Bioengineering and Bioprinting: metrology to improve innovation

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Senior Researcher,
Bioengineering Laboratory,
Directory of Life Sciences Applied Metrology, INMETRO

FLORIANÓPOLIS
2019

Team

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

Inmetro

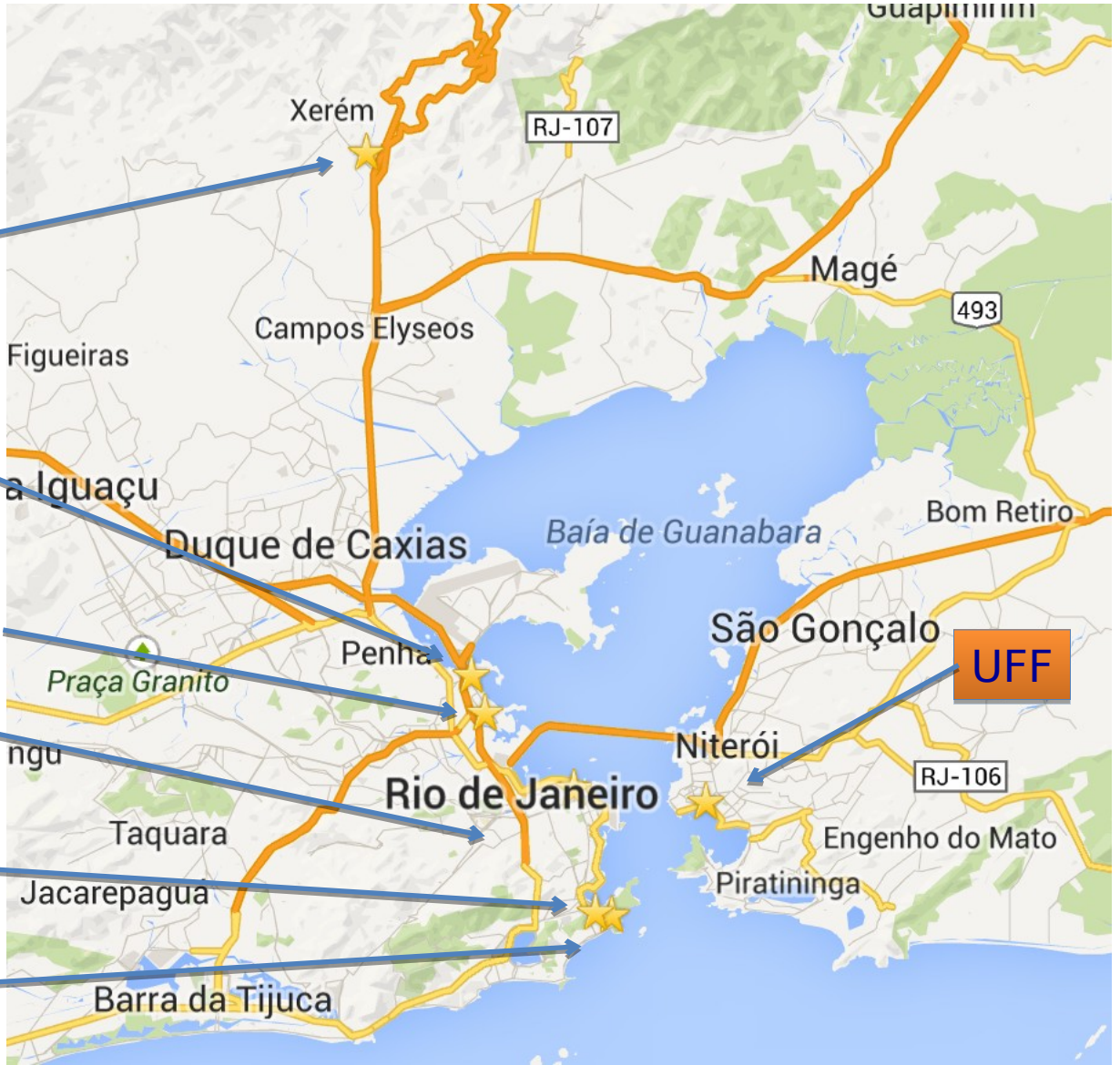
UFRJ:
Embryology and Histology
Chemical School
Pharmaceutical School

Engineering School / Coppe

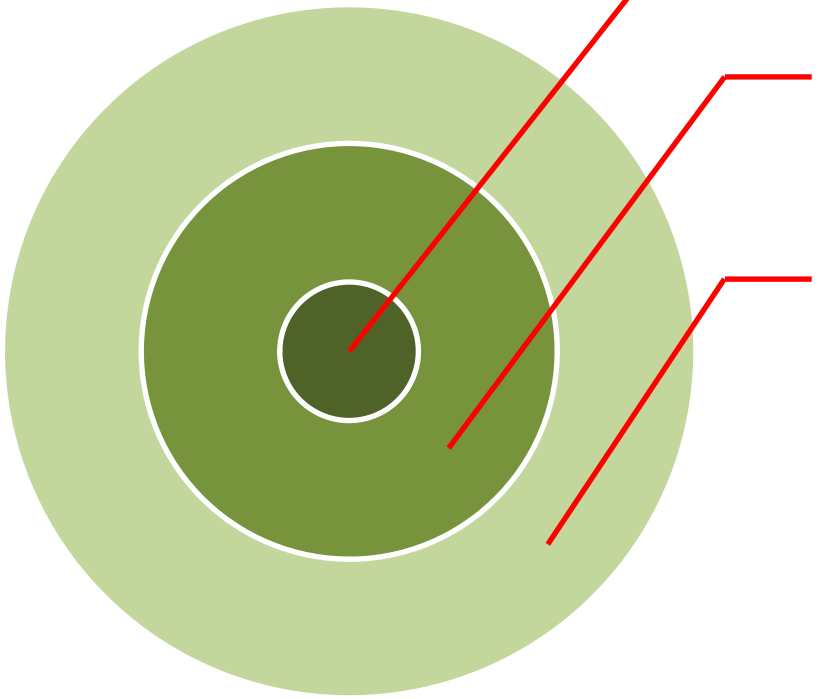
National Institute of Techcnology (INT)

Brazilian Center of Physic Research

Military Institute of Engineering



Team Networks – funding and cooperation

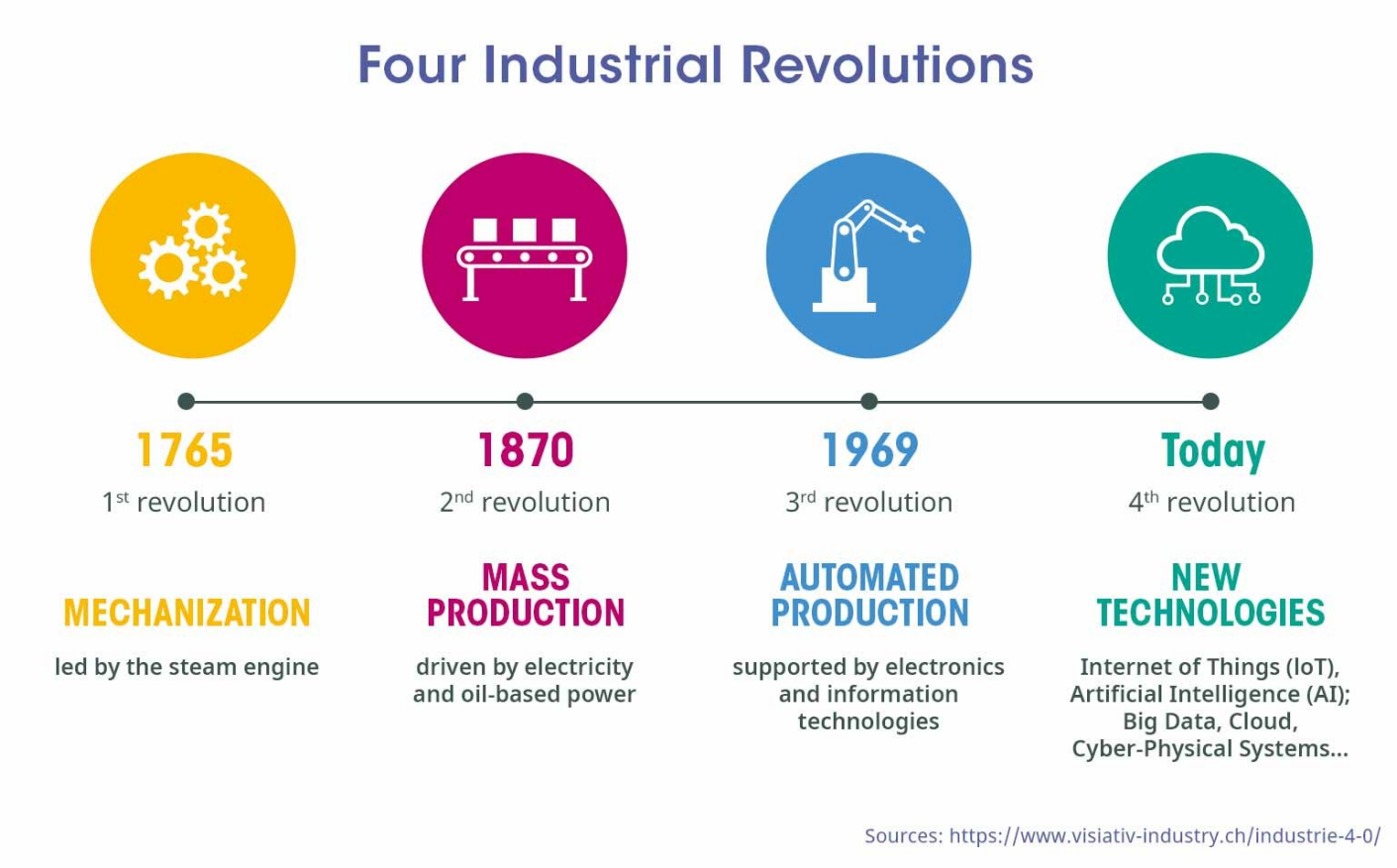


Rio de Janeiro Bioengineering Network

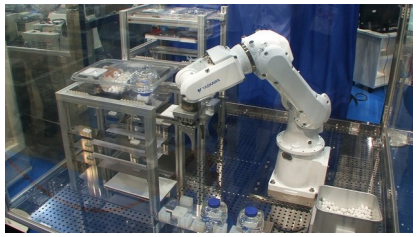
Nano Health Network (Rio de Janeiro - Faperj)

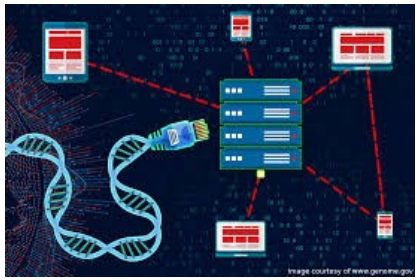
National Institute of Science and Technology on Regenerative Medicine (CNPq/Faperj - Brazil)

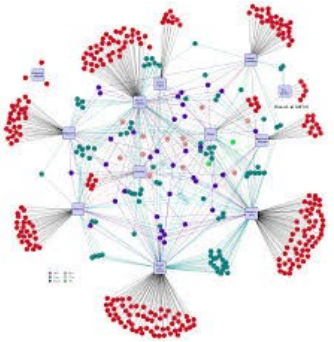
Context


Four Industrial Revolutions
1765
1st revolution
MECHANIZATION
led by the steam engine
1870
2nd revolution
MASS PRODUCTION
driven by electricity and oil-based power
1969
3rd revolution
AUTOMATED PRODUCTION
supported by electronics and information technologies
Today
4th revolution
NEW TECHNOLOGIES
Internet of Things (IoT), Artificial Intelligence (AI); Big Data, Cloud, Cyber-Physical Systems...
Sources: https://www.visiativ-industry.ch/industrie-4-0/

Industry 4.0 and Biotecnology



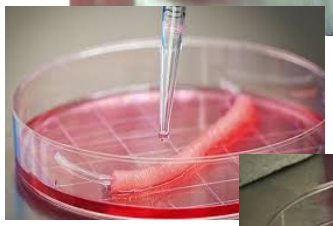




Biotecnologia: tecidos para a vida

Bioengineerd tissues

Bladder



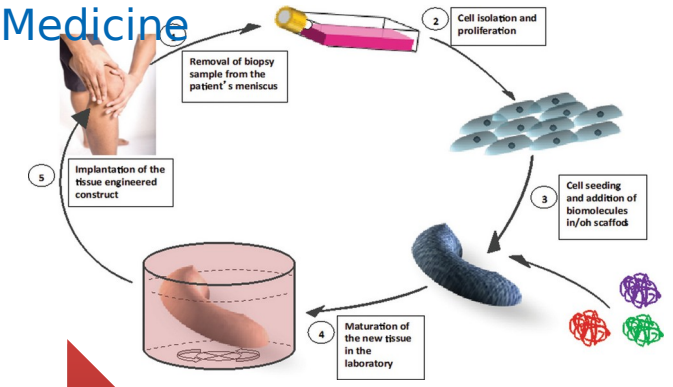
Blood vessel

Ear

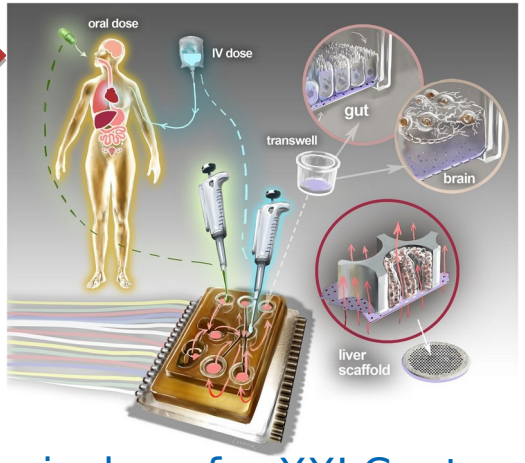
Mandible

Cardiac valve

Regenerative Medicine



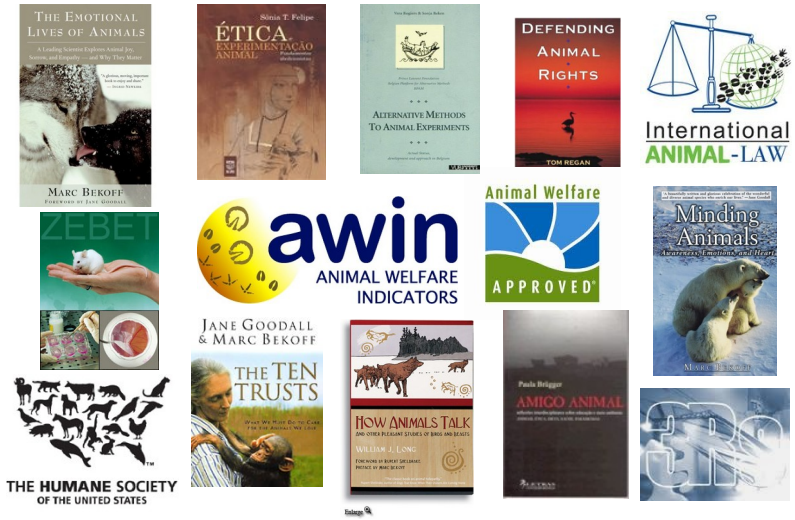
For what?



