

Novel strategies for 3D cell culture & developmental tissue engineering



NUMPEX-BIO

Núcleo Multidisciplinar de Pesquisa em Biologia
UFRJ - Xerém

Leandra S. Baptista

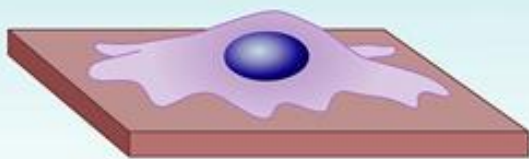
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3D cell culture

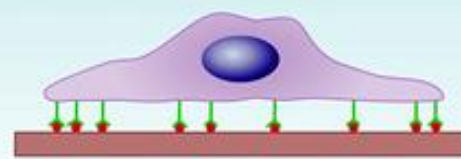
2D

3D

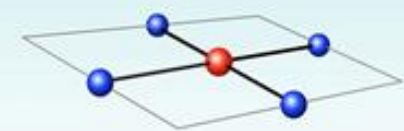
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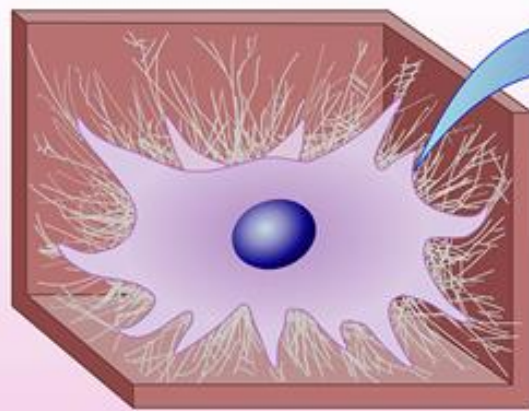
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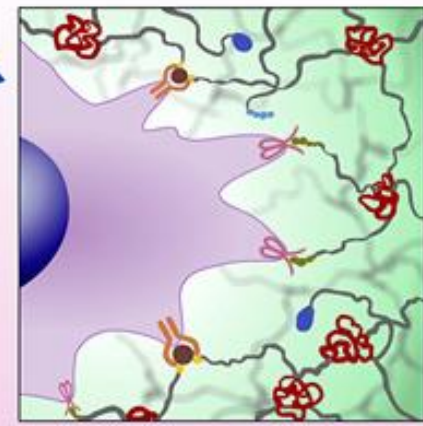
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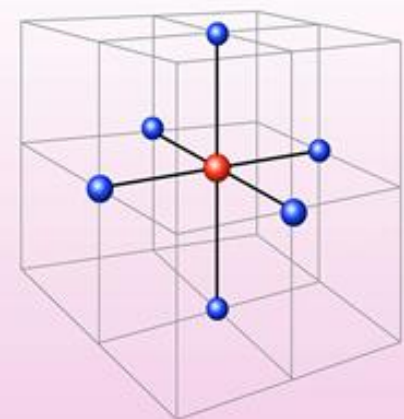
D



E



F

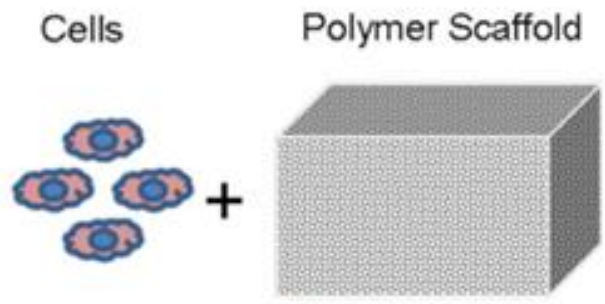


Baptista LS et al. *Frontiers In Bioscience, Landmark*, 23, June 1, 2018. DOI No:10.2741/4683]

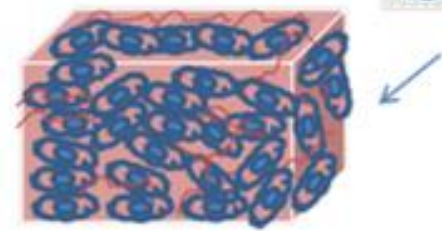
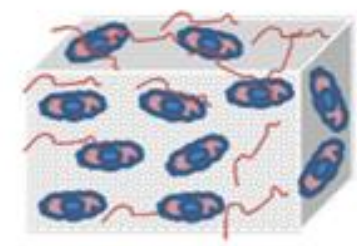
3D cell culture and Tissue Engineering Approaches

Scaffolds provide anchoring sites for individual cells.

“Top-down” Approach



Cell Proliferation + Scaffold Degradation

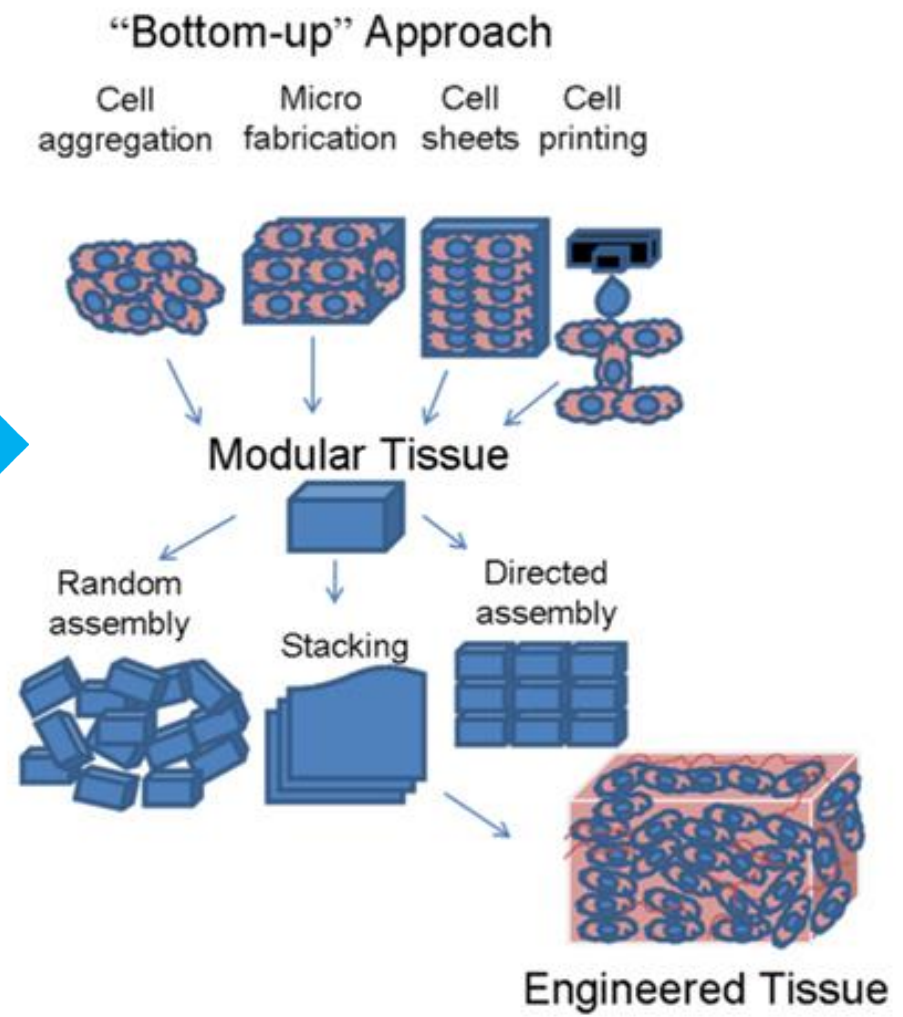


Engineered Tissue

Adapted from Nichol JW, Khademhosseini A. *Soft Matter*. 2009;5(7):1312-1319. doi: 10.1039/b814285h

