



Università degli Studi di Napoli Federico II
Polo delle Scienze e delle Tecnologie per la Vita

La medicina rigenerativa: approccio multidisciplinare

Napoli 16 aprile 2011

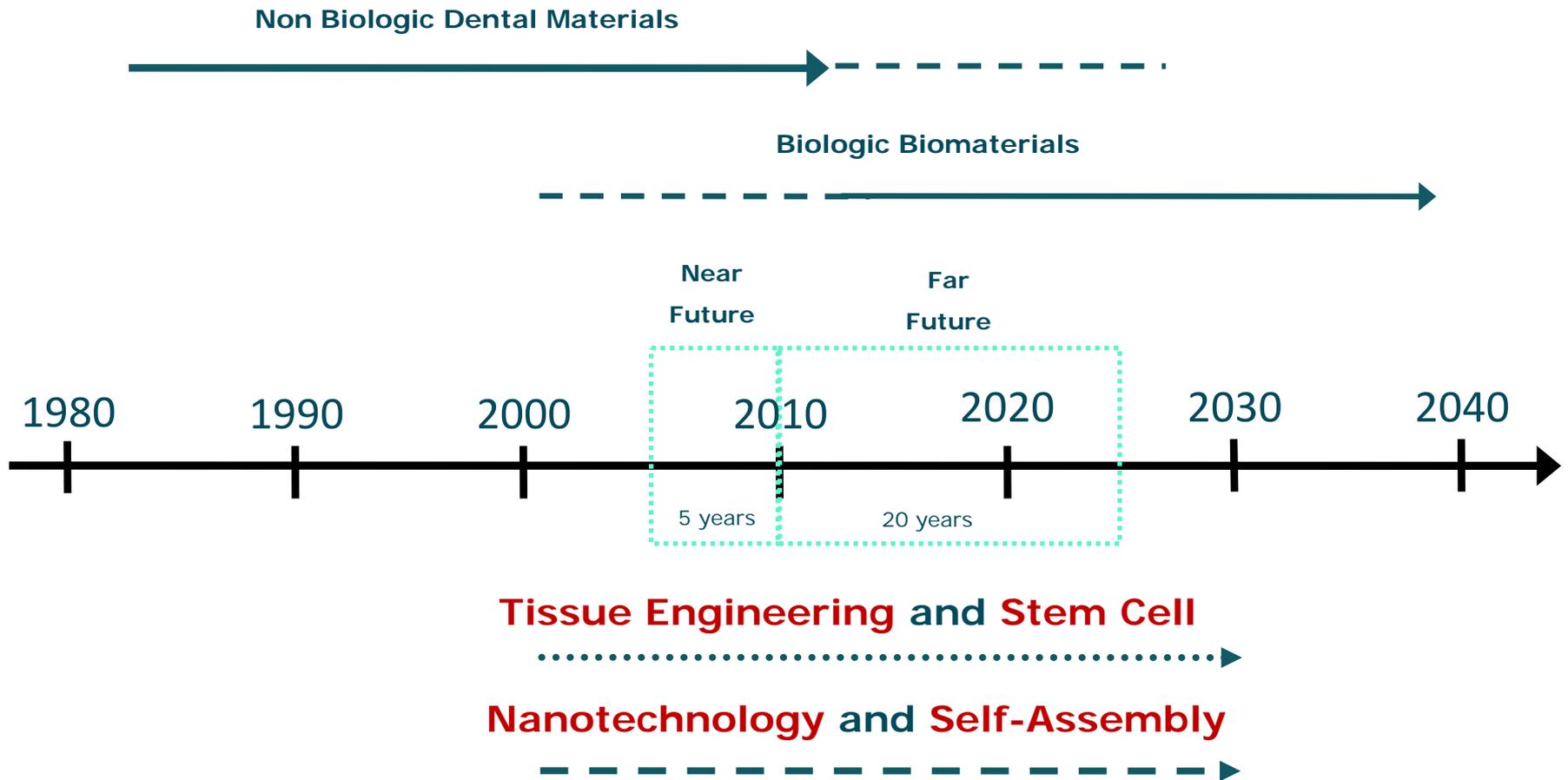
Centro Congressi Università "Federico II"
Via Partenope

Nuove frontiere in odontoiatria

Vincenzo D'Antò, DDS PhD



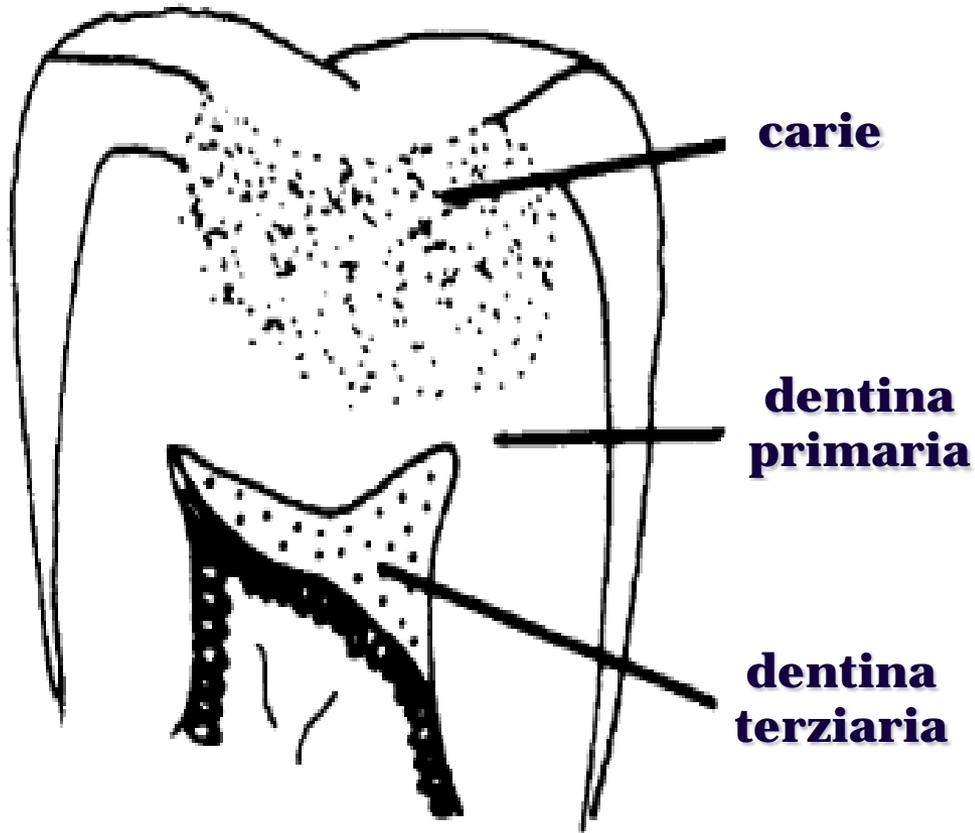
Il futuro dei materiali



Bayne SC. J Dent Educ (2005)



Deposizione della dentina terziaria

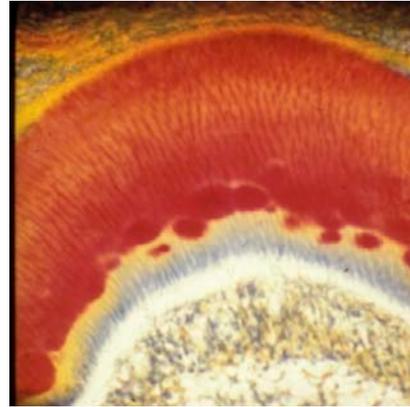


Tziafas et al., 2000



courtesy of Nicola Perrini





Dentine bridge

**Riassorbimenti
radicolari**

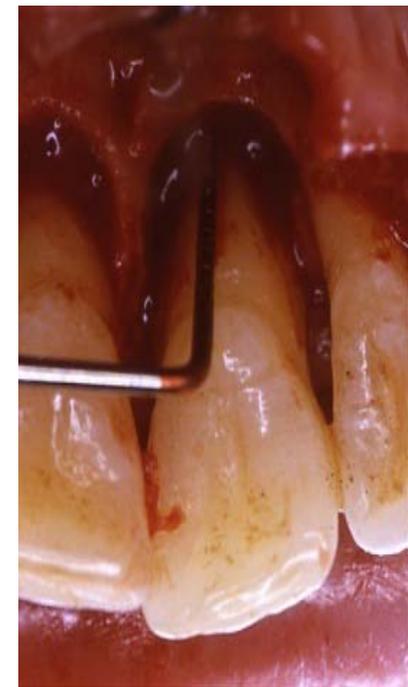


**Perforazioni
radicolari**



Apicogenesi





courtesy of prof. Sergio Matarasso





9-YEARS LATER ...

CLINICAL VIEW



CLINICAL VIEW



courtesy of prof. Sergio Matarasso



9-YEARS LATER ...

INTRA-SURGICAL VIEW



1998

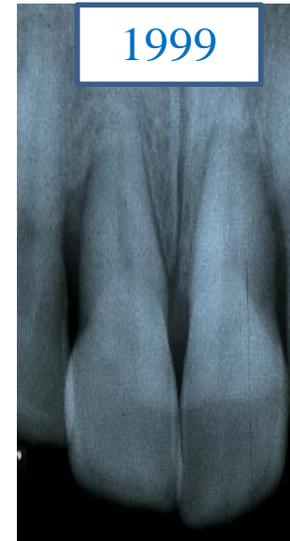


2007

RX- EXAMINATION



1998



1999



2007



courtesy of prof. Sergio Matarasso



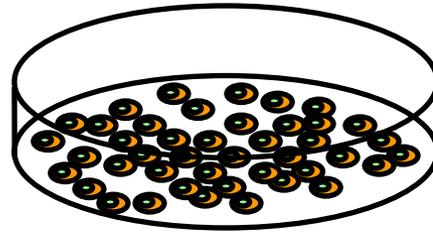
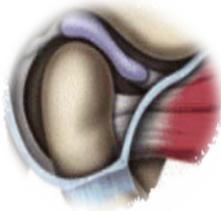
Medicina rigenerativa: *applicazioni in campo odontoiatrico*

Creste
alveolari



Ripristino elementi
dentari mancanti

ATM

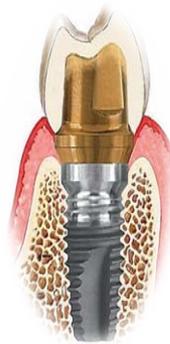


Endodonzia

Parodontologia



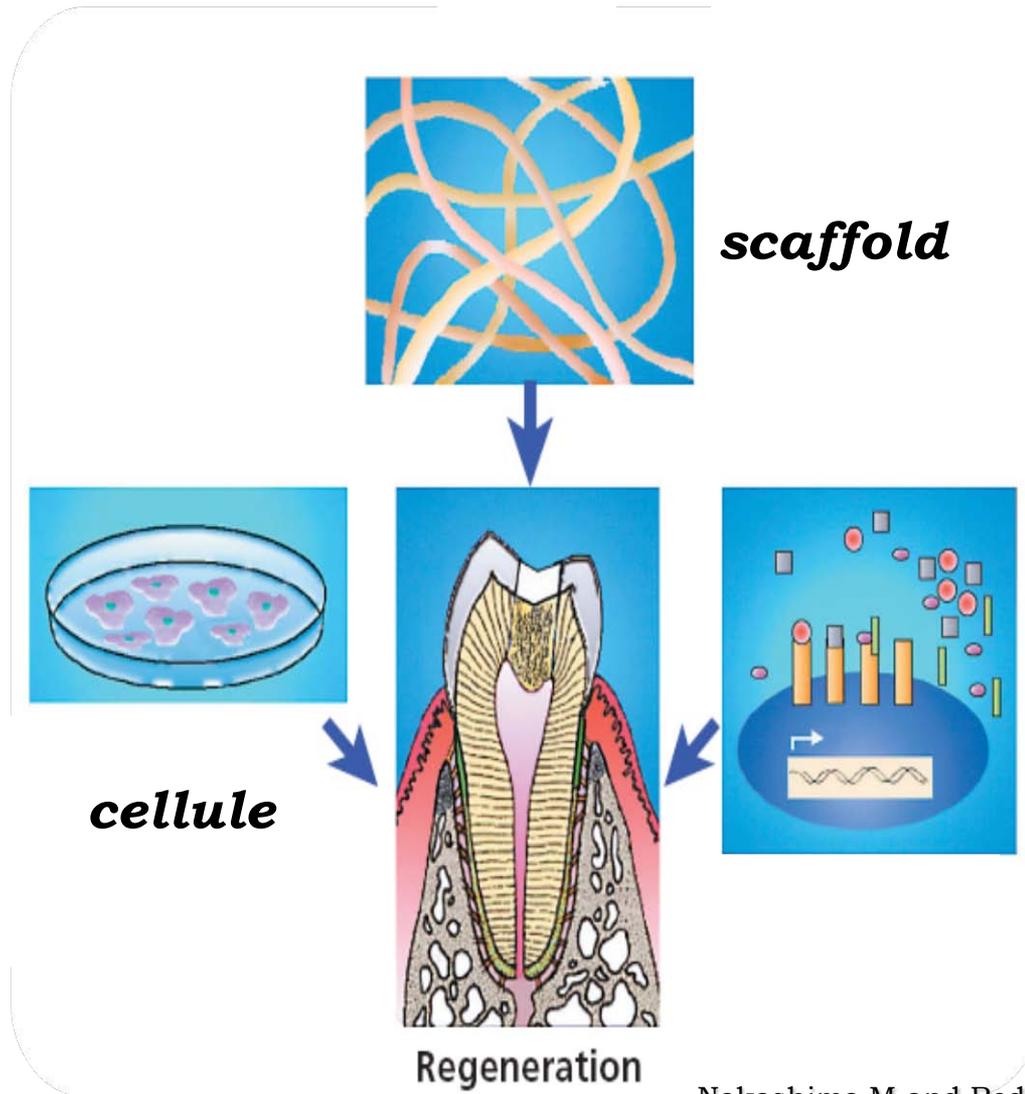
Implantologia



Fratture
ossee

INGEGNERIA DEI TESSUTI

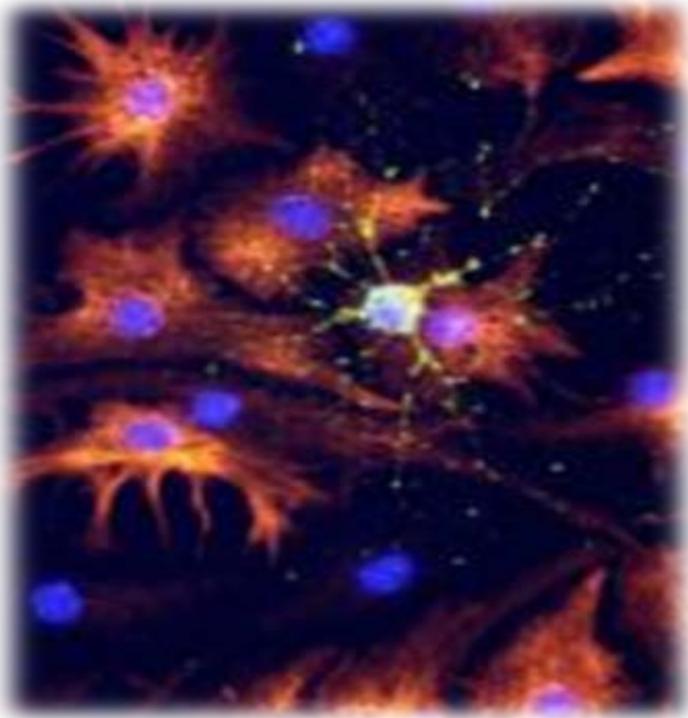




Nakashima M and Reddi AH, Nature Biotechnology 2003



Ingegneria dei tessuti: *cellule staminali adulte*



- epidermide
- midollo osseo
- tessuto muscolare
- tessuto adiposo
- tendini
- cartilagine articolare
- milza e timo
- pancreas
- fegato
- rene....

Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*

S. Gronthos, M. Mankani, J. Brahim, P. Gehron Robey, and S. Shi*

Craniofacial and Skeletal Diseases Branch, National Institute on Dental Research, National Institutes of Health, Bethesda, MD 20892

Edited by Darwin J. Prockop, Tulane University, New Orleans, LA, and approved September 26, 2000 (received for review July 5, 2000)

SHED: Stem cells from human exfoliated deciduous teeth

Masako Miura*, Stan Gronthos†, Mingrui Zhao‡, Bai Lu‡, Larry W. Fisher*, Pamela Gehron Robey*, and Songtao Shi*⁵

*Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892; †Mesenchymal Stem Cell Group, Division of Haematology, Institute of Medical and Veterinary Science, Frome Road, Adelaide 5000, South Australia, Australia; and ‡Section on Neural Development and Plasticity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892

Edited by Anthony P. Mahowald, University of Chicago, Chicago, IL, and approved March 12, 2003 (received for review December 16, 2002)

Investigation of multipotent postnatal stem cells from human periodontal ligament

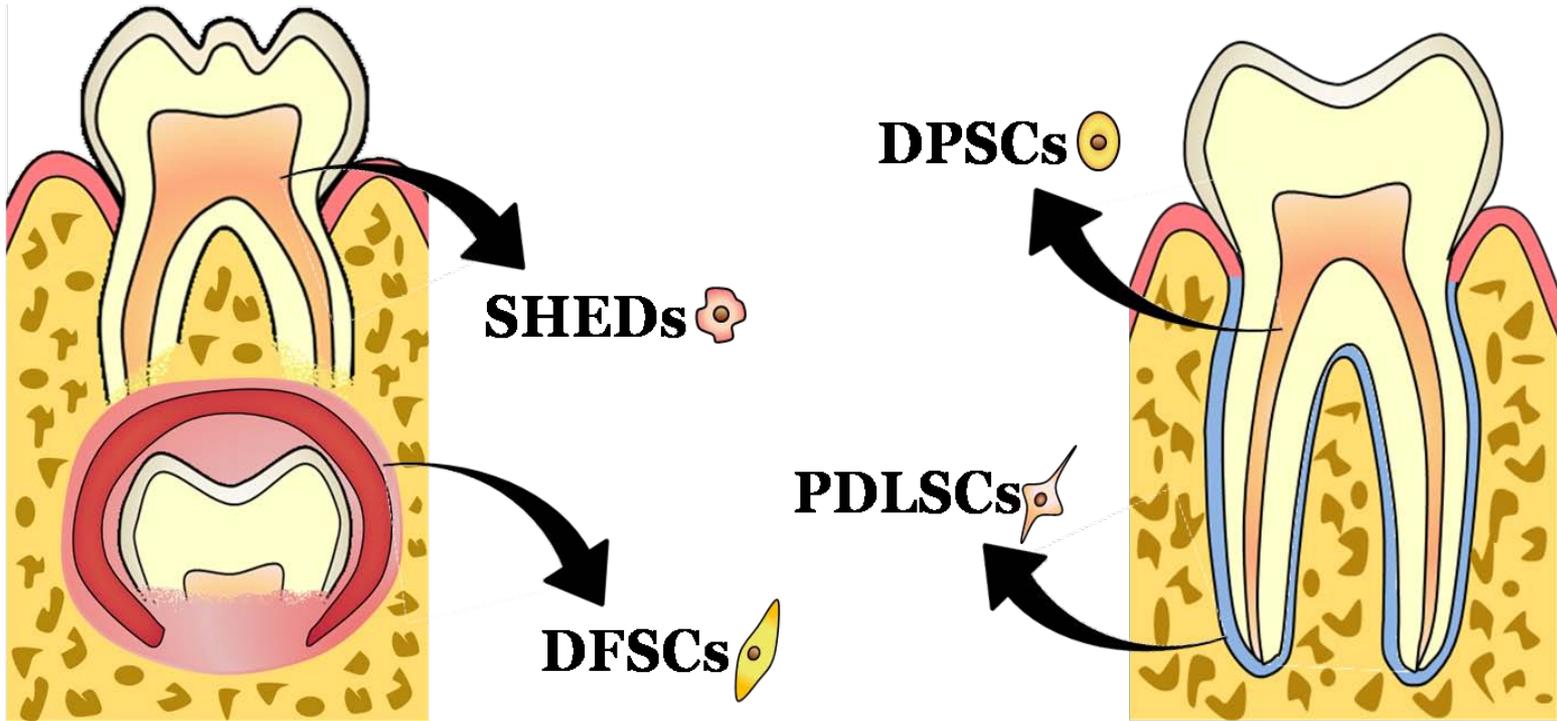
scienceserver.cilea.it

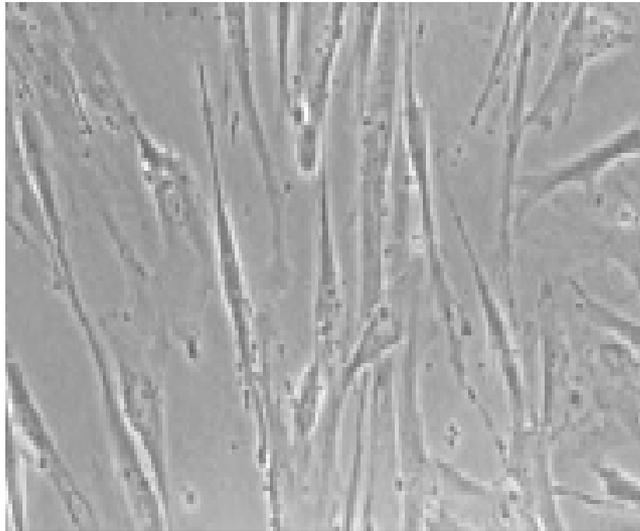
Byoung-Moo Seo, Masako Miura, Stan Gronthos, Peter Mark Bartold, Sara Batouli, Jaime Brahim, Marian Young, Pamela Gehron Robey, Cun-Yu Wang, Songtao Shi

Lancet 2004; 364: 149-55

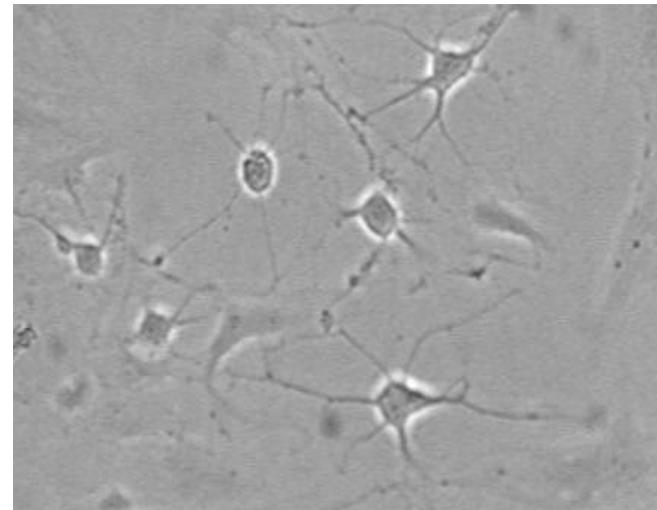
Craniofacial and Skeletal
Disease Branch (B. M. Seo, DDS)







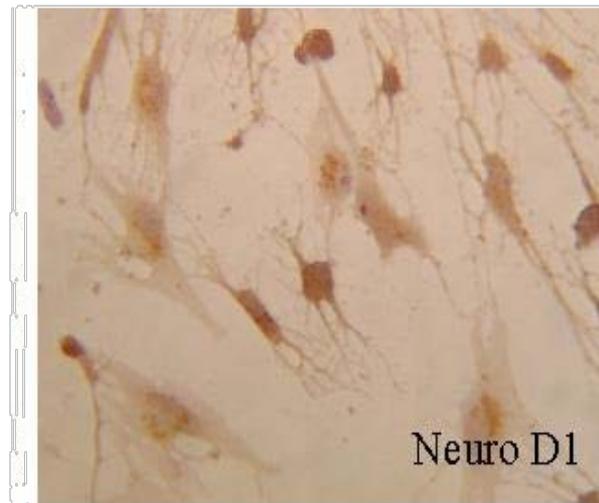
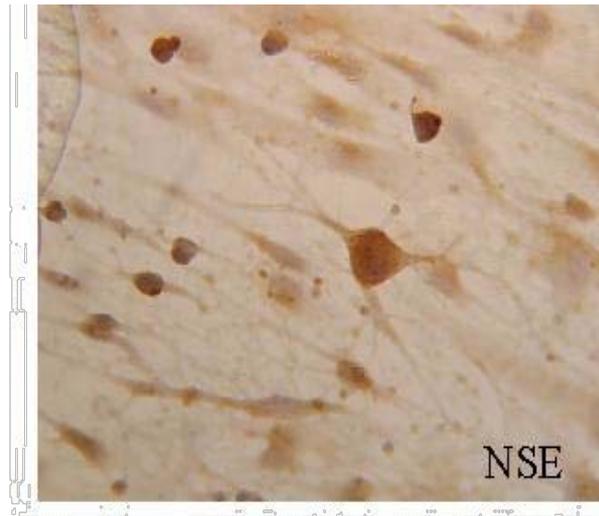
**Morfologia delle cellule
pulpari**



**Morfologia delle cellule
pulpari stmolate con db-
cAMP**

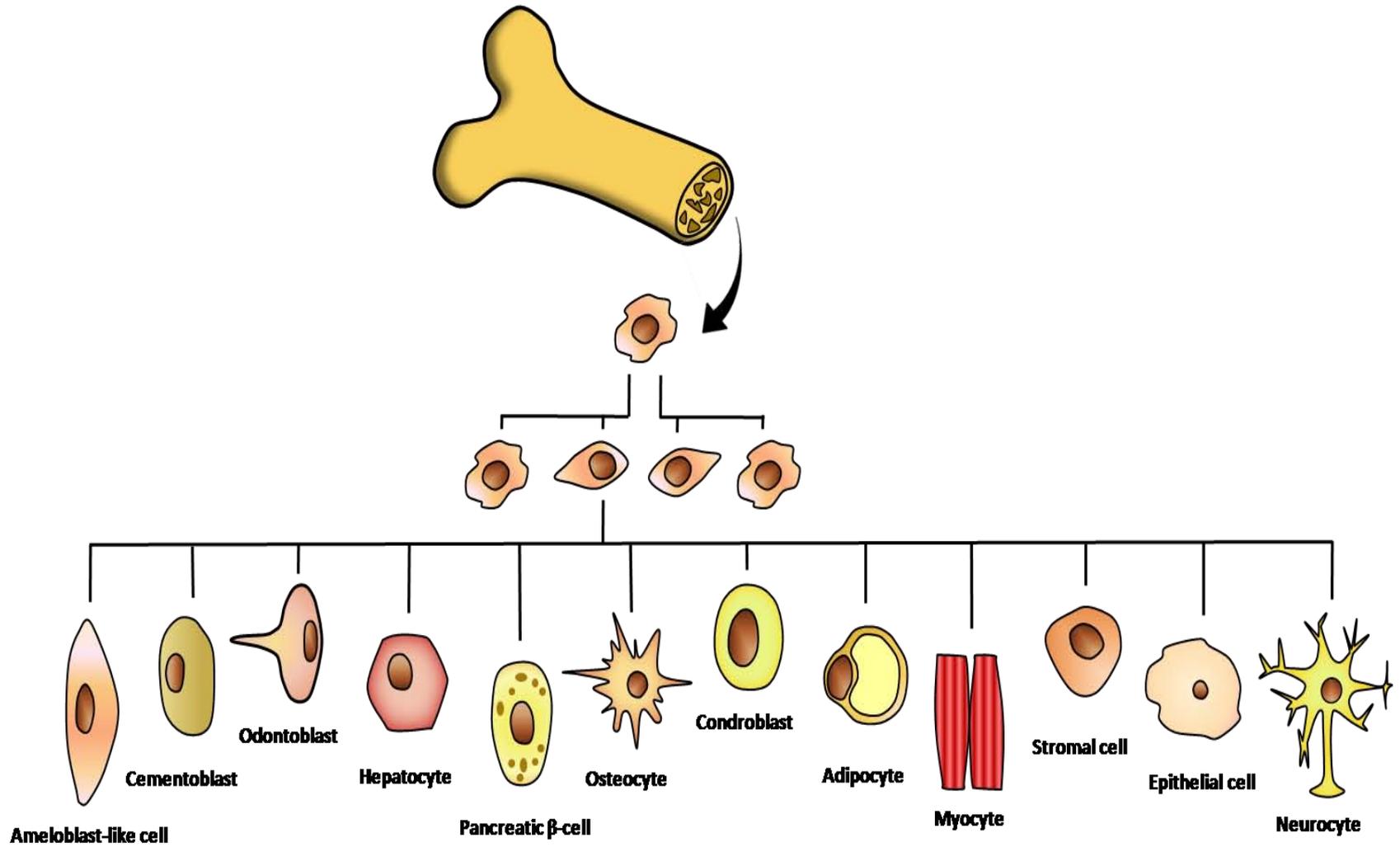
D'Antò et al., 2006

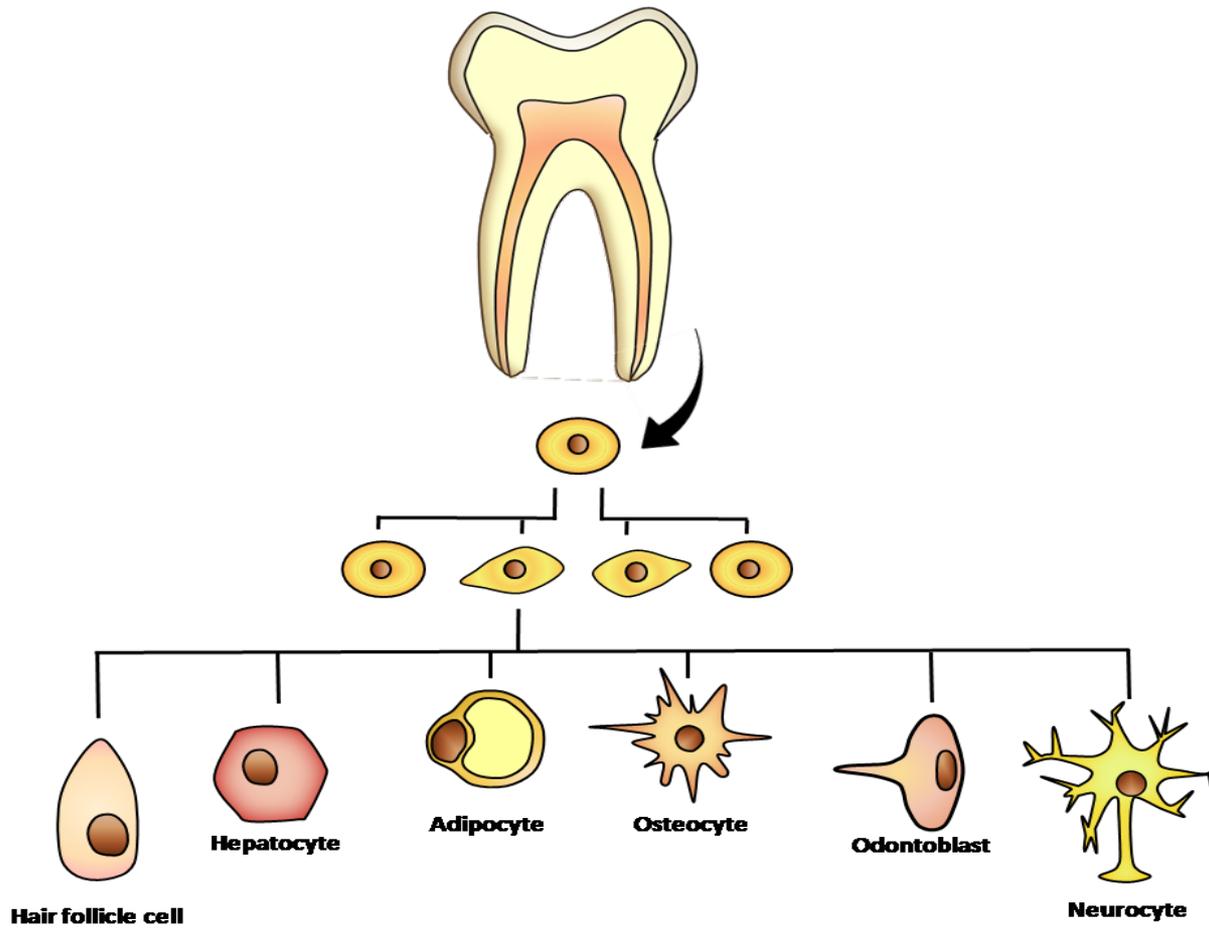




D'Antò et al., 2006









CORD BLOOD ITALY

DENTALSTEMCELLS



Your Stem Cells. Your Future Health.

