



Health & Wealth

Tecnologie per la Salute



Development of a 3D Skin Model based on Biocompatible Polymeric Hydrogel Scaffolds (HyPSs), bioengineered with Human Mesenchymal Stem Cells (hMSCs) with or without fibroblast, keratinocytes and lymphocytes for healing of skin wounds
“SKINFOLDS”

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Lab CREA
DPT of Clinical and Experimental Sciences
University of Brescia - ASST Spedali Civili of Brescia*



Project Aim

Design and develop a 3D Culture Model for studying the mechanisms of tissue repair for : **Skin**

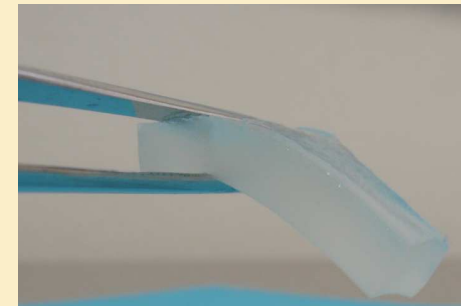
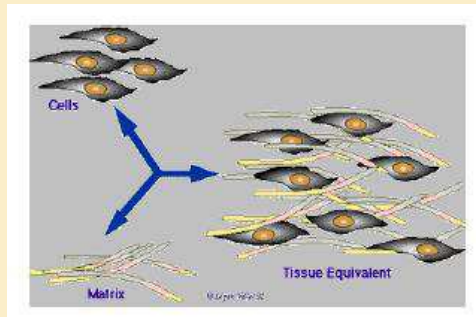
**Human
Mesenchymal
Stem Cells
(hMSCs)**

+

**Human Platelet
Lysate (hPL)**

+

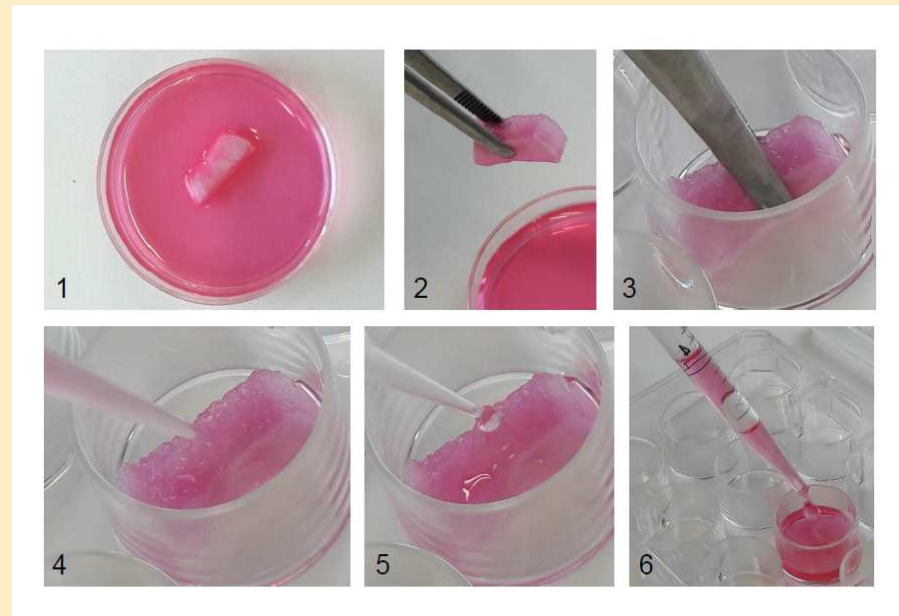
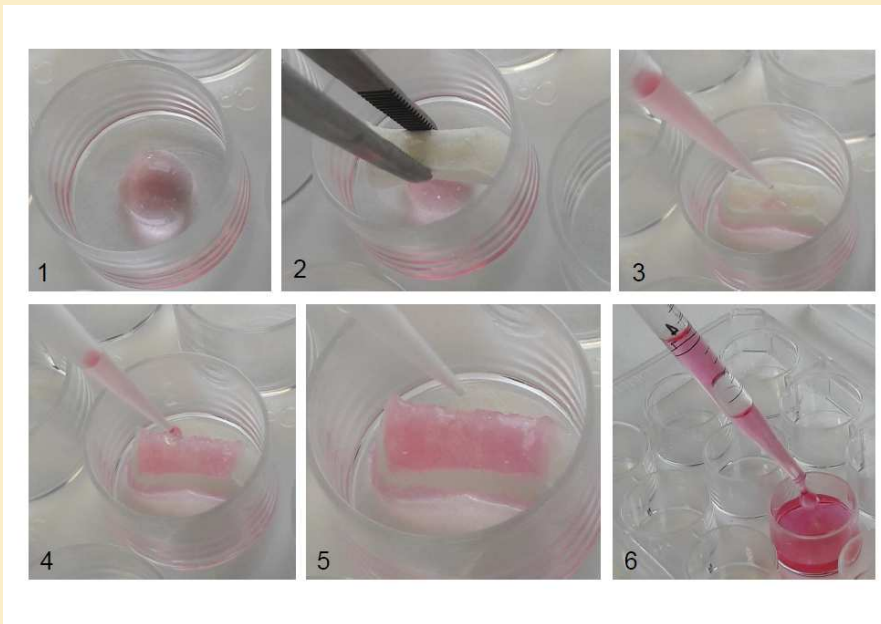
**Hydrogel
Polymeric
Scaffolds
(HyPSs)**



