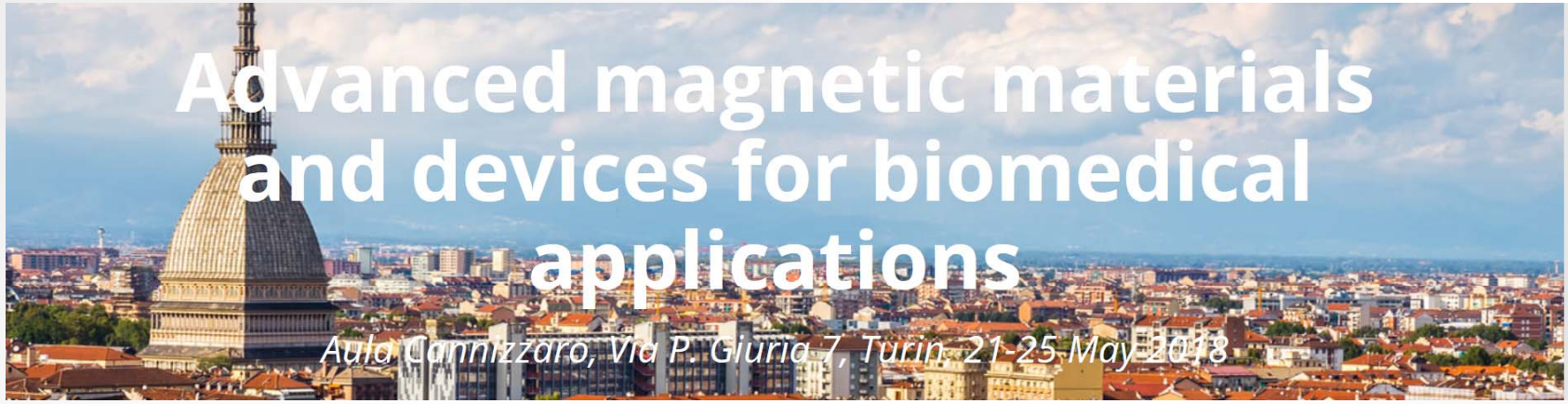
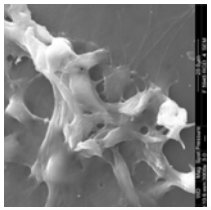
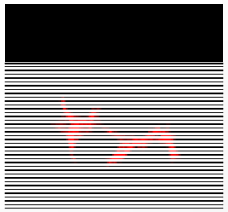
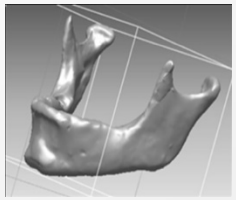
Italian School on Magnetism

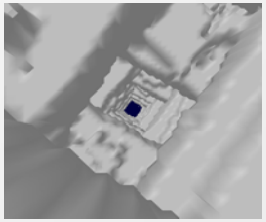


Design and Additive Manufacturing of Scaffolds for Bone Tissue Engineering





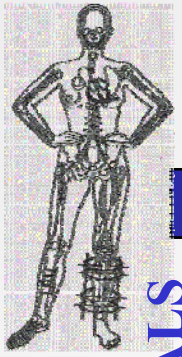




Antonio Gloria

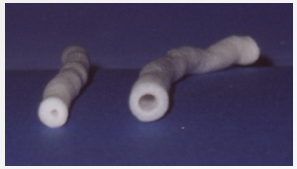
Institute of Polymers, Composites and Biomaterials (IPCB)
National research Council of Italy

Biomaterials and Tissue Engineering



BIOMATERIALS

1993



ligament

IVD

meniscus

2003

2005

hip

tooth

Restorative Materials

disc

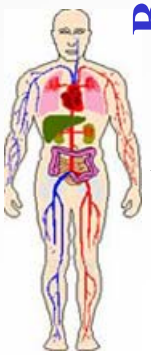
cage

mandible

custom made and morphologically-controlled scaffolds

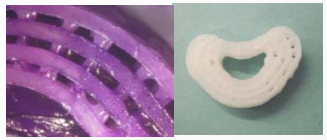
2007

2016

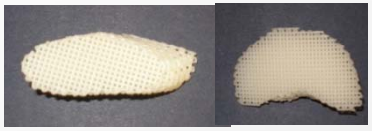


SCAFFOLDS

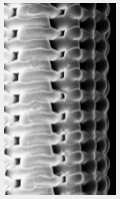
Magnetic Scaffold



IVD



Mandibular ramus and symphysis



Stereolithograhy

Envisiontec® Perfactory Mini Multi lens

3D-printing

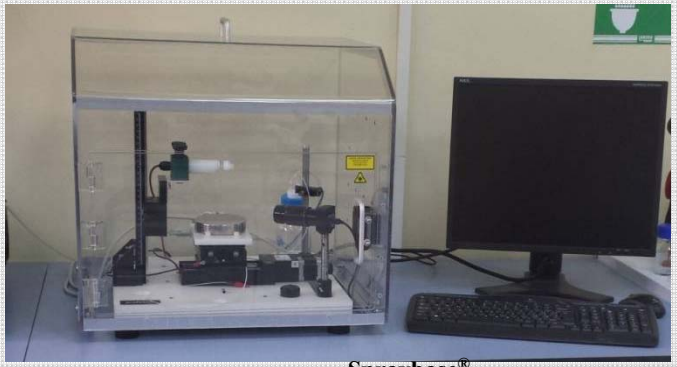
ZPrinter® 310

Bioplotter

Envisiontec® Bioplotter

HomeMade Bioextruder/Bioinjector system (National Instrument™/Labview™)

CAD-CAM System

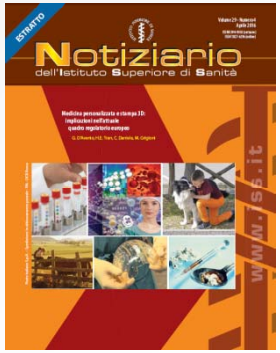


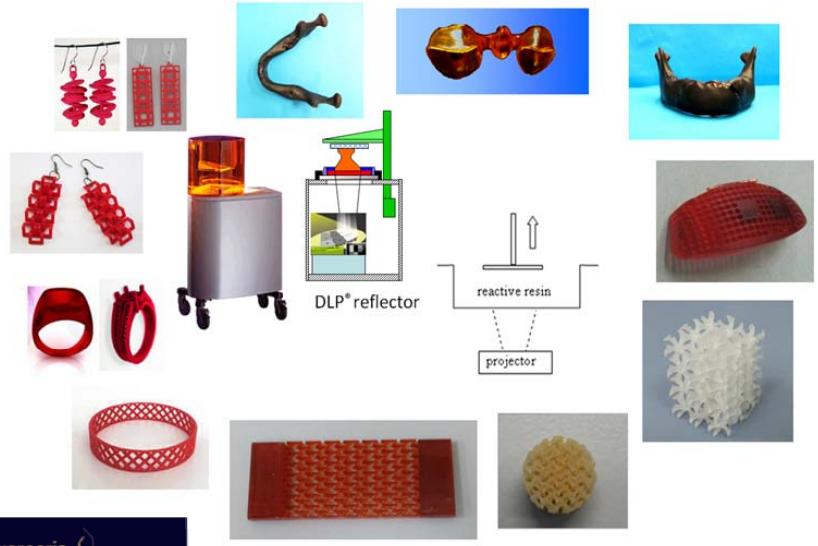
Spraybase®

Fused Deposition Modeling

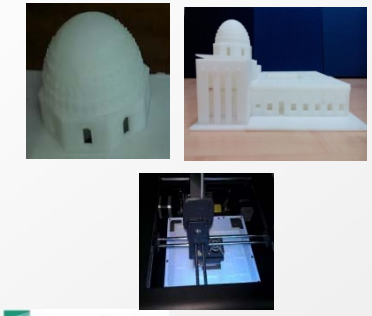
Shoe Factory

Cultural Heritage





iPCB INSTITUTE FOR POLYMERS, COMPOSITES & BIOMATERIALS

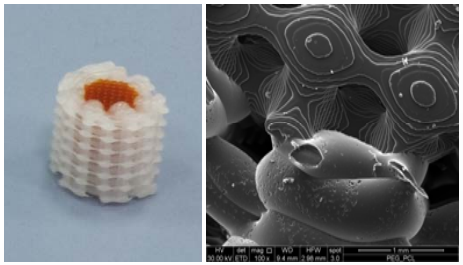


Fraunhofer

Fraunhofer

ideas INTERACTIVE DESIGN AND SIMULATION

CENTER OF DIVERSE MANUFACTURING ADDITIVE MANUFACTURING INNOVATIONS creamni

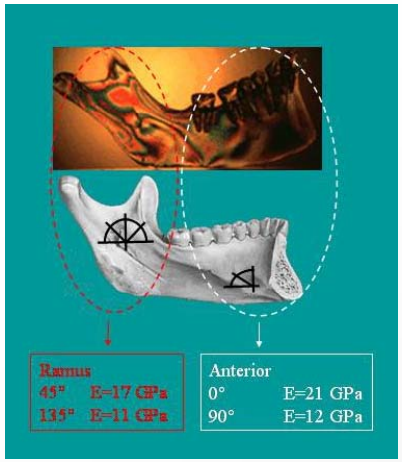


Materials – Design - Technology

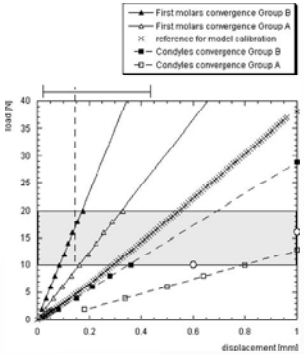
Experimental and theoretical composite model of the human mandible

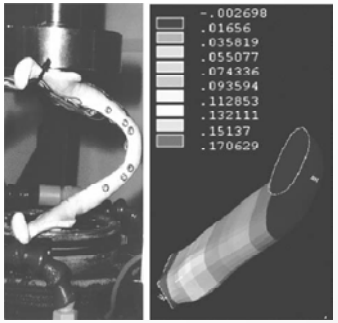
Composite Model

Additive Manufacturing
& Composite Materials Technology



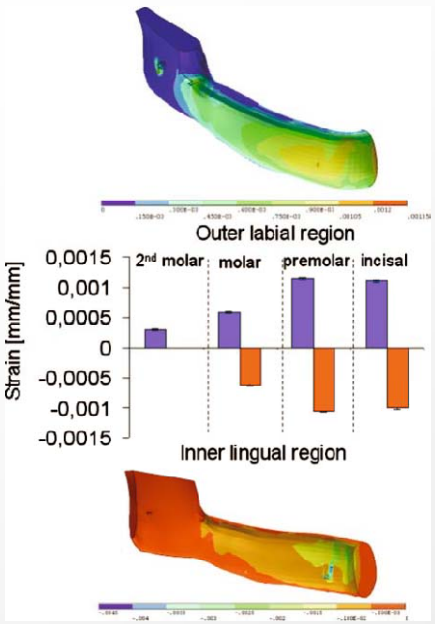
PMMA-based inner core
Composite outer shell (glass fibre reinforced epoxy)