Benvenuti

Fonti di cellule staminali

Guida per i nostri odontoiatri

Programmi di stoccaggio

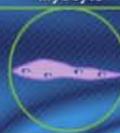
Contatti

Benvenuti

Mesenchymal Stem Cell



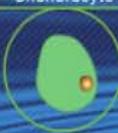
Myocyte



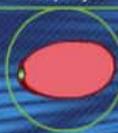
Osteocyte



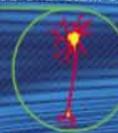
Chondrocyte



Adipocyte



Neuronal stem cell



Con il primato assoluto in Europa la Cord Blood Italy è lieta di presentare il suo nuovo servizio di crioconservazione delle cellule staminali della polpa dentale.

La Cord Blood Italy nata a Torino nel 2006 consente già ai genitori Italiani la conservazione delle cellule staminali del cordone ombelicale presso le strutture della prestigiosa banca New England Cord Blood Bank a Boston Massachusetts-USA.

Dal mese di aprile 2008, diamo anche la possibilità a tutti gli Italiani di conservare le cellule staminali della polpa del dente. La Cord Blood Italy è la prima e unica in Europa a dare questa meravigliosa e innovativa possibilità a chi è interessato ad investire nella salute futura di se stesso e della propria famiglia.

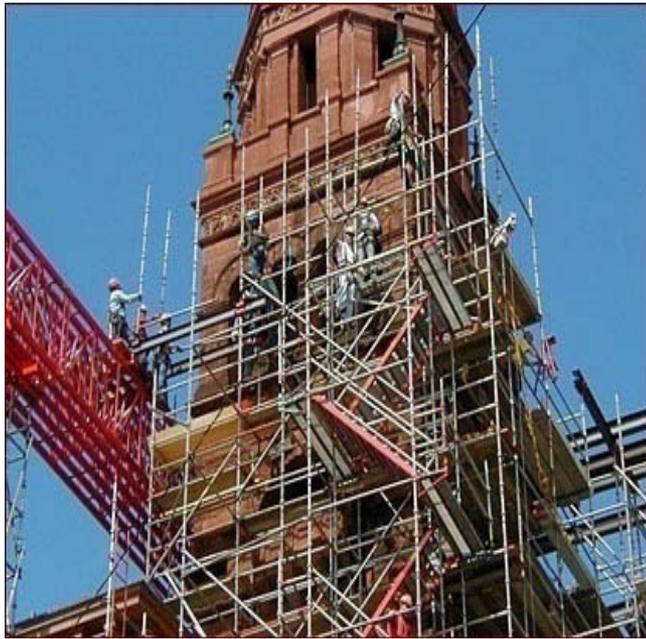
Il National Dental Pulp Laboratory, Inc. (NDPL)

La nostra banca è una delle banche per la crioconservazione di cellule e tessuti più antiche e prestigiose al mondo. Presso la nostra banca ci sono strutture dedicate alla crioconservazione di diverse cellule e tessuti; nella struttura della New England Cord Blood Bank vengono crioconservate le cellule staminali del cordone ombelicale, nella

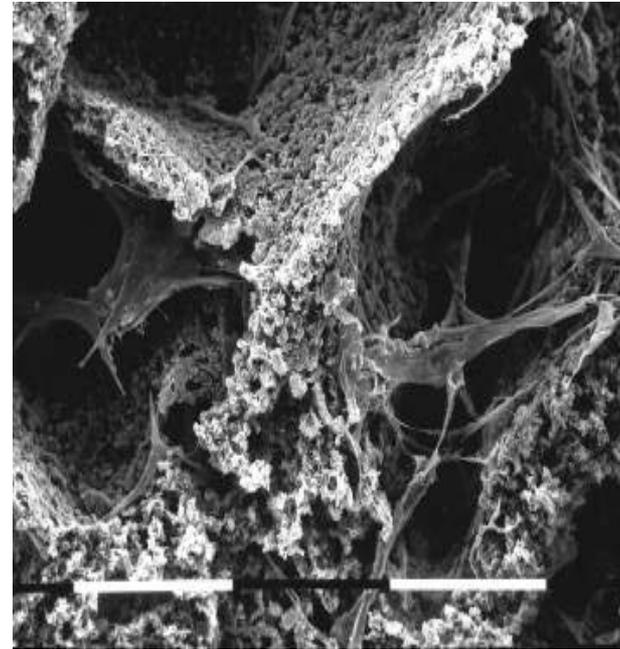


Ingegneria dei tessuti: *scaffold*

Uno scaffold combina diverse proprietà strutturali e funzionali

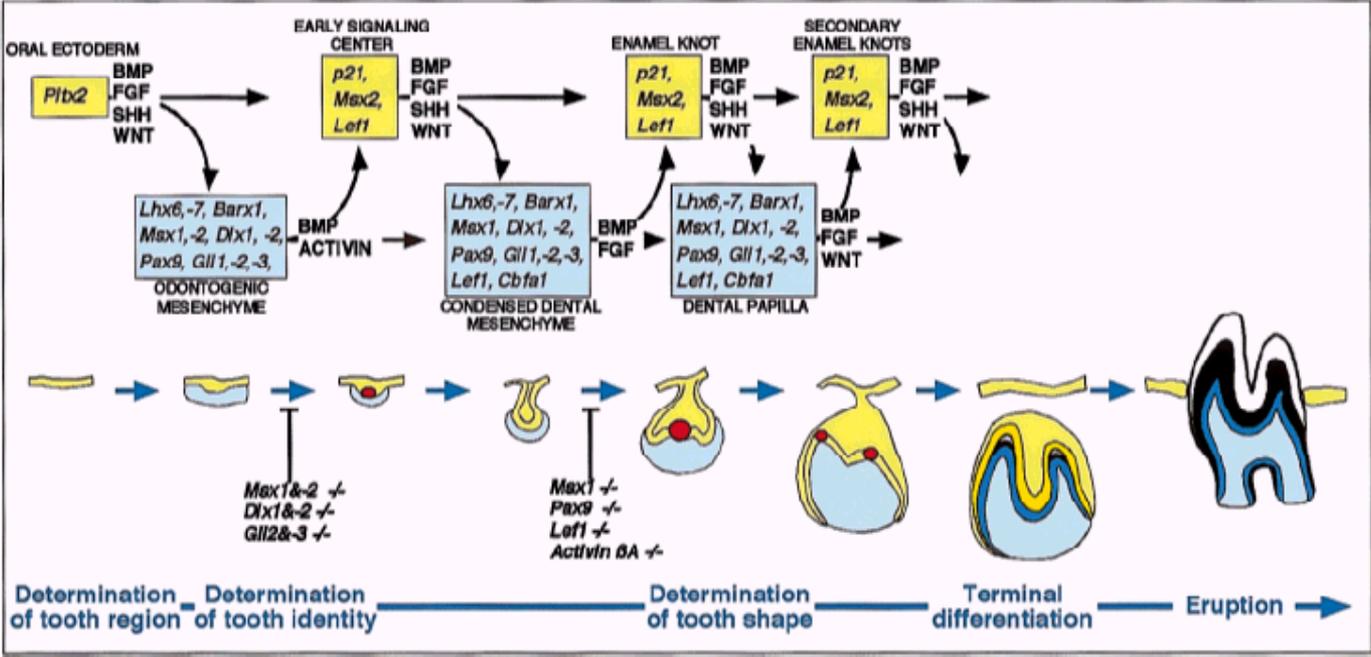
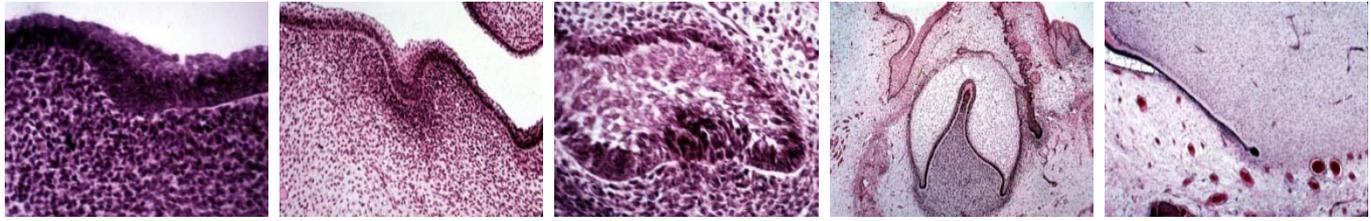


1m



10⁻⁴m

Segnali



Jernvall, J. AND Thesleff, I. (2000). Reiterative signalling and patterning in mammalian tooth morphogenesis. *Mech. Dev.* 92, 19-29.



RAPID COMMUNICATION

Biomaterials & Bioengineering

C.S. Young¹, S. Terada², J.P. Vacanti², M. Honda³, J.D. Bartlett^{1*}, and P.C. Yelick^{1*}

¹Department of Cytokine Biology and Harvard-Forsyth Department of Oral Biology, The Forsyth Institute, Boston, MA 02115, USA; ²Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA; and ³Department of Oral and Maxillofacial Surgery, Nagoya University School of Medicine, Nagoya, Japan; *corresponding authors, pyelick@forsyth.org, jbartlett@forsyth.org

J Dent Res 81(10):695-700, 2002

Tissue Engineering of Complex Tooth Structures on Biodegradable Polymer Scaffolds

RESEARCH REPORTS

Biomaterials & Bioengineering

M.T. Duailibi⁴, S.E. Duailibi⁴, C.S. Young², J.D. Bartlett², J.P. Vacanti³, and P.C. Yelick^{2*}

¹University Federal of São Paulo, Department of Otorhinolaryngology and Human Communication Disorders, São Paulo, Brazil; ²Department of Cytokine Biology, The Forsyth Institute, 140 The Fenway, and Department of Oral and Developmental Biology, Harvard Medical School, Boston, MA 02115, USA; and ³Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA; ⁴authors contributing equally to this paper; *corresponding author, pyelick@forsyth.org

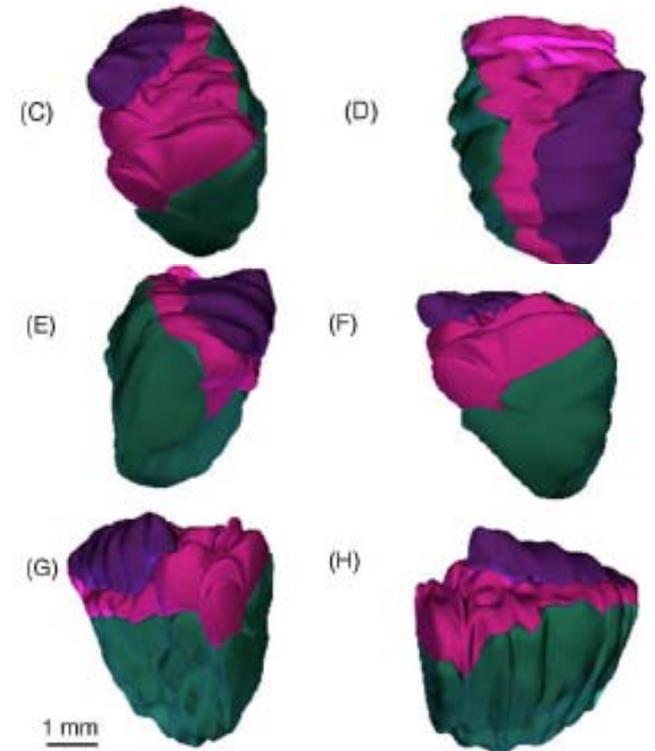
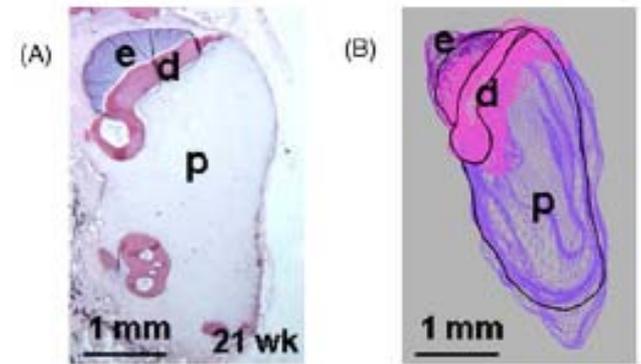
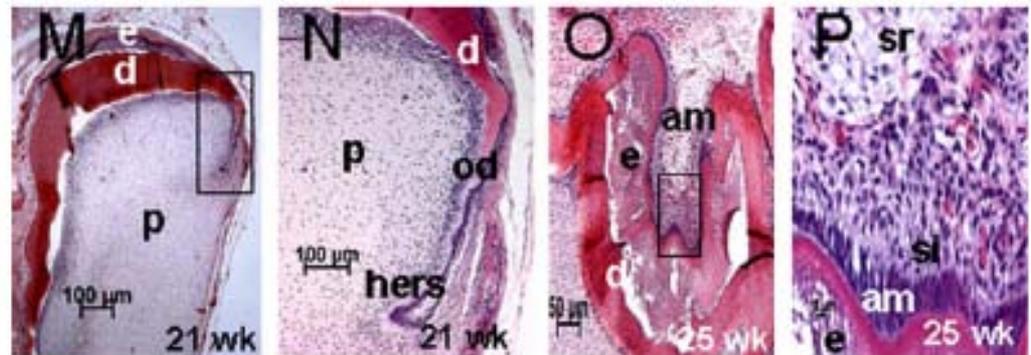
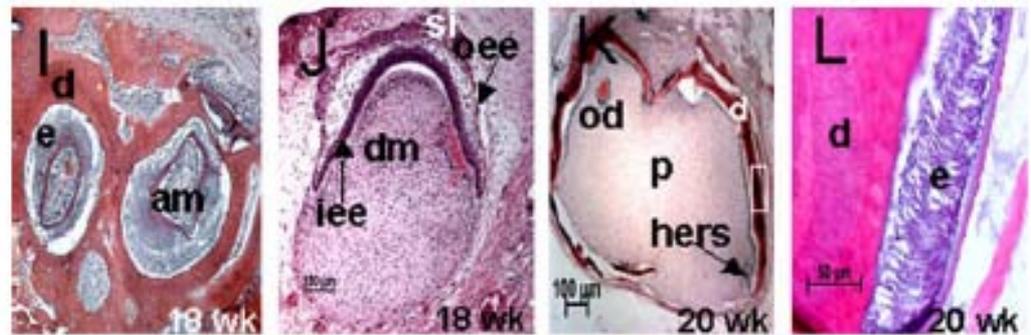
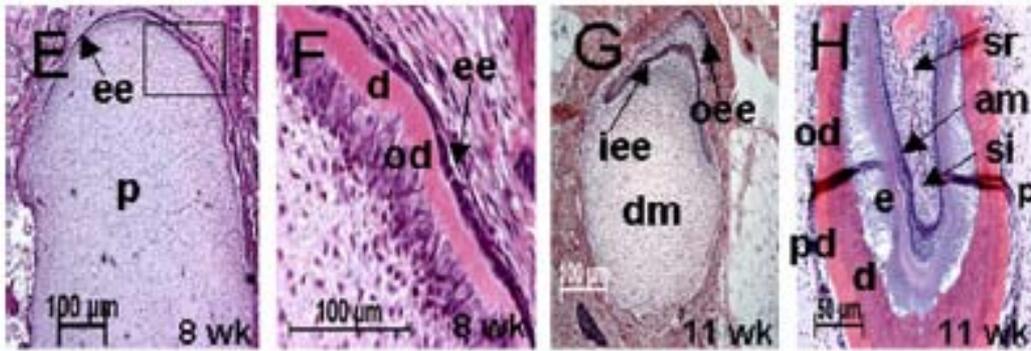
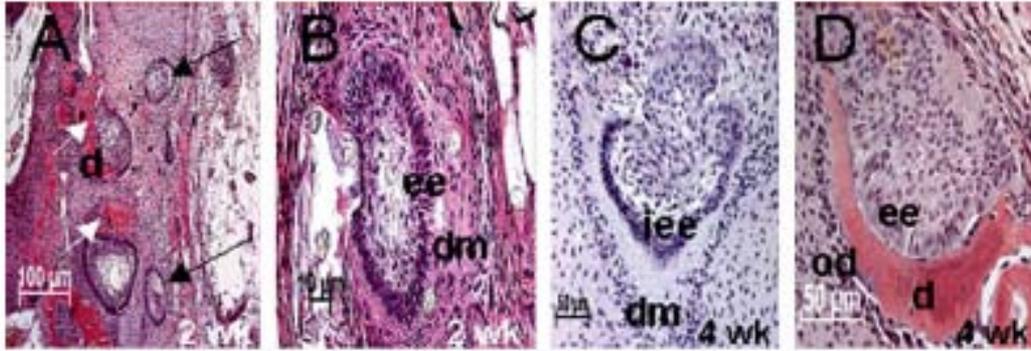
J Dent Res 83(7):523-528, 2004