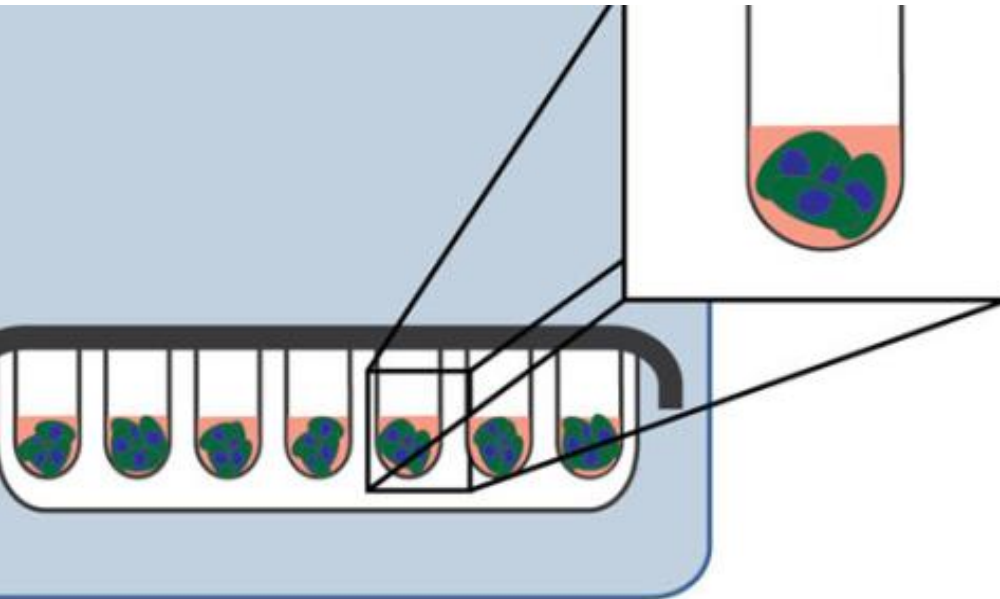
3D cell culture and Tissue Engineering Approaches

Bottom-up starts from microtissues



Adapted from Nichol JW, Khademhosseini A. *Soft Matter*. 2009;5(7):1312-1319. doi: 10.1039/b814285h

3D cell culture technologies



Scaffold-free 3D cell culture

- Most of all support organogenesis
- Cells are immersed within a extracellular matrix, produced by them.
- Provides physical support for multiple cell layers

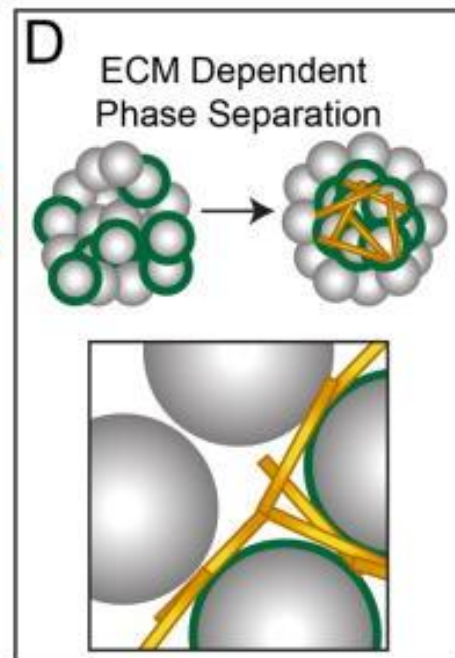
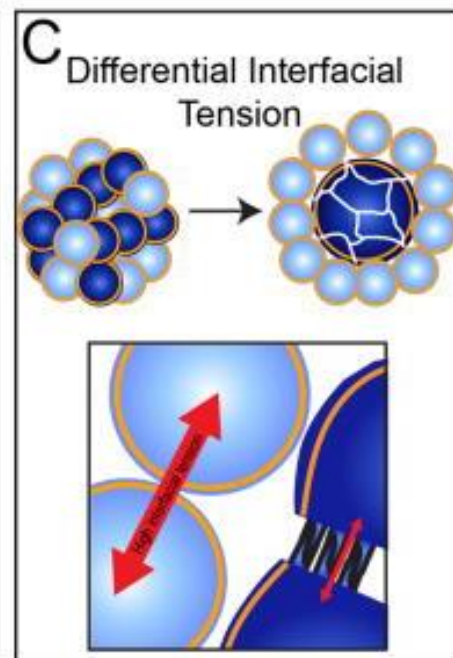
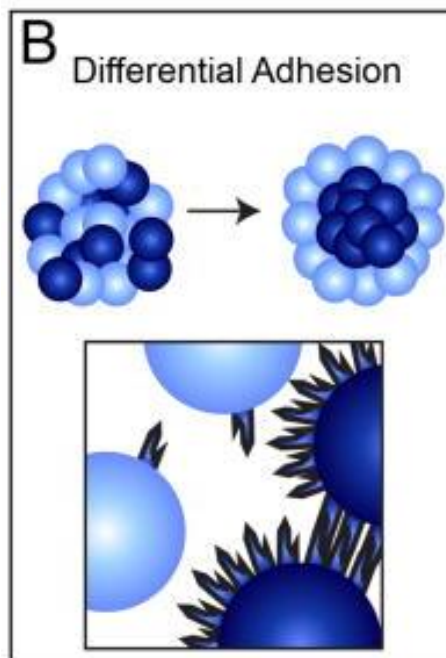
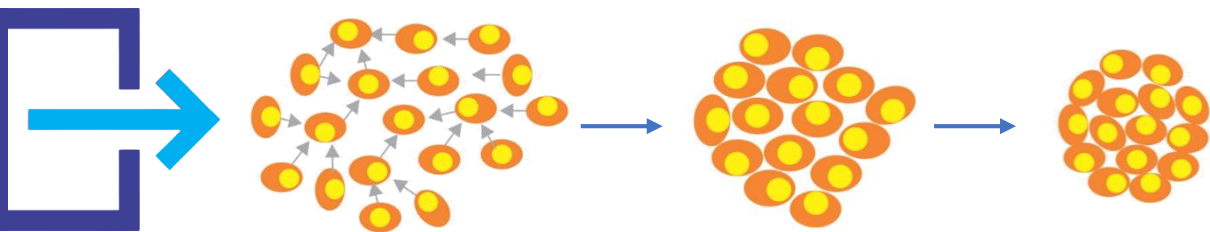
Microfluidics-based 3D cell culture
Magnetic levitation
3D bioprinting

Scaffold-based 3D cell culture

3D cell culture Technologies

3D spheroids

Self-assembly
(spheroids and organoids)

