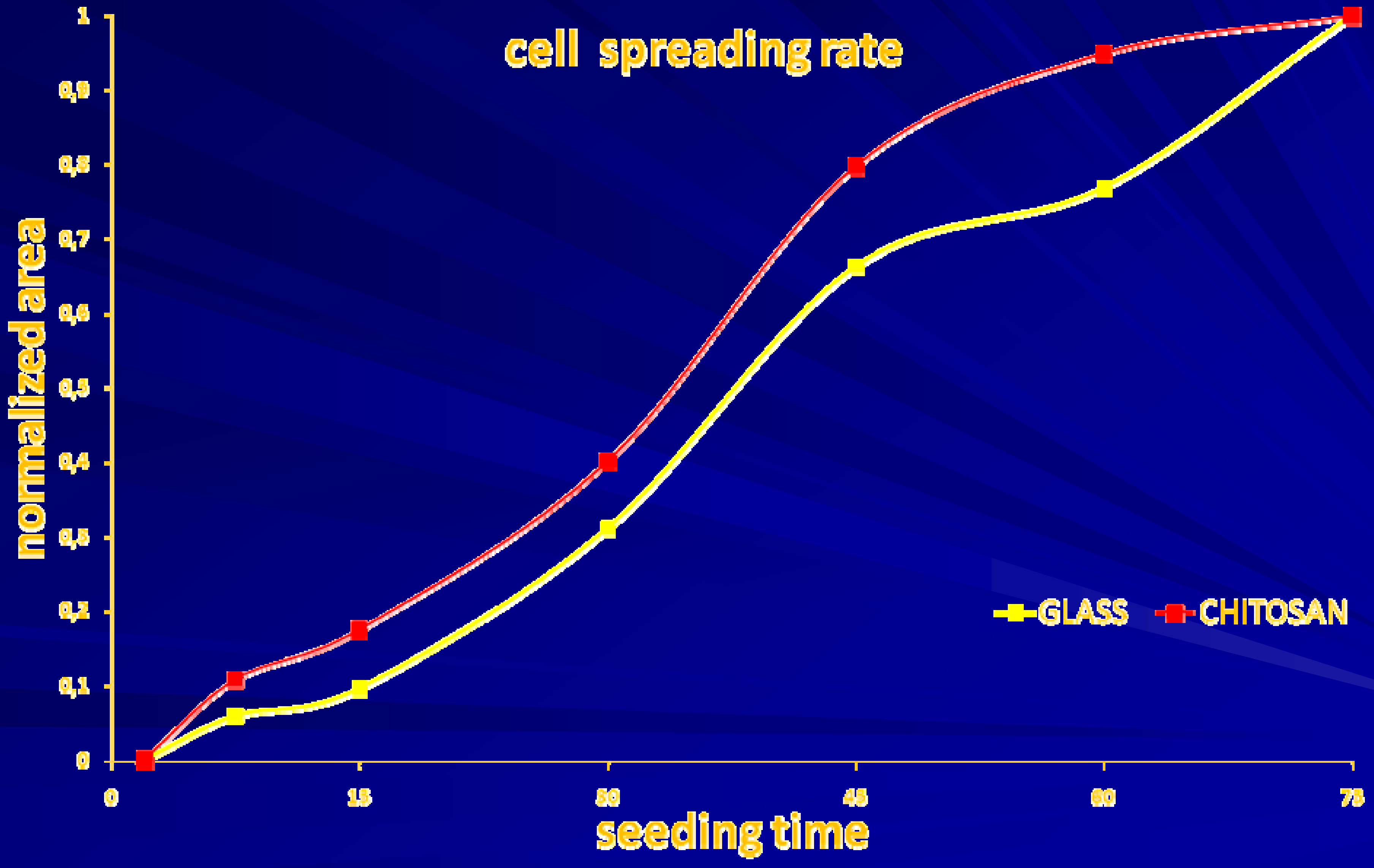


Figure 5. Mean ± SD values of average cell area attached on control and chitosan surfaces. Values are calculated using IMAGE PRO for equal random SEM images of the samples.

control vs chitosan: $p<0.05$ for 15 and 30 minutes and $p<0.002$ for 45 and 60 min. (Student's t-test)

Results


cell spreading rate
normalized area
seeding time
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
15
30
45
60
75
GLASS
CHITOSAN

Results

B. 3. Cell detachment strength

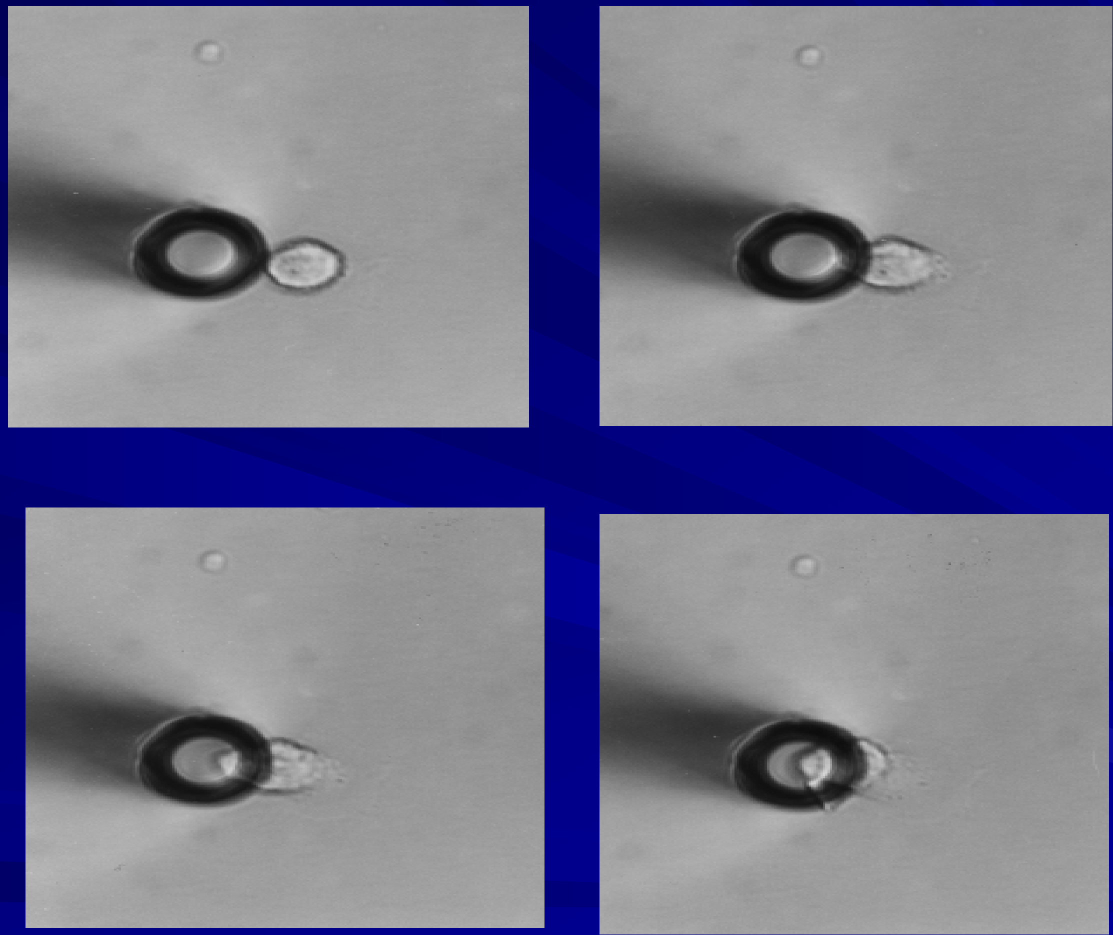


Figure 8. Four successive stages of the detachment process of an osteoblast.