Bioengineered Teeth from Cultured Rat Tooth Bud Cells

INTRODUCTION

The incidence of children born with missing primary and/or adult teeth is

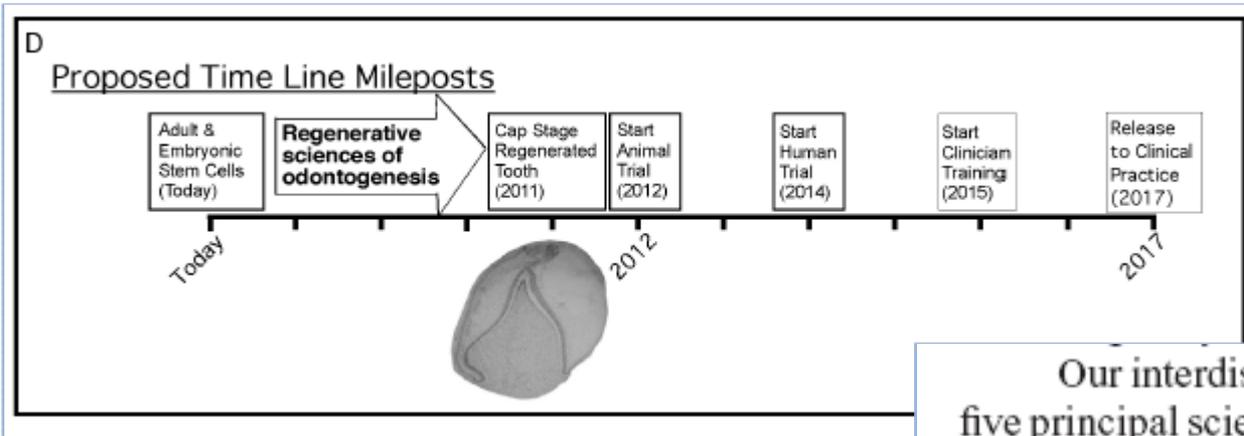




Young CS et al.,
Arch Oral Biol 2005

Whole-Tooth Regeneration: It Takes a Village of Scientists, Clinicians, and Patients

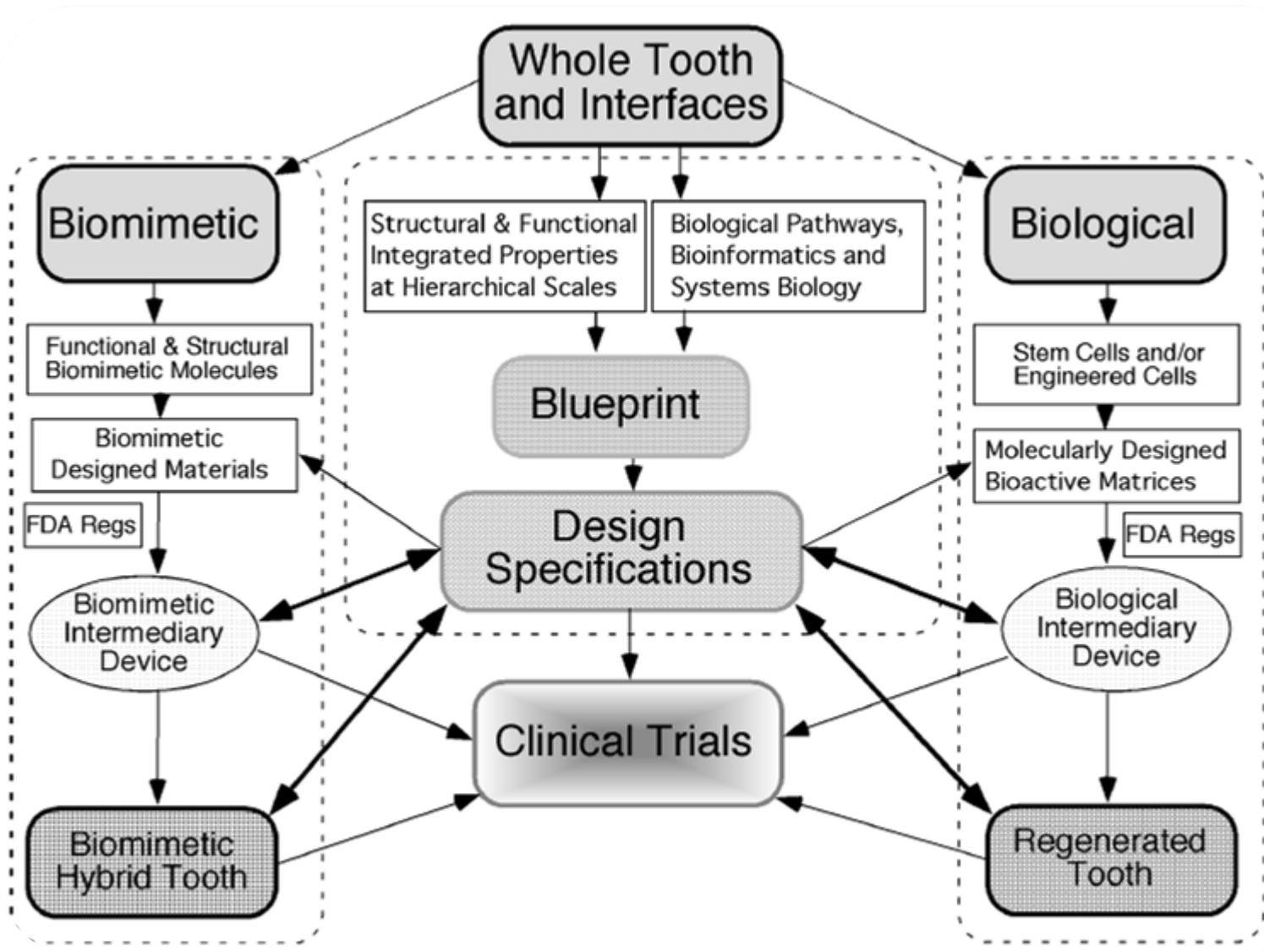
Malcolm L. Snead, D.D.S., Ph.D.



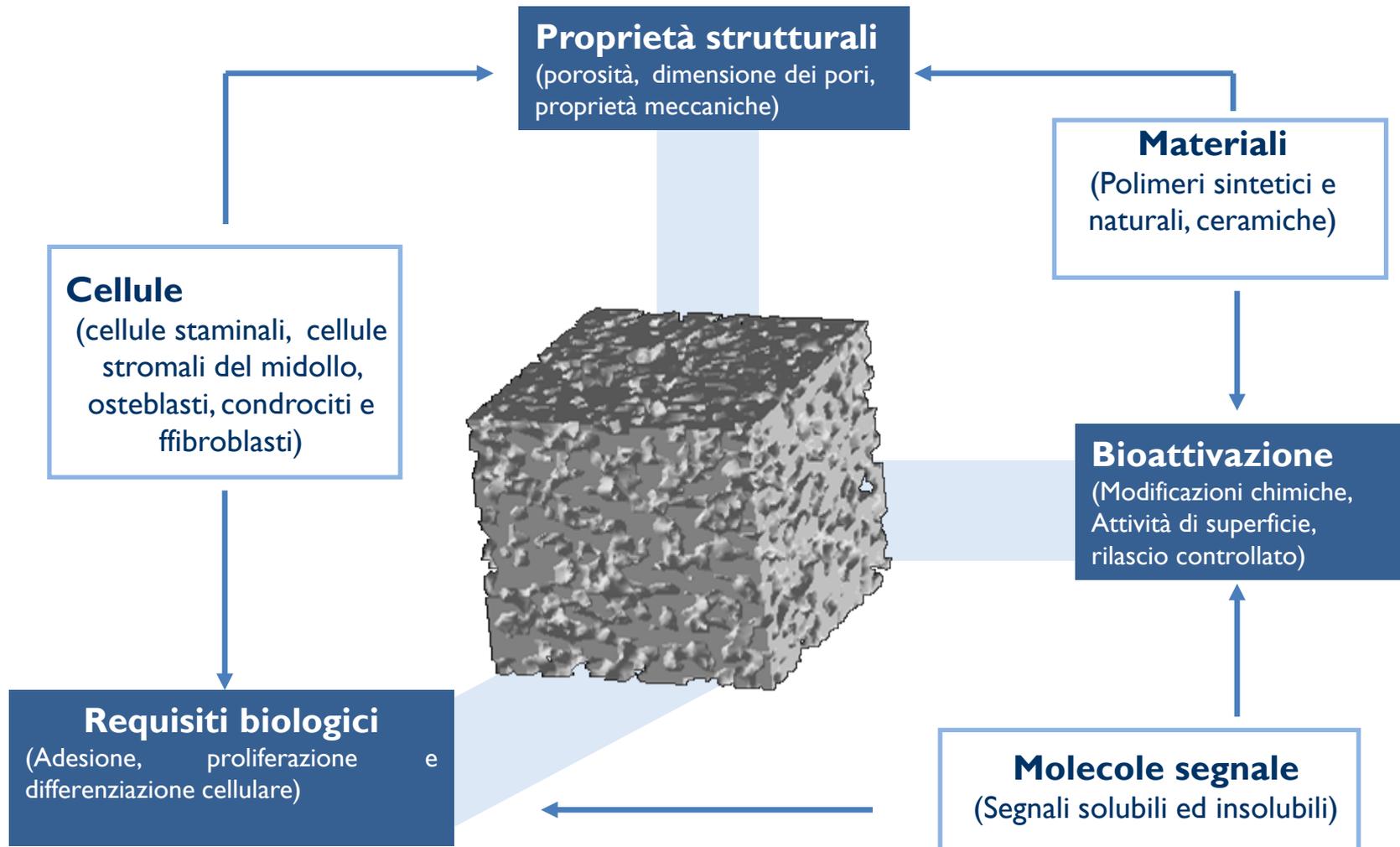
National Center focused on Building a Tooth by Bridging Biology and Material Sciences (BTBBMS)

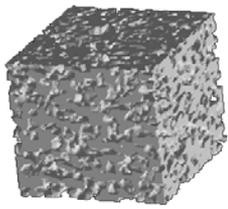
Our interdisciplinary team consisted of twenty-five principal scientists in the fields of developmental biology, stem cell biology, engineering, chemistry, structural biology, computational biology, tissue engineering, high performance teams, crisis management, systems biology, genomics, proteomics, oral surgery, wound repair, periodontology, material sciences, angiogenesis, the FDA regulatory system, and systems engineering.





GLI SCAFFOLD PER L'INGEGNERIA DEI TESSUTI



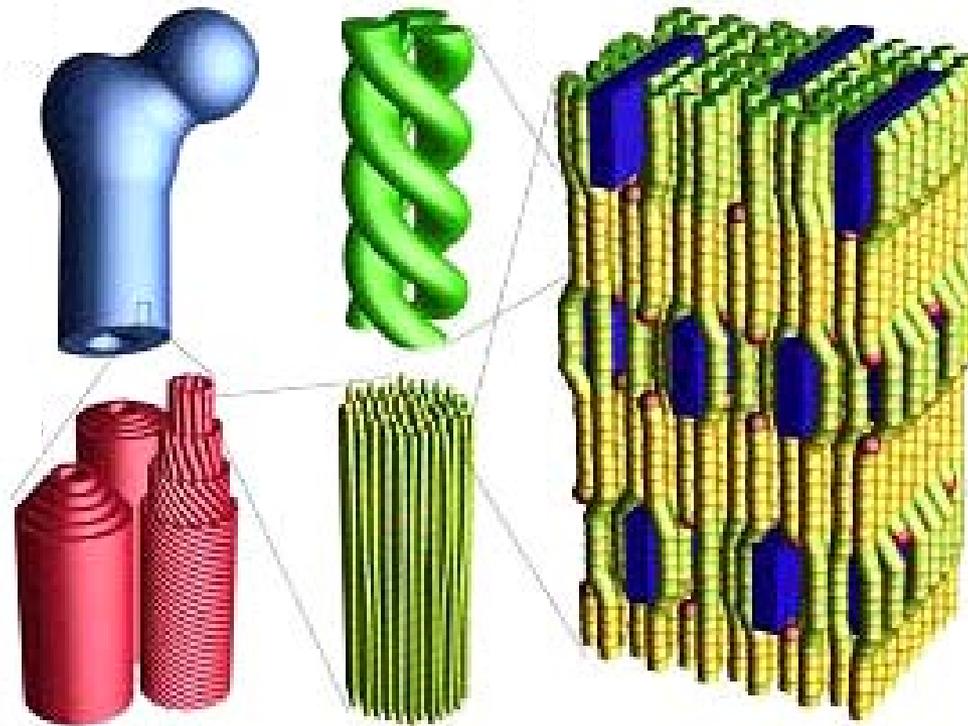


MATERIALI COMPOSITI IBRIDI

Osso
macroscopico

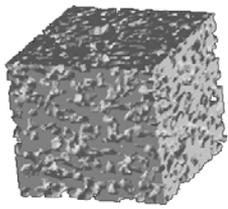
Osteoni

Cristalli minerali incorporati



Fibre
striate
di collagene

Fibre di collagene

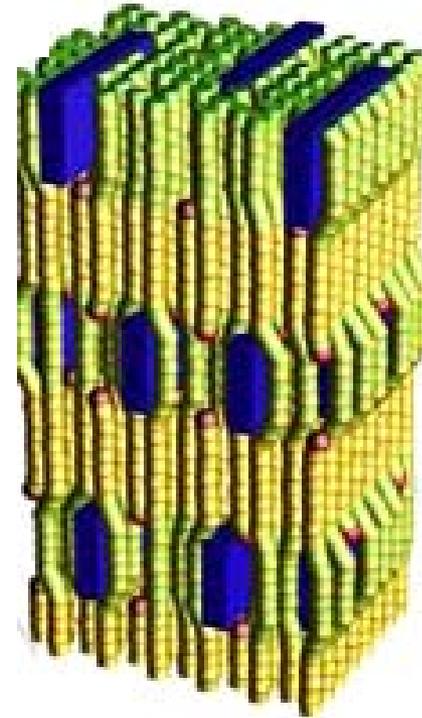


MATERIALI COMPOSITI IBRIDI

Cristalli minerali incorporati

Le ceramiche di **idrossiapatite** sono conosciute per la loro biocompatibilità, ma le loro proprietà meccaniche limitano le loro applicazioni cliniche