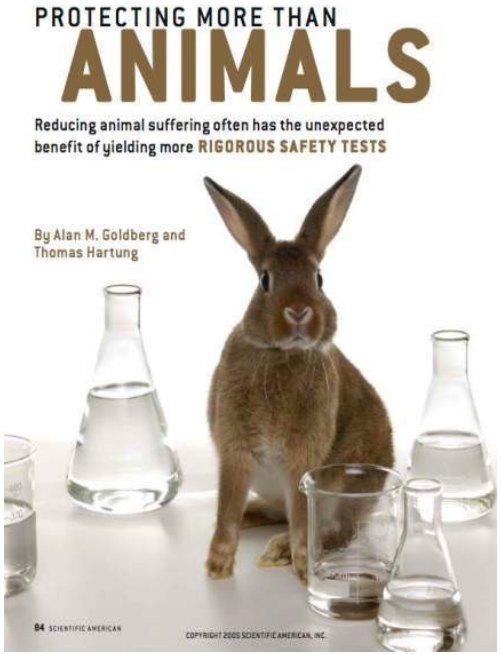
Toxicology for XXI Century
(*human on a chip*)

Alternative Methods to Animal tests

Ethical issues



Scientific issues



- Better science
- Less animals
- Human relevance
- Faster and cheaper results

3R

Replacement
Reduction
Refinement

Hughe challange

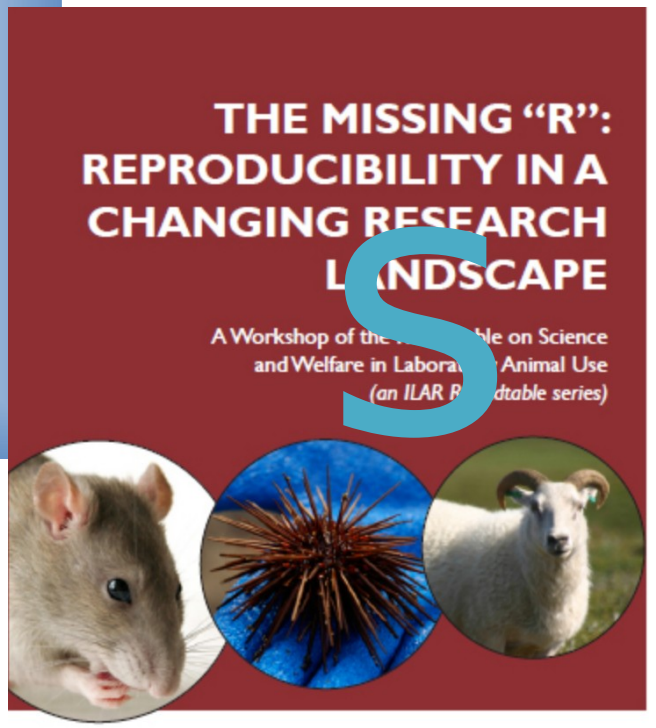
Challenge

- Quality
- Reliability
- Traceability

Bias

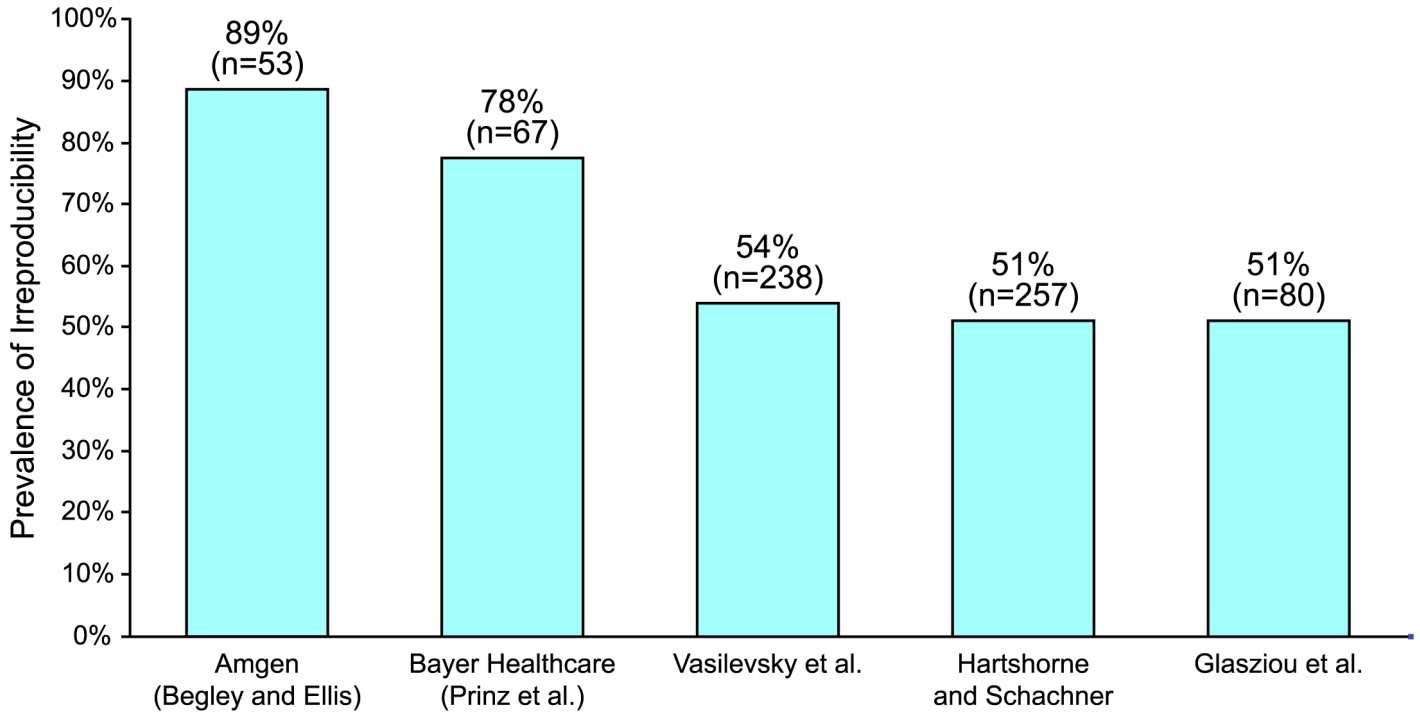
- Publish
- Funding
  - Private
  - Public

4R



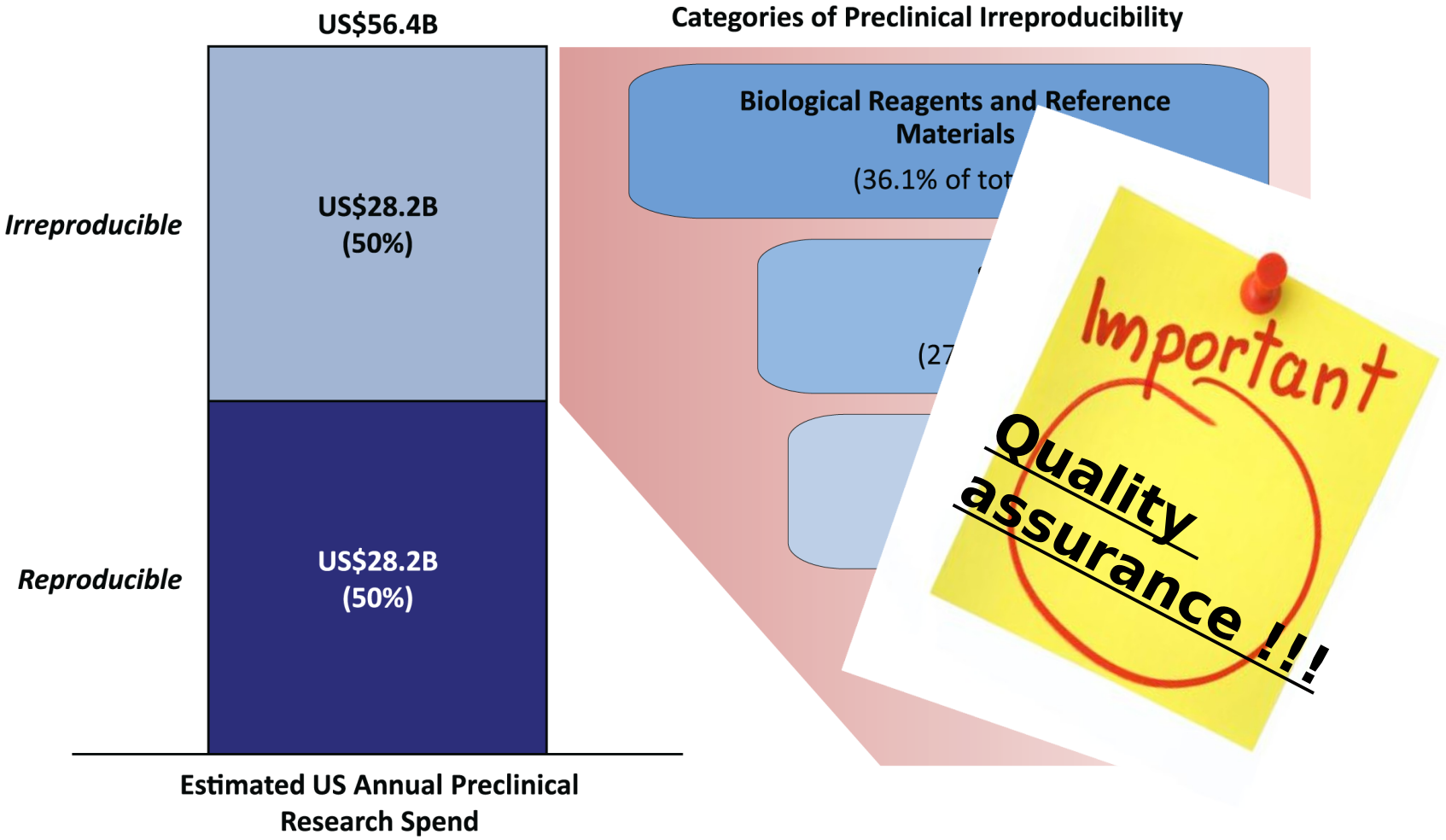
The Economics of Reproducibility in Preclinical Research

Leonard P. Freedman , Iain M. Cockburn, Timothy S. Simcoe. **PLOS**
https://doi.org/10.1371/journal.pbio.1002165

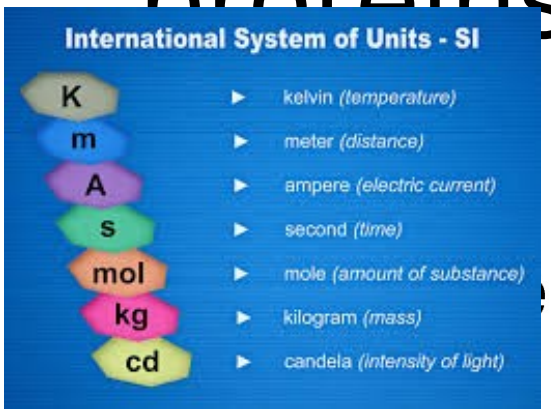


Reliability....