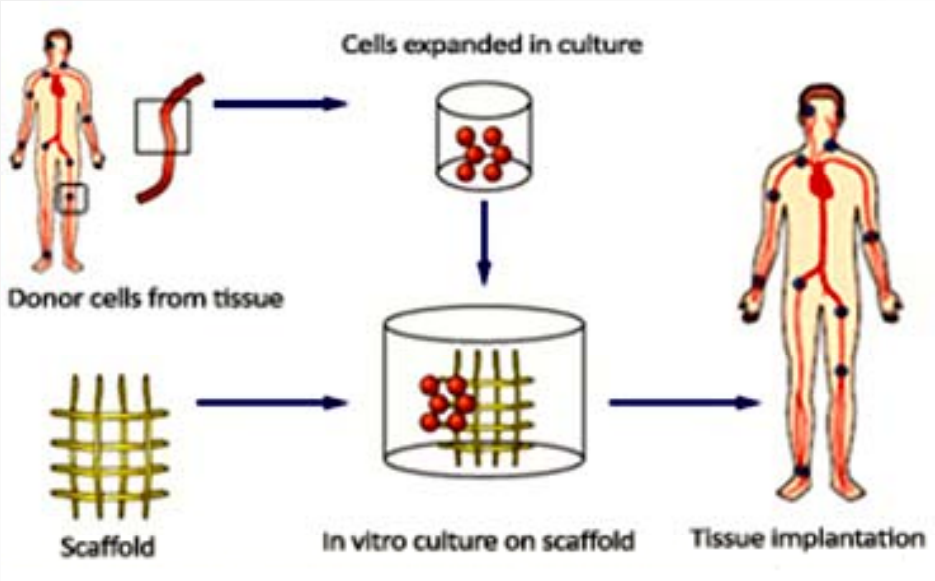


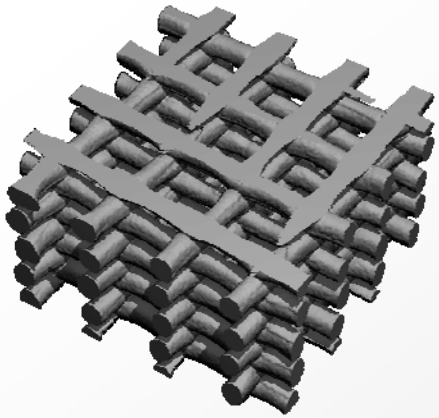
Suitable mechanical properties …

Osteon orientation and mechanical properties



Tissue Engineering & Scaffolds

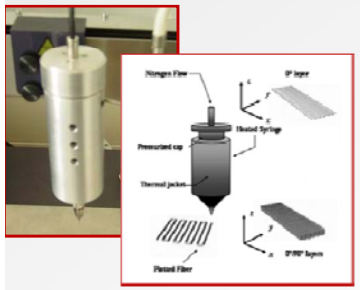


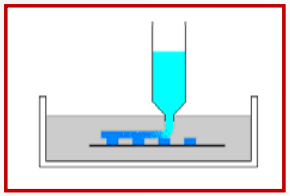


Source: *www.hinnovic.org*

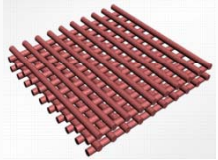
FDM/3D Fiber Deposition: Technical Features

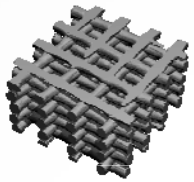
BIOPLOTTER

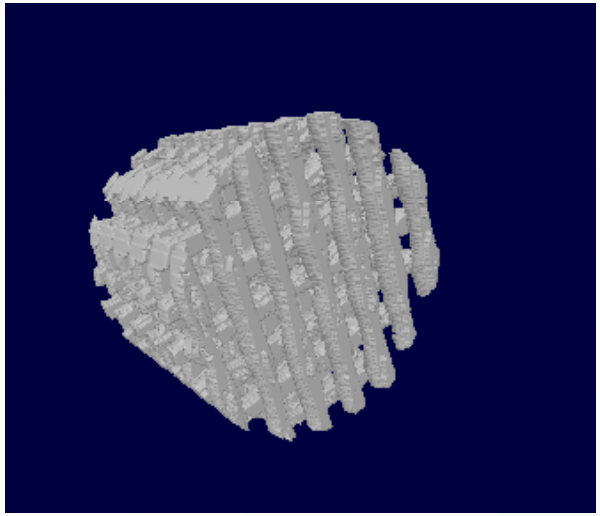


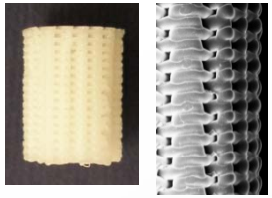


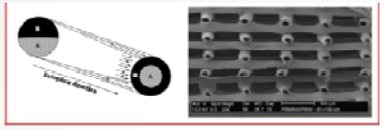
3D interconnected pore network







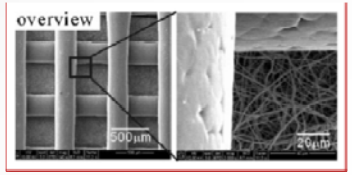


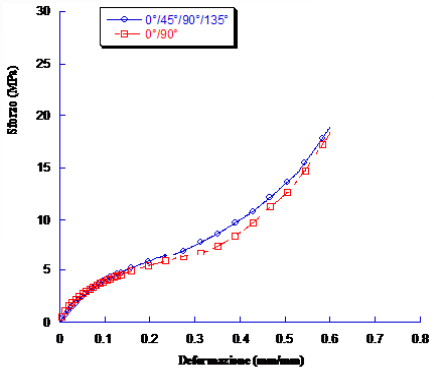


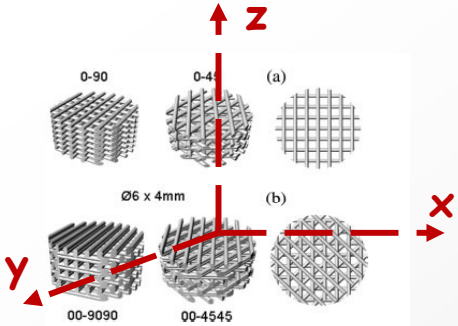
Hollow Fibers

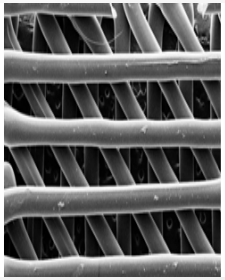
&

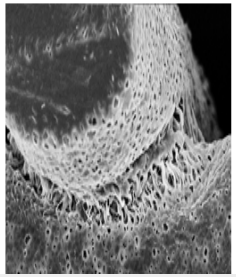
Shell-Core configuration









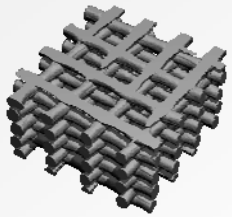


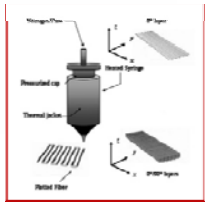
… to tailor architecture/morphology, mechanical and mass transport properties

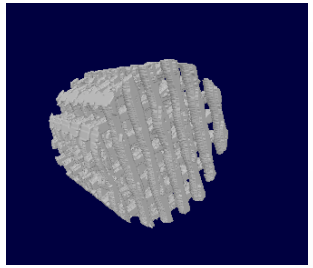
Design of Scaffolds for Tissue Engineering

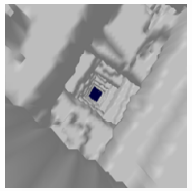
FDM/3D Fiber Deposition: Technical Features

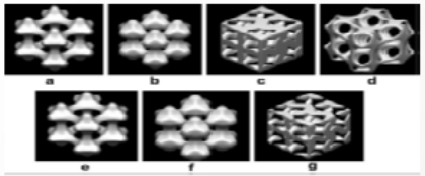
Design-Material-Technology





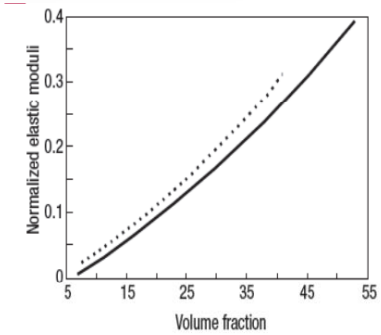






Topology Optimization

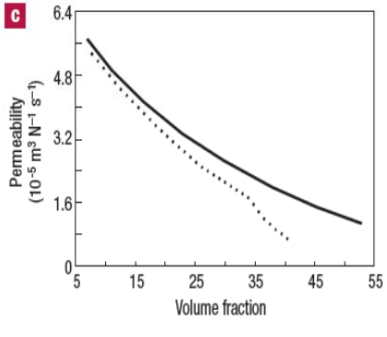
Stiffness/Elastic Modulus



$$C_{ijkl} = \rho^p C_{ijkl}^{\text{base}},$$

$$C_{ijkl}^{\text{macro}} = \frac{1}{|V_{\text{unit cell}}|} \int_V C_{ijpm}^{\text{micro}} \, M_{pmkl} \, \mathrm{d}V_{\text{unit cell}}$$