Il **poly- ϵ -caprolattone** è conosciuto per la sua biodegradabilità, biocompatibilità e proprietà meccaniche adeguate



Fibre
striate
di collagene

Composito organico/inorganico



PREPARAZIONE DEI MATERIALI COMPOSITI IBRIDI

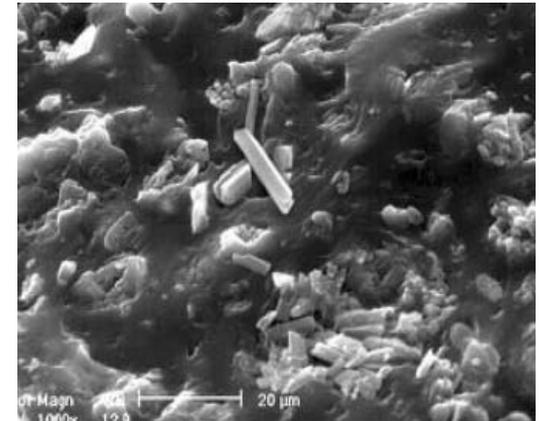
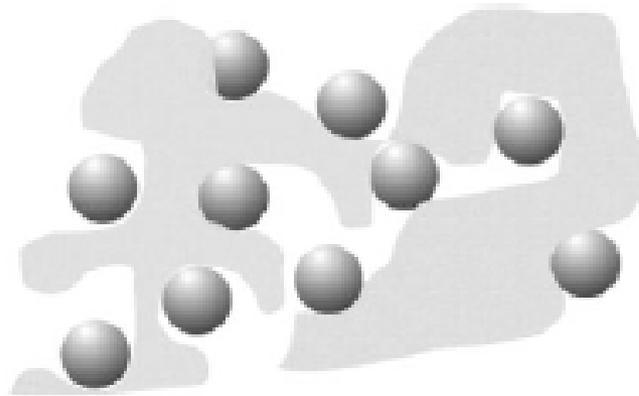
MODI DIFFERENTI

Miscelazione fisica

Sintesi idrotermale

Gas Foaming

Sol-gel



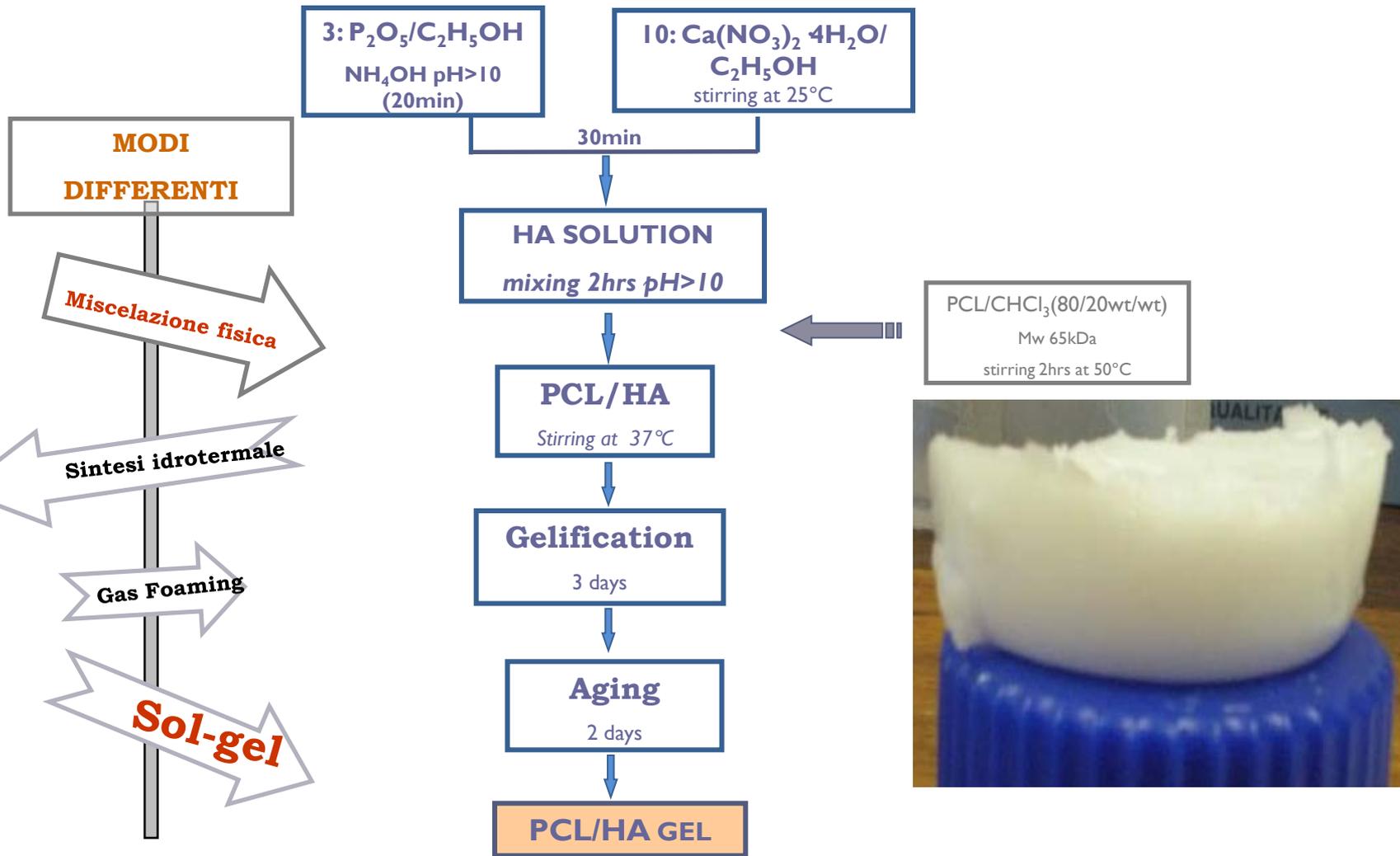
A. La Gatta et al / *Macromol. Biosci.* 5 (2005)

Metodo SOL-GEL

- Bassa temperatura di sintesi;
- Alta purezza del prodotto;
- Composizione omogenea;
- Microstruttura di grani fini contenente un misto di cristalli di varia grandezza;
- Sintesi di compositi ibridi organici/inorganici.

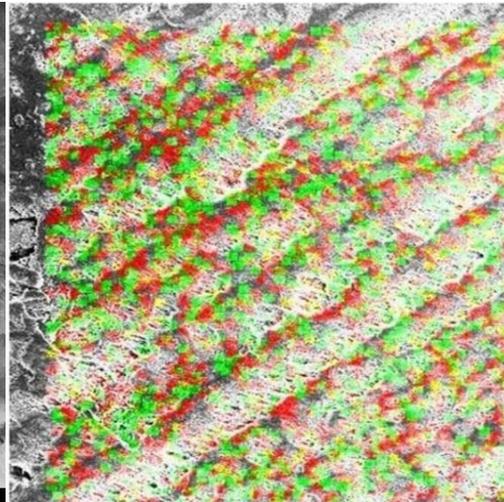
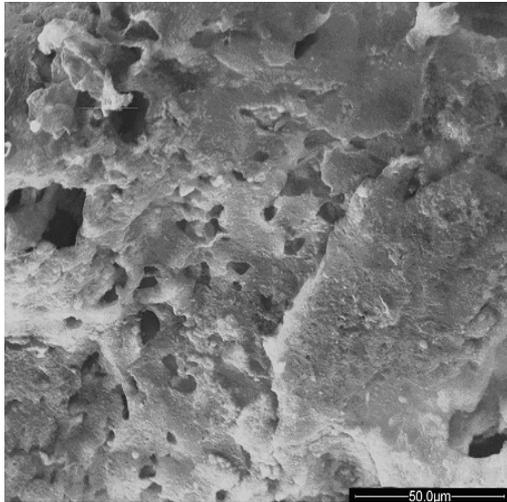


SINTESI CHIMICA tramite la tecnica Sol-gel



CARATTERIZZAZIONE DEI COMPOSITI HA/PCL

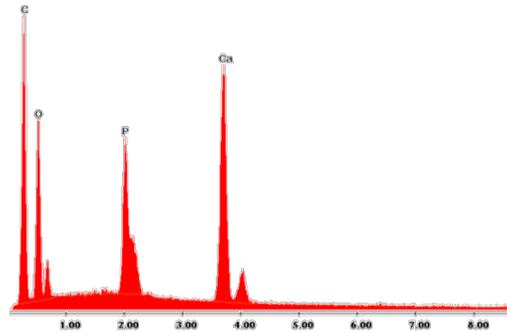
SEM analysis



Mapping Test

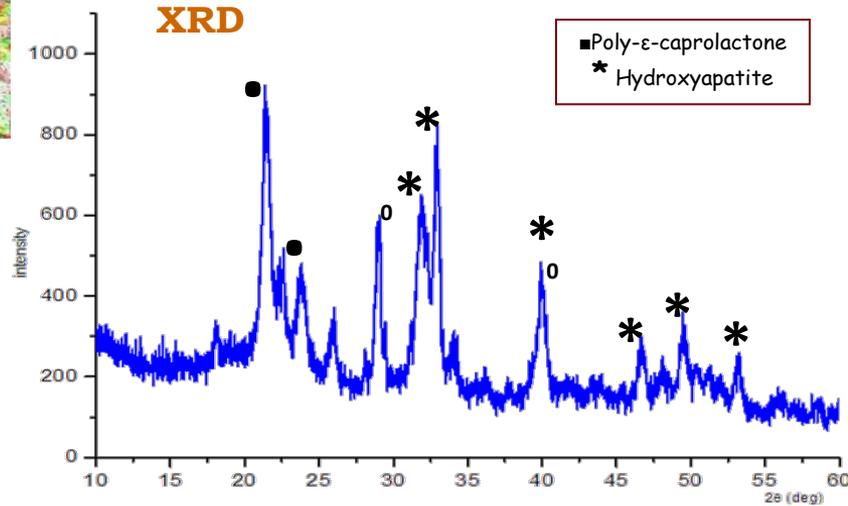
Le particelle di HA (P) sono uniformemente distribuite nella matrice polimerica (C).

EDS analysis



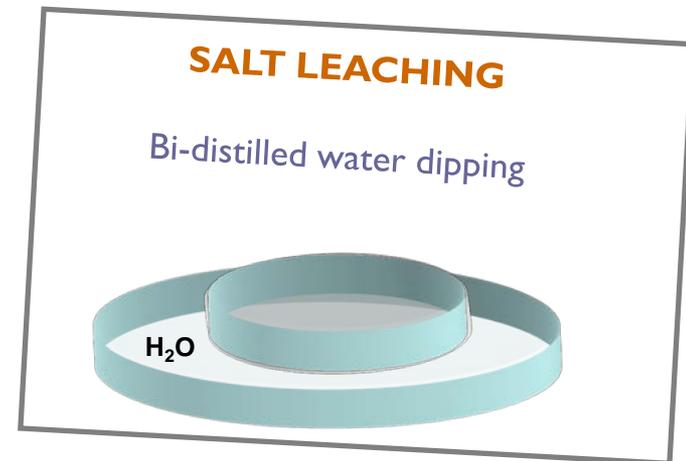
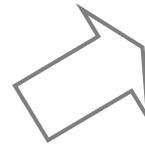
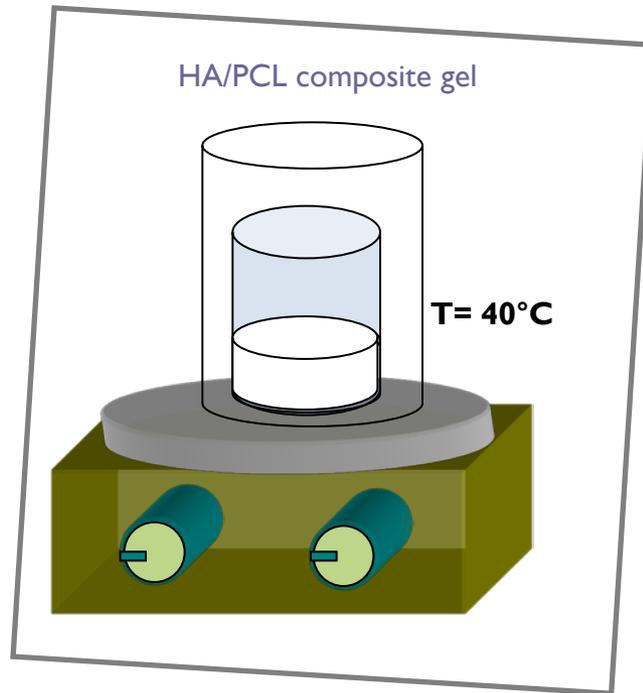
Elem	Wt%	At%
C K	29.10	37.92
O K	58.24	56.79
P K	4.18	1.98
Ca K	8.49	3.30
Ca/P		1.66

Rapporto Ca/P tipico dell'idrossiapatite



Presenza di idrossiapatite con bassa cristallinità

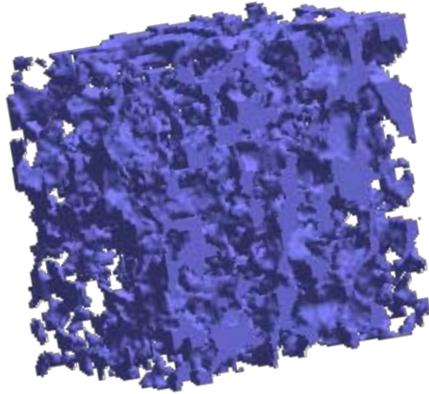




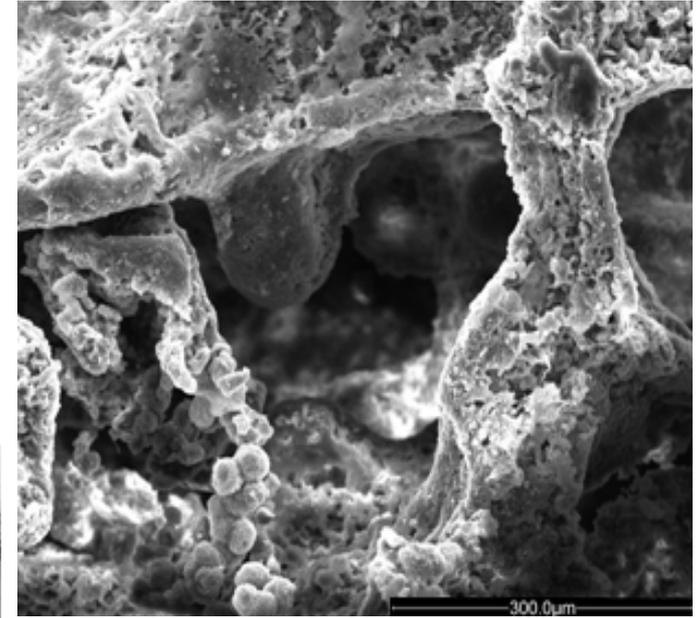
*Inversione di fase/
salt leaching*

MORFOLOGIA 3D DEGLI SCAFFOLD HA/PCL

Analisi μ CT

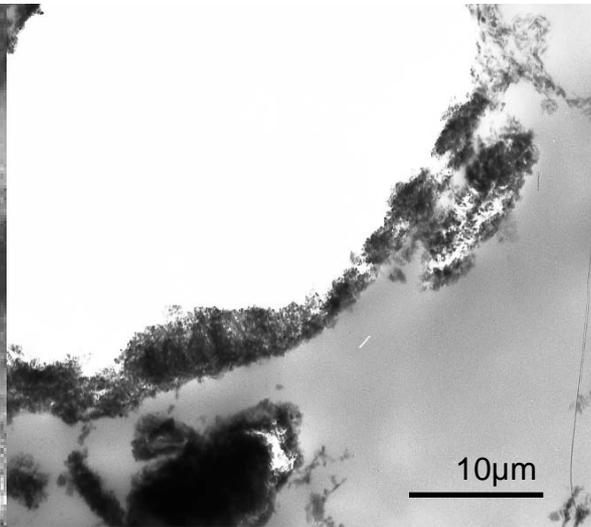
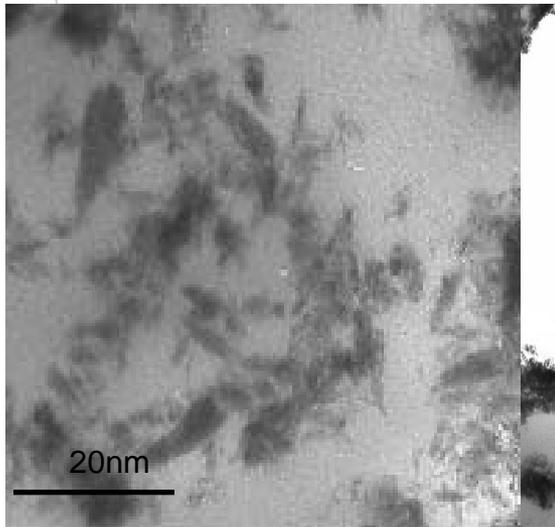