Irreproducibility cost

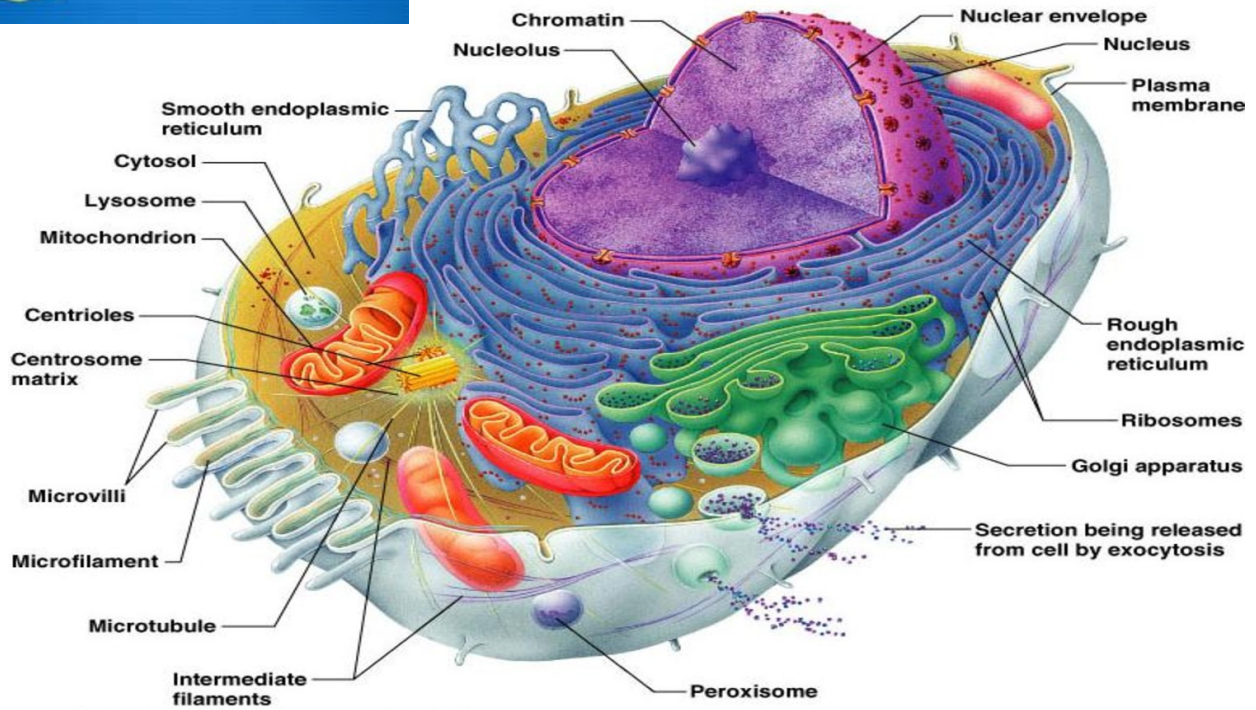


Metrology of cells, nucleic acids and proteins

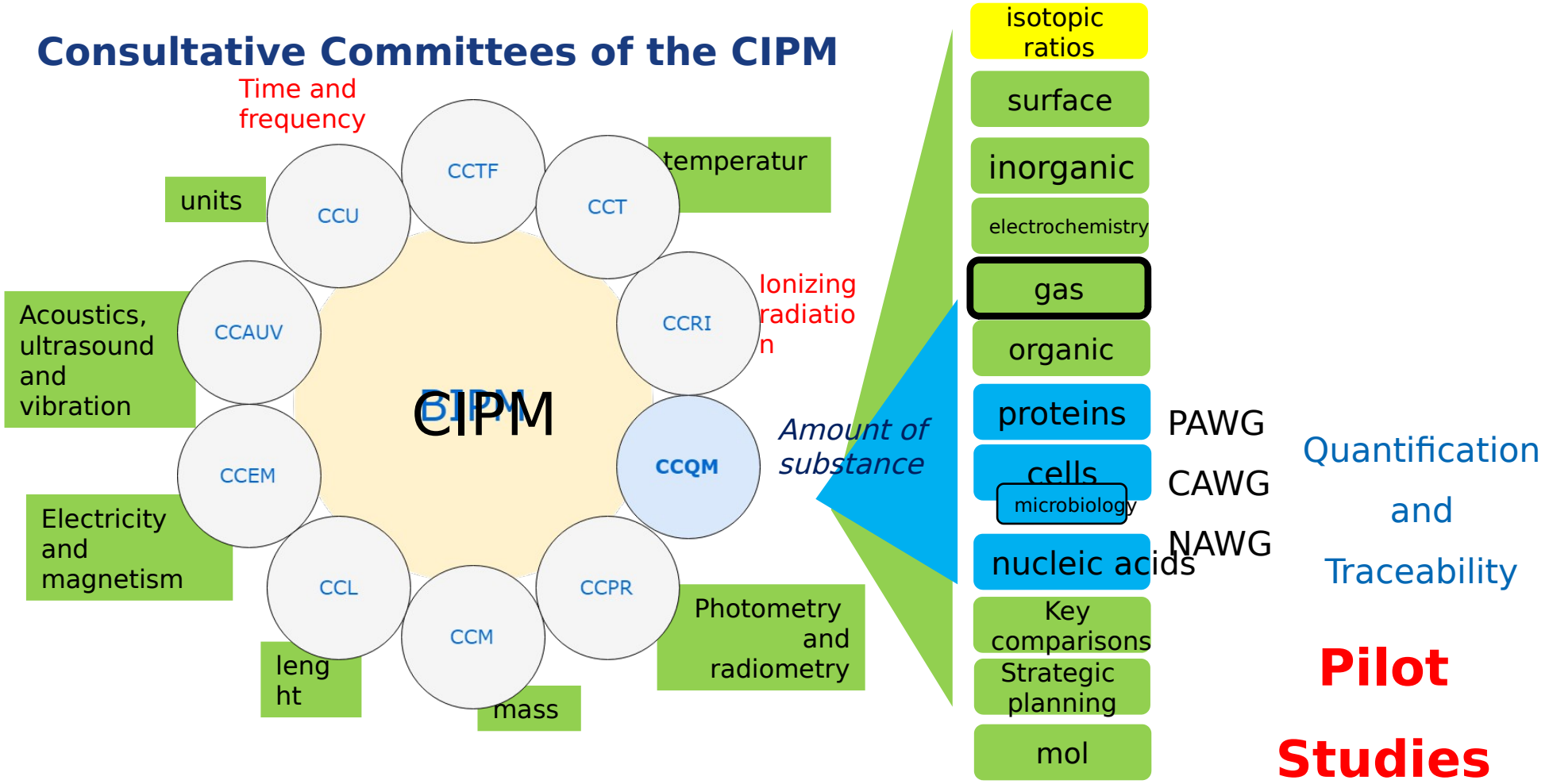


1. Counting viable cells
2. Identification and quantification of amino acids and proteins
3. Identification and quantification of nucleic acids (DNA, RNA, microRNA)

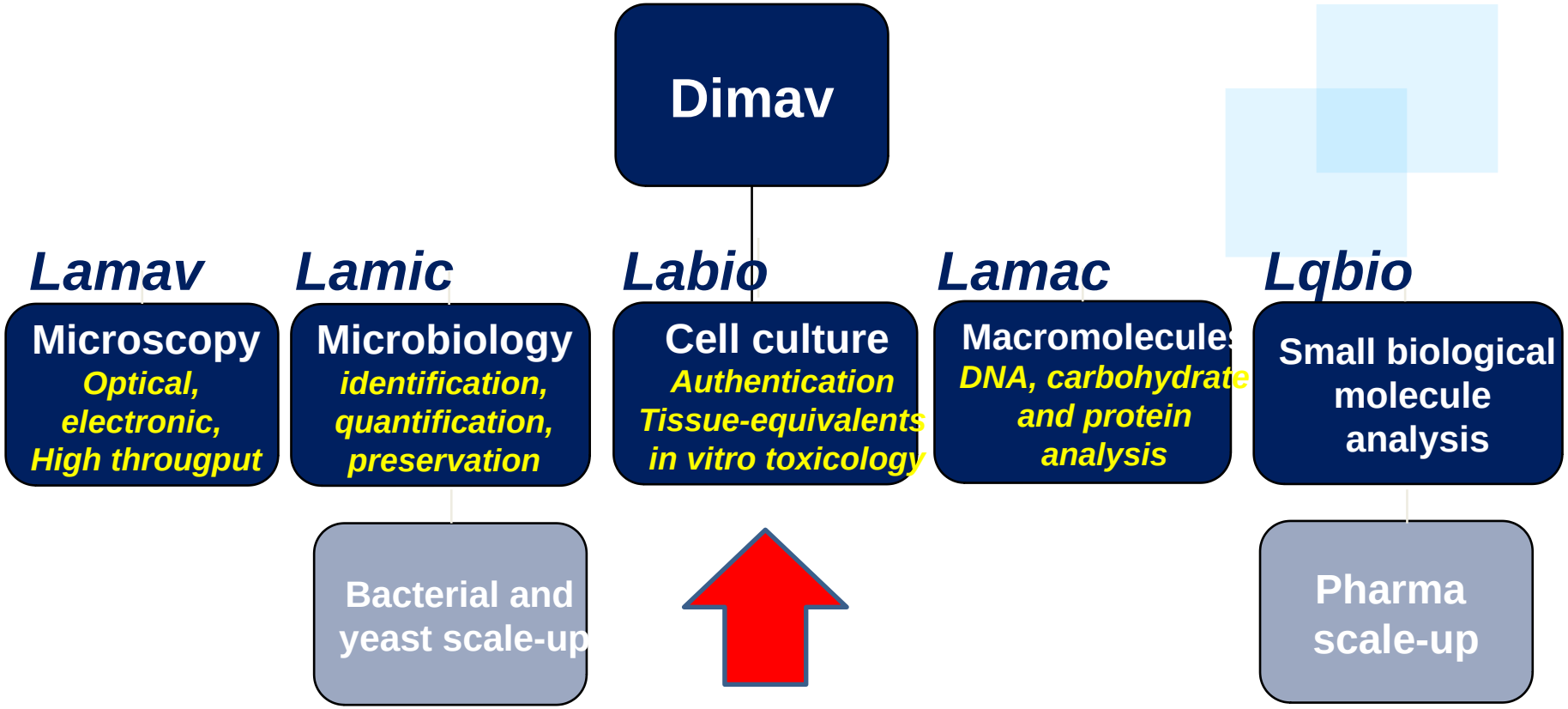
of a Generalized Cell



Biometrology – 2001 BSWG/BIPM)



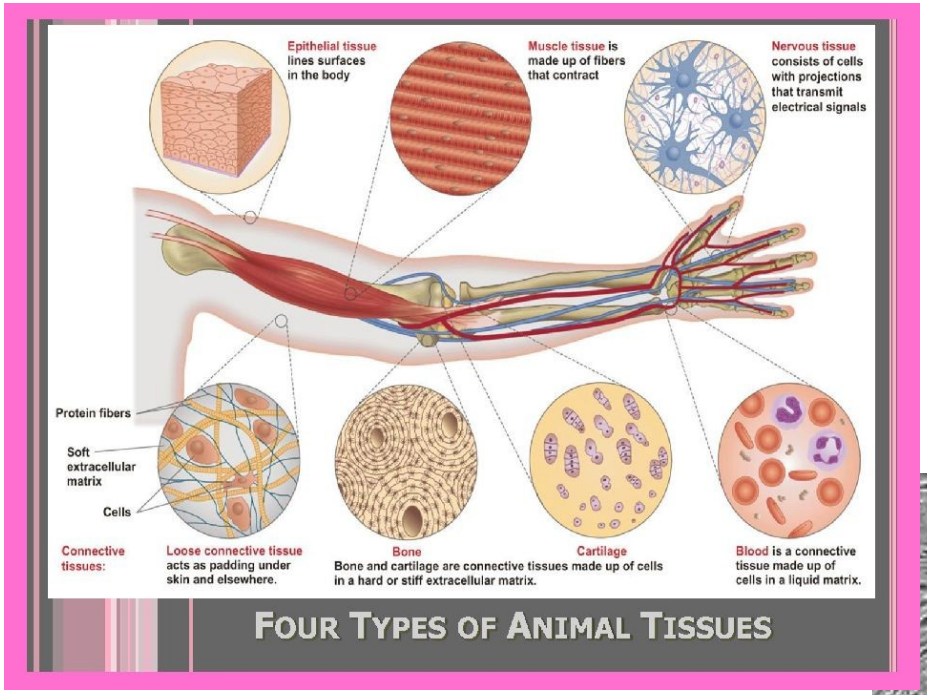
Life Sciences Applied Metrology

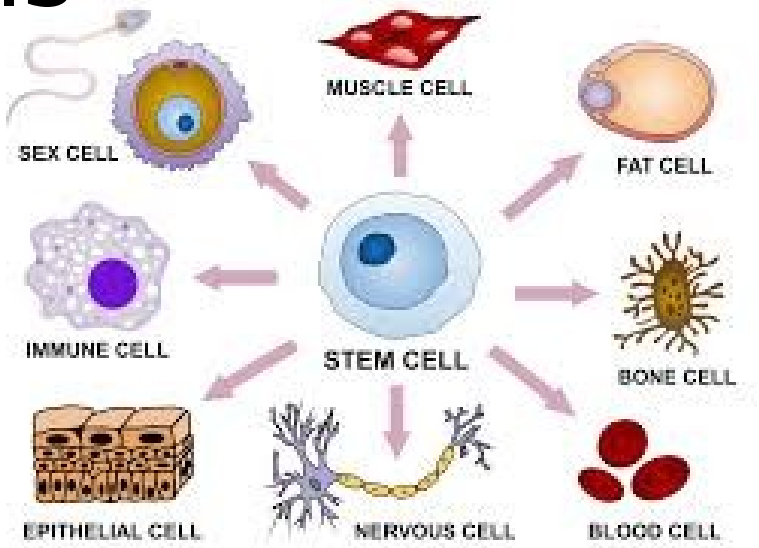


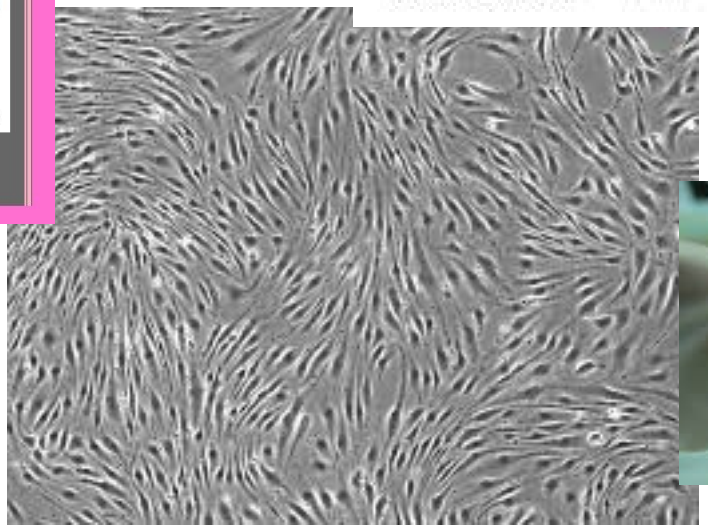
Staff (researchers, technicians)

7 8 6 8 3

Human cells


Epithelial tissue lines surfaces in the body
Muscle tissue is made up of fibers that contract
Nervous tissue consists of cells with projections that transmit electrical signals
Protein fibers
Soft extracellular matrix
Cells
Connective tissues:
Loose connective tissue acts as padding under skin and elsewhere.
Bone
Cartilage
Bone and cartilage are connective tissues made up of cells in a hard or stiff extracellular matrix.
Blood is a connective tissue made up of cells in a liquid matrix.
FOUR TYPES OF ANIMAL TISSUES


SEX CELL
MUSCLE CELL
FAT CELL
IMMUNE CELL
STEM CELL
BONE CELL
EPITHELIAL CELL
NERVOUS CELL
BLOOD CELL



Tissue Engineering - spheroids

- Human cells
  - Blood vessels
  - Mucosa
    - Gastric
    - Respiratory
    - Oral
    - Intestine;
  - Bone
  - **Cartilage**

BCRJ BANCO DE CÉLULAS DO RIO DE JANEIRO

UNIVERSIDADE UNIGRANRIO
Vá além da sala de aula!

UNIVERSIDADE FEDERAL DO RIO DE JANEIRO
UFRJ

uff
Universidade Federal Fluminense

FIOCRUZ
FUNDAÇÃO OSWALDO CRUZ

SOCIAL AND ECONOMIC IMPACT

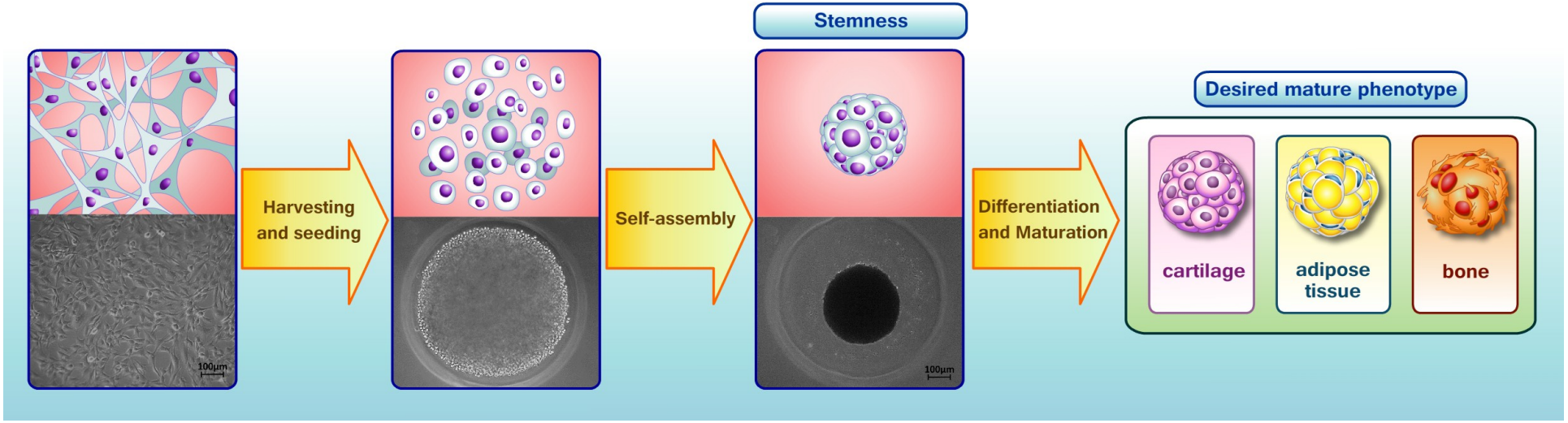
- IN 2013, ABOUT $ 304 BILLION WAS SPENT ON MEDICAL EXPENSES AND SICK LEAVE IN THE UNITED STATES.

