Bioengineered scaffolds in head and neck surgery: optimization of bone regeneration devices and development of local release systems

PhD in Artificial Intelligence in Medicine and Innovation in Clinical Research and Methodology - Coordinatore: Prof. Domenico Russo

UNIVERSITÀ DEGLI STUDI DI BRESCIA

Alessandra Ruaro MD
Supervisor: Prof. Luciana Sartore

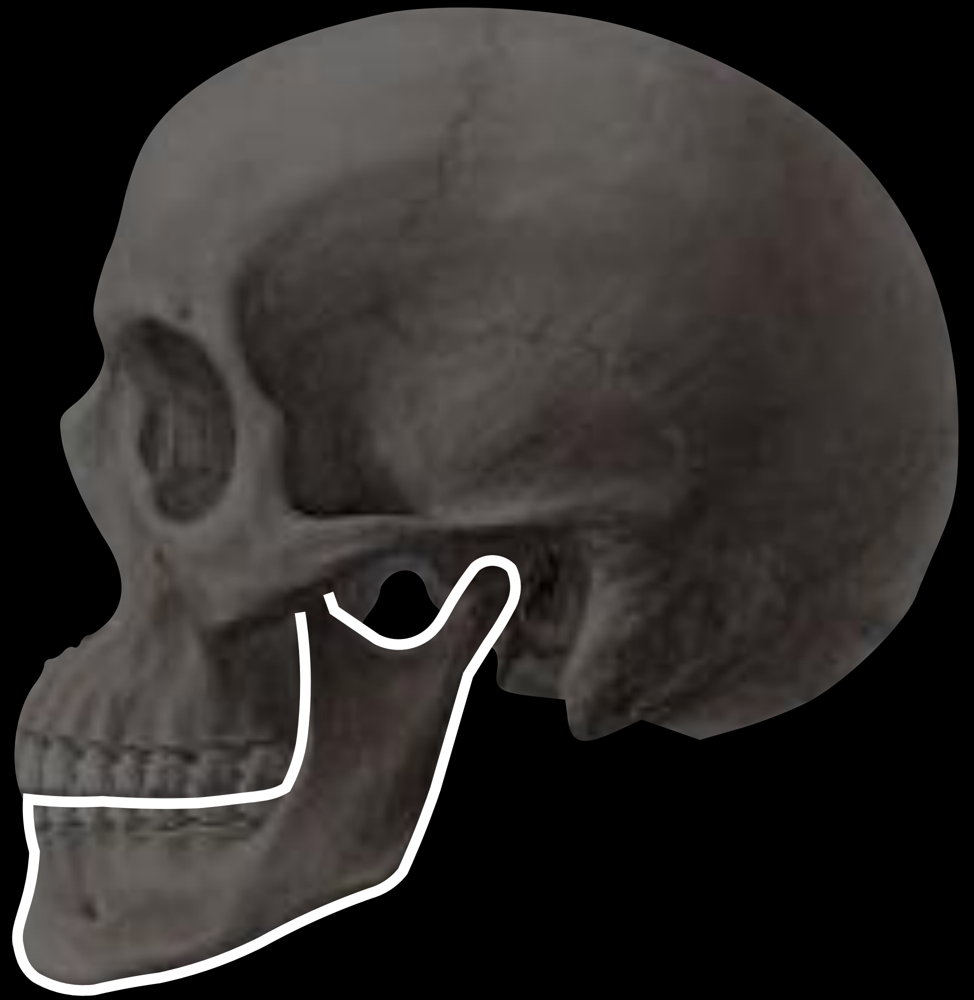
24 Aprile 2025

Bioengineered scaffolds for bone regeneration

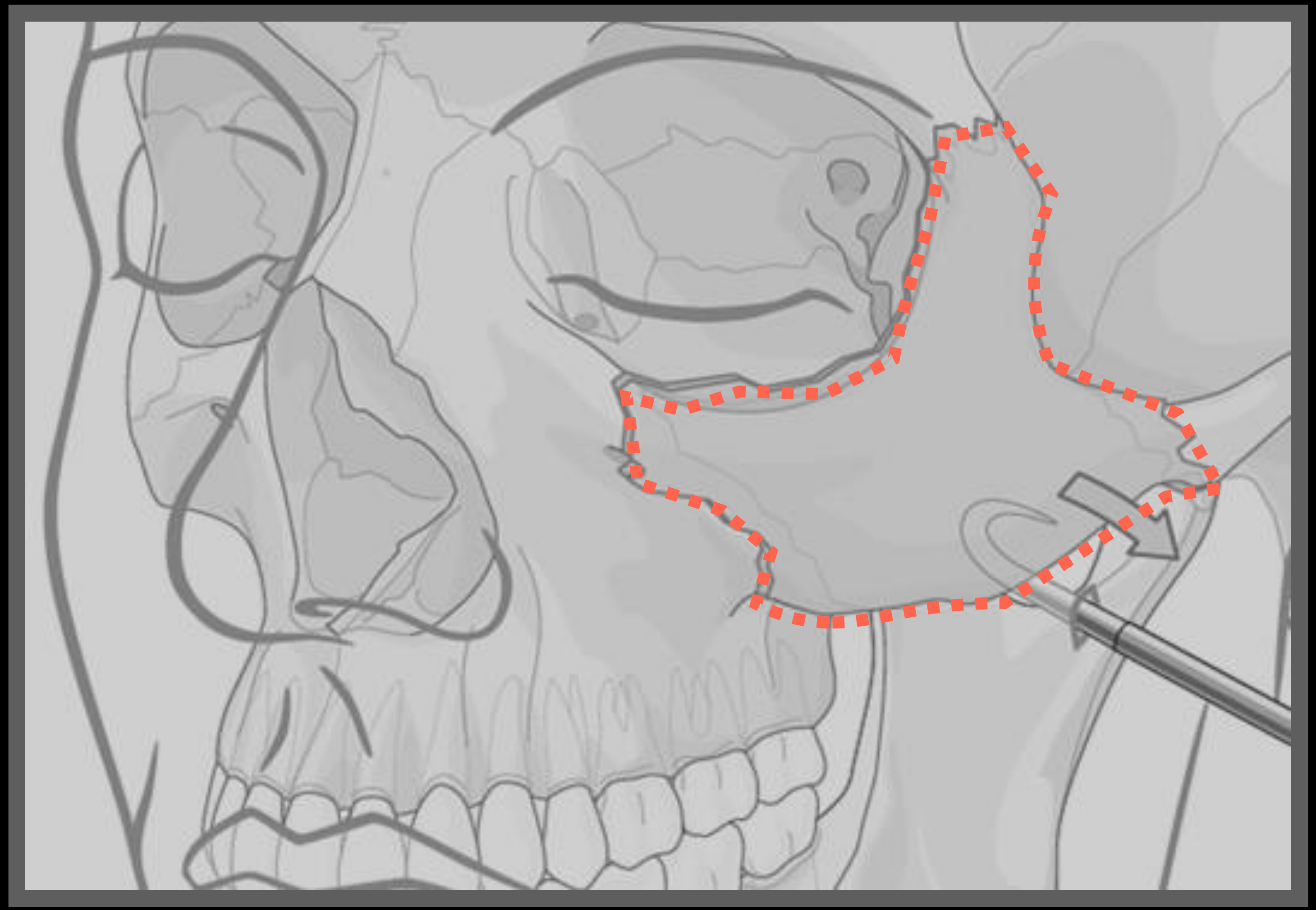
- Background - literature review
- Research updates
- Ancillary projects

Reconstruction in head and neck surgery

CLINICAL SCENARIOS



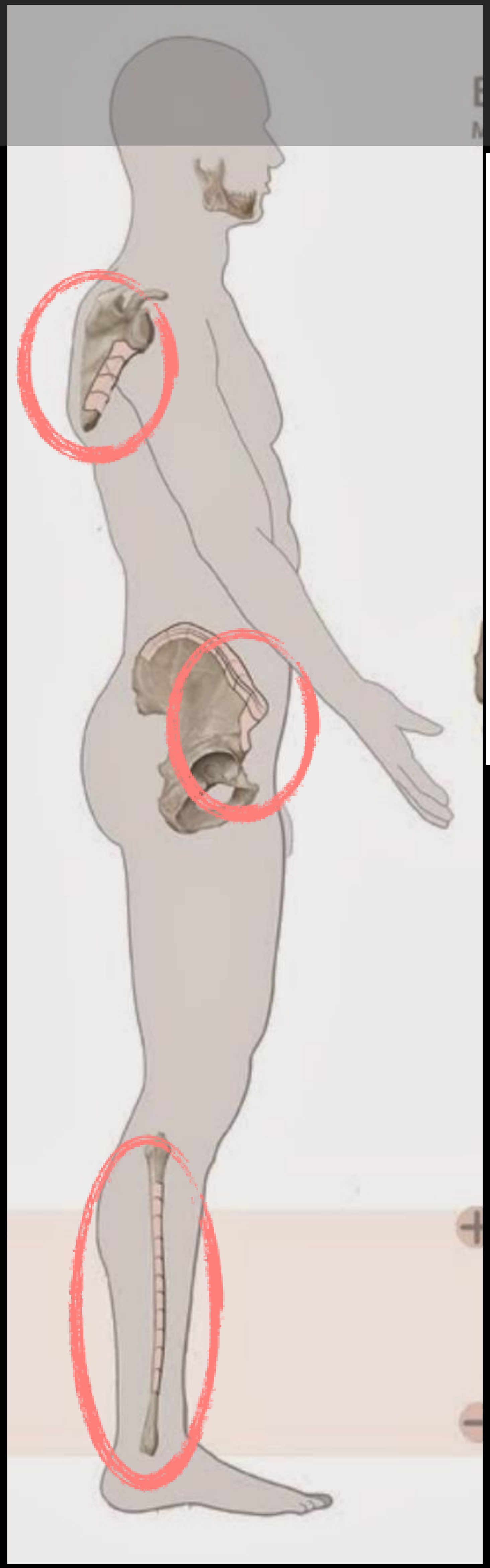
BONE-INCLUDING FREE FLAP RECONSTRUCTION

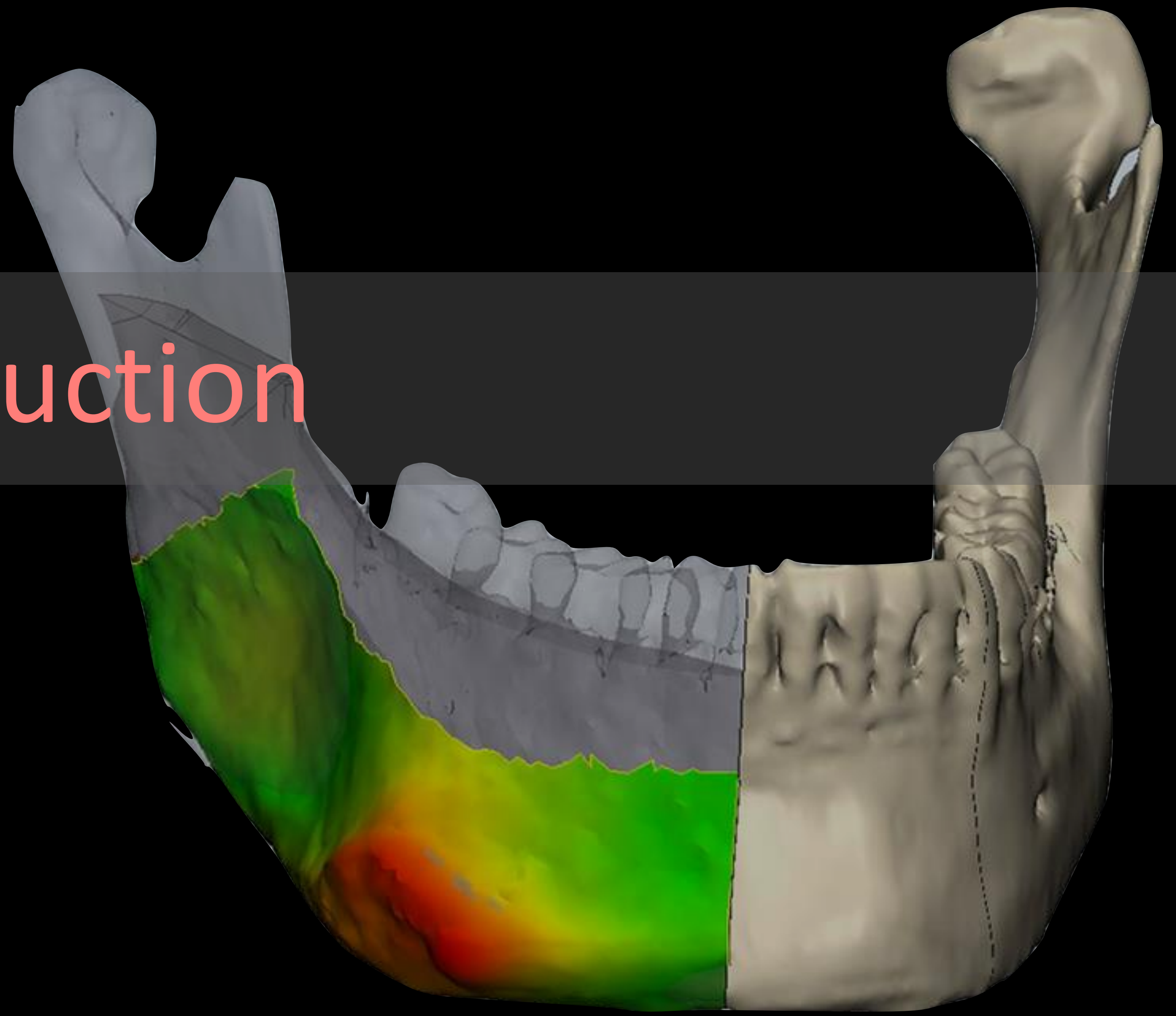


TRAUMA

BONE AUGMENTATION

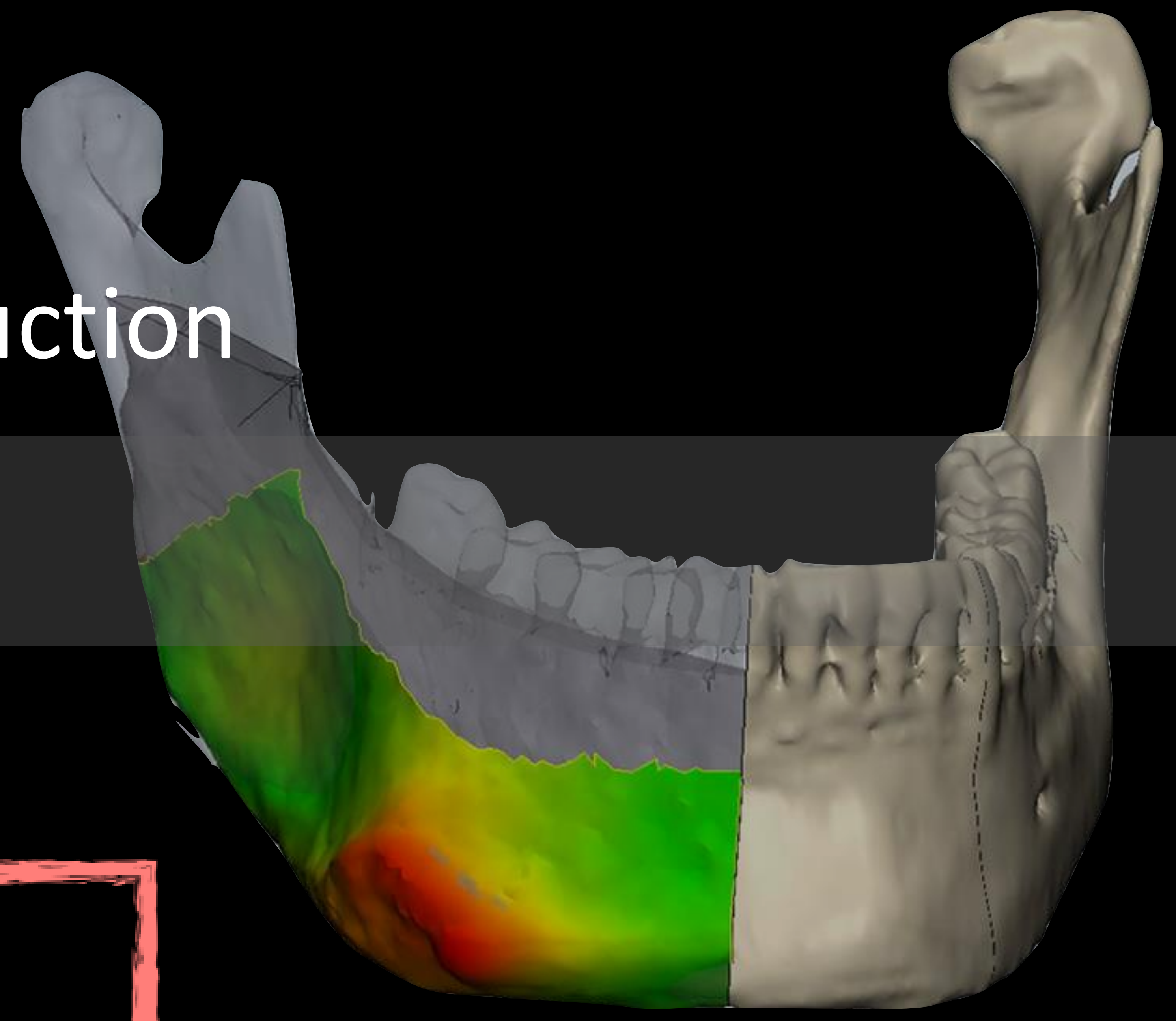
Current gold standard in HN reconstruction





Reconstruction in head and neck surgery

Current gold standard in HN reconstruction



Is this always possible?

MAIN ISSUES:

Need for specific expertise

Relatively long surgery

Donor site morbidity

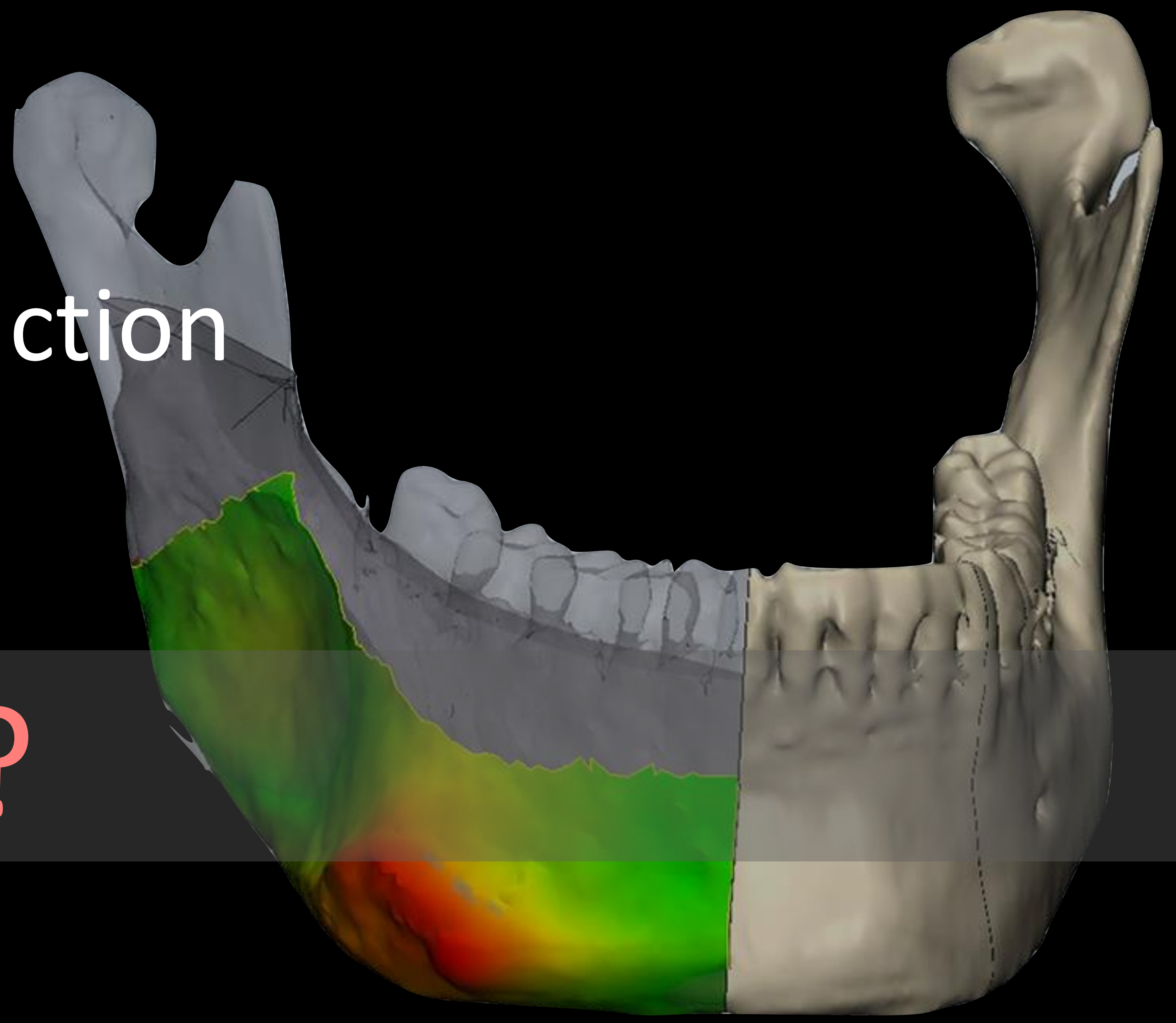
Risk of total flap failure

Reconstruction in head and neck surgery

Current gold standard in HN reconstruction

Is this always possible?

Are there alternatives?