Results

B. 3. Cell detachment strength

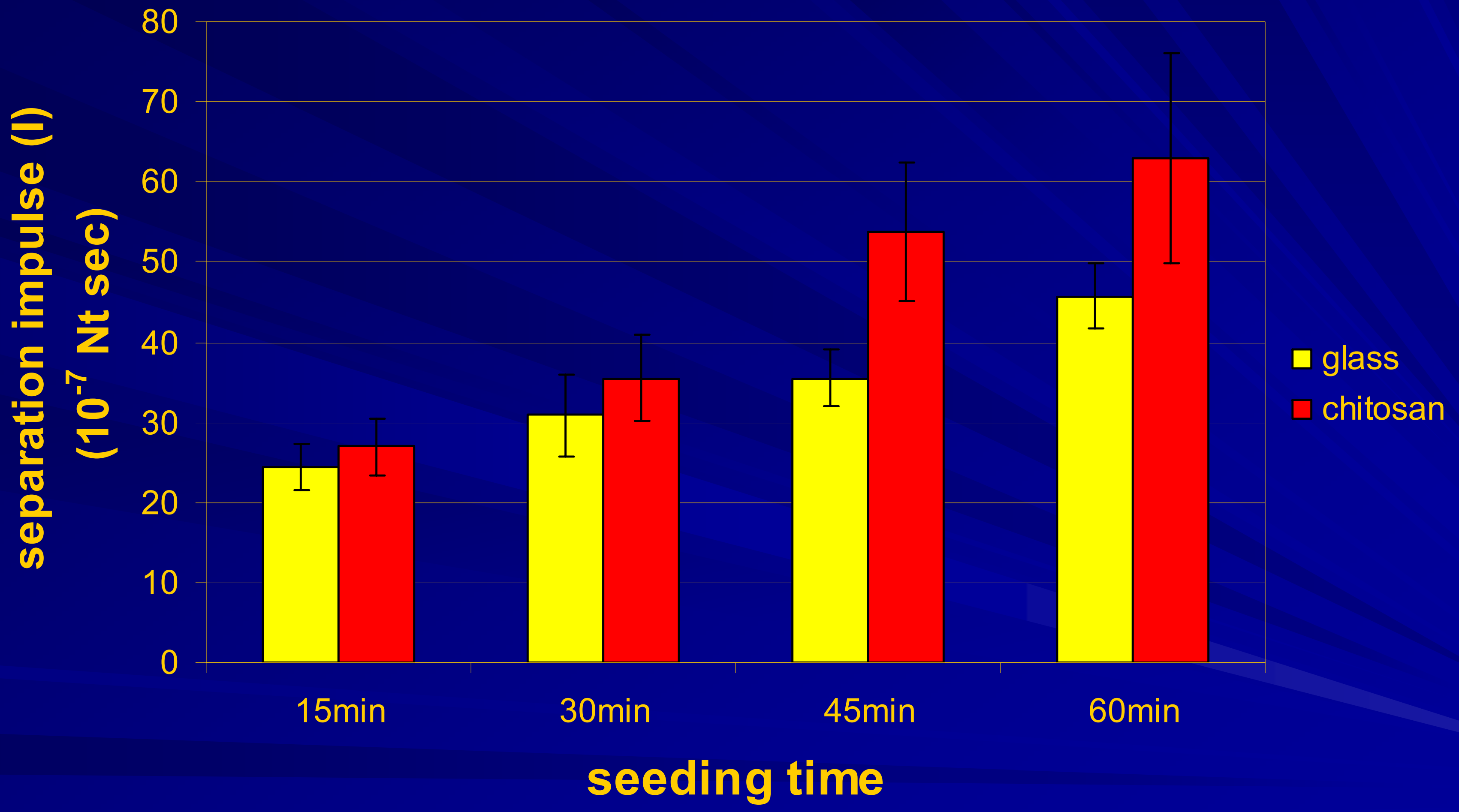


Figure 7. Detachment strength of osteoblasts at different attachment times for non-coated and chitosan-coated glass. Differences between substrates were statistically significant ($p<0.05$) for 45 and 60min

Conclusions

- Enhancement of attachment strength is in agreement with previous reported results indicating high attachment and growth rates of osteoblasts or fibroblasts on highly de-acetylated chitosan films.

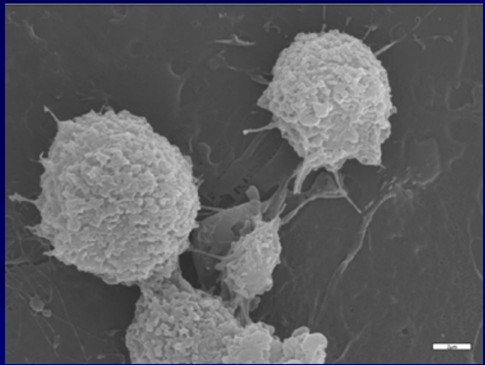
Bumgardner J et al, J Biomater Sci Polymer Edn (2003)

Fang N et al, Macromol Biosci (2005)

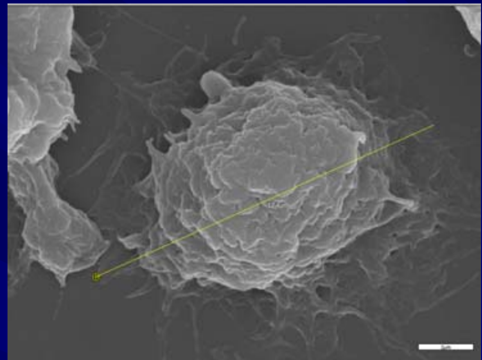
- **The results support the hypothesis that chitosan has the potential to be used as a coating for tissue engineered scaffolds.**

- **Methods of determining cell attachment. Precise with what is measured:**
  - **Number of cells**
  - **Strength**
  - **Cell Area**

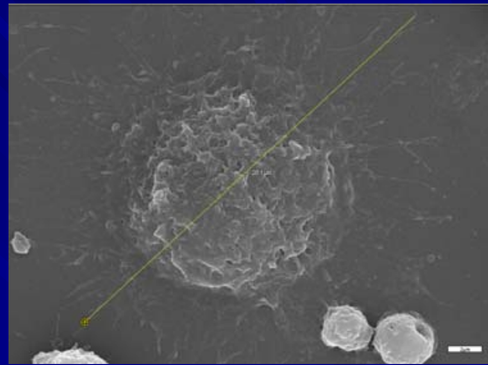
Osteoblasts on chitosan in different phase of adhesion