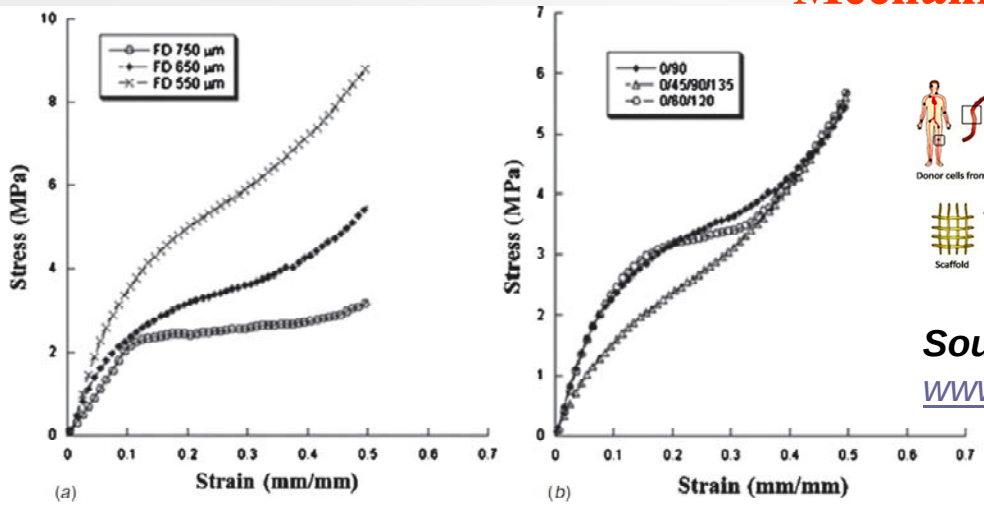
Permeability

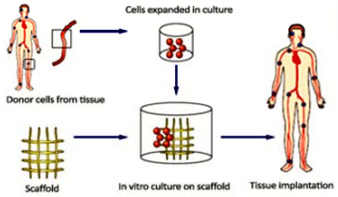


Nature Materials; 4: **518 – 524**

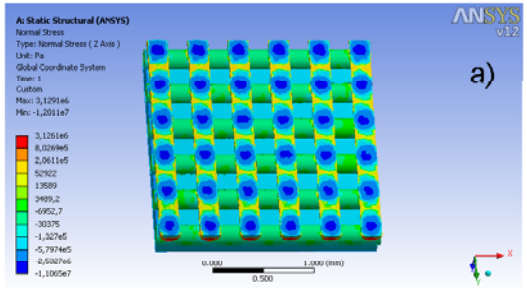
PCL Scaffolds

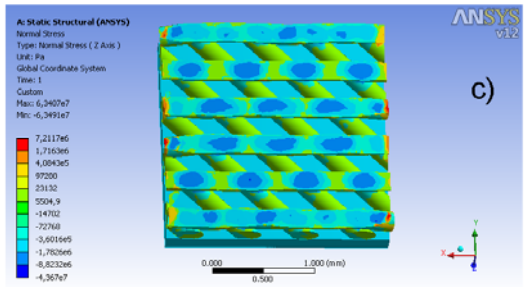
From Design to Mechanical properties …





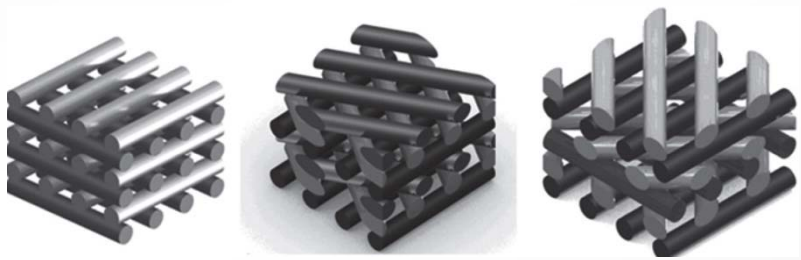
Source:
www.hinnovic.org





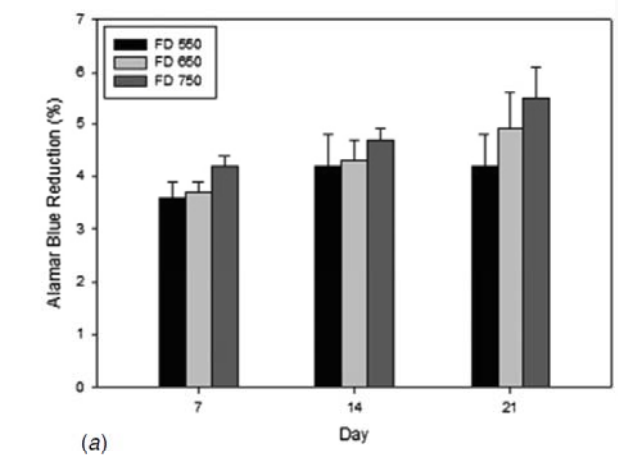
Lay-down pattern	Compressive modulus E (MPa)	Maximum stress σ_{max} (MPa)
0°/90°	34.2 ± 3.8	5.6 ± 0.2
0°/60°/120°	30.5 ± 4.5	5.0 ± 0.6
0°/45°/90°/135°	19.1 ± 2.8	4.9 ± 0.3

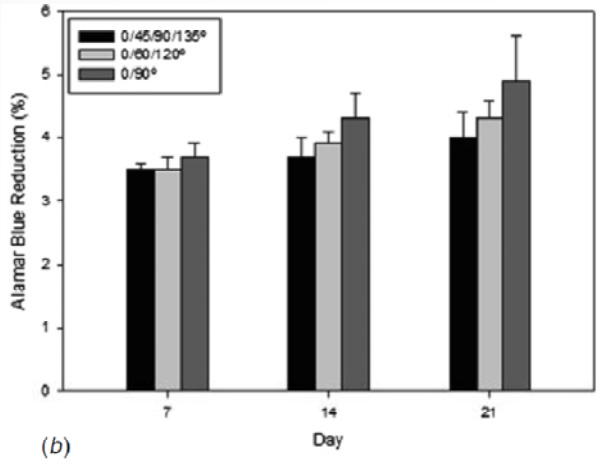
Effect of architectures/lay-down pattern, pore size & shape



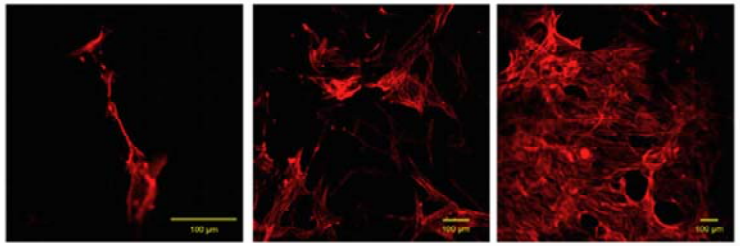
Filament distance FD (μm)	Compressive modulus E (MPa)	Maximum stress σ_{max} (MPa)
750	23.1 ± 2.8	2.7 ± 0.5
650	34.2 ± 3.8	5.6 ± 0.2
550	52.5 ± 4.5	8.7 ± 1.2

From Design to Mechanical/Functional properties and Biological Properties …



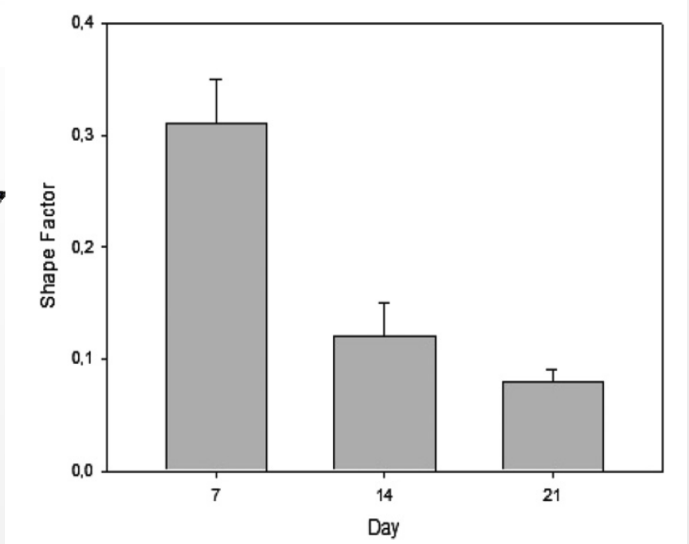


Cell Viability/Proliferation

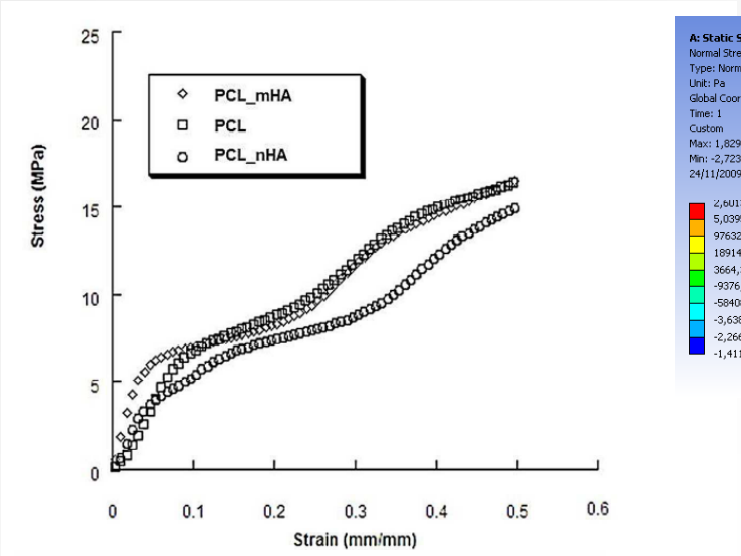


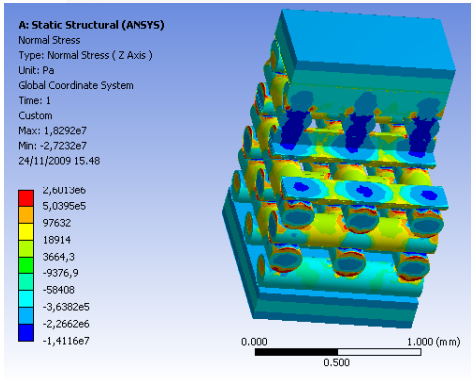
$$\Phi = \frac{\Theta A}{P^2}$$

Shape Factor



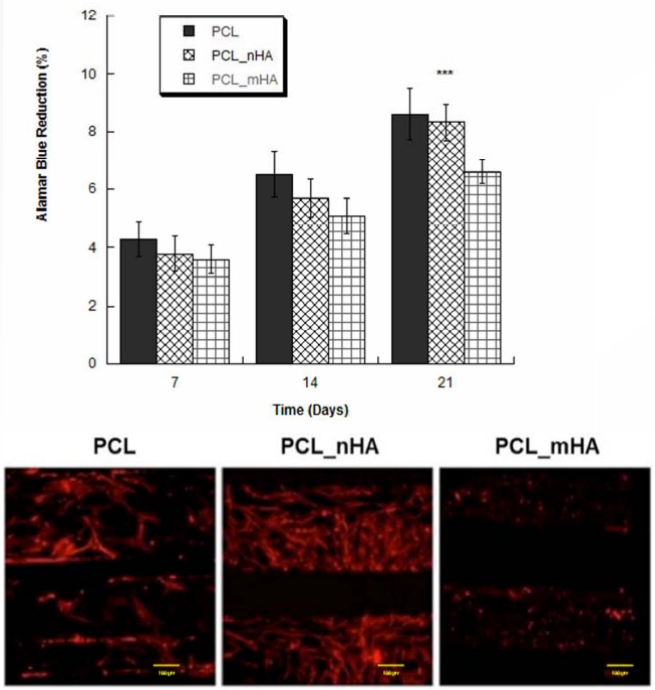
Micro/nanocomposite Scaffolds





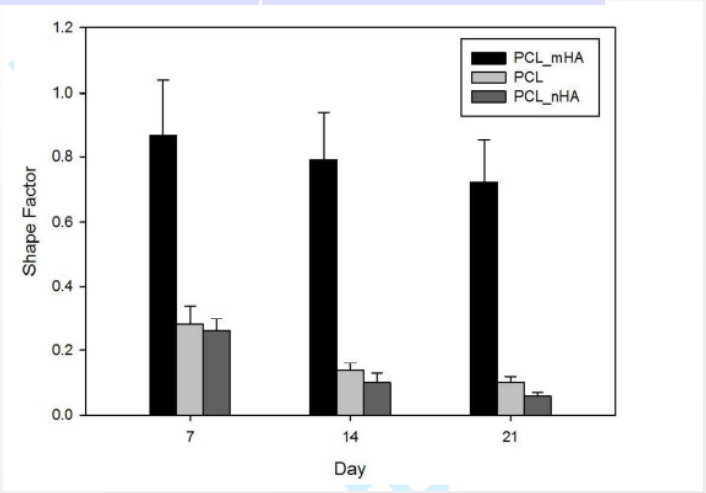
From Design to Mechanical/Functional properties and Biological Properties …

Scaffold	Compressive Modulus (E) (MPa)	Maximum stress (σ_{max}) (MPa)
PCL	105.5 ± 11.2	16.5 ± 1.4
PCL_nHA	138.8 ± 12.9	15.3 ± 1.7
PCL_mHA	217.2 ± 21.8	17.4 ± 1.8

