Materials Today Virtual Conference: Biomaterials

Nanoindentation testing of a chitosan-*graft*-poly(ε-caprolactone) copolymer scaffold for soft tissue engineering

A.Skarmoutsou,[1] D. Dragatogiannis,[1] M. Kaliva,[2,3] C. Pontikoglou,[4] M. Vamvakaki ,[2,3] M. Chatzinikolaidou,[2,3] C.A. Charitidis [1]

[1] *Department of Materials Science and Technology, School of Chemical Engineering, National Technical University of Athens, Greece*

[2] *Department of Materials Science and Technology, University of Crete, Greece*

[3] *Institute of Electronic Structure and Laser (IESL), Foundation for Research and Technology Hellas (FORTH), Greece*

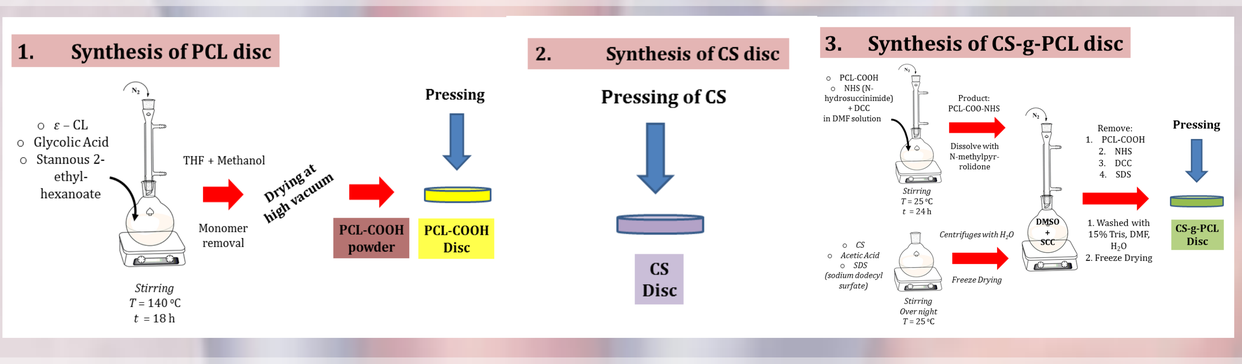
[4] *School of Medicine, Haematology Laboratory, University of Crete, Greece*

Introduction

Tissue engineering is a promising therapeutic approach in medicine for many diseases and injuries, which through a suitable scaffold cells attachment, proliferation and differentiation and tissue development I achieved. Mixing two of the most widely used components in bio-applications, such as chitosan (CS) and poly(ε-caprolactone) (PCL), is of great interest, due to the unique properties and advantages that CS and PCL present. This study aimed at the synthesis of a CS-*g*-PCL graft copolymer in order to investigate its nanomechanical properties, during the degradation process and its *in vitro* activity for the development of myocardium tissue.

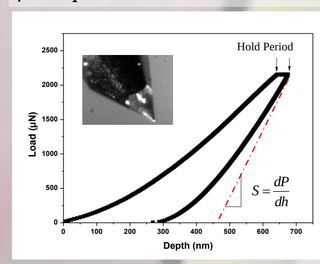
Experimental Procedure

Materials' Synthesis [1]



Nanoindentation Testing

The indentation tests were performed with a Hysitron TriboLab® test instrument. Conospherical tip with 50 μm tip radius.



Oliver & Pharr Model [2]

$$P = A(h-h_f)^m$$

$$S = \frac{dP}{dh}(h_{max}) = mA(h_{max}-h_f)^{m-1}$$

$$h_c = h_{max} - \varepsilon\frac{P_{max}}{S}$$

$$E_r = \frac{S\sqrt{\pi}}{2\sqrt{A_c}}$$

$$H = \frac{P_{max}}{A_c}$$

Hertz Model [5]

$$P = \frac{4\sqrt{R}\,E}{3(1-\nu^2)}h^{3/2}$$

Creep-based analysis [3,4]