Scaffold con uniforme distribuzione dei pori e buona interconnessione



SEM analysis

Analisi TEM



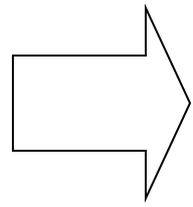
Piccoli grappoli di particelle di nano-Idrossiapatite a margine dei pori: effetto idrofilico

Step 3

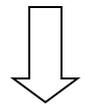
VALUTAZIONE DELLA BIOATTIVITA'

TRATTAMENTO CON SBF

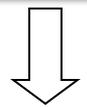
	Blood plasma	SBF	1.5 SBF
Na ⁺	142.0	142.0	213.0
K ⁺	5.0	5.0	7.5
Mg ²⁺	1.5	1.5	2.25
Ca ²⁺	2.5	2.5	3.75
Cl ⁻	103.0	147.8	221.7
HCO ₃ ⁻	27.0	4.2	6.3
HPO ₄ ²⁻	1.0	1.0	1.5
SO ₄ ²⁻	0.5	0.5	0.75



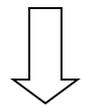
Incubazione in 1xSBF
pH=7 per 7 giorni a 37°C



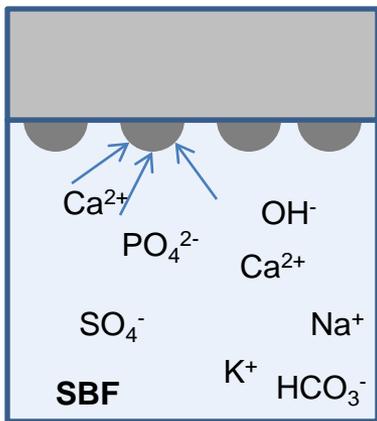
**Precipitazione dell'apatite
sulla superficie del materiale**



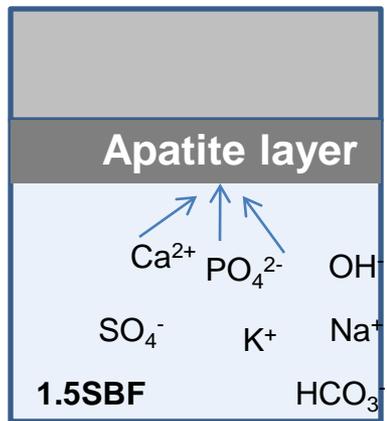
Incubazione in 1.5xSBF
pH=7 per 14 giorni a 37°C



**Crescita dei nuclei di apatite
sulla superficie del materiale**

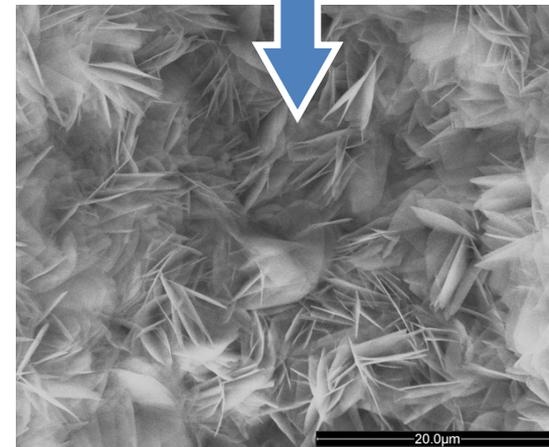
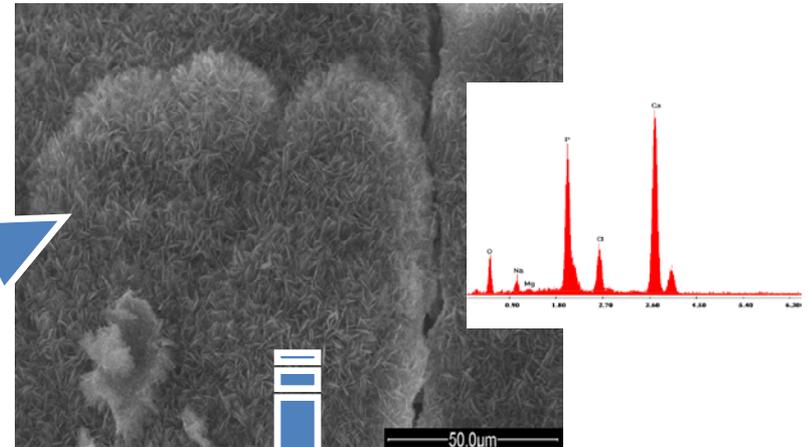
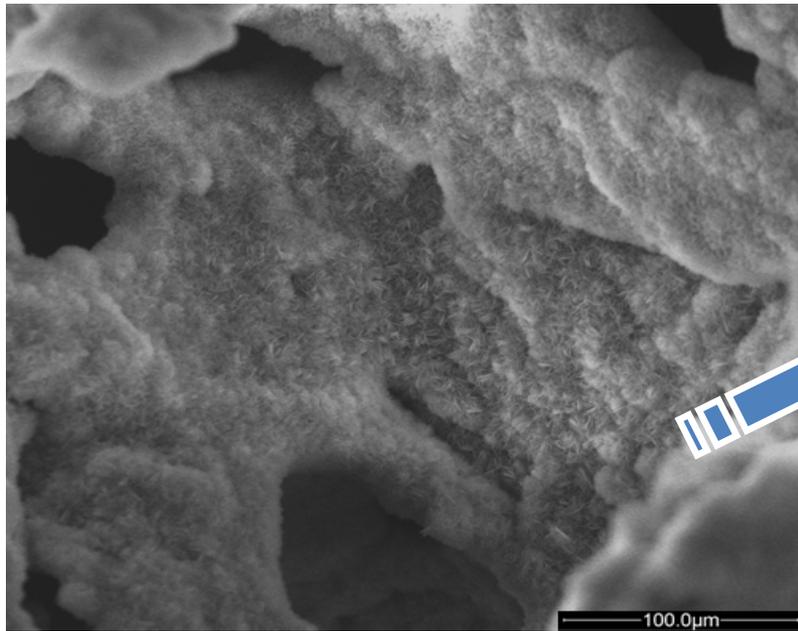


Nucleazione dell'apatite



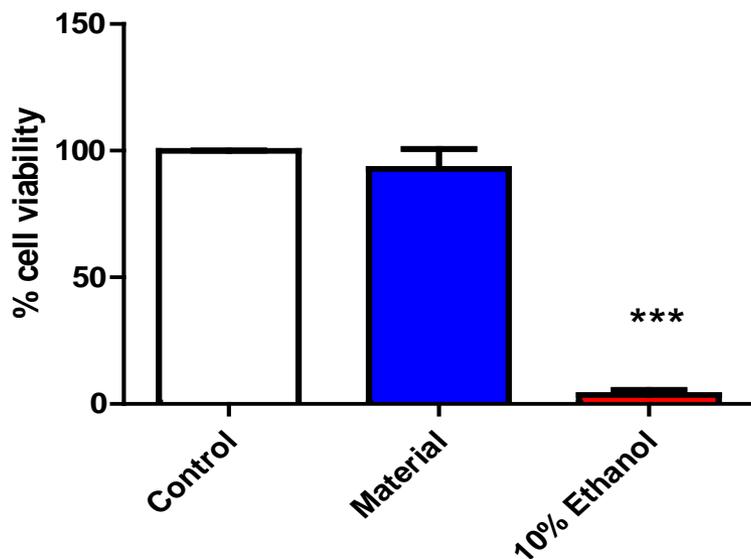
Crescita dell'apatite



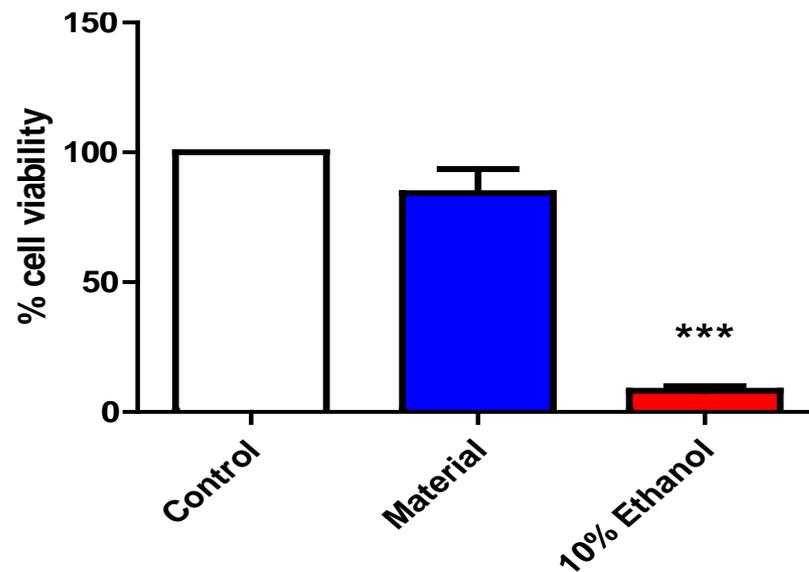


La presenza di nanoparticelle di HA uniformemente disperse nella matrice polimerica consente un miglioramento Della bioattività del composito

Alamar blue

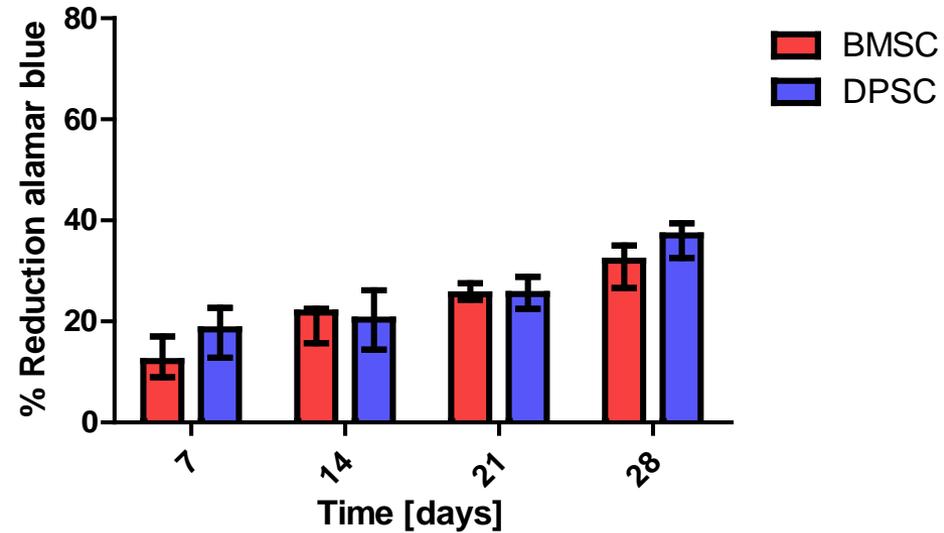
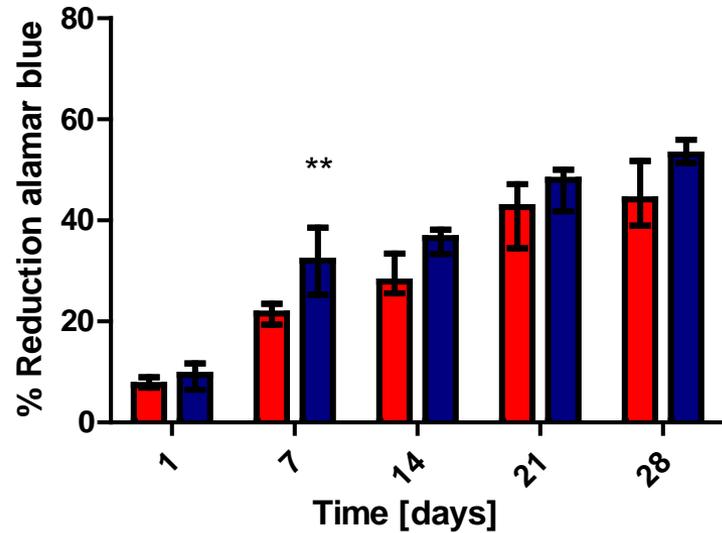


Saggio MTT



Il materiale non ha effetti negativi sulla vitalità cellulare dei fibroblasti di topo Balb/c 3T3.

PROLIFERAZIONE CELLULARE



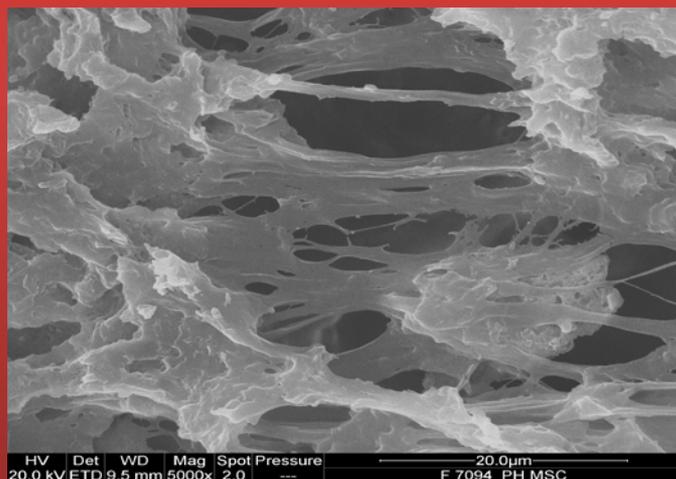
La proliferazione cellulare aumenta durante il tempo di coltura



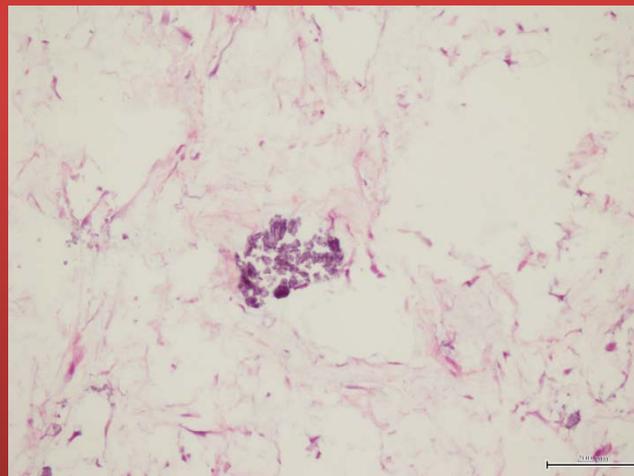
ANALISI MORFOLOGICA (15 giorni)