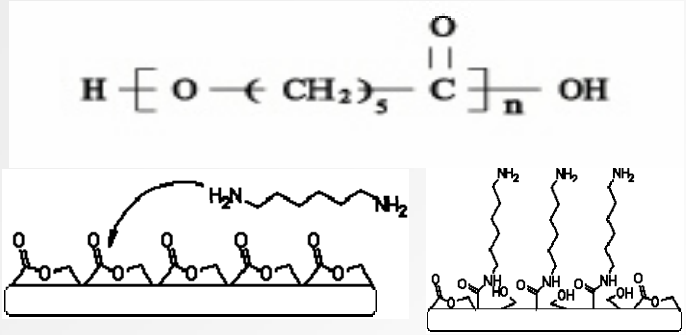


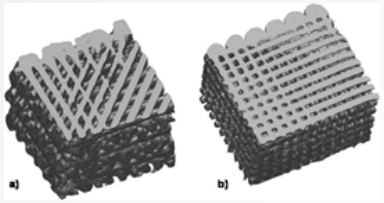
$$\Phi = \frac{\Theta A}{P^2}$$

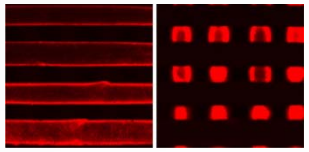
Shape Factor

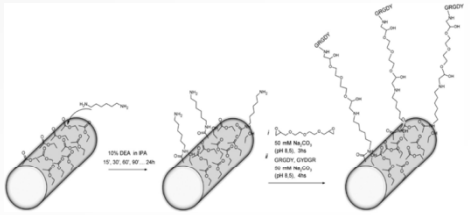


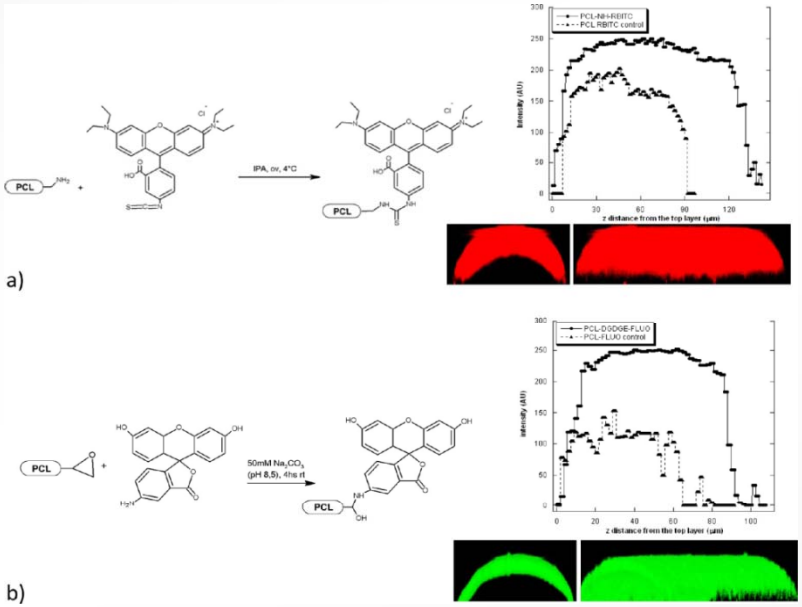
Additive Manufacturing & Surface Modification

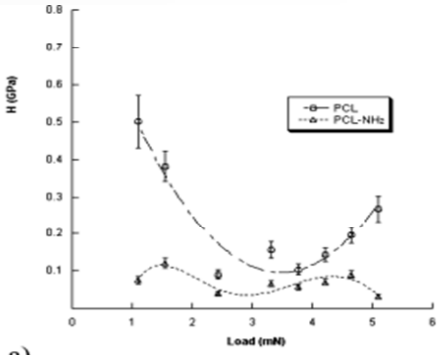




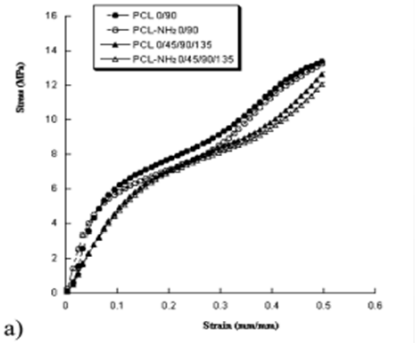




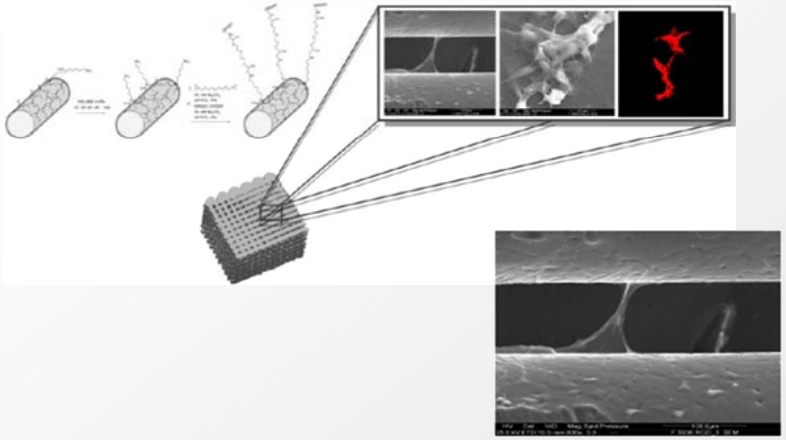




a)

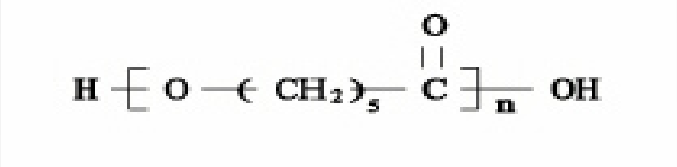


a)



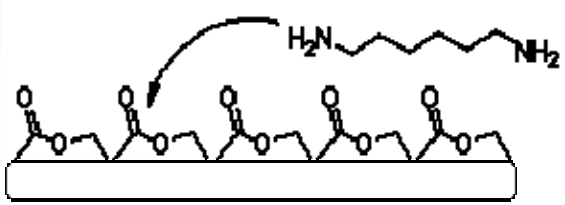
3D Fiber Deposition Technique & Surface Modification

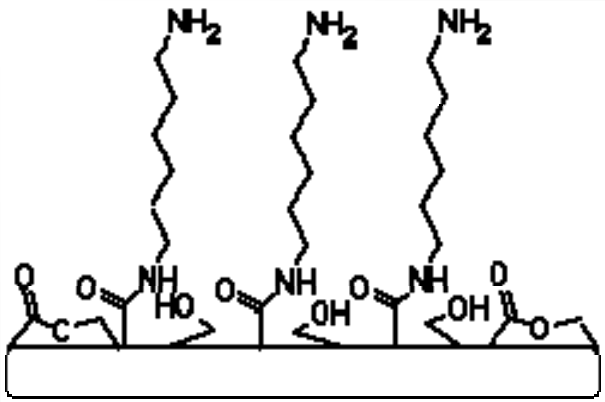
PCL molecules are characterized by a great number of ester groups (−COO−) that can be hydrolyzed to carboxylic acid under alkaline conditions.
Moreover, amino groups can be introduced onto the polyester surface through a reaction with diamine, providing that one amino group reacts with the −COO− group to form a covalent bond, −CONH−, while the other amino group is unreacted and free



Polycaprolactone (PCL)

Aminolysis



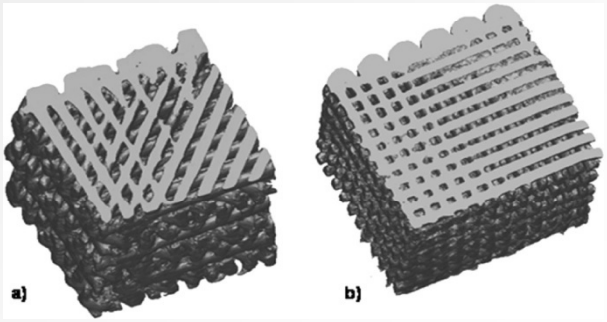


RGD-modified 3D PCL scaffolds

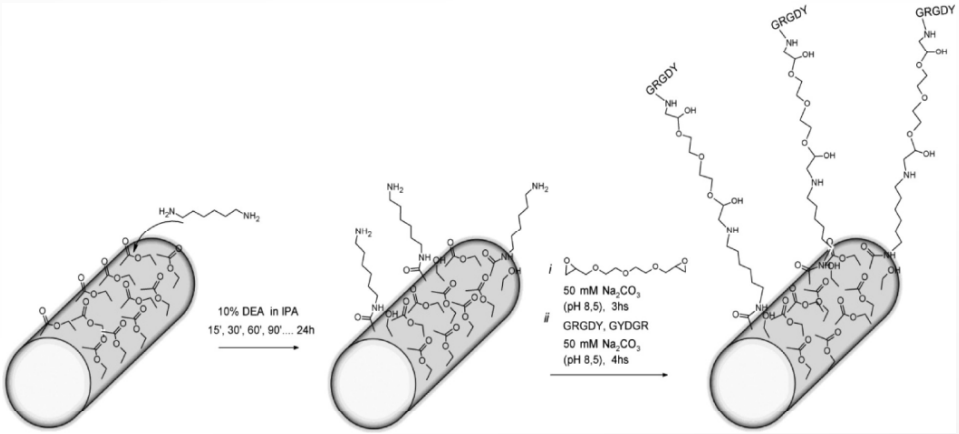
…to control and guide cell-material interactions

3D Fiber Deposition Technique & Surface Modification

Aminolysis & Bioactivation with RGD-like peptides…

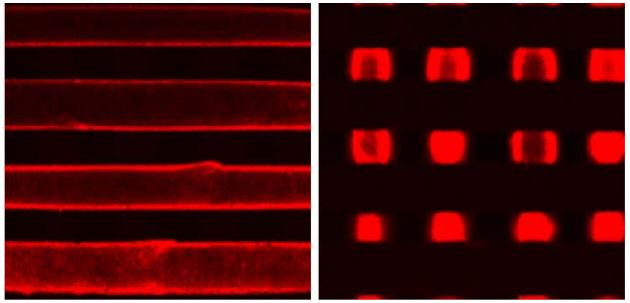


Different lay-down patterns

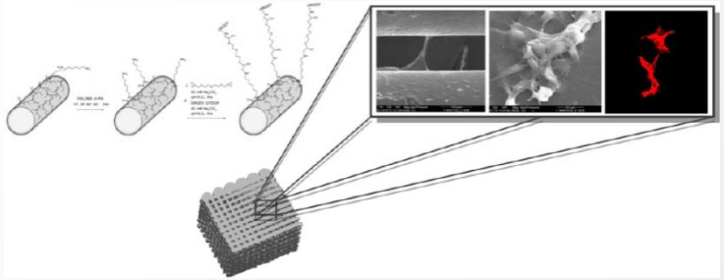


Scheme of the two-step procedure used to graft GRGDY peptides to PCL fibers of the 3D scaffolds

Amino groups were uniformly distributed within the 3D structure

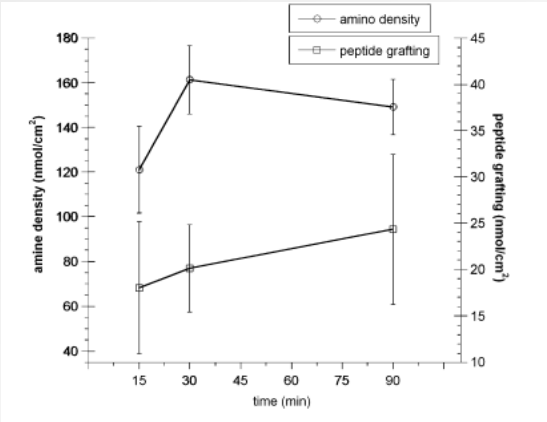


CLSM analysis: images of fibers belonging to two layers at different depths within a PCL-NH-RBITC scaffold characterized by a specific lay-down pattern (0°/90°)



Gloria et al., "Three-Dimensional Poly(ε-caprolactone) Bioactive Scaffolds with Controlled Structural and Surface Properties", *Biomacromolecules*, vol. 13, p. 3510-3521, 2012.