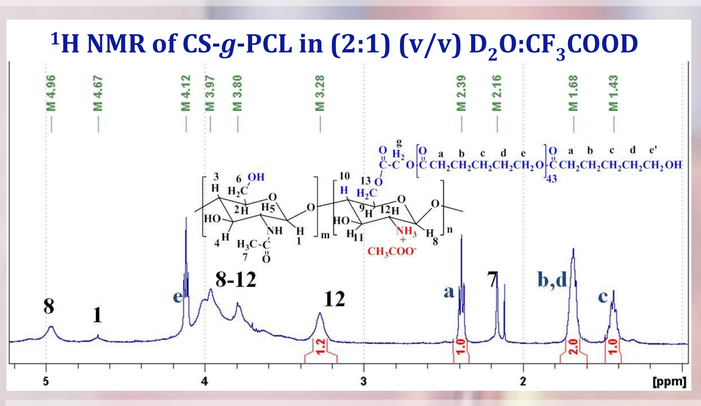
$$h^{3/2} = \frac{3k}{8\sqrt{R}}\left[C_0 t - \sum C_i\tau_i\left(1-\exp\left(\frac{-t}{\tau_i}\right)\right)\right], 0<t<t_R$$

$$h^{3/2} = \frac{3k}{8\sqrt{R}}\left[C_0 t_R - \sum C_i\tau_i\exp\left(\frac{-t}{\tau_i}\right)\left(\exp\left(\frac{-t_R}{\tau_i}\right)-1\right)\right], t>t_R$$

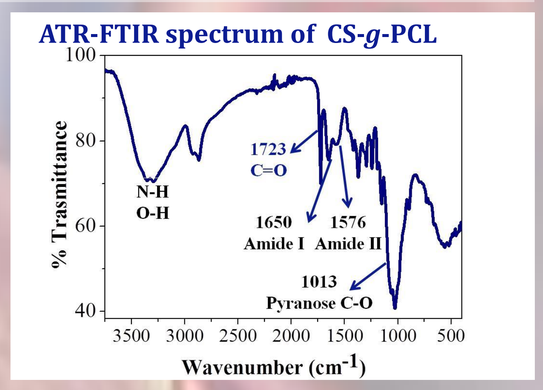
$$G_0 = \frac{1}{2J(0)} = \frac{1}{2(C_0-\sum C_i)} \longrightarrow G = \frac{E}{2(1-\nu)}$$

Results & Discussion

^{1}H NMR and FTIR spectra of CS-g-PCL



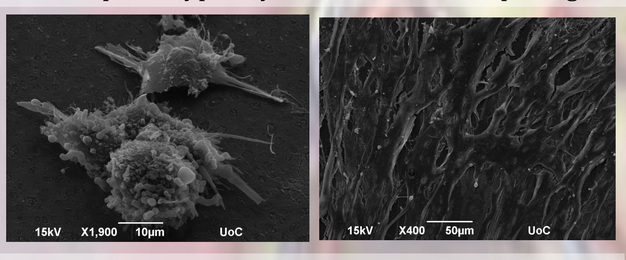
✓ The PCL grafting along the CS backbone was calculated to ~ **0.71 %**, by rationing the peak integral of **a** over **12**.



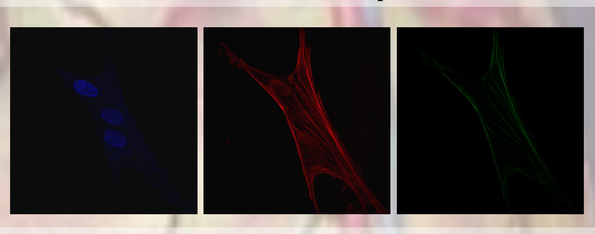
✓ The appearance of the characteristic peaks of both PCL and CS confirms the grafting of PCL along the CS backbone.

In vitro interactions of Wharton's jelly (WJ) mesenchymal stem cells (MSCs) with the CS-g-PCL copolymer

Wharton's jelly (WJ) mesenchymal stem cells (MSCs) were isolated from umbilical cord samples collected from 4 full-term neonates delivered by normal labour at the University Hospital of Heraklion, after written informed consent of the family. Samples were processed within 24 h after delivery. Cells were immunophenotypically characterized, and passages 2 and 3 were cultured in IMDM complete medium.



