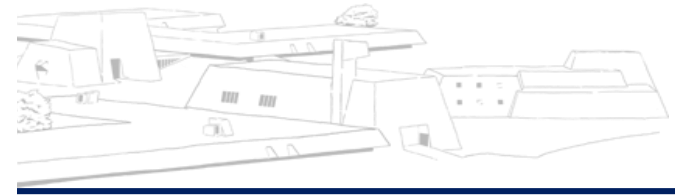




2nd  
**PAN-AMERICAN**  
Conference for Alternative Methods

August 23-24, 2018  
Rio de Janeiro



# **INMETRO activities in Alternative Methods**

***José Mauro Granjeiro***

*Senior Researcher – Bioengineering Group*

*Associate Professor – Dental School / Fluminense Federal University*

*[jmgranjeiro@inmetro.gov.br](mailto:jmgranjeiro@inmetro.gov.br)*



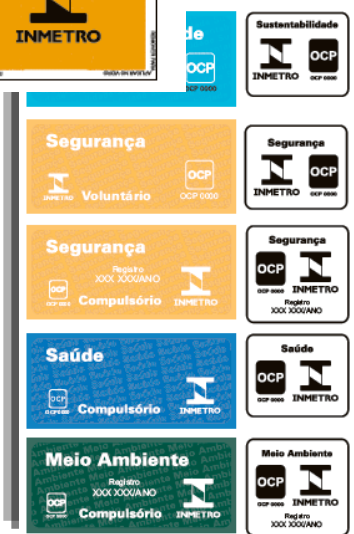
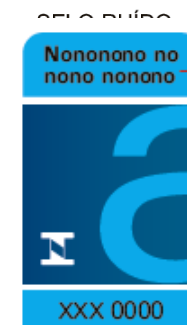
# Mission

PROVIDE CONFIDENCE TO THE SOCIETY  
CONCERNING  
MEASUREMENTS AND PRODUCTS

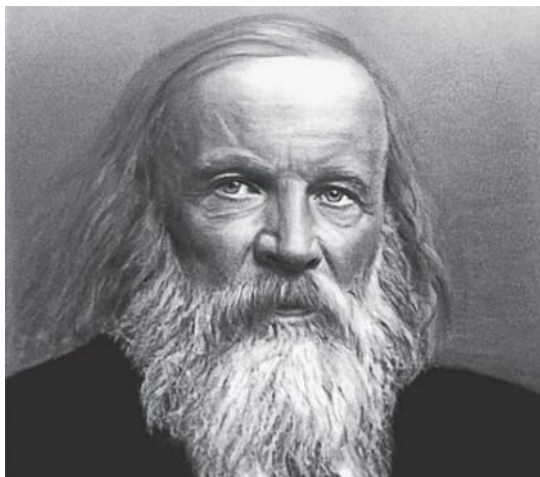
PROMOTING HARMONIZATION IN CONSUMPTION  
RELATIONS, INOVATION AND COMPETITIVENESS

through:

METROLOGY and CONFORMITY ASSESSMENT



# Metrology is about measurement



Famous Russian pioneer  
of metrology in chemistry:  
Science starts from the  
measurement  
(*Dmitry Ivanovich Mendeleev*)

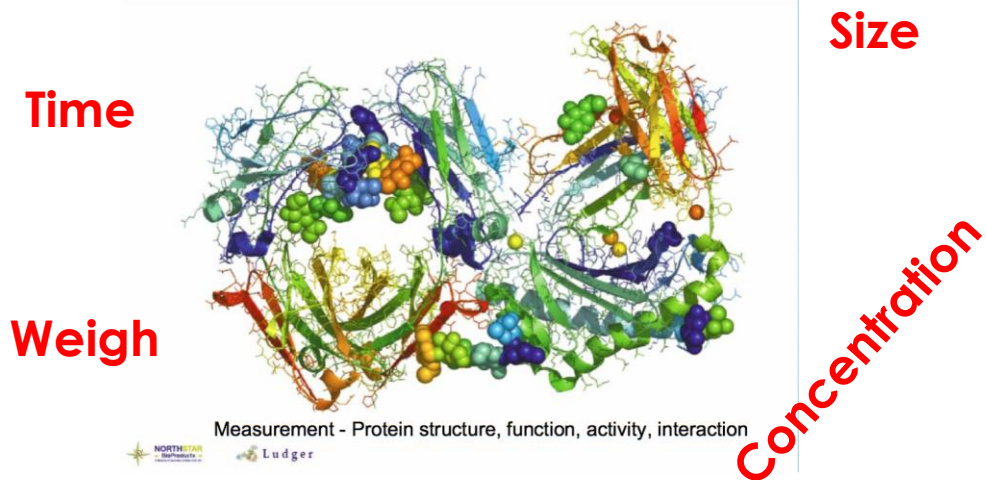
D.I. Mendeleev  
Institute for Metrology  
(VNIIM)



**Lord Kelvin**

Standardization is fundamental

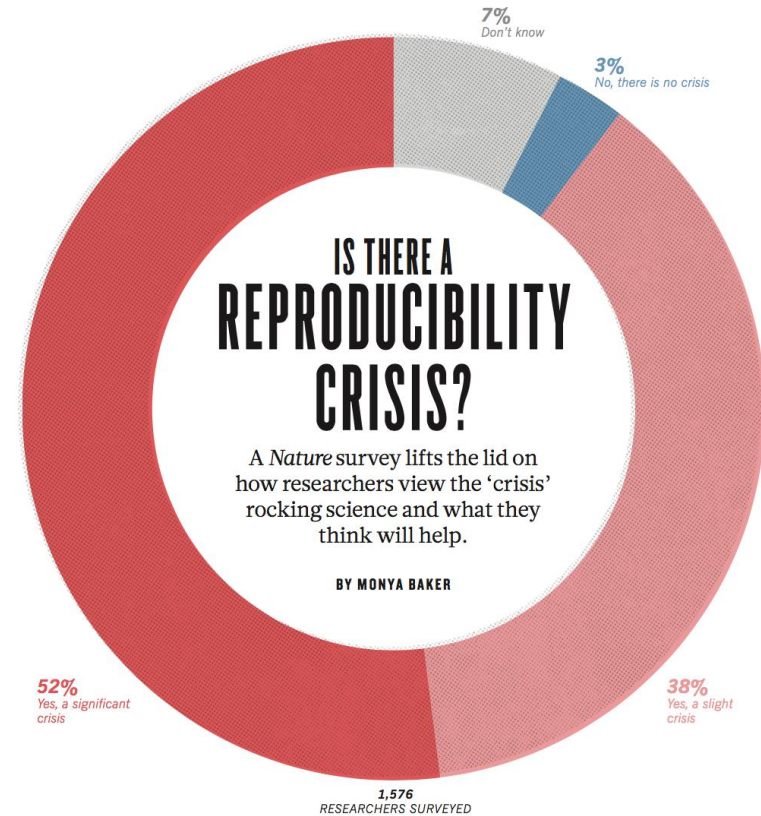
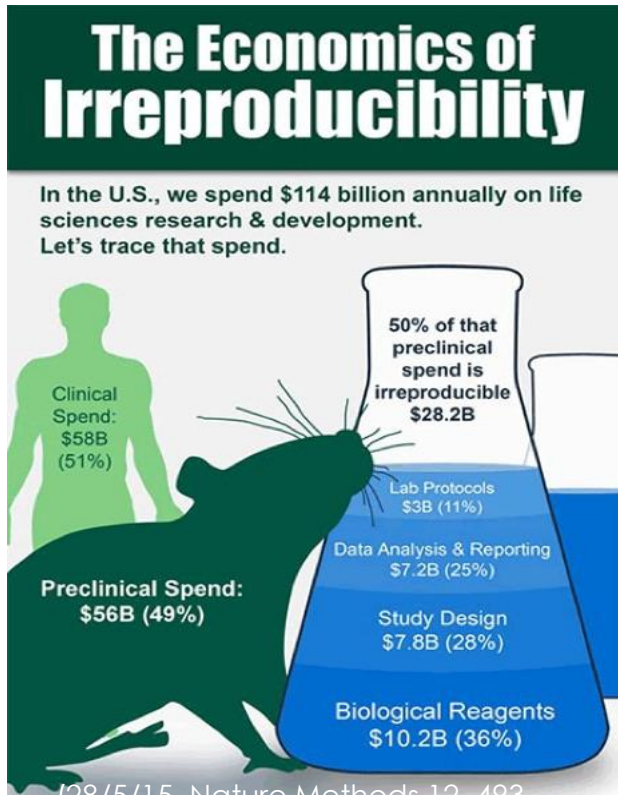
Antibody Fc - Fc Receptor Complex Showing Glycosylation