Life Quality

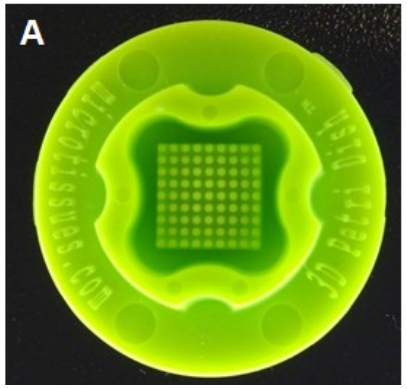
- **7.5% OF LEAVES OF WORK.**
- **10.5% OF SICK LEAVE APPLICATIONS.**
- **6.2% EARLY RETIREMENT.**

https://www.vectorstock.com/royalty-free-vector/sad-people-black-icon-vector-19729271

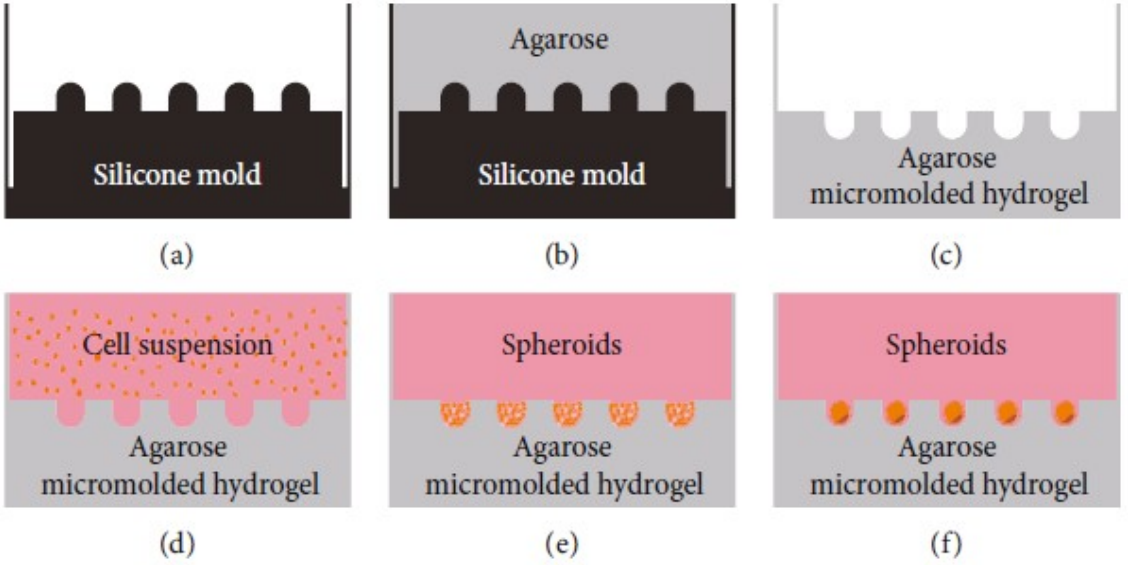
Tissue Engineering for bone and cartilage injury



Human adipose tissue derived stem cells spheroids



Micro-molded non-adhesive hydrogel



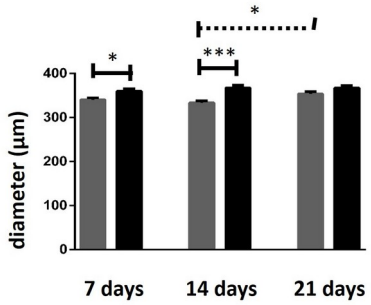
Stuart MP et al. Stem Cells International. 2017. https://doi.org/10.1155/2017/7053465

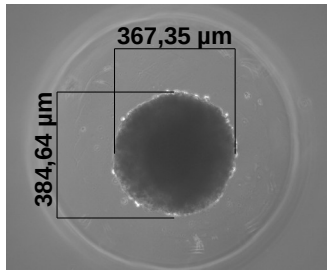
End points (analysis) of 3D spheroids

From spheroids

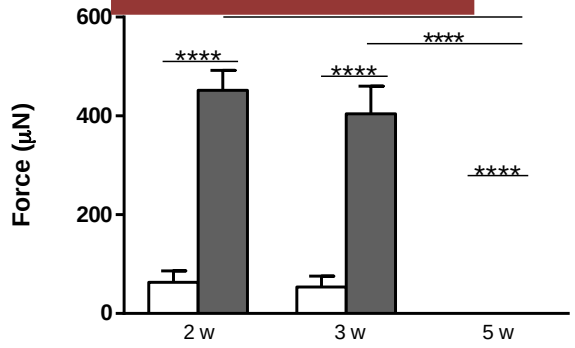
- Diameter measurement (viability)
- Eletronic microscopy (morphology)
- Biomechanical assay
- Histology/Specific targets (immunofluorescence, immunohistochemistry)
- Molecular assays (qPCR

REPRODUCIBILITY



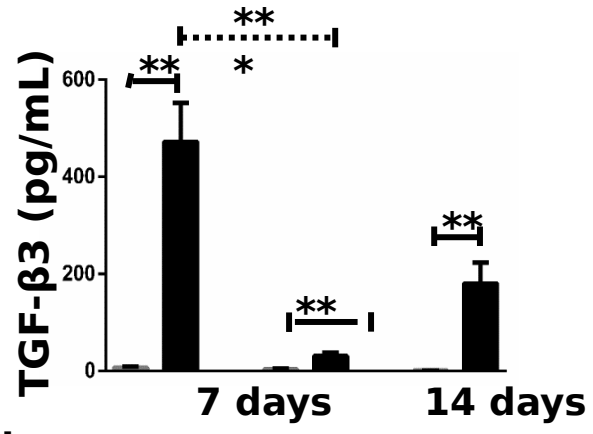


RASTREABILITY



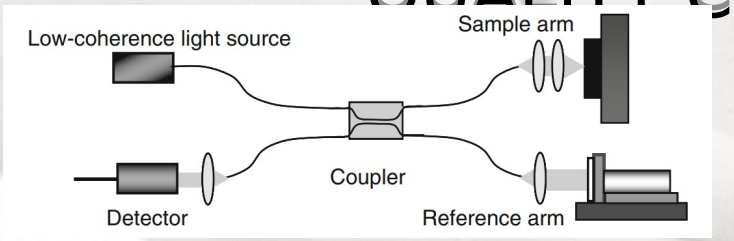
From spheroid culture supernatant

- Biochemistry assays (viability)
- Non-specif targets (secretome)
- Specific targets (multiplex, CBA)

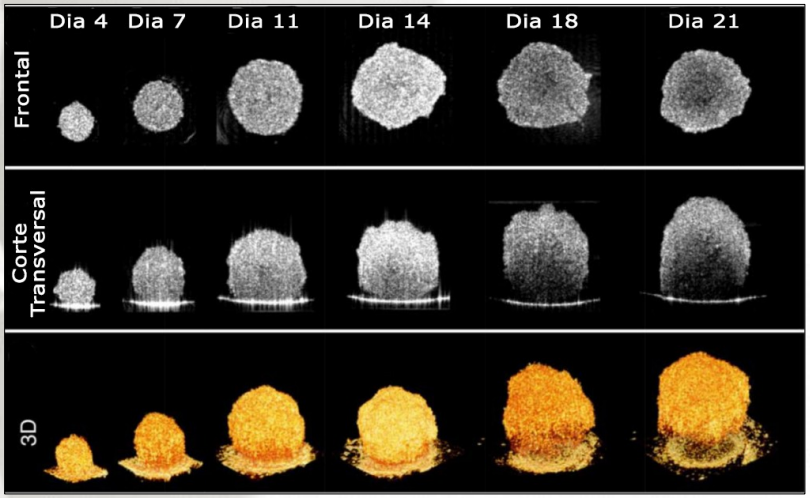


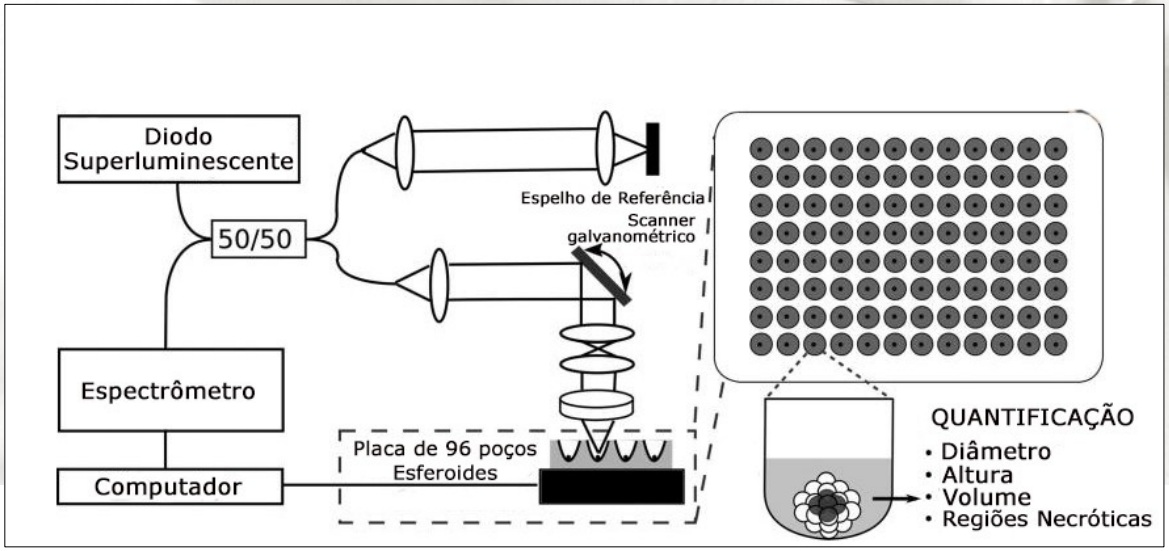
OPTICAL COHERENCE TOMOGRAPHY - OCT (HUANG ET AL, 2017)