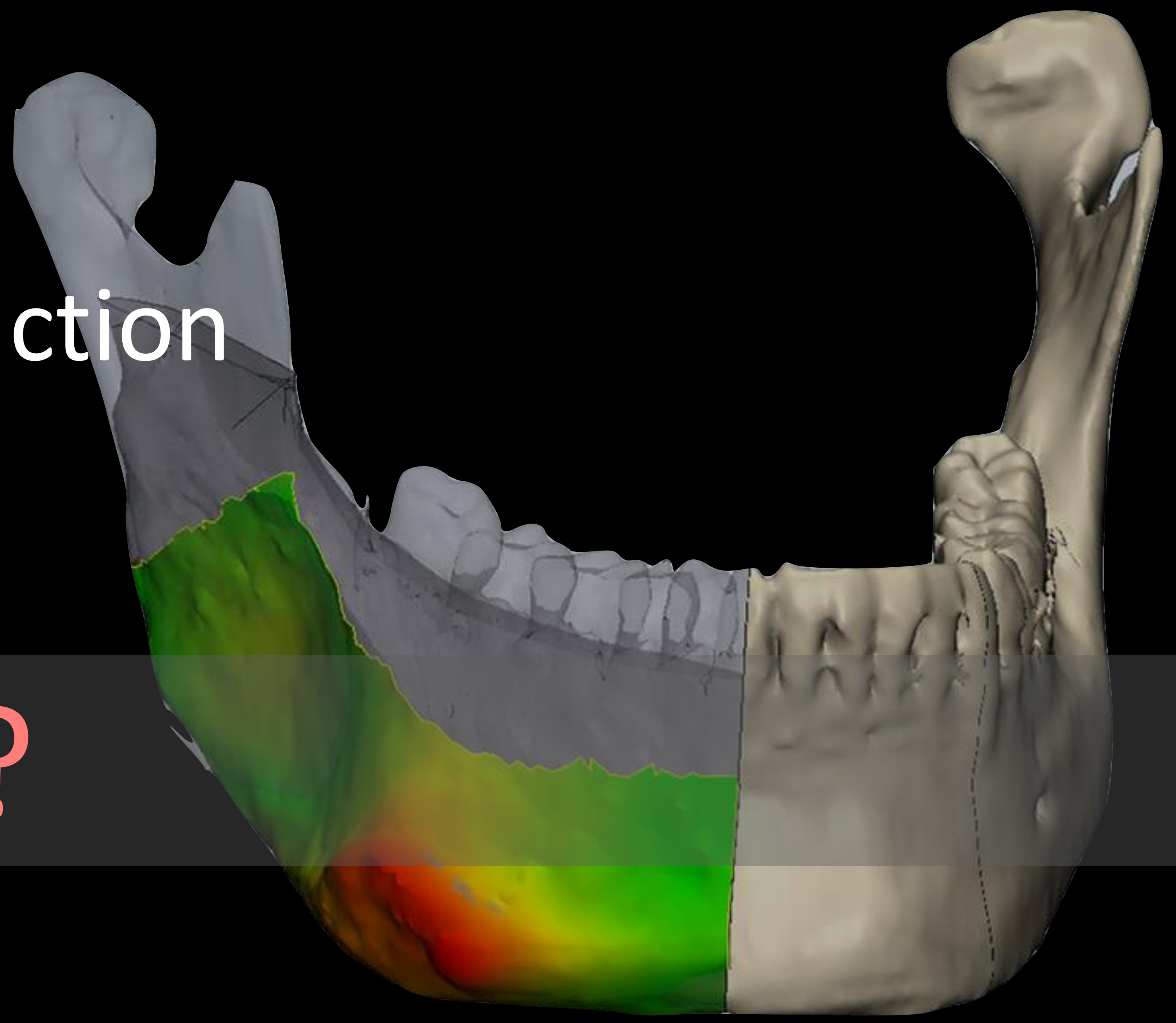


Reconstruction in head and neck surgery

Current gold standard in HN reconstruction

Is this always possible?

Are there alternatives?



.. NOT YET

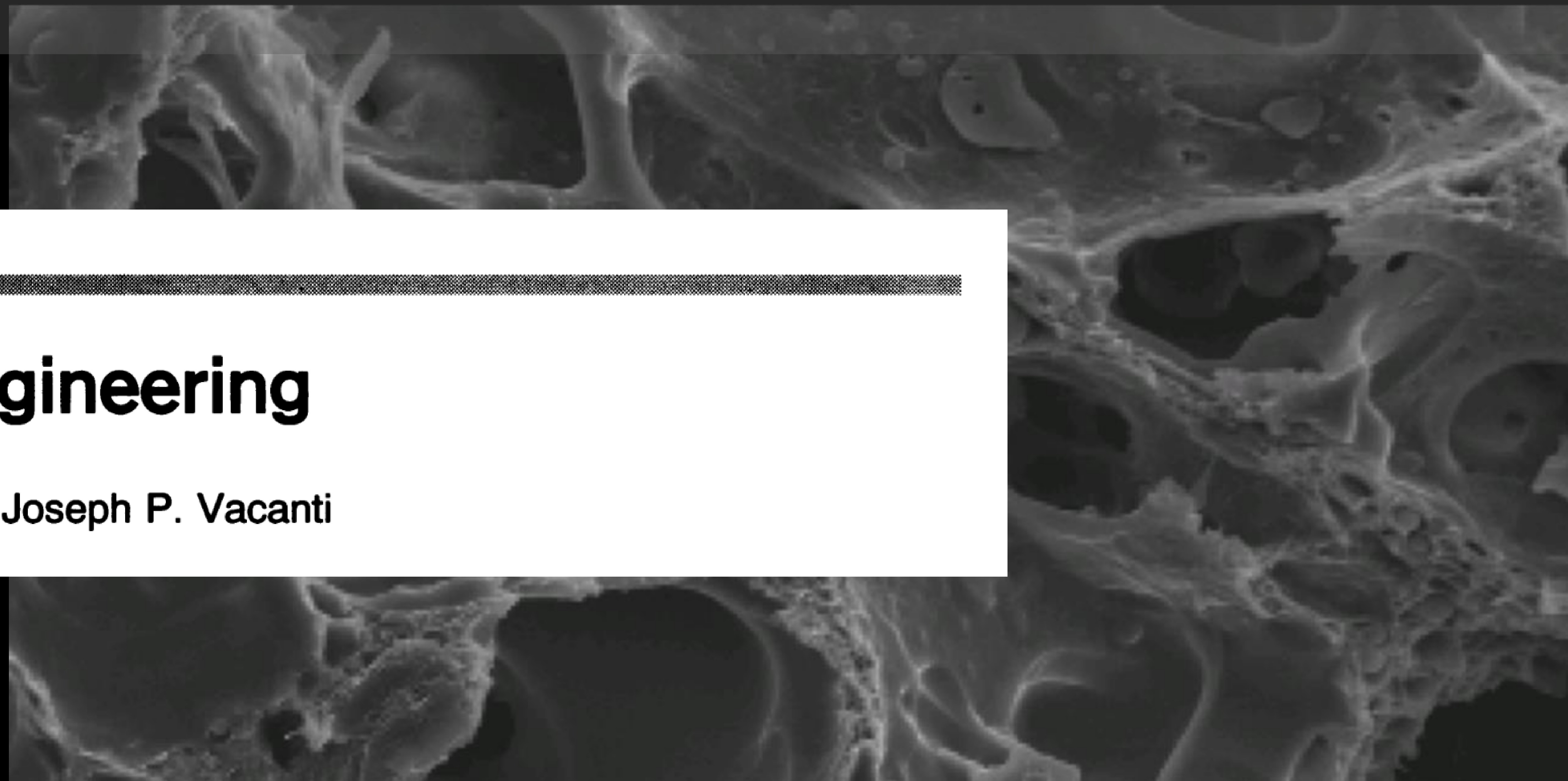
Reconstruction in head and neck surgery

Current gold standard in HN reconstruction

Is this always possible?

Are there valid alternatives?

BONE TISSUE ENGINEERING



ARTICLES

Tissue Engineering

Robert Langer* and Joseph P. Vacanti

Seminal paper, 1993

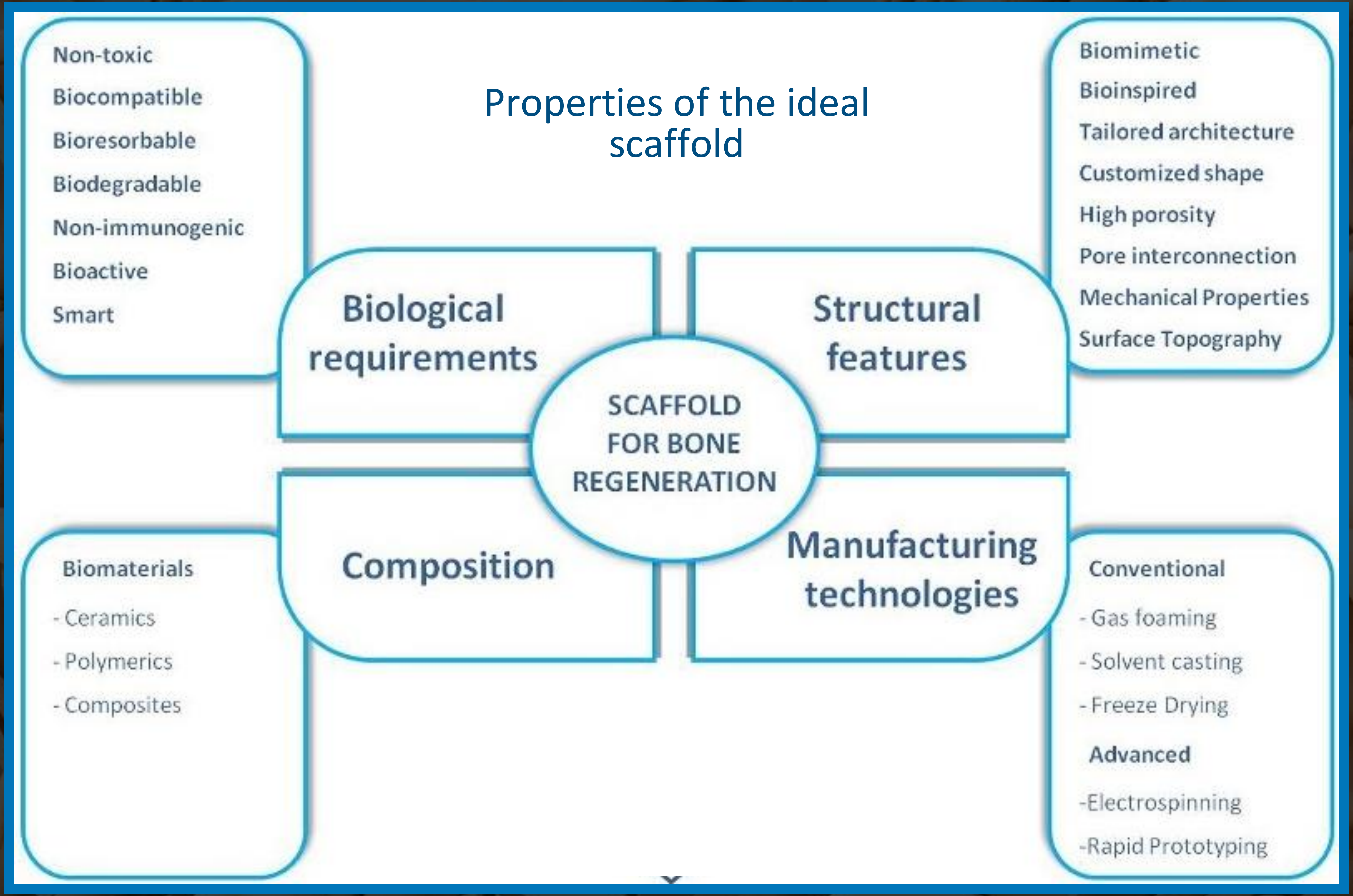
ARTICLES

Tissue Engineering

Robert Langer* and Joseph P. Vacanti

The loss or failure of an organ or tissue is one of the most frequent, devastating, and costly problems in human health care. A new field, tissue engineering, applies the principles of biology and engineering to the development of functional substitutes for damaged tissue. This article discusses the foundations and challenges of this interdisciplinary field and its attempts to provide solutions to tissue creation and repair.

Essential ingredients of tissue regeneration



"Functional scaffold"

Review

Functional Scaffolds for Bone Tissue Regeneration: A Comprehensive Review of Materials, Methods, and Future Directions

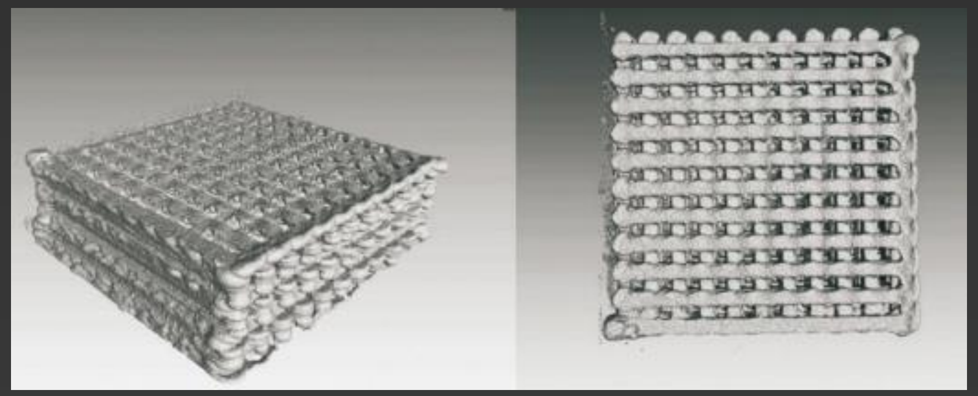
Emily Ann Todd [1], Nicholas A. Mirsky [1], Bruno Luís Graciliano Silva [2,3], Ankita Raja Shinde [2,4], Aris R. L. Arakelians [5], Vasudev Vivekanand Nayak [6], Rosemary Adriana Chiérici Marcantonio [3], Nikhil Gupta [4], Lukasz Witek [2,7,8] and Paulo G. Coelho [5,6,*]

Journal of Functional Biomaterials, 2024

Essential ingredients of tissue regeneration

“Functional scaffold”

Biomaterials



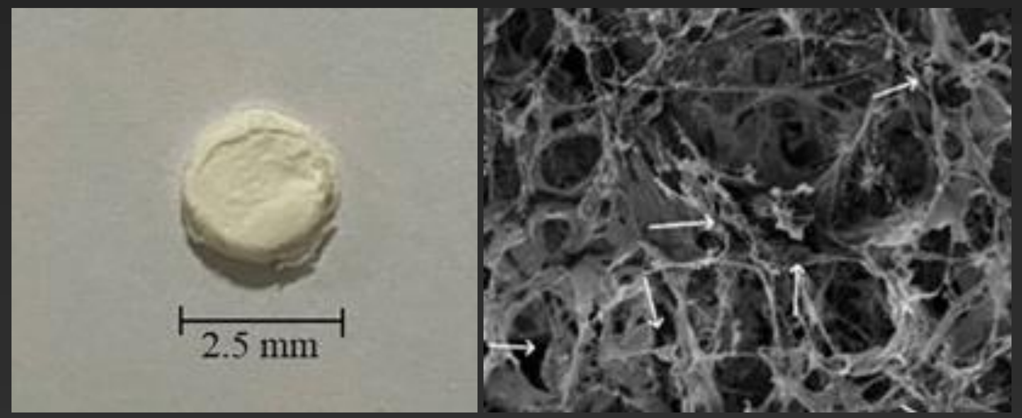
Bioceramics *(hydroxyapatite, TCP..)*

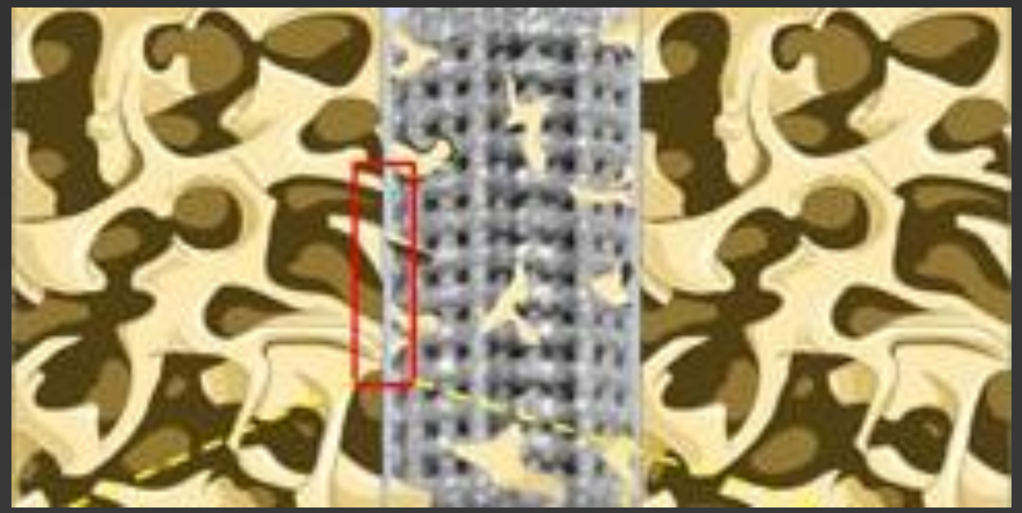
Synthetic polymers *(PLA, PCL)*

Natural polymers *(chitosan, fibrin, hylyronic acid, collagen)*

Metals

Hydrogels





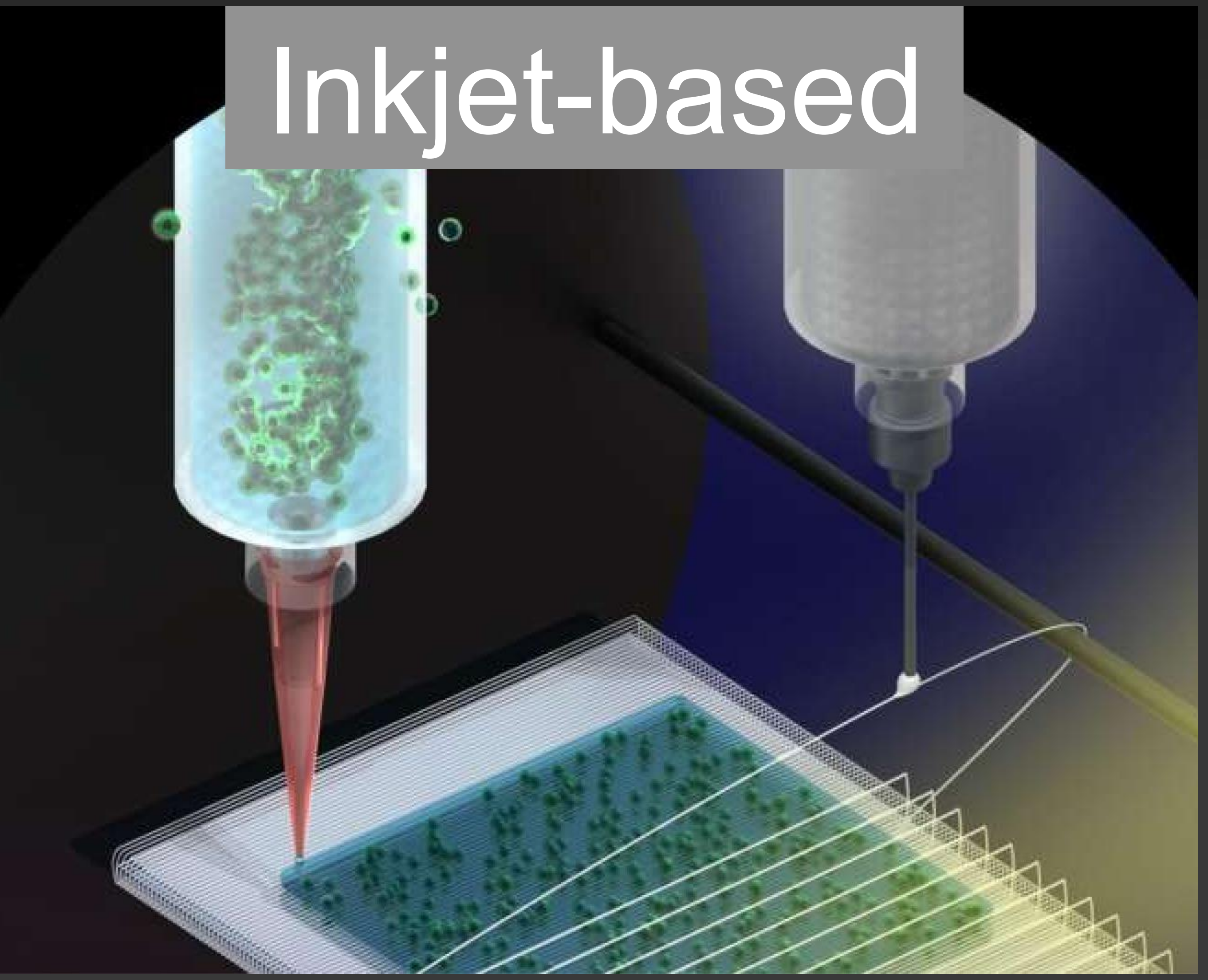
Functional Scaffolds for Bone Tissue Regeneration: A Comprehensive Review of Materials, Methods, and Future Directions, *Journal of Functional Biomaterals, 2024*

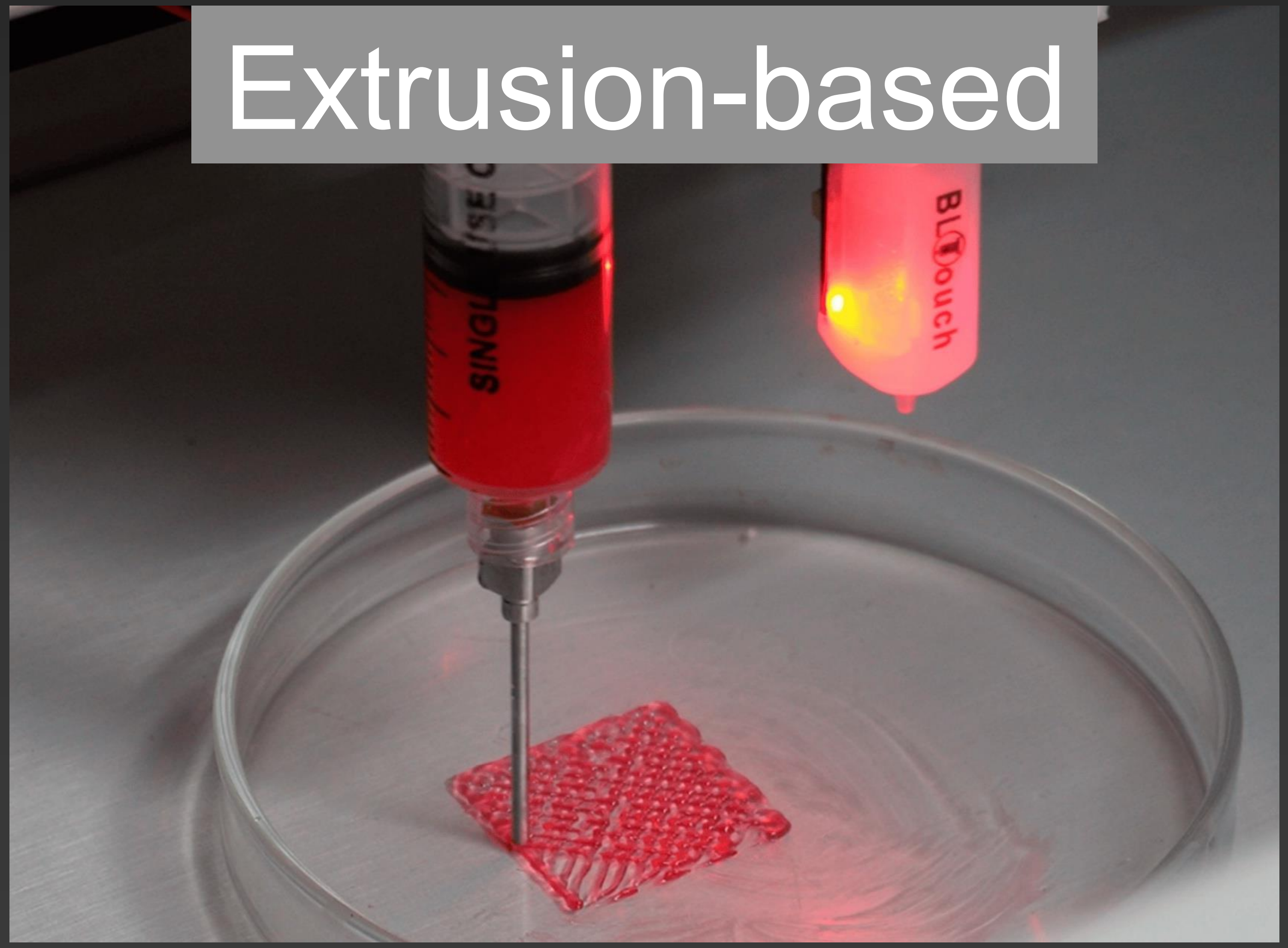
Essential ingredients of tissue regeneration