# Nature 533, 452–454 (26 May 2016)

## doi:10.1038/533452a



# Reliability....



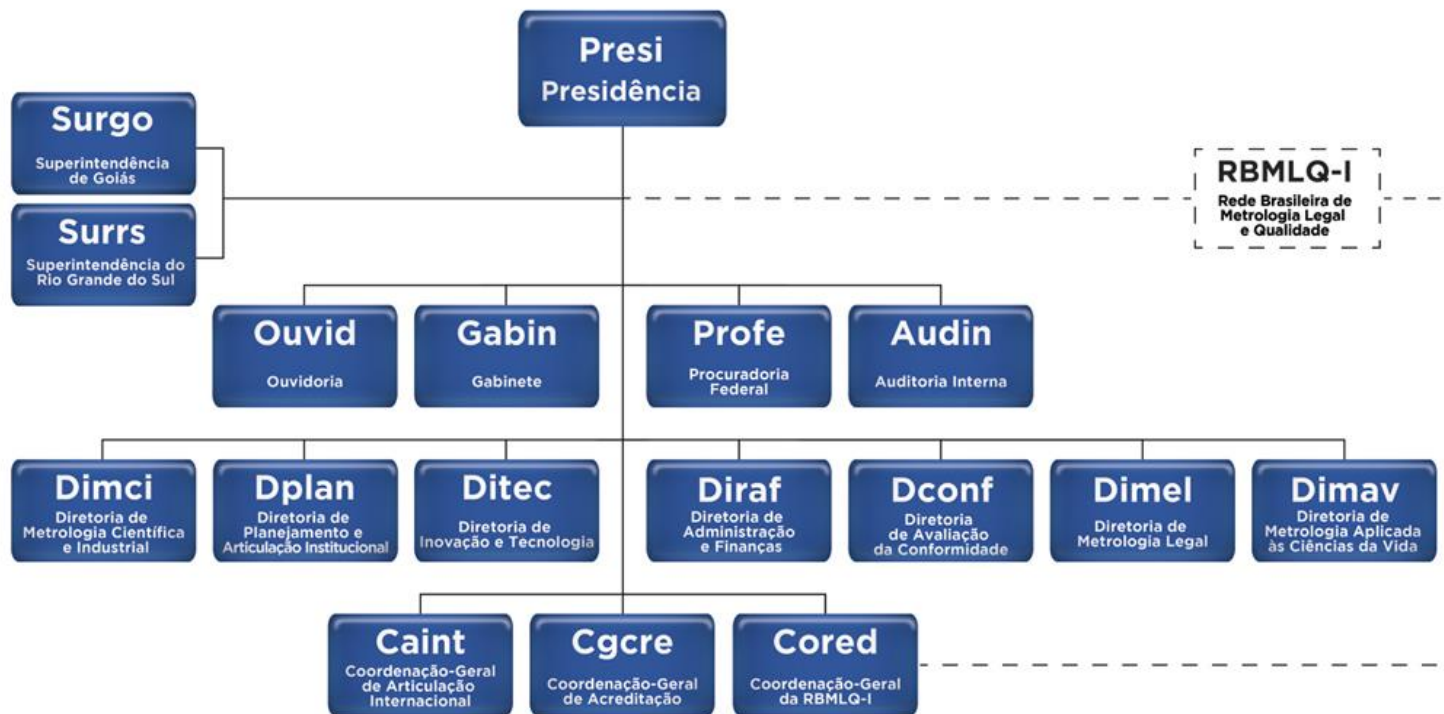
# Is there a reproducibility crisis?

## Requirements

- **Standardization**
  - SOP – Standard Operational Procedures
- **Calibration**
- **Traceability**
- **Interlaboratory comparisons**

GLP

ISO / OECD Guides  
Standards (SOPs)





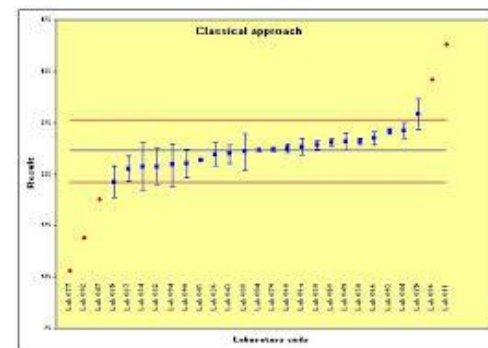
# Inmetro and Renama

(Science, Technology and Innovation Ministry Act 491/2012)

Central Labs:



Associated Labs:  
**2012 = 27**



- Implementation and dissemination of validated alternative methods
- OECD series on principles of good laboratory practice and compliance monitoring

# RENAMA\_41 LABORATÓRIOS

3 CENTRAIS

38 ASSOCIADOS



- 1 BAHIA
- 1 GOIAS
- 1 PERNAMBUCO
- 1 SANTA CATARINA
- 2 MINAS GERAIS
- 5 PARANÁ
- 10 RIO DE JANEIRO
- 19 SÃO PAULO

Fonte: Vanessa Rocha





## **Inmetro Activities in AM**

- **Central laboratory in RENAMA**
  - Dissemination of OECD guidelines
  - Training on OECD guidelines (Luciene Ballotin)
  - Interlaboratory comparisons – training and competence assessment
  - Research on cell quality and purity (CNPq/2016)
  - Production of cells master banks (CNPQ/2016)
  - Training and interlab comparisons in cell purity
  - Harmonized SOPs.....



## **Inmetro Activities in AM**

- Masterbanks (1 batch = 10 criotubes; free of mycoplasma and authentic human cells):
  - A549 – 4 batches
  - HEPG2 – 2 batches
  - MDCK2 – 3 batches
  - V79-4 – 1 batch
  - BALB3T3 A31 Clone – 2 batches
  - SIRC do labio (in development).



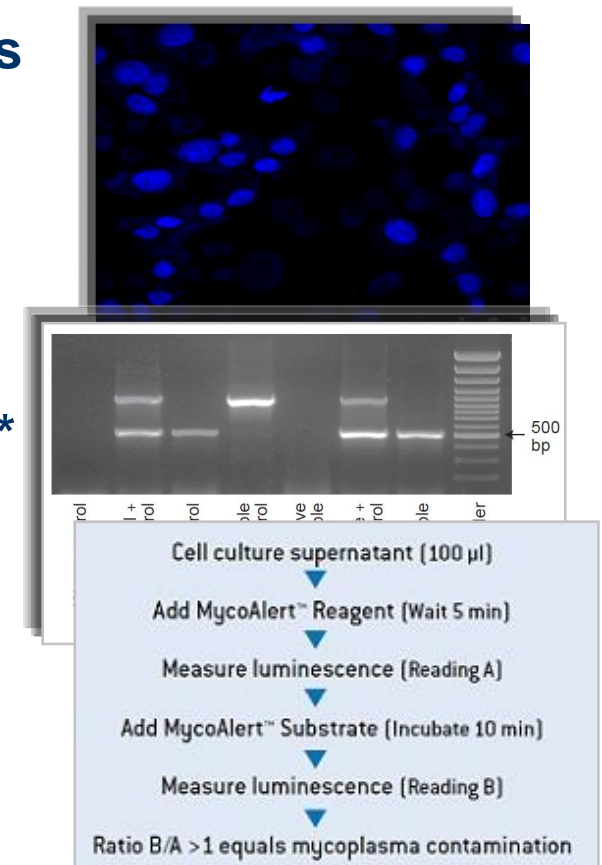
## Inmetro Activities in AM

- Scientific research in tissue engineering aiming (see posters 18, 54, 94, 99, 102, 103, 121, 128):
    - Regenerative medicine
    - In vitro tissue models for tox test
  - Models:
    - Cartilage
    - Bone
    - Lung
- Bioprinting + 3D scaffolds