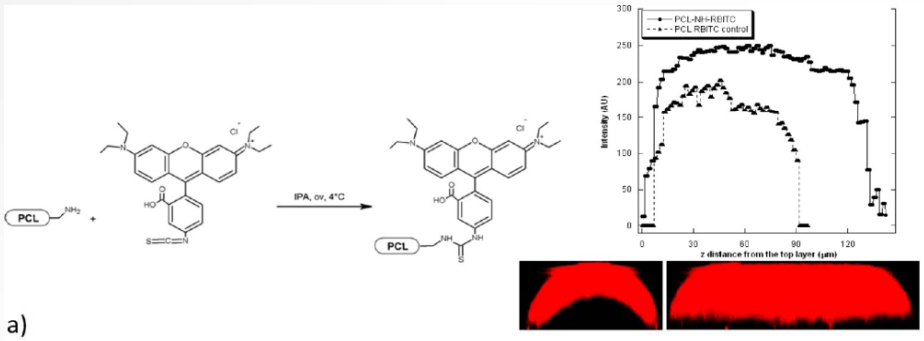
3D Fiber Deposition Technique & Surface Modification



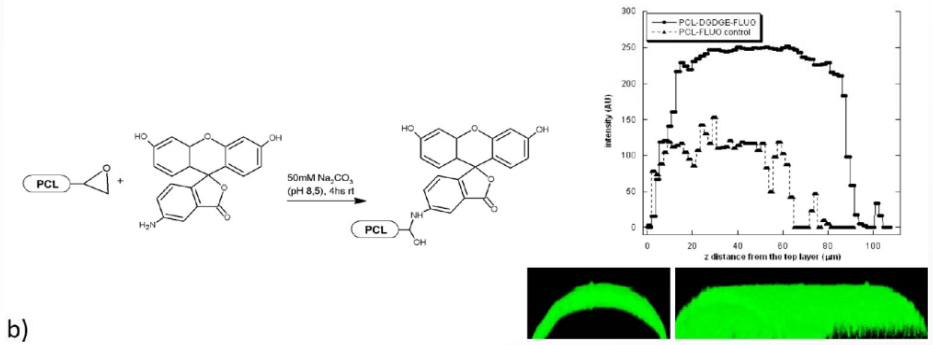
Amine and peptide density as a function of time of aminolysis treatment

(amino-density of 161.3 ± 15.3 nmol/cm^2 and peptide density of 20.13 ± 4.68 nmol/cm^2 were reached at 30 min…)

OPTIMIZATION PROCESS



a)



b)

Intensity profiles of fluorescent molecules in the depth during the two-step conjugation procedure:

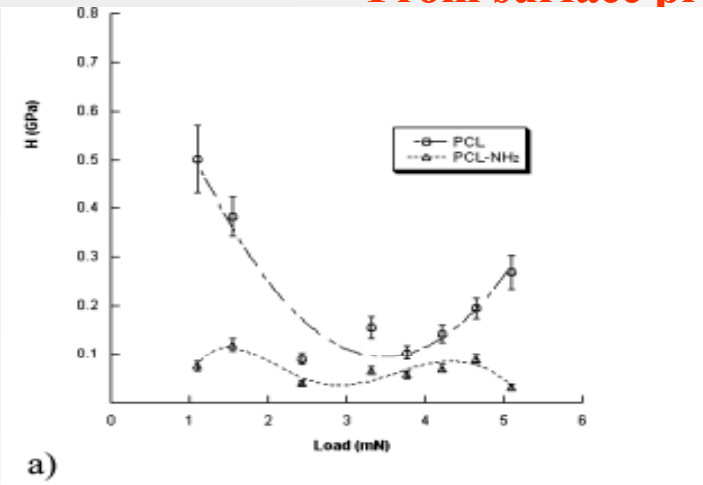
(a) CLSM intensity profile as afunction of depth in the z-direction of a PCL-NH-RBITC fiber

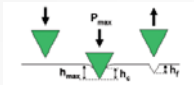
(b) CLSM intensity profile as a function of depth in the z-direction of a PCL-DGDGE-FLUO fiber.

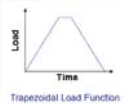
Penetration Depth within the fiber: 140 µm and 80 µm

Gloria et al., "Three-Dimensional Poly(ε-caprolactone) Bioactive Scaffolds with Controlled Structural and Surface Properties", *Biomacromolecules*, vol. 13, p. 3510-3521, 2012.

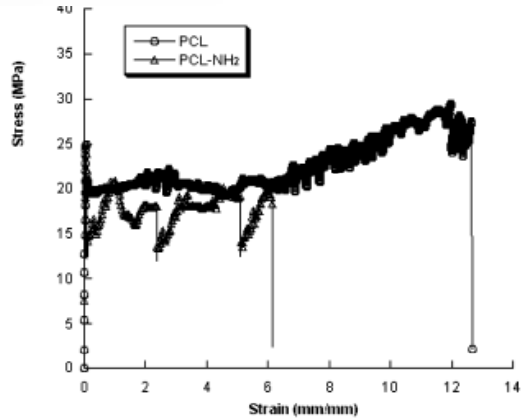
Effects of surface modification via aminolysis
From surface properties to macro-mechanical features...







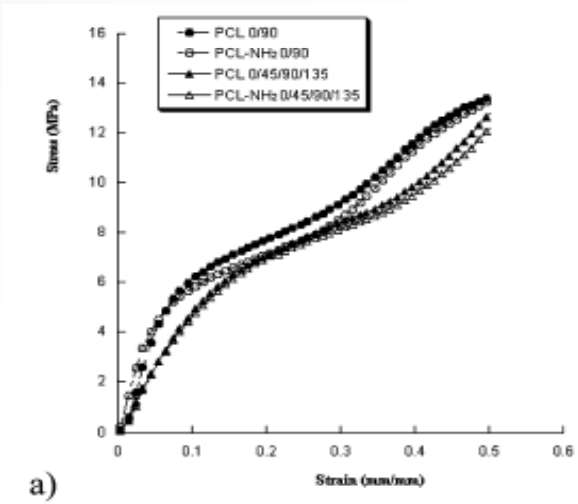
$$H = \frac{P_{max}}{A(h_c)}$$



Nanoindentation tests on fibers have sown a reduction of hardness from 0.50−0.27 GPa to 0.1−0.03 GPa due to the surface treatment

Tensile tests on PCL fibers have evidenced how treatment strongly reduces themaximum strain without altering the values of modulus and yield stress

Fibers	E (MPa)	σ_y (MPa)	ε_{max} (mm/mm)
PCL	570.5 ± 50.1	25.0 ± 3.5	12.7 ± 1.1
PCL-NH_2	550.0 ± 48.6	24.2 ± 3.7	6.5 ± 0.5



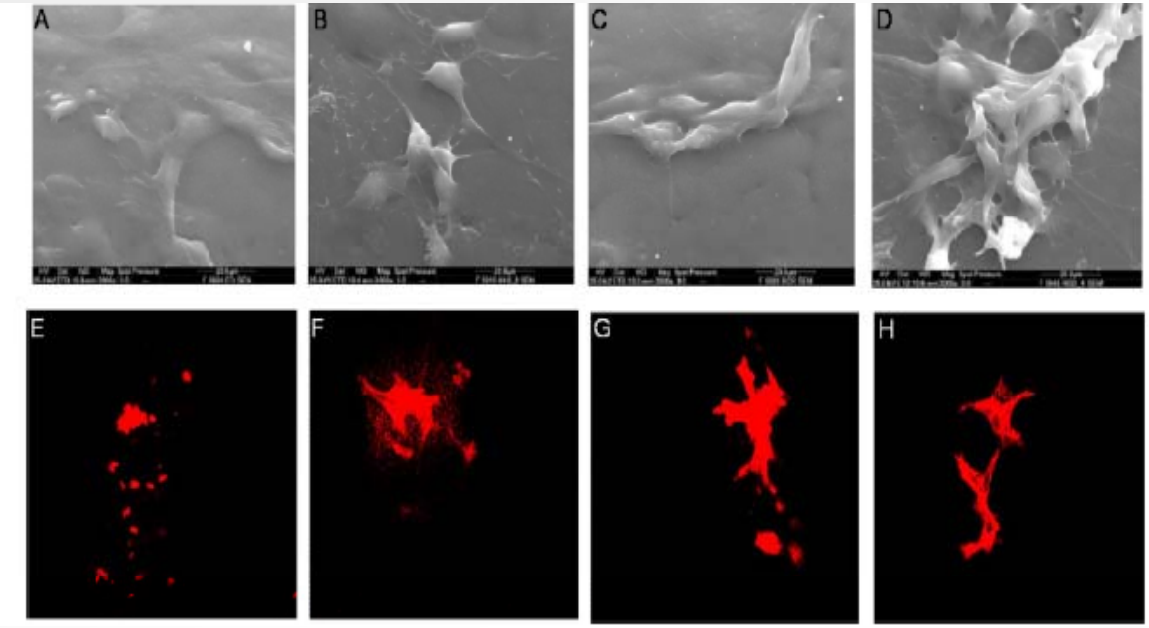
Although nanoindentation and tensile measurements on PCL fibers have shown some differences, results from compression tests on 3D scaffolds have suggested that the surface treatment does not negatively affect the macromechanical behaviour

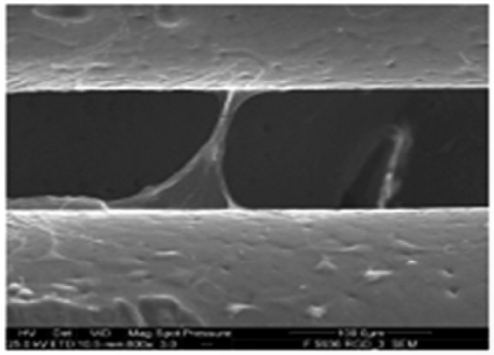
	compressive modulus E (MPa)		maximum stress σ (MPa)	
lay-down pattern	PCL	PCL-NH_2	PCL	PCL-NH_2
0°/45°/90°/135°	63.0 ± 4.7	61.1 ± 5.1	12.3 ± 1.1	12.0 ± 1.3
0°/90°	89.1 ± 6.9	87.9 ± 8.1	13.5 ± 1.3	13.2 ± 1.5

Gloria et al., "Three-Dimensional Poly(ε-caprolactone) Bioactive Scaffolds with Controlled Structural and Surface Properties", *Biomacromolecules*, vol. 13, p. 3510-3521, 2012.

3D Fiber Deposition Technique & Surface Modification

Biological Performances…





Cell adhesion study after 48 h from seeding: SEM micrograph (PCL-DGDGE-GRGDY), bar 50 µm.