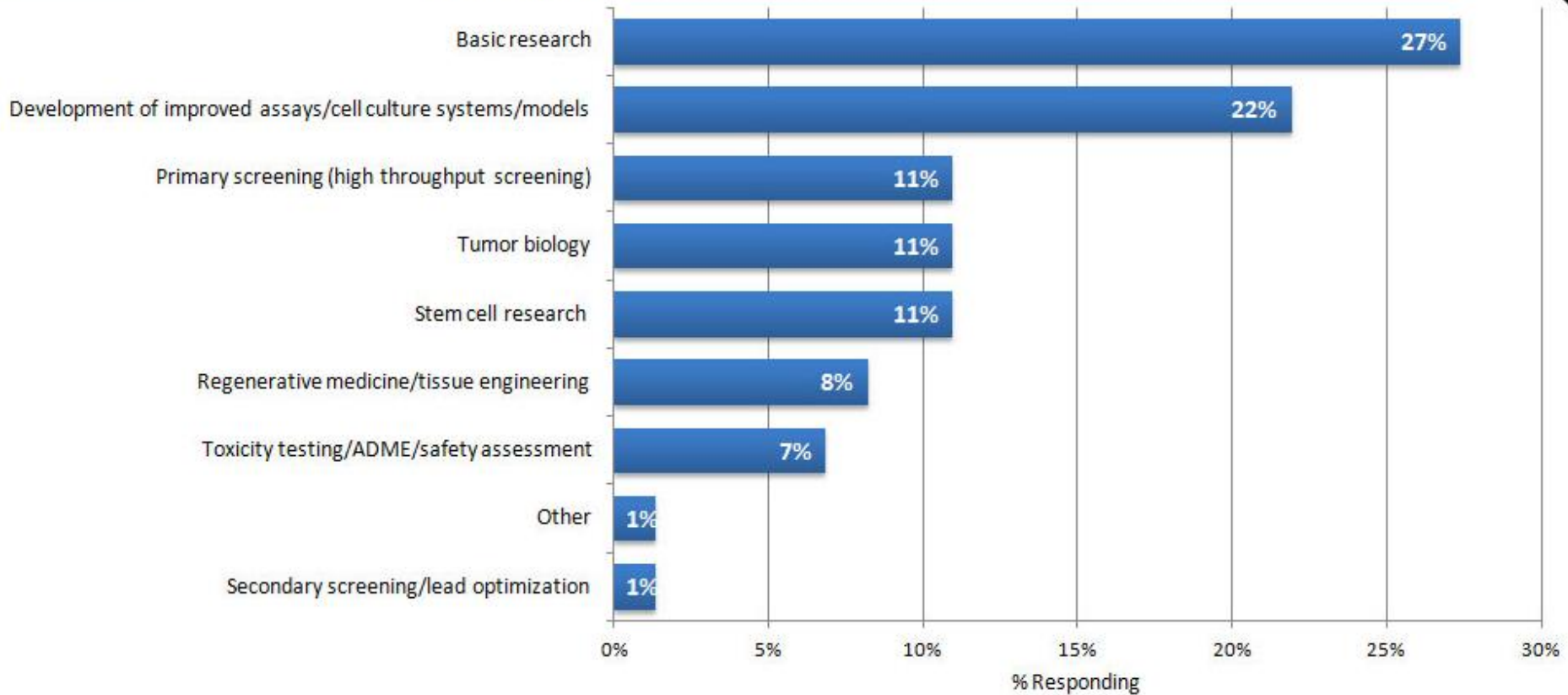


→ McMillen P, Holley SA. *Curr Opin Cell Biol.* 2015 Oct;36:48-53. doi: 10.1016/j.ceb.2015.07.002.

3D spheroids applications

HTStec 2015

Figure 2. Main Area Of Interest/Activity Involving 3D Spheroids

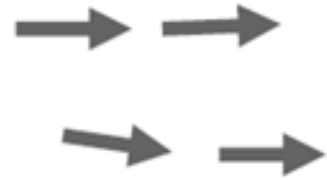


<https://www.technologynetworks.com/drug-discovery/articles/3d-spheroid-culture-trends-184234>

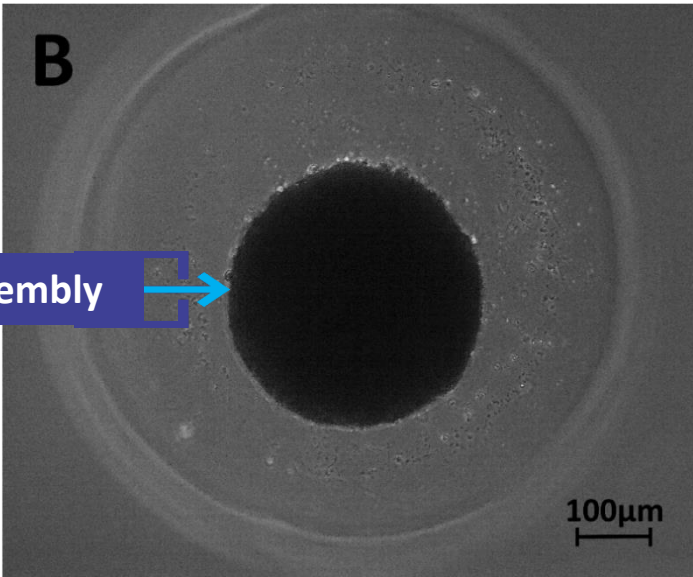
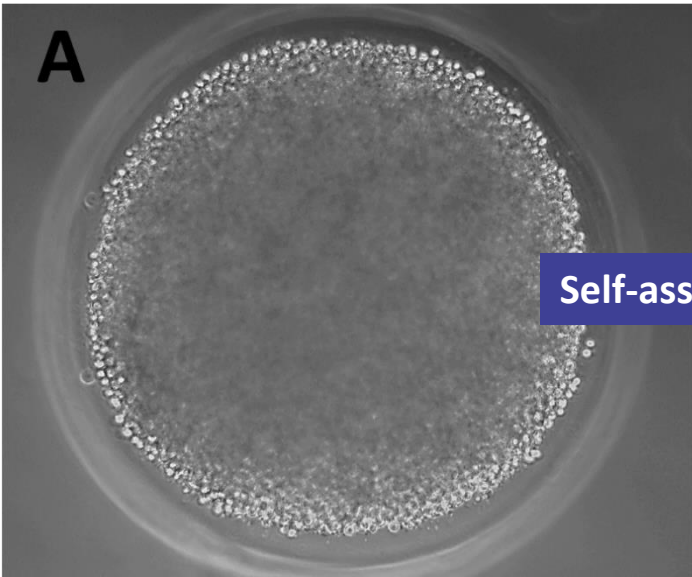
3D spheroids from adult stem cells Developmental tissue engineering