## ***Interlaboratorial Comparisons - Reproducibility*** ***Quality of cell lines***

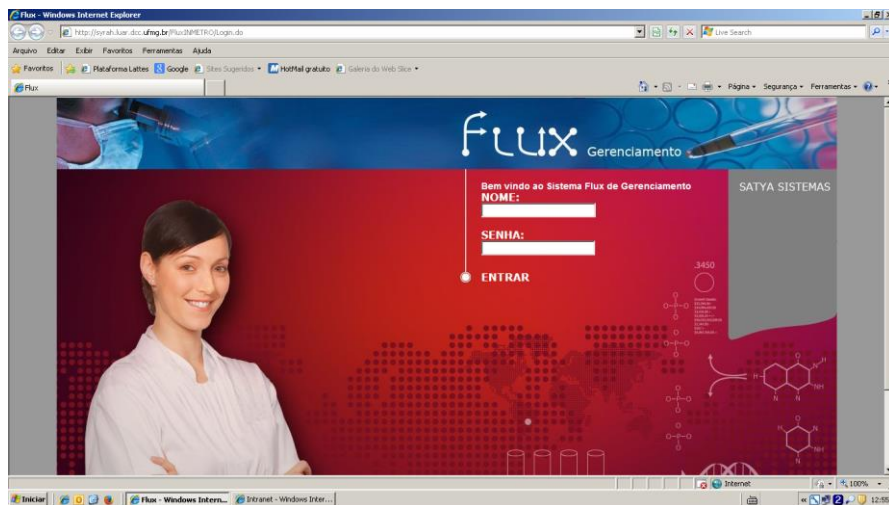
- a) **Authenticity assay – STR for human cell lines**
- b) **Environmental monitoring \***  
(the lab, surfaces, incubators and hoods are tested)
- c) **Microbiological testing (bacteria and fungi) \*\***  
(all cells and solutions are tested)
- d) **Mycoplasma testing**  
(all cells and solutions are tested)
  - **Bioluminescence (biochemical assay) \*\*\***
  - **PCR \*\*\*\***





# *Interlaboratory Comparisons (see poster 54)*

## *The Principles of Good Laboratory Practices (OECD)*



**Assay Protocol (TG OECD)**



**GLP based**





## ***Inmetro/General Coordination for Accreditation (GCA) and GLP***

**GCA as the Brazilian Compliance Monitoring Authority for the Principles of Good Laboratory Practices (Nov 26th 2007)**

**in May 2011, Brazil, through GCA, obtained the full adherence to the OECD acts related to the system of mutual acceptance of GLP data (MAD) including the products "pesticides, their components and related products" and "industrial chemical products".**

**OECD TG + GLP Facility = acceptance by OECD member and non-member countries**



## Activities 2016-2017

- Technical Training (NR18 Conceca/ Cell quality)
- GLP Workshop
- Test method implementation (NR18 Conceca/Cell quality)
- Increase network integration





# **Research at Inmetro on Alternative Methods**





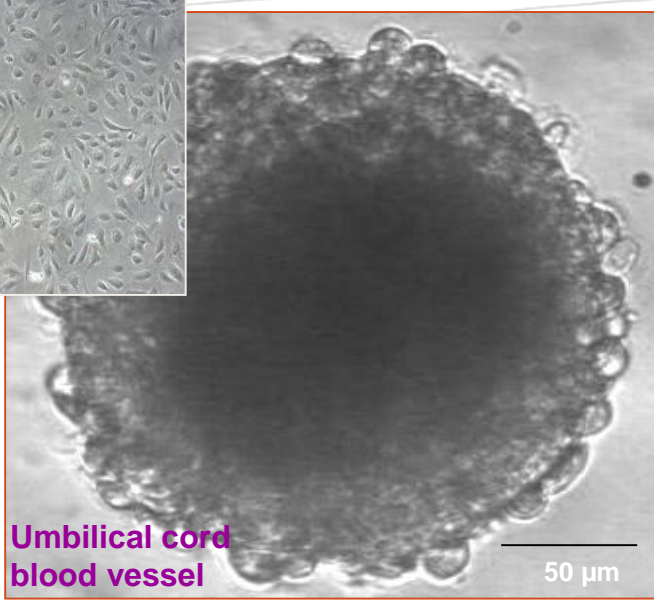
# Engineering spheroids to mimic human tissues

- Human
  - Blood vessels
  - Gastric mucosa
  - Respiratory mucosa
  - Oral mucosa
  - Intestinal mucosa
  - Osteoblasts
  - Cartilage

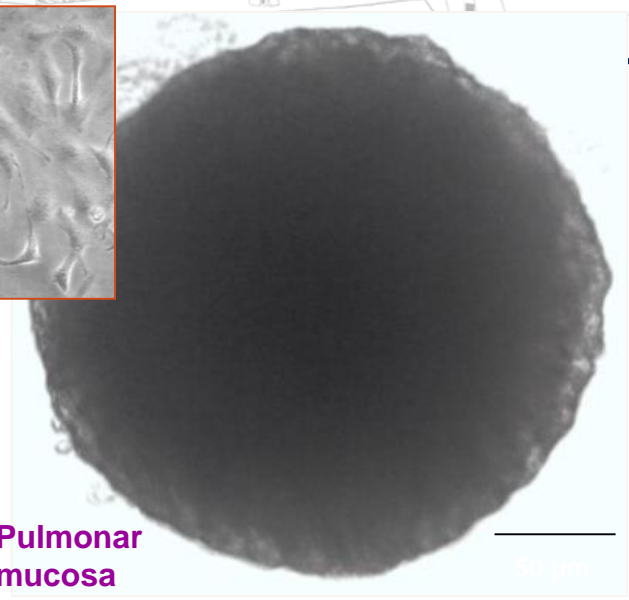
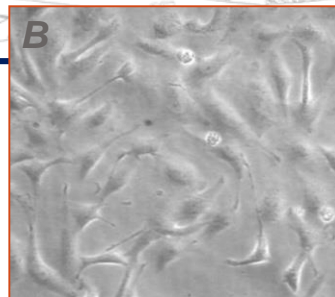




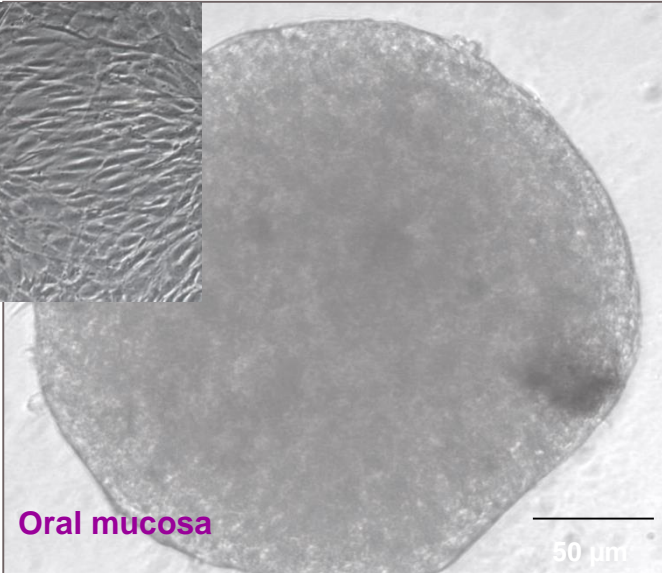
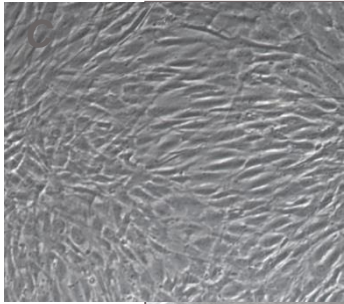
Aug



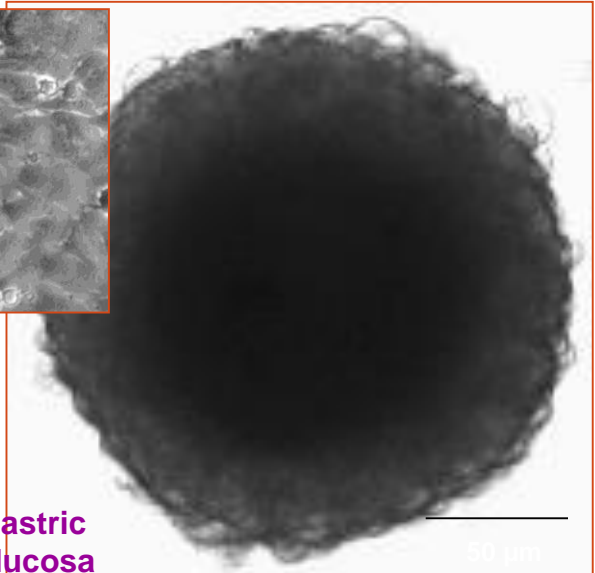
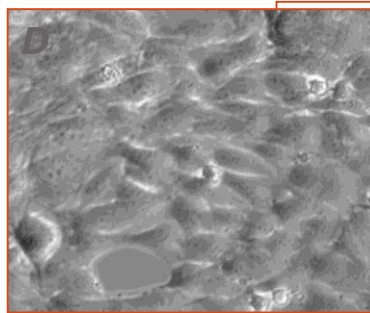
Umbilical cord  
blood vessel



