BA00505-Módulo Transversal: Inovações Tecnológicas na Área da Saúde

Engenharia de Tecidos e aspectos translacionais da pesquisa básica-clínica.

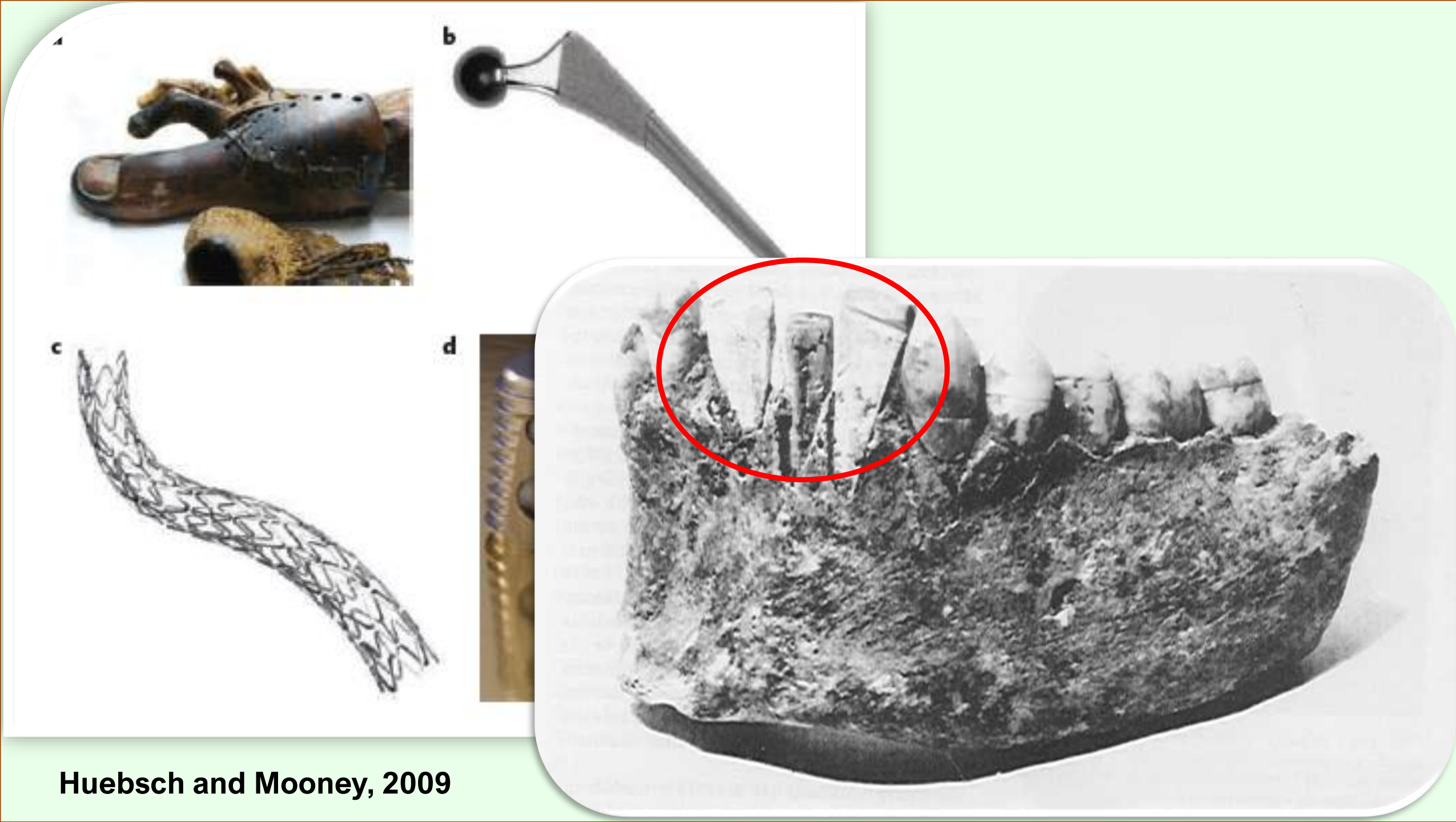
Prof. Dr. Rodrigo Cardoso de Oliveira

Depto de Ciências Biológicas da FOB-USP

rodrigocardoso@usp.br

Objetivos de Aprendizagem

- Reconhecer as principais técnicas envolvidas na Engenharia de Tecidos
- Reconhecer as diferentes áreas beneficiadas e as potencialidades práticas da Engenharia de Tecidos



Huebsch and Mooney, 2009

Definições

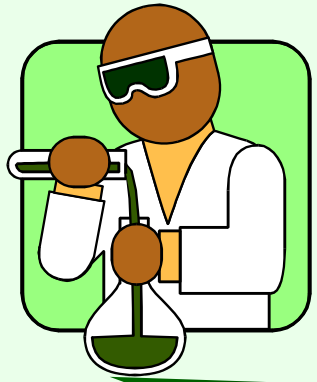
- Langer e Vacanti (1993) "Campo interdisciplinar no qual os princípios de engenharia e biologia são aplicados em razão da geração de substitutos biológicos com o objetivo de criar, preservar, ou restaurar uma função perdida."

- Ueda (2000) a engenharia de tecidos se apoia em três elementos fundamentais: carreadores, células, moléculas sinalizadoras.

Dr. Robert Langer

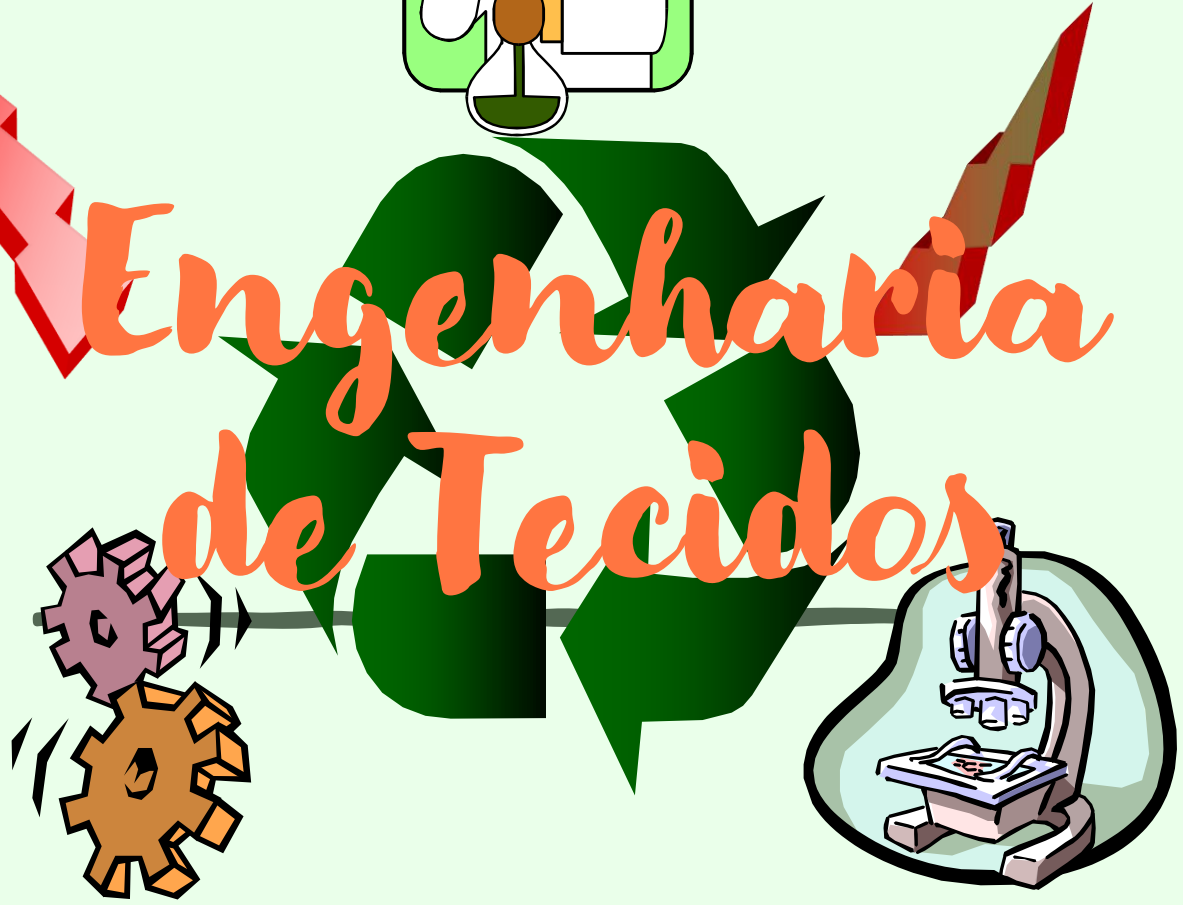
Dr. Joseph Vacanti

Compreensão:
estrutura-função
normais-patológicos



Substitutos
Biológicos

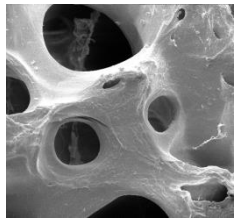
Engenharia de Tecidos



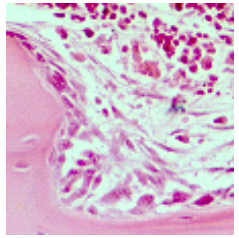
Evolução do conhecimento

- Novas descobertas
- Ferramentas mais precisas no estudo de eventos fisiológicos e patológicos
- Desenvolvimento de técnicas que visam modular a resposta celular e molecular do organismo

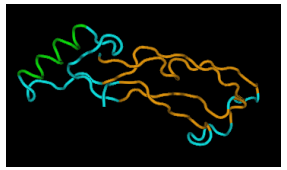
Os Elementos da Engenharia de Tecidos



Suporte:
Colágeno, osso,
Minerais, polímeros



Células:
Queratinócitos, osteoblasto
Fibroblasto, condrócitos

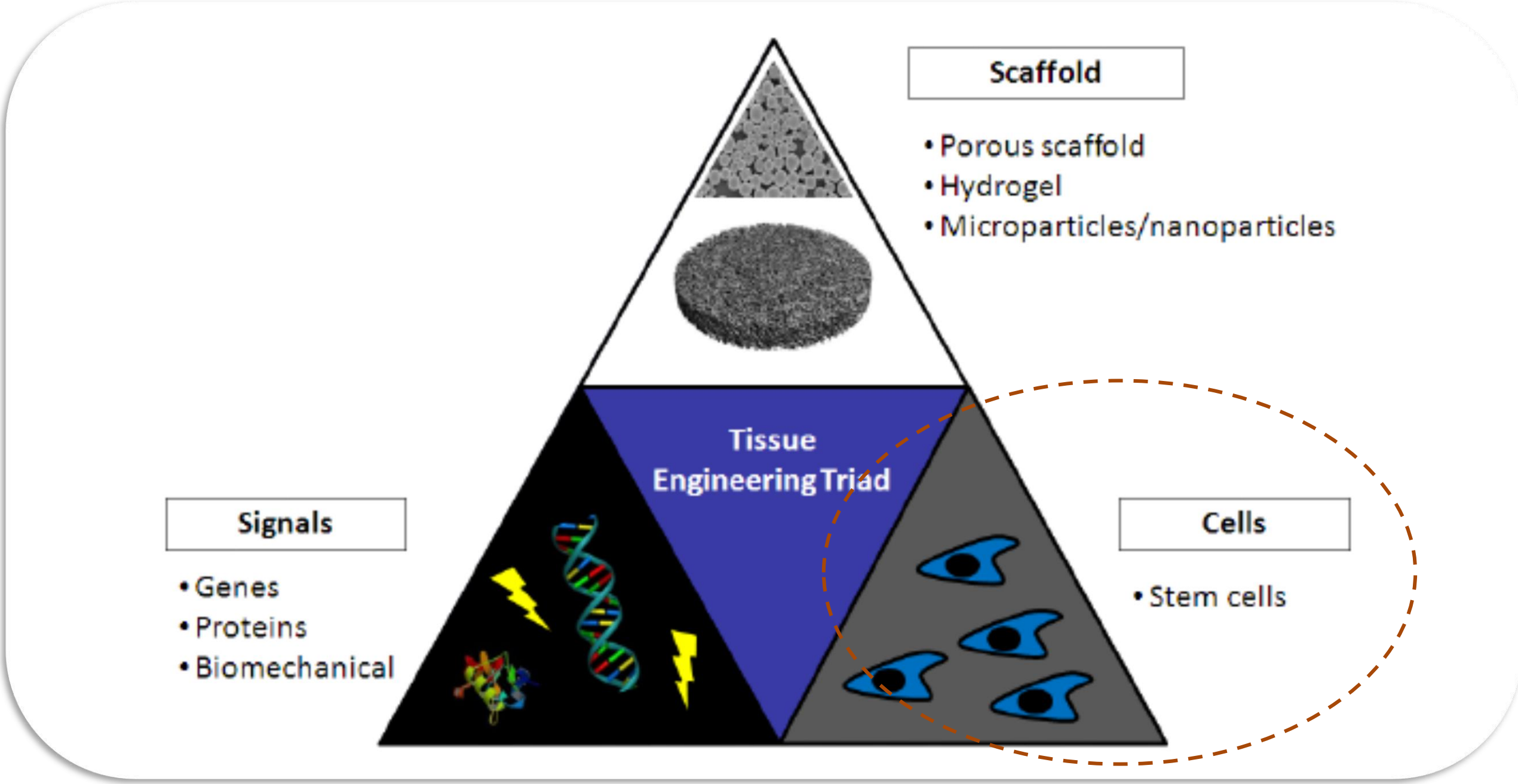


Moléculas Sinalizadoras:
Fatores de crescimento,
Morfogenes, adesinas

Tempo

Ambiente

Regeneração do Tecido/Órgão



Raftery et al. 2013, *18*, 5611-5647; doi:10.3390/molecules18055611

Cultura celular – Histórico

- Wilhen Roux (1885): Células de embrião de aves em solução salina aquecida.

- Ross Harrison (1907): Vários estudos com células animais. Considerado o pioneiro no cultivo celular.

- Alexis Carrel (1912/1913): Desenvolvimento de metodologias para assepsia e subcultivo.

Freshney, 2010.