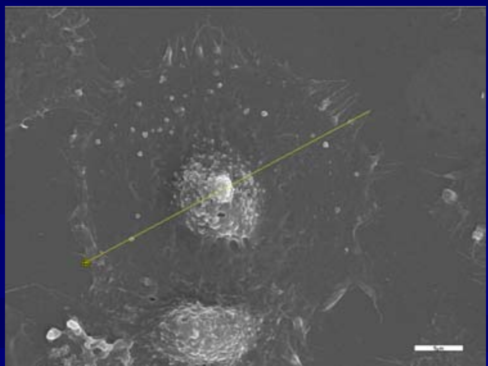
7,5 min (x 5000)



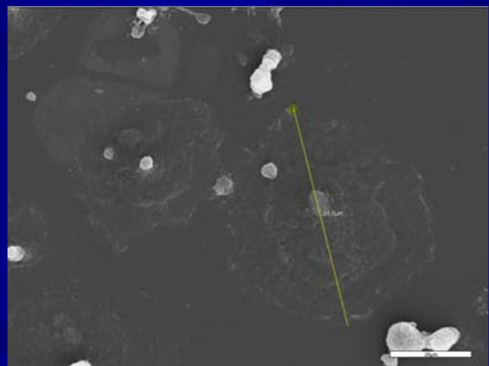
15 min (x 5000)



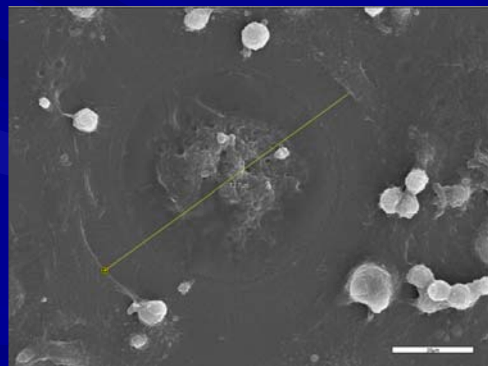
30 min (x 5000)



45 min (x 2000)



60 min (x 1000)



75 min (x 1000)

Bovine pericardial tissue scaffold

- Acellular xenogeneic tissues have been used as alternatives to polymeric scaffold materials in TE research. Benefits:
  - Extracellular matrix similar in composition-structure with tissues to be replaced
  - Availability (animal tissues)
  - Biocompatibility

- Cardiovascular TE scaffolds need to meet specific biomechanical characteristics. Special focus has to be directed in:
  - Dynamic mechanical behavior
  - Balanced cell-tissue growth vs. scaffold degradation-remodeling
  - Quality factor higher – high risk of cardiovascular system malfunction.

Decellularization protocols

Mechanical characteristics

BPF :

- Fresh bovine pericardial tissue (control)
- Storage: 0,9% NaCl, 4^{o}C

BP1: Triton® solution, 12 hr., 4°C

- Deionized water, 2 hr., 4°C
- 1% Triton® X- 100, 0.1% SDS, 150mM NaCl και deoxycholic acid 1%, in 10Mm Tris buffered solution, pH 7.4 , 12 hr., 4°C.
- Washout : dH_2O

BP2 : Trypsin/EDTA solution, 48 hr., 37°C

- (0,5%/0,2%), 10Mm Tris, pH 7,5, Rnase A (20μg/ml) και DNase (0,2 mg/ml),.
- Washout : PBS 1x (3 times)

Ref: - USPTO Patent Application 20090041729 (modified)
- Jun Liao , Erinn M. Joyce , Michael S. Sacks. Biomaterials 29 1065–74, 2008.

Experimental setup

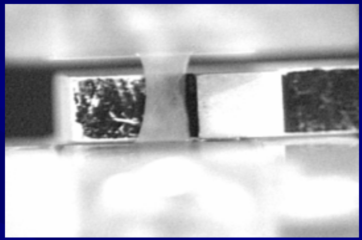
- Rectangular specimens 2,5x20 mm, cut in longitudinal, apex to base (L) and transverse (90° - T) directions
- Dynamic tensile mechanical testing (Test Resources® 800 LM electromechanical testing device)
- Cyclic loading-unloading, 1Hz, wetting by saline, 37°C.

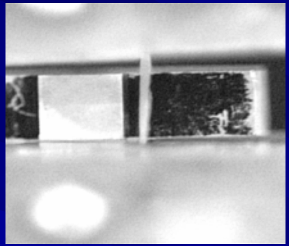
Measured data

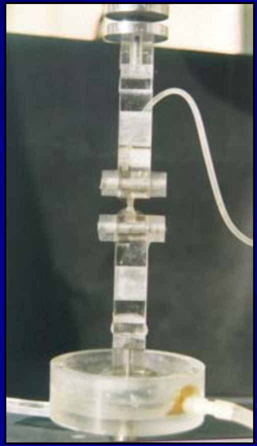
- "Free length" l_0
- Force
- Elongation
- Width
- Thickness

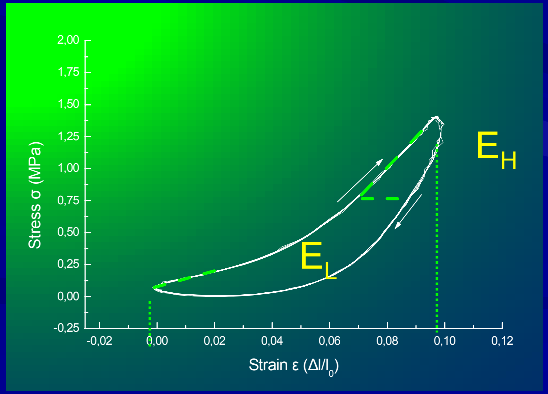
Biomechanical characteristics Computed

- Elastic modulus high (collagen phase) (E_H)
- Elastic modulus low (elastin phase) (E_L)
- Mechanical damping (hysteresis ratio) (h)