Pulmonar  
mucosa



Oral mucosa



Gastric  
Mucosa

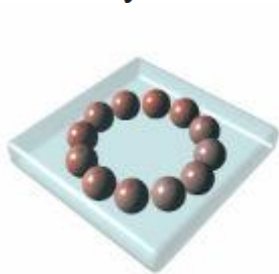
**Epithelial cells**

Original Article

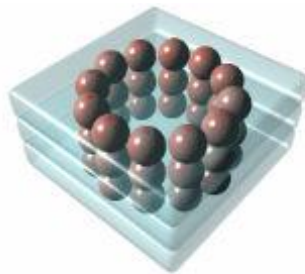
## The fusion of tissue spheroids attached to pre-stretched electrospun polyurethane scaffolds

Journal of Tissue Engineering  
Volume 5: 1–11  
© The Author(s) 2014  
DOI: 10.1177/2041731414556561  
tej.sagepub.com  

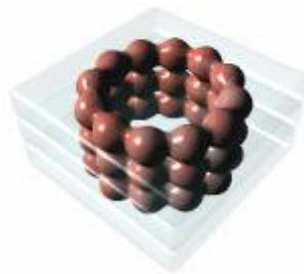

Vince Beachley<sup>1</sup>, Vladimir Kasyanov<sup>2</sup>, Agnes Nagy-Mehesz<sup>3</sup>,  
Russell Norris<sup>3</sup>, Iveta Ozolanta<sup>2</sup>, Martins Kalejs<sup>2,4</sup>, Peteris  
Stradins<sup>2,4</sup>, Leandra Baptista<sup>5</sup>, Karina da Silva<sup>5</sup>, Jose Grainjero<sup>5</sup>,  
Xuejun Wen<sup>6</sup> and Vladimir Mironov<sup>3,7</sup>



[A]  
Bioink spheroids  
printed into layer  
of biopaper gel



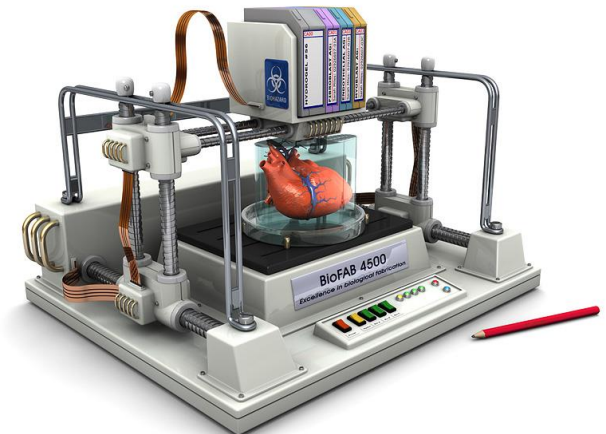
[B]  
Additional layers  
printed to build  
object



[C]  
Bioink spheroids  
fuse together and  
biopaper dissolves

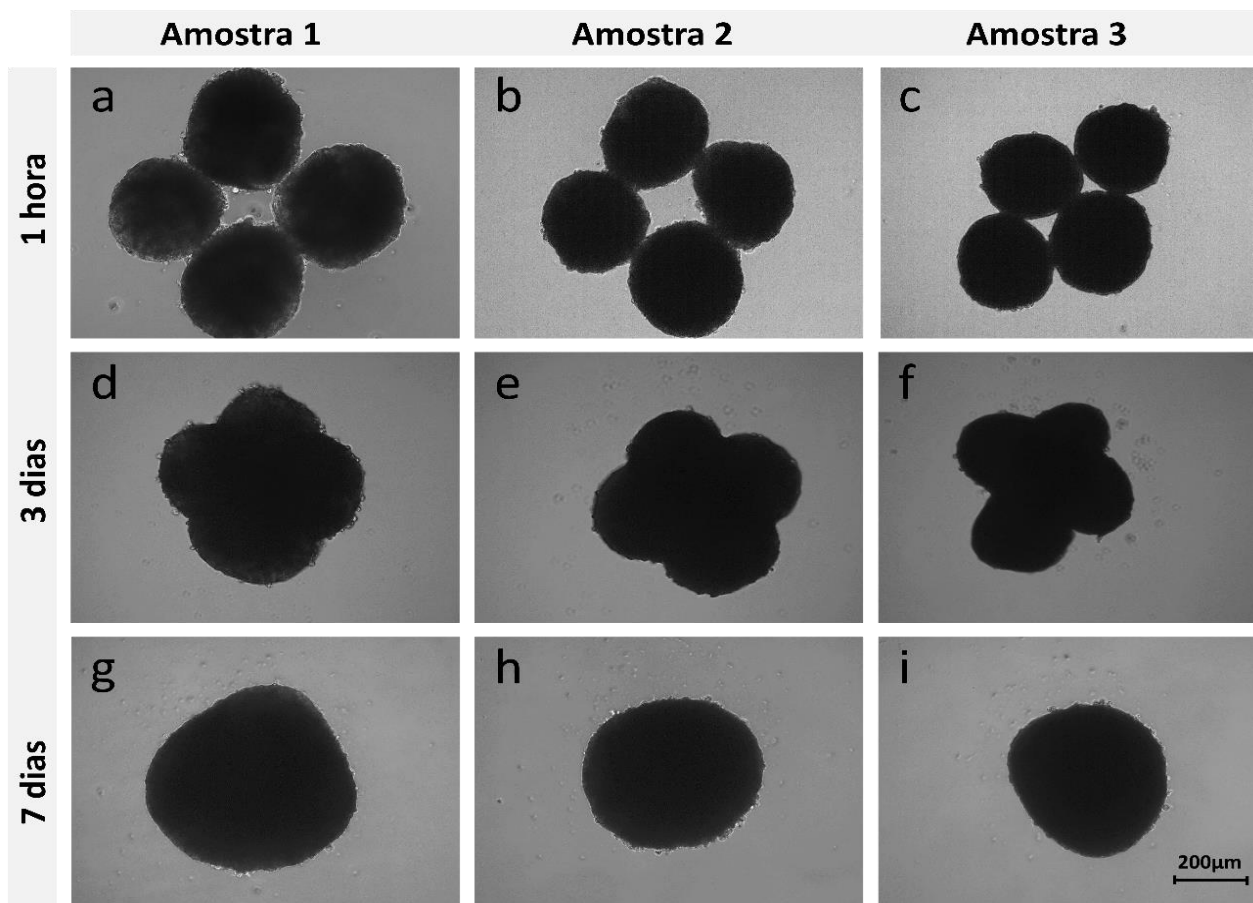


[D]  
Final living  
tissue



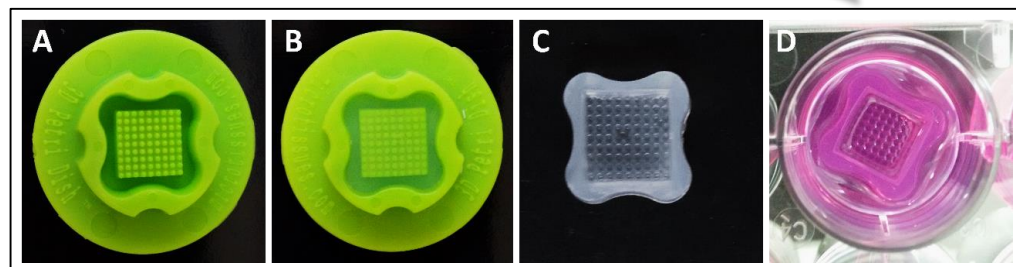
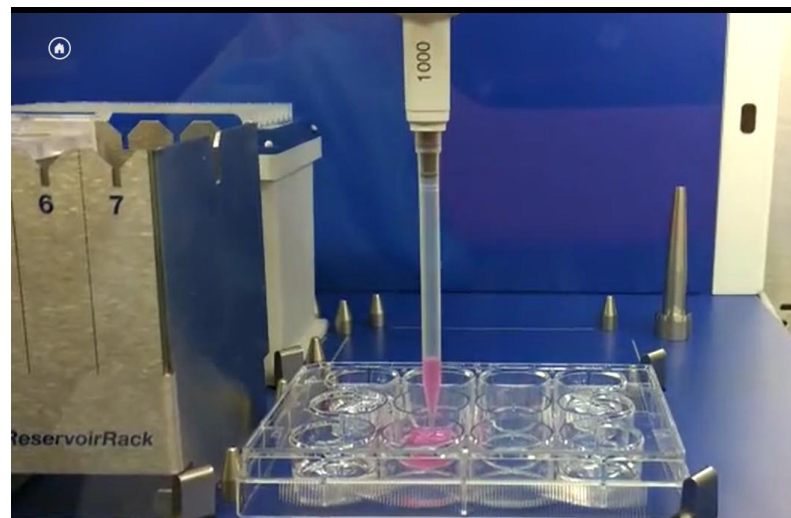
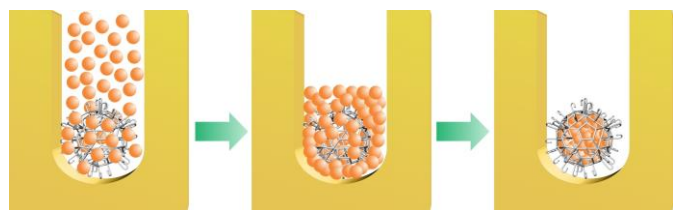
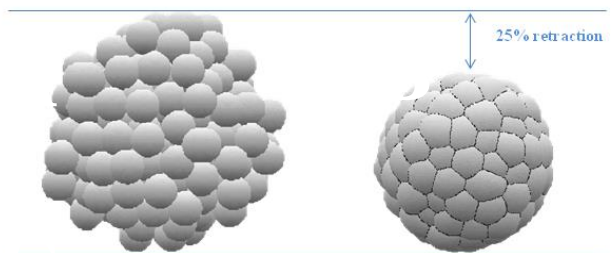