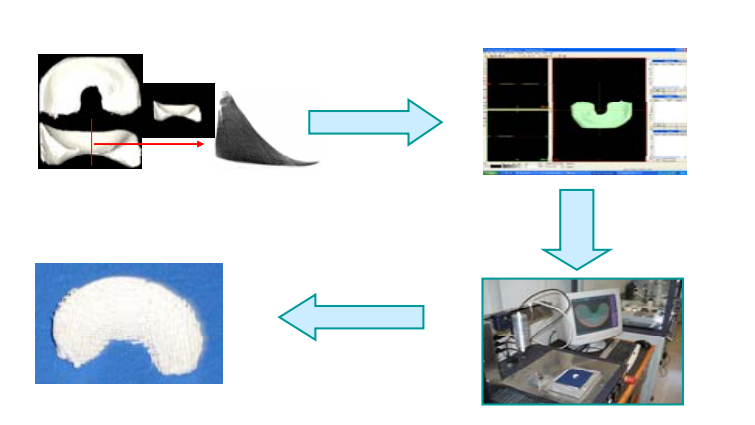
Cell adhesion study after 24 h from seeding: SEM micrographs (A: PCL; B: PCL-NH_2; C: PCL-DGDGE-GYDGR; D: PCL-DGDGE-GRGDY),bar 20 µm; CLSM images of phalloidin staining of microfilaments (E: PCL; F: PCL-NH_2; G: PCL- DGDGE-GYDGR; H: PCL-DGDGE-GRGDY)

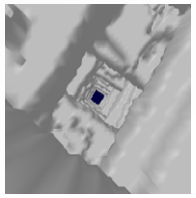
Possibility to enhance cell adhesion

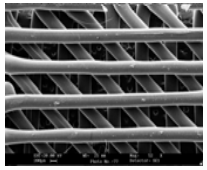
Cells better adhered on RGD bioactivated scaffolds…

Gloria et al., "Three-Dimensional Poly(ε-caprolactone) Bioactive Scaffolds with Controlled Structural and Surface Properties", *Biomacromolecules*, vol. 13, p. 3510-3521, 2012.

Innovative Materials and Technologies for a Bio-engineered Meniscus Substitute

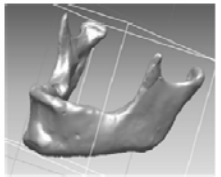


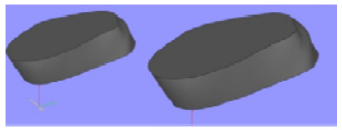


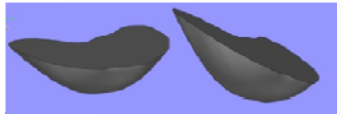


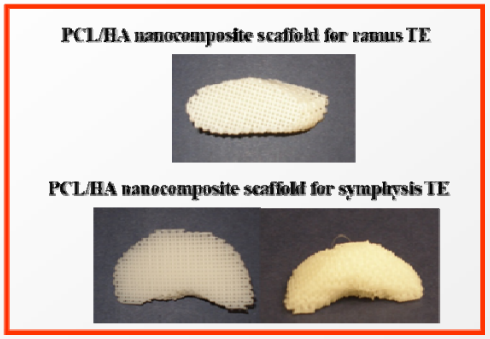
From 3D Modelling and AM

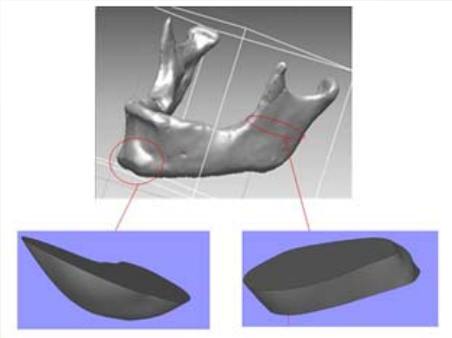
to 3D Custom-Made Nanocomposite Scaffolds for Mandibular Ramus and Symphysis TE…





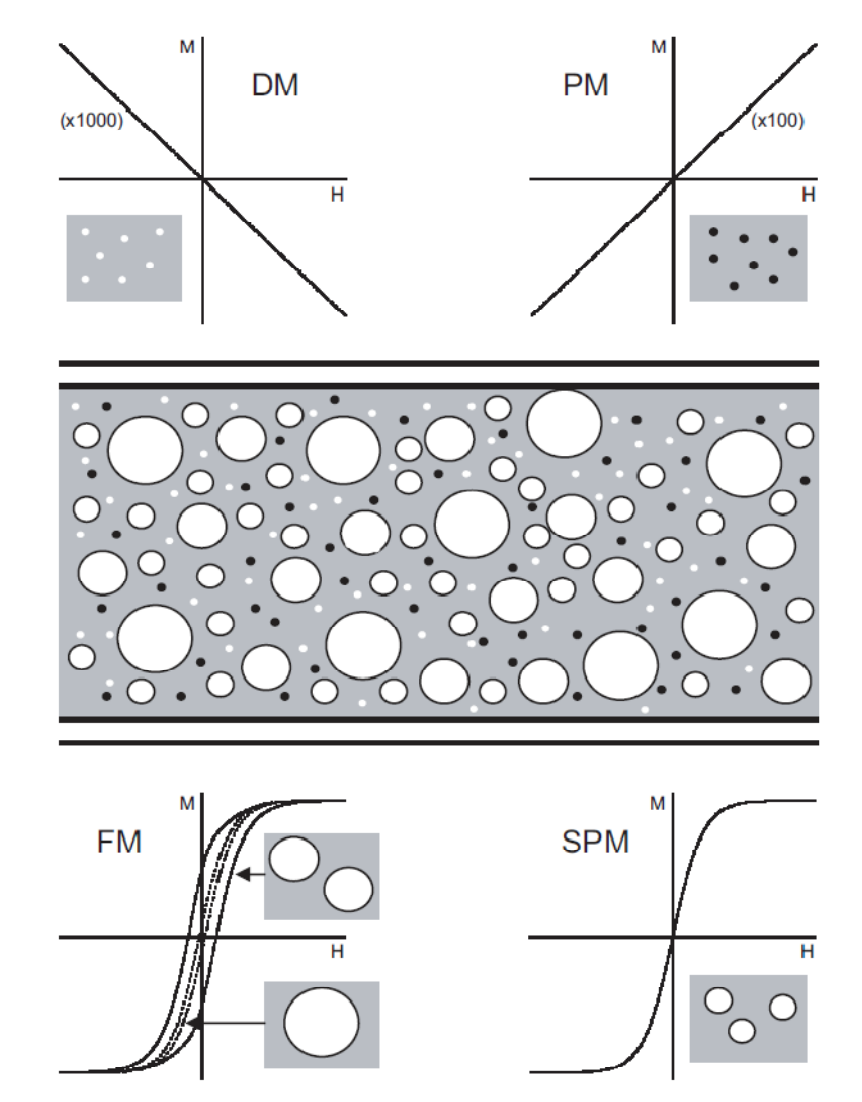






Applications of magnetic nanoparticles in biomedicine

J. Phys. D: Appl. Phys. 36 (2003) R167–R181



Magnetic responses associated with different classes of magnetic material, illustrated for a hypothetical situation in which ferromagnetic particles of a range of sizes from nanometre up to micron scale are injected into a blood vessel. *M–H* curves are shown for diamagnetic (DM) and paramagnetic (PM) biomaterials in the blood vessel, and for the ferromagnetic (FM) injected particles, where the response can be either multi-domain (- - - - in FM diagram), single-domain (—— in FM diagram) or superparamagnetic (SPM), depending on the size of the particle.

Applications of magnetic nanoparticles in biomedicine

J. Phys. D: Appl. Phys. 36 (2003) R167–R181

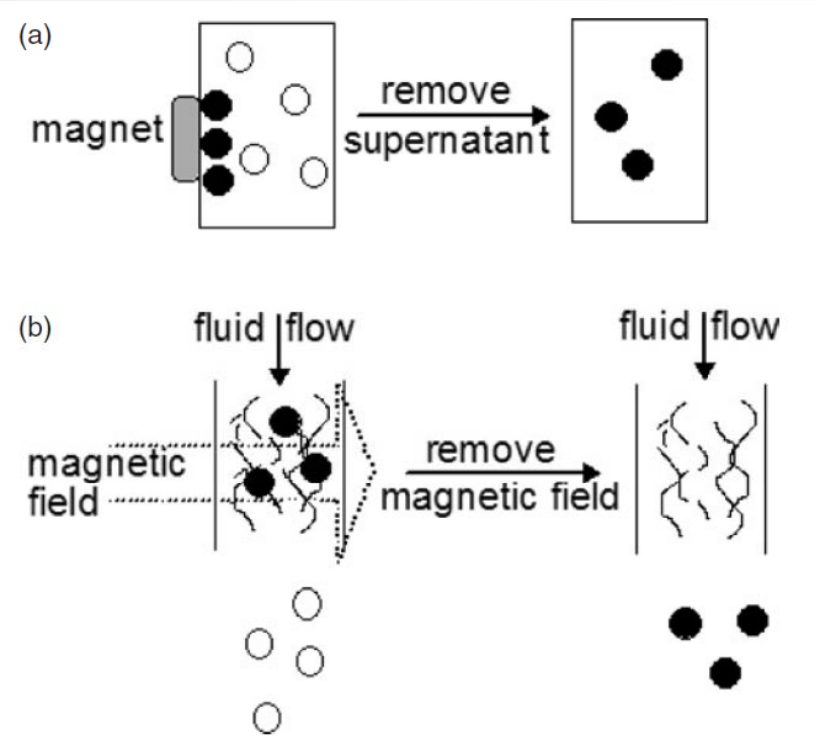
Cell labelling and magnetic separation

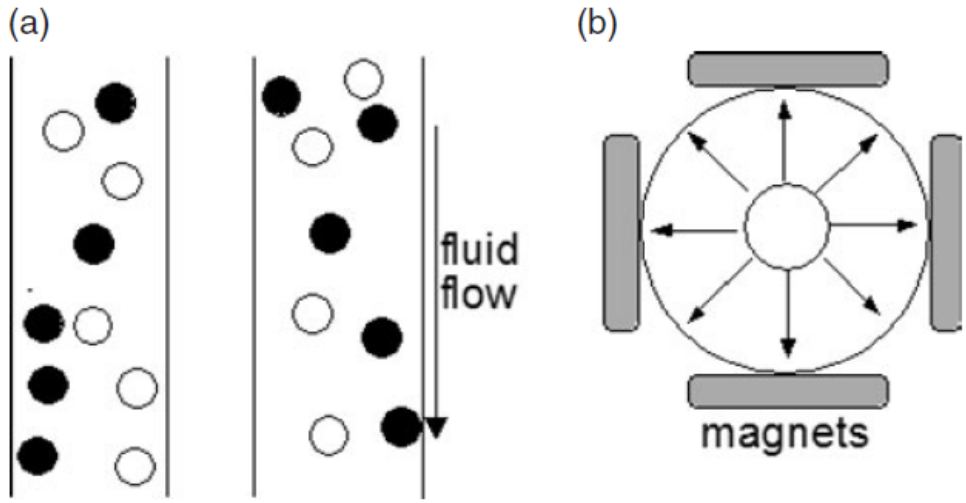
$$F_{\mathrm{d}} = 6\pi\eta R_{\mathrm{m}}\Delta v$$

$$\Delta v = \frac{R_{\mathrm{m}}^2 \Delta\chi}{9\mu_0\eta}\nabla(B^2) \qquad \text{or} \qquad \Delta v = \frac{\xi}{\mu_0}\nabla(B^2)$$