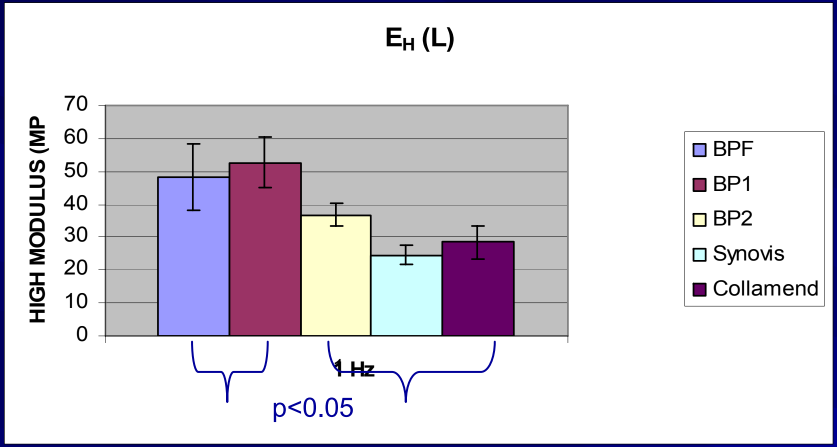


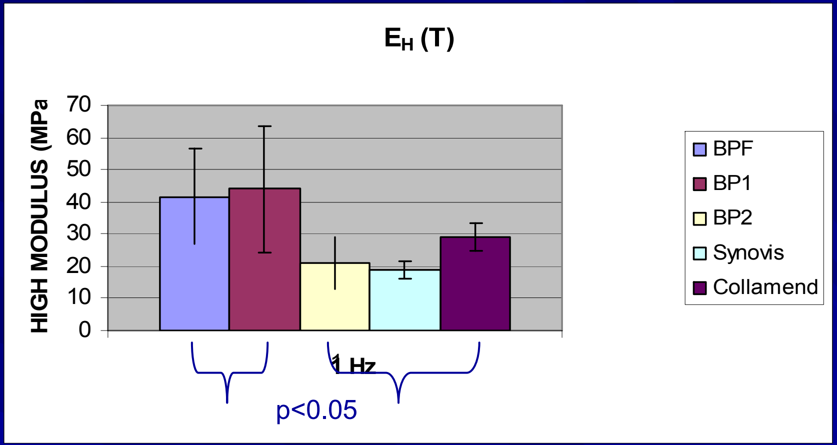




D. Mavrilas et.al. J. *Biomechanics*, 38;761-68, 2005

Results
Collagen phase E_H

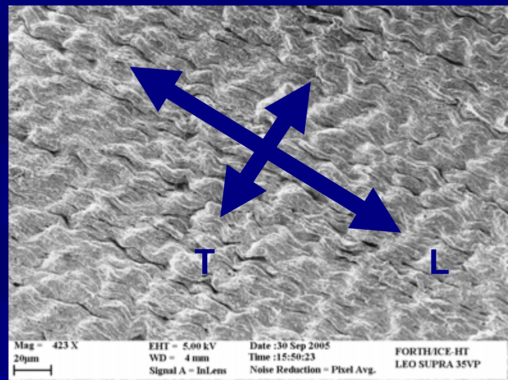


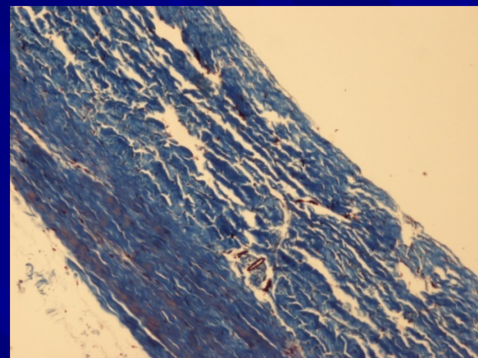


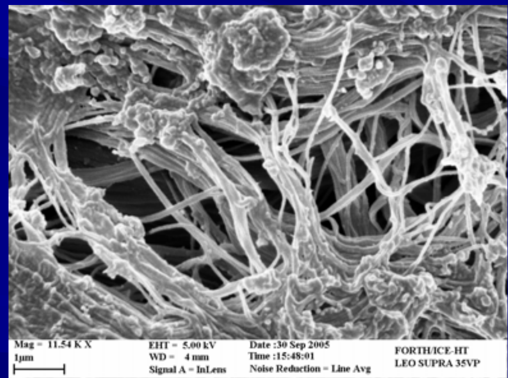
- Mean ± sdev
- Groups: BP1 similar to BPF. Statistical differences between BPF-BP1 and other groups in the same direction (One-way ANOVA, $p<0.05$).
- Anisotropy of BP, isotropy of porcine dermal tissue.

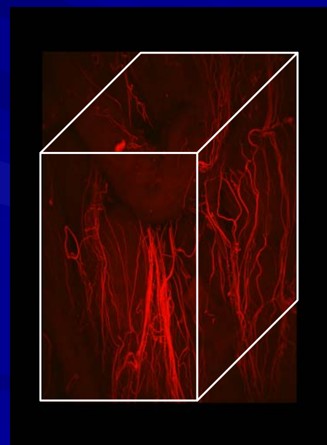
Histology-characterization

- Bovine pericardium, like other **s**oft tissues, can be mechanically considered as multilaminate reinforced composite materials.
- Collagen-elastic fibers in amorphous organic matrix (proteoglycans, glukosaminoglycans, water, soluble proteins-electrolytes.
- Undergo Cyclic Deformations at very high strains
- Exhibits hardly anisotropic, non–linear, viscoelastic mechanical characteristics.



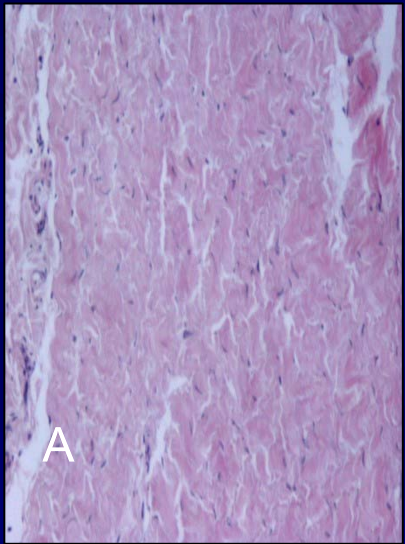


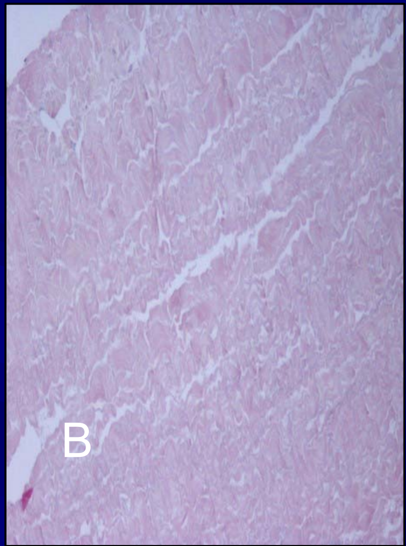


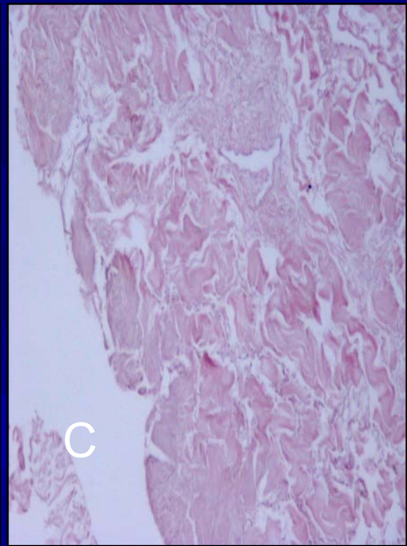


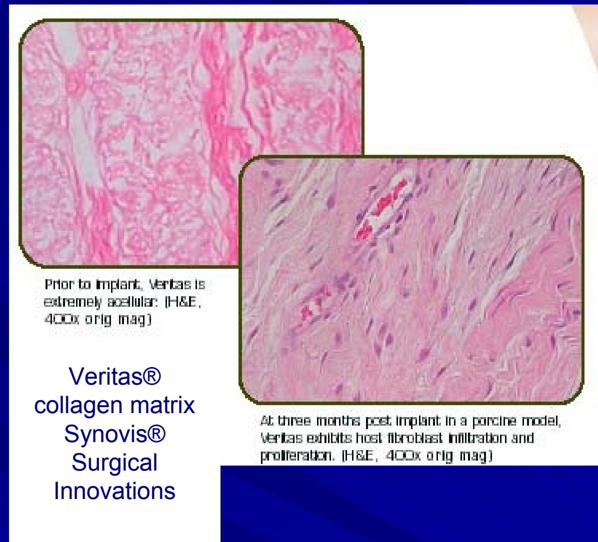
D. Mavrilas et.al. UNIVERSITY OF PATRAS, GR ESB 2010