HyPSs Cutting and 3D Culture Seeding



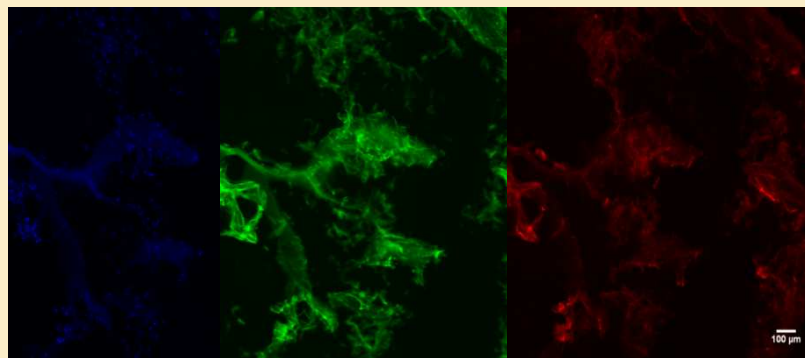
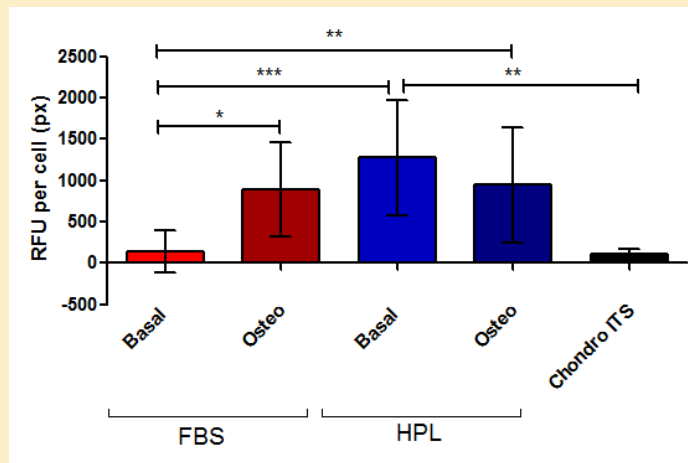
Skin → Bone - Cartilage



Scaffold 27: Osteogenic Differentiation

BM-hMSCs: osteogenic differentiation (**OSTEOCALCIN**)

S-27



DAPI

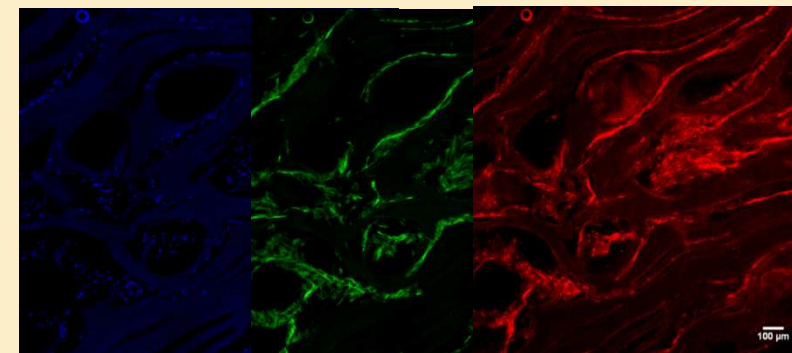
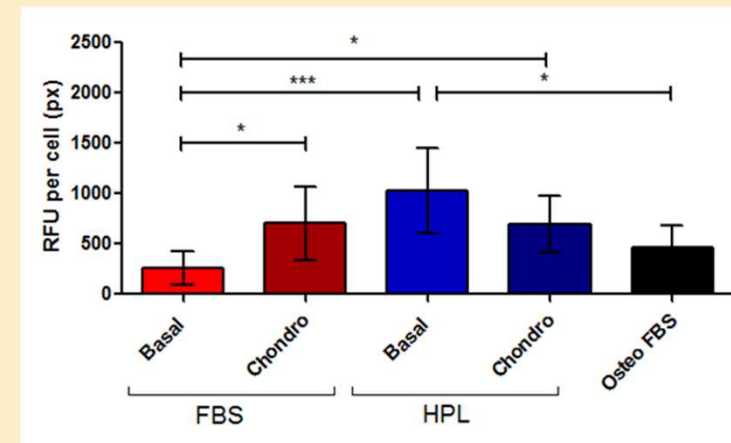
ACTIN

OSTEOCALCIN

Scaffold 26 : Chondrogenic Differentiation

BM-hMSCs: chondrogenic differentiation (**AGGRECAN**)