MSC

SEM

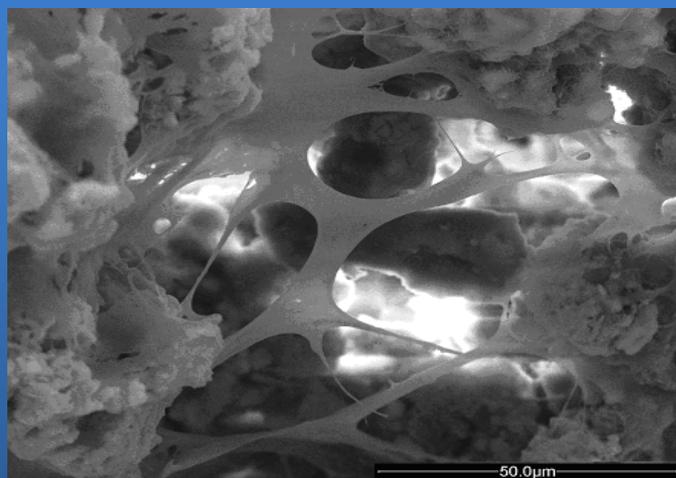


H & E

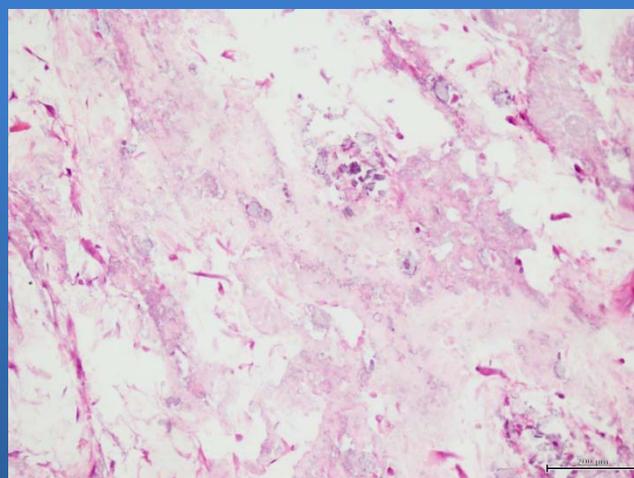


DPSC

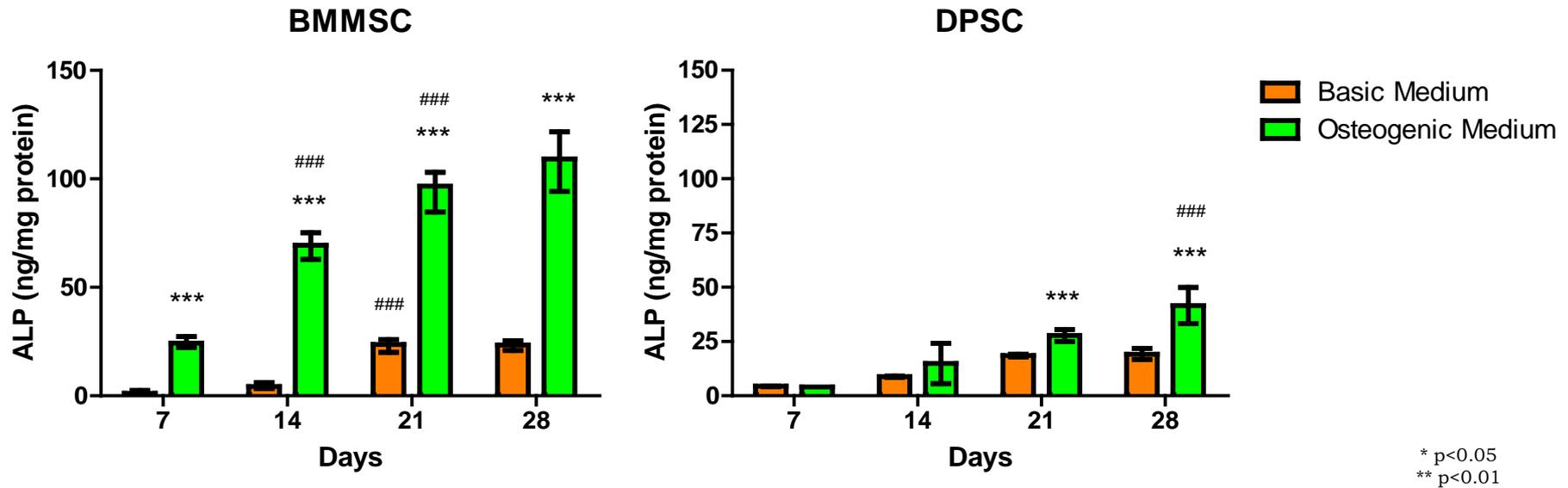
SEM



H & E



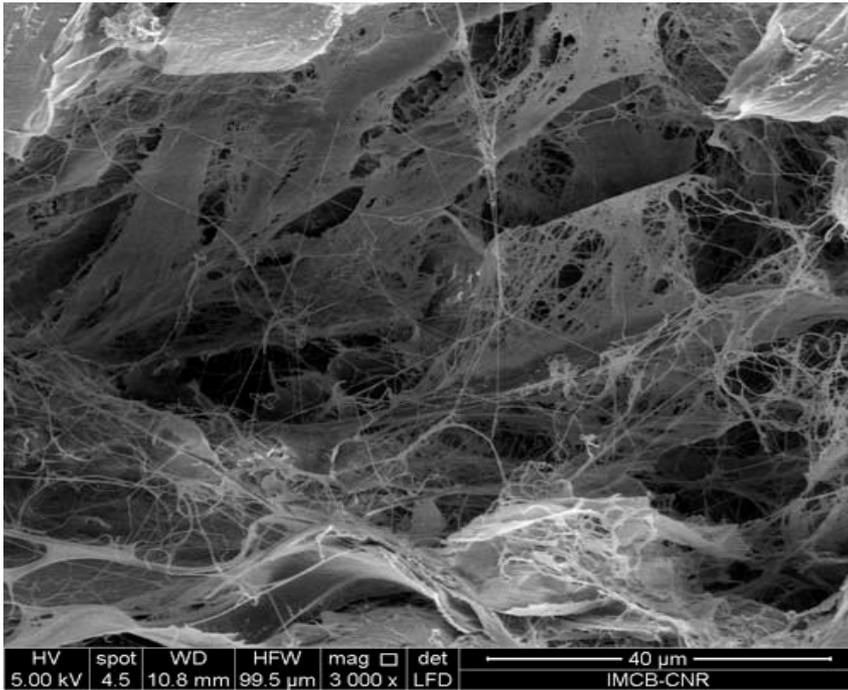
ATTIVITA' DELL'ALP



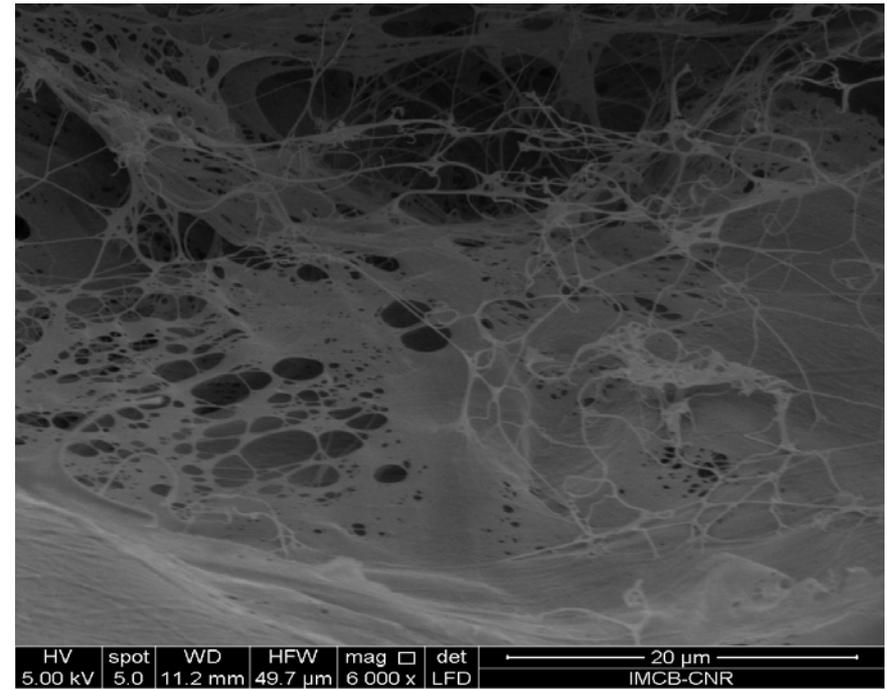
I valori dell'ALP aumentano in maniera significativa fino al 21° giorno di crescita delle hMSC sullo scaffold e raggiungono un plateau tra il 21° e il 28° giorno.

ANALISI MORFOLOGICA (35 giorni)

MSC

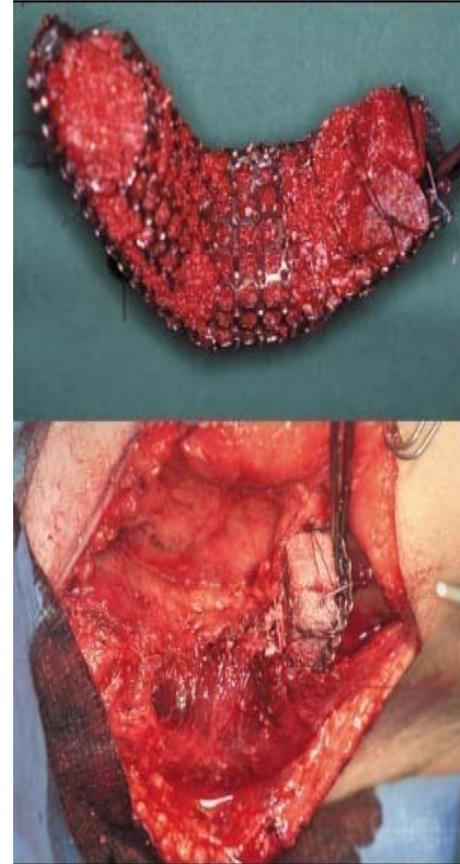
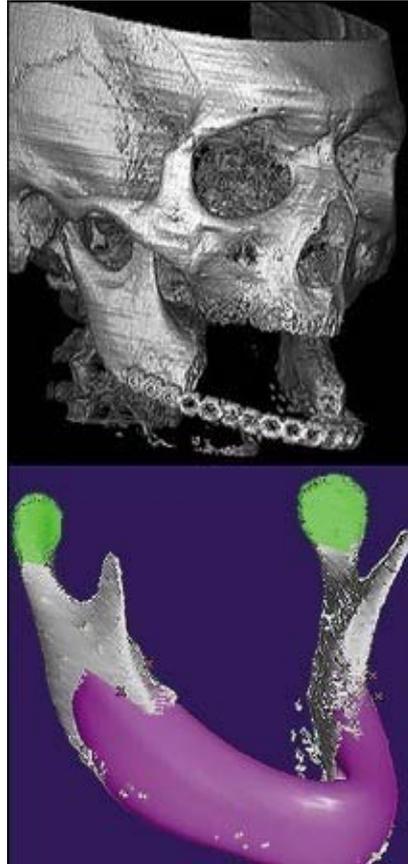


DPSC



Growth and transplantation of a custom vascularised bone graft in a man

Lancet 2004; 364: 766-70 P H Warnke, I N G Springer, J Wiltfang, Y Acil, H Eufinger, M Wehmöller, P A J Russo, H Bolte, E Sherry, E Behrens, H Terheyden

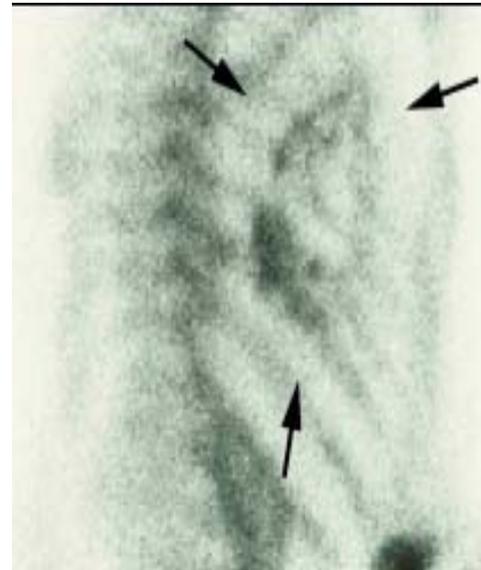


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Three weeks after implantation inside latissimus muscle



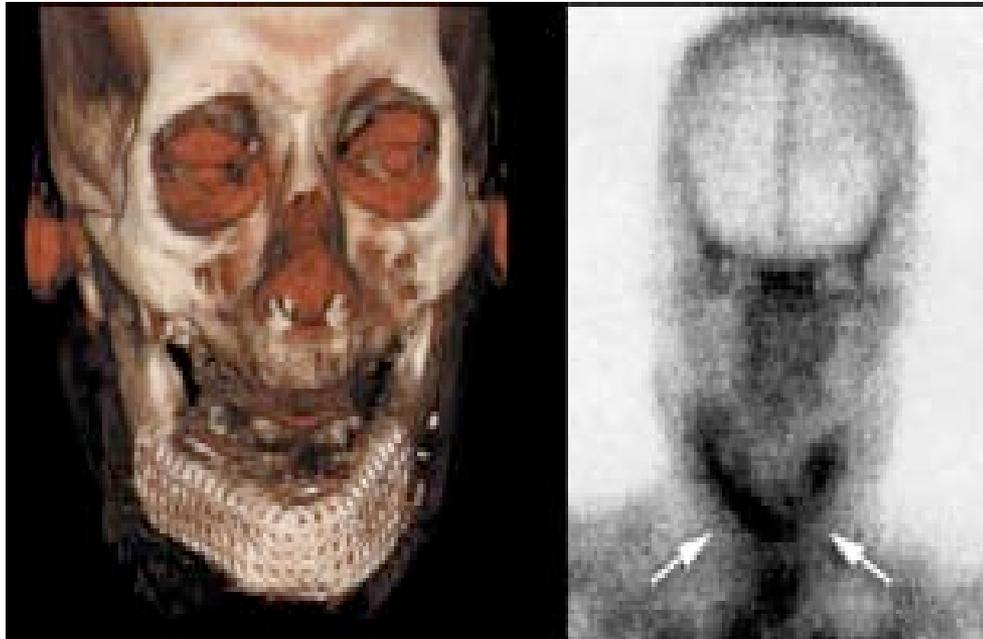
Four weeks later

Warnke PH et al., Lancet 2004



Growth and transplantation of a custom vascularised bone graft in a man

Lancet 2004; 364: 766-70 P H Warnke, I N G Springer, J Wiltfang, Y Acil, H Eufinger, M Wehmüller, P A J Russo, H Bolte, E Sherry, E Behrens, H Terheyden