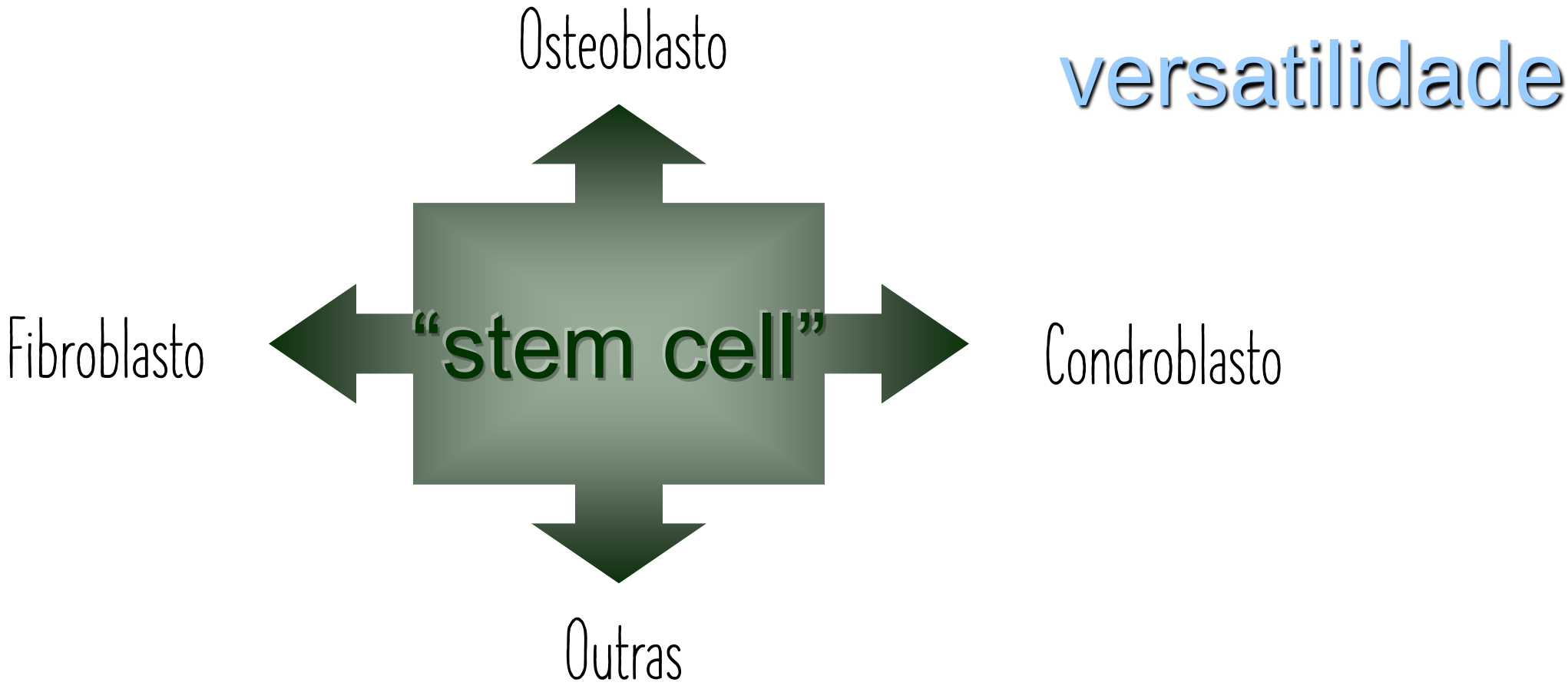
1940 - antibióticos

Composição do meio de cultura (variável!)

- Sais
- Amino Ácidos
- Vitaminas
- Glicose
- Hormônios
- Antibióticos

- Proteínas
- Fatores de crescimento
- Nutrientes e metabolitos
- Lipídeos
- Bicarbonato

CULTURA DE "STEM CELL"

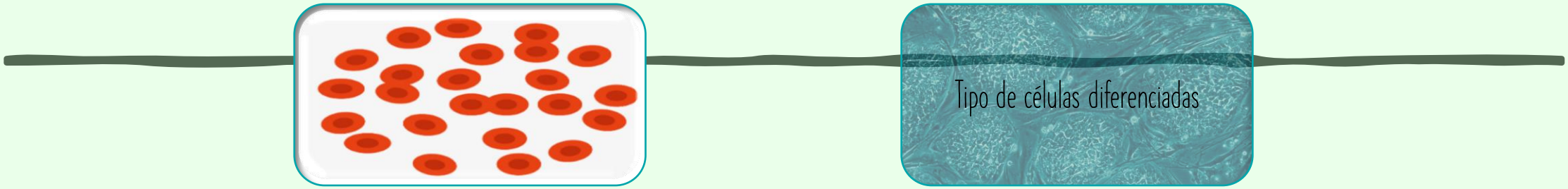


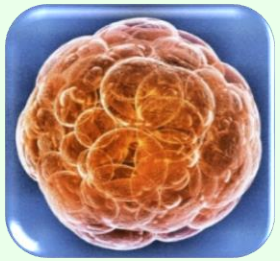
EMBRIONÁRIAS

ADULTAS

PLURIPOTENTES INDUZIDAS

Naturalmente, as células tronco adultas e embrionárias diferem:

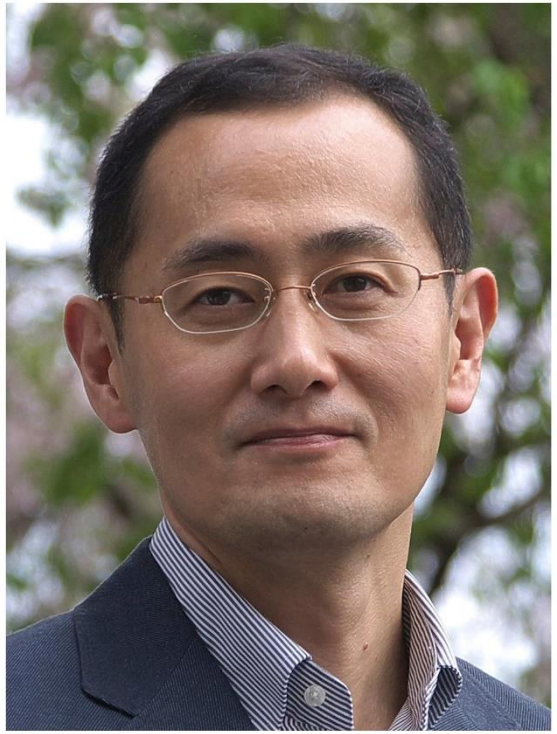


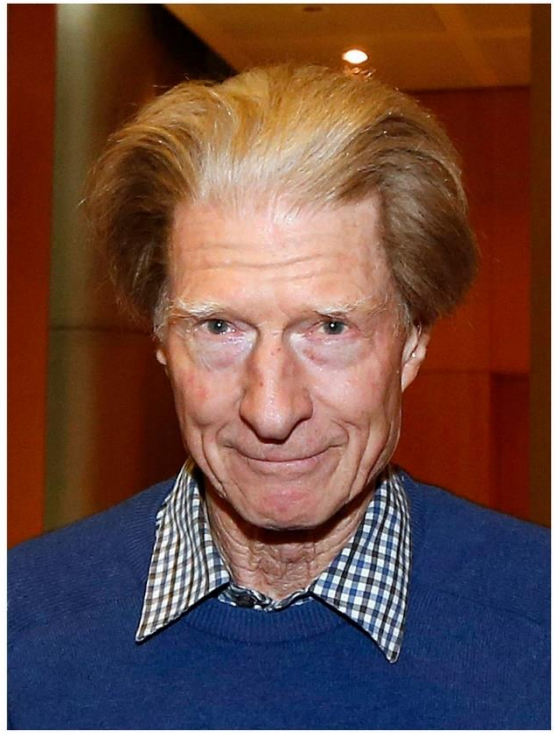


As células-tronco embrionárias podem transformar-se em todos os tipos celulares do corpo porque são pluripotentes

As células-tronco adultas são geralmente limitadas para diferenciar-se em tipos diferentes de células de seu tecido de origem

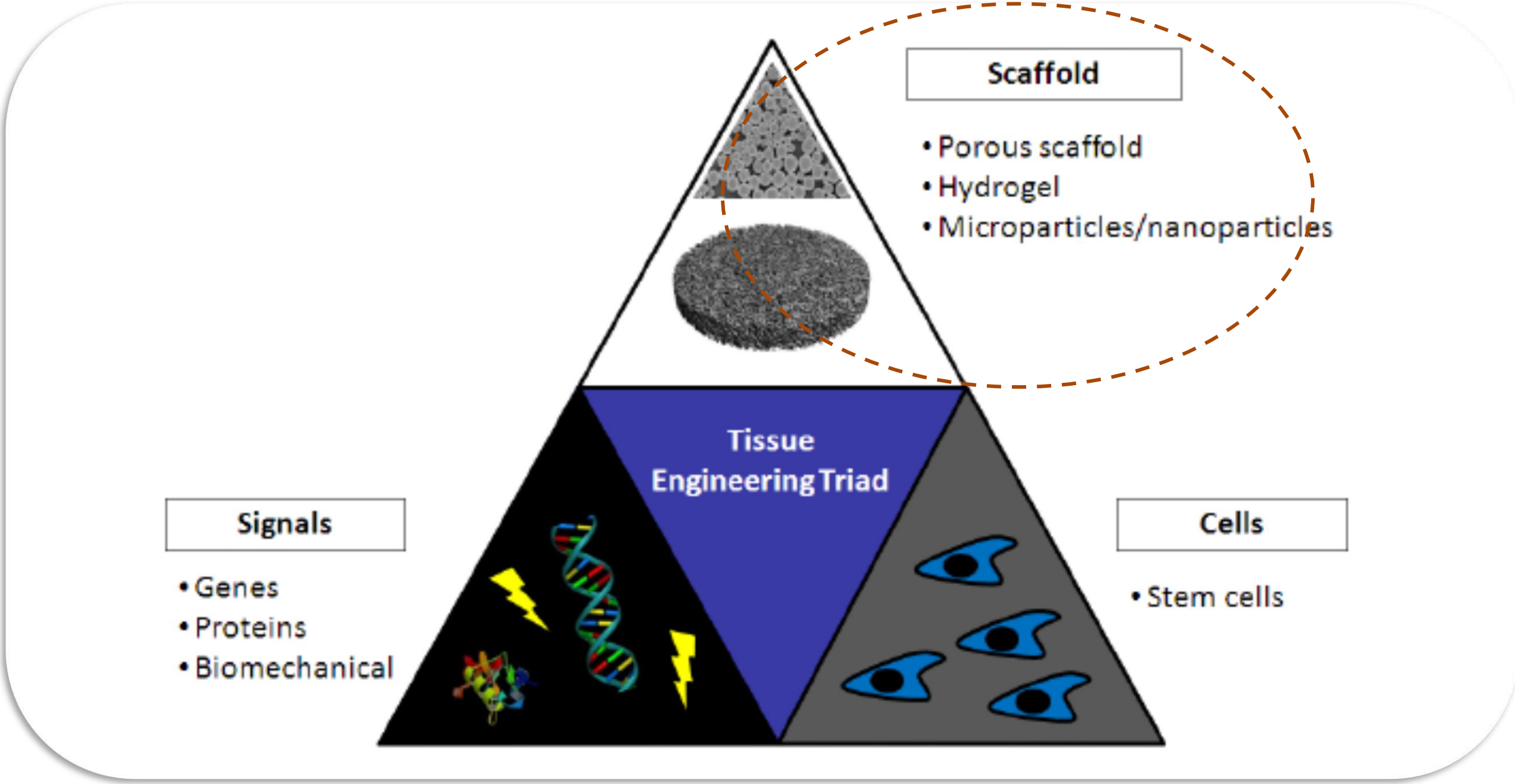
Nobel de Medicina 2012 vai para a descoberta de reprogramação de células





Shinya Yamanaka & John B. Gurdon

"Descoberta de que células adultas podem ser reprogramadas e se tornarem pluripotentes, células-tronco capazes de se converter em qualquer outro tipo de célula do corpo"



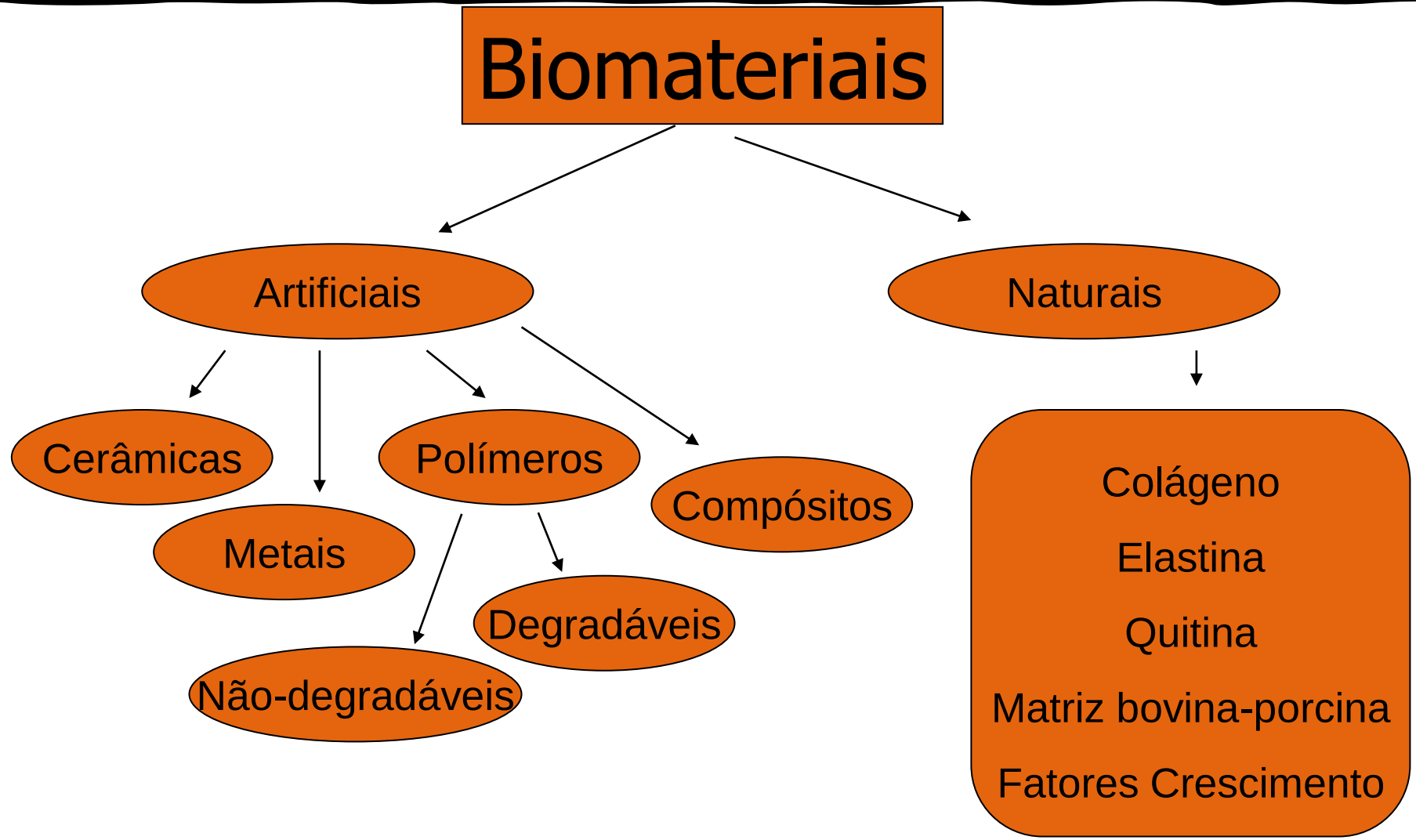
Raftery et al. 2013, *18*, 5611-5647; doi:10.3390/molecules18055611