# Spheroids fusion assay





# General Objective





## Just before 3D ... Growth factors

- Human growth factors
  - Platelet-rich plasma
  - BMP's
- Cell source and characterization
  - Human discarded tissues
    - Adult cells
    - Mesenchymal stem cells
  - Induced Pluripotent Stem Cells – IPS

**Standards (SOPs)  
Quality Control check points**



Amable *et al. Stem Cell Research & Therapy* 2013, **4**:67  
<http://stemcellres.com/content/4/3/67>



**RESEARCH**

**Open Access**

# Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors

Paola Romina Amable<sup>1\*</sup>, Rosana Bizon Vieira Carias<sup>1</sup>, Marcus Vinicius Telles Teixeira<sup>1</sup>, Ítalo da Cruz Pacheco<sup>1</sup>, Ronaldo José Farias Corrêa do Amaral<sup>2</sup>, José Mauro Granjeiro<sup>3</sup> and Radovan Borojevic<sup>1</sup>



# Bioengineered Cartilage in a Scaffold-Free Method by Human Cartilage-Derived Progenitor Cells: A Comparison With Human Adipose-Derived Mesenchymal Stromal Cells

\*†Leandra S. Baptista, †‡Karina R. Silva, †§Carolina S.G. Pedrosa, §Ronaldo J.F.C. Amaral, †João Vitor Belizário, ‡§Radovan Borojevic, and †José Mauro Granjeiro

**TABLE 1.** *Scoring for the evaluation of Safranin O-Fast Green-stained cartilaginous pellet culture sections based on Safranin O staining and cell morphology (minimum score = 0; maximum score = 6)*

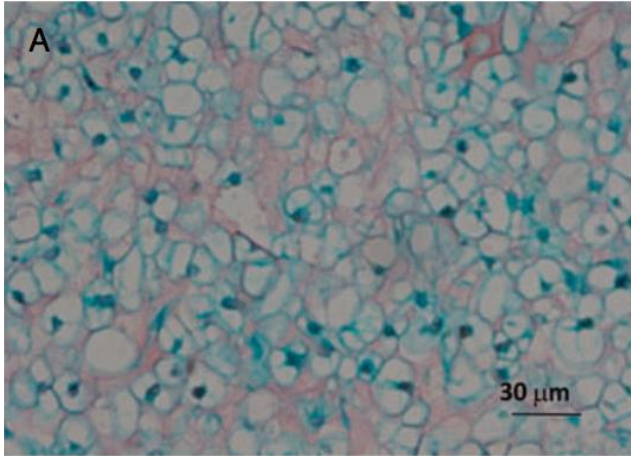
Intensity* of Safranin O stain	Score	Cell morphology	Score
No staining (blue)	0	Condensed/necrotic/pyknotic bodies	0
Weak staining (rose)	1	Spindle/fibrous	1
Moderate staining (orange)	2	Mixed spindle/fibrous with a rounded periphery	2
Dark staining (dark orange)	3	Majority with a rounded periphery	3

\* Sections 5  $\mu$ m thick.

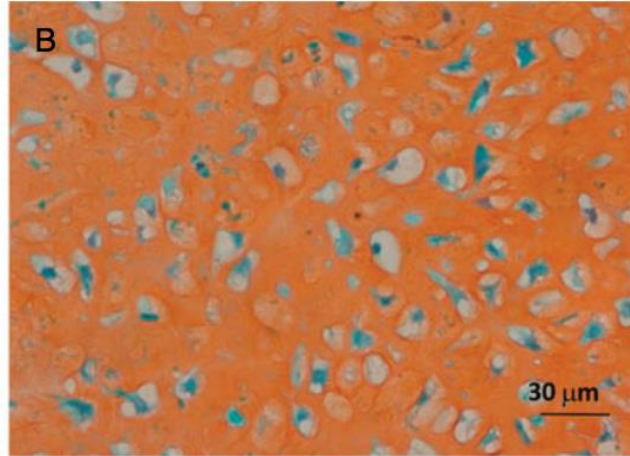


# Representative Safranin O images (after 21 days)

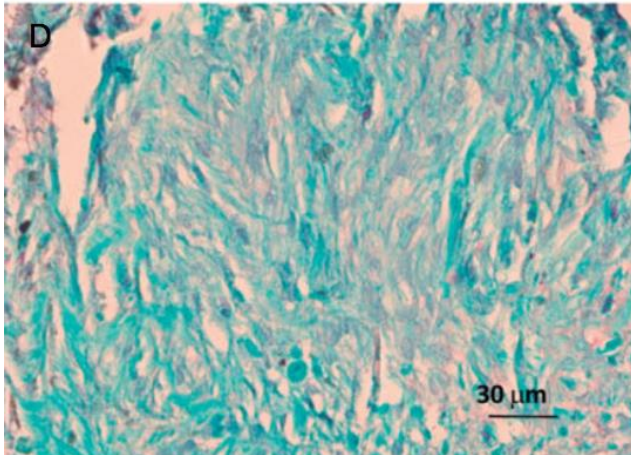
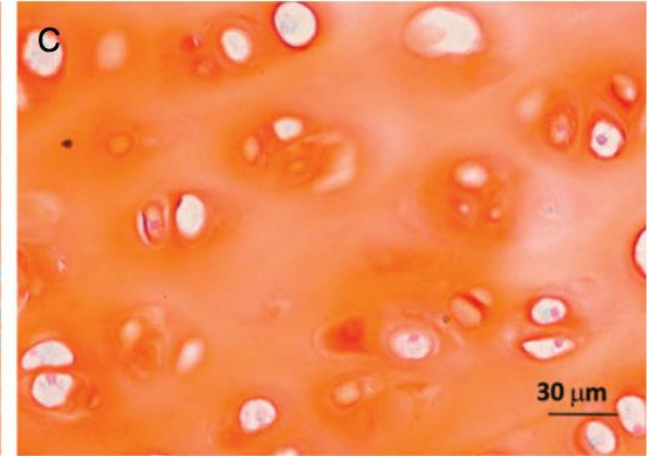
PCs not induced



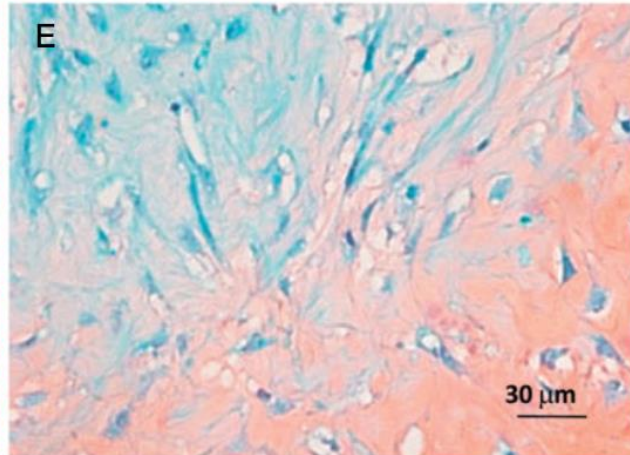
PCs induced



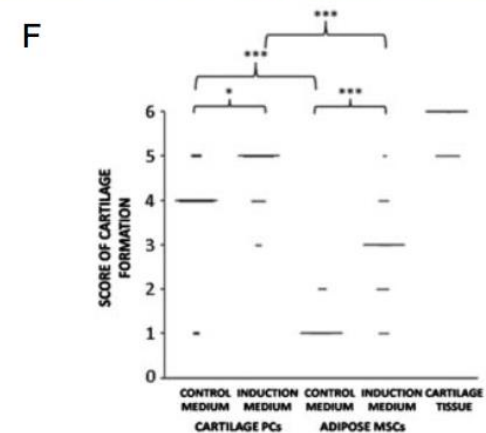
Natural cartilage



MSCs not induced

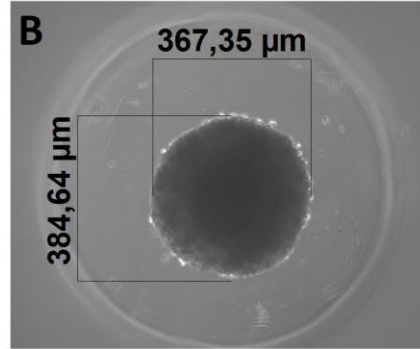
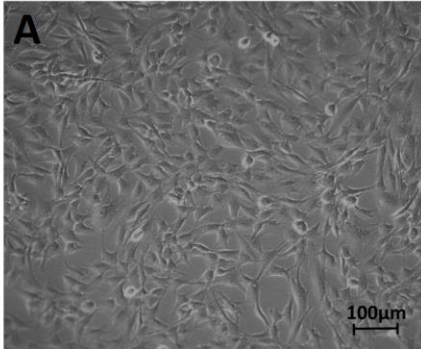