Differentiation and maturation

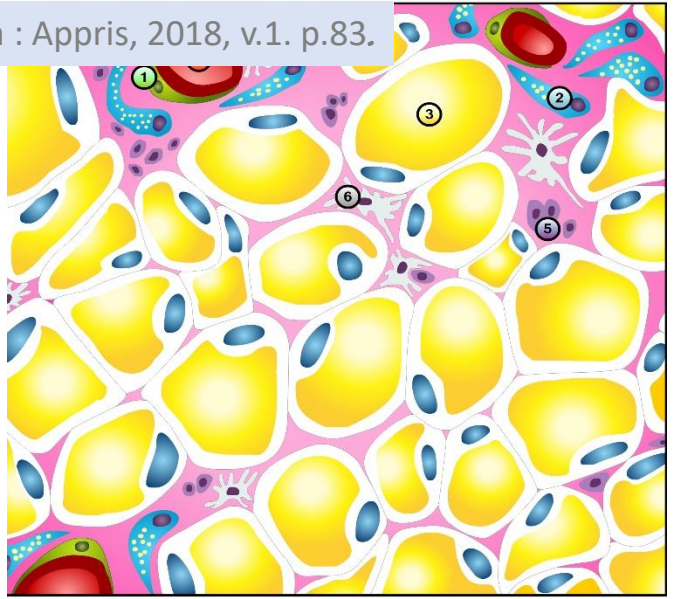


- Disease models
- Biomarkers
- Desired mature phenotype



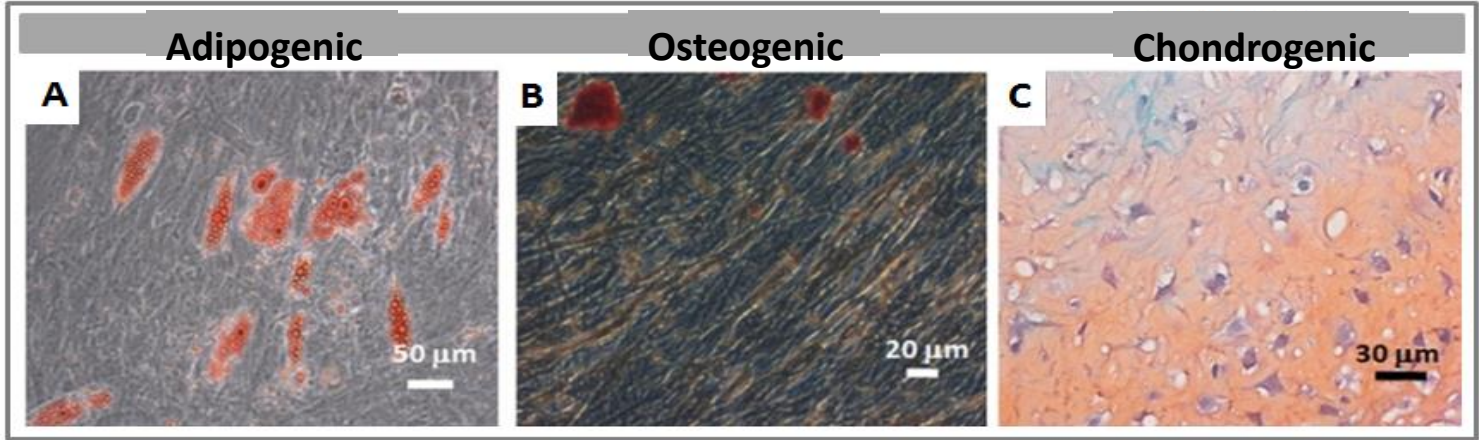
Adipose tissue derived stem cells

→ COSTA, C. C. P.; BAPTISTA, L. S. Tecido adiposo: vilão ou herói?. Curitiba : Appris, 2018, v.1. p.83.



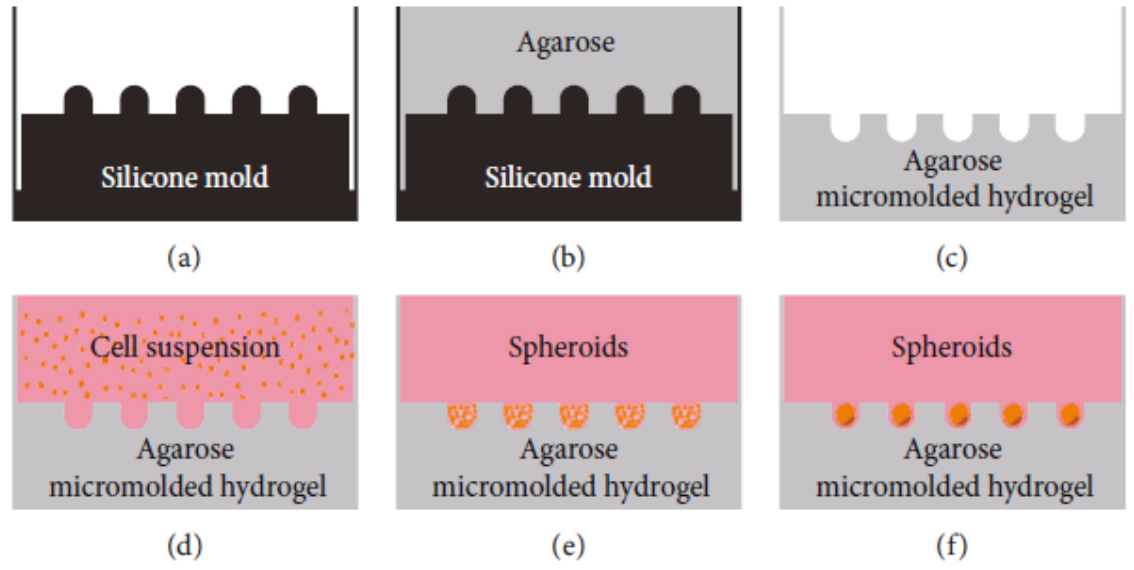
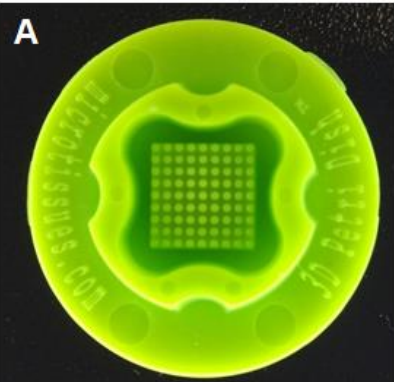
Stromal vascular fraction →

← *In vitro* multilineage potential



→ Baptista et al., Processing of Lipoaspirate Samples for Optimal Mesenchymal Stem Cells Isolation. 2011.

Human adipose tissue derived stem cells spheroids



Micro-molded non-adhesive hydrogel
SERUM-FREE MEDIUM

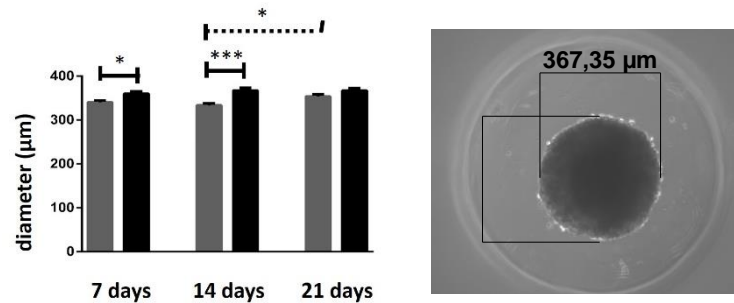
→ Stuart MP et al. Stem Cells International. 2017. <https://doi.org/10.1155/2017/7053465>

End points (analysis) of 3D spheroids

From spheroids

- Diameter measurement (viability)
- Electronic microscopy (morphology)
- Biomechanical assay
- Histology/Specific targets (immunofluorescence, immunohistochemistry)
- Molecular assays (qPCR, transcriptome)
- Flow cytometry (viability, cell cycle and cell phenotype)