Histology-decellularization





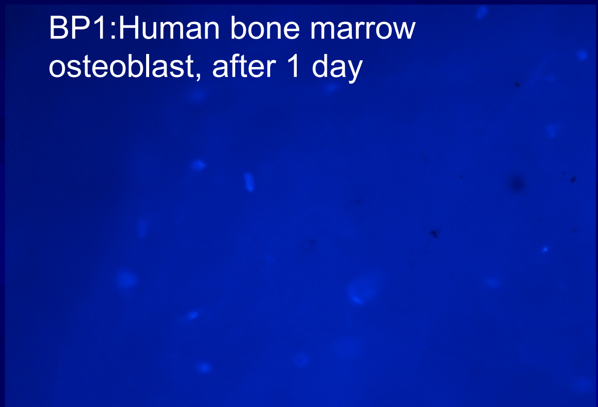




A-BPF B- BP1 C – BP2

Biomech.Characteristics-Biocompatibility

cell culture-viability



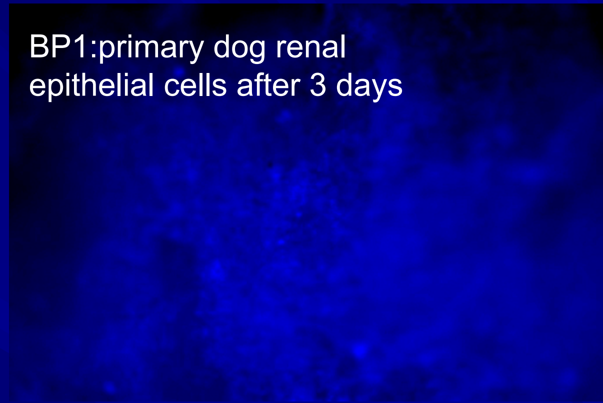
BP1:Human bone marrow osteoblast, after 1 day

Osteoblasts

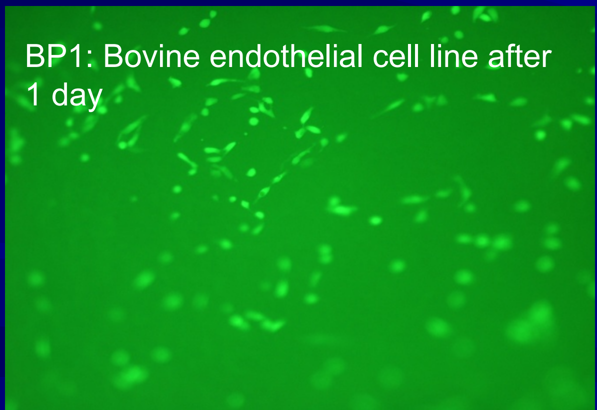
Alpha Medium + antibiotics + vitamins

Fluorescence microscopy : Dapi dye 10x

(epithelial cells)



BP1:primary dog renal epithelial cells after 3 days

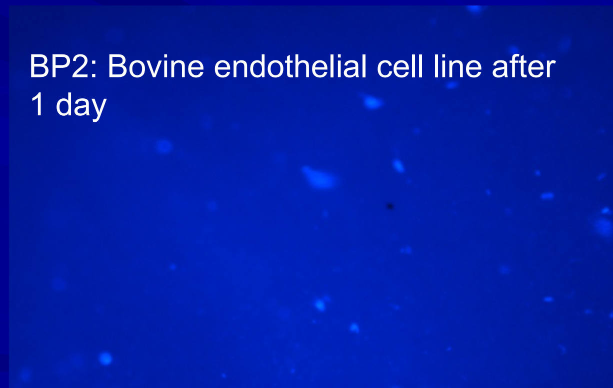


BP1: Bovine endothelial cell line after 1 day

epithelial cells

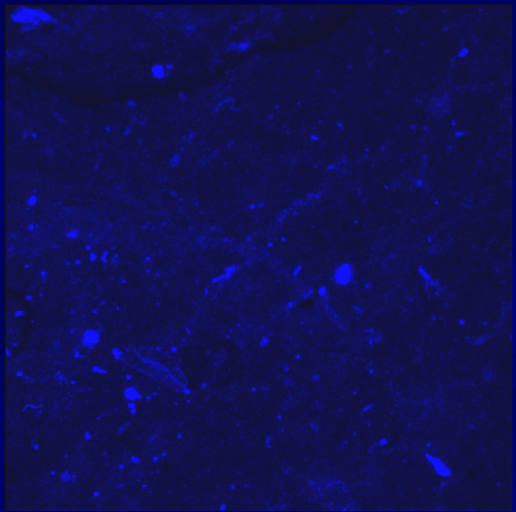
- DMEM
- + 10% FBS (fetal bovine serum)
- + 1% L-glutamine
- +1% Pen/ Strep (penicillin / streptomycin)

Fluorescence microscopy : FDA dye 10x

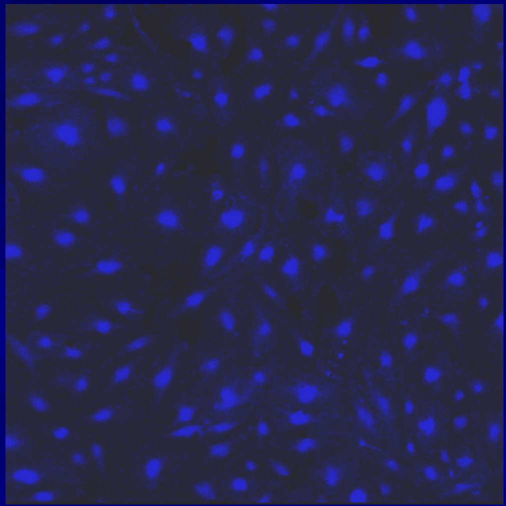


BP2: Bovine endothelial cell line after 1 day

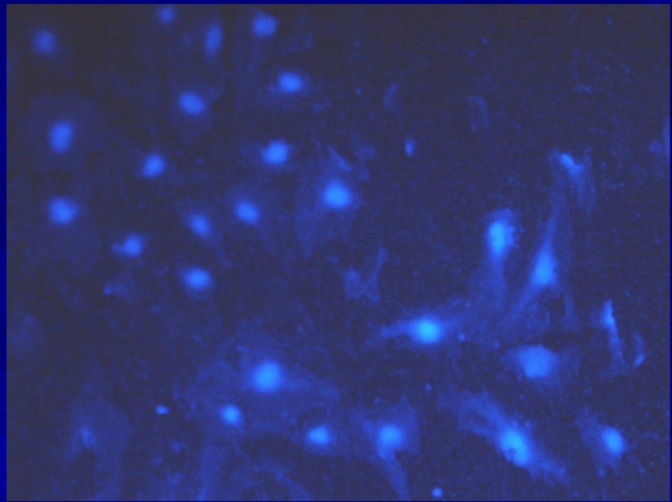
Biocompatibility fibroblast cell culture