CARREADORES PARA BIOENGENHARIA

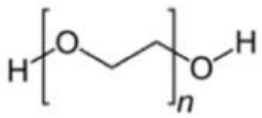
CARACTERISTÍCAS DESEJÁVEIS:

- BIOCOMPATIVEL
- POROSO
- ABSORVÍVEL EM TEMPO ADEQUADO
- ESTERELIZAVEL
- MIMETIZAR O TECIDO ALVO (MEC)
- DELIVERY
- PERMITIR ADESÃO E DIFERENCIAÇÃO CELULAR
- FÁCIL MANUSEIO CLÍNICO

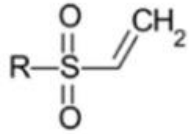
Classificação



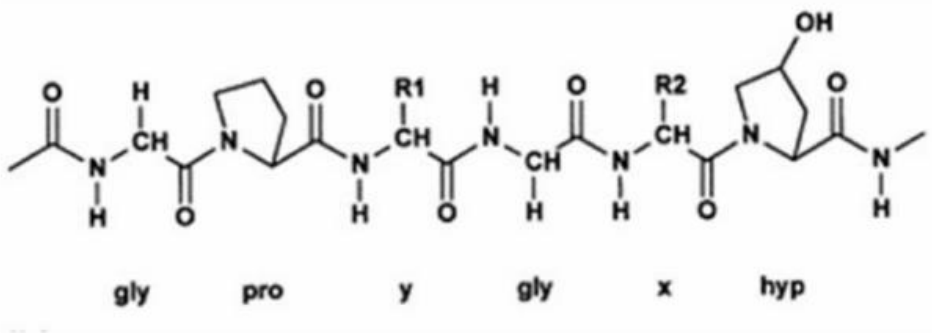
Polyethylene-glycol PEG



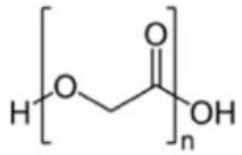
Vinyl-sulfone VS



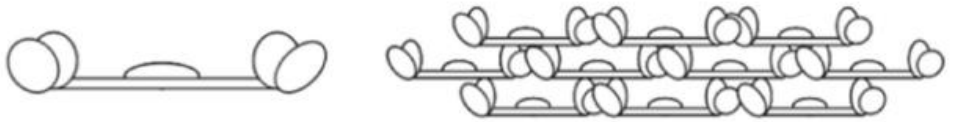
Collagen



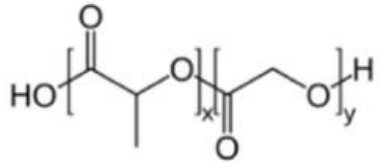
Poly(glycolic acid) PGA



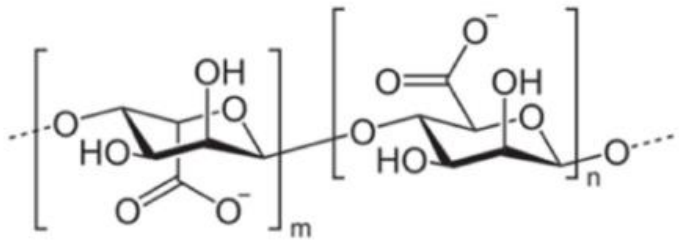
Fibrinogen/Fibrin



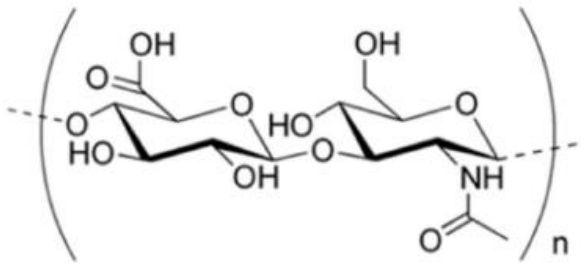
Poly(lactic-co-glycolic acid) PLGA



Alginate



Hyaluronic acid



Brownell et al. (2022). Tissue Engineering in Gynecology. Int J Mol Sci. doi: 10.3390/ijms232012319.

Materials Science and Engineering C 71 (2017) 498–503

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Biophysical and biological characterization of intraoral multilayer membranes as potential carriers: A new drug delivery system for dentistry

Mariana dos Santos Silva [a], Natalino Lourenço Neto [a], Silgia Aparecida da Costa [b], Sirl
Thais Marchini Oliveira [a], Rodrigo Cardoso de Oliveira [c,*], Maria Aparecida Andrade M

[a] Department of Pediatric Dentistry, Orthodontics and Community Dentistry, Discipline of Pediatric Dentistry, Bauru School of Dentistry, University
[b] Course on Textiles and Fashion, School of Arts, Sciences and Humanities, University of São Paulo, Brazil
[c] Department of Biological Sciences, Discipline of Biochemistry, Bauru School of Dentistry, University of São Paulo, Brazil

ARTICLE INFO

Artide history:
Received 26 August 2016
Received in revised form 3 October 2016
Accepted 16 October 2016
Available online 17 October 2016

Keywords:
Layer-by-layer coatings
Alginate
Chitosan
Cell viability
Chlorhexidine
Controlled drug release

ABSTRACT

The current study developed through layer-by-layer deposition a multilay
ery and analyzed the biochemical, functional, and biological properties of
designed a three-layer chlorhexidine-incorporated membrane composed b
chemical, functional, and biological properties were analyzed by the follov
um; controlled drug release; water absorption, mass loss; pH analysis; an
cell viability by MTT assay. All tests were conducted at three different peri
demonstrated that hybrid membranes composed by alginate and chitosa
sorption and mass loss in buffer solution and in artificial saliva. The cont
the hybrid membrane exhibited greater drug release (0.075%). All chlorh
duced the cell viability, and chitosan membranes with and without glycer
ability. The biochemical and biophysical characteristics of the designed
viability tests indicate great potential for application in Dentistry.

2.5. Controlled chlorhexidine release testing

In vitro release tests were performed for semi-quantitative evaluation of the release of chlorhexidine digluconate in phosphate buffer solution (PBS), pH 7.4 ± 0.2, according to the methodology of AKAKI (2005) with some modifications. The chlorhexidine-incorporated membranes were placed in Falcon tubes and 14 mL of PBS were added

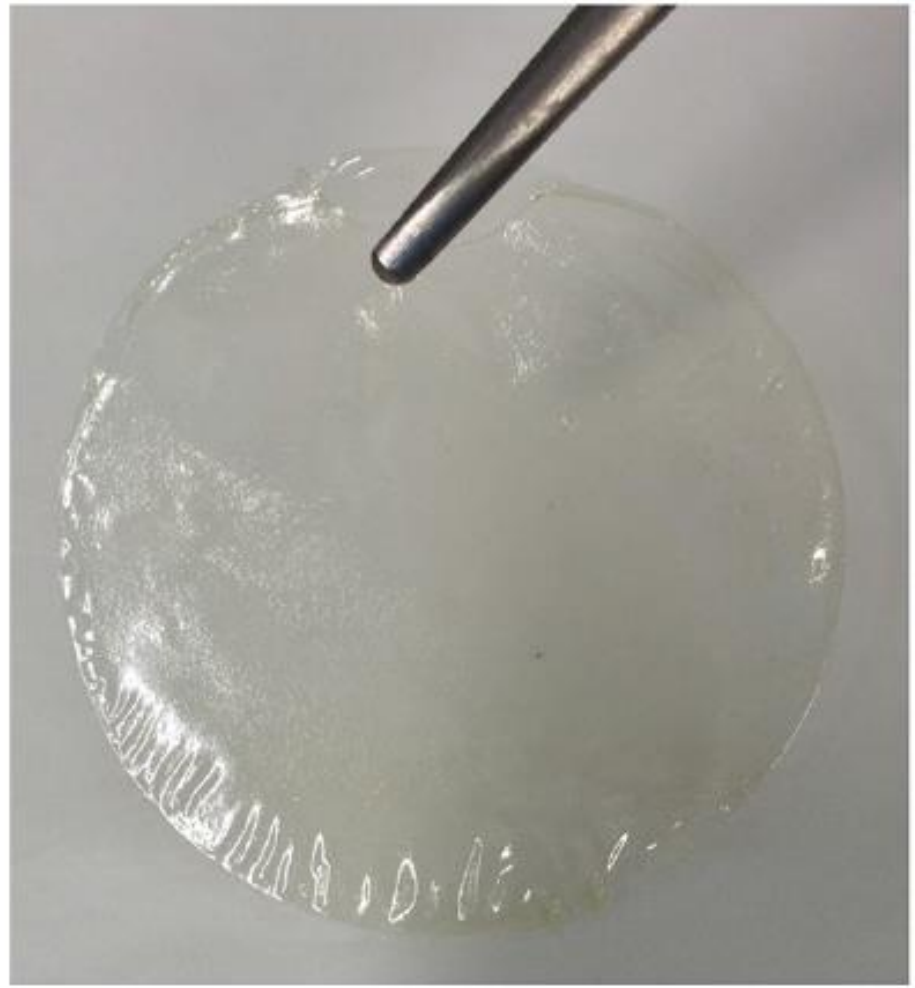


Fig. 1. Final aspect of tested membranes.

Materials Research. 2017; 20(2): 377-386
DOI: http://dx.doi.org/10.1590/1980-5373-MR-2016-0509

Fibers Obtained from Alginate, Chitosan and

Daniela Camargo Furuya[a], Silgia Aparecida da

Gomes Ferraz[c], Adalberto Pessoa

[a] School of Arts, Sciences and Humanities, Textile an
Béttio, 1000, 03828-000, J
[b] Department of Biological Sciences, Bauru School
Octávio Pinheiro Brisolla, 9-75, Vila Un
[c] Department of Pharmacy, School of Pharmaceu
Lineu Prestes, 580, Cidade Universit
[d] Department of Biochemical and Pharmaceutical
University of São Paulo, Av. Prof. Lineu Prestes, 58
Bra

Received: July 07, 2016; Revised: Octobe

The main aim of this study was to develop scaf
with and without glycerol. The scaffolds developed u
ratio and weight loss, cellular viability, degradation a
analysis. Tenacity values showed that use of glycerc
as a hybrid fiber were associated with increasing ten
the scaffolds containing glycerol presented lower w
scaffolds, compared to scaffolds without glycerol,
of the alginate, chitosan and hybrid scaffolds, with
third day of the biomineralization assay, chitosan
crystals. The degradation study showed that glycer

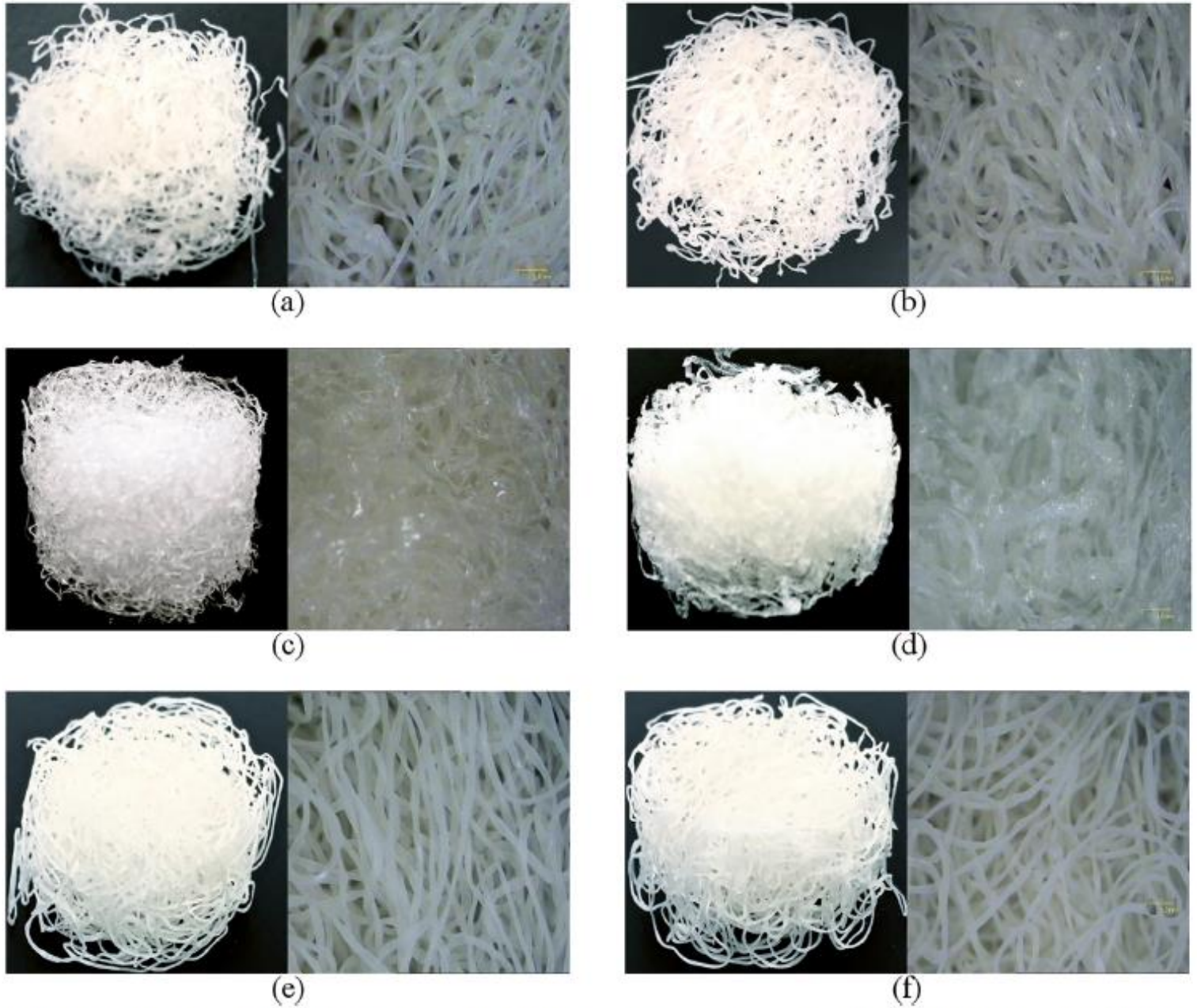
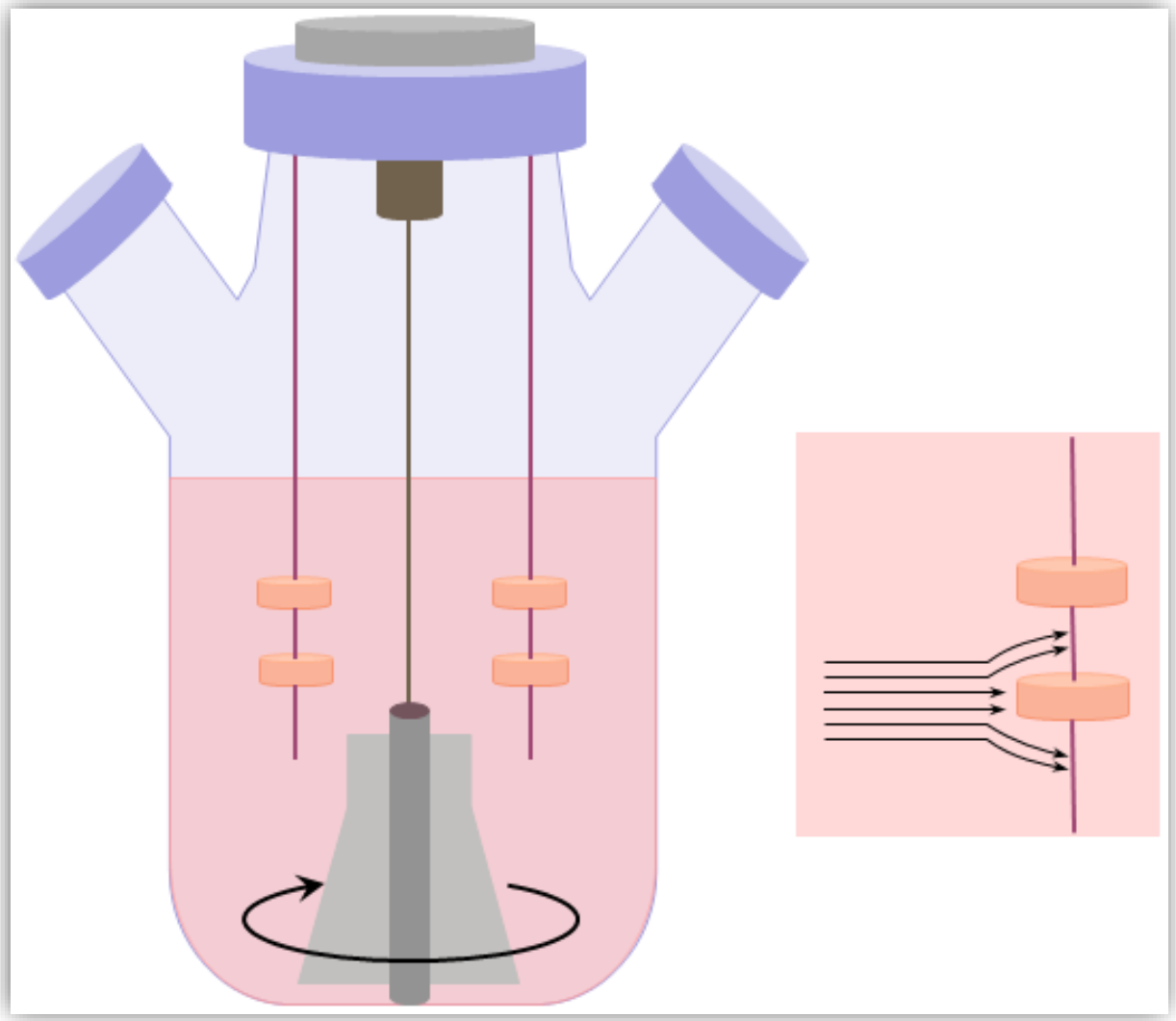