S-26



DAPI

ACTIN

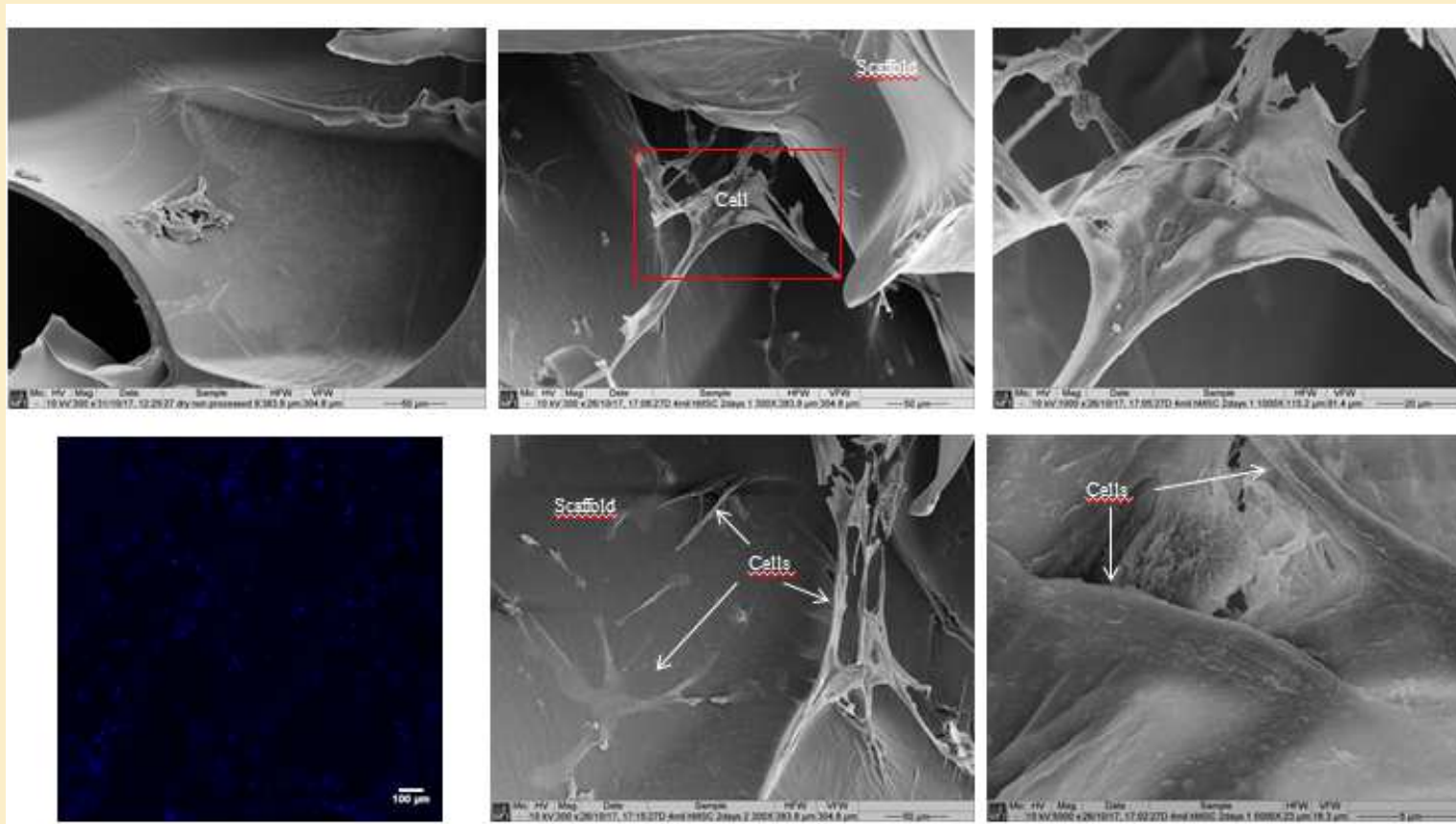
AGGRECAN

Scanning Electron Microscope (SEM) Analysis

Scaffold 27 and BM-hMSCs: adhesion capabilities after 2 days of cell culture in growth medium