OCT USES LIGHT WAVES TO TAKE CROSS-SECTION IMAGES OF TISSUE: NON-INVASIVE, REAL TIME, QUALITY CONTROL OF SPHEROIDS



POPESCU et al, 2011

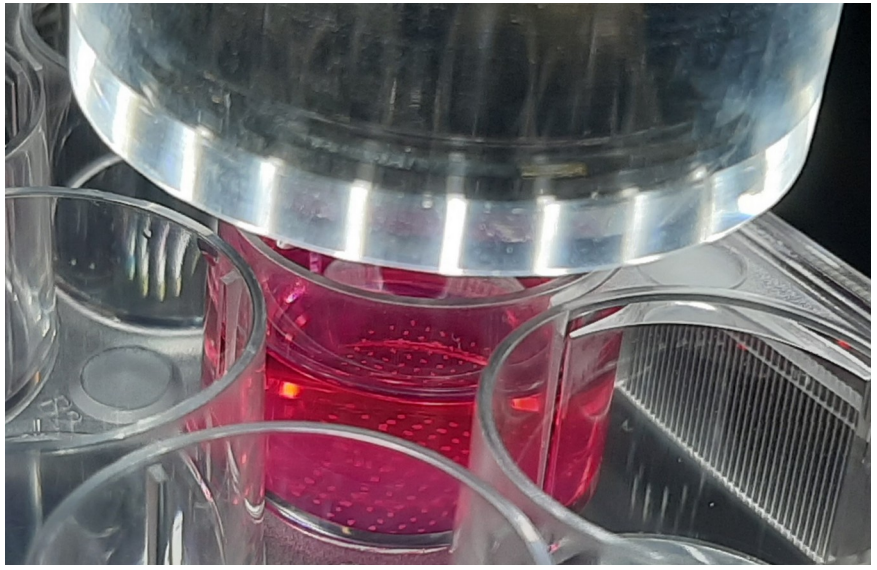


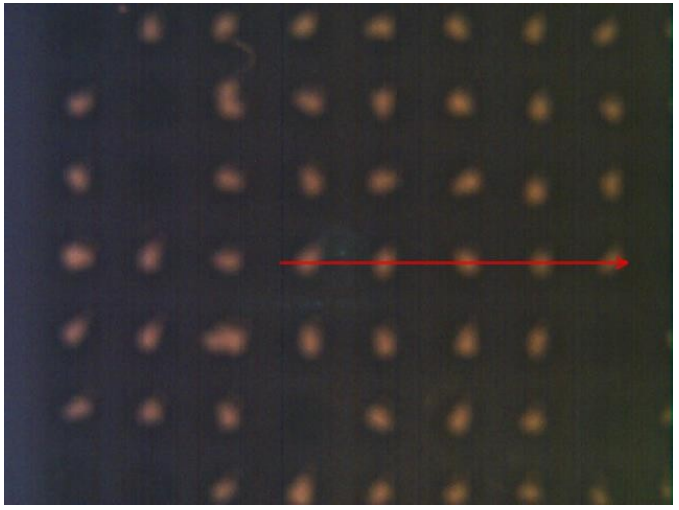


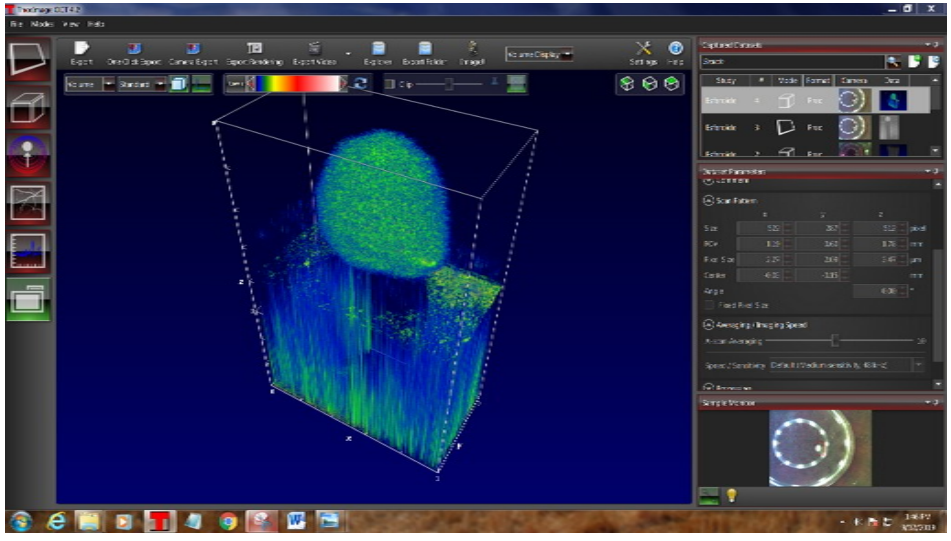
HUANG et al, 2007

HUANG et al, 2017

PRELIMINARY RESULT







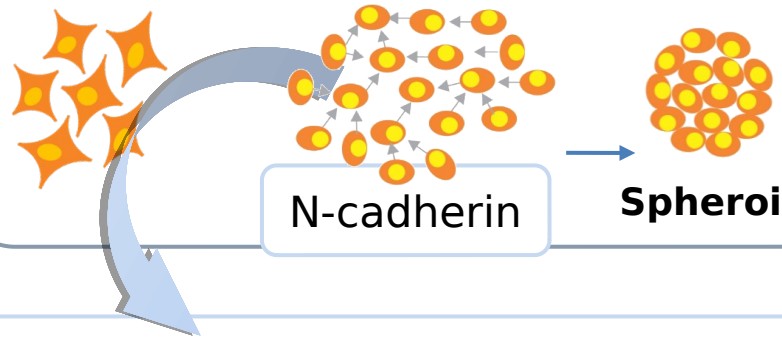
Metrologia Florianópolis - SC 2019

CAMPUS DE INOVAÇÃO E METROLOGIA

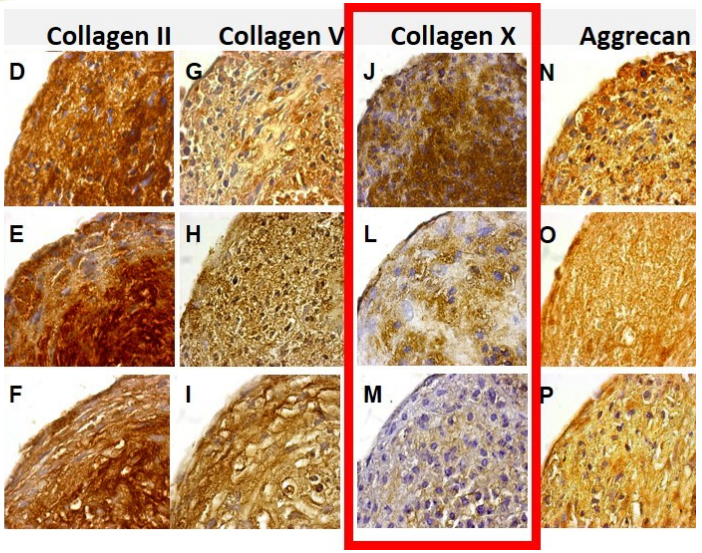
INMETRO

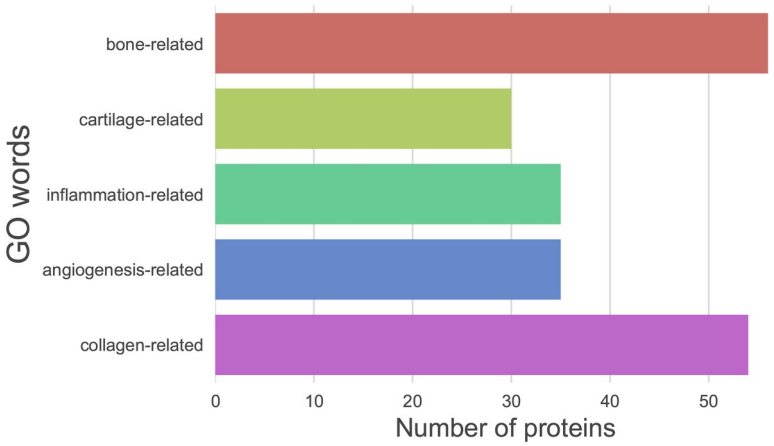
Cartilage bioengineering

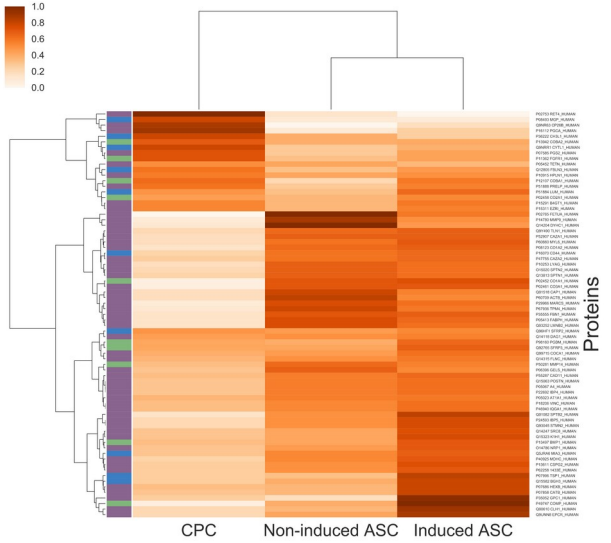
Biological advantages of self-assembly



Embryological development of stable cartilage



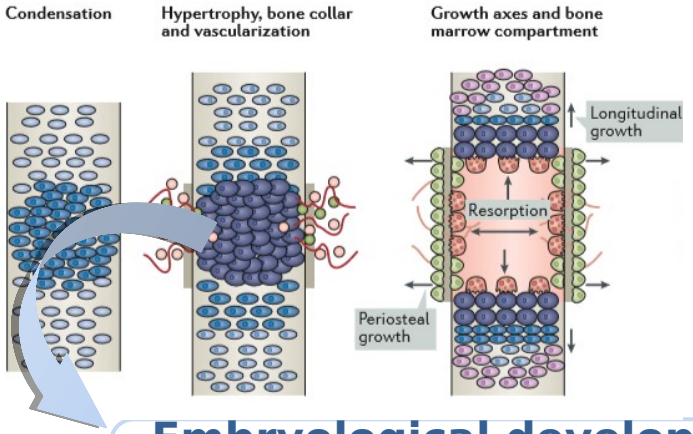


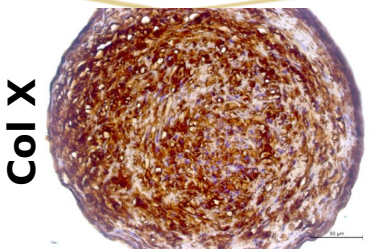


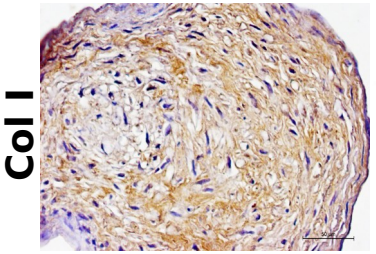
Lyon 1

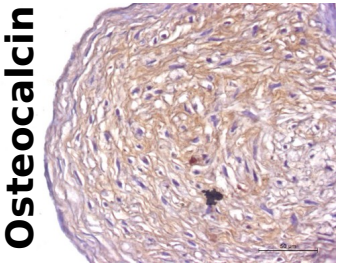
Côrtes et al. Tissue Eng Part A. 2019 Feb 8. doi: 10.1089/ten.TEA.2018.0311

Bone bioengineering

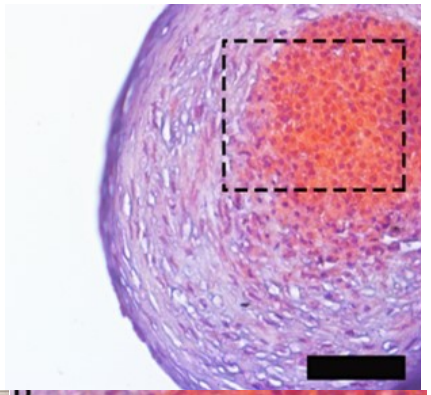


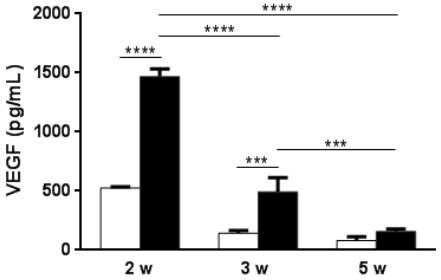


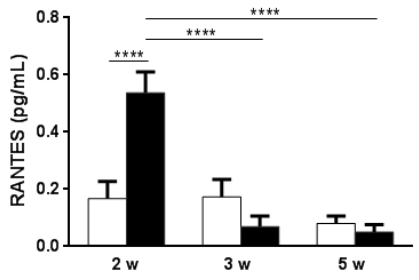


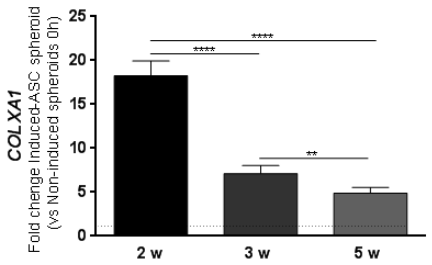


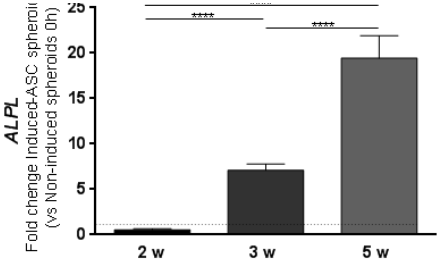
Embryological development of endochondral ossification
Engineered hypertrophic cartilage

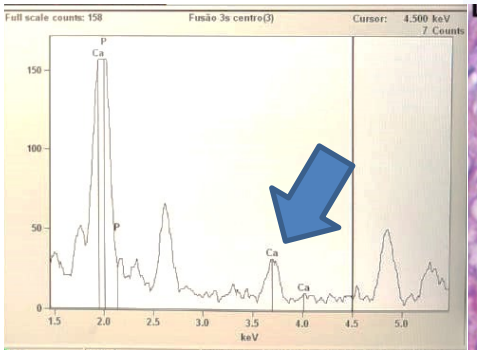


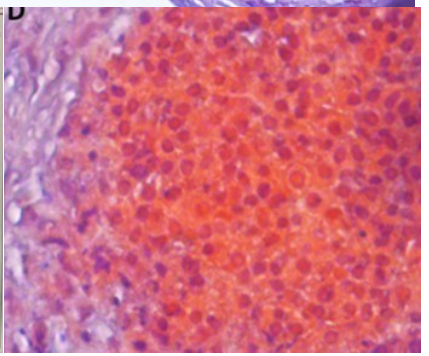












Master Thesis
Kronemberger, 2018
Submitted results

Bioprinting

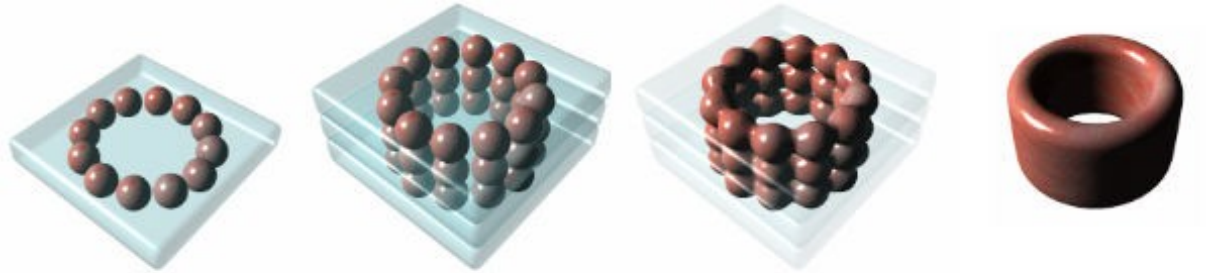
Original Article

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

Journal of Tissue Engineering
Volume 5: 1–11

DOI: 10.1177/2041731414556561
tej.sagepub.com
SAGE

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

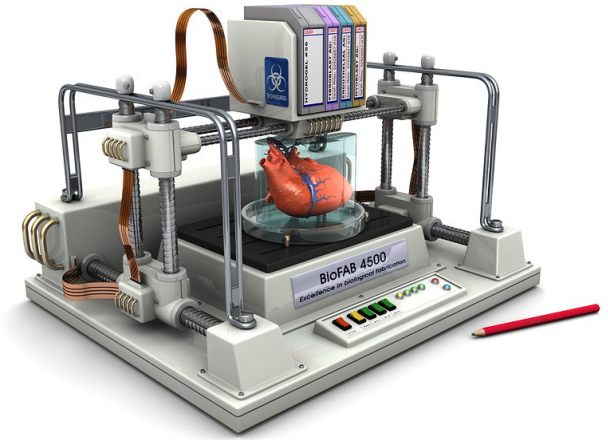


[A] Bioink spheroids printed into layer of biopaper gel

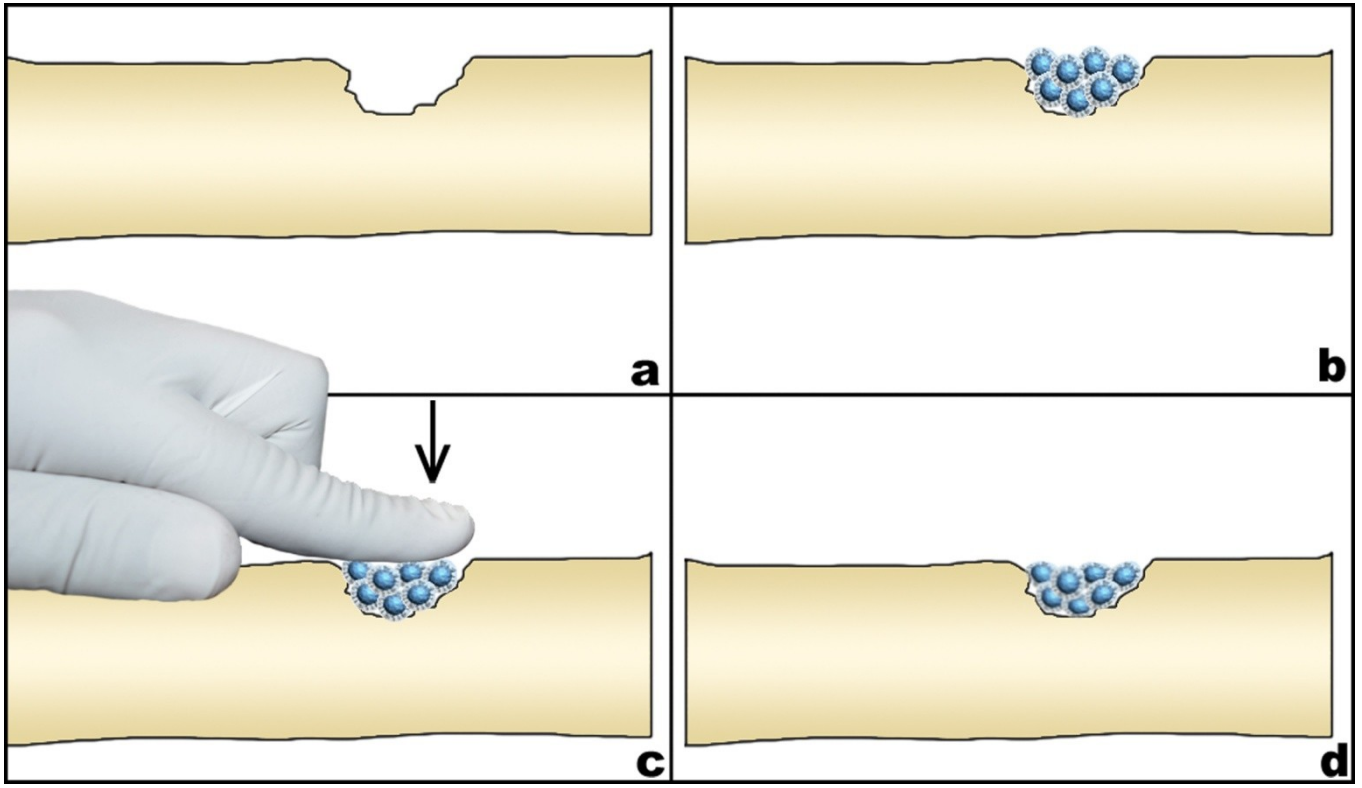
[B] Additional layers printed to build object