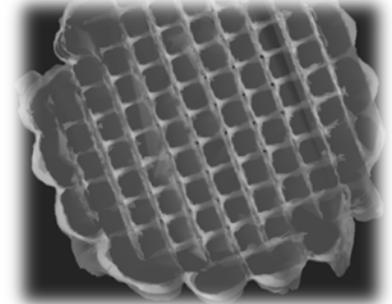
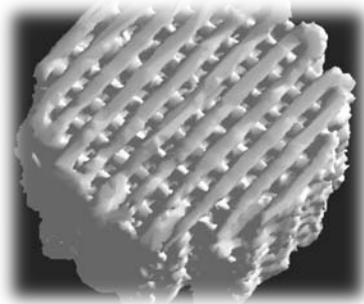
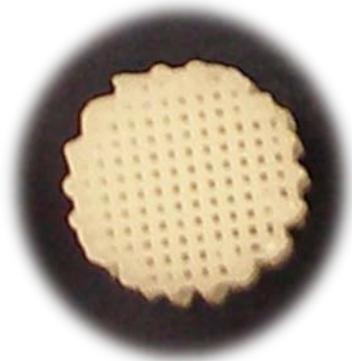
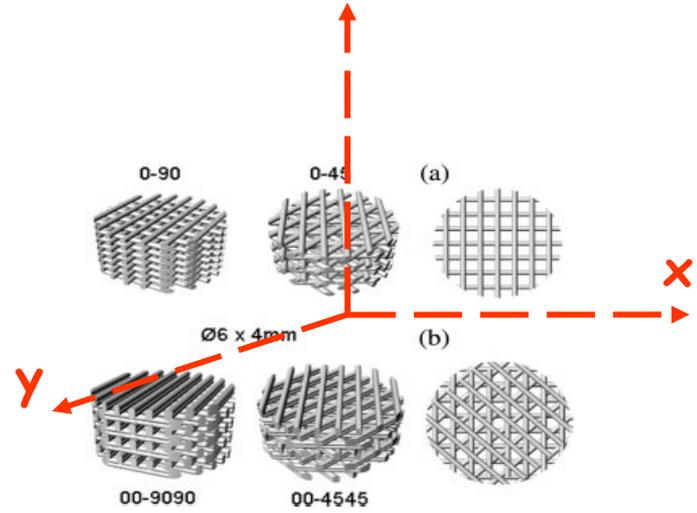
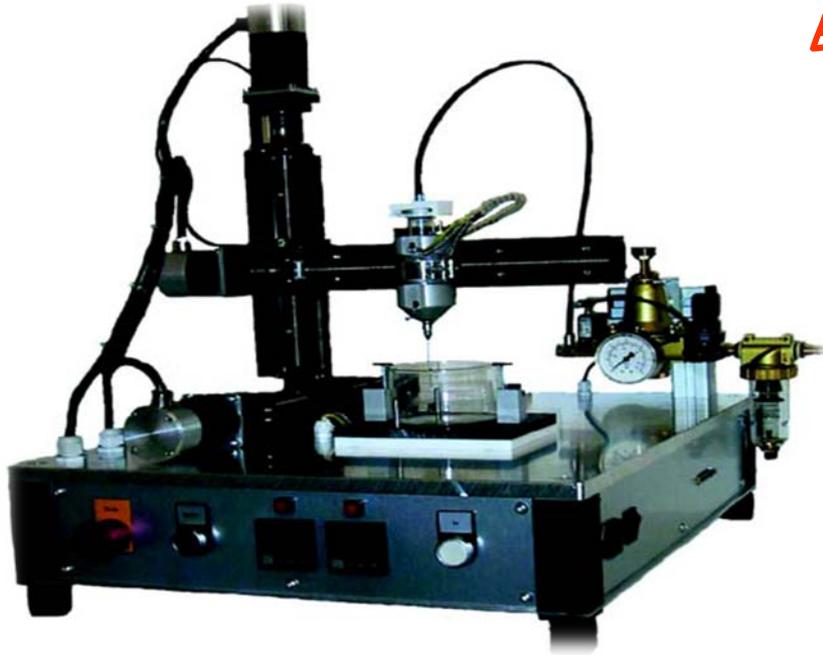


Seven weeks later...

Warnke PH et al., *Lancet* 2004

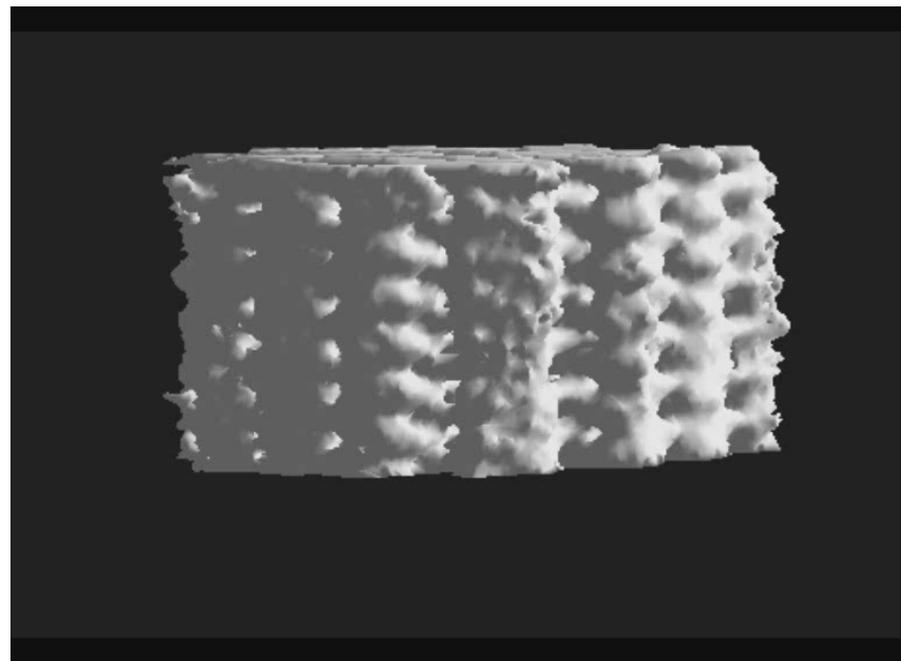
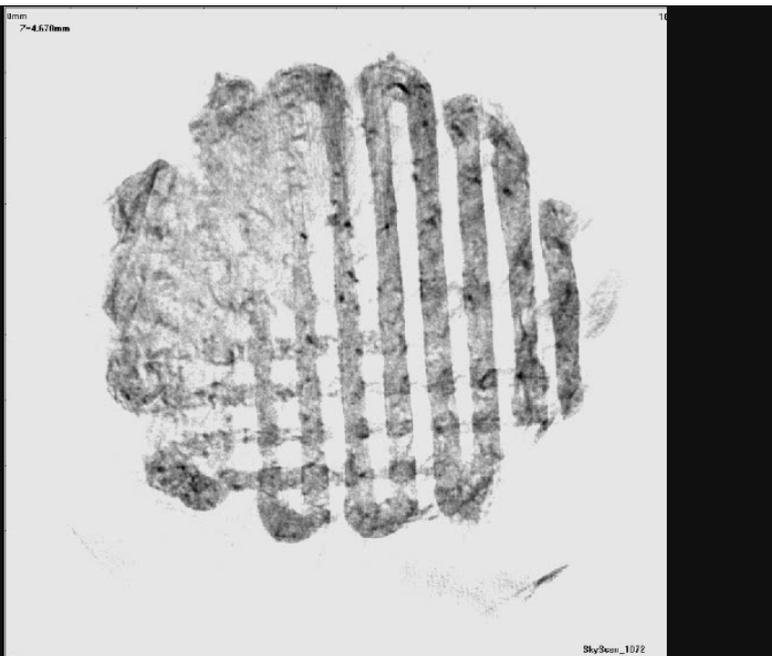


BIOPLOTTER



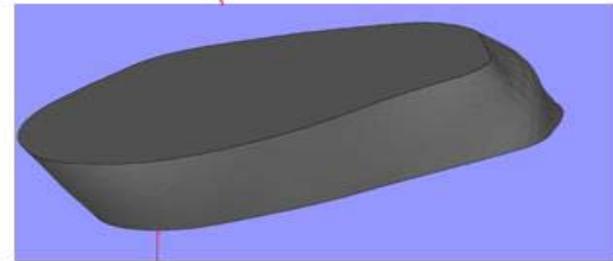
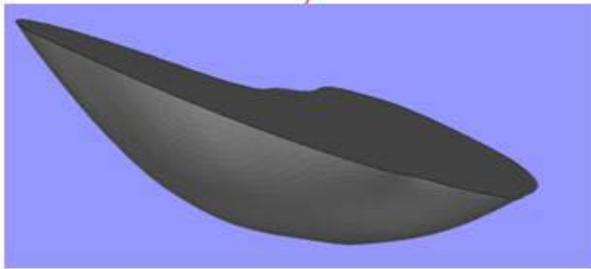
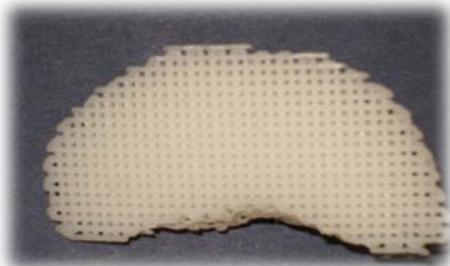
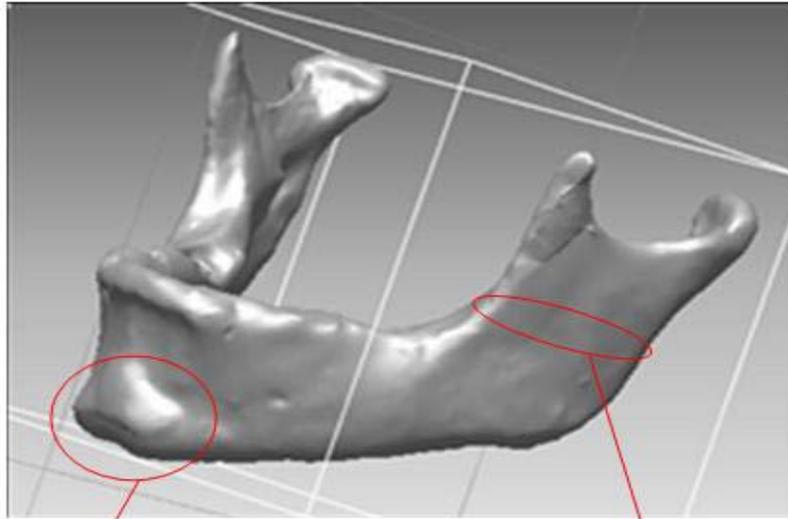


Ingegneria dei tessuti: *segnali*



Scaffold in Policaprolattone (PCL)

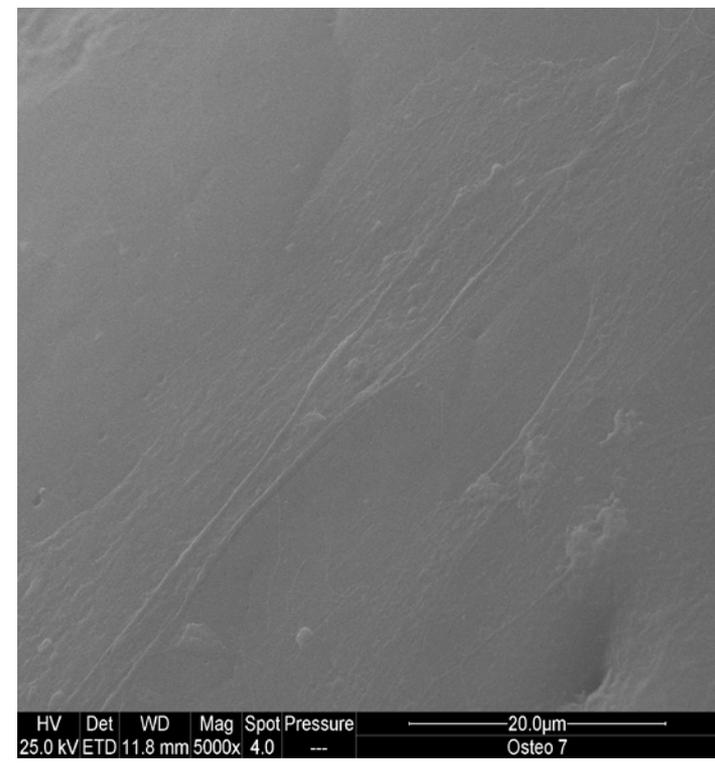
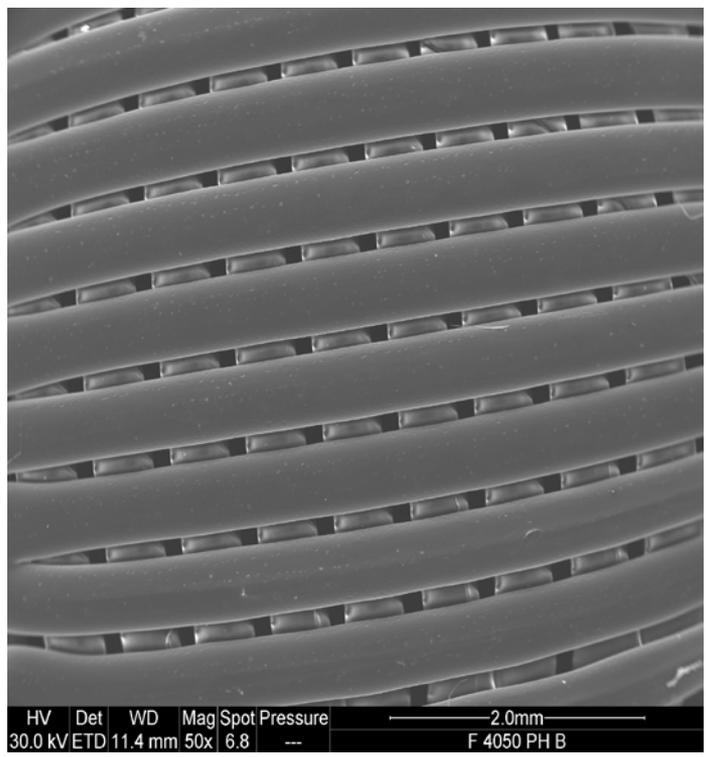






Ingegneria dei tessuti: **scaffold**

SEM

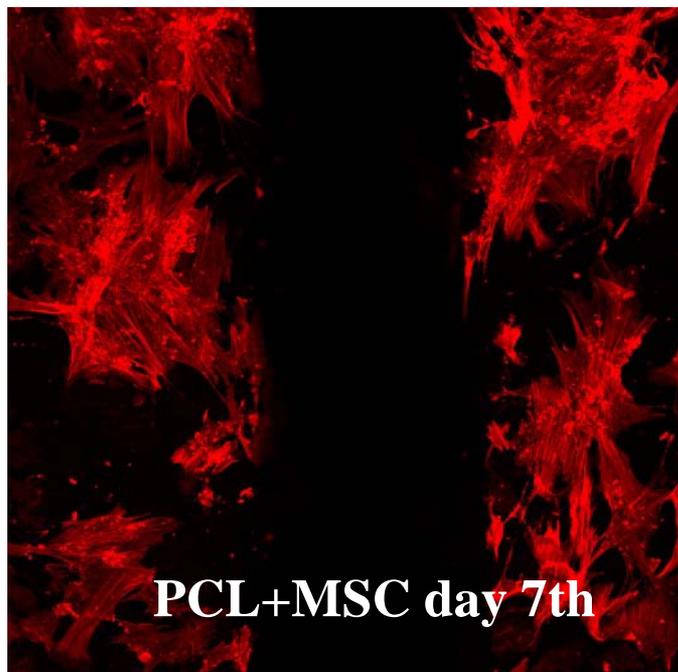


Adesione e spreading cellulare sullo scaffold in PCL

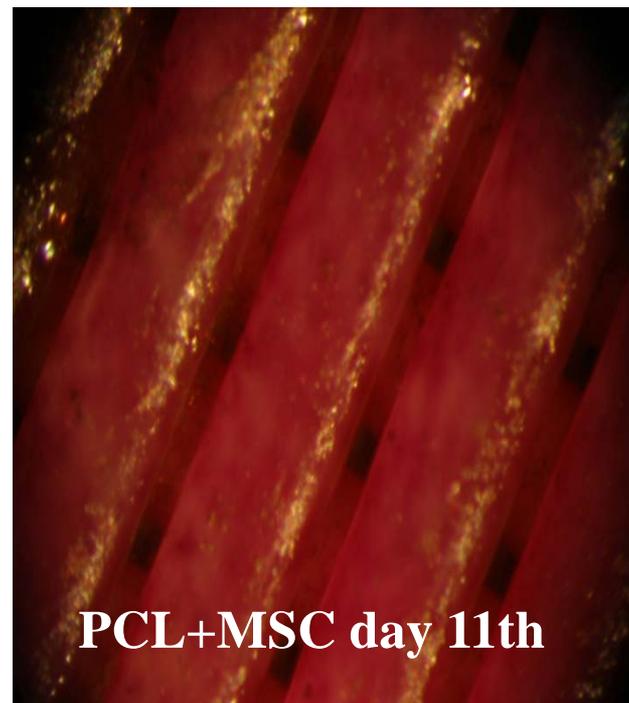




Adesione delle cellule sullo scaffold



PCL+MSC day 7th



PCL+MSC day 11th

Ringraziamenti