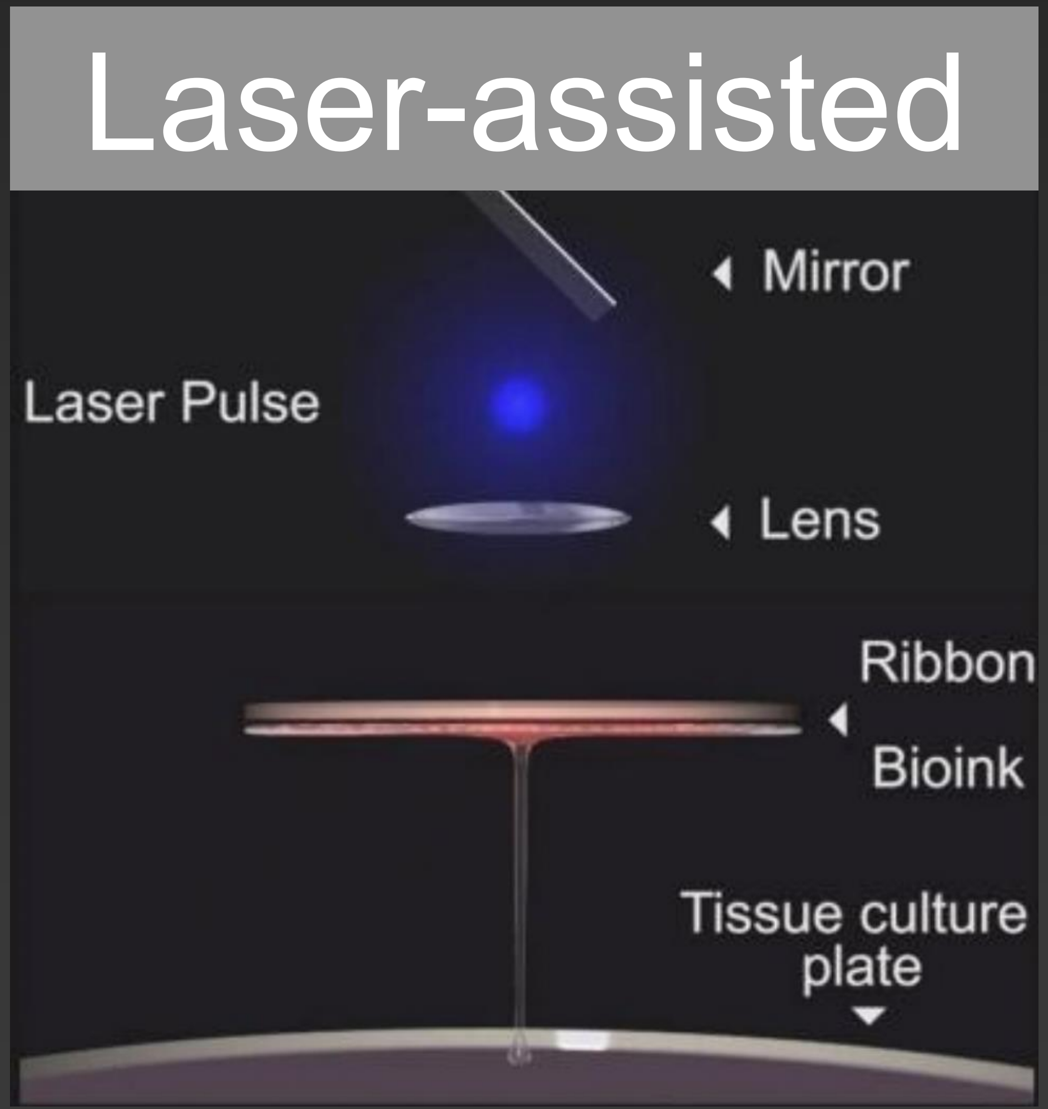
“Functional scaffold”

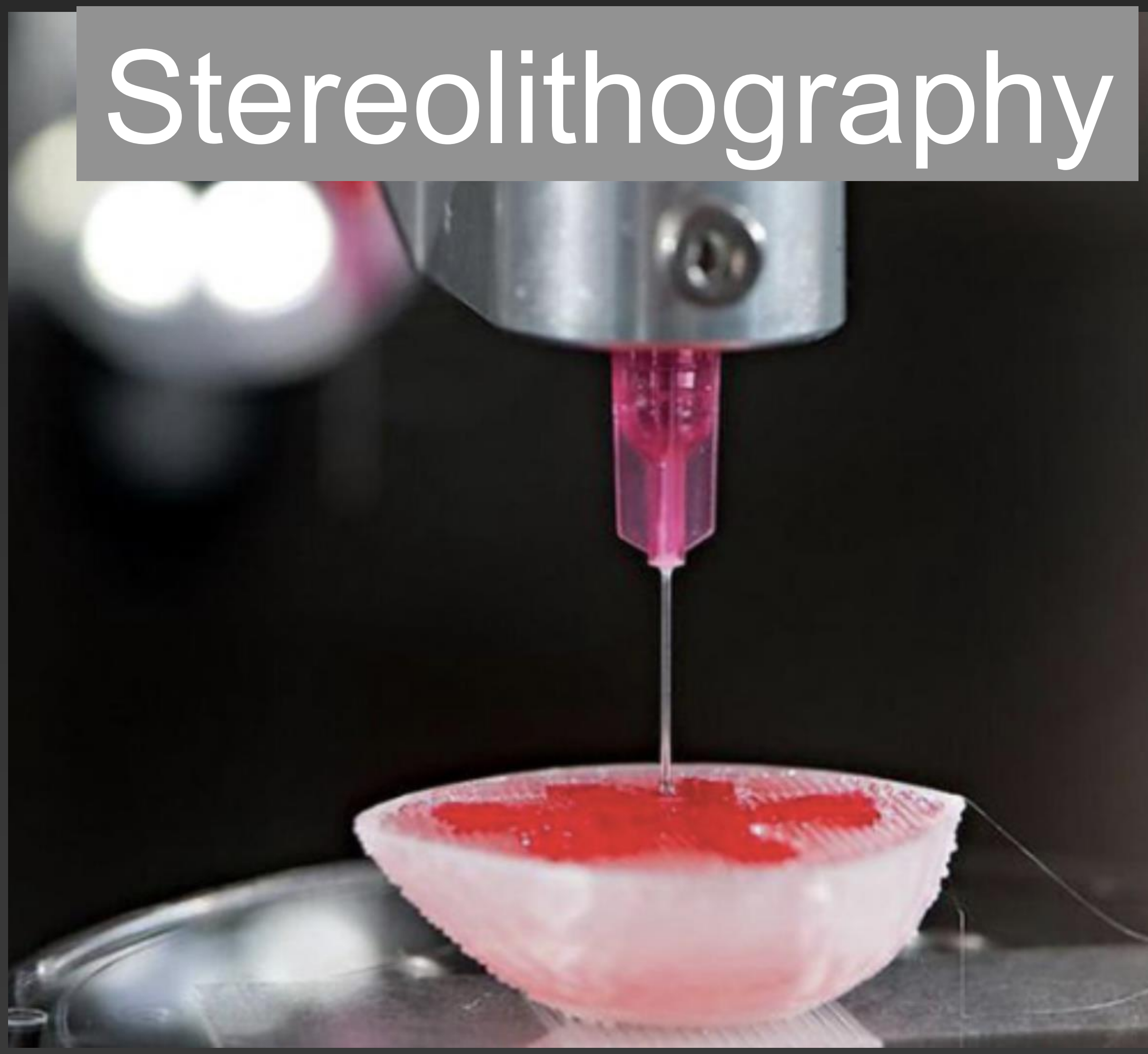
Biomaterials

3D printing









Functional Scaffolds for Bone Tissue Regeneration: A Comprehensive Review of Materials, Methods, and Future Directions, *Journal of Functional Biomaterals, 2024*

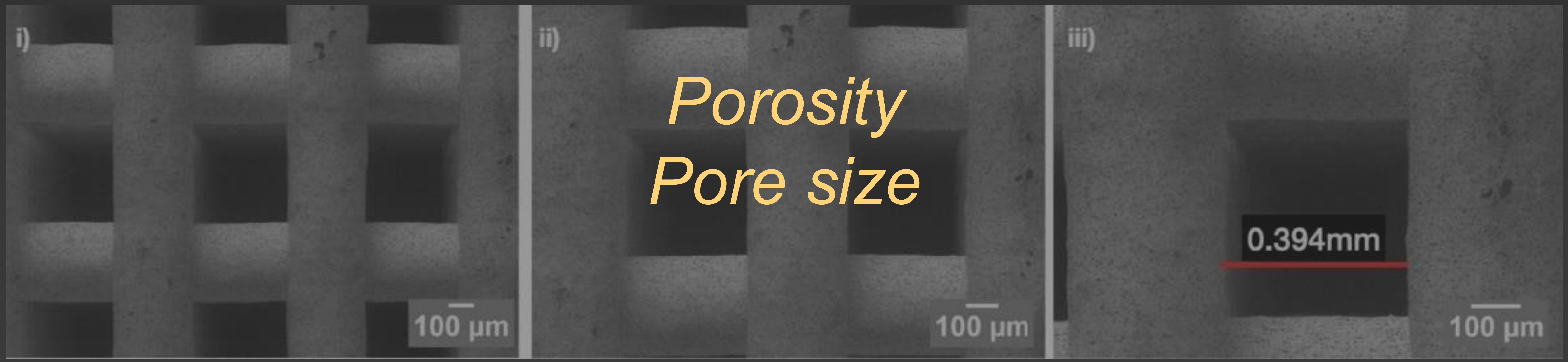
Essential ingredients of tissue regeneration

“Functional scaffold”

Biomaterials

3D printing

Microarchitectural Design and Surface Topography



Functional Scaffolds for Bone Tissue Regeneration: A Comprehensive Review of Materials, Methods, and Future Directions, *Journal of Functional Biomaterals, 2024*

Essential ingredients of tissue regeneration

"Functional scaffold"

Biomaterials

3D printing

Microarchitectural Design and
Surface Topography

Bioactive molecules

Functional Scaffolds for Bone Tissue Regeneration: A Comprehensive Review of Materials, Methods, and Future Directions, *Journal of Functional Biomaterals, 2024*

Essential ingredients of tissue regeneration

“Functional scaffold”

Biomaterials

3D printing

Microarchitectural Design and
Surface Topography

Bioactive molecules

BMP2 and BMP7, IGF,
VEGF, PDGF,

Functional Scaffolds for Bone Tissue Regeneration: A Comprehensive Review of Materials, Methods, and Future Directions,
Journal of Functional Biomaterals, 2024

Essential ingredients of tissue regeneration

“Functional scaffold”

Biomaterials

3D printing

Microarchitectural Design and Surface Topography

Bioactive molecules

Incorporation of stem cells

Functional Scaffolds for Bone Tissue Regeneration: A Comprehensive Review of Materials, Methods, and Future Directions, *Journal of Functional Biomaterals, 2024*

BONE TISSUE ENGINEERING

BIOMATERIALS

Essential ingredients of tissue regeneration

STEM CELLS

STIMULATING FACTORS

Current application

SCIENCE ADVANCES | REVIEW 2024

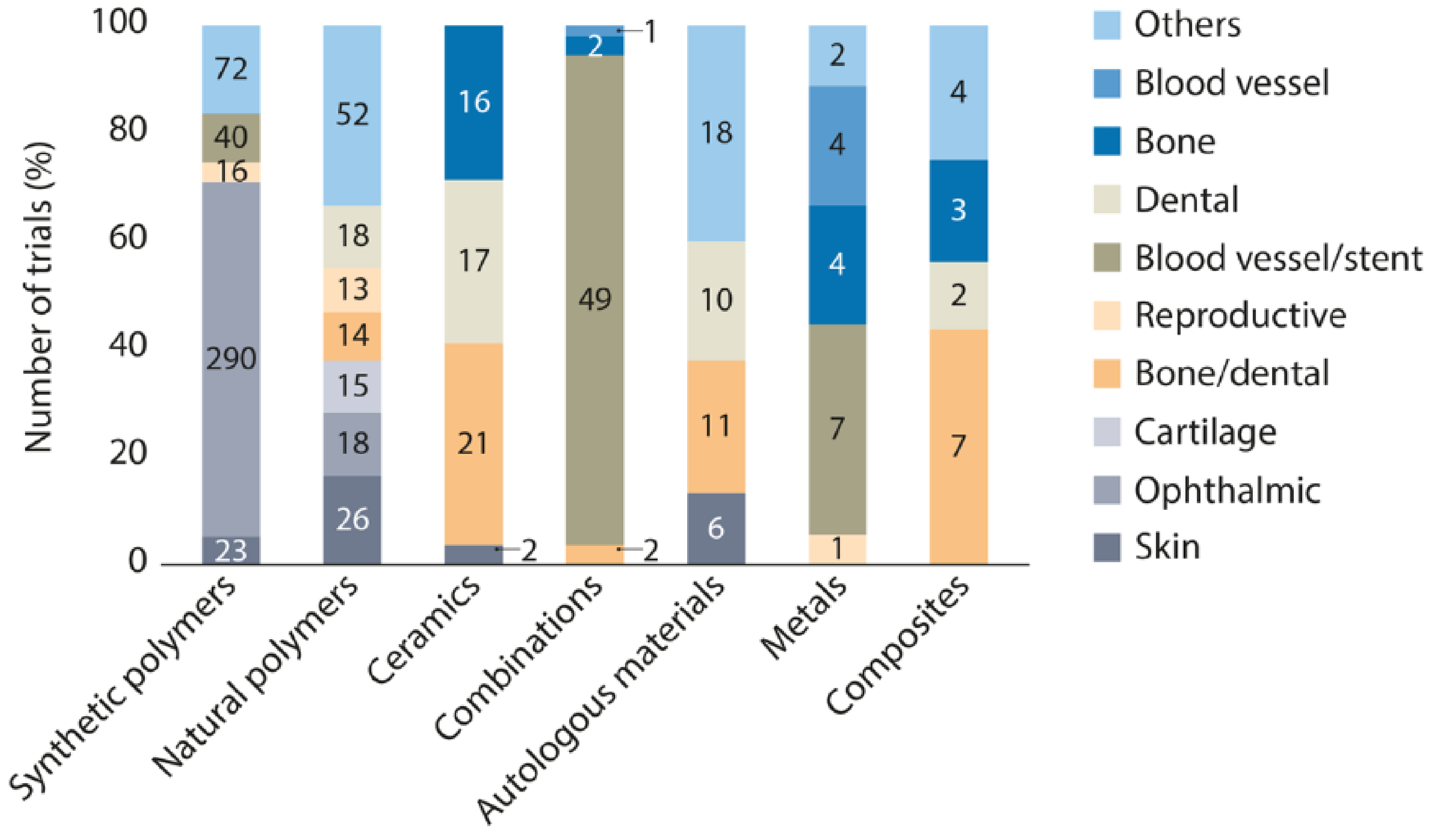
APPLIED SCIENCES AND ENGINEERING

Global trends in clinical trials involving engineered biomaterials

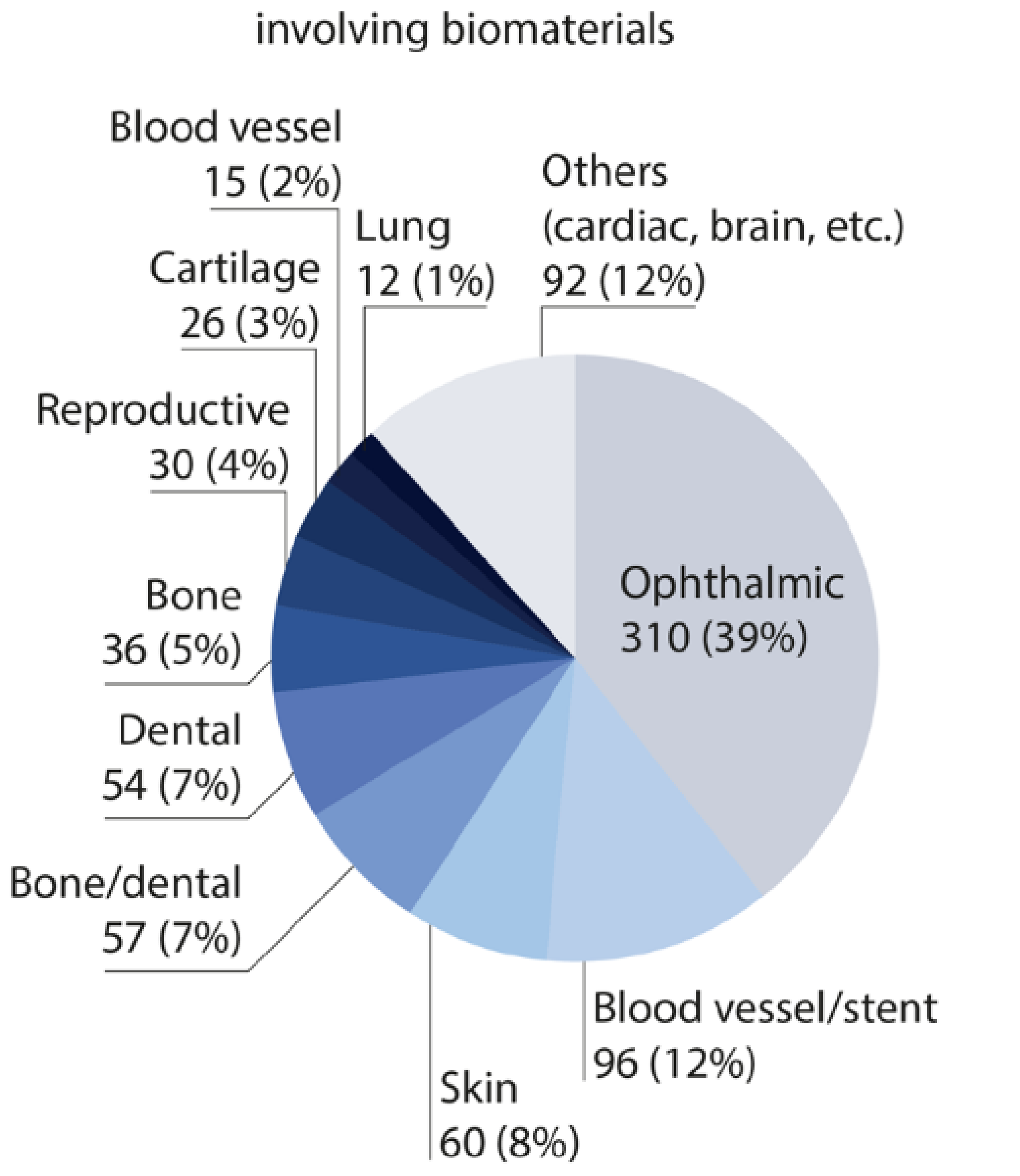
Mahima Lele[1], Shaunak Kapur[2], Sarah Hargett[3], Nivedhitha Malli Sureshbabu[3]*, Akhilesh K. Gaharwar[3,4,5,6]*

Comprehensive analysis global trial trends involving biomaterials
Included 834 CTs