BP2-confocal microscopy

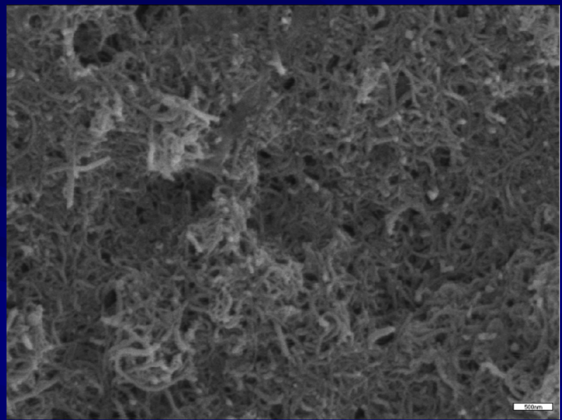


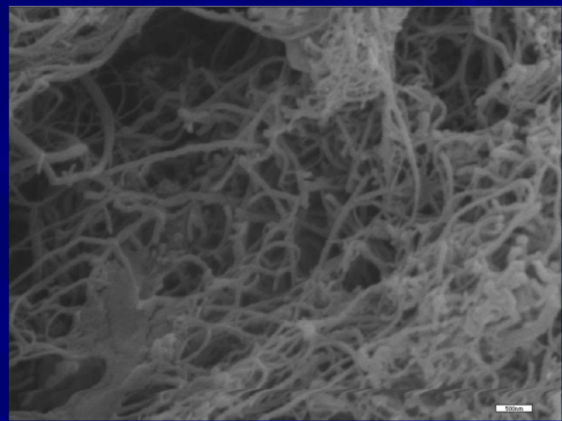
Control-confocal microscopy



Control-fluorescence microscopy

MULTI WALLED CARBON NANOTUBES (MWCNTs) for tissue engineering Scaffolds





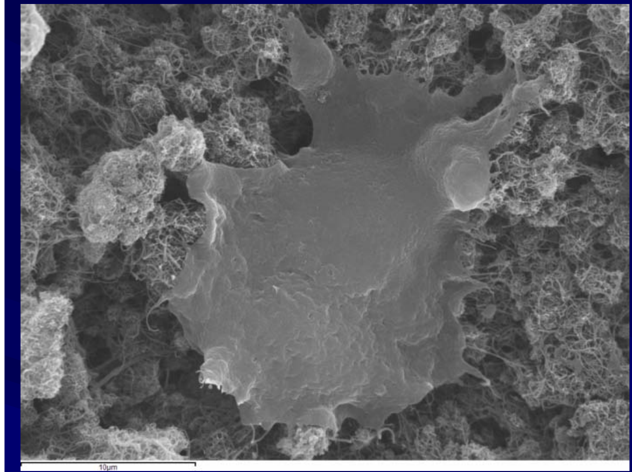
Design & synthesis of orthopedic CNTs scaffolds ------->

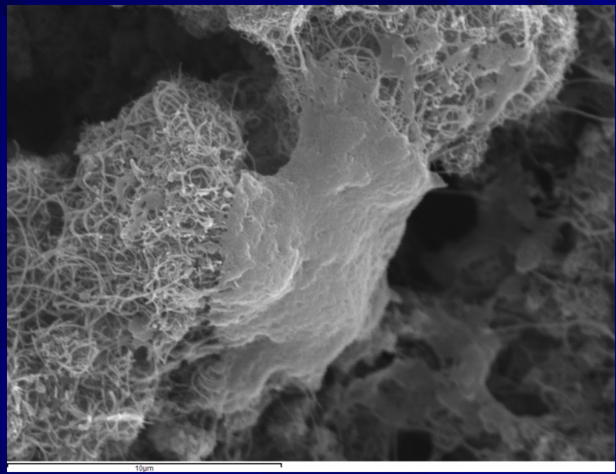
Bone properties simulation in synthetic implant formulation