MSCs induced

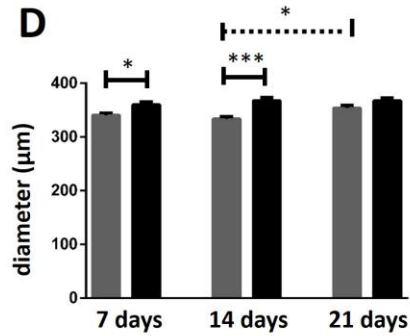
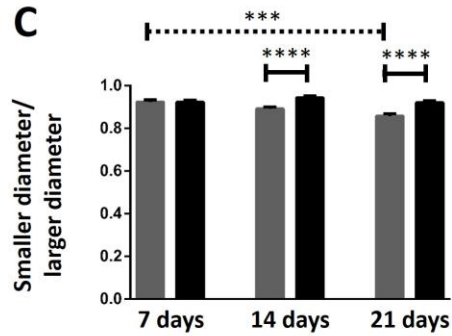




**A1**

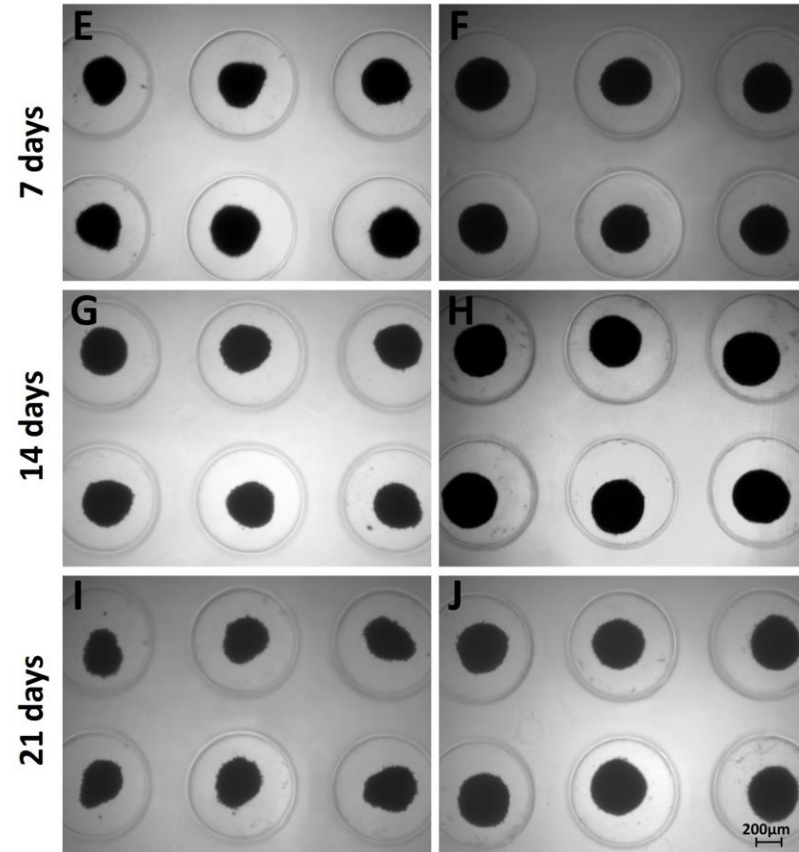


■ Non-induced ASC spheroids  
■ Induced ASC spheroids



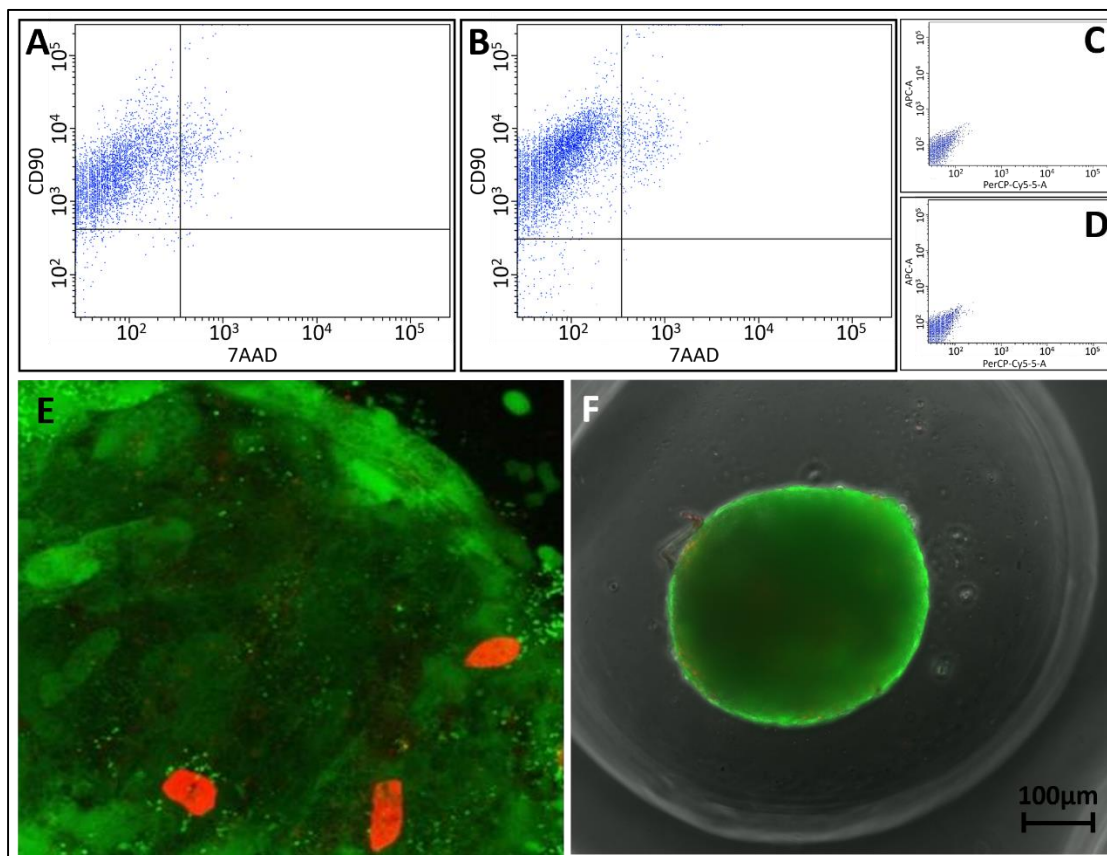
**Non-induced  
ASC spheroids**

**Induced  
ASC spheroids**





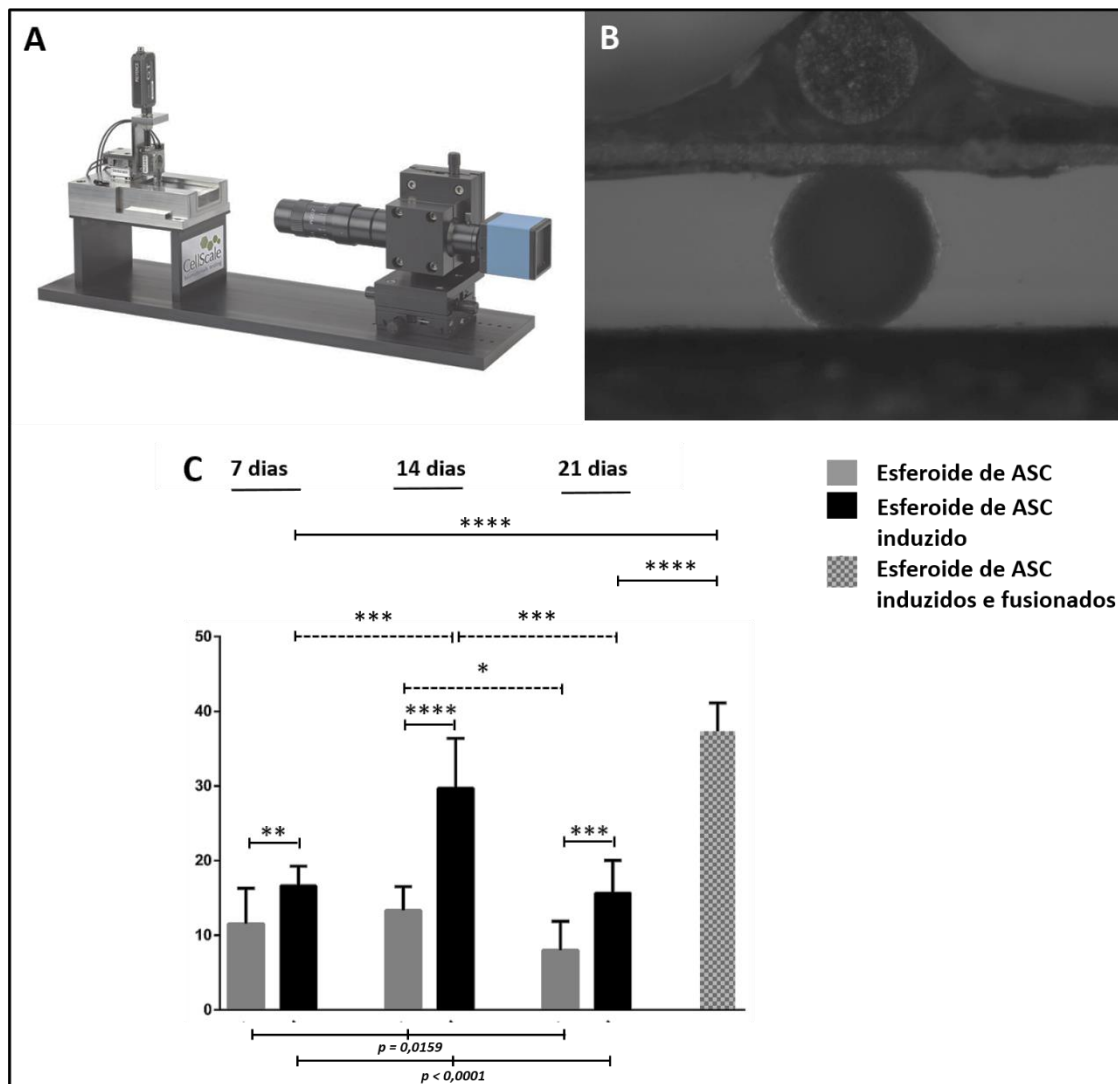
# Cell viability – flow cytometry / immunocytochemistry



Calcein (green)  
Etidium  
homodimer (red)



# Mechanical analysis

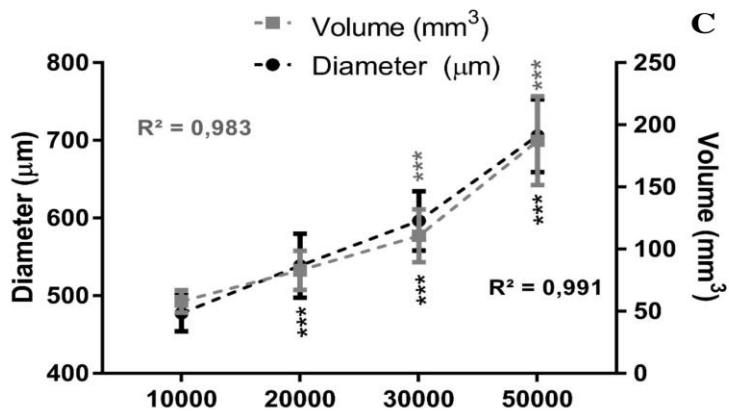
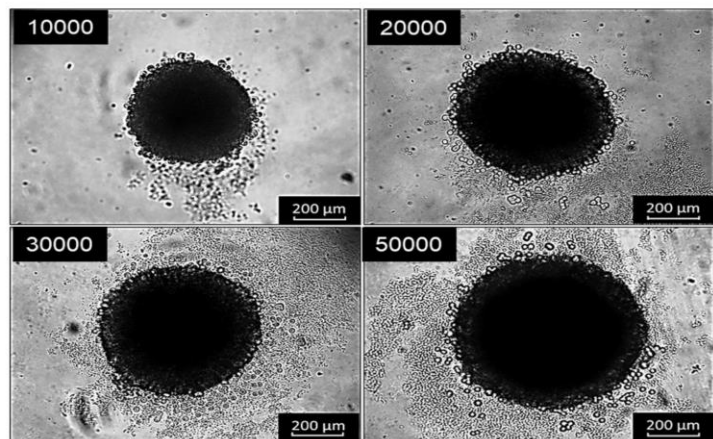