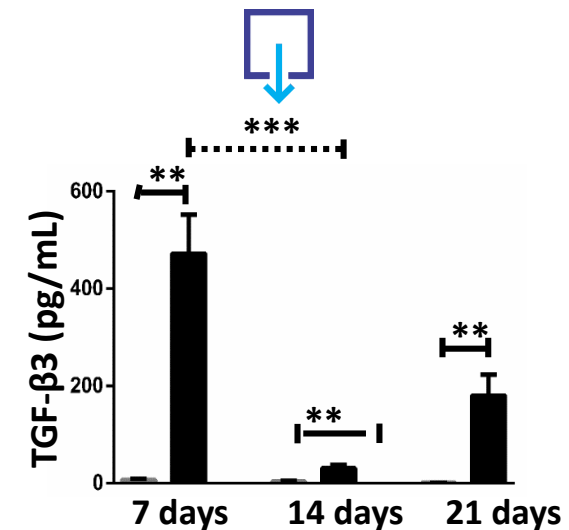
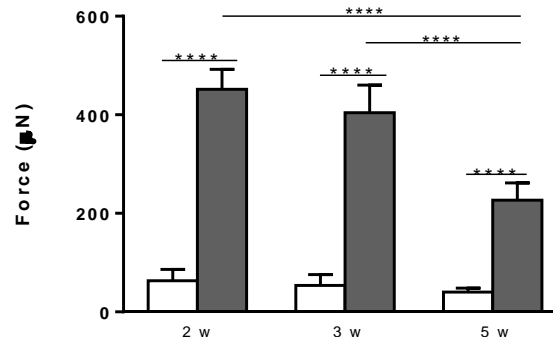
REPRODUCIBILITY



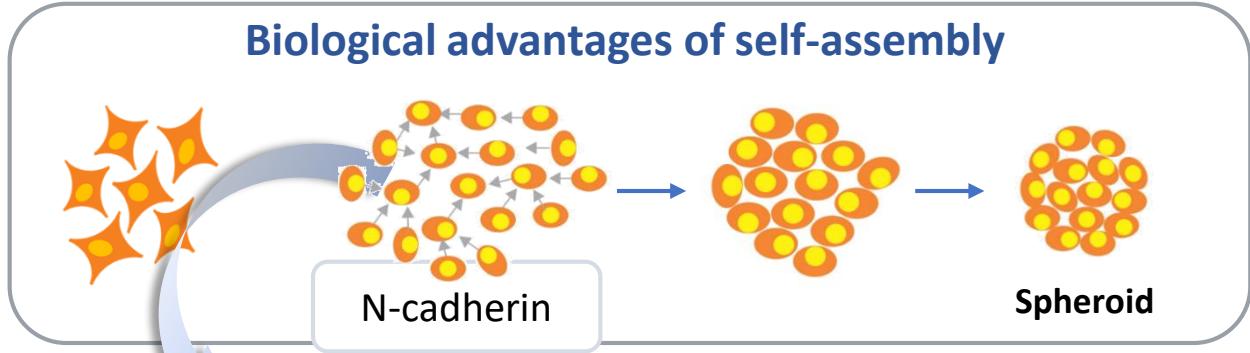
From spheroid culture supernatant

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

RASTREABILITY

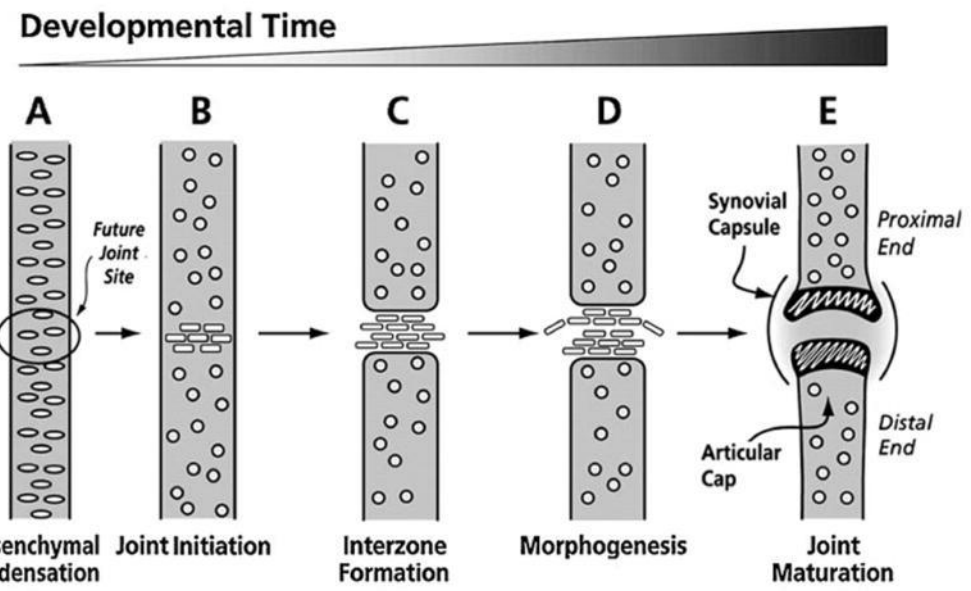


Adipose tissue derived stem cells spheroids to cartilage



Embryological development of cartilage

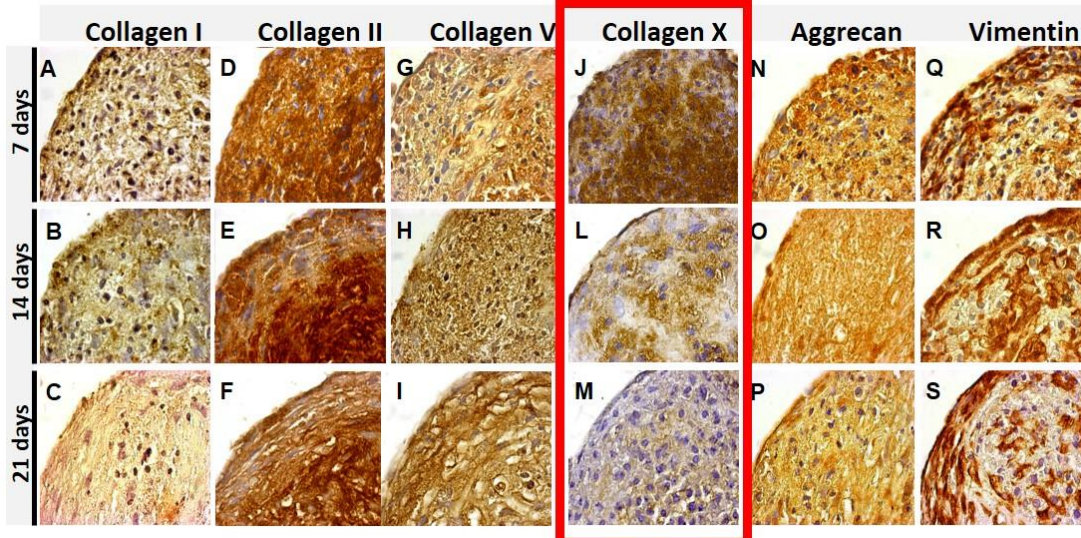
Mesenchymal condensation –started by cell-cell interaction



Pacifici M et al. Ann N Y Acad Sci. 2006 Apr;1068:74-86. DOI: 10.1196/annals.1346.010