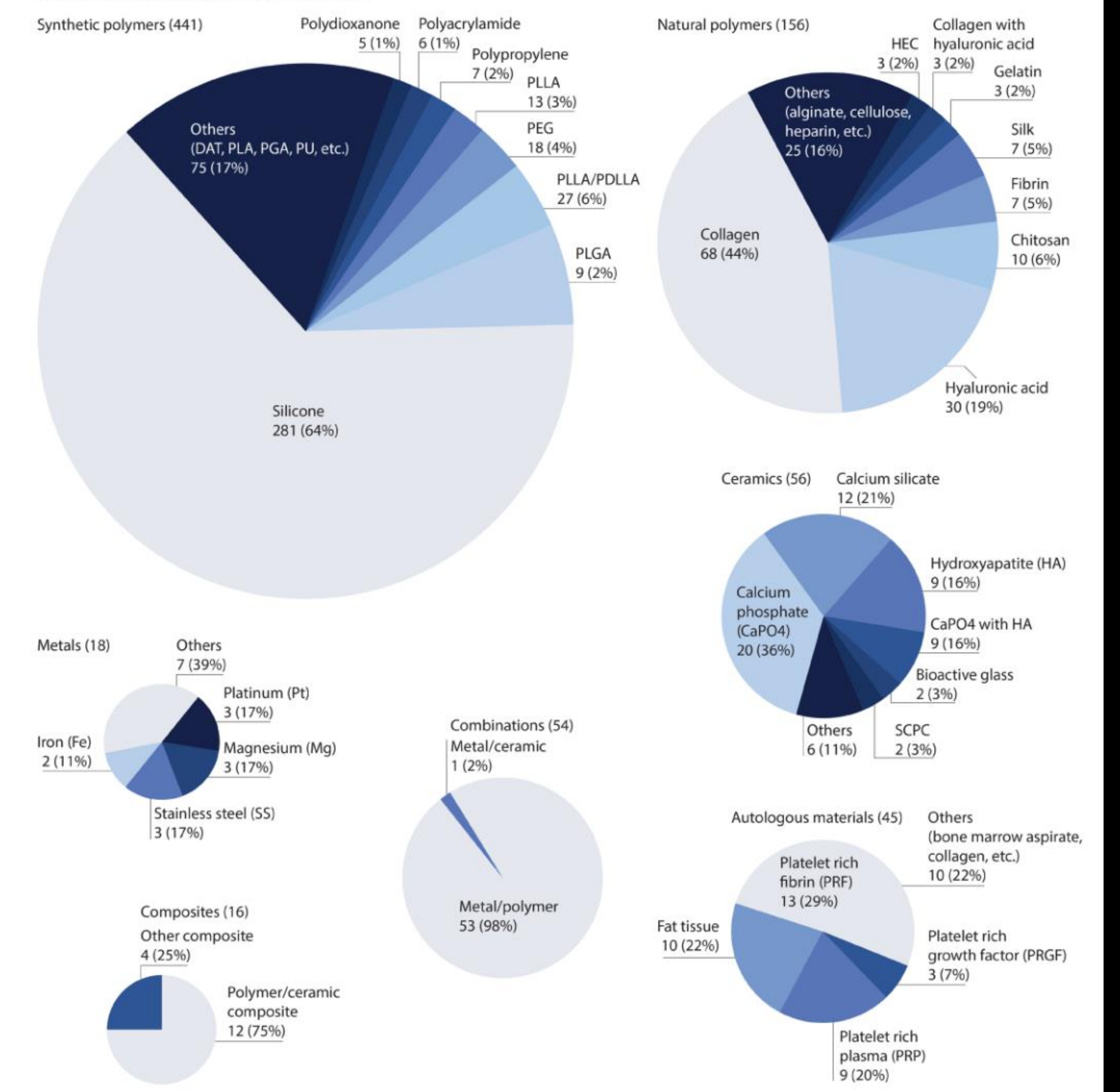
Biomaterial vs target tissue



Target tissue distribution

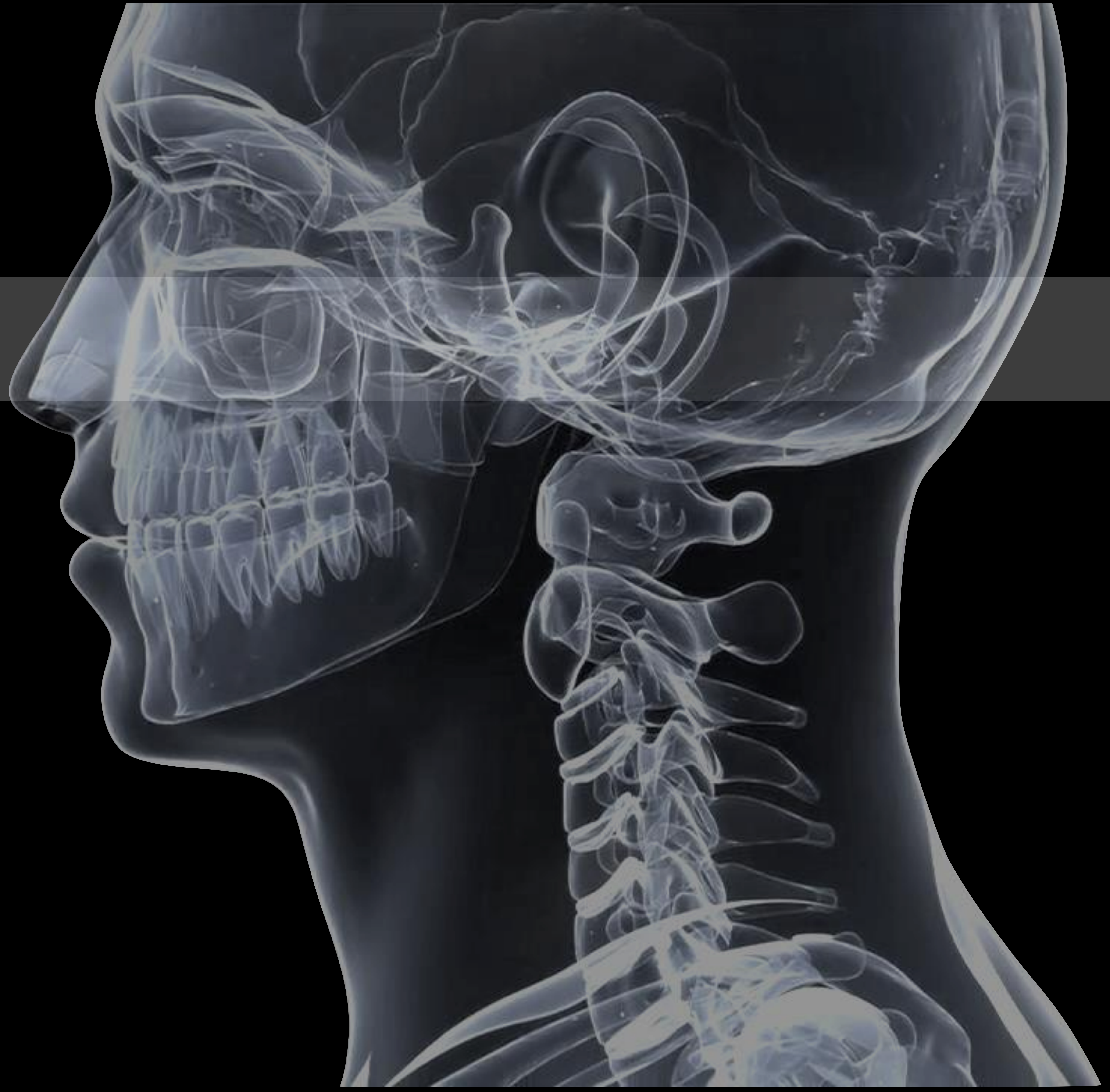


Distribution of biomaterials



Bioengineered scaffolds in head and neck surgery

- Background - literature review
- Research updates
- Ancillary projects

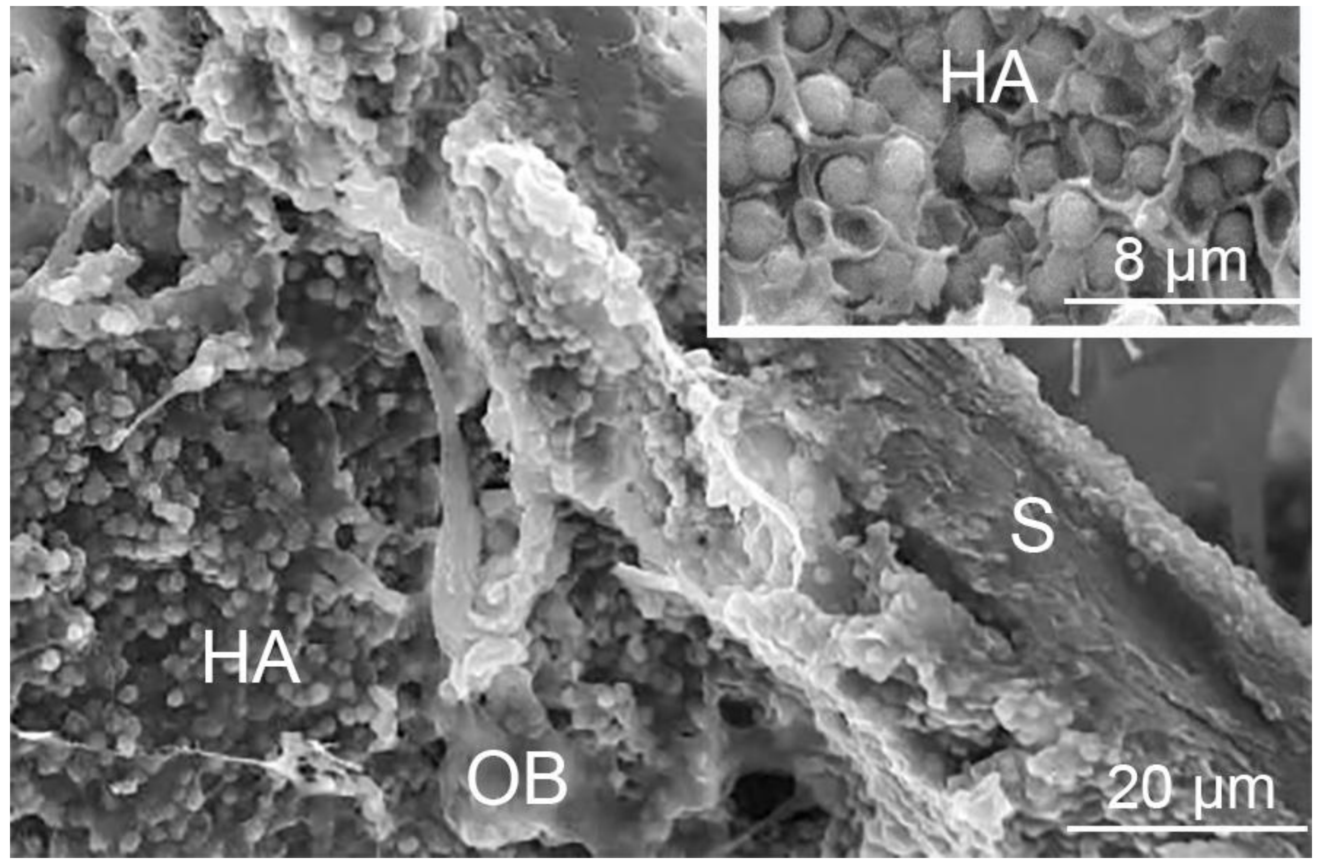


A collaborative international project

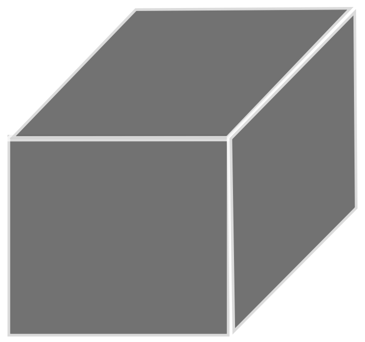
UHN
Toronto General
Toronto Western
Princess Margaret
Toronto Rehab
Michener Institute

UNIVERSITÀ DEGLI STUDI DI BRESCIA

800 ANNI 1222·2022
UNIVERSITÀ DEGLI STUDI DI PADOVA



To develop a model for bone regeneration using bioengineered scaffolds and stem cells



UHN
Toronto General
Toronto Western
Princess Margaret
Toronto Rehab
Michener Institute

Essential ingredients of tissue regeneration

BIOMATERIALS

Chitosan-polyethylene glycol

Blend of PLA-PCL-chitosan-polyethylene glycol

STEM CELLS

hMSC

rMSC

STIMULATING FACTORS

BMP2

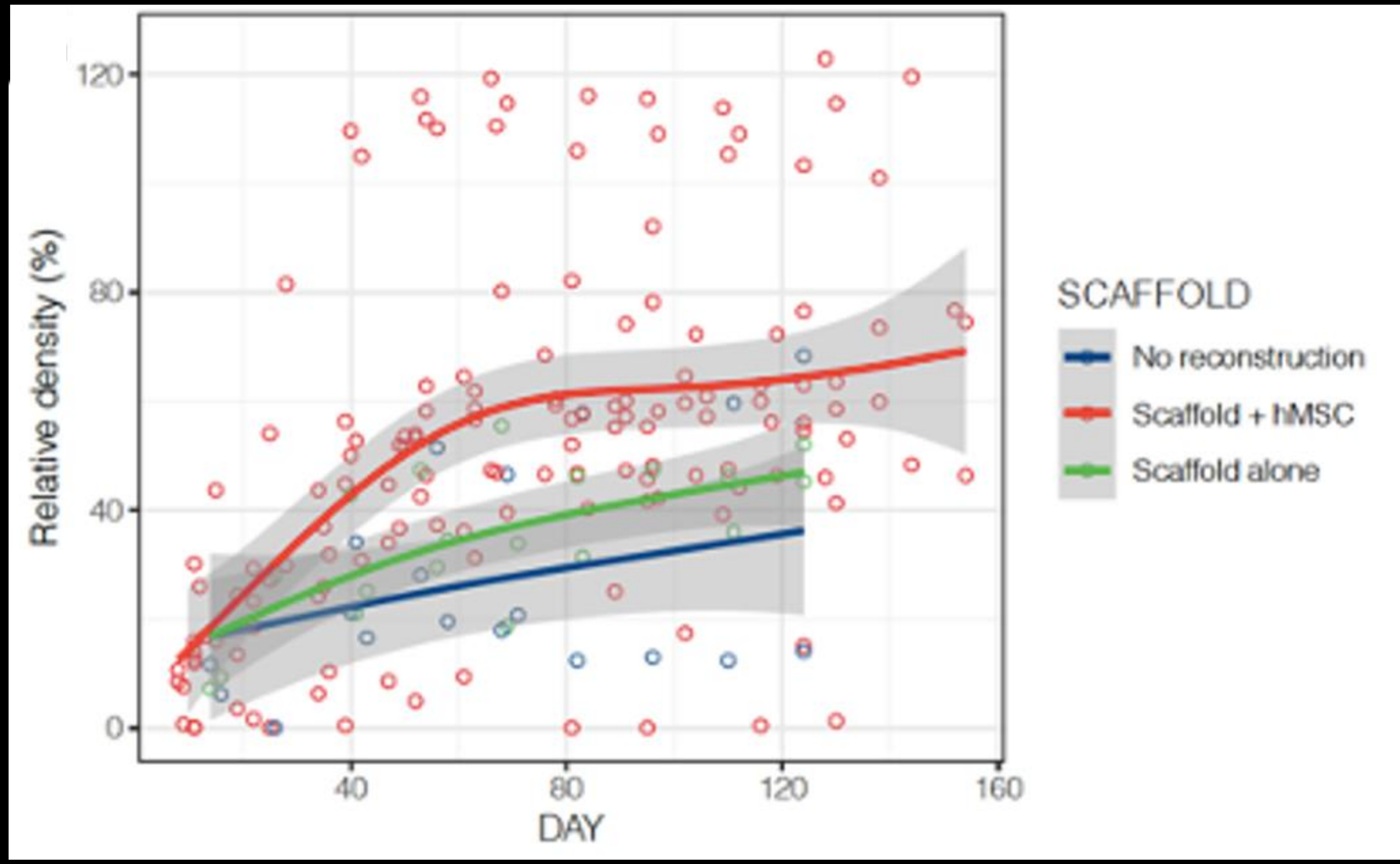
Our experience: results

Restoration of the presurgical bone density in 120 days

frontiers | Frontiers in Bioengineering and Biotechnology 2024

Hydrogel-chitosan and polylactic acid-polycaprolactone bioengineered scaffolds for reconstruction of mandibular defects: a preclinical *in vivo* study with assessment of translationally relevant aspects

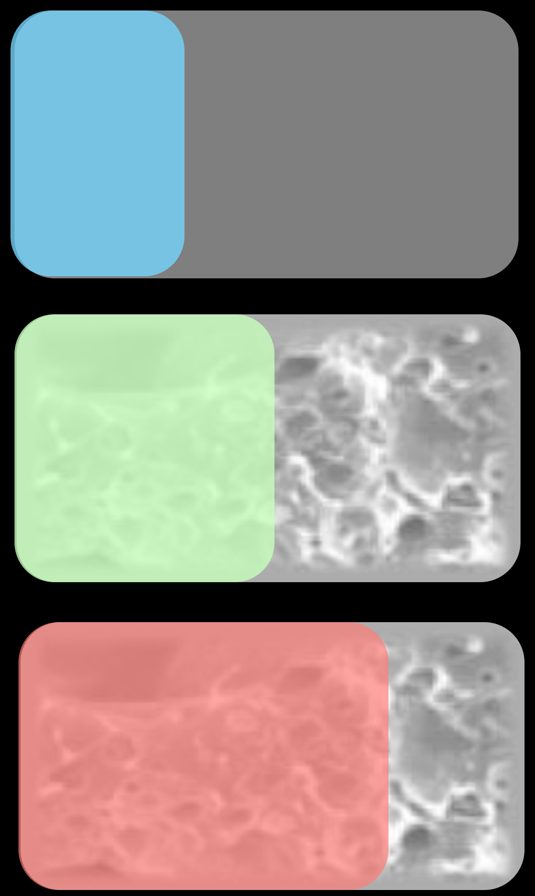
Marco Ferrari[1,2,3*†], Stefano Taboni[1,2,3,4*†], Harley H. L. Chan[5], Jason Townson[5], Tommaso Gualtieri[1,6], Leonardo Franz[1,2], Alessandra Ruaro[1,2,3], Smitha Mathews[7], Michael J. Daly[5], Catriona M. Douglas[5,8,9], Donovan Eu[5,8,10], Axel Sahovaler[5,8,11], Nidal Muhanna[5,12], Manuela Ventura[13,14], Kamol Dey[15,16], Stefano Pandini[15], Chiara Pasini[15], Federica Re[17,18], Simona Bernardi[17,18], Katia Bosio[17,18], Davide Mattavelli[19,20], Francesco Doglietto[21,22], Shrinidh Joshi[7], Ralph W. Gilbert[8‡], Piero Nicolai[2,3‡], Sowmya Viswanathan[7‡], Luciana Sartore[15‡], Domenico Russo[17‡] and Jonathan C. Irish[5,8‡]



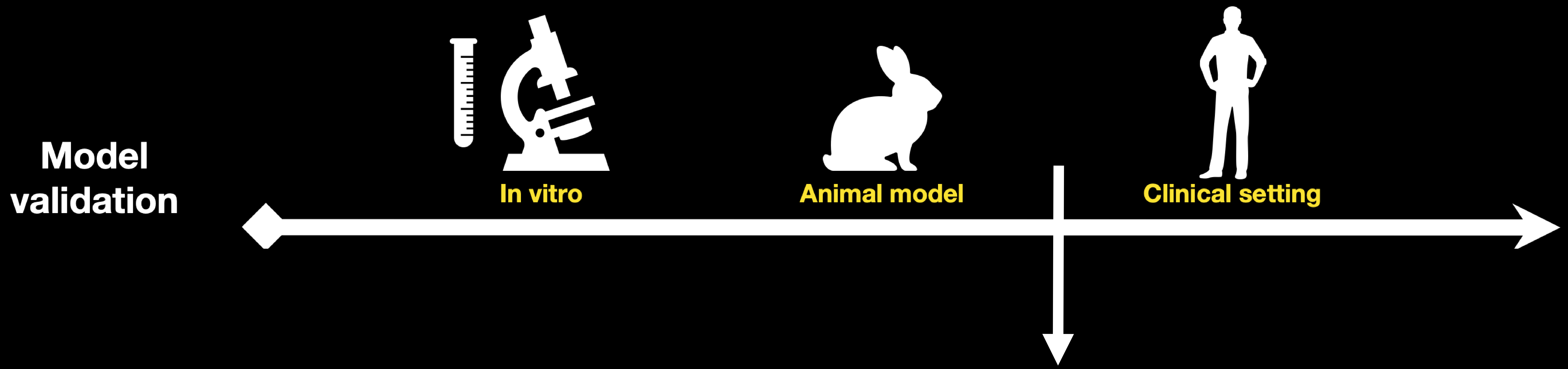
Spontaneous regeneration of bone 40%

Scaffold only- reconstruction 50%

hMSC Seeded scaffold-reconstruction 70%



How can we optimise our model?