Applications of magnetic nanoparticles in biomedicine

J. Phys. D: Appl. Phys. 36 (2003) R167–R181



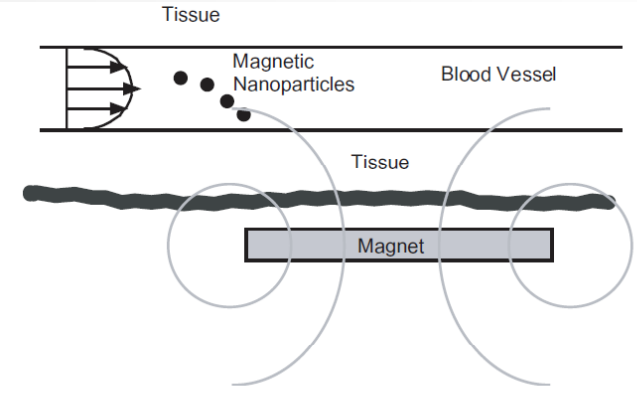


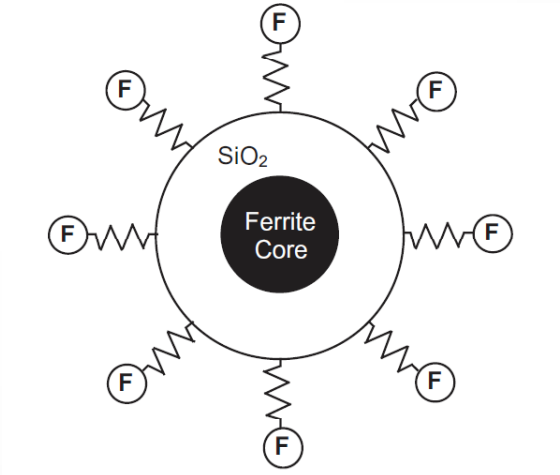
Applications of magnetic nanoparticles in biomedicine

J. Phys. D: Appl. Phys. 36 (2003) R167–R181

Drug Delivery

Motivation and physical principles





Magnetic drug carriers

Targeting studies

Hyperthermia

MRI contrast enhancement

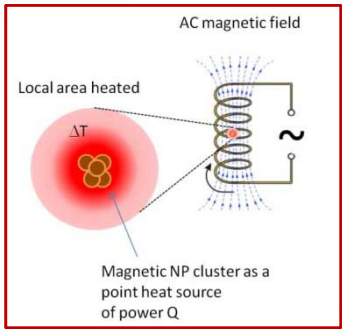
MAGNETIC SCAFFOLDS

The main driving idea of the work is the creation of a conceptually new type of bioactive and highly porous *scaffold* able to be manipulated *in situ* by means of magnetic forces in order to repair large bone defects and osteochondral lesions.

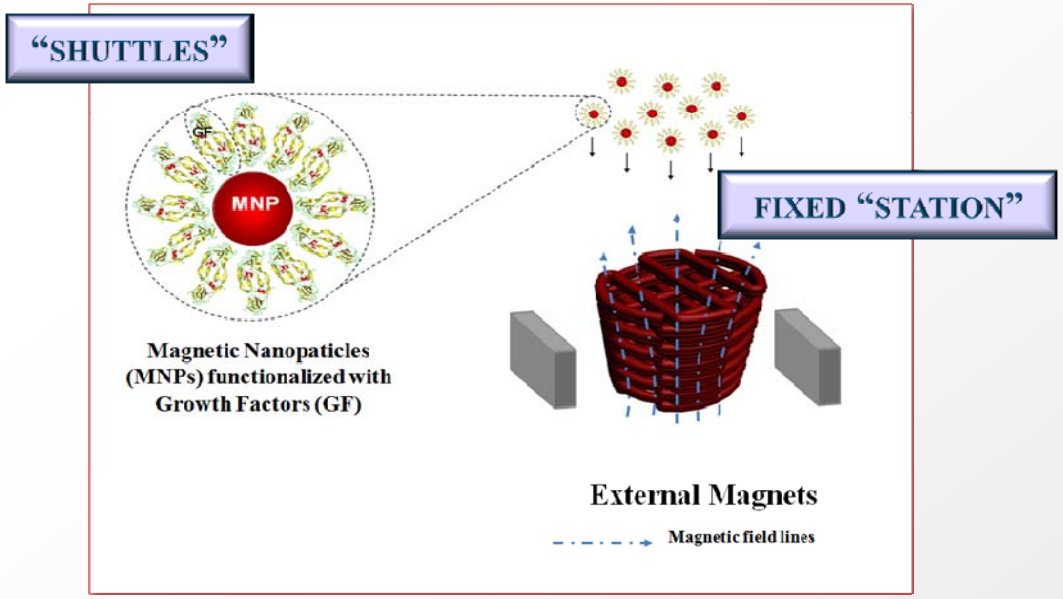
MAGNETIC SCAFFOLD

Magnetic Fixation

Hyperthermia Treatment



On demand release of angiogenic factors or stem cells through a switch-on/switch-off command scheme

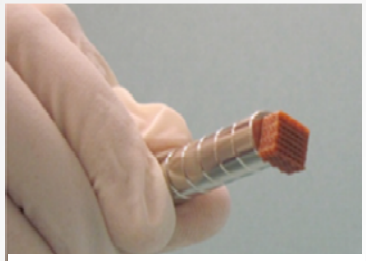


Magnetic guiding of magnetic functionalized bioaggregates and cells

- *Tampieri A., Landi E., Valentini F., et al. Nanotechnology. 22: 1-82, 2011.*
- *Bock N., Riminucci A., Dionigi C., Russo A., Tampieri A., et al. Acta Biomaterialia. 6: 786, 2010.*
- *Pankhurst Q.A., Connolly J., Jones S.K., Dobson J. J. Phys. D: Appl. Phys. 36: R167-R181, 2003.*
- *Russo A., Shelyakova T., Casino D., et al. Med. Eng. Phys. 34: 1287– 1293, 2012.*

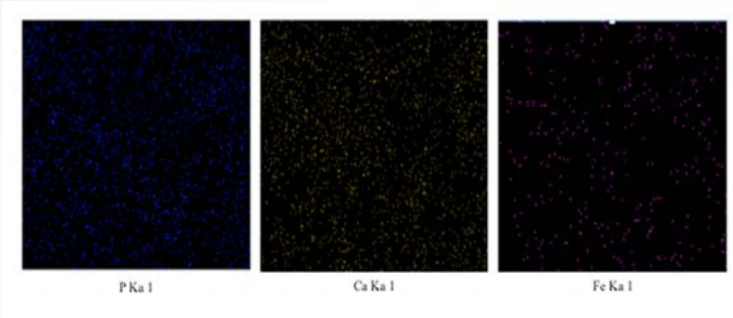
MAGnetIc Scaffolds for in vivo Tissue EngineeRing

3D Magnetic PCL/ FeHA Nanocomposite Scaffolds

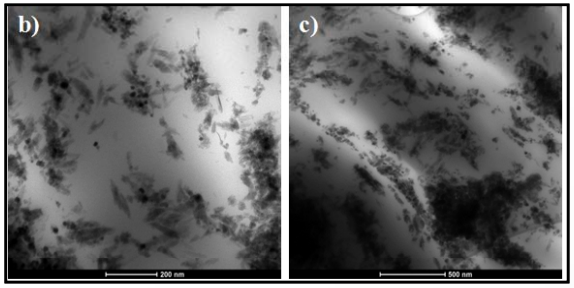


PCL/ FeHA (80/20 w/w)

SEM-EDS mapping photograph

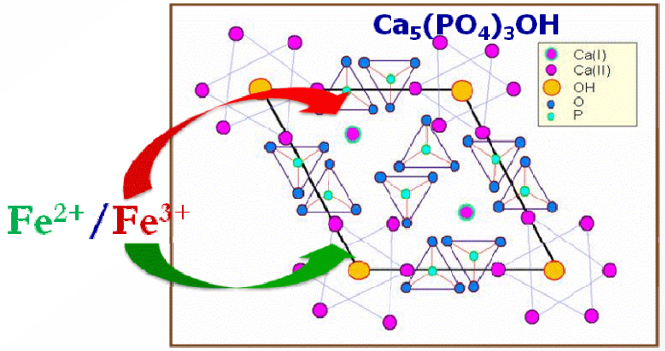


TEM images



NPs are uniformly and randomly distributed in the matrix

Bioresorbable iron-doped hydroxyapatite (FeHA) nanoparticles

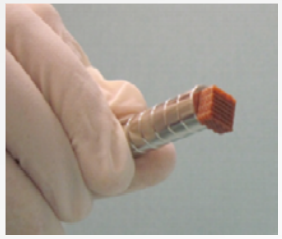


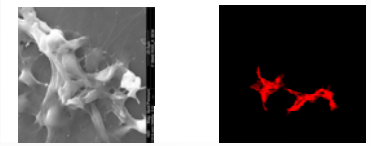
Fe/Ca=0.20 mol and a Fe(III)/Fe(II) ratio of 1:1, prepared through neutralization method in presence of Fe(III) and Fe(II) doping ions

Tampieri A., D'Alessandro T., Sandri M., Sprio S., Landi E., Bertinetti L., Panseri S., Pepponi G., Goettlicher J., Bañobre-López M., Rivas J. *Intrinsic magnetism and hyperthermia in bioactive Fe-doped hydroxyapatite.* Acta Biomater. 8: 843-51, 2012.

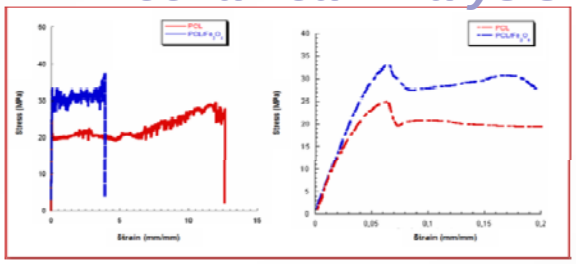
A. Tampieri et al., Italian Patent No. MI2010A001420, 2010.

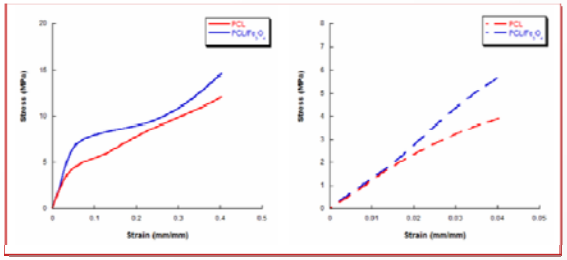
Additive Manufactured Magnetic Structures ...