Scanning electron microscopy images of WJ-MSCs on the CS-*g*-PCL material: Cells extend protrusions and adhere strongly onto the material after 1 h in culture (left), and proliferate after 4 weeks (right).



Confocal fluorescence microscopy images of WJ-MSCs on CS-*g*-PCL after 24 h. Cell morphology is shown following triple staining with TOPRO for cell nucleus (purple), TRITC-phalloidin (red), and FITC-conjugated anti-vinculin antibody (green).

Conclusions

✓ The as prepared CS-*g*-PCL sample revealed low nanomechanical properties as a result of the poor mixing of the two components and the lower crystallinity of the copolymer. After submersion in the cell culture medium a sharp reduction of *H* and *E* values was observed attributed to water uptake by CS, PCL hydrolysis and increase of sample's porosity. ⇨ Stable under any applied load and mechanical properties are close to those reported for soft tissues, such as the myocardium [12], rendering the scaffold suitable for cardiac tissue regeneration.

✓ Different models were applied for the calculation of the *E* values that suggest that viscous materials have to be carefully tested (certain experimental procedure) and analyzed.

Nanomechanical Properties

As prepared samples

Load-unload curves of all samples at *P* = 500 μN. High resistance is attributed to the CS and PCL samples ⇨ higher nanomechanical properties.

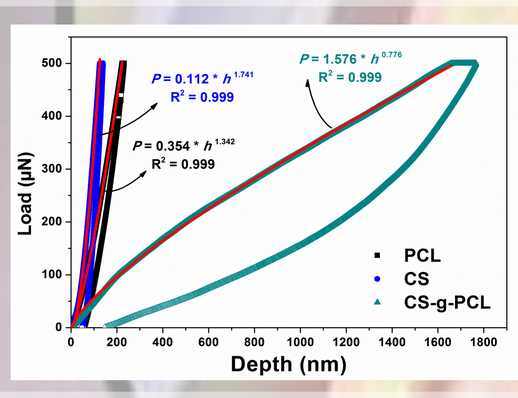


Table Young's Modulus and hardness values at 1000 nm indentation depth.

Sample	Young's modulus (MPa)	Hardness (MPa)
PCL	443 ± 1	13 ± 1
CS	615 ± 9	54 ± 1
CS-*g*-PCL	87 ± 9	4 ±1

Low resistance and nanomechanical properties of CS-*g*-PCL because of:

✓ Poor mixing between the two components,

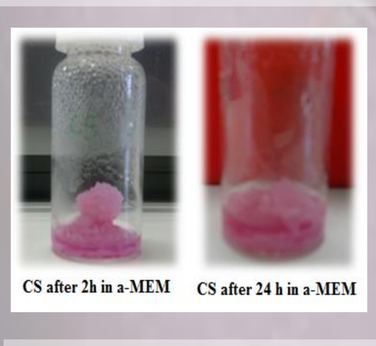
✓ High porosity of the sample and

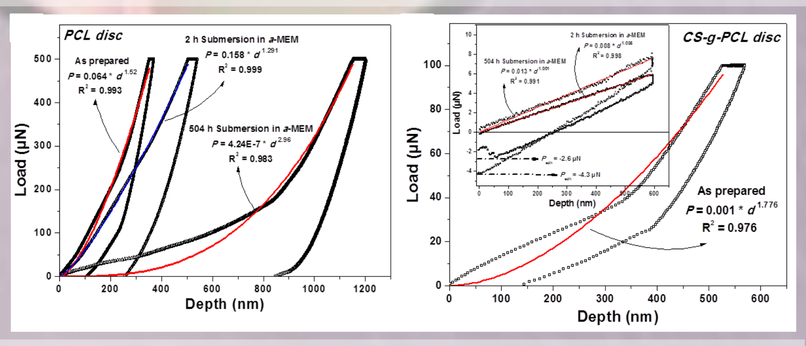
✓ Lower crystallinity due to the graft nature of the copolymer [6-9].