Aims

↑ % of BONE REGENERATION

↓ TIMING

↑ DEFECT SIZE

Essential ingredients of tissue regeneration

BIOMATERIALS

2 new materials

STEM CELLS

STIMULATING FACTORS

1

3D PRINTED CORE-SHELL PLA-Hydrogel

Materials and microstructure

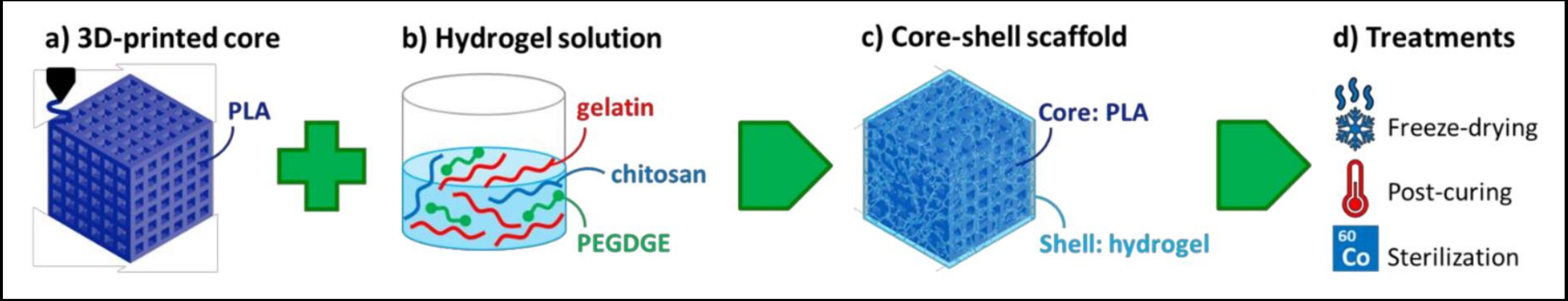
Hybrid
Core-shell structure
3D printed

polymers 2023

Article

New Poly(lactic acid)–Hydrogel Core–Shell Scaffolds Highly Support MSCs' Viability, Proliferation and Osteogenic Differentiation

Chiara Pasini [1], Stefano Pandini [1], Federica Re [2], Matteo Ferroni [3,4], Elisa Borsani [5], Domenico Russo [2] and Luciana Sartore [1,*]

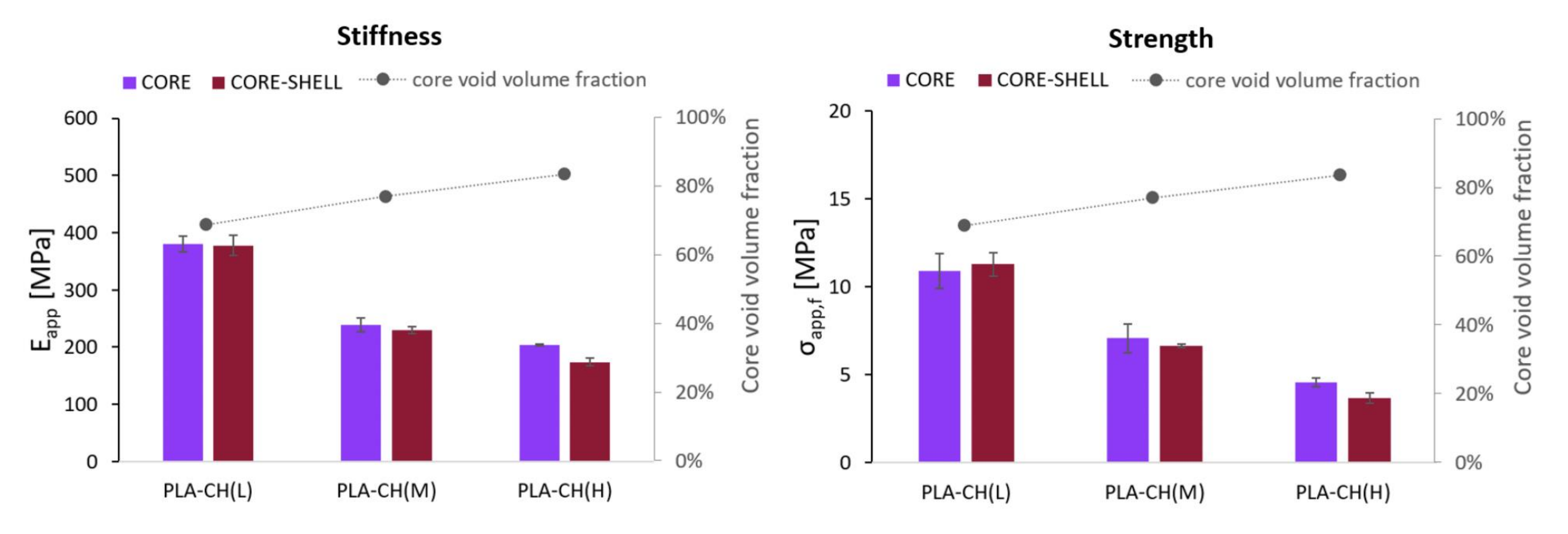


BIOMATERIALS

1

3D PRINTED CORE-SHELL PLA-Hydrogel

In vitro results

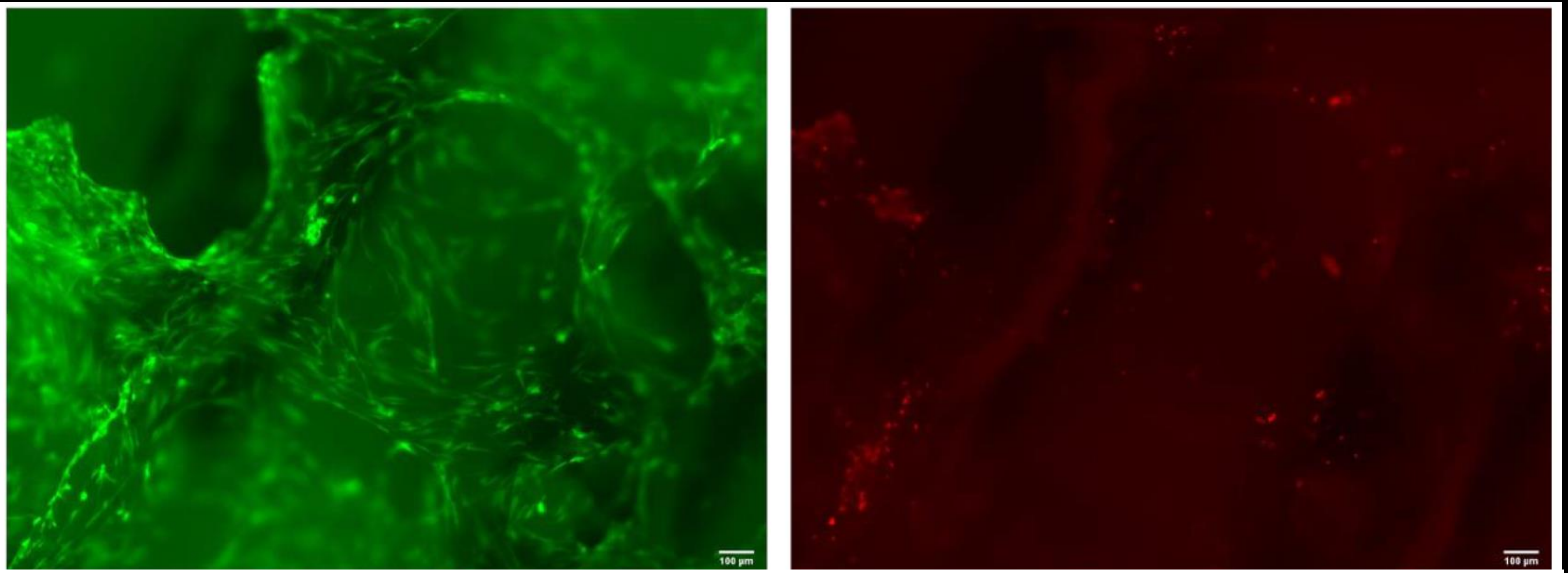


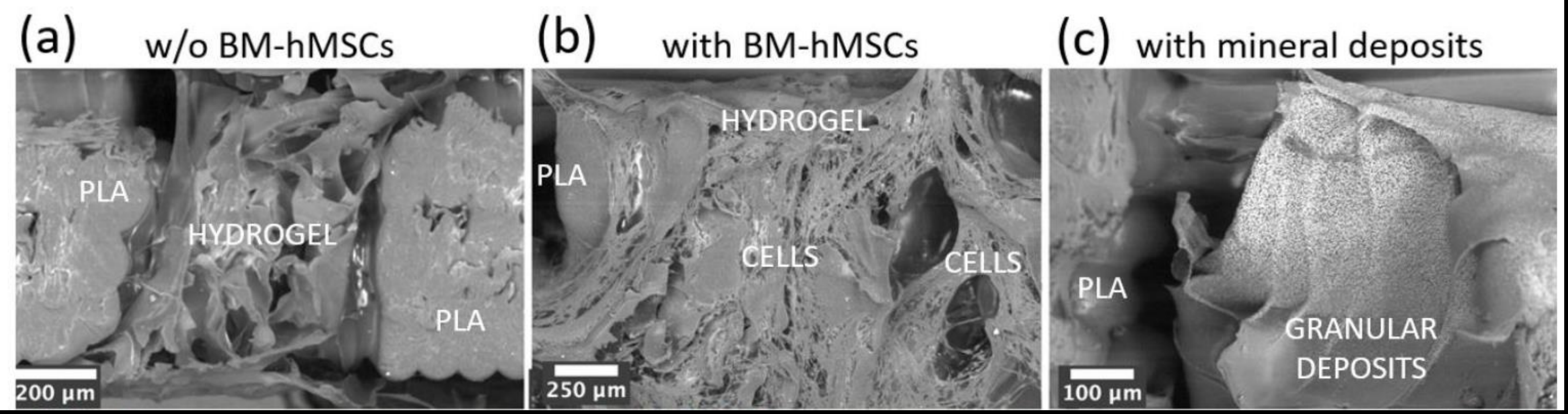
polymers 2023

Article

New Poly(lactic acid)–Hydrogel Core–Shell Scaffolds Highly Support MSCs' Viability, Proliferation and Osteogenic Differentiation

Chiara Pasini [1], Stefano Pandini [1], Federica Re [2], Matteo Ferroni [3,4], Elisa Borsani [5], Domenico Russo [2] and Luciana Sartore [1,*]



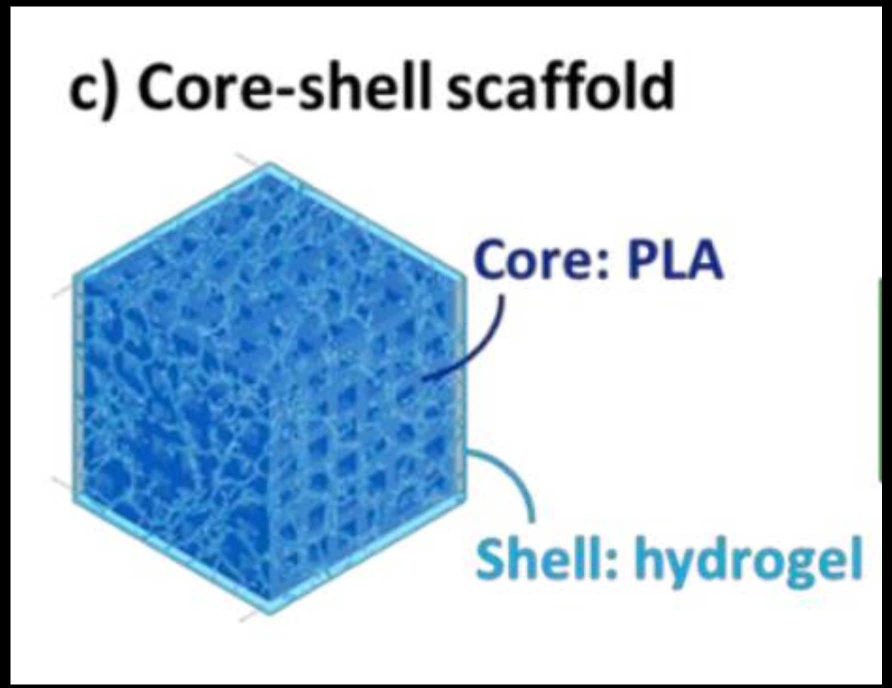


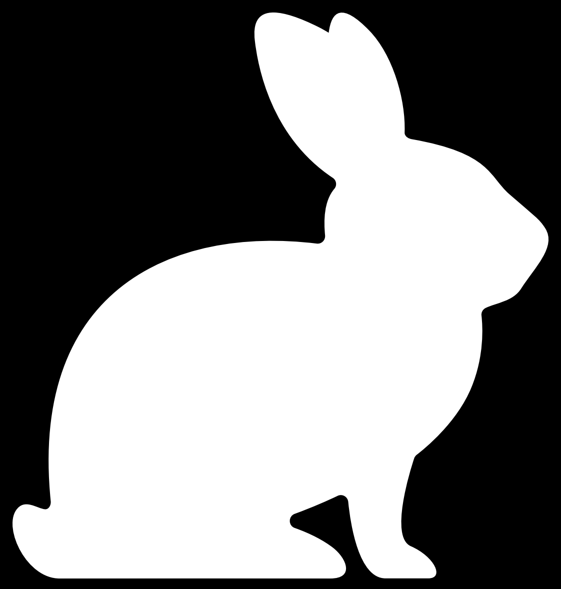
1

3D PRINTED CORE-SHELL PLA-Hydrogel

In vivo experiments

Study groups
1. Defect only
2. Scaffold reconstruction
3. hMSC-seeded scaffold reconstruction



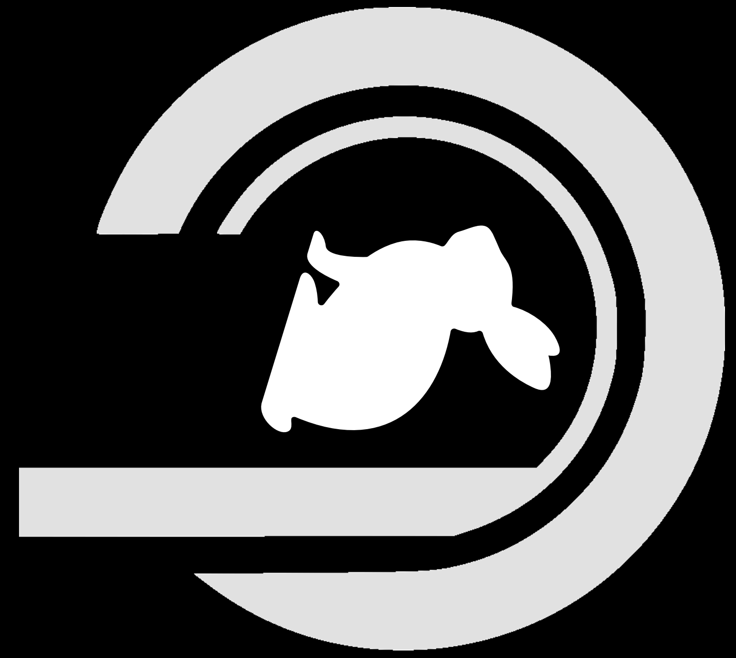


Every 2 weeks

For 5 months

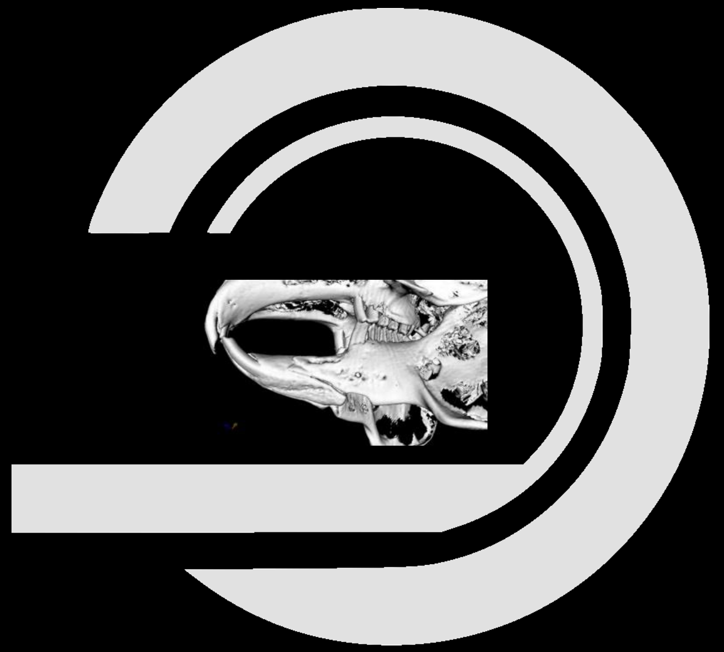
In Vivo phase

CE-CT scan

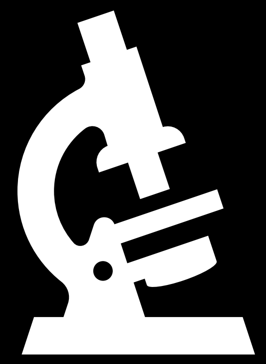


Euthanasia

Ex Vivo phase

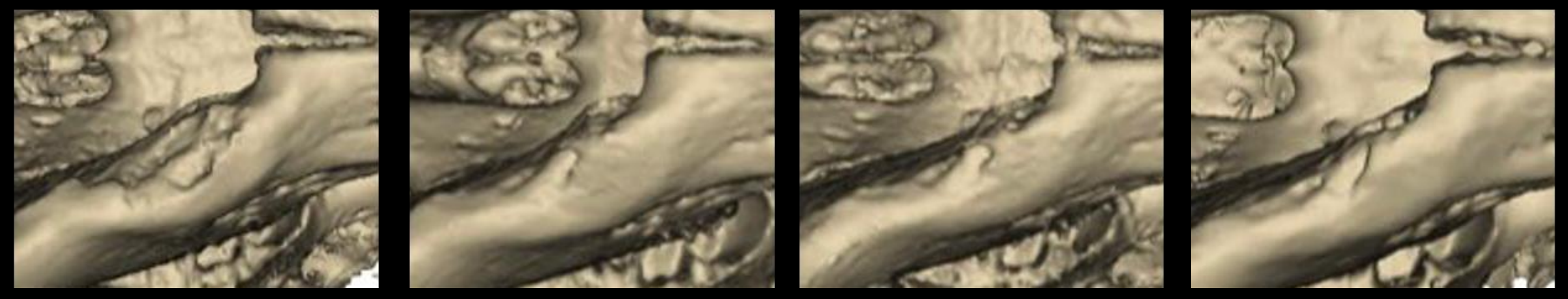


UltraHD CT scan



H&E
HC/IHC:
- TRAP
- HNA
- VEFG-A
- Bone-SP
- Osteocalcin
- Osteopontin

30 days | 60 days | 90 days | 120 days



Density of the surgical site (HU)

2

A NEW HYBRID CORE-SHELL SCAFFOLD: MONETITE + HYDROGEL

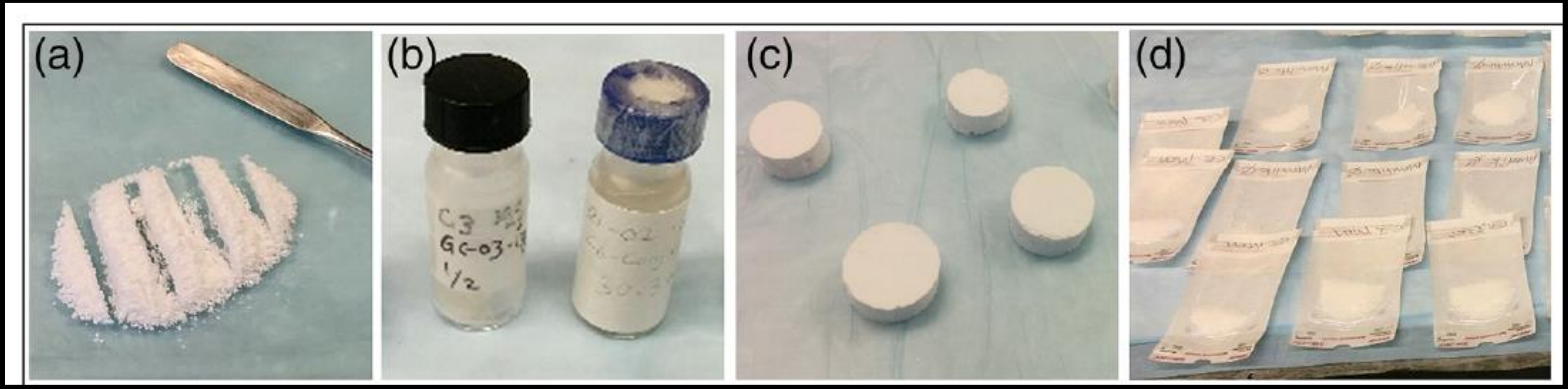
Dicalcium phosphates

Bioactivity

Bioconductivity

Completely resorbs and do not convert in hydroxyapatite

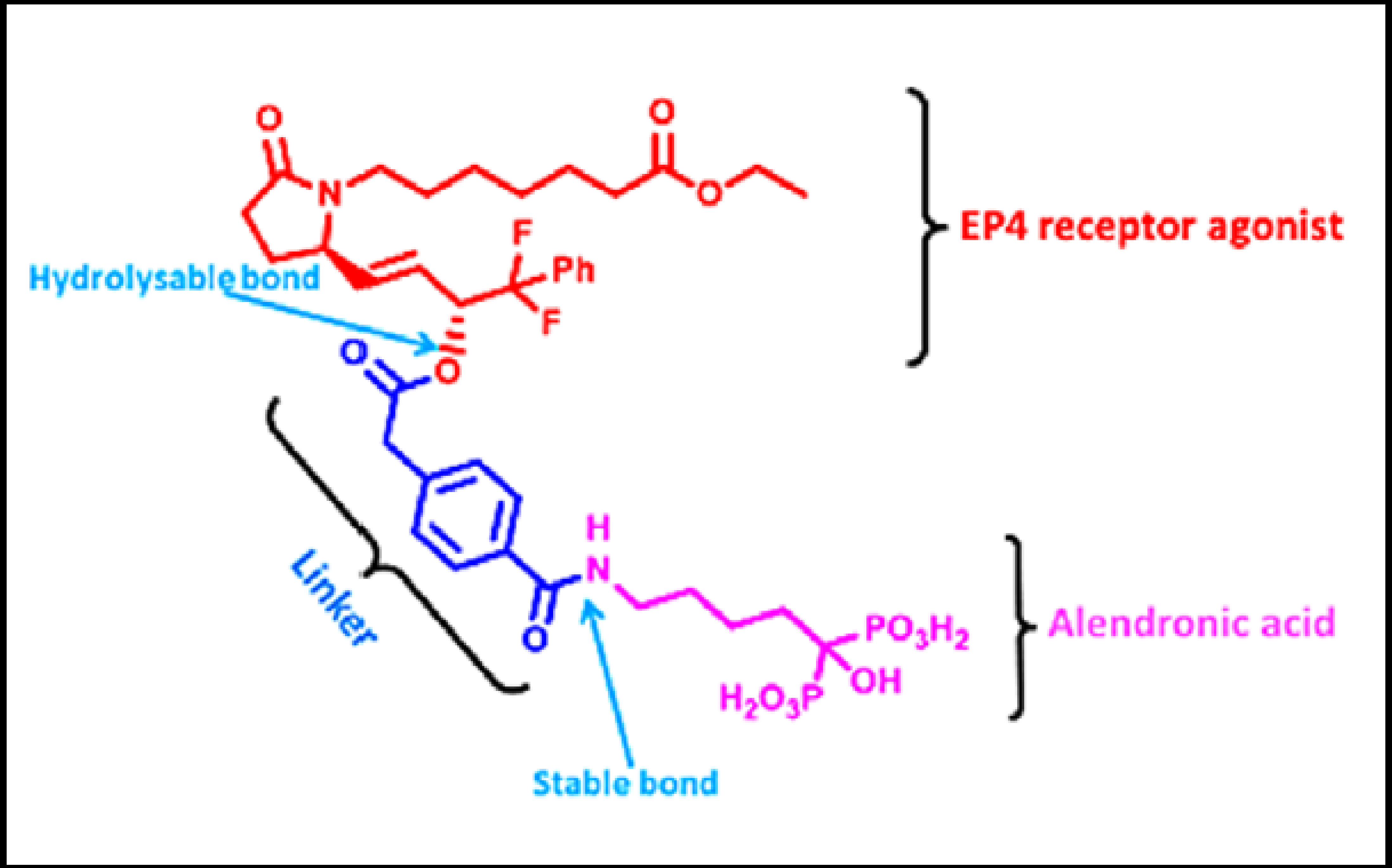
Lacks in osteoinductivity



2

A NEW HYBRID CORE-SHELL SCAFFOLD: MONETITE + HYDROGEL

+ stimulating factor promoting bone anabolism



EP4-selective agonist
induces anabolic effects by activating EP4 receptors in bone tissues

To avoid the systemic side-effects, the conjugated drugs is linked to inactive alendronic acid which has a strong bone targeting ability

2

A NEW HYBRID CORE-SHELL SCAFFOLD: MONETITE + HYDROGEL

+ stimulating factor promoting bone anabolism

Achieving enhanced bone regeneration using monetite granules with bone anabolic drug conjugates (C3 and C6) in rat mandibular defects

Zeeshan Sheikh[1,2,3,4**] | Mohamed-Nur Abdallah[1,2**] | Faik Al-Jaf[1] | Gang Chen[5] | Nader Hamdan[4] | Robert N. Young[5] | Marc D. Grynpas[2,3,6] | Michael Glogauer[1,7]

A Novel Anabolic Conjugate (C3) in the Matrix of Dicalcium Phosphate Onlay Block Grafts for Achieving Vertical Bone Augmentation: An Experimental Study on Rabbit Calvaria