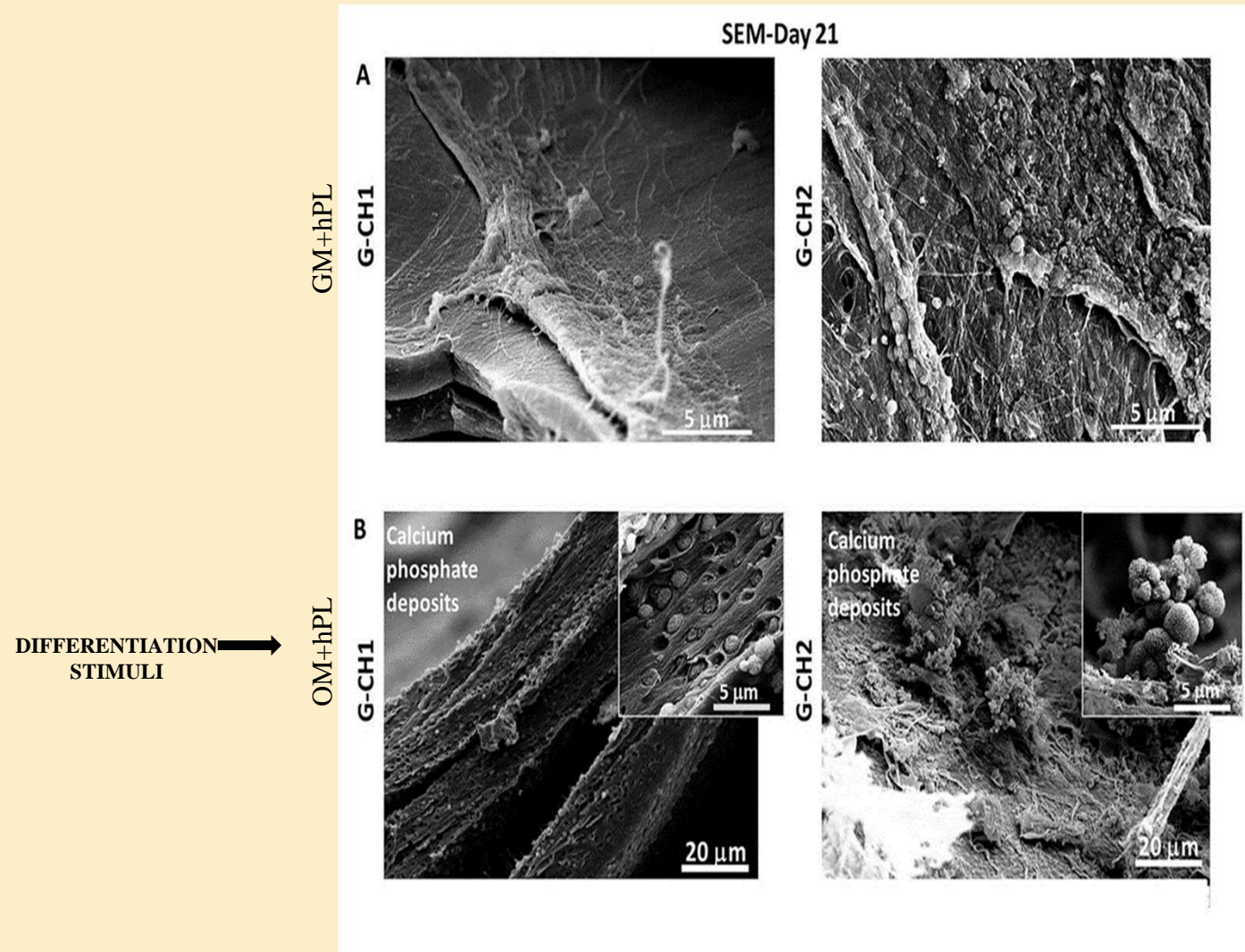
Scaffold w/o hMSC

Scaffold with hMSCs

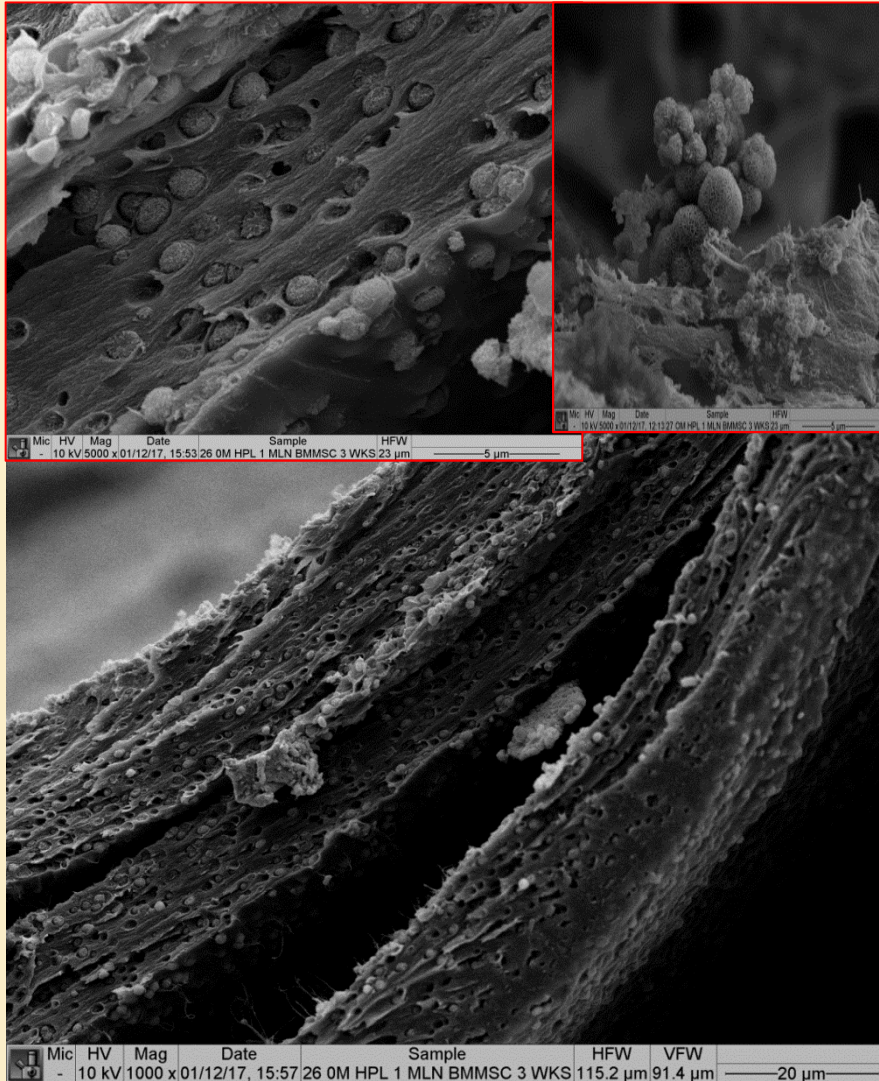


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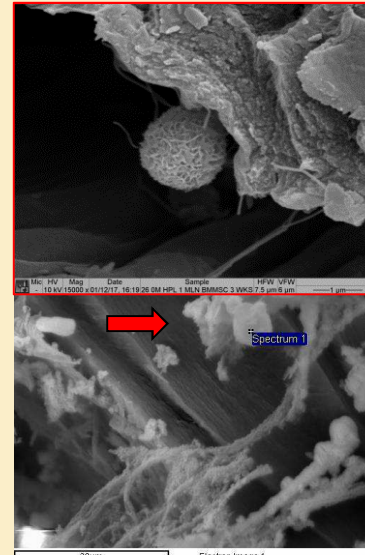
SEM ANALYSIS OF THE BM-hMSCs AND AT-hMSCs ADHESION AND OSTEOGENIC DIFFERENTIATION AT DAY 21



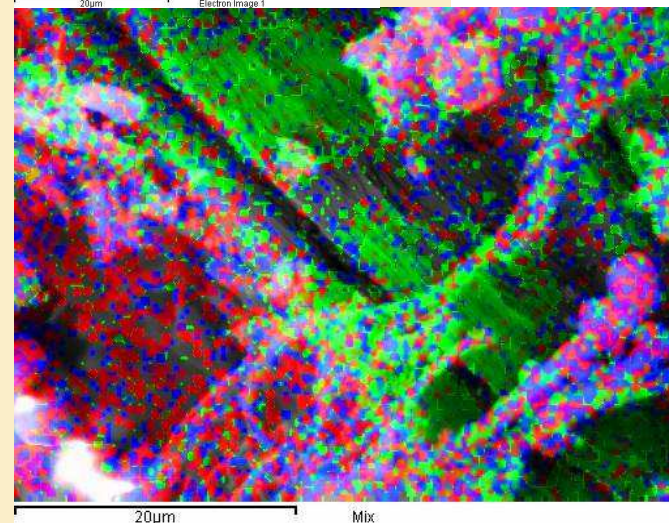
Scanning Electron Microscope (SEM)/ Energy-Dispersive X-ray Spectroscopy (EDX) Analysis