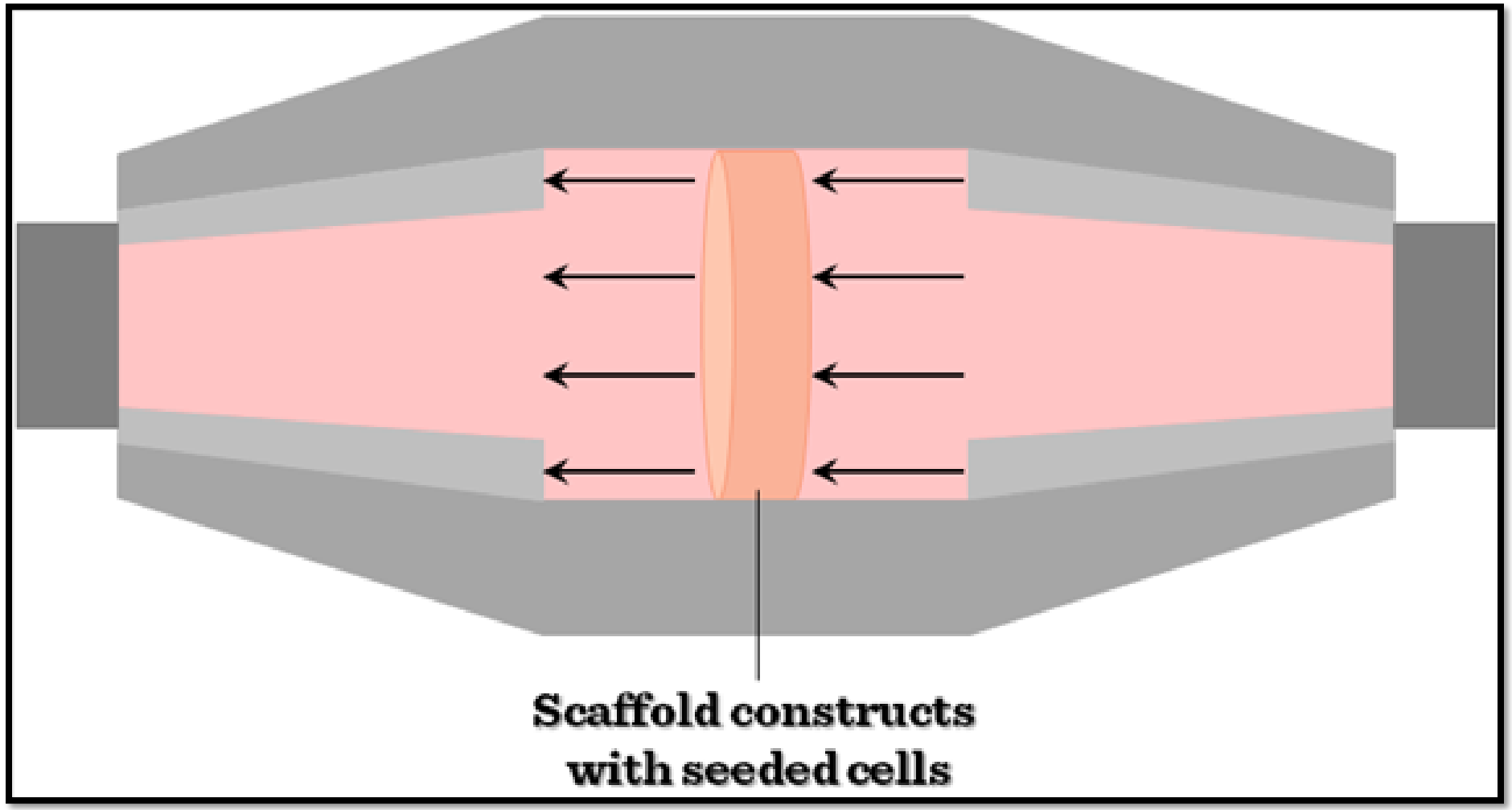
Figure 1: Scaffolds of (a) alginate without glycerol, (b) alginate with glycerol, (c) chitosan without glycerol, (d) chitosan with glycerol, (e) hybrid without glycerol and (f) hybrid with glycerol produced in mold of 15 mm per well in digital image and optical microscopy with 20x magnification.