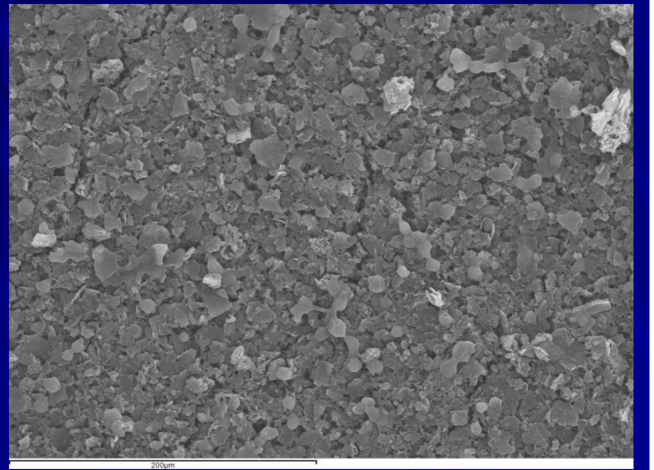
MWCNTs: -PRISTINE, -NH2, -OH, -COOH

A Kroustalli, S. Kourkouli, J. Kallitsis, D. Deligianni,

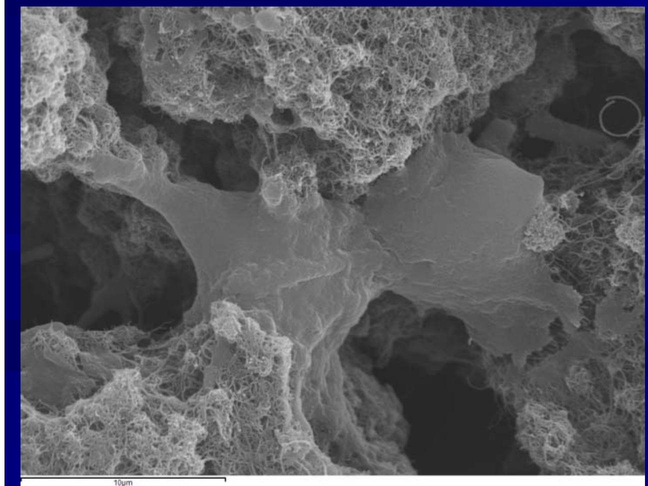
MWCNTs -OH

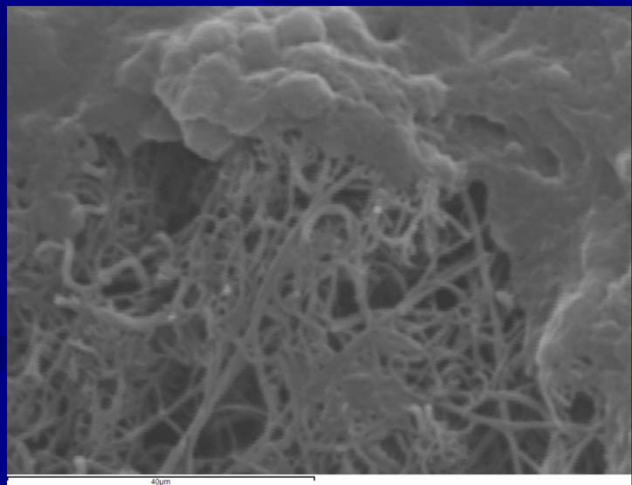


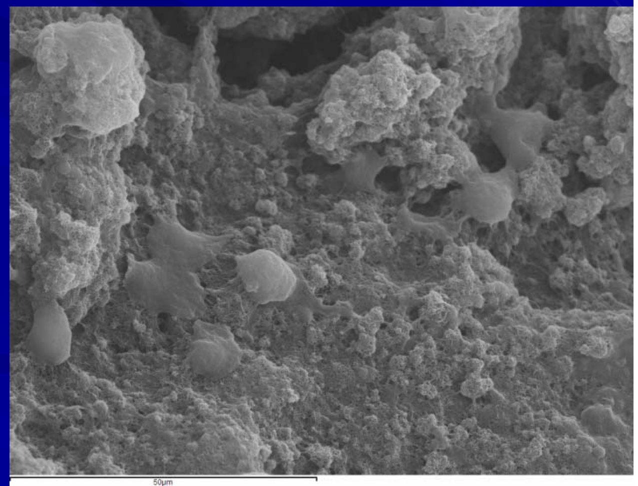




MWCNTs -COOH

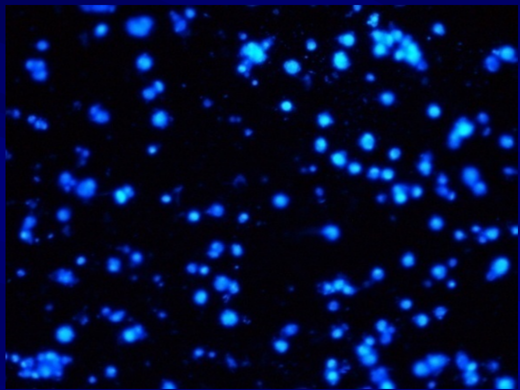




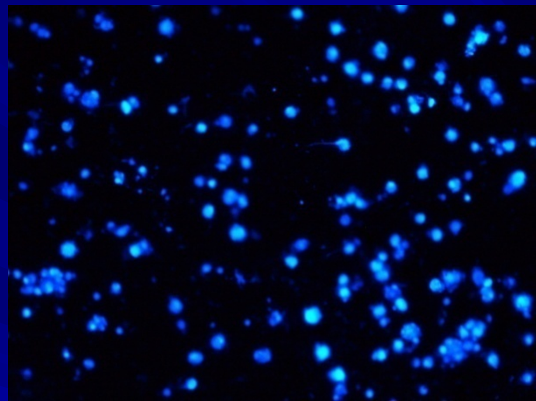


Biocompatibility

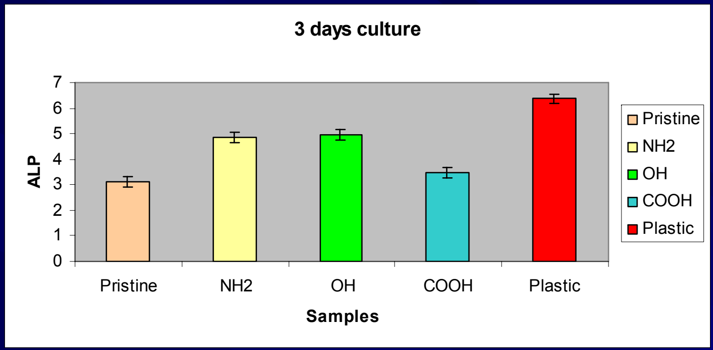
Estimation of biomechanical characteristics by Adult Stem Cells Differentiation