S-26: hMSCs in osteogenic medium with hPL



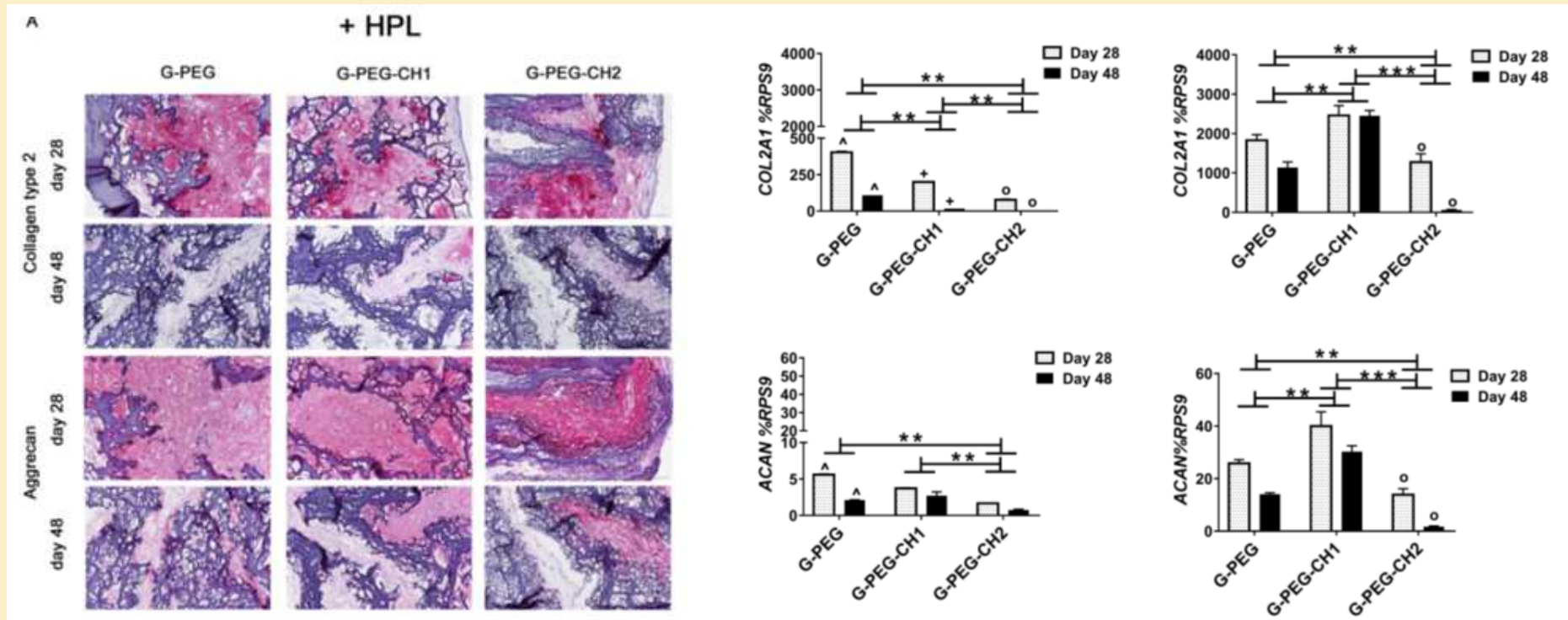
Element	Weight%	Atomic%
C K	40.55	49.80
O K	50.44	46.50
Si K	0.72	0.38
P K	4.52	2.15
Ca K	2.50	0.92
As L	1.26	0.25
Totals	100.00	



■ C
 ■ Ca
 ■ P

Re F et al., J Tissue Eng, 2019

HyPs Scaffolds differently affect chondrogenic differentiation of human mesenchymal stromal cells

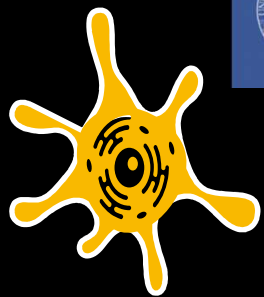


Immunohistochemical and *gene expression*
 evaluation of chondrogenic markers in differentiated hBM-MSCs:
Collagen type 2 and Aggrecan

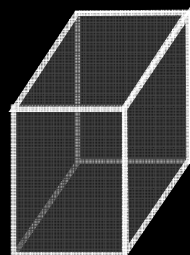


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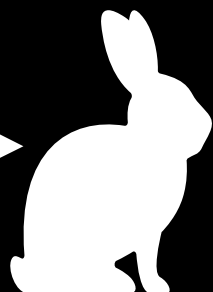
Study workflow



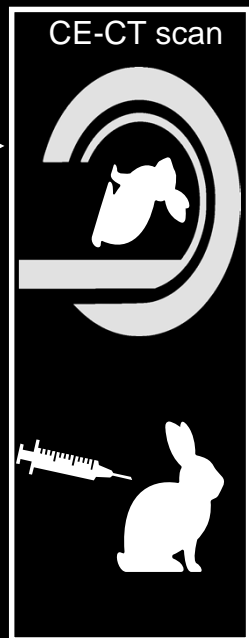
hMSC - P3



Initial 1:1
randomization



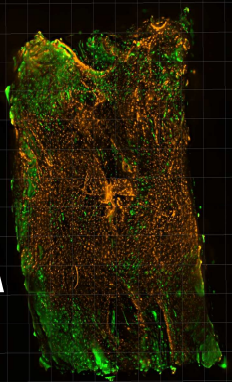
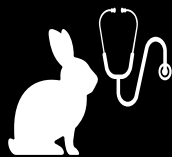
Every 2 weeks



CBC and basic
biochemistry
examination

Daily examination:

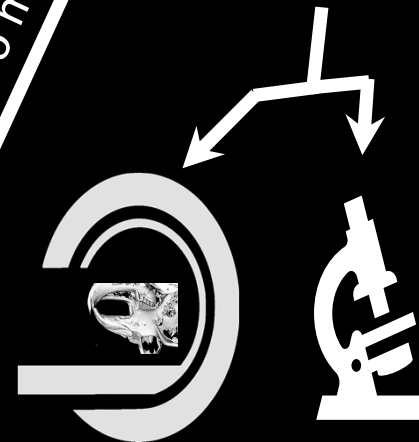
- Overall status
- Activity
- Ability to eat and drink
- Signs of pain
- Surgical wound status
- Urinary and fecal output
- Body temperature