Adipose tissue derived stem cells spheroids to cartilage

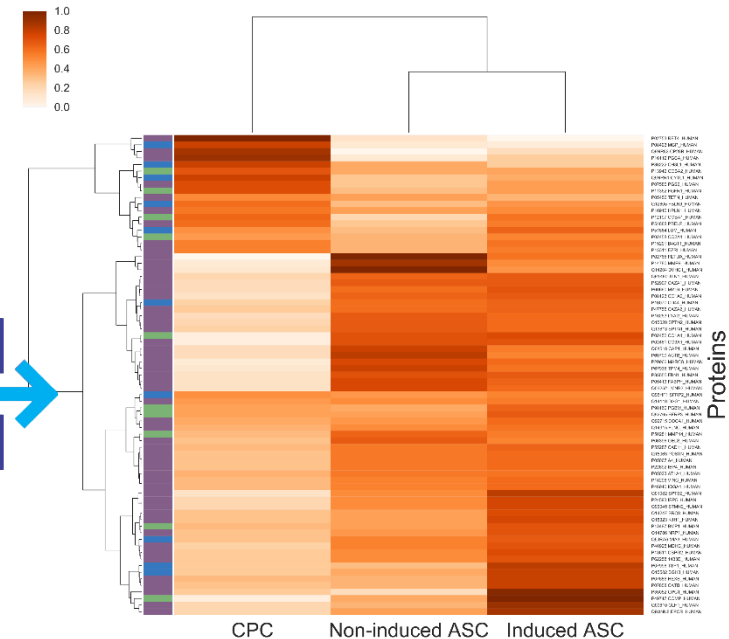
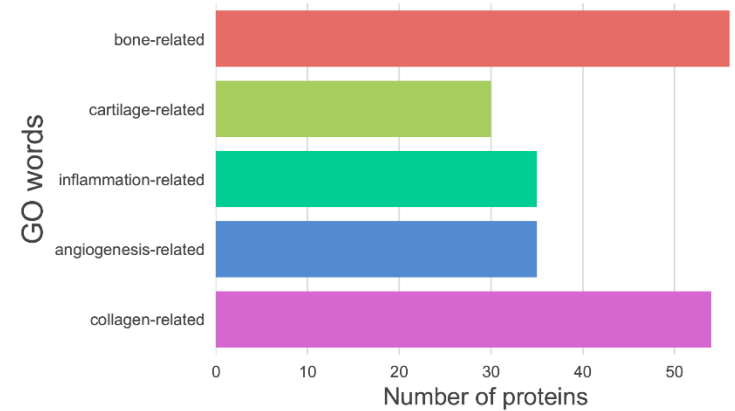
Immunohistochemistry



- Decreases marking intensity throughout the differentiation process
- It was not detected in secretome analysis

New biomarkers

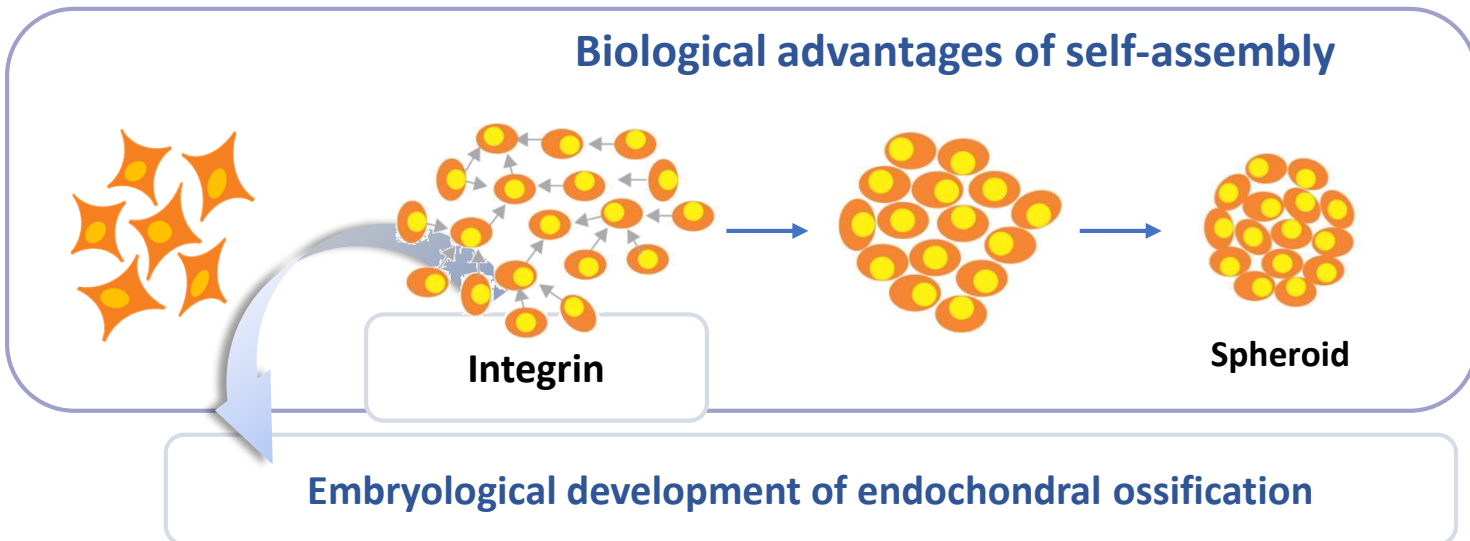
Secretome



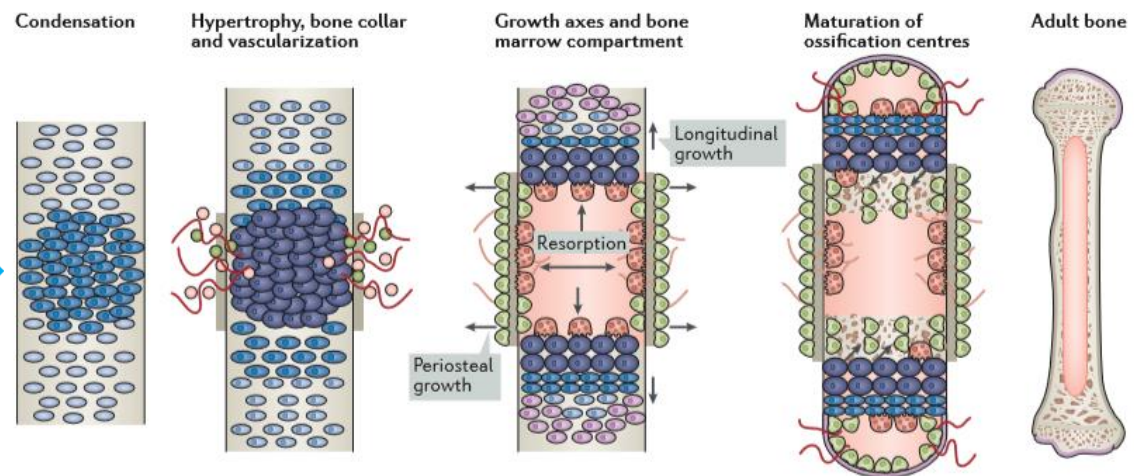
Côrtes, master thesis, 2017 / Matsui, master thesis, 2017