Mechanical Analysis

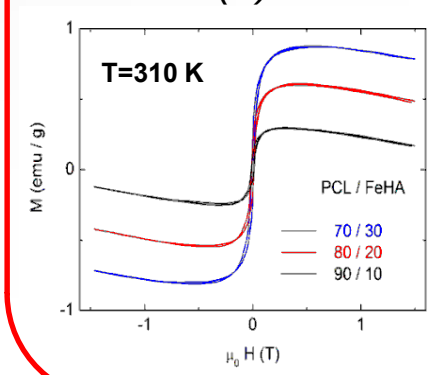




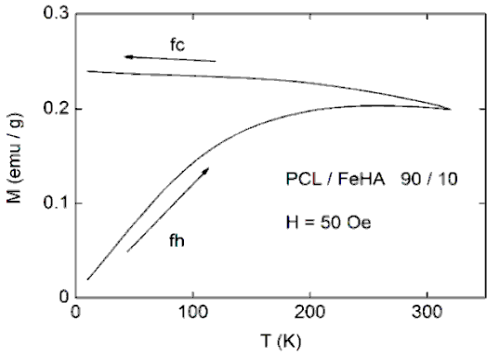
Magnetic Analysis

$$M = M_s\left(\coth(\mu_p H / k_B T) - k_B T / \mu_p H\right)$$

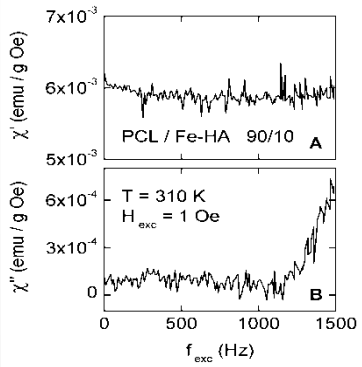
M (H)



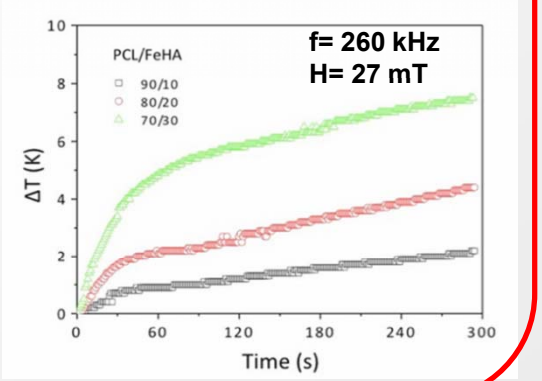
M (T)



χ(*f*)



ΔT(*t*)

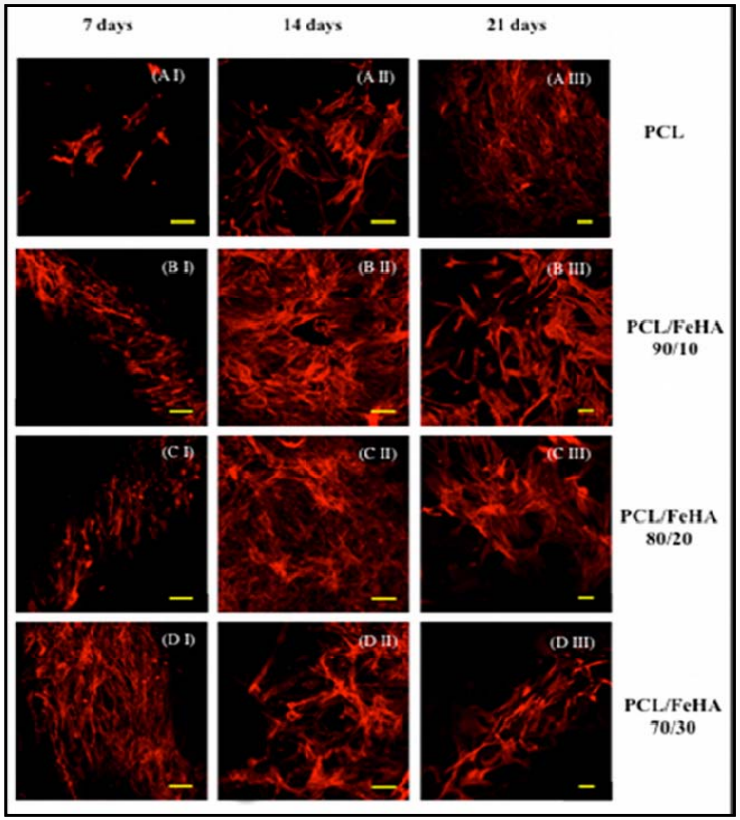


MAGnetic Scaffolds for in vivo Tissue EngineeRing

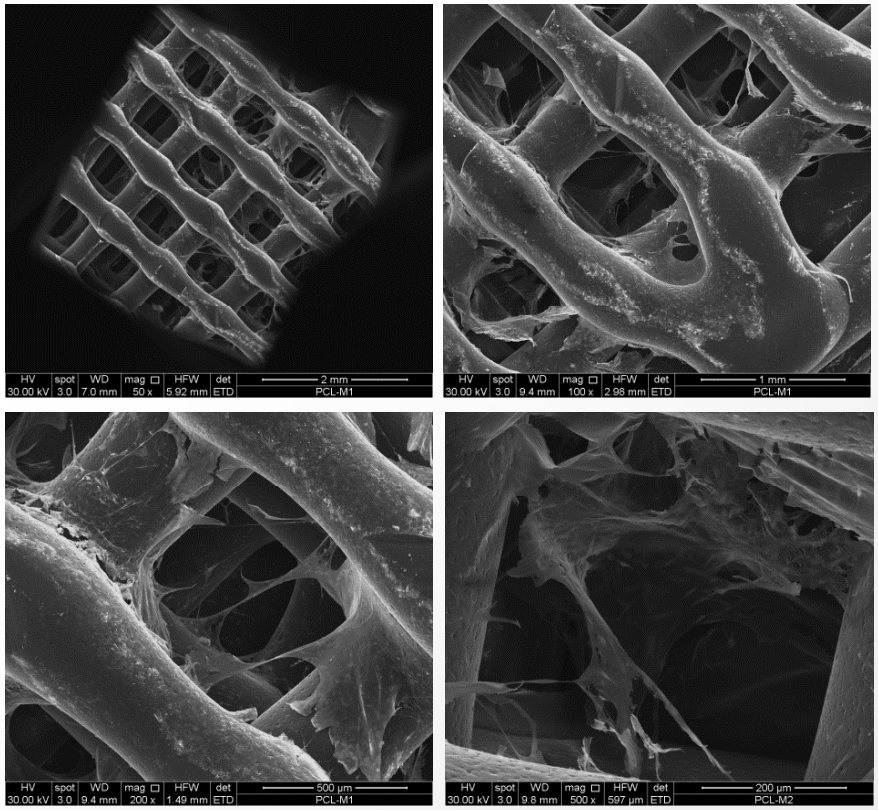
3D Magnetic PCL/ FeHA Nanocomposite Scaffolds

Biological Analyses

Confocal Laser scanning microscopy was carried out to study hMSCs adhesion and spreading on the PCL/FeHA nanocomposites at 7,14, 21 days after seeding.



Scanning Electron Microscopy was carried out to study hMSC adhesion and spreading on the PCL/FeHA nanocomposites at 21days from cell seeding



… TO ENHANCE CELL SPREADING

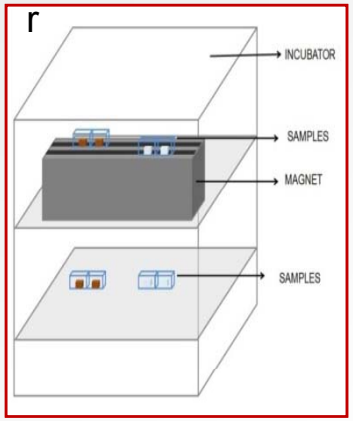
R. De Santis, A. Gloria, et al. *Virtual and Physical Prototyping*, 6(4), 189-195, 2011
Gloria A et al. *J. R. Soc. Interface* 2013 10, 20120833, published online 9 January 2013

MAGnetIc Scaffolds for in vivo Tissue EngineeRing

3D Magnetic PCL/ FeHA Nanocomposite Scaffolds

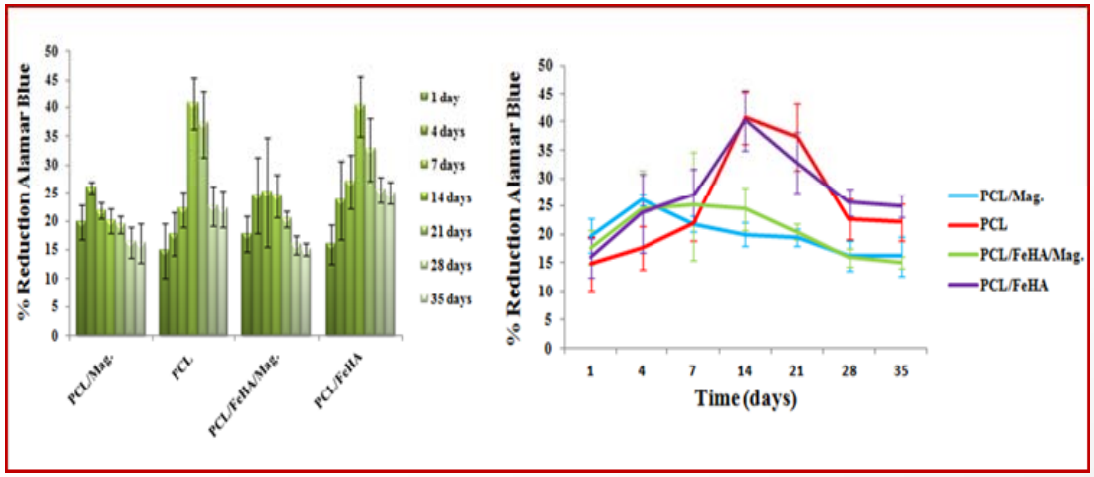
Biological Analyses

3D additive-manufactured nanocomposite magnetic scaffolds: Effect of the application mode of a time-dependent magnetic field on hMSCs behavior



Sinusoidal magnetic field

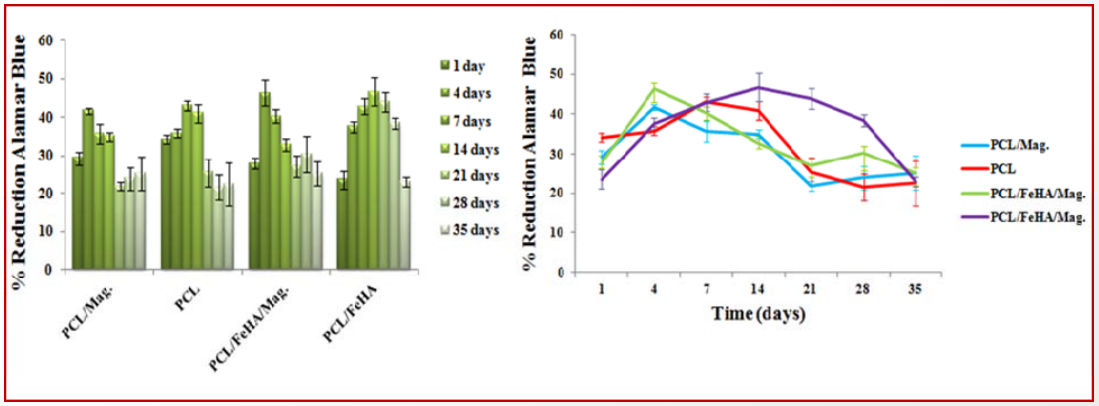
Group 1



- **Group 1:** continuous exposure (6 h per day)

- **Group 2:** discontinuous exposure (6 h/day distributed in 18 minute time intervals, followed by 54 minutes of stasis)

Group 2



… differences in terms of cell viability/proliferation

Bioactive Materials , 2017

Bio-chemical Functionalisation Methods

MNPs

Magnetic Field

Sensors

Microfluidic Device

MADIA project

Magnetic DIagnostic Assay for neurodegenerative diseases

732678 — MADIA — H2020-ICT-2016-2017/H2020-ICT-2016-1

Thank you for the attention