[C] Bioink spheroids fuse together and biopaper dissolves

[D] Final living tissue

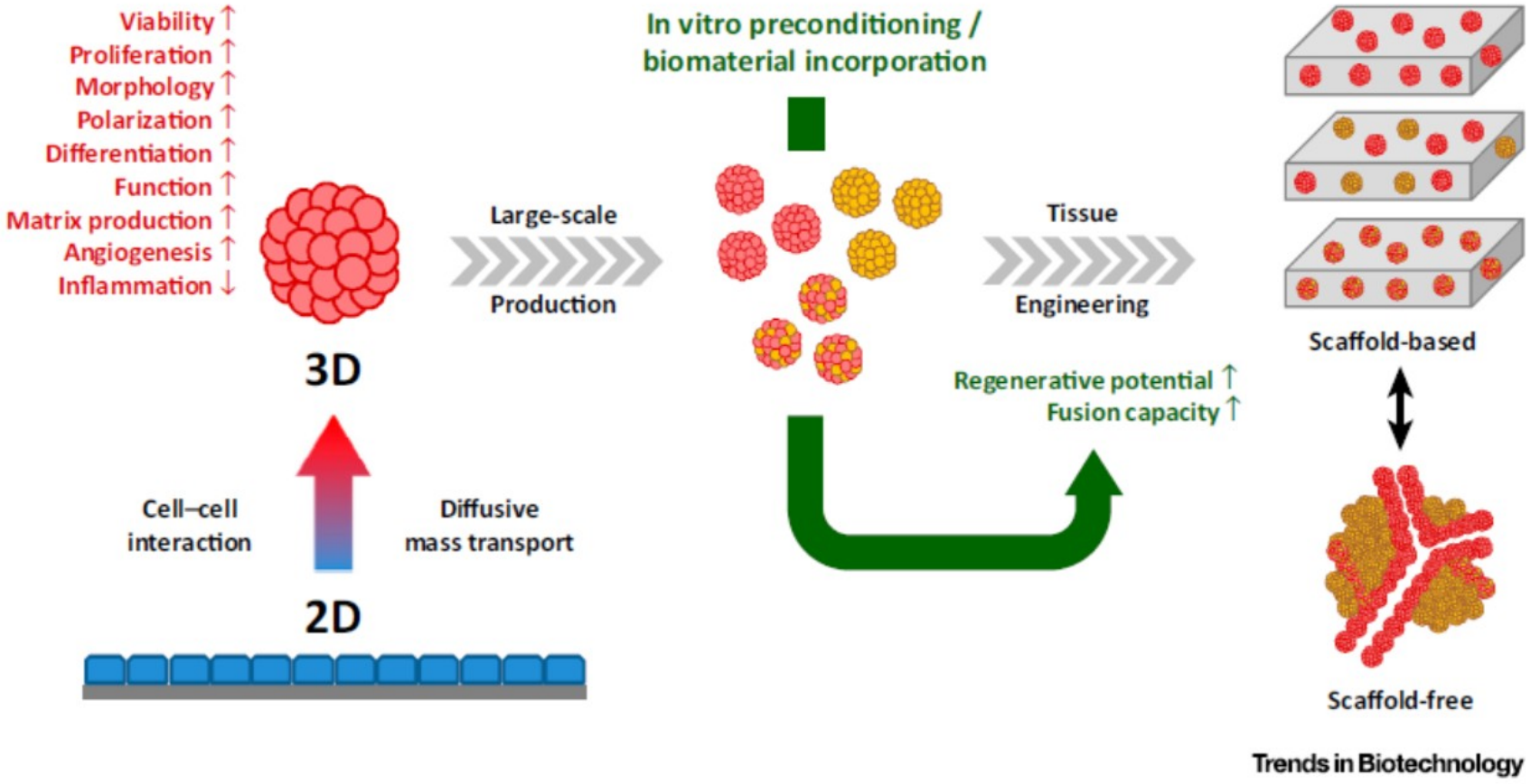


CLINICAL CONCEPT: IN SITU RAPID TISSSUE BIOFABRICATION USING SCAFFOLDS



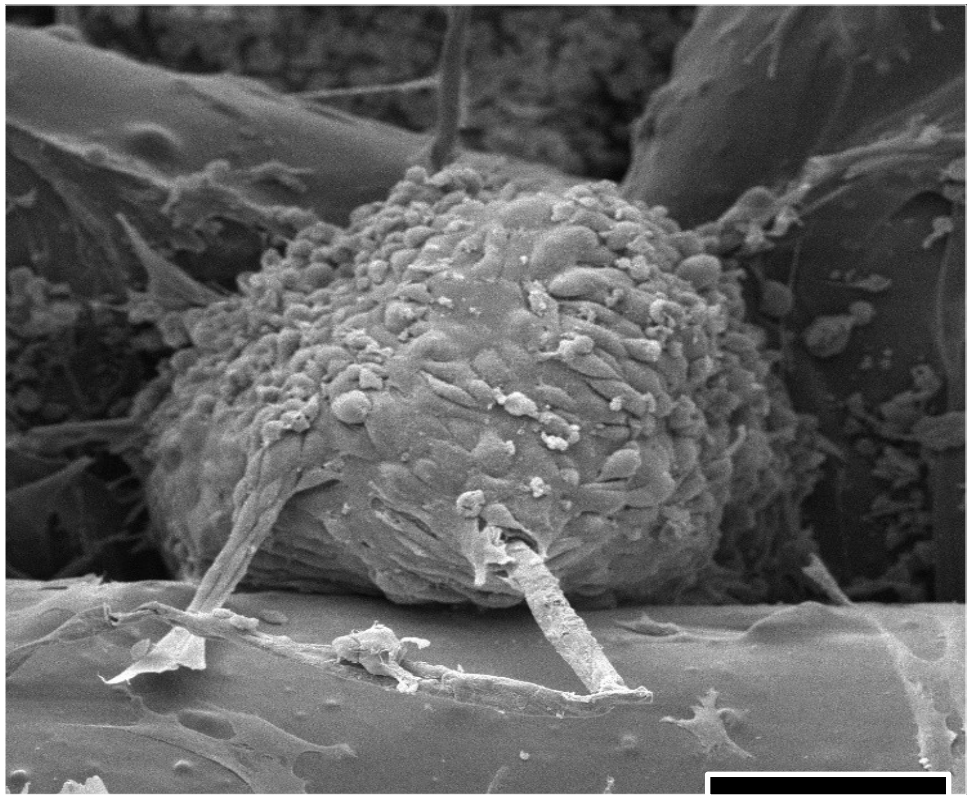
Scheme demonstrating in vivo rapid 3D tissue biofabrication using tissue spheroids

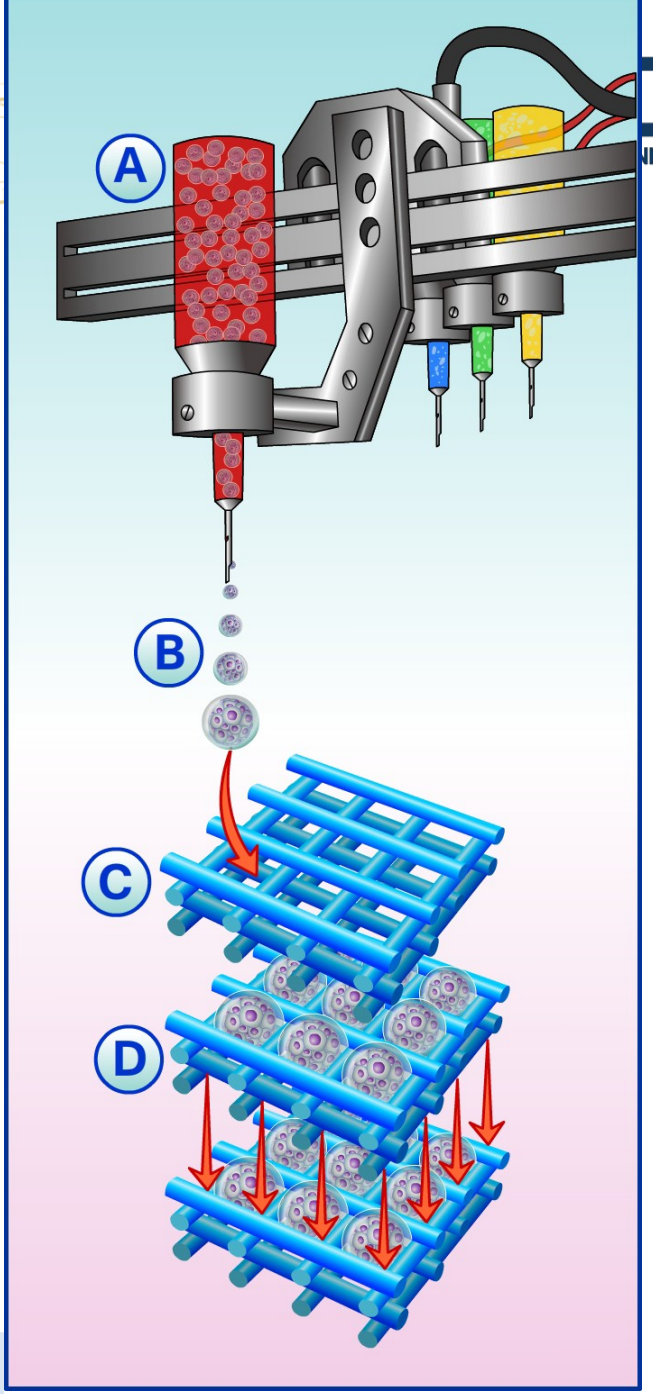
Bottom-up tissue engineering: spheroids



Laschke MW, Menger MD. Trends Biotechnol. 2017 Feb;35(2):133-144. doi: 10.1016/j.tibtech.2016.08.004.

Cartilage and bone bioprinting

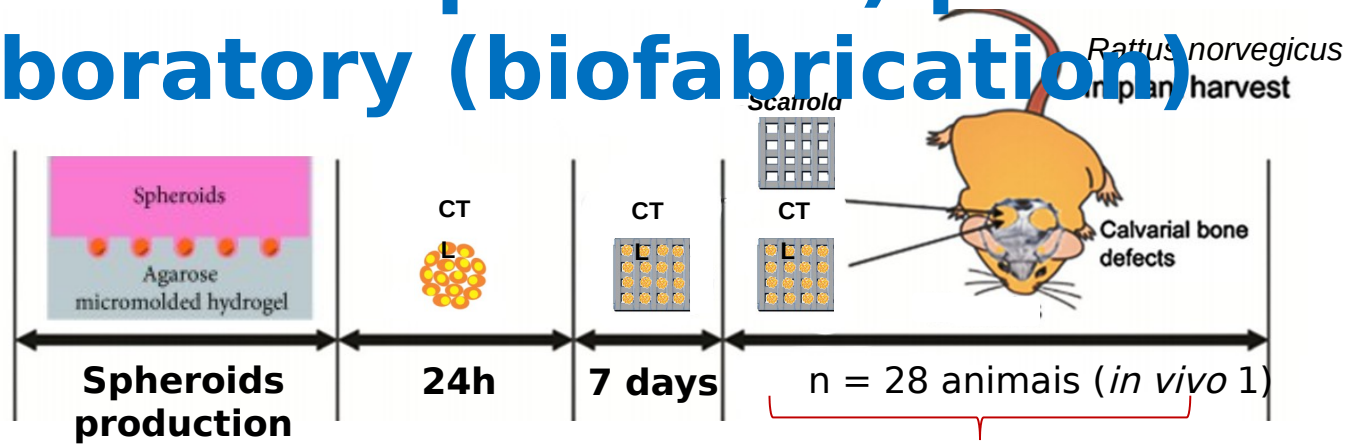




Baptista LS et al. Int. J. Mol. Sci. 2018, 19, 1285; doi:10.3390/ijms19051285

Our first bioprinting test with cartilage spheroids

Bone constructs (biomaterial+spheroids) produced in the laboratory (biofabrication)