Euthanasia

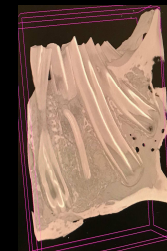


3-5 months

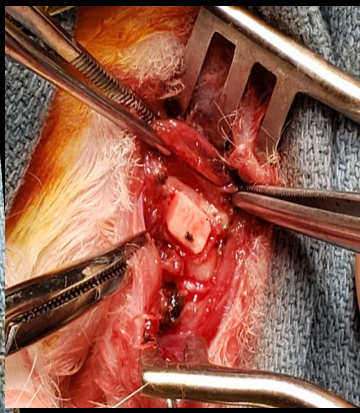
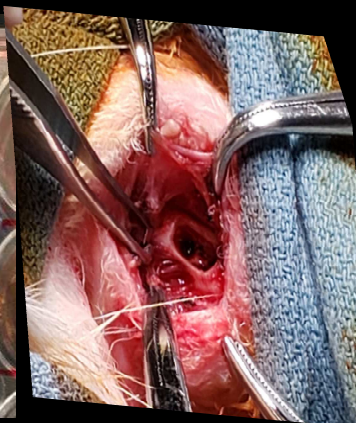


UltraHD CT scan

Spatial resolution:
10 microns

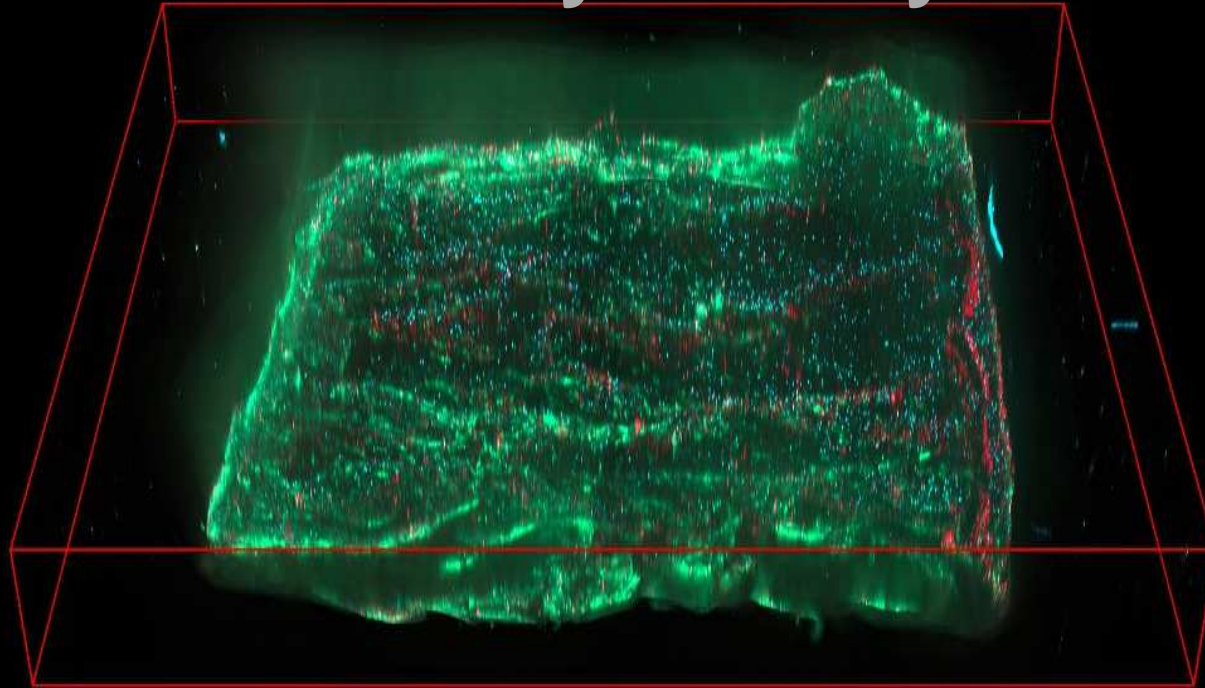


- H&E
HC/IHC:
- TRAP
 - HNA
 - CD44
 - VEGF-A
 - CD31
 - Bone-SP
 - Osteocalcin
 - Osteopontin



Proliferation assay

HyCh - Day 11



1000.00 μm

Legend:

Red = Ki67

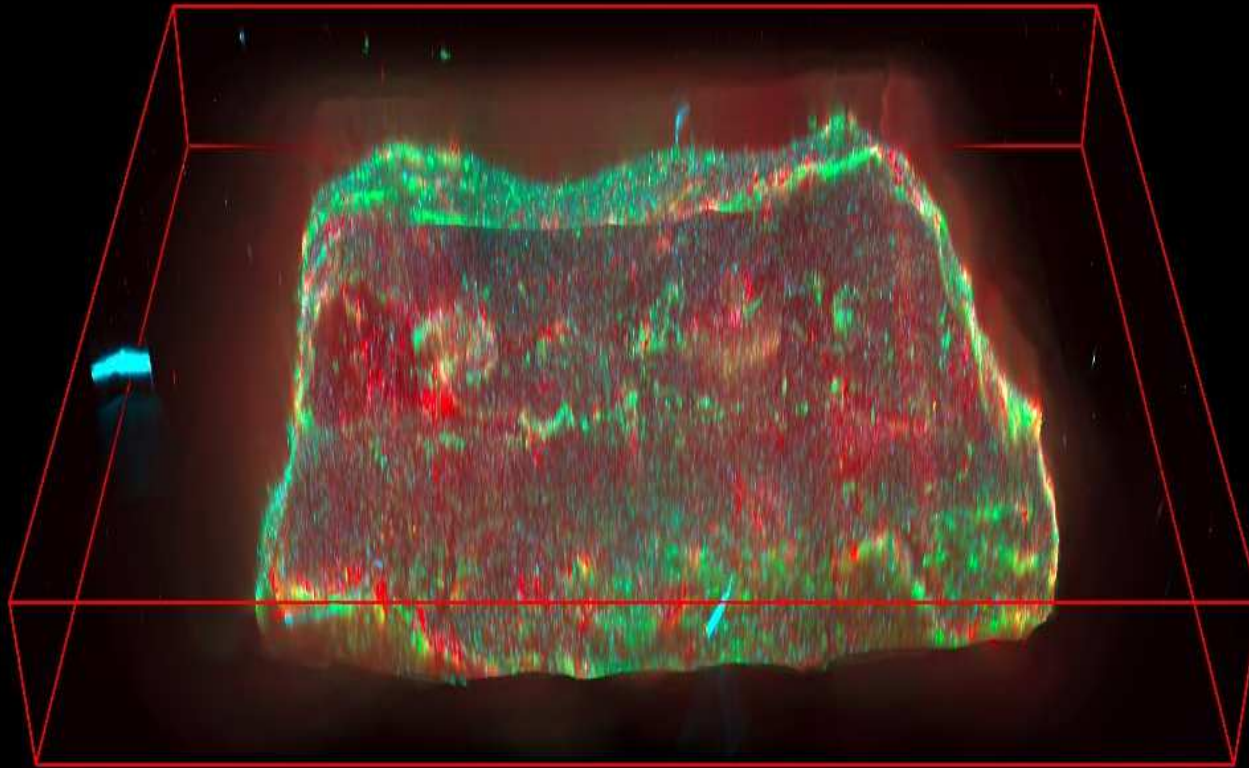
Blue = Nuclei

Green = Cytoplasm

XYZ coordinates

Osteodifferentiation assay

HyCh - Day 11 Osteogenic Medium



500.00 μm
| | | | |

Legend:

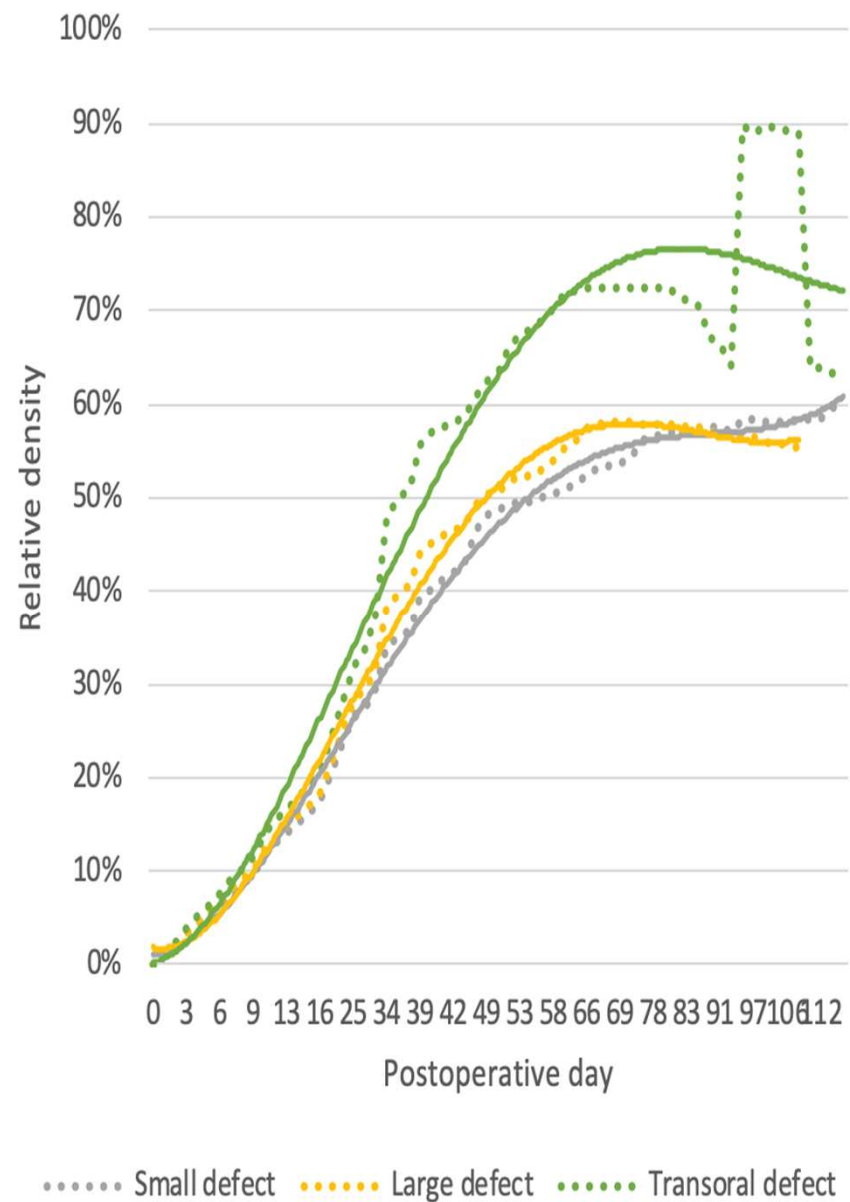
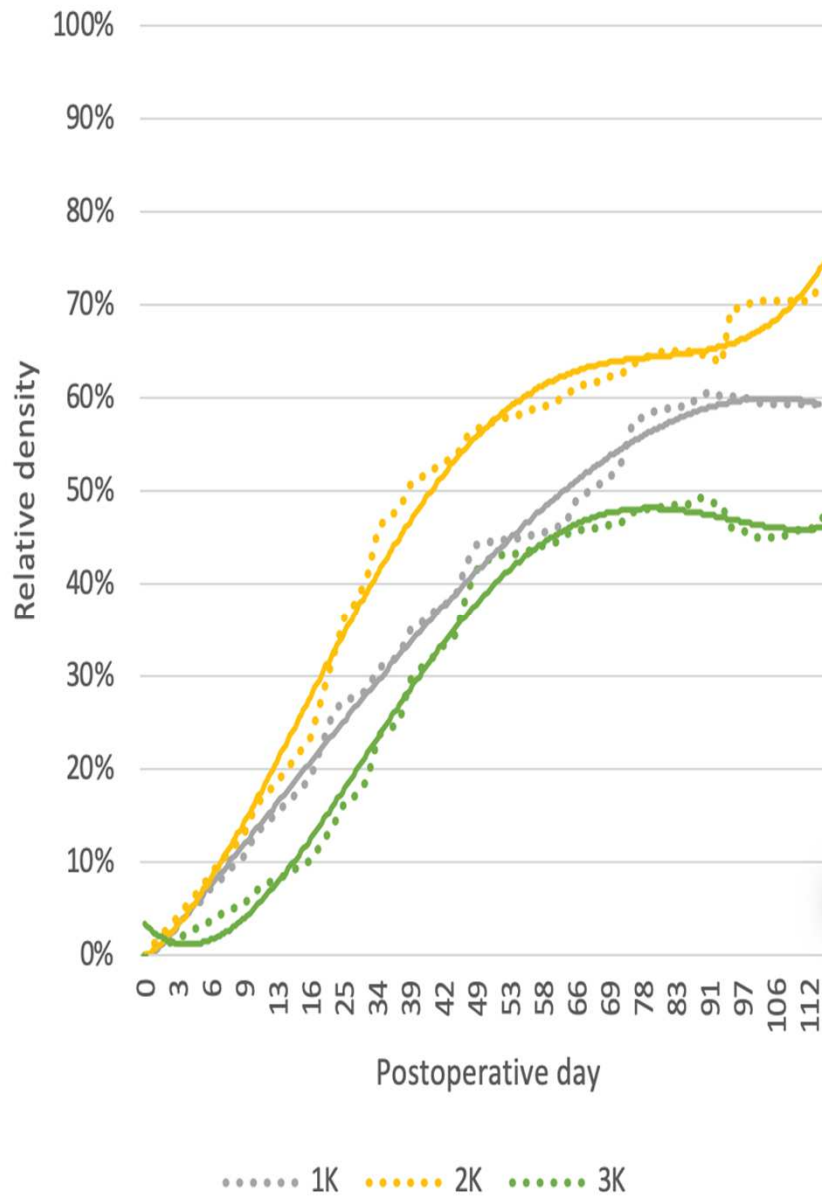
Red = Osteocalcin