Biorreatores

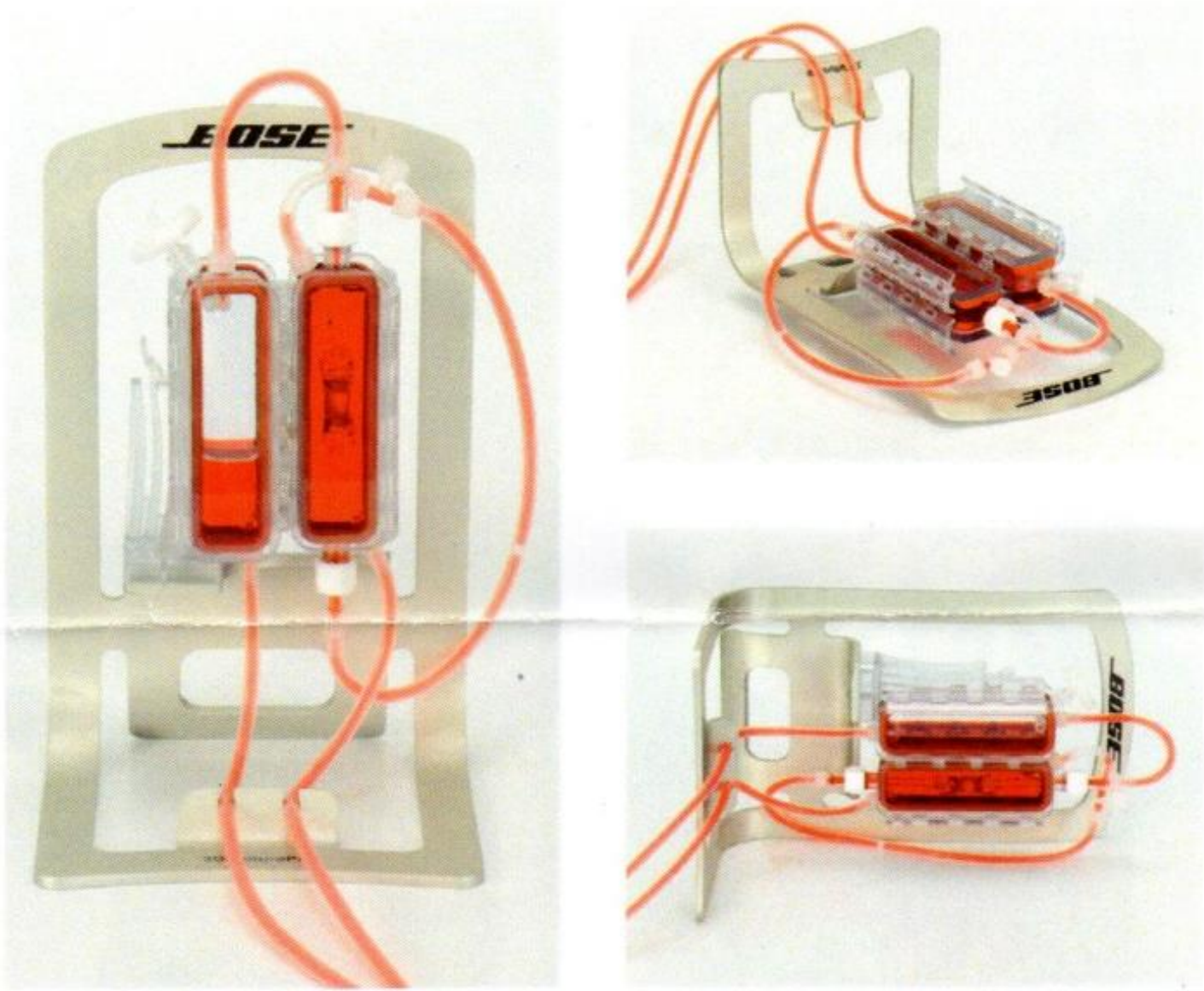


Biorreatores

Biorreatores

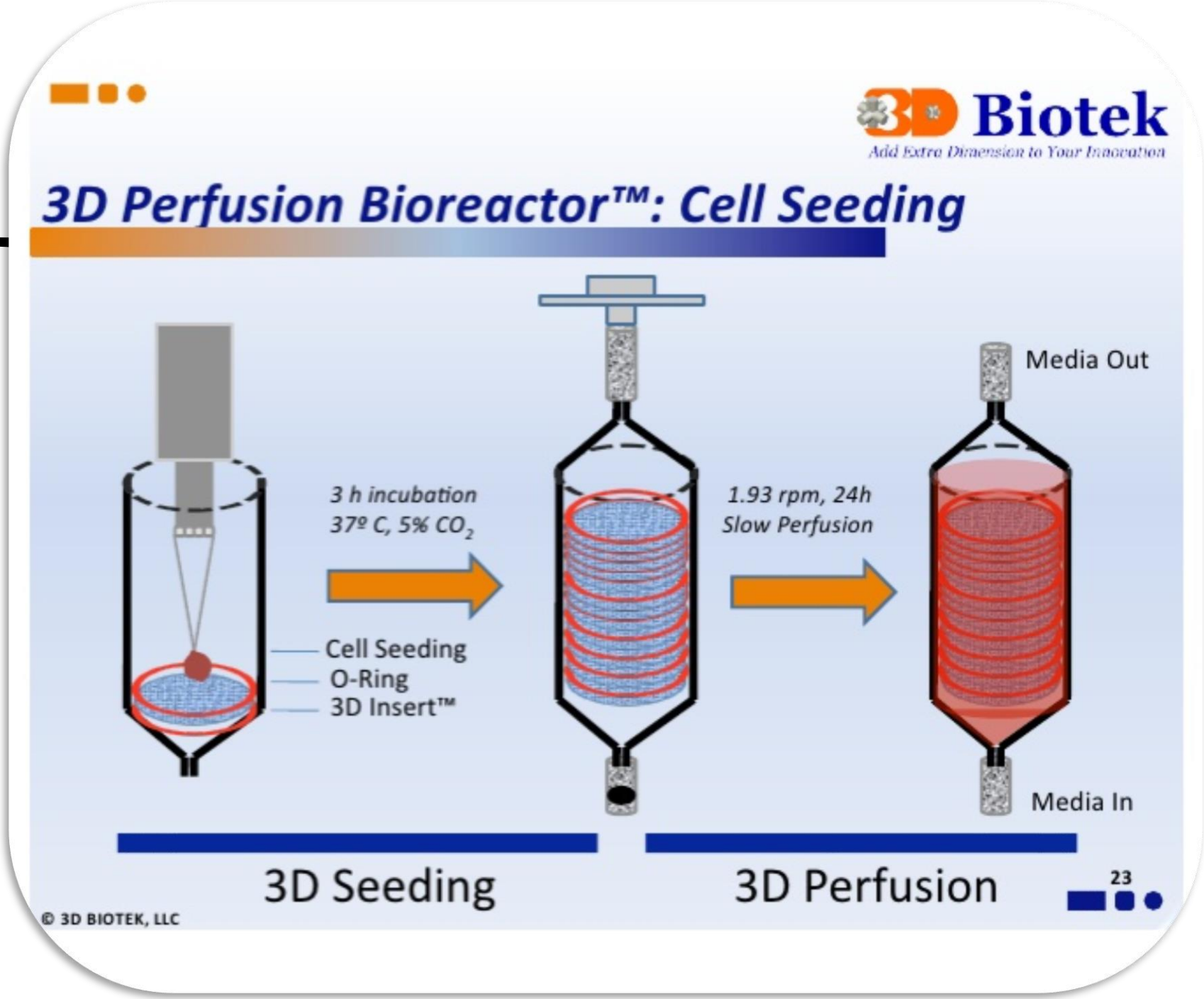


Biorreatores



Position vertically, horizontally, or on the side

Biorreatores

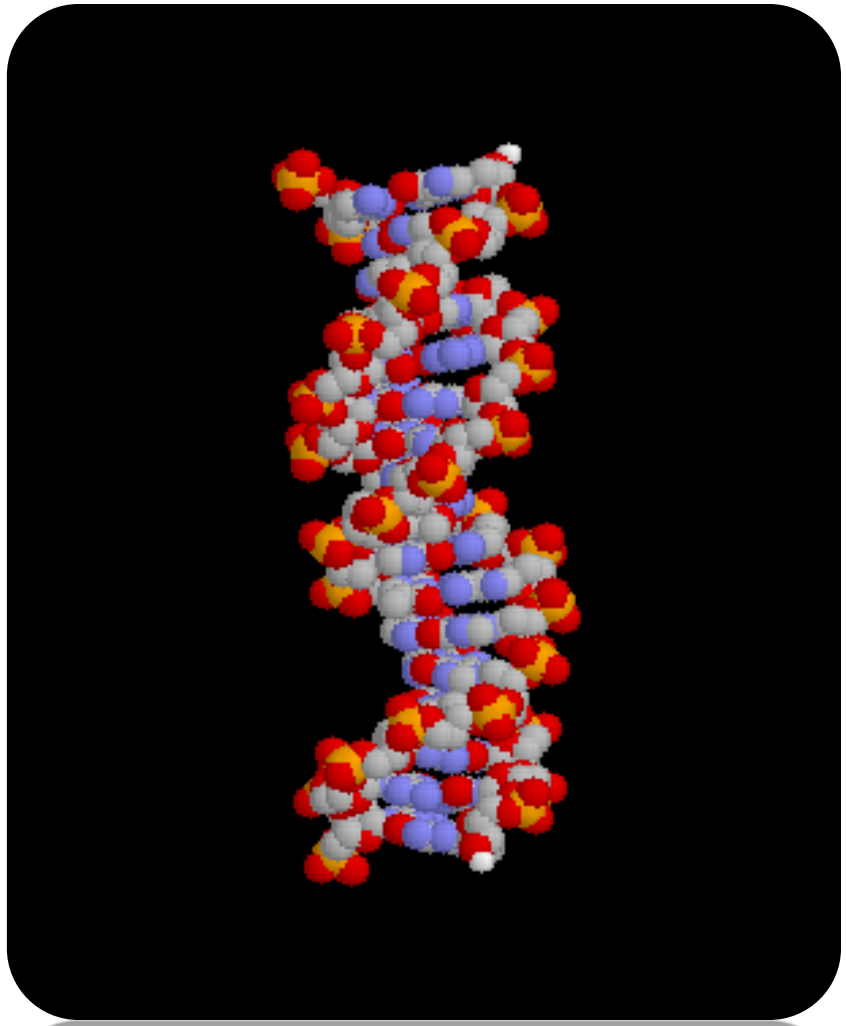


FUNÇÕES DO FATOR DE CRESCIMENTO

Sinais Moleculares

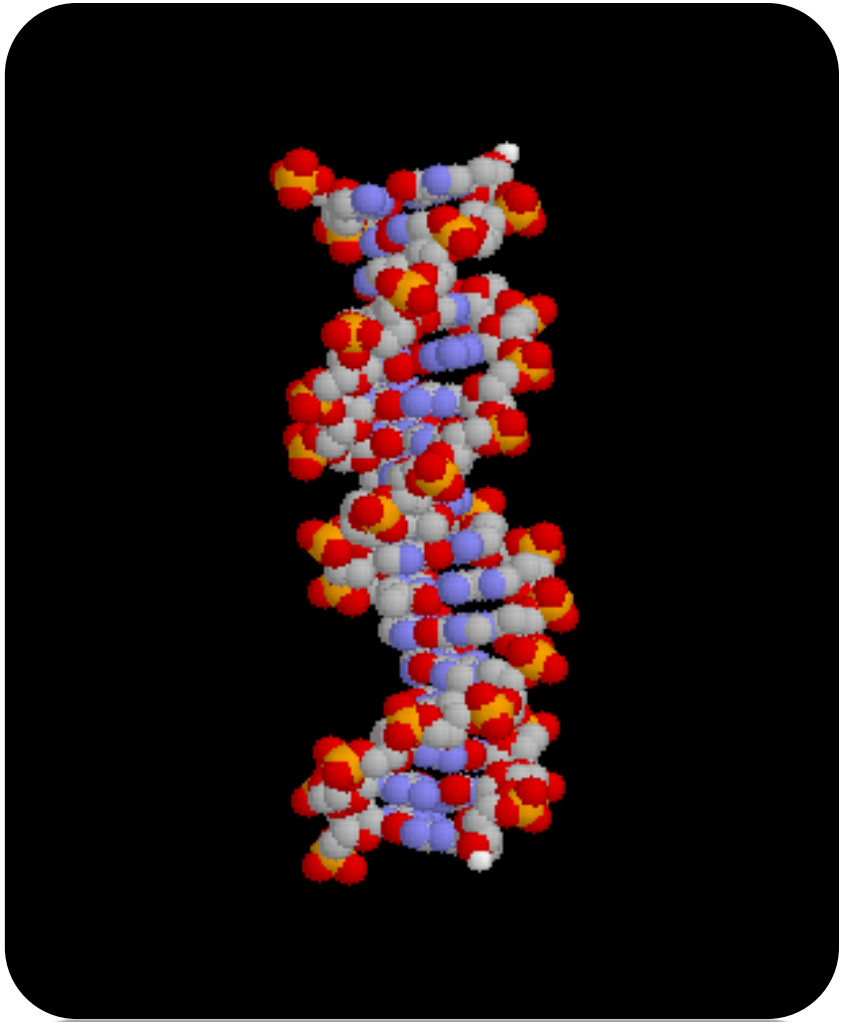
FATORES DE CRESCIMENTO

- Polipeptídeo > sinal afeta atividade celular
- Fator de crescimento > inadequado
  - Nem sempre promove crescimento
  - Inibição às vezes
  - Controle metabolismo
  - Na verdade: moduladores de crescimento



FATORES DE CRESCIMENTO

- BMPs
- PDGF
- TGF - beta
- IGF (insulin-like growth factor)
- FGF
- VEGF



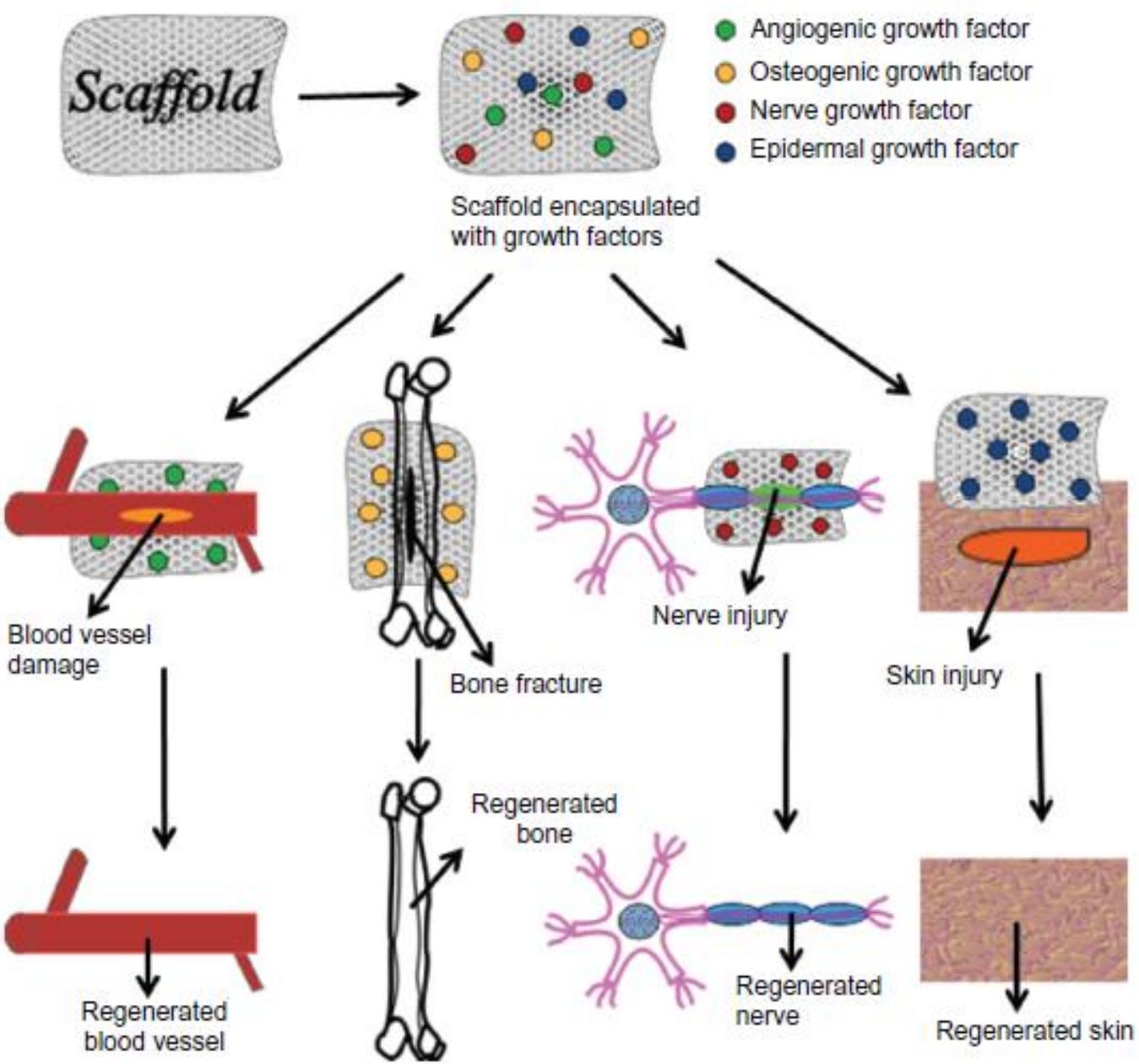


Figure 8 Specific growth factor–loaded scaffolds used for regeneration of various tissues.

Rajangam and An. Int J Nanomedicine. 2013;8:3641-62.

npj | Regenerative Medicine

www.nature.com/npjregenmed

REVIEW ARTICLE OPEN

Check for updates

Cellularized small-caliber tissue-engineered vascular grafts: looking for the ultimate gold standard

Adrien Fayon[1], Patrick Menu[1,2]✉ and Reine El Omar[1,2]

Due to the lack of efficacy of synthetic vascular substitutes in the replacement of small-caliber arteries, vascular tissue engineering (VTE) has emerged as a promising solution to produce viable small-caliber tissue-engineered vascular grafts (TEVG). Previous studies have shown the importance of a cellular intimal layer at the luminal surface of TEVG to prevent thrombotic events. However, the cellularization of a TEVG seems to be a critical approach to consider in the development of a TEVG. To date, no standard cellularization method or cell type has been established to create the ideal TEVG by promoting its long-term patency and function. In this review, advances in VTE are described and discussed with a particular focus on the construction approaches of cellularized small-caliber TEVGs, the cell types used, as well as their preclinical and clinical applications.

npj Regenerative Medicine (2021)6:46 ; https://doi.org/10.1038/s41536-021-00155-x

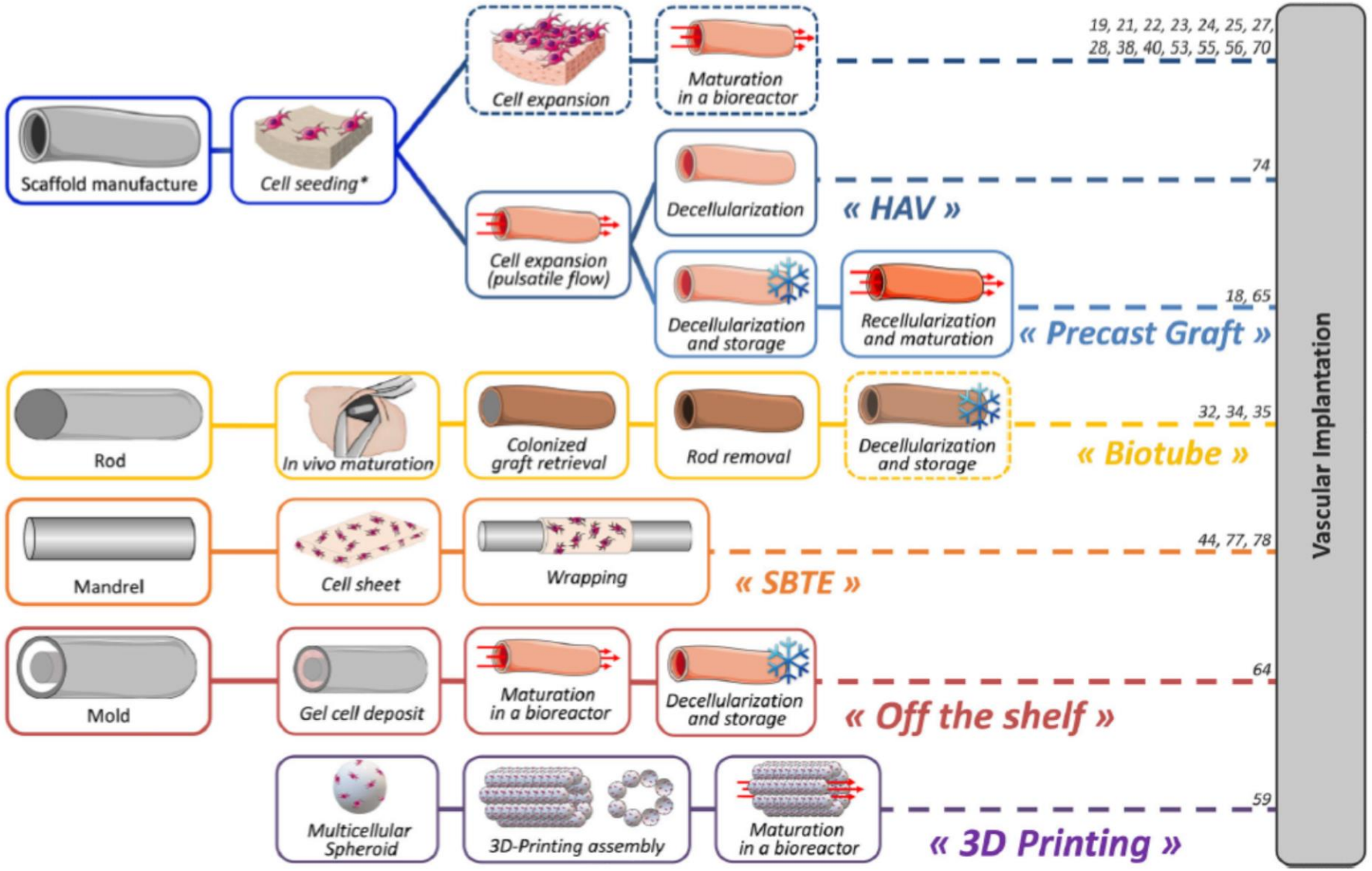


Fig. 1 Fabrication approaches to produce a small-diameter TEVG. Dotted frames represent optional steps found in the literature. *Cells can be seeded using cellularized gel or cell suspension. TEVG Tissue-Engineered Vascular Graft, HAV Human Acellular Vessel, SBTE Sheet-Based Tissue Engineering.