Unsubmitted manuscript

Adipose tissue derived stem cells spheroids to bone

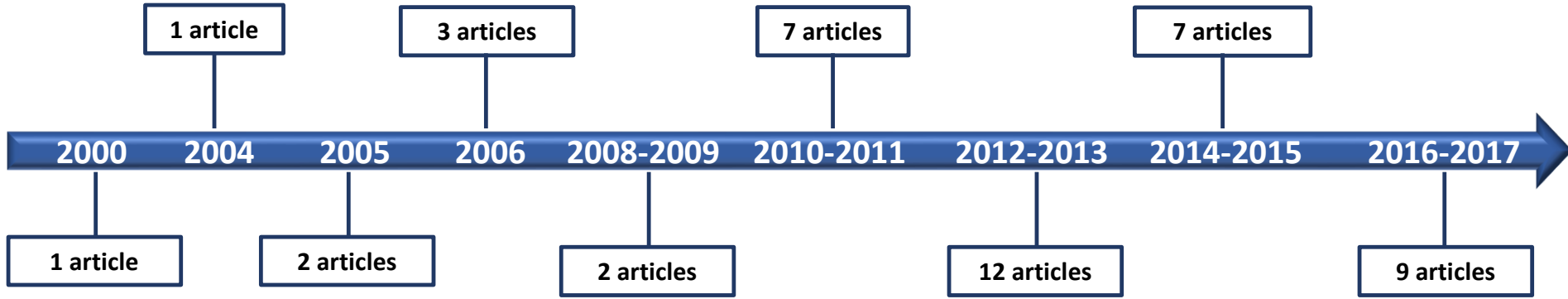


Mesenchymal condensation –started by cell-cell interaction

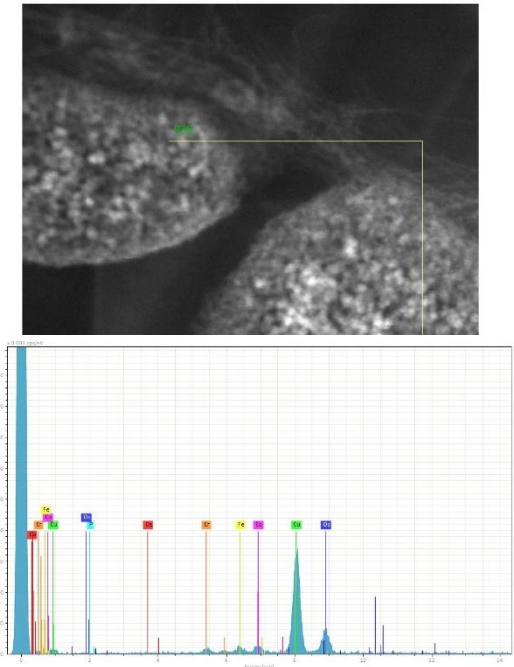
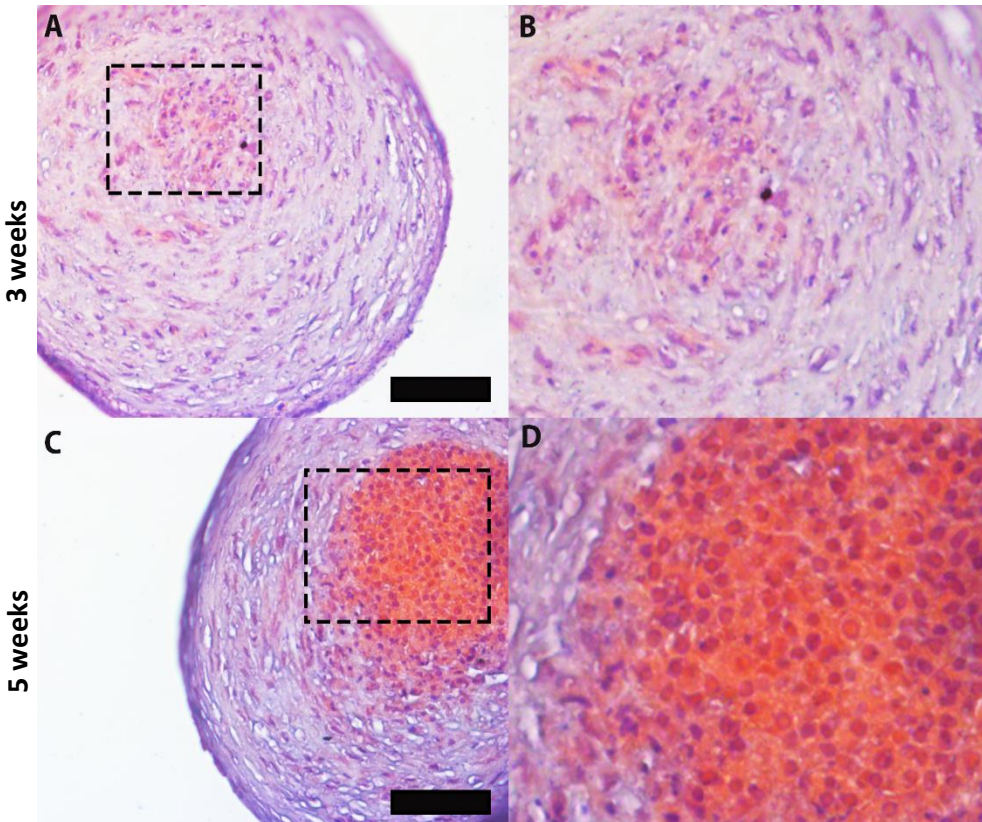


→ Salazar VS., et al. Nature reviews - Endocrinology. 2016; 1068:1-19. DOI:10.1038/nrendo.2016.12

→ Adipose tissue derived stem cells spheroids to bone



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



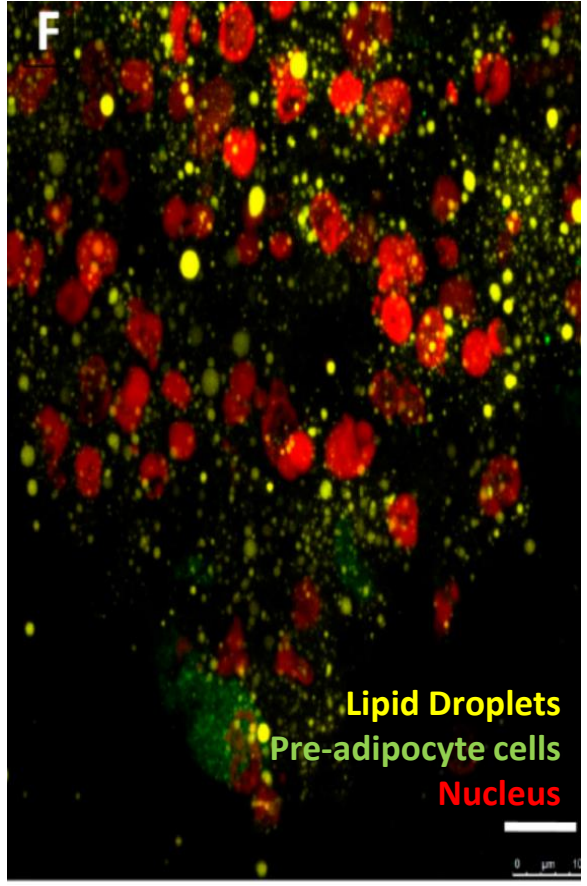
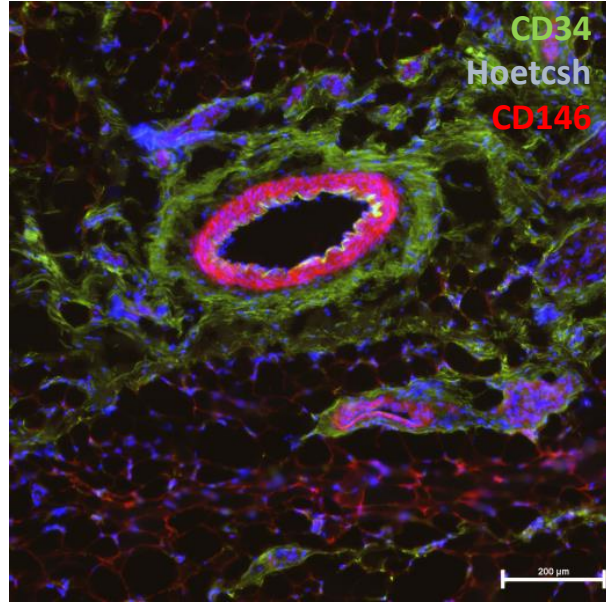
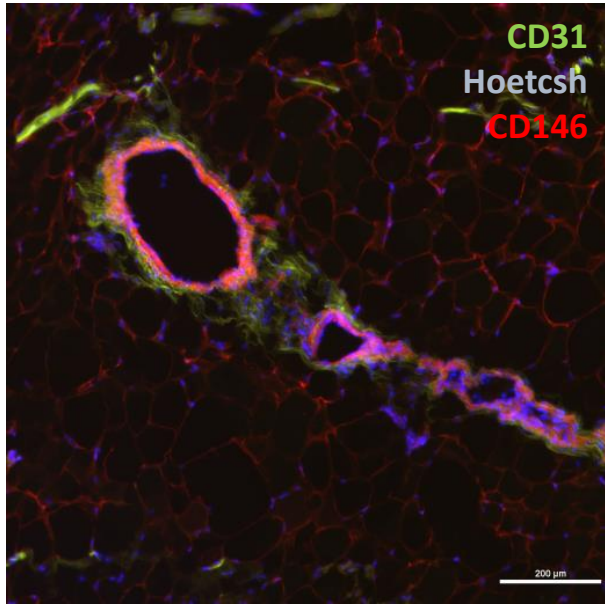
→ Kronemberger, master thesis, 2017
Unsubmitted manuscript