Zeeshan Sheikh, Dip Dh, BDS, MSc, PhD[1]/Gang Chen, PhD[2]/Marion Thévenin, PhD[2]/
Robert N. Young, PhD[2]/Marc D. Grynpas, PhD[3]/Michael Glogauer, DDS, Dip Perio, PhD[1]

ORIGINAL ARTICLE JBMR PLUS Open Access ASBMR

In Vivo Bone Effects of a Novel Bisphosphonate-EP4a Conjugate Drug (C3) for Reversing Osteoporotic Bone Loss in an Ovariectomized Rat Model

Zeeshan Sheikh,[1,2,3,4] Gang Chen,[5] Faik Al-Jaf,[3] Marion Thévenin,[5] Kate Banks,[6,7] Michael Glogauer,[3,8]
Robert N Young,[5] and Marc D Grynpas[1,2,9]

RESULTS:

At 2 weeks:

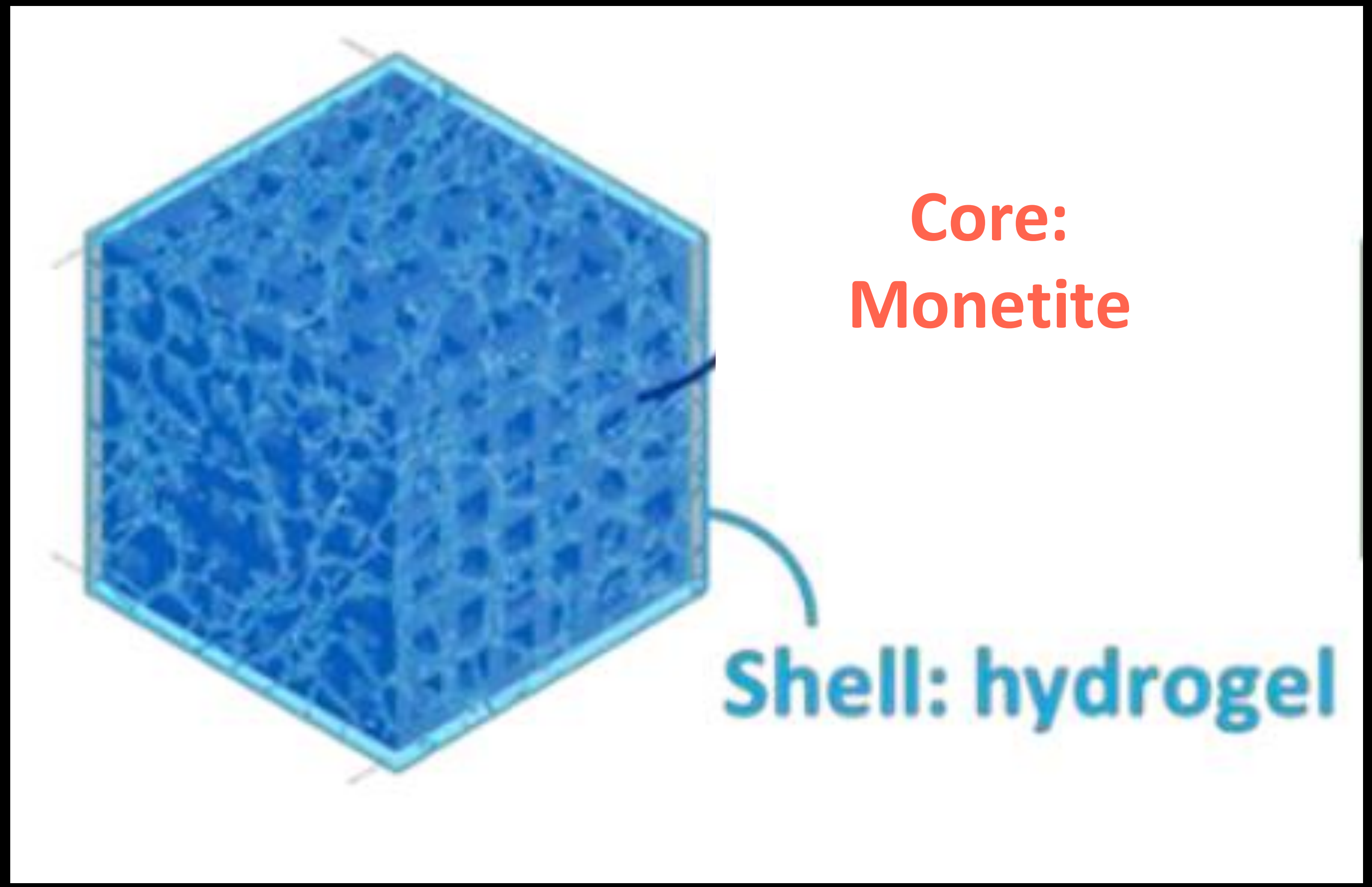
- % of new bone tissue: 21% (vs 7% control)

At 4 weeks:

- % of new bone tissue: 42% (vs 22% control)

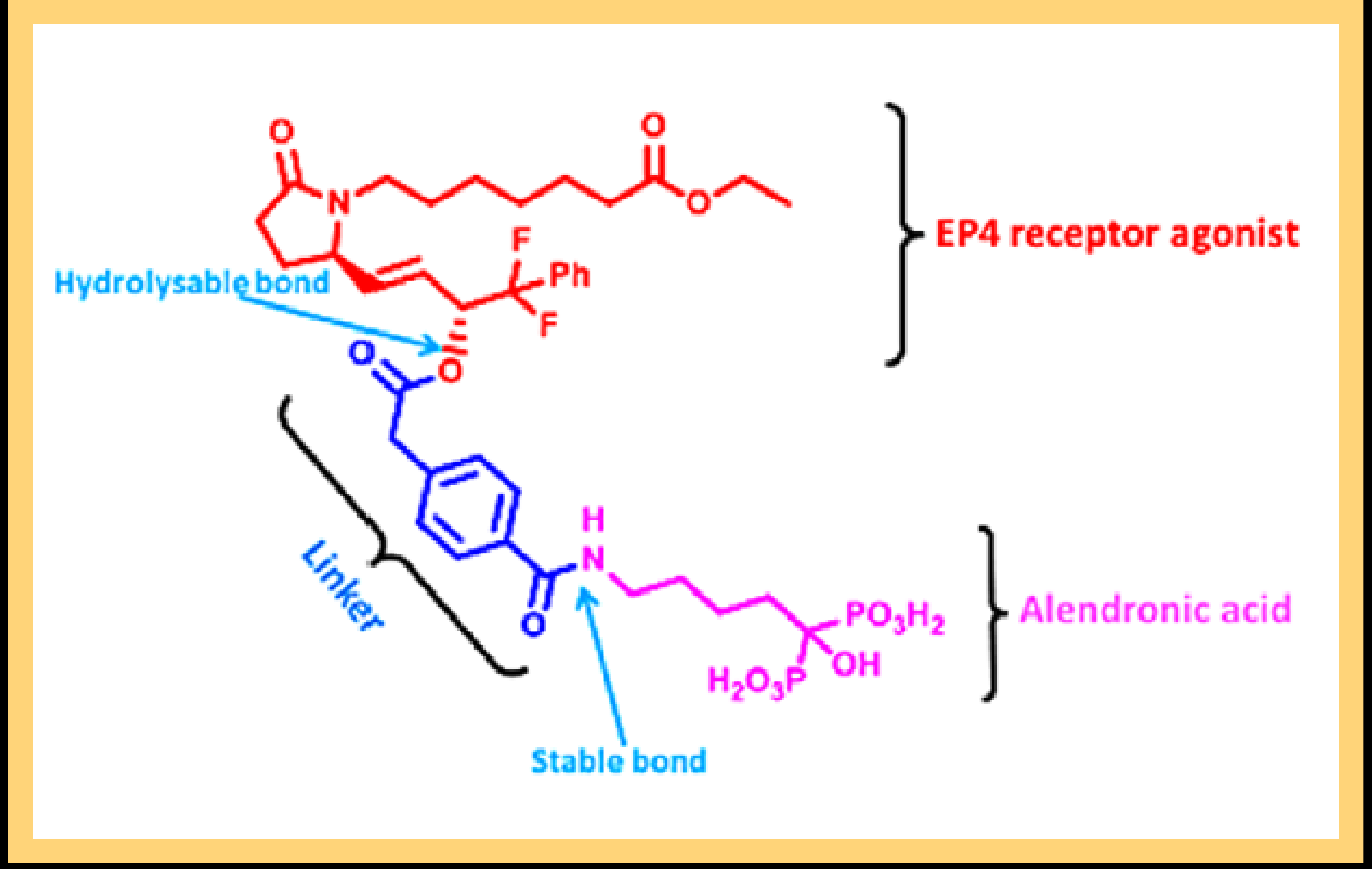
2

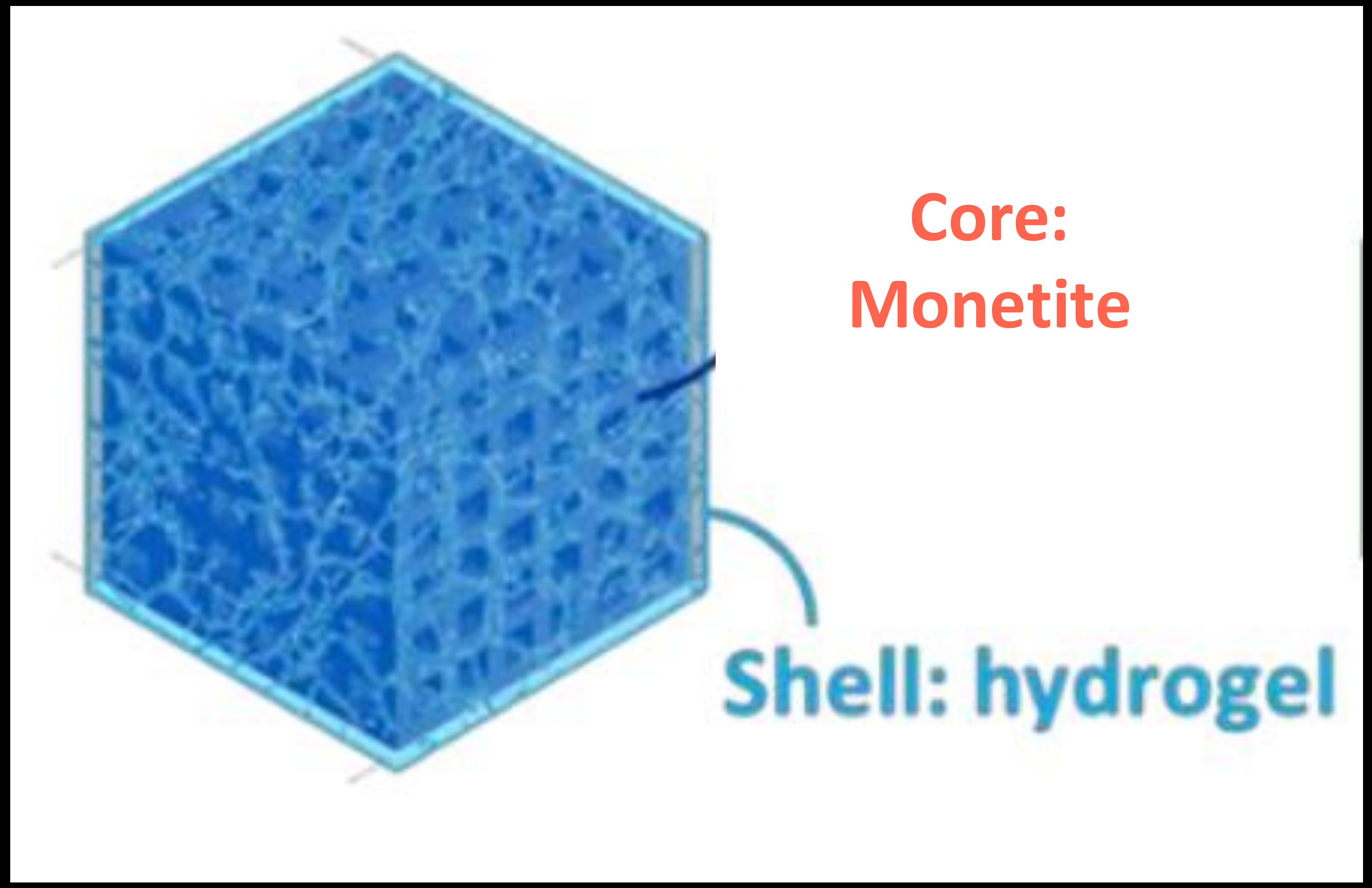
A NEW HYBRID CORE-SHELL SCAFFOLD: MONETITE + HYDROGEL



2

A NEW HYBRID CORE-SHELL SCAFFOLD: MONETITE + HYDROGEL





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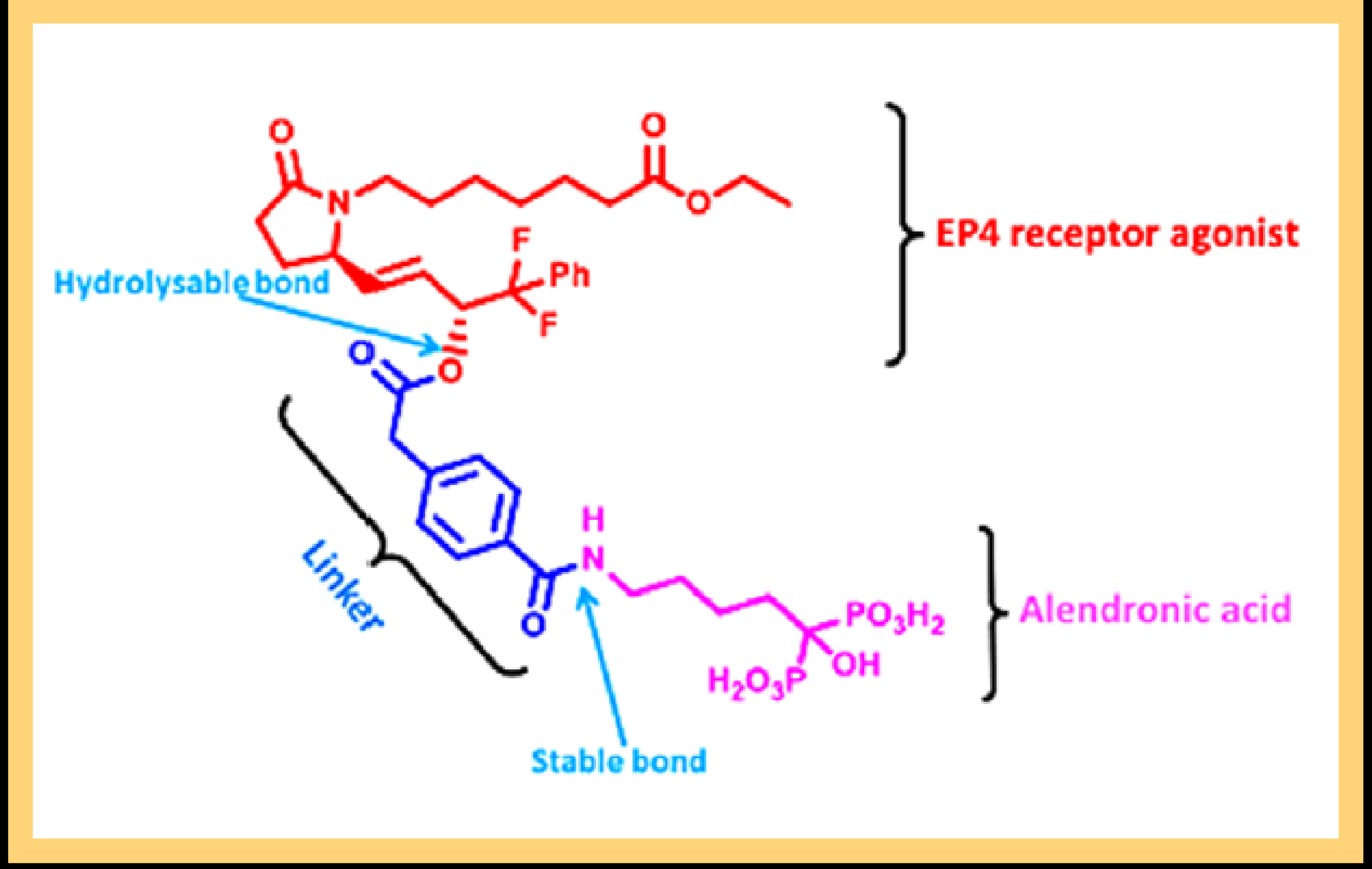
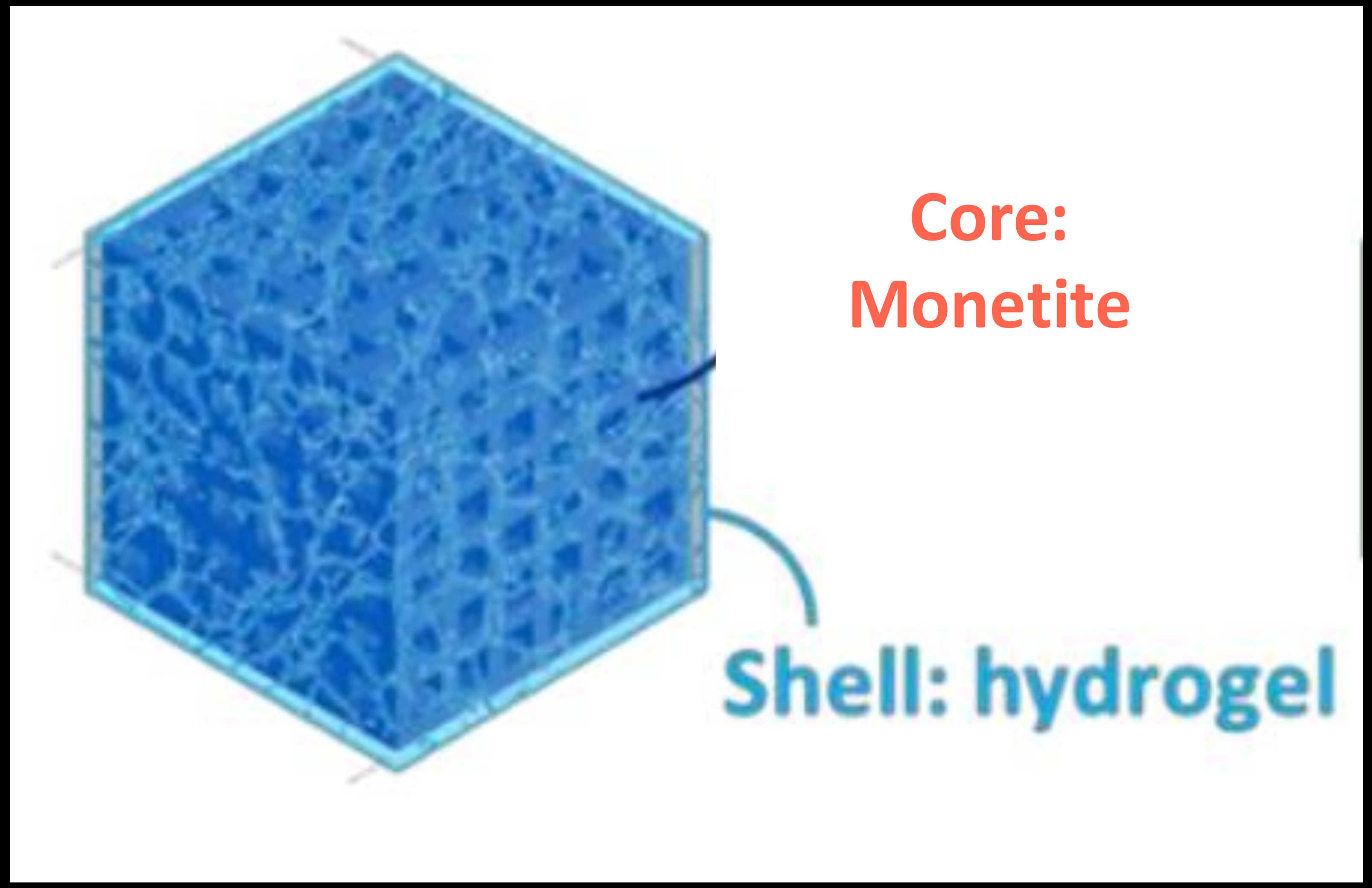
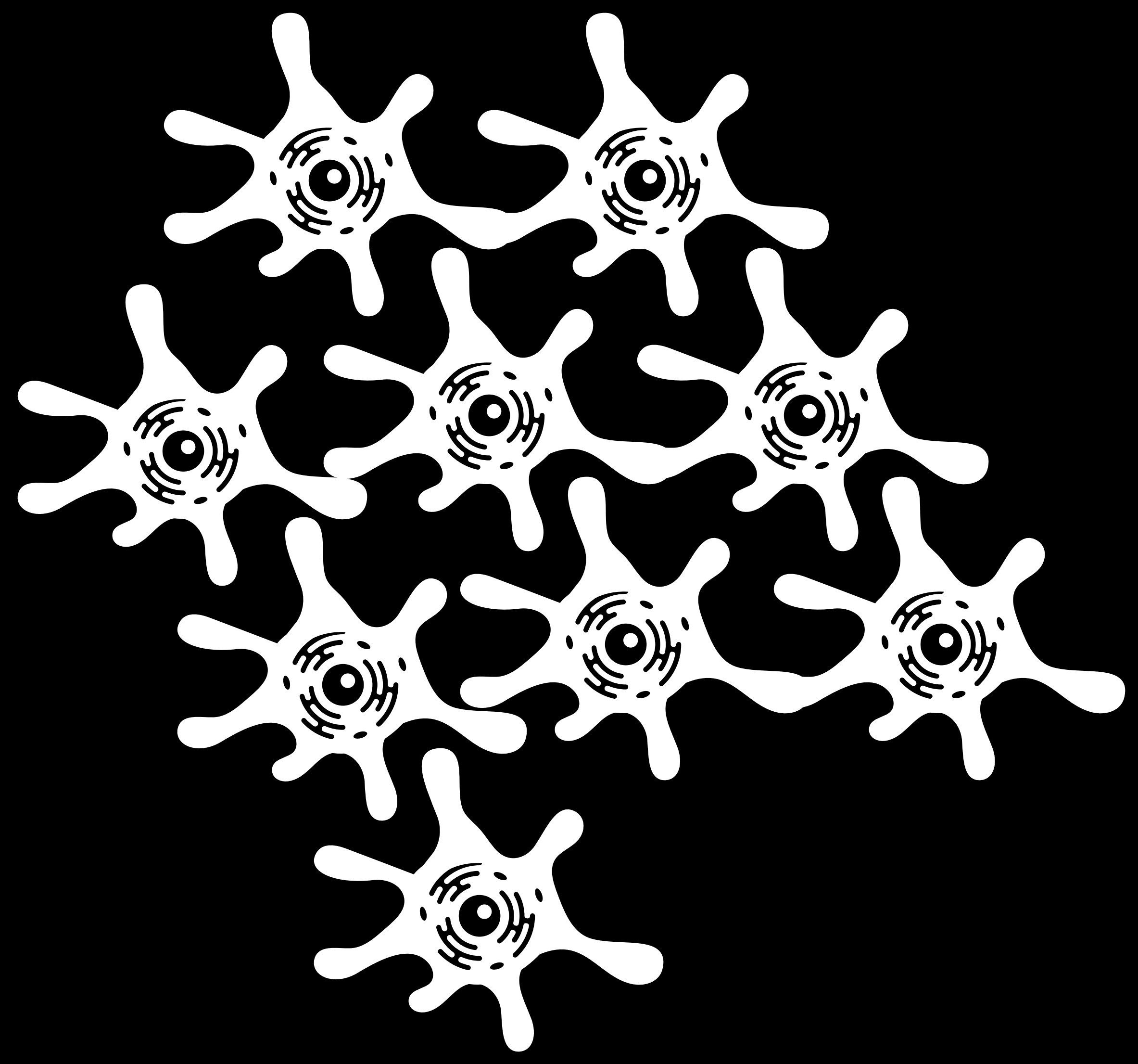
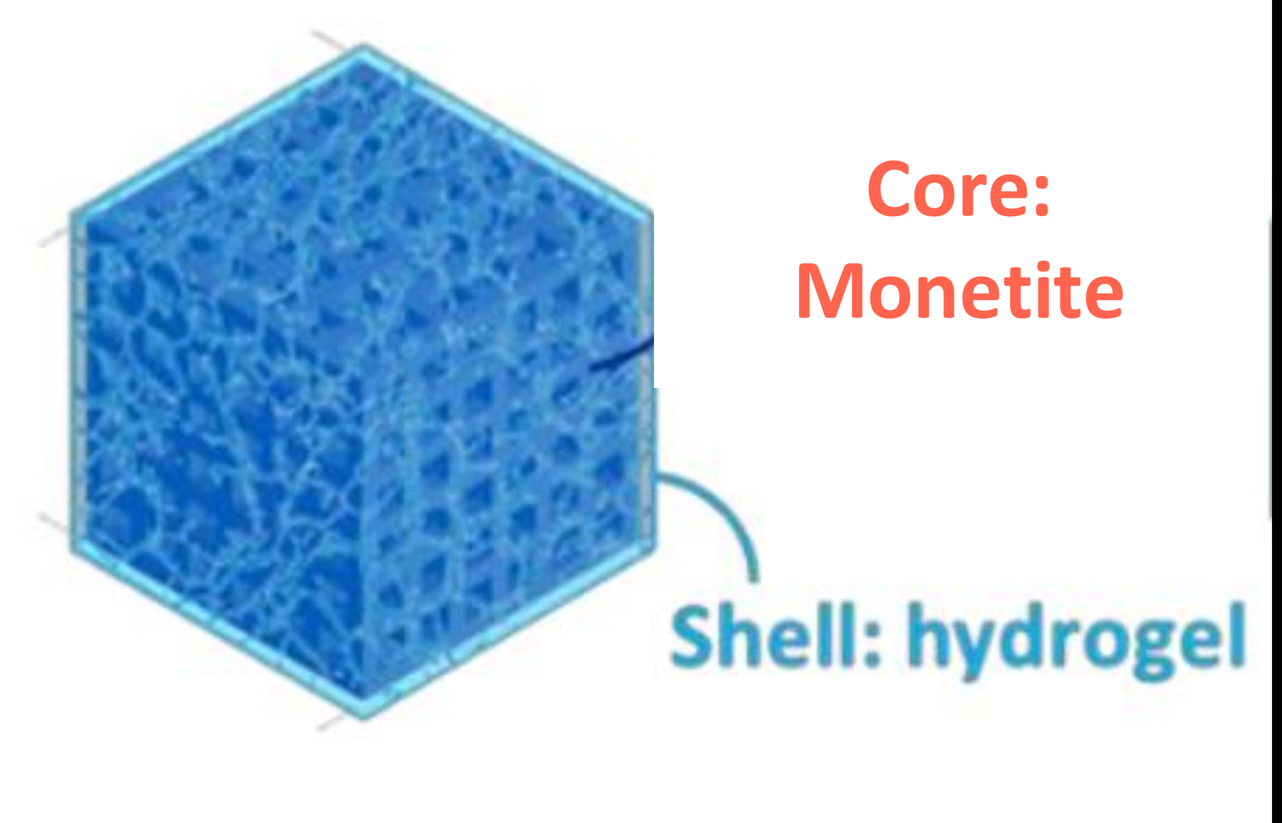