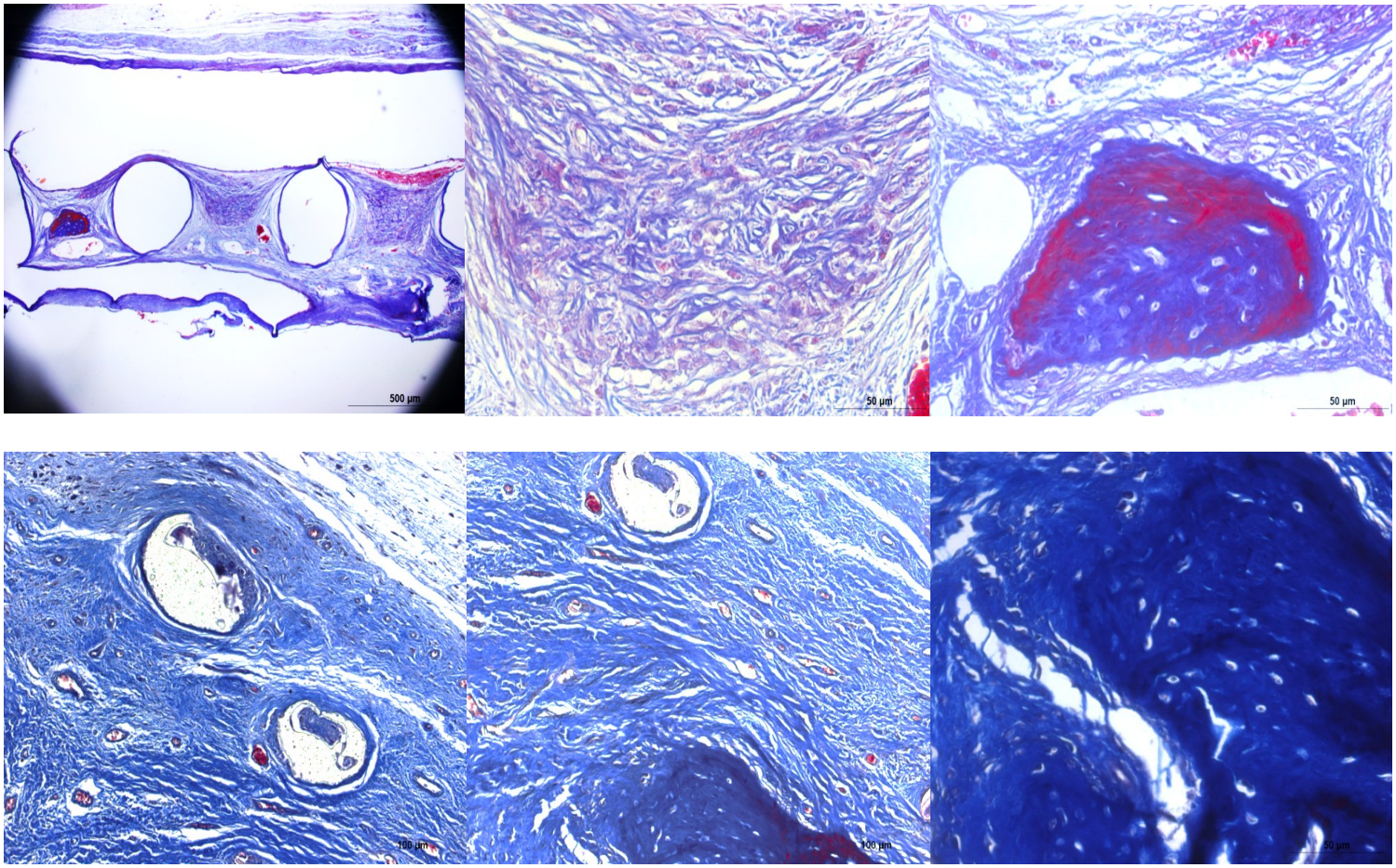


n = 1 (Cloth group)
n = 6 (Biomaterial group)
n = 6 (Bomaterial + spheroids group)

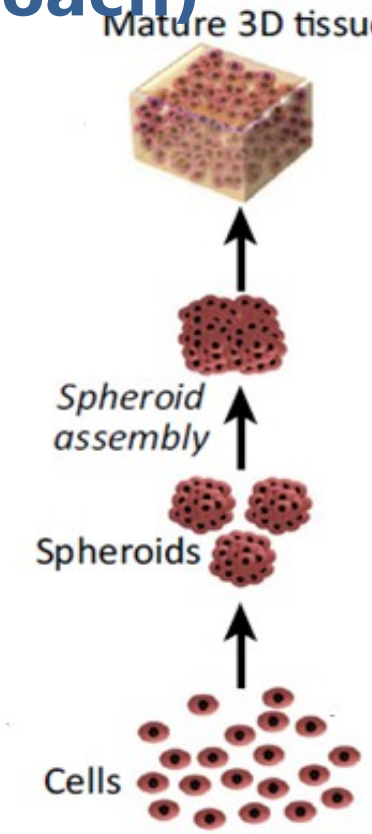
Experimental period = 3 months

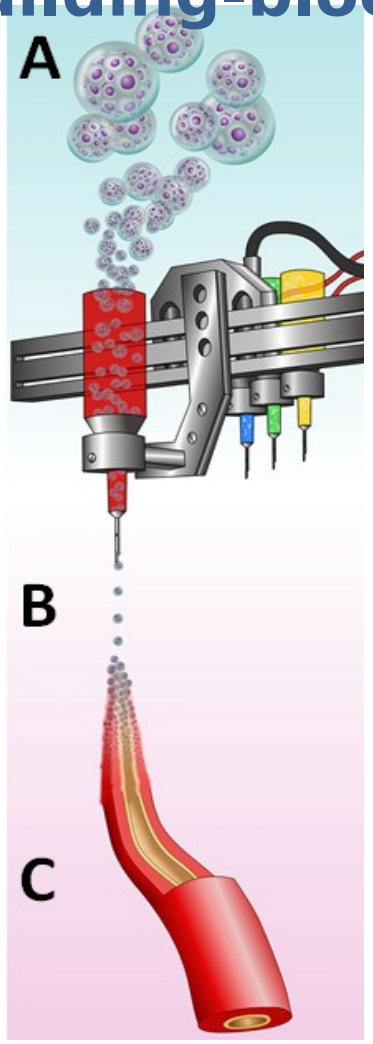
Paulo A de O, Castro-Silva II, Oliveira DF, Machado ME, Bonetti-Filho I, Granjeiro JM. Repair of critical-size defects with autogenous periosteum-derived cells combined with bovine anorganic apatite/collagen: an experimental study in rat calvaria. Braz Dent J. 2011;22(4):322–328. doi:10.1590/s0103-64402011000400011

3 months after implantation: biocompatibility, no immunological rejection – **new bone formed**



3D cell culture perpective: Bioprinting using spheroids as building-blocks (bottom-up approach)

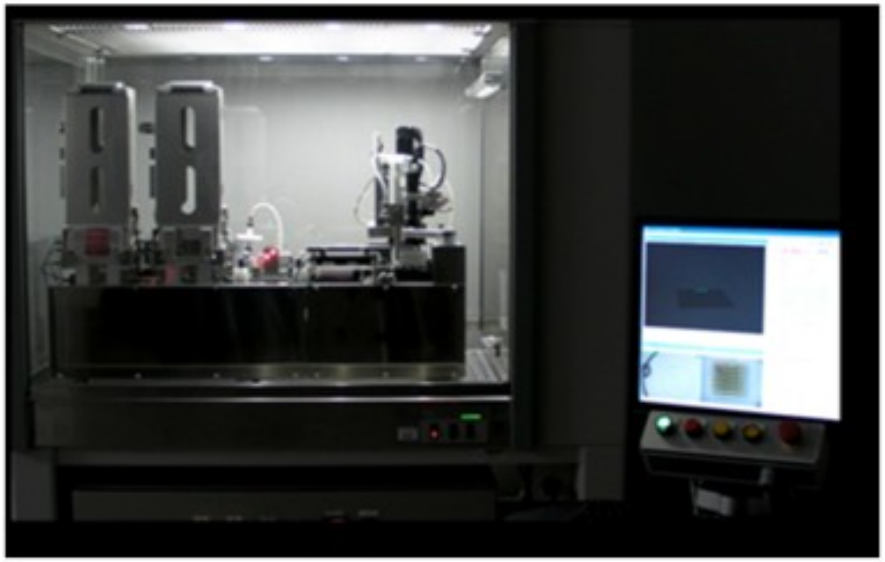


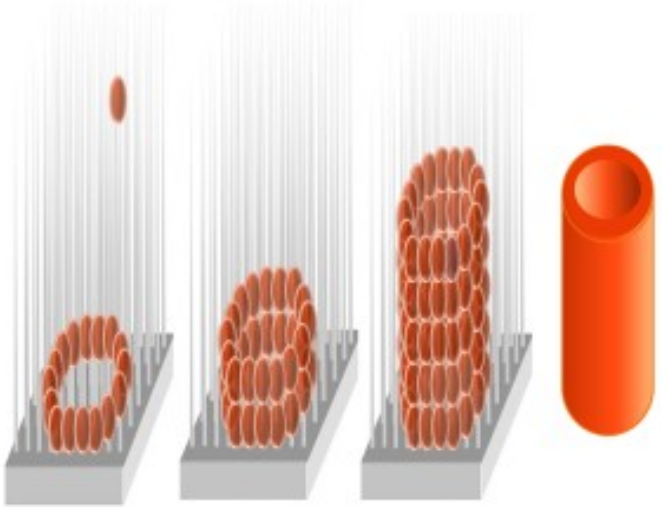


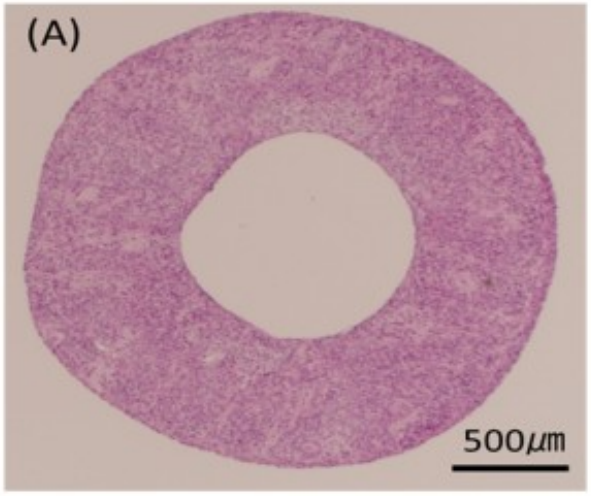
Adaptado de Guven S et al., Trends Biotechnol. 2015, 33:269-279. doi: 10.1016/j.tibtech.2015.02.003.

Baptista LS et al. Frontiers In Bioscience, Landmark, 23, 1969-1986, June 1, 2018. DOI No:10.2741/4683]

Bioprinting using spheroids as building-blocks



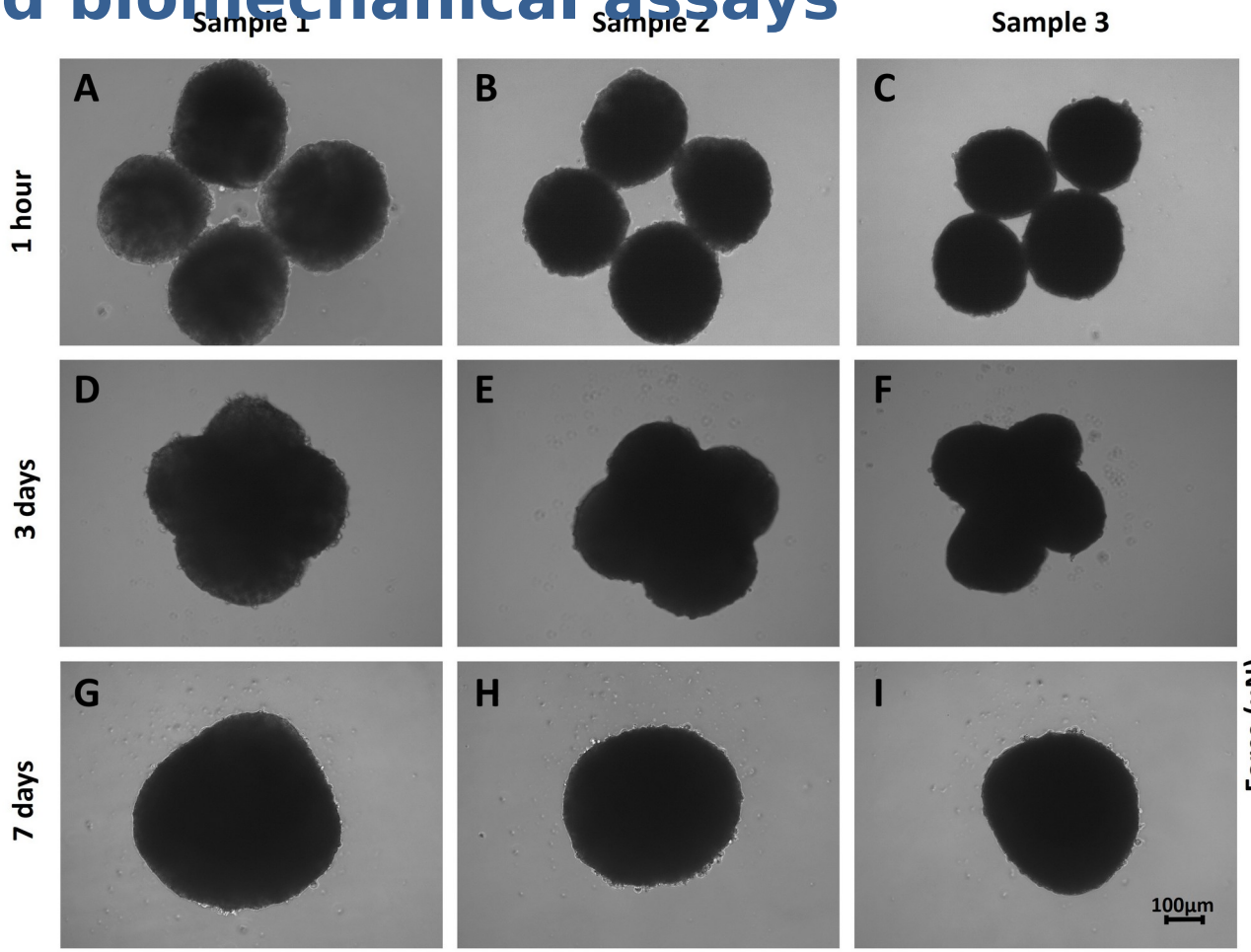


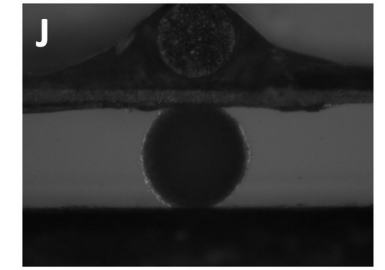


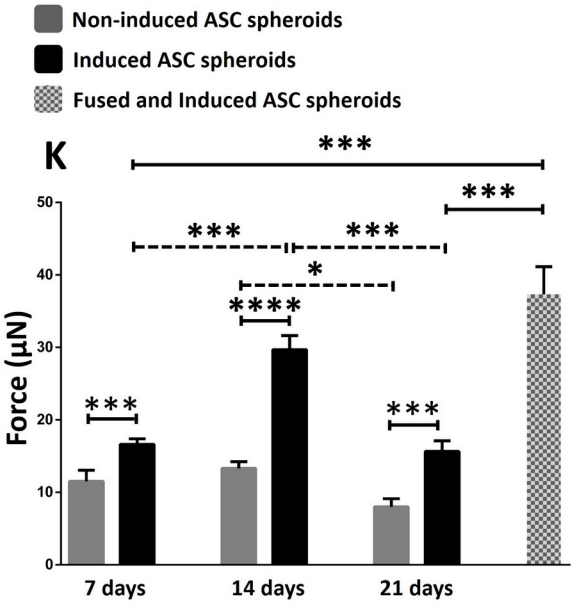
1.5mm diâmetro por 7mm comprimento

Itoh M et al. PLoS One. 2015 1;10(9):e0136681. doi:10.1371/journal.pone.0136681.