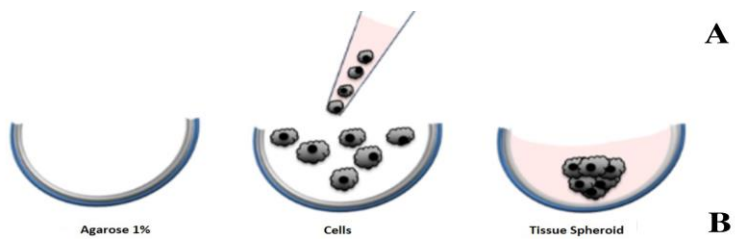




## The two faces of titanium dioxide nanoparticles bio-camouflage in 3D bone spheroids¶



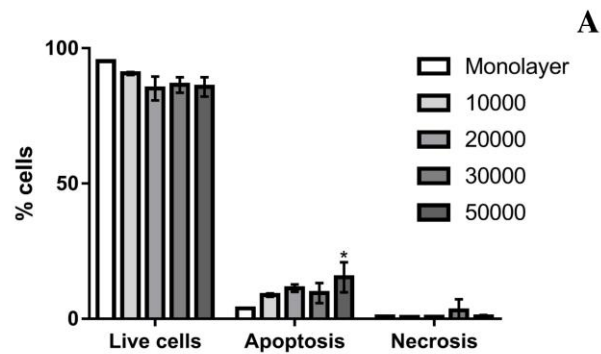
W. Souza<sup>1,2,3</sup>, S. G. Piperni<sup>3,4</sup>, P. Laviola<sup>1,3,5</sup>, A.L. Rossi<sup>4</sup>, Maria Isabel D. Rossi<sup>6</sup>, Bráulio  
S. Archanjo<sup>7</sup>, P.E. Leite<sup>1,2,8</sup>, R. Borojevic<sup>1,3,9</sup>, L. A. Rocha<sup>3,10</sup>, J.M. Granjeiro<sup>1,2,3,11</sup> A. R.  
Ribeiro<sup>1,2,3,5</sup>¶



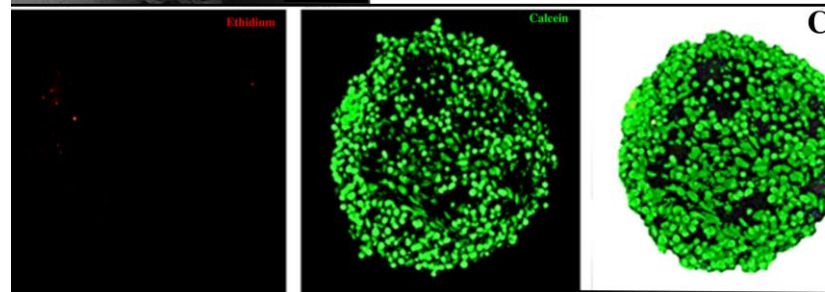
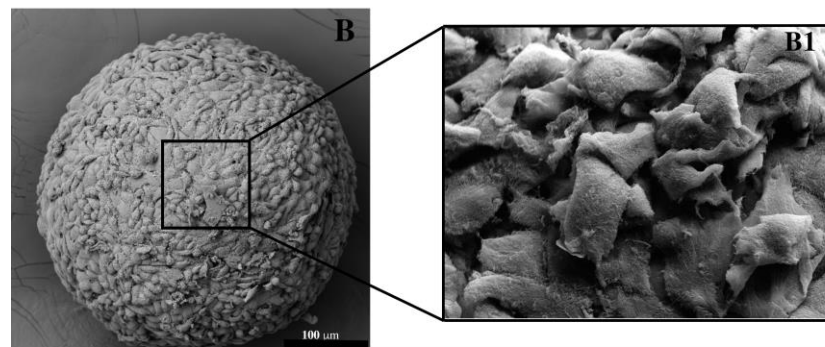
A