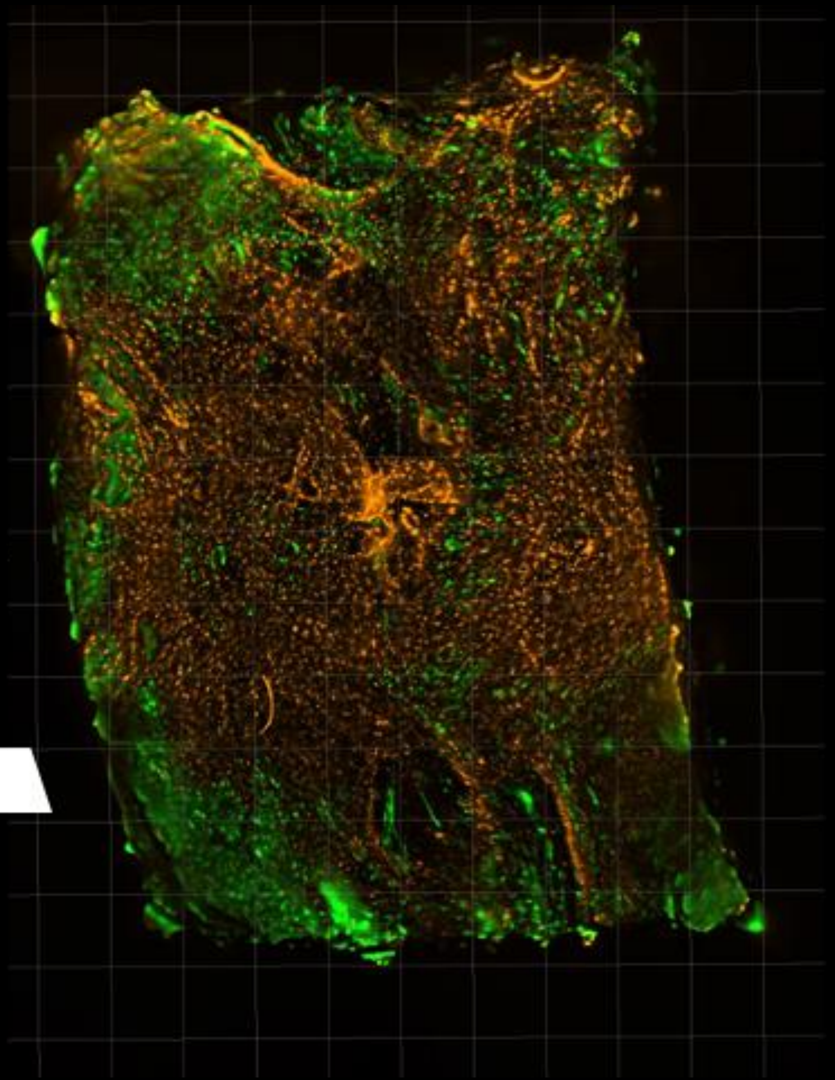
BIOMATERIALS

2 NEW HYBRID SCAFFOLD: MONETITE + HYDROGEL



+ stimulating factor

+ stem cells



In vitro phase

In vivo phase

Essential "ingredients" of tissue regeneration

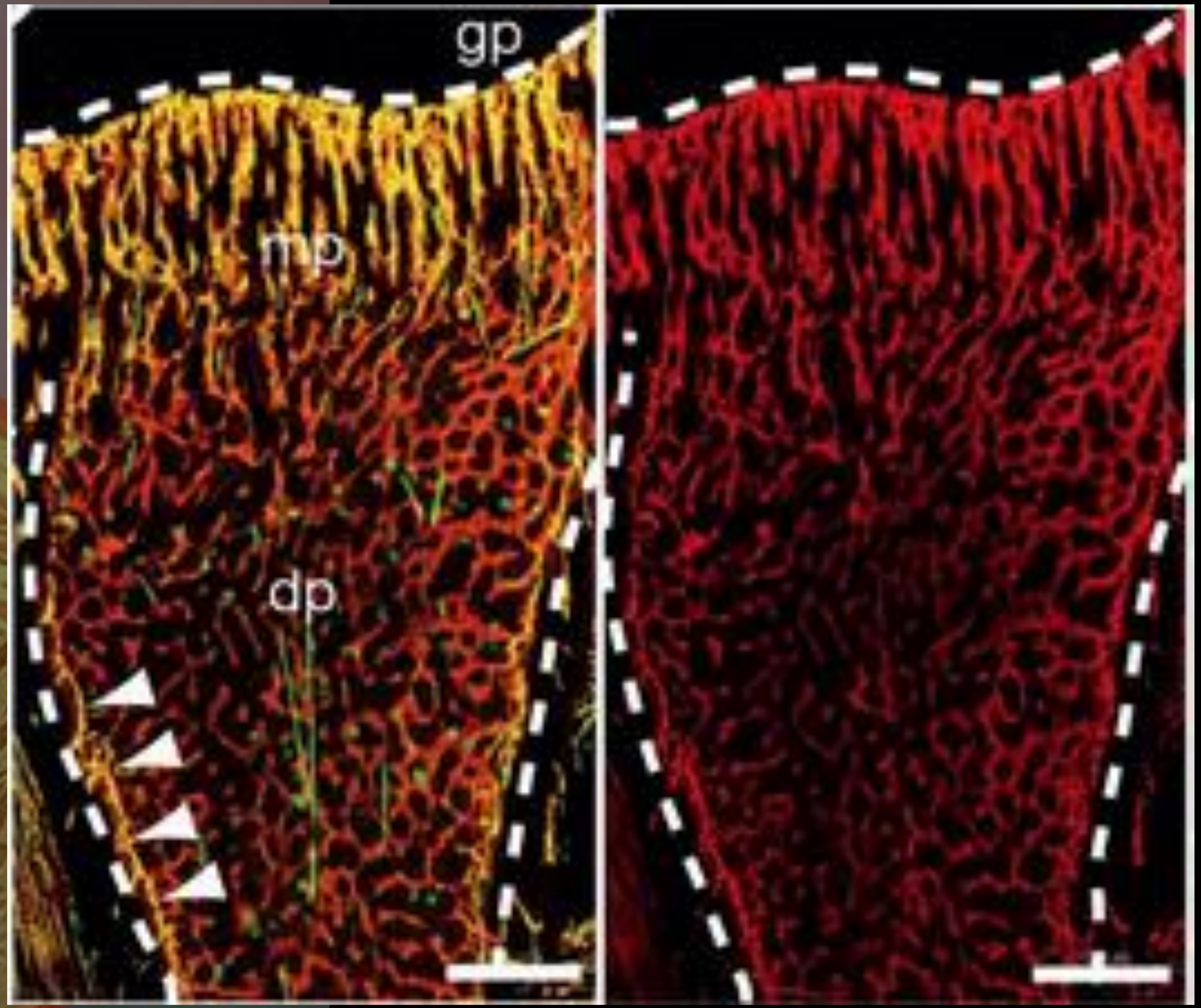
BIOMATERIALS

STEM CELLS

STIMULATING FACTORS

Co-colture systems

THE CRUCIAL ROLE OF VASCULARIZATION



The Role of Vasculature and Angiogenic Strategies in Bone Regeneration

by Hye-Jeong Jang and Jeong-Kee Yoon *

CO-CULTURE SYSTEMS

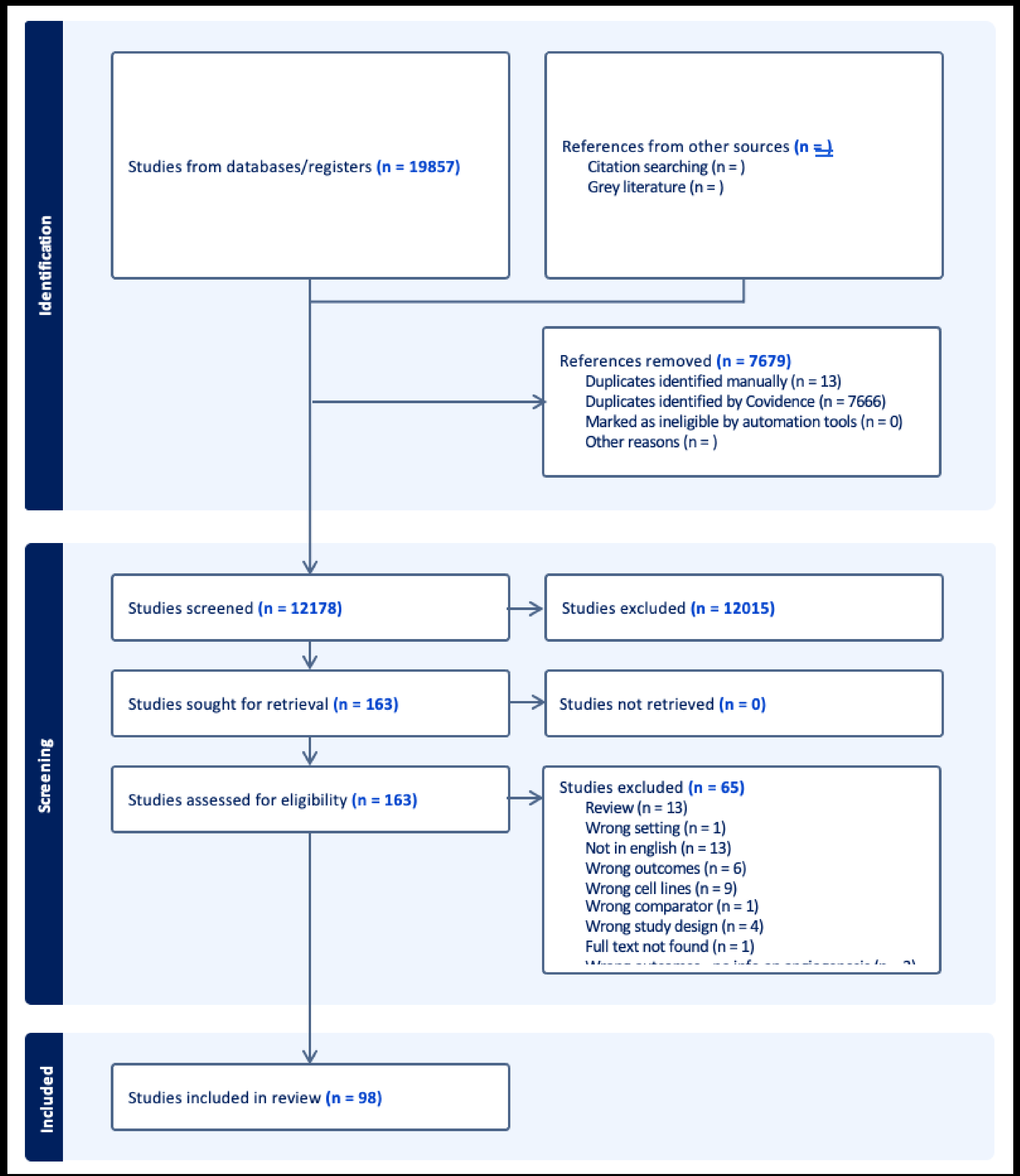
covidence

The World's #1
Systematic Review Tool

Bone / Tissue engineering / Tissue Scaffolds / Mesenchymal stem cells / Endothelial cells / coculture techniques /Bone generation

19.857 results

STEM CELLS



CO-CULTURE SYSTEMS

19.857 results

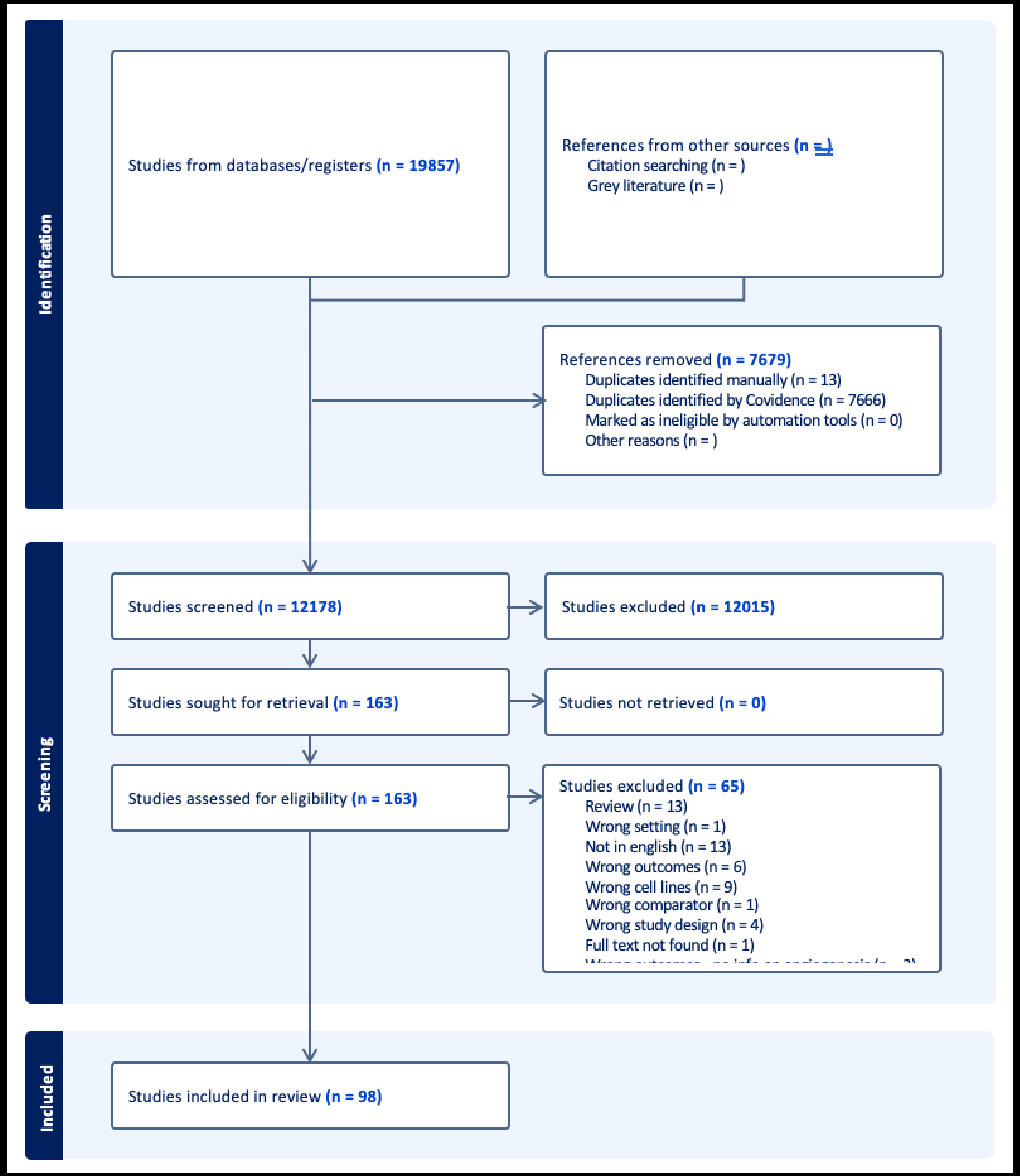
7679 duplicates

12.178 studies screened

12015 excluded

163 studies for eligibility

STEM CELLS



CO-CULTURE SYSTEMS

163 studies for eligibility

65 excluded

98 studies included

CO-CULTURE SYSTEMS

98 studies included

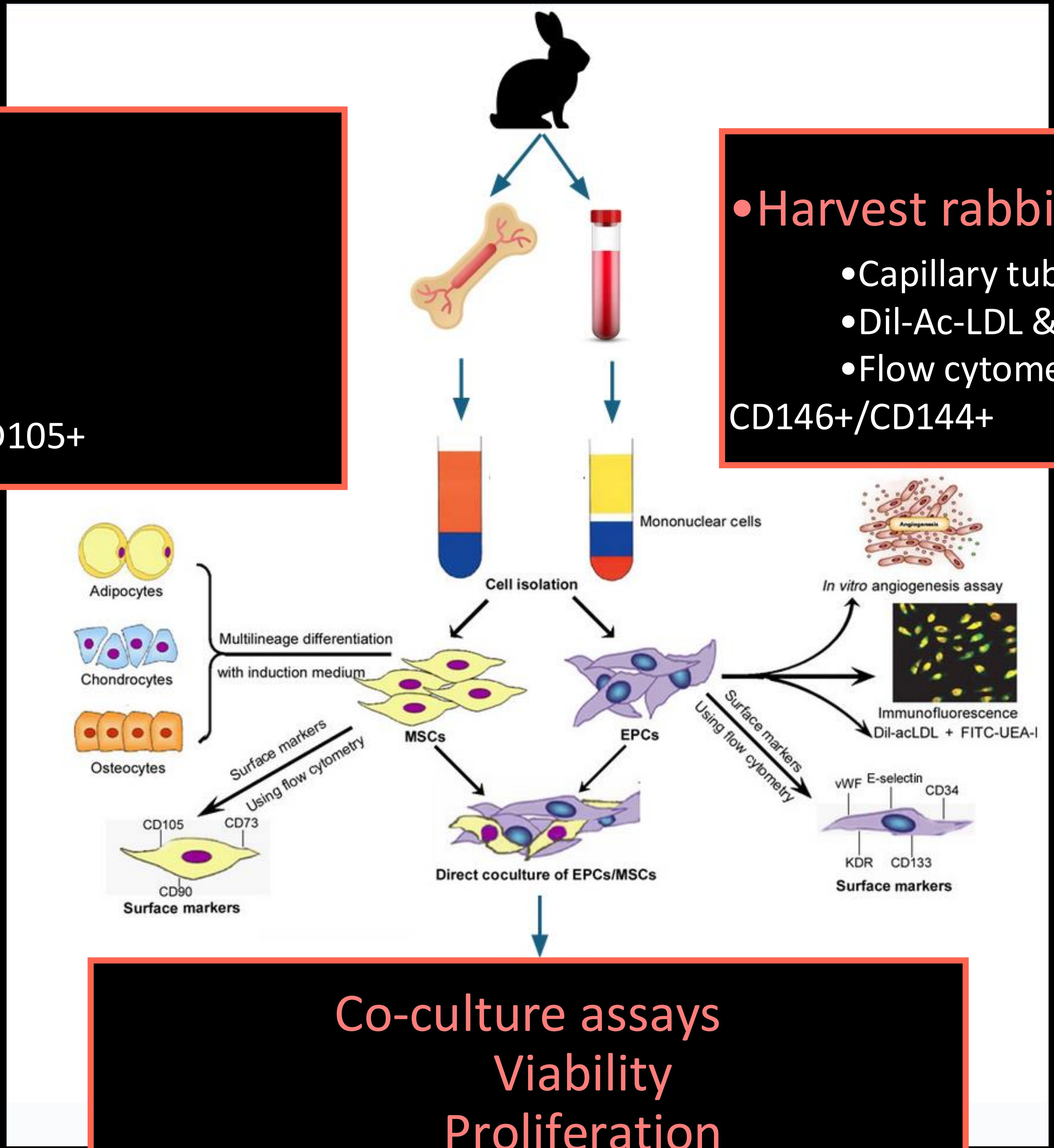
In *Vitro* only	In Vitro + In Vivo	In *Vivo* only
48	35	15