Chia and Wu *Journal of Biological Engineering* (2015) 9:4
DOI 10.1186/s13036-015-0001-4

JOURNAL OF BIOLOGICAL ENGINEERING

REVIEW Open Access

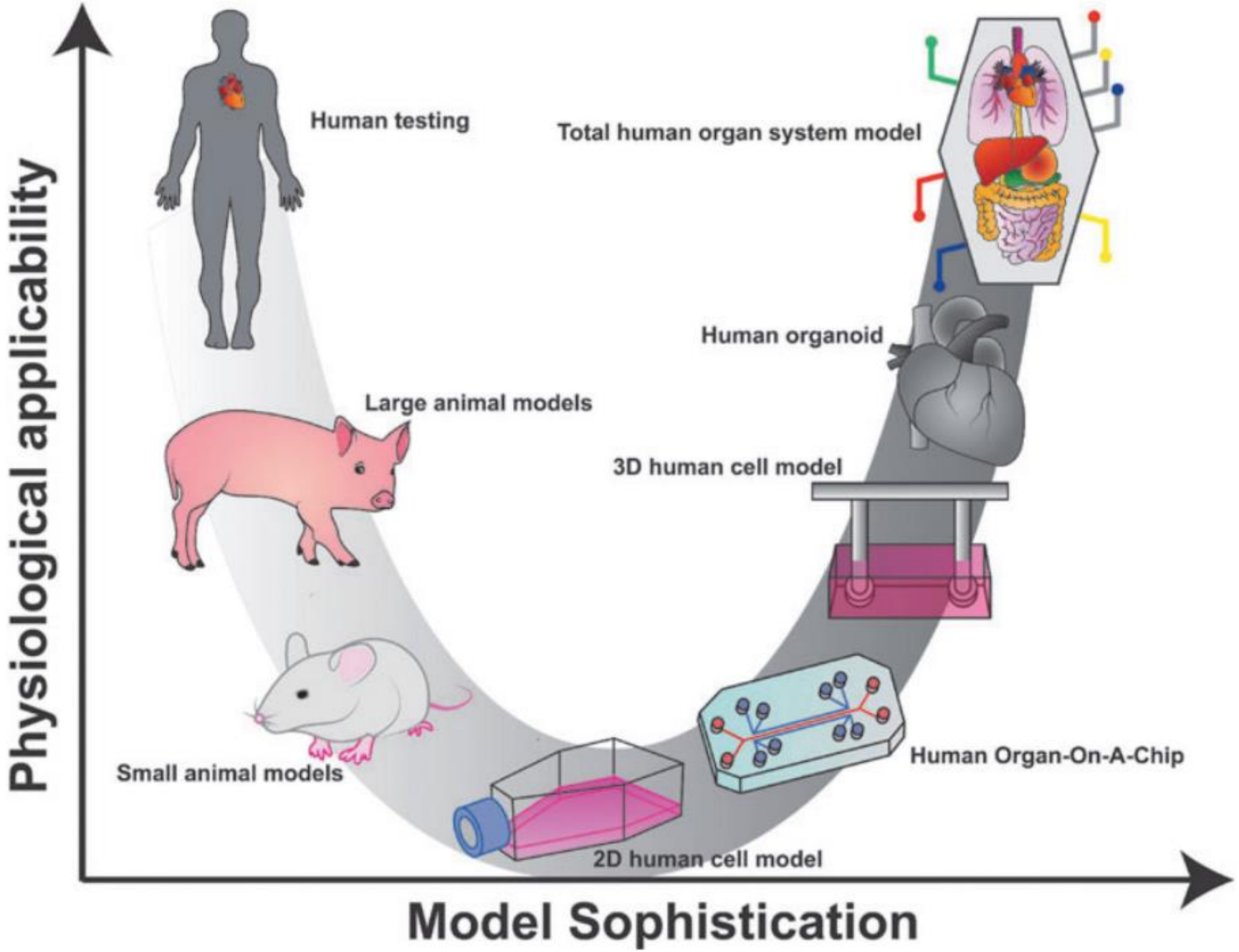
Recent advances in 3D printing of biomaterials

Helena N Chia[1] and Benjamin M Wu[1,2,3,4*]

Abstract

3D Printing promises to produce complex biomedical devices according to computer design using patient-specific anatomical data. Since its initial use as pre-surgical visualization models and tooling molds, 3D Printing has slowly evolved to create one-of-a-kind devices, implants, scaffolds for tissue engineering, diagnostic platforms, and drug delivery systems. Fueled by the recent explosion in public interest and access to affordable printers, there is renewed interest to combine stem cells with custom 3D scaffolds for personalized regenerative medicine. Before 3D Printing can be used routinely for the regeneration of complex tissues (e.g. bone, cartilage, muscles, vessels, nerves in the craniomaxillofacial complex), and complex organs with intricate 3D microarchitecture (e.g. liver, lymphoid organs), several technological limitations must be addressed. In this review, the major materials and technology advances within the last five years for each of the common 3D Printing technologies (Three Dimensional Printing, Fused Deposition Modeling, Selective Laser Sintering, Stereolithography, and 3D Plotting/Direct-Write/Bioprinting) are described. Examples are highlighted to illustrate progress of each technology in tissue engineering, and key limitations are identified to motivate future research and advance this fascinating field of advanced manufacturing.

Keywords: 3D Printing, Fused deposition modeling, Selective laser sintering, Stereolithography, Computer-aided tissue engineering, 3D plotting, Bioprinting



Salem et al. (2022). Tissue Eng Part B Rev. doi: 10.1089/ten.TEB.2021.0088.

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

Lancet 2004; 364: 766–70

See Comment page 735

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

Department of Oral and Maxillofacial Surgery, University of Kiel, Arnold-Heller-Str 16, 24105 Kiel, Germany (P H Warnke MD, I N G Springer MD, Prof J Wiltfang MD, Prof Y Acil PhD, E Behrens DMD, Prof H Terheyden MD); Department of Oral and Maxillofacial Surgery, Knappschaftskrankenhaus Recklinghausen, Recklinghausen, Germany (Prof H Eufinger MD, M Wehmöller PhD); Department of Rheumatology, Australian National University, Canberra, Australia (P A J Russo MD); Department of Diagnostic Radiology, University of Kiel, Kiel, Germany (H Bolte MD); and Department of Orthopaedic Surgery, University of Sydney, Penrith, Australia (E Sherry MD)

Correspondence to:

Summary

Background A major goal of research in bone transplantation is the ability to avoid creation of secondary bone defects. We aimed to repair an extended mandibular discontinuity defect by growth of a custom bone transplant inside the latissimus dorsi muscle of an adult male patient.

Methods Three-dimensional computed tomography (CT) scanning and computer-aided design techniques were used to produce an ideal virtual replacement for the mandibular defect. These data were used to create a titanium mesh cage that was filled with bone mineral blocks and infiltrated with 7 mg recombinant human bone morphogenetic protein 7 and 20 mL of the patient's bone marrow. Thus prepared, the transplant was implanted into the latissimus dorsi muscle and 7 weeks later transplanted as a free bone-muscle flap to repair the mandibular defect.

Findings In-vivo skeletal scintigraphy showed bone remodelling and mineralisation inside the mandibular transplant both before and after transplantation. CT provided radiological evidence of new bone formation. Postoperatively, the patient had an improved degree of mastication and was satisfied with the aesthetic outcome of the procedure.

Interpretation Heterotopic bone induction to form a mandibular replacement inside the latissimus dorsi muscle in a human being is possible. This technique allows for a lower operative burden compared with conventional techniques by avoiding creation of a secondary bone defect. It also provides a good three-dimensional outcome.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 27 (2006) 3163–3167
Biomaterials
www.elsevier.com/locate/biomaterials

Leading Opinion

Man as living bioreactor: Fate of an exogenously prepared customized tissue-engineered mandible ☆

Patrick H. Warnke[a,*], Jörg Wiltfang[a], Ingo Springer[a], Yahya Acil[a], Hendrik Bolte[b], Markus Kosmahl[c], Paul A.J. Russo[a,d], Eugene Sherry[e], Ulf Lützen[f], Stefan Wolfart[g], Hendrik Terheyden[a]

[a]*Department of Oral and Maxillofacial Surgery, University of Kiel, Arnold-Heller-Str. 16, 24105 Kiel, Germany*
[b]*Department of Diagnostic Radiology, University of Kiel, Germany*
[c]*Department of Pathology, University of Kiel, Germany*
[d]*Royal North Shore Hospital, University of Sydney, Australia*
[e]*Department of Orthopaedic Surgery, Bond University, Australia*
[f]*Department of Nuclear Medicine, University of Kiel, Germany*
[g]*Department of Prosthodontics and Materials Research, University of Kiel, Germany*

Received 28 November 2005; accepted 25 January 2006

Abstract

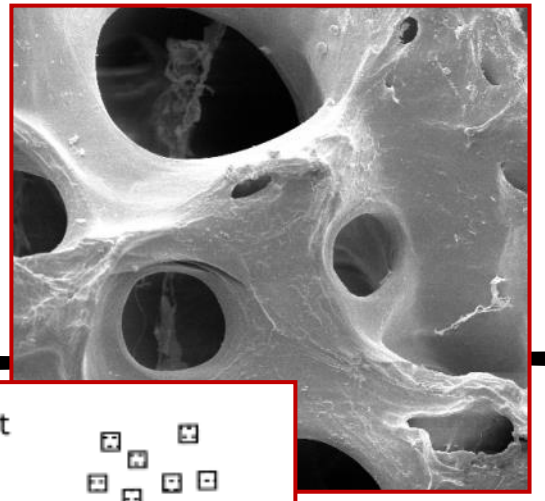
In 2004, we reported a novel method of repairing a human mandible by in vivo tissue engineering. The patient served as his own bioreactor as the exogenously prepared customized mandible replacement was grown inside his latissimus dorsi muscle prior to transplantation to repair the existing defect. Our technique was developed through extensive experience with an animal model. We describe our and the patient's experiences with this procedure. We give details to the benefits and limitations of this technique as it stands and outline issues that should be addressed in future human clinical trials.

Keywords: In vivo tissue engineering; Bone regeneration; Replacement; Bioreactor; Mandible reconstruction; BMP

Metodologia

- 7 mg de rhBMP-7
- 20 mL de aspirado da medula óssea
- 5 mg de Bio-oss (Bloco)
- Durante 7 semanas

Protocolo Sugerido



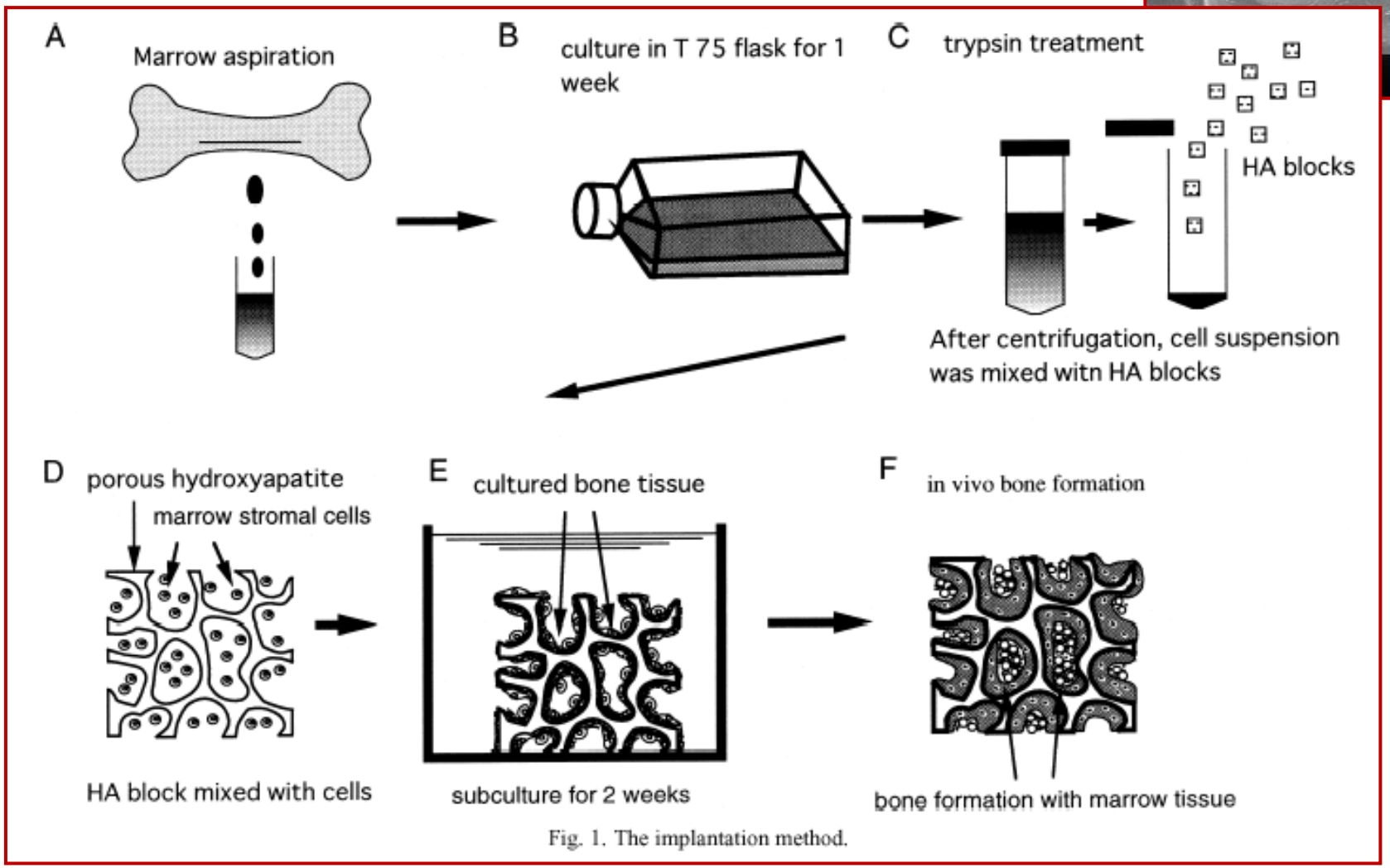
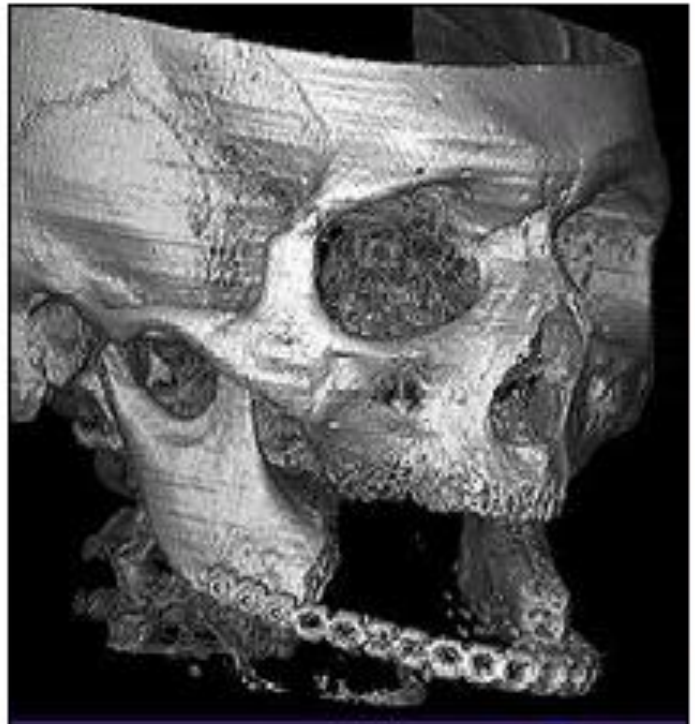


Fig. 1. The implantation method.