B

C



A

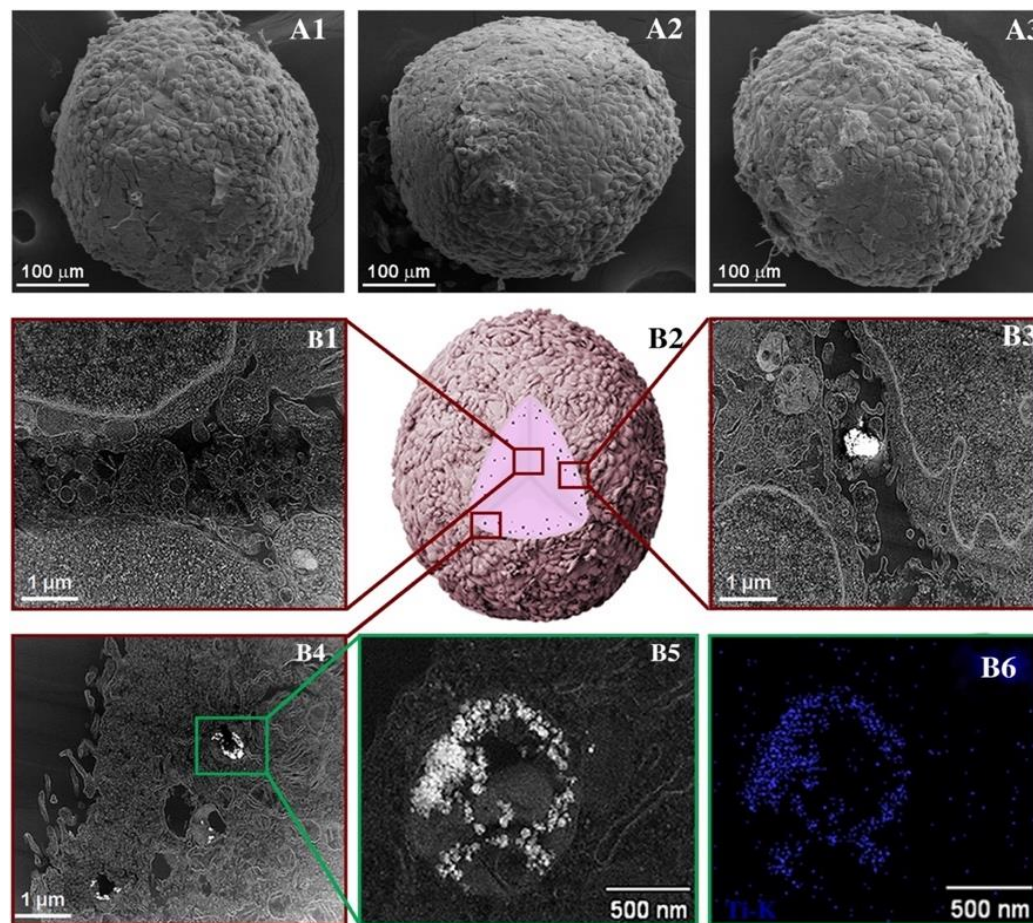


C



## Exposition do TiO<sub>2</sub>

**Figure 5:** Spheroid organization and NPs internalization: **(A1)** SEM micrograph of spheroids without and with **(A1)** 5, 10 **(A2)** and **(A3)** 100  $\mu\text{g/ml}$  TiO<sub>2</sub> NPs exposure during 72 hours. **(B1)** Scanning TEM (STEM) micrograph of the interior of spheroids with 100  $\mu\text{g/ml}$  TiO<sub>2</sub> NPs. **(B2)** Schematic illustration of the penetration behavior of TiO<sub>2</sub> NPs, **(B3)** STEM micrograph of the outer layers of the spheroid showing NPs in the space between cells (arrow), **(B4)** and in membrane-vesicle (arrowhead), **(B5)** high magnification of the membrane vesicle, **(B6)** STEM/EDS map of Ti-K X-ray line confirming the presence of NPs. Image are representative of four independent analysis.



# Team

UNICAMP, USP, UNESP,  
UFMG, UFRG, Centro  
Renato Archer, NYU,  
HU, PU, FP7

Inmetro

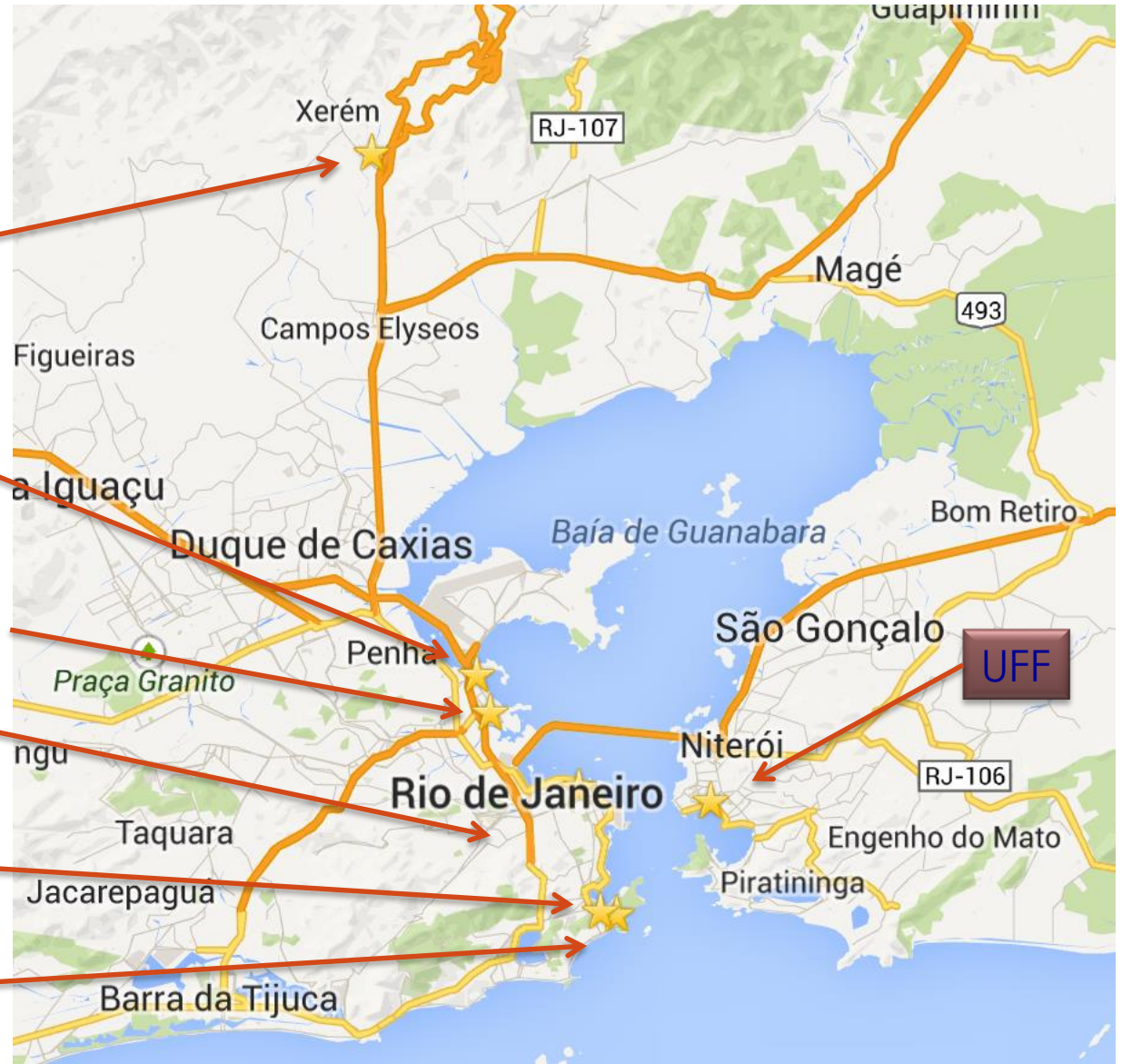
UFRJ:  
Embryology and Histology  
Chemical School  
Pharmaceutical School

Engineering School / Coppe

National Institute of  
Technology (INT)

Brazilian Center of  
Physic Research

Military Institute of  
Engineering







# Funding





# Thank you

- [www.inmetro.gov.br/](http://www.inmetro.gov.br/)
- [www.renama.org.br](http://www.renama.org.br)
- José Mauro Granjeiro
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  - [jmgranjeiro@inmetro.gov.br](mailto:jmgranjeiro@inmetro.gov.br) or [jmgranjeiro@gmail.com](mailto:jmgranjeiro@gmail.com)
  - +55 021 2679 9834
  - +55 021 999883498
  - <http://scholar.google.com.br/citations?user=5peGDJgAAAAJ&hl=pt-BR>
  - <http://www.researcherid.com/rid/D-8289-2012>