Adipose tissue derived stem cells spheroids to adipose tissue

In situ



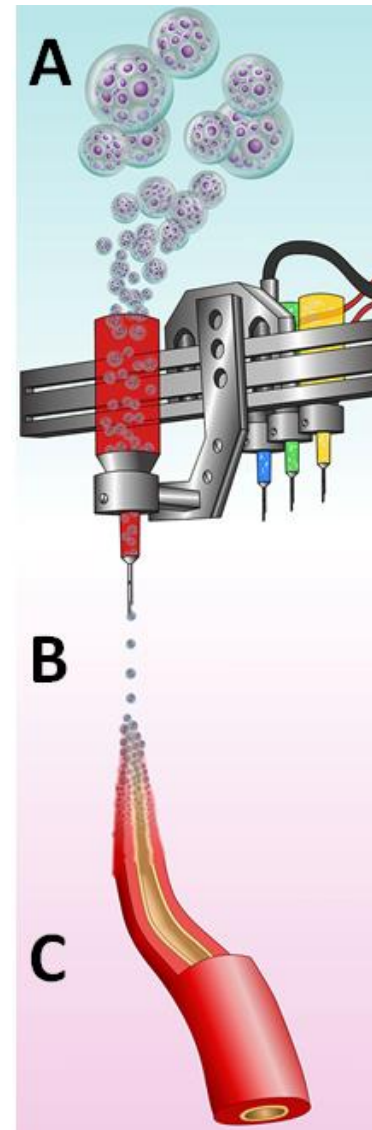
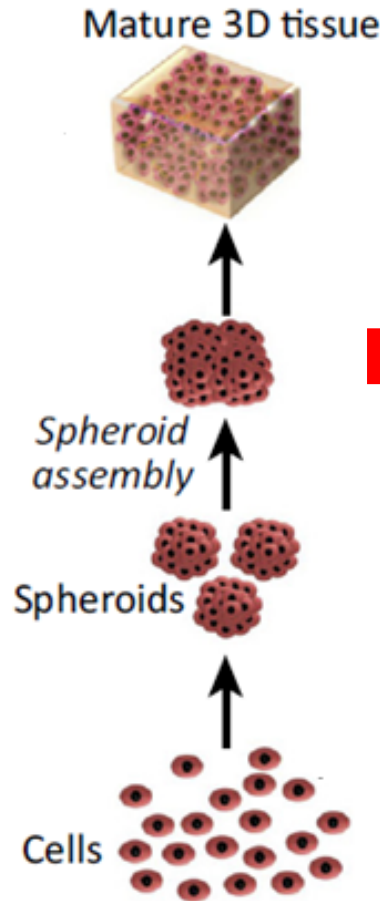
In vitro



- Obesity
- Lost and re-gain of weight

Unsubmitted results

3D cell culture perspectives Bioprinting



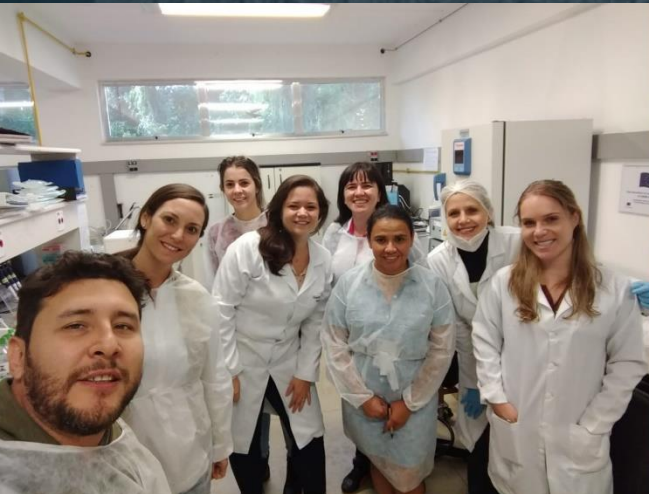
Adapted from Guven S et al., Trends Biotechnol. 2015. 33(5):269-279. doi: 10.1016/j.tibtech.2015.02.003.

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

CURSO DE CULTIVO CELULAR EM 3D



Information:
www.grupoctab.com
qualidade@bcrj.org.br



Workshop Impressão 3D e Bioimpressão

Oportunidades e desafios das convergências tecnológicas na Indústria 4.0

Palestrantes confirmados:

- Prof. Dr. Ricardo Michel (Instituto de Química – UFRJ)
- Profa. Dra. Rossana Thiré (MetalMat – UFRJ)
- M.SC. José Manuel Baena (CEO REGEMAT 3D)
- Profa. Dra. Leandra Baptista (Numpex-Bio – UFRJ)
- Prof. Dr. Tiago Albertini (PENt/COPPE – UFRJ)
- Dra. Janaina Dernowsek (CTI Renato Archer)

Data: 03 de agosto de 2018

Local: Campus do Inmetro (Xerém) – Auditório do Prédio 6

Inscrições gratuitas pelo link:
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Apoio



Realização

ConvergeLab
Grupo de Pesquisa
UFRJ

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→ CTAB Research group

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- Karina Ribeiro, Pos-doc, Inmetro

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- Isis Côrtes, Inmetro
- Renata Matsui, Inmetro
- Gabriela Kronemberger, Inmetro

Master student:

- Letícia Charelli, Inmetro

Undergraduate students:

- Guilherme Miranda, UFRJ-Xerém
- Thais Barreto, UFRJ-Xerém
- Bianca Montenegro, UFRJ-Xerém
- Marcela Marins, UFRJ-Xerém



Acknowledgments ;)



Bioengineering Networking – Rio de Janeiro
Tissue Bioengineering laboratory
Prof. Dr. José Mauro Granjeiro, Inmetro/UFRJ
Dr. Leonardo Boldrini, Inmetro

Thank you!

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