⇨ ***Amorphous state*** ⇨ *Suppression of PCL crystallization.*

Submersion in a-MEM buffer at 37°C for a period of 4 weeks

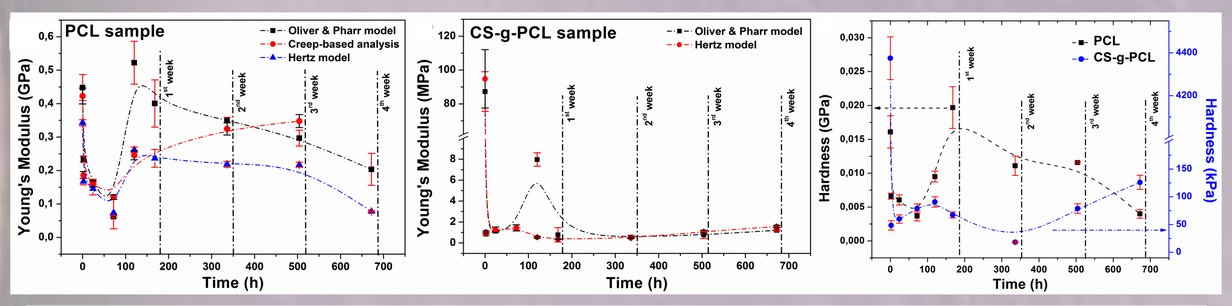
All samples were submerged in *a*-MEM buffer and their nanomechanical properties at certain periods were evaluated. CS was completely dissolved within 24 h.





PCL: exhibits softening, i.e. reduction of resistance at the same applied load.

CS-*g*-PCL: more softening ⇨ reduction of nanomechanical properties by almost one order of magnitude.



Decrease of *H* and *E* values due to degradation.

Creep-based analysis: takes into consideration the viscous behavior of PCL and provides more accurate *E* values.

Hertz model: same trend but lower *E* values than the Oliver & Pharr model.

PCL: heterogeneities in the sample ⇨ amorphous regions [10] + random chain fracture result in porosity [11] ⇨ reduction of *H* and *E* values.

CS-*g*-PCL: water uptake by CS + hydrolysis of PCL + pores ⇨ reduction of *H* and *E* values.

European Union
European Social Fund
OPERATIONAL PROGRAMME
EDUCATION AND LIFELONG LEARNING
investing in knowledge society
MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS, CULTURE & SPORTS
MANAGING AUTHORITY
NSRF 2007-2013
EUROPEAN SOCIAL FUND
Co-financed by Greece and the European Union

References

1. Cai G., Jiang H., Tu K., Wang L., Zhu K., *Macromol. Biosci.* **9**:256 (2009).
2. Oliver W.C. and Pharr G.M., *J. Mater. Res.* **7**:1564 (1992).
3. Bembey A.K., Oyen M.L., Bushby A.J., Boyde A., *Philosoph. Magaz.* **86**(33-35):5691(2006)
4. Oyen M.L., *J. Mater. Res.* **20**:2094 (2005).
5. Johnson KL. Contact mechanics. Cambridge: Cambridge University Press; 1985.
6. Honma T. , Senda T., Inoue Y., *Polym Int.* **52**:1839 (2003).
7. Wan Y., Wu H., Cao X., Dalai S., *Polym. Degrad. Stabil.* **93**(10):1736 (2008).
8. Cai G., Jiang H., Chen Z., Tu K., Wang L., Zhu K., *Europ. Polym. J.* 45(6):1674 (2009).
9. García Cruz D.M., Gómez Ribelles J.L., Salmerón Sánchez M., *J. Biomed. Mater. Res. B* **85**:303 (2008).
10. Shalaby S.W., Biomedical polymers, Hanser Publishers, New York (1994).
11. Zhang Q., Jiang Y., Zhang Y., Ye Z., Tan W., Lang M., *Polym. Degrad. Stabil.* **98**:209 (2013).
12. Hiesinger W. et al., *J. Thorac. Cardiovasc. Surg.* 143(2):962 (2012).