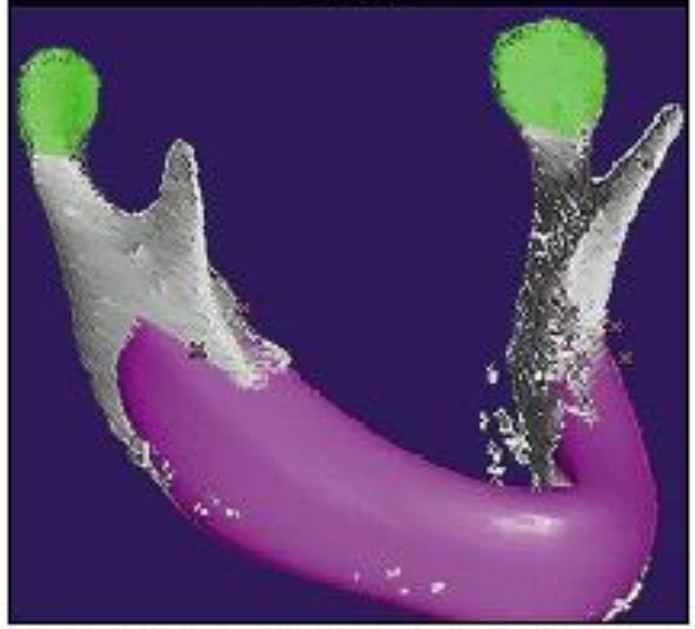


Figure 1: Three-dimensional CT scan of size defect (upper) and CAD plan of ideal mandibular transplant (lower)

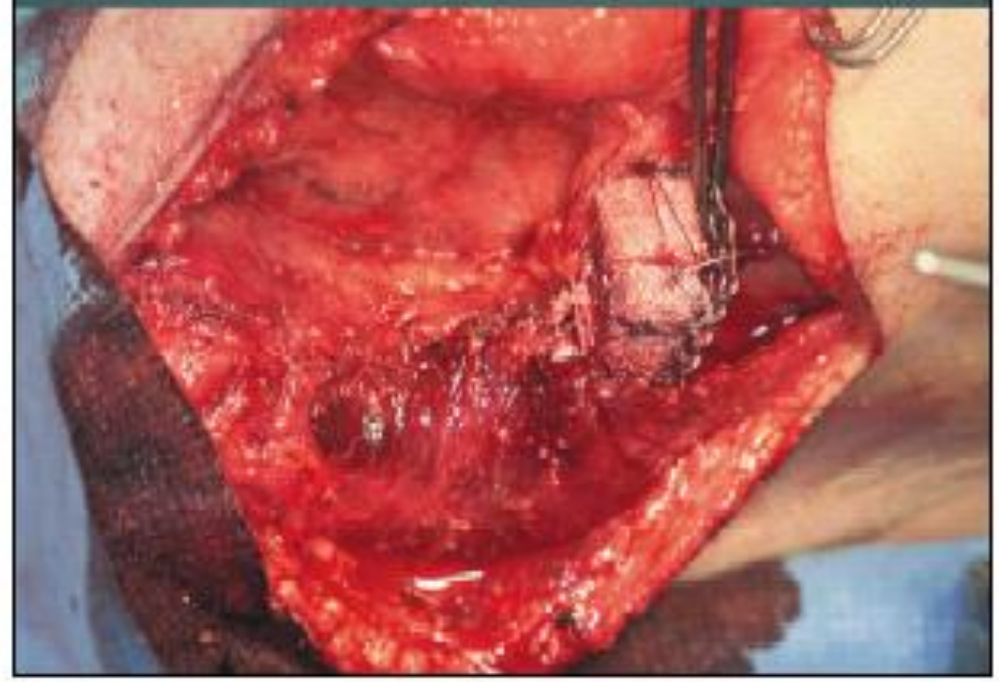


Figure 2: Titanium mesh cage filled with bone mineral blocks infiltrated with recombinant human BMP7 and bone-marrow mixture (upper) and implantation into right latissimus dorsi muscle (lower)

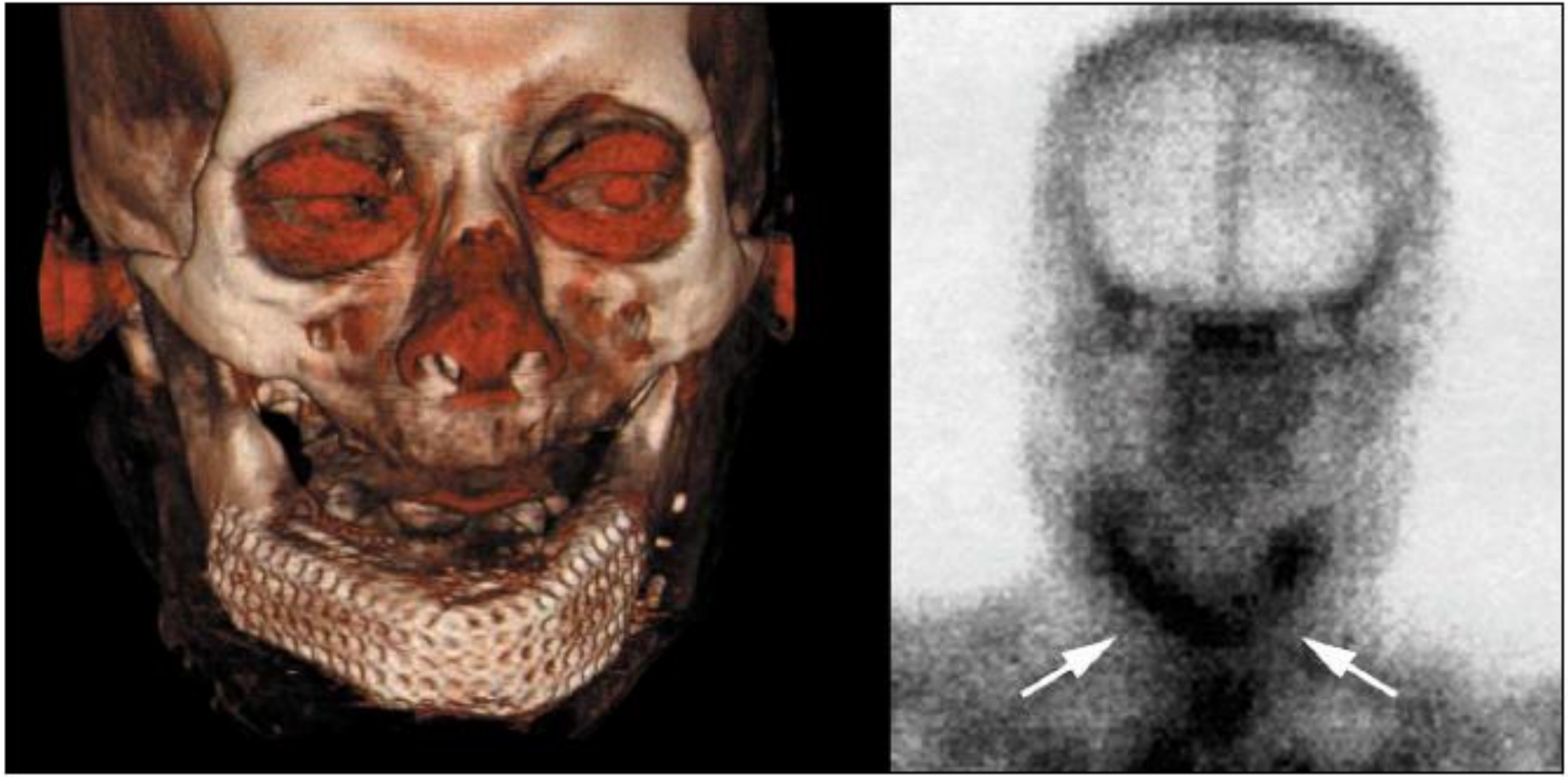
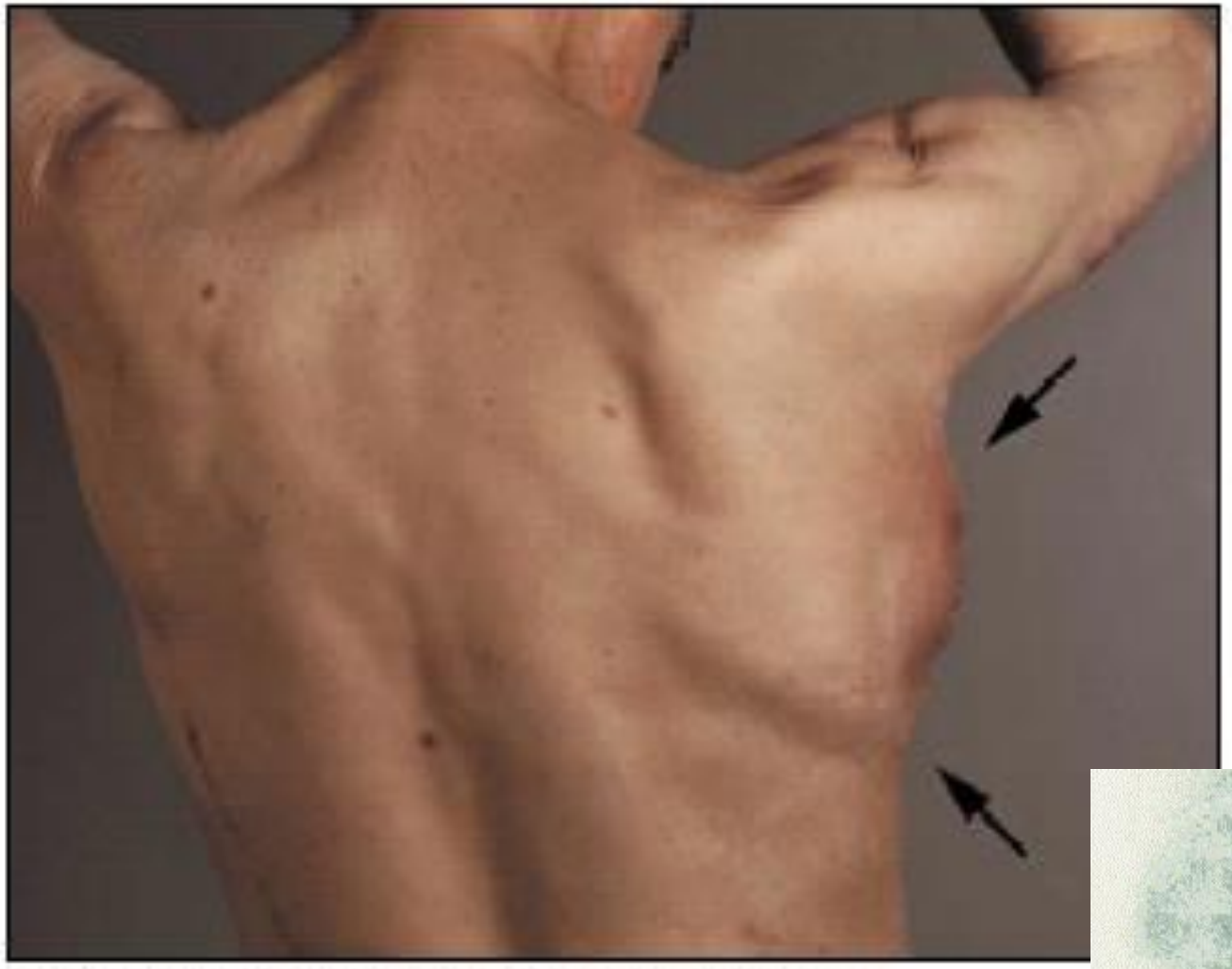


Figure 5: Three-dimensional CT scan (left) after transplantation of bone replacement with enhancement of soft tissue (red) and repeat skeletal scintigraphy (right) with tracer enhancement showing continued bone remodelling and mineralisation (arrows)