Green = Cytoplasm

Blue = Nuclei

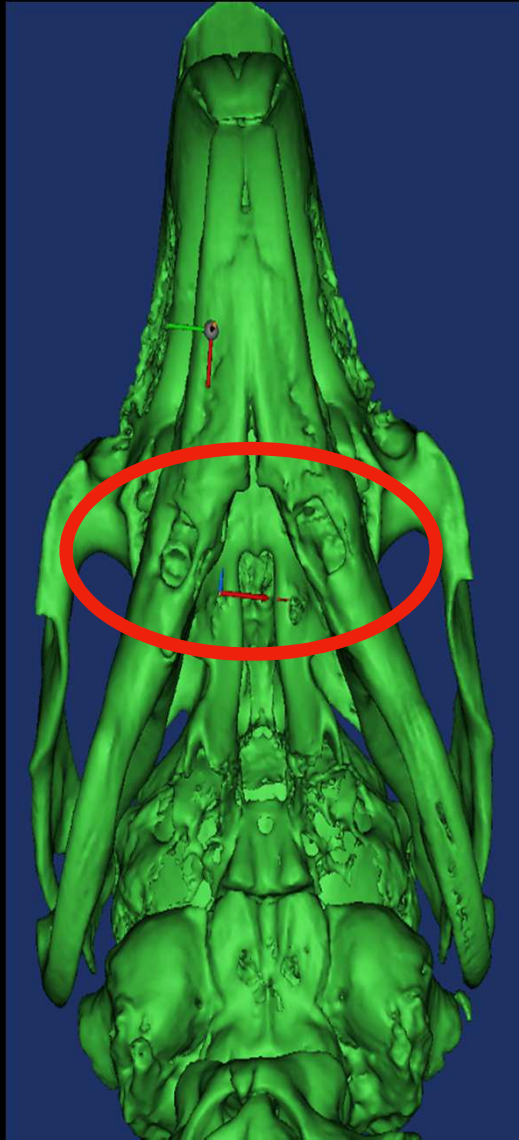
XYZ coordinates

Density of the surgical site



Allograft model - Rabbit Mesenchymal Stem Cells

I° Week POSTOP

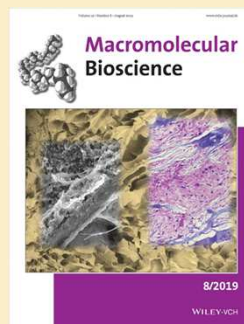


IV° Week POSTOP



Scientific Production

2 PATENTS on HyPSs SCAFFOLDS



10 SCIENTIFIC ARTICLES

1 SPECIAL ISSUE: *MATERIALS* (Guest Editors: D. Russo, L.Sartore, G. Lisignoli)

10 ORAL PRESENTATION AT NATIONAL AND INTERNATIONAL CONGRESS

3 Prin (2017 n° 2 and 2020)

1 RTA → RTDB

4 PhD Students



Under UniBS International Program



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L. Sartore - Kamol Dey - S. Agnelli
Dpt of Mechanical and Industrial Engineering



**Manuel Salmeron-Sanchez –
M. Cantini - V. Moulisova**
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C. Almici – A. Bianchetti
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Cells Laboratory*



**P Nicolai –
S. Taboni – M. Ferrari**
University of Padova

L. Rodella – E. Borsani
Dpt of Clinical and Experimental Sciences



F. Magni - C. Chinello
Dpt of Medicine and Surgery

E. Sardini – N. Lopomo
Dpt of Information Engineering



**F. Doglietto –D. Mattavelli - C. Paganelli –
F. Savoldi**
DPT of Medical and Surgical Specialities



Ralph Gilbert - Jonathan Irish
*Head and Neck Surgery, University Health
Network – Toronto, Canada*

Thank you for your attention



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