Bioprinting using spheroids as building-blocks: fusion and biomechanical assays

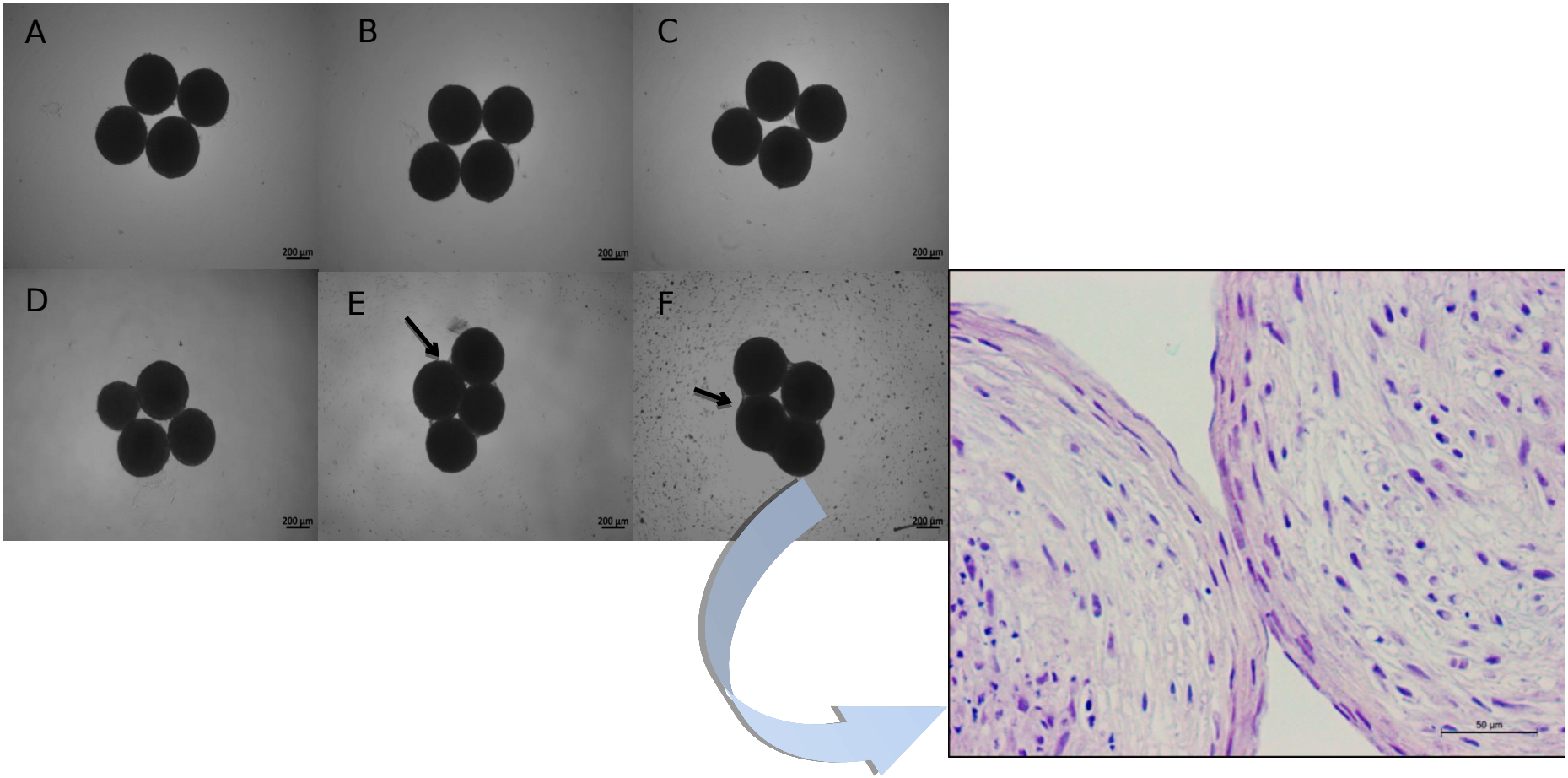






Côrtes et al. Tissue Eng Part A. 2019 Feb 8. doi: 10.1089/ten.TEA.2018.0311

Bioprinting using spheroids as building-blocks: fusion and biomechanical assays



Unsubmitted results

Motivation


UNILATERAL CLEFT LIP
CLEFT LIP & CLEFT PALATE
(ROOF OF MOUTH)
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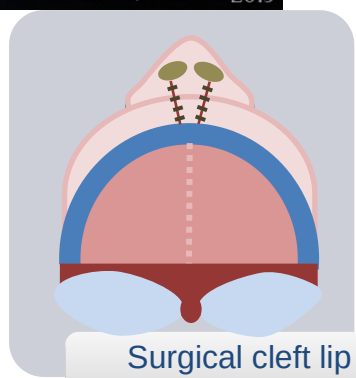
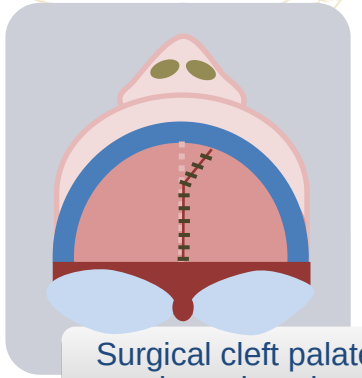
CENTRINHO-USP
HOSPITAL DE REABILITAÇÃO DE ANOMALIAS CRANIOFACIAIS
UNIVERSIDADE DE SÃO PAULO

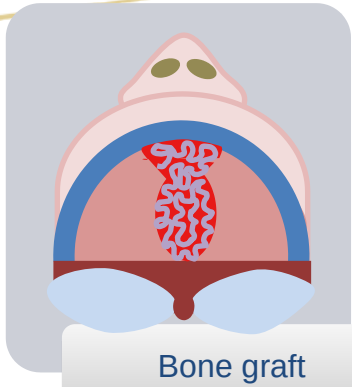
A



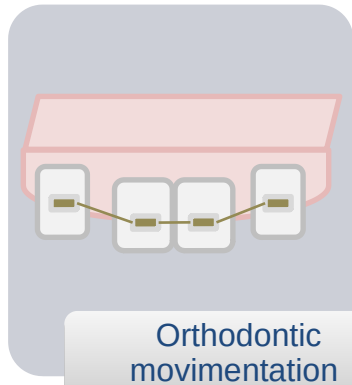
Surgical cleft lip repair - cheiloplasty (3 months)



Surgical cleft palate repair - palatoplasty (6-12 months)



Bone graft (8-11 years)



Orthodontic movimentation (2-15 years)

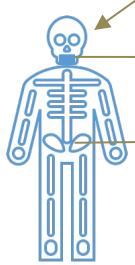
B

1st Generation → 2nd Generation → 3rd Generation

Allogenous Graft

Donor: Human cadaveric bone

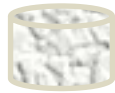
Autologous Graft



Receptor site: palate

Donor site: iliac crest/hip (most commum)

Alloplastic Graft



Osteconductive Biomaterials/scaffold (hydroxyapatite, TCP)

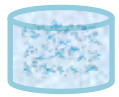
Growth Factors

PRP

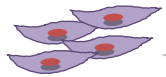
PRF

BMPs

Bone Bioengineering



Biomaterials/scaffold



Stem Cells

Signaling molecules

Engineered bone graft

patient

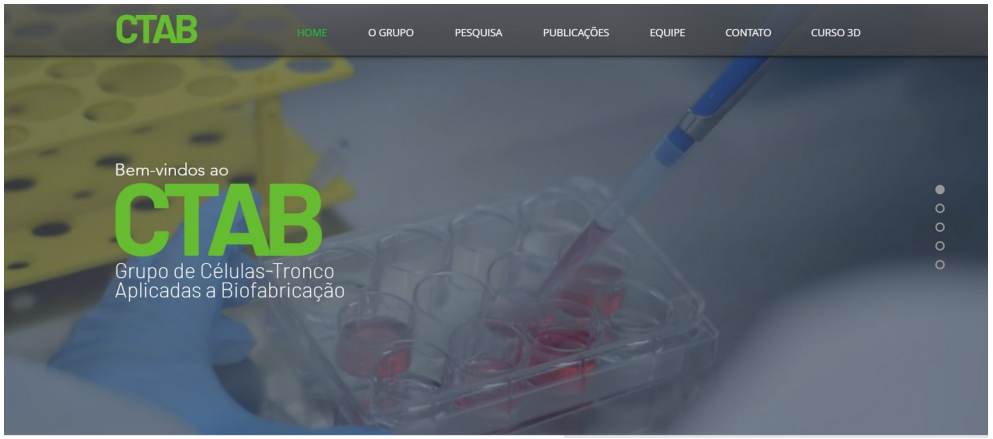
Biometrology: the new frontier to be explored!

MISSÃO DO INMETRO

A **medida certa** para promover confiança à sociedade e competitividade ao setor produtivo.

Agradecimentos ;)

https://www.grupoctab.com/



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Tathiana Proença, UFRJ-

CAMPUS DE INOVAÇÃO E METROLOGIA

INMETRO

DIA 10

8:30 - ABERTURA

9:00 - A ENGENHARIA DE TECIDOS E AS METODOLOGIAS ALTERNATIVAS AO USO DE ANIMAIS
Dr. José Mauro Granjeiro
Inmetro, Duque de Caxias, RJ, Brasil

10:00 - REGULAMENTAÇÃO DOS PRODUTOS DE TERAPIA CELULAR AVANÇADA
Joao Batista da Silva Junior
Anvisa, Brasília, DF, Brasil

11:00 - O EMPREENDEDORISMO NA UNIVERSIDADE
Dr. Paulo Afonso Granjeiro UFSJ
São João del-Rei, MG, Brasil

12:00 / 13:00 - ALMOÇO

13:00 - IMPRESSÃO 3D EM MEDICINA REGENERATIVA
Dr. José Manuel Baena
CEO REGEMAT, Espanha

14:00 / 15:30 - APRESENTAÇÃO DE PÔSTERES
Alunos de graduação, pós-graduação e pós-doutorado Premiação em insumos de laboratórios para os 3 primeiros colocados.

15:30 - TECIDO EQUIVALENTE DE PELE PARA TESTES *IN VITRO*
Dr. Rodrigo de Vecchi, Episkin, L'Oréal do Brasil
Rio de Janeiro, RJ, Brasil

16:30 - ENCERRAMENTO

DIA 11

8:30 - BIOIMPRESSÃO DE PELE
Dra. Ana Luiza 3DBS,
Campinas, SP, Brasil

9:30 - DESENVOLVIMENTO DE BIOTINTAS
Dr. Gabriel Liguori, TissueLabs
São Paulo, SP, Brasil

10:30 - CAPACITAÇÃO EM BIOIMPRESSÃO
Dra. Janaina Dernowsek, Bio3DTech,
São Paulo, SP, Brasil

12:00 / 13:00 - ALMOÇO

13:00 - VENTURE BUILDER NANOBUINESS E SEUS PROJETOS DEEP TECH
Dr. Ronaldo Pedro da Silva, Nanobusiness
Duque de Caxias, RJ, Brasil

14:00 - IMPULSIONANDO O SEU PROJETO DE PESQUISA COM O CULTIVO 3D
Dra. Leandra Baptista, GCell,
Duque de Caxias, RJ, Brasil

15:00 - CHALLENGES IN BIOPRINTING
Dr. Vladimir Mironov Chief Scientific Officer at 3D Bioprinting Solutions (3D Bio), Rússia

16:00 - ENCERRAMENTO E PREMIAÇÃO

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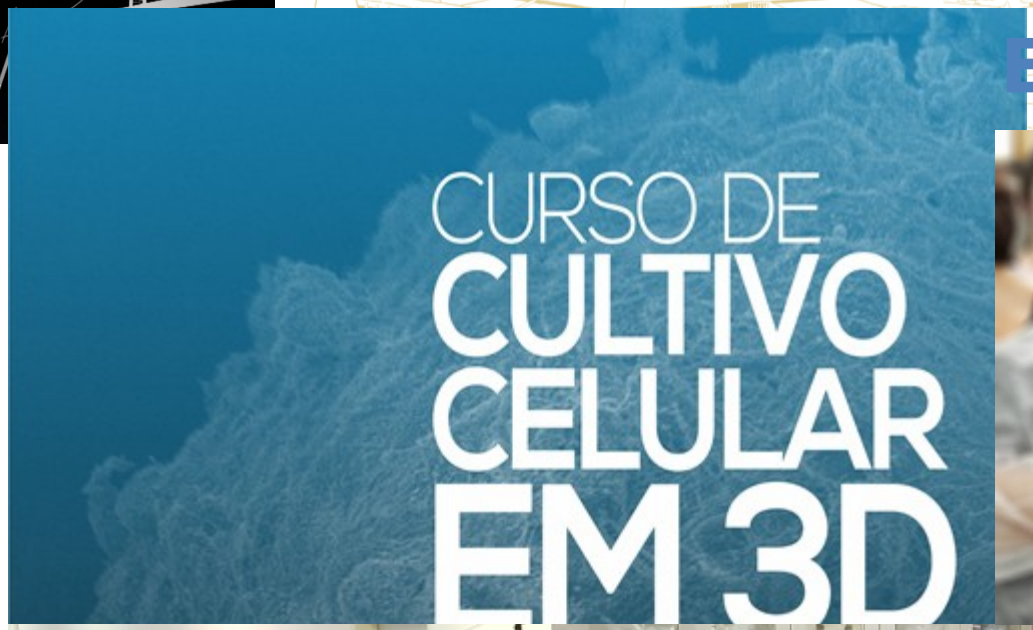
CURSO DE CULTIVO CELULAR EM 3D

Episkin, L´Oreal, Brazil



Próxima edição – São Paulo, Merck, Barueri ;)

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Thanks

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