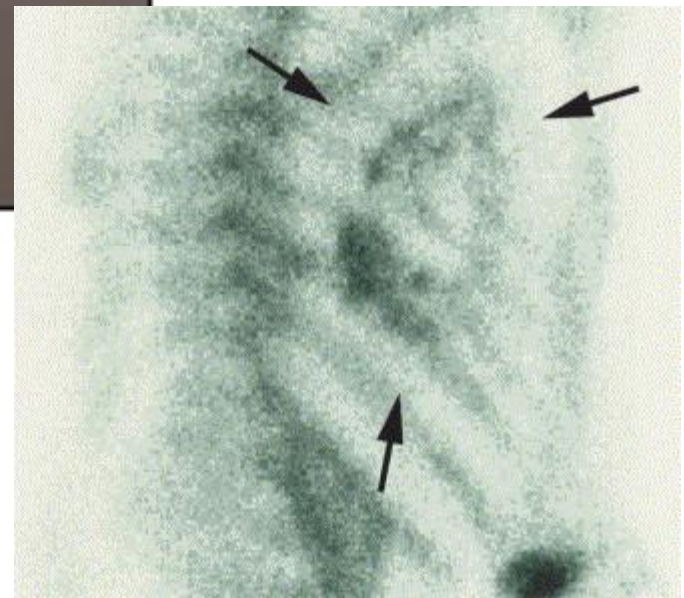
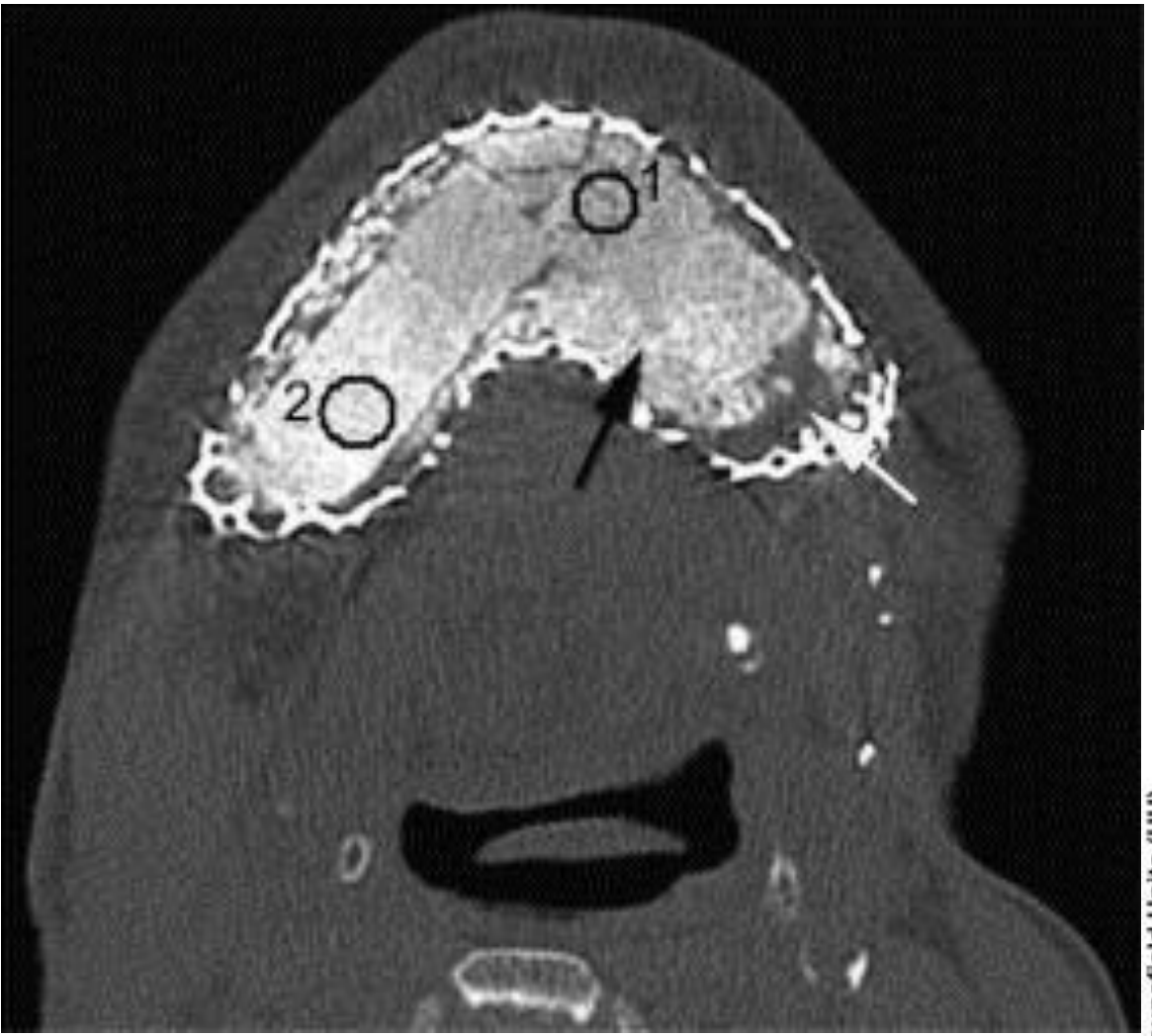
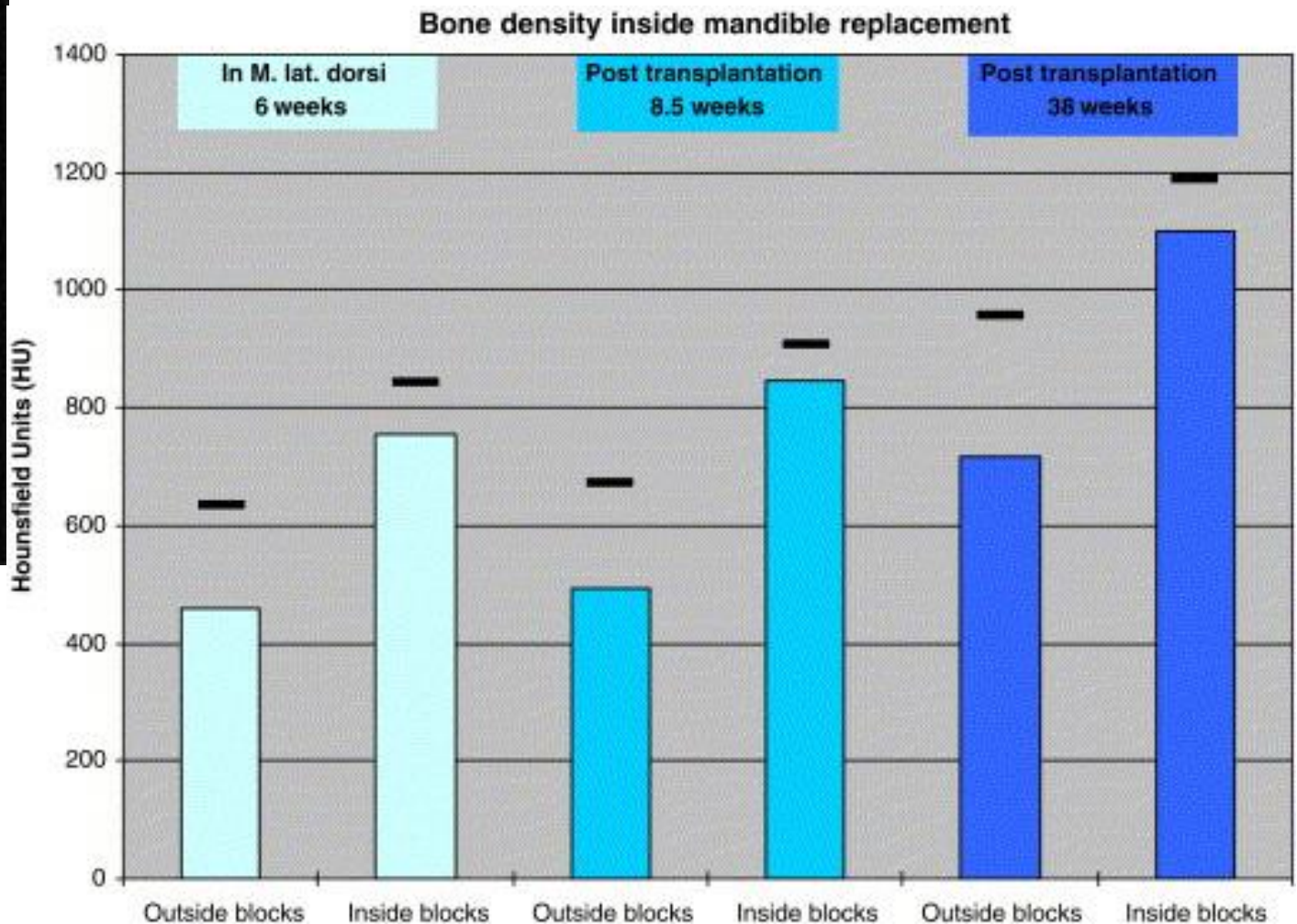
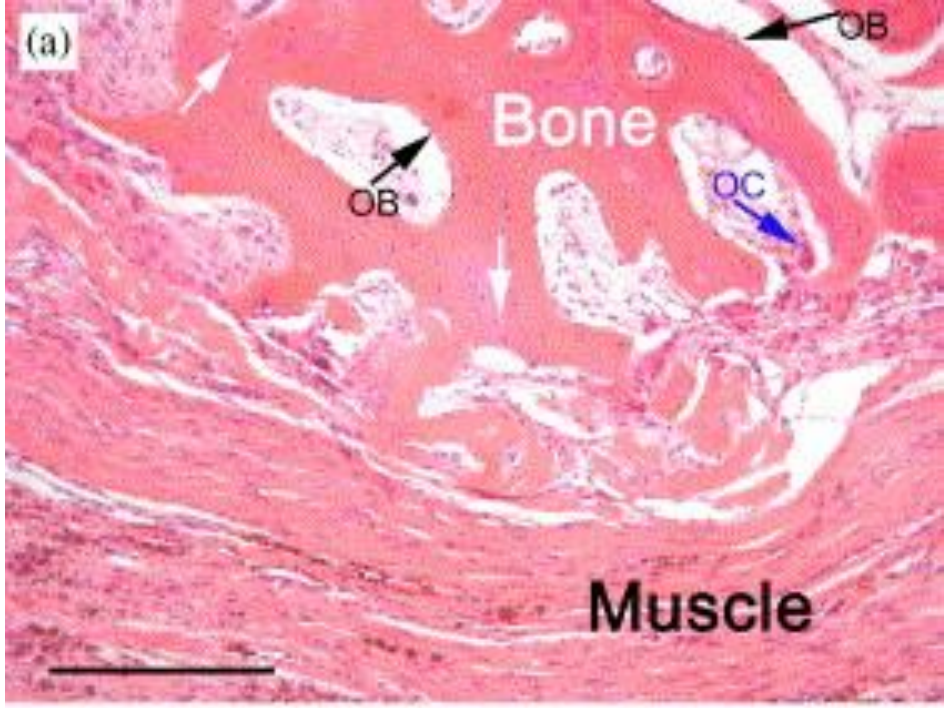
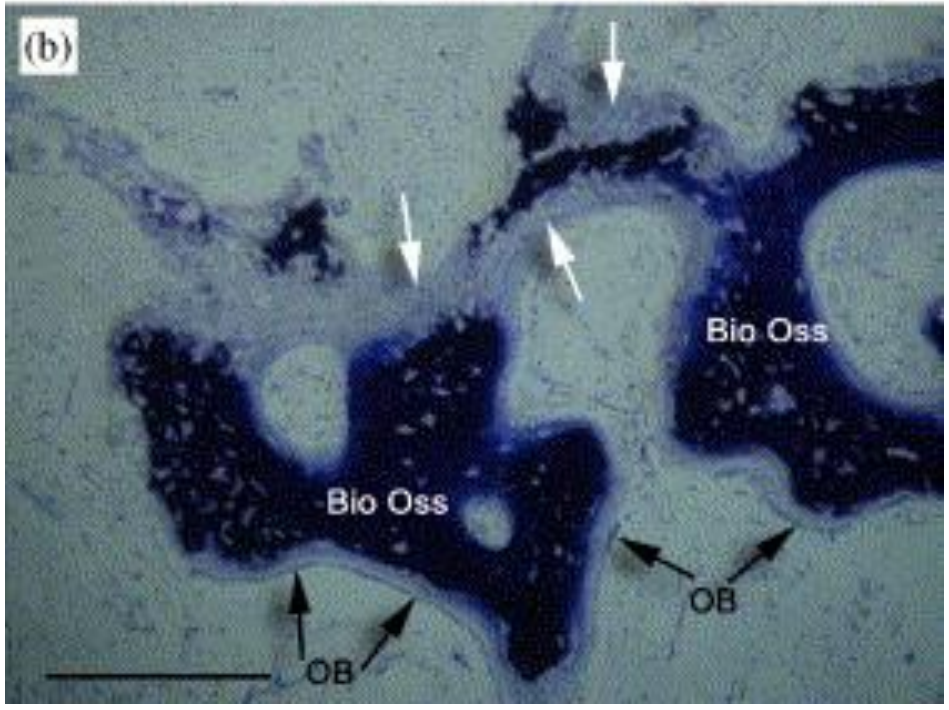


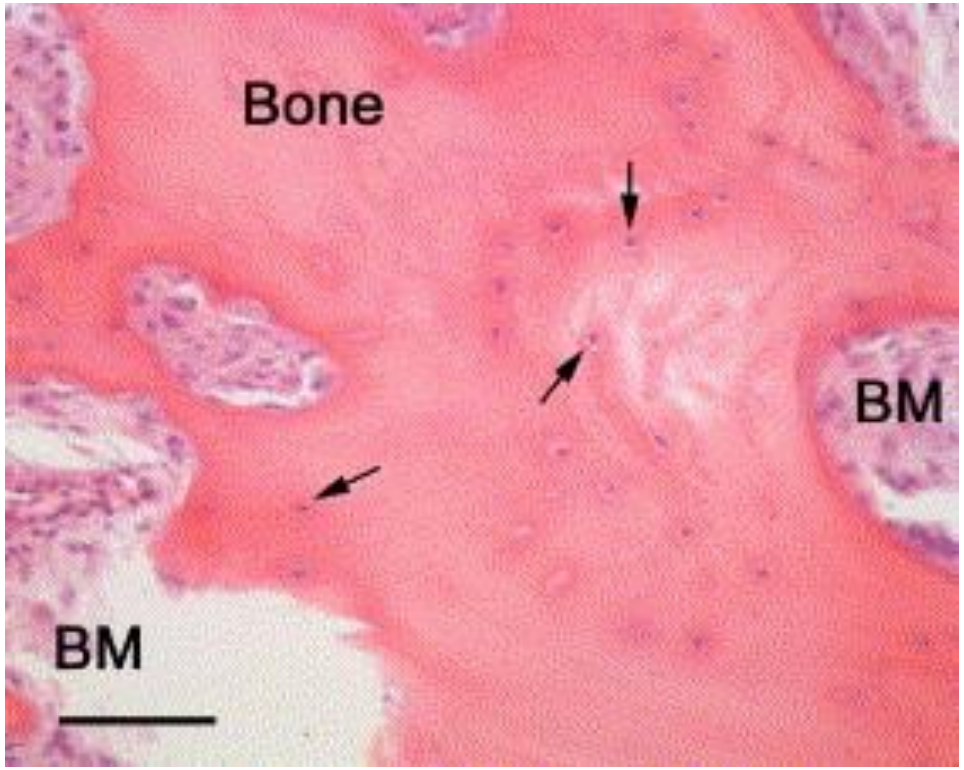
Figure 3: Dorsal view of mandibular replacement 3 weeks after implantation
Arrows show area of implantation inside latissimus dorsi muscle.


1
2


Bone density inside mandible replacement
In M. lat. dorsi
6 weeks
Post transplantation
8.5 weeks
Post transplantation
38 weeks
Hounsfield Units (HU)
1400
1200
1000
800
600
400
200
0
Outside blocks
Inside blocks
Outside blocks
Inside blocks
Outside blocks
Inside blocks


(a)
OB
Bone
OB
OC
Muscle


(b)
Bio Oss
Bio Oss
OB
OB


Bone
BM
BM

Perspectivas

- Mimetizar ao máximo a resposta do próprio organismo (~ autógeno).
- Devolver a função de células ou órgãos perdidos.
- Associar a bioengenharia a tratamentos tradicionais.
- Tornar as técnicas mais acessíveis.

CONCLUSÃO

O uso de diferentes linhagens celulares e modelos experimentais tem ampliado o leque de opções terapêuticas. A manipulação de células para obtenção de informações básicas até o uso direto em pacientes é uma realidade na área da saúde.

Obrigado!