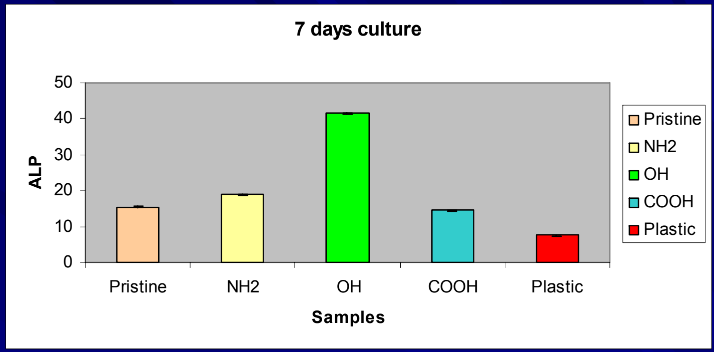


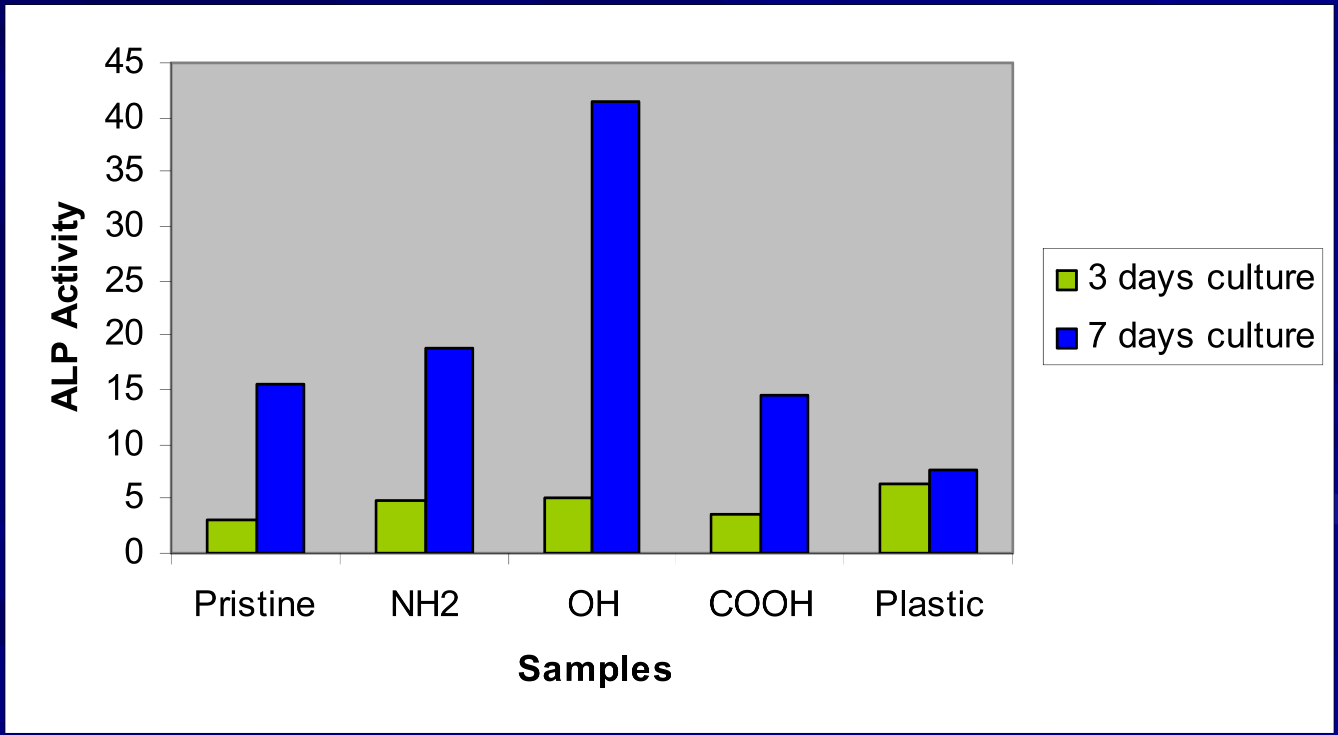
Pristine MWCNT



$-NH_2$ MWCNT


3 days culture
ALP
Pristine
NH2
OH
COOH
Plastic
Samples


7 days culture
ALP
Pristine
NH2
OH
COOH
Plastic
Samples


ALP Activity
Pristine
NH2
OH
COOH
Plastic
Samples
3 days culture
7 days culture

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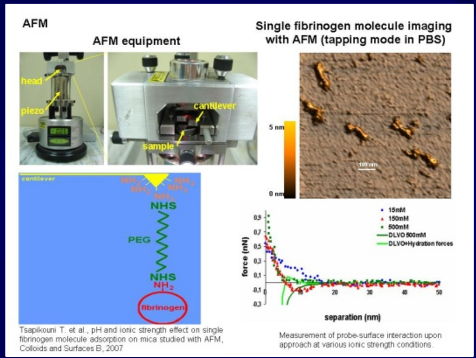
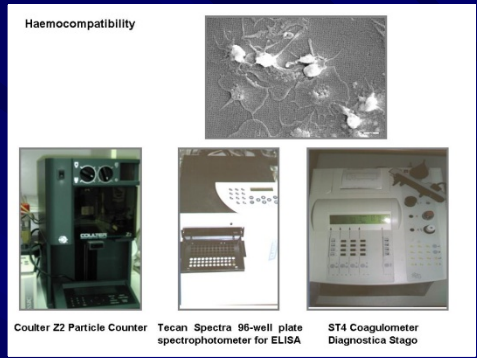
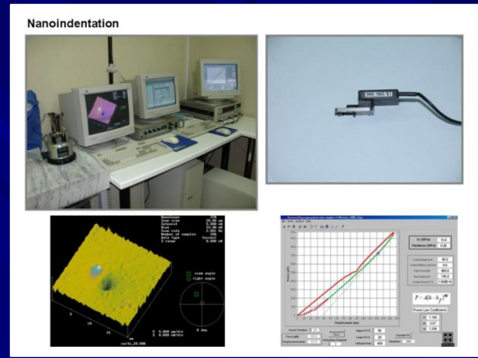


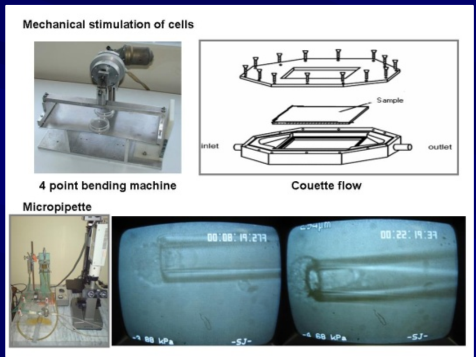
AFM



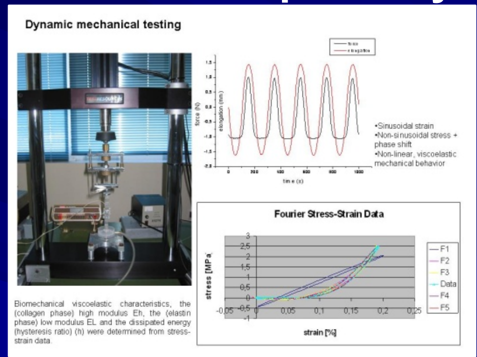
Haemocompatibility



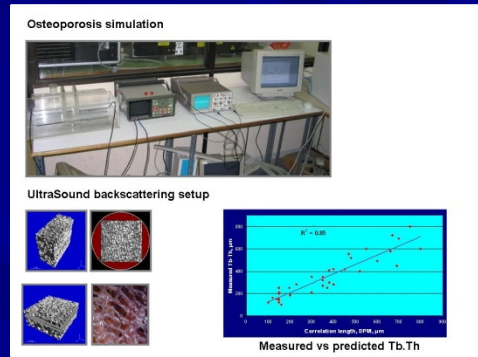
Nanoindentation



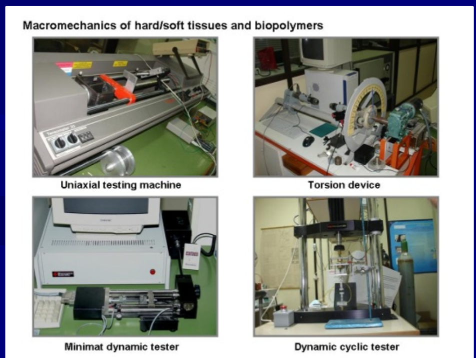
Mechanical stimulation of cells + Micropipette



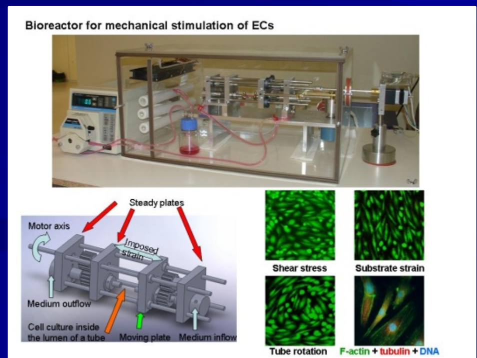
Dynamic mechanical testing



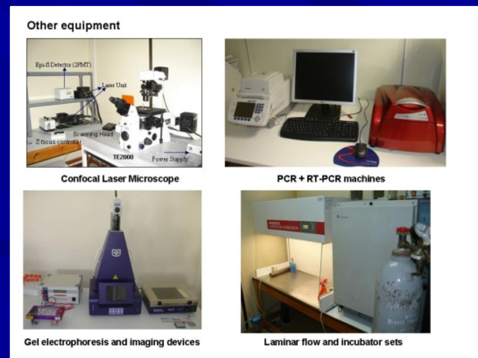
Osteoporosis simulation



Macromechanics of hard/soft tissues and biopolymers



Bioreactor for mechanical stimulation of ECs



Other equipment

- Thank you for your attention