CO-CULTURE SYSTEMS

Preliminary results

- In *vitro* angiogenesis and osteogenic differentiation significantly higher in co-colture systems
- Different cell ratio tested (1:1 ratio mostly favoured)
- In *vivo* bone regeneration markedly improved with co-colture seeded scaffold
- End point mostly adopted was 12 weeks
- Mice and rats model predominantly used - few tested in rabbit model critical size defect

Next phase - in *vitro* experiments

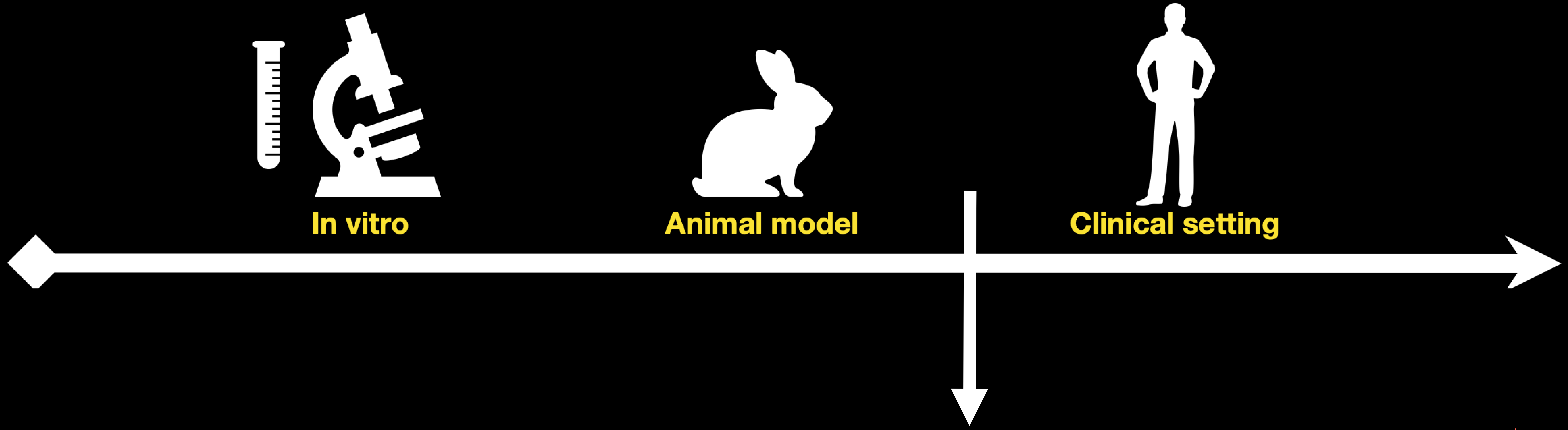


- Harvest rabbit MSCs from bone marrow
  - Assessment of multipotentiality
    - Alizarin Red S (in osteogenic culture)
    - Oil red O (in adipogenic culture)
    - Alcian blue (in chondorgenic culture)
  - Flow cytometry: CD34-, CD45-, CD90+, CD29+, CD105+

- Harvest rabbit EPCs from peripheral blood
  - Capillary tube formation assay in Matrigel
  - Dil-Ac-LDL & FITC-CEA-1 staining
  - Flow cytometry: CD34+, CD45-, CD31+, VEGFR2+, CD146+/CD144+

Co-culture assays
Viability
Proliferation
Cell differentiation assays

Model validation



Aims

% of BONE REGENERATION

TIMING

DEFECT SIZE

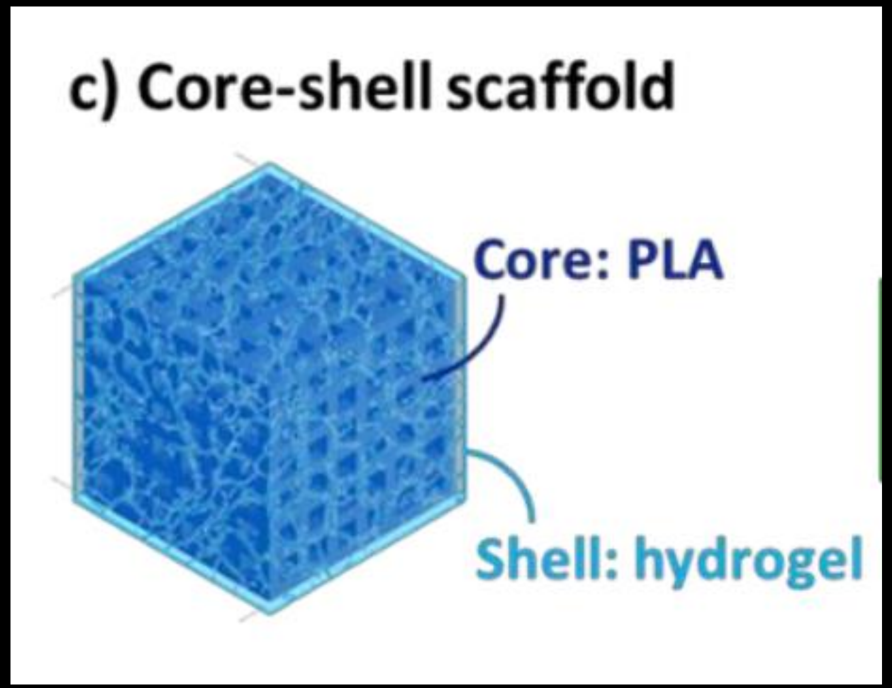
STEP 1

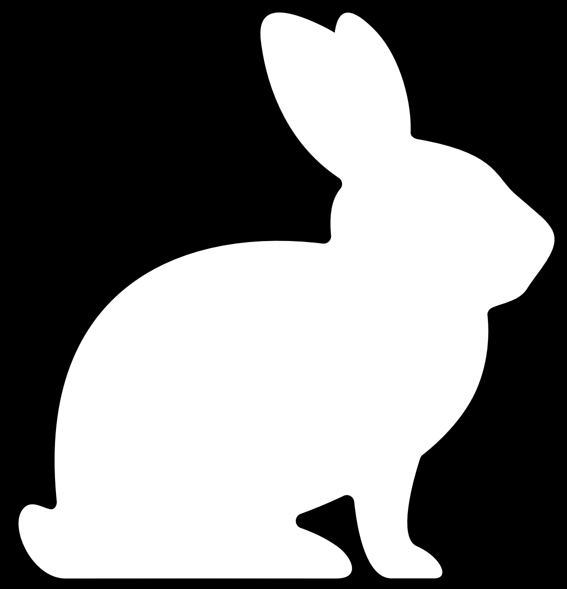
DEFECT SIZE

3D PRINTED CORE-SHELL PLA-Hydrogel

In vivo experiments

Study groups
1. Defect only
2. Scaffold reconstruction
3. hMSC-seeded scaffold reconstruction



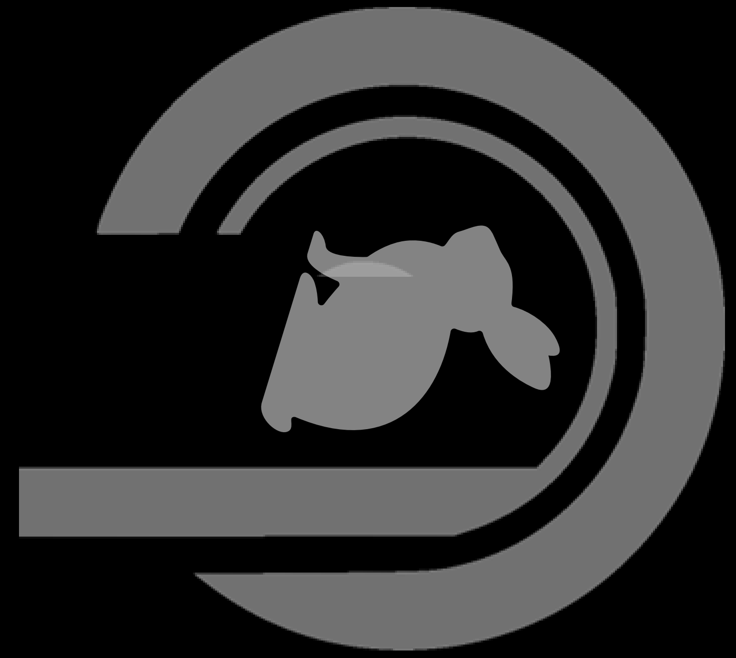


Every 2 weeks

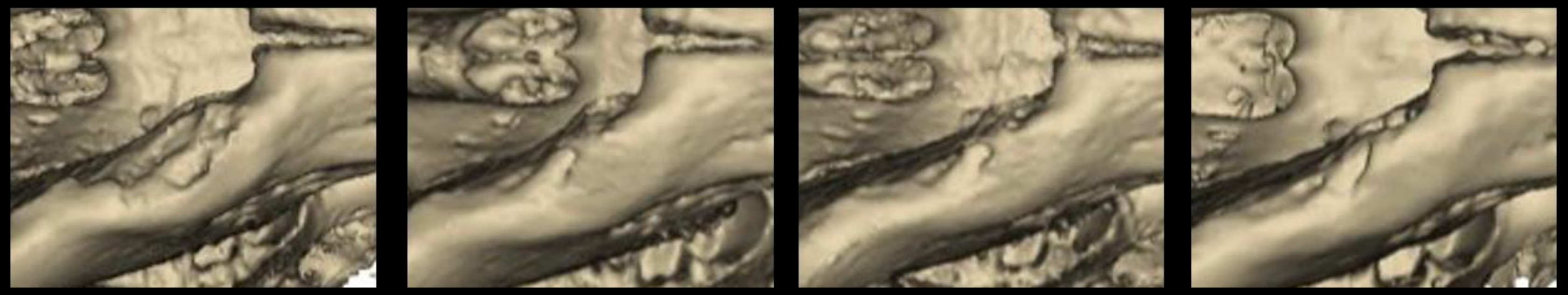
For 5 months

In Vivo phase

CE-CT scan

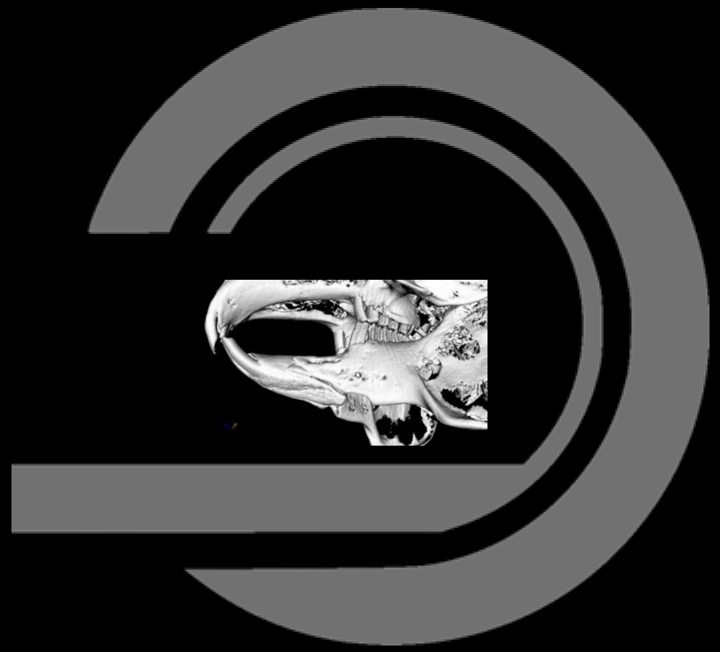


30 days
60 days
90 days
120 days



Euthanasia

Ex Vivo phase



UltraHD CT scan

H&E
HC/IHC:
- TRAP
- HNA
- VEFG-A
- Bone-SP
- Osteocalcin
- Osteopontin

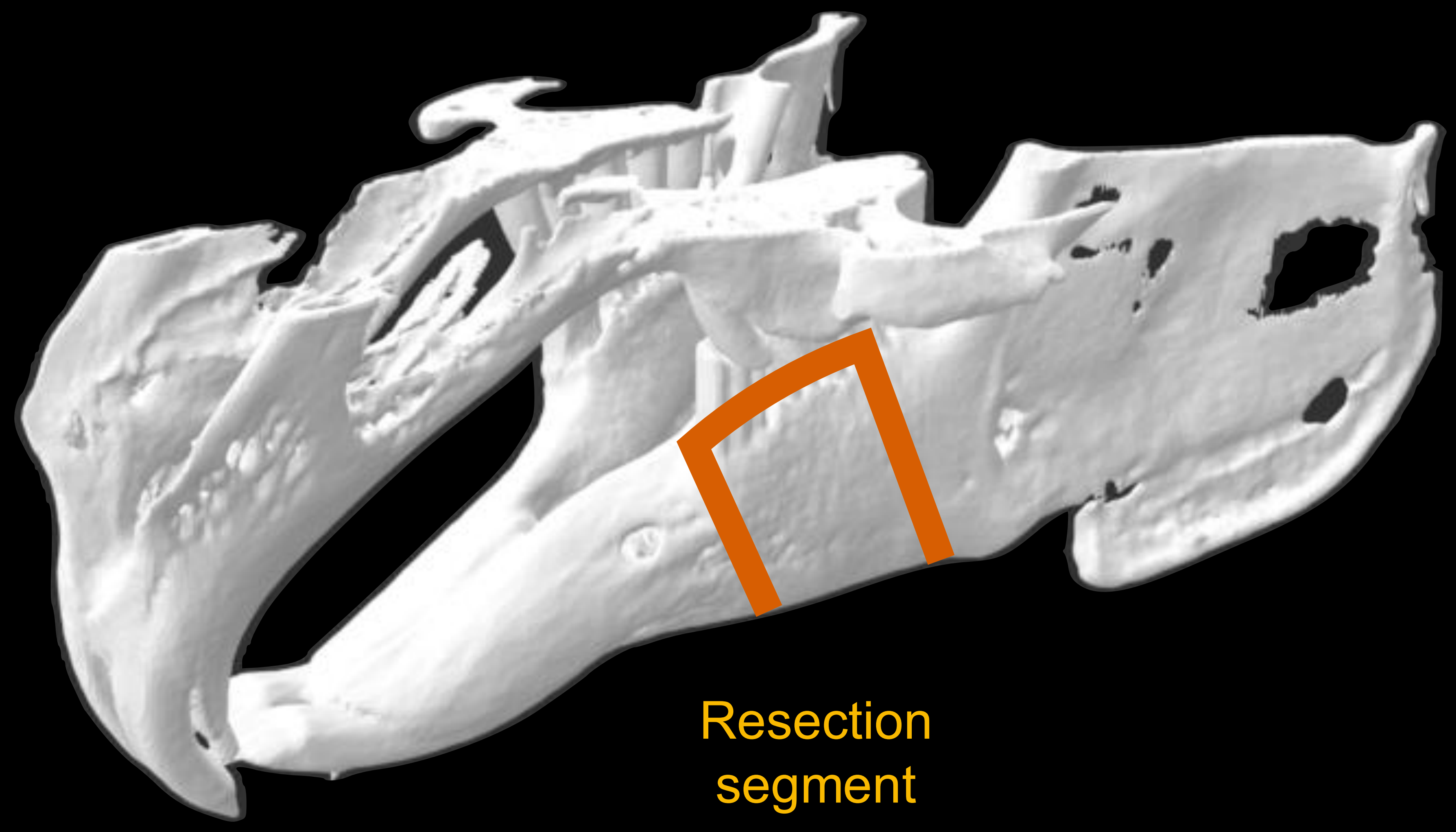
Defect size: 10 x 5 x5 mm
(Previously 5x3x3)

MARGINAL MANDIBULECTOMY

STEP 2

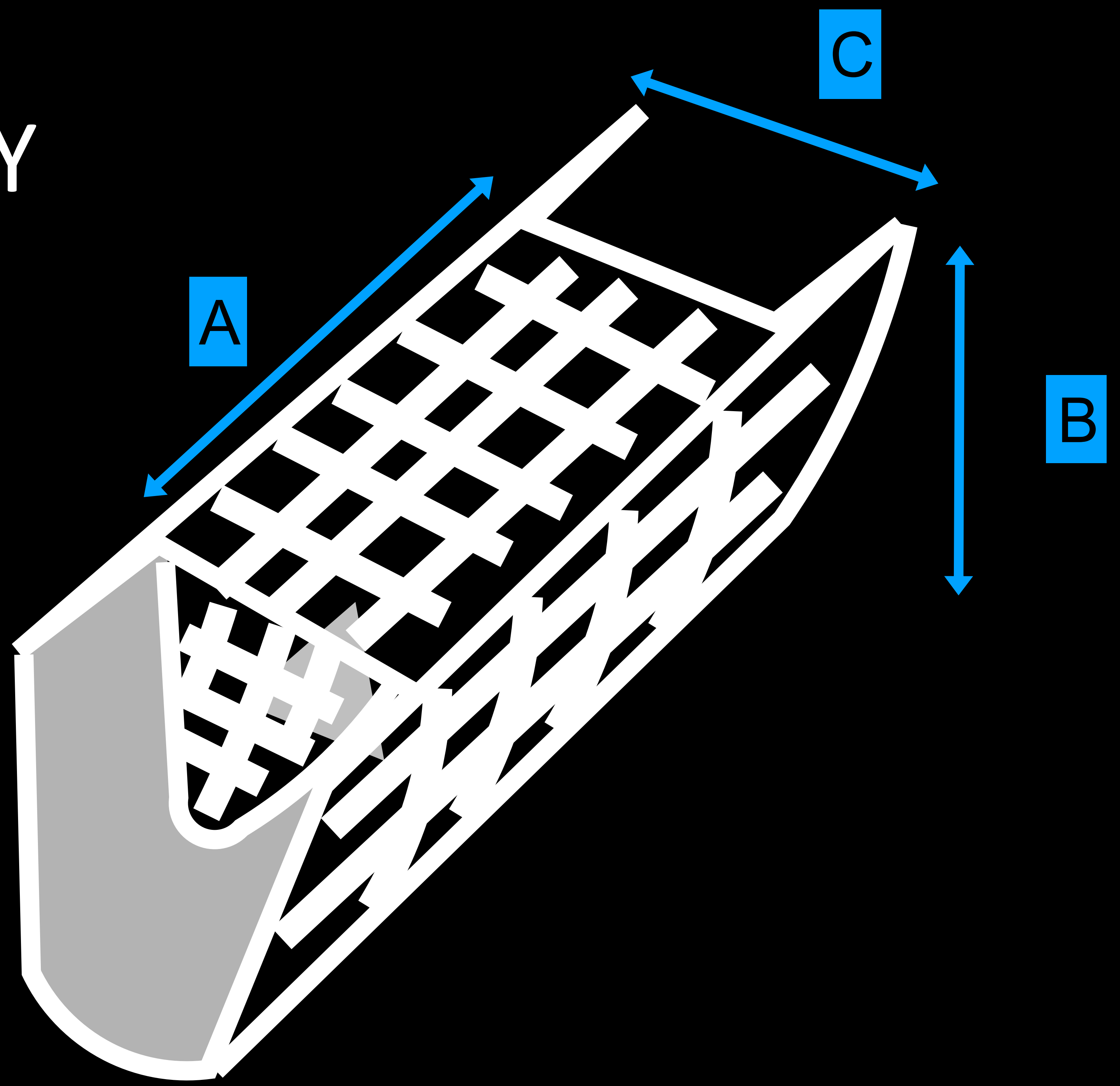
DEFECT SIZE

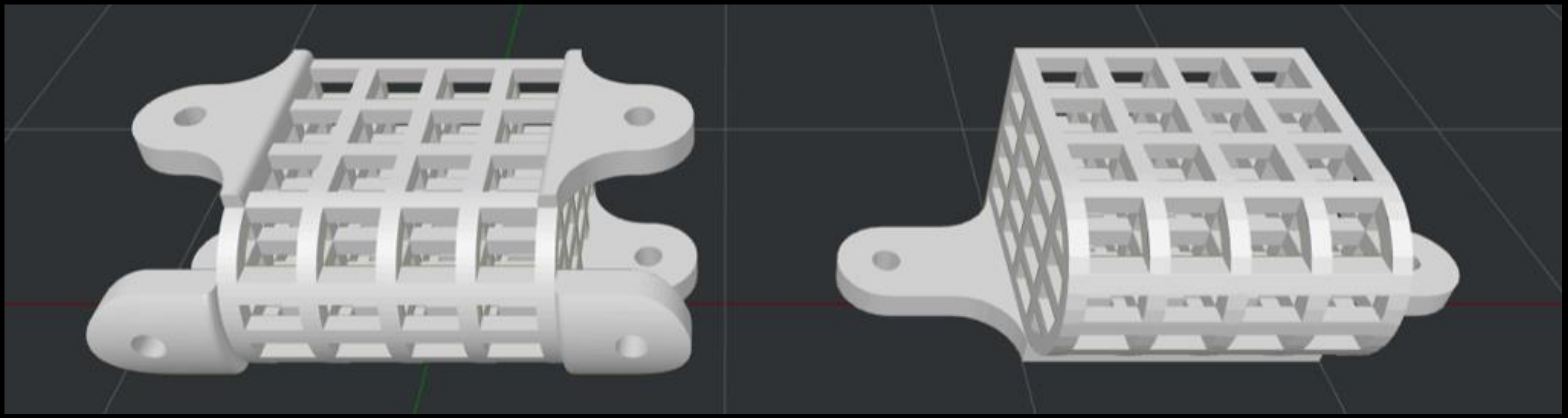
SEGMENTAL MANDIBULECTOMY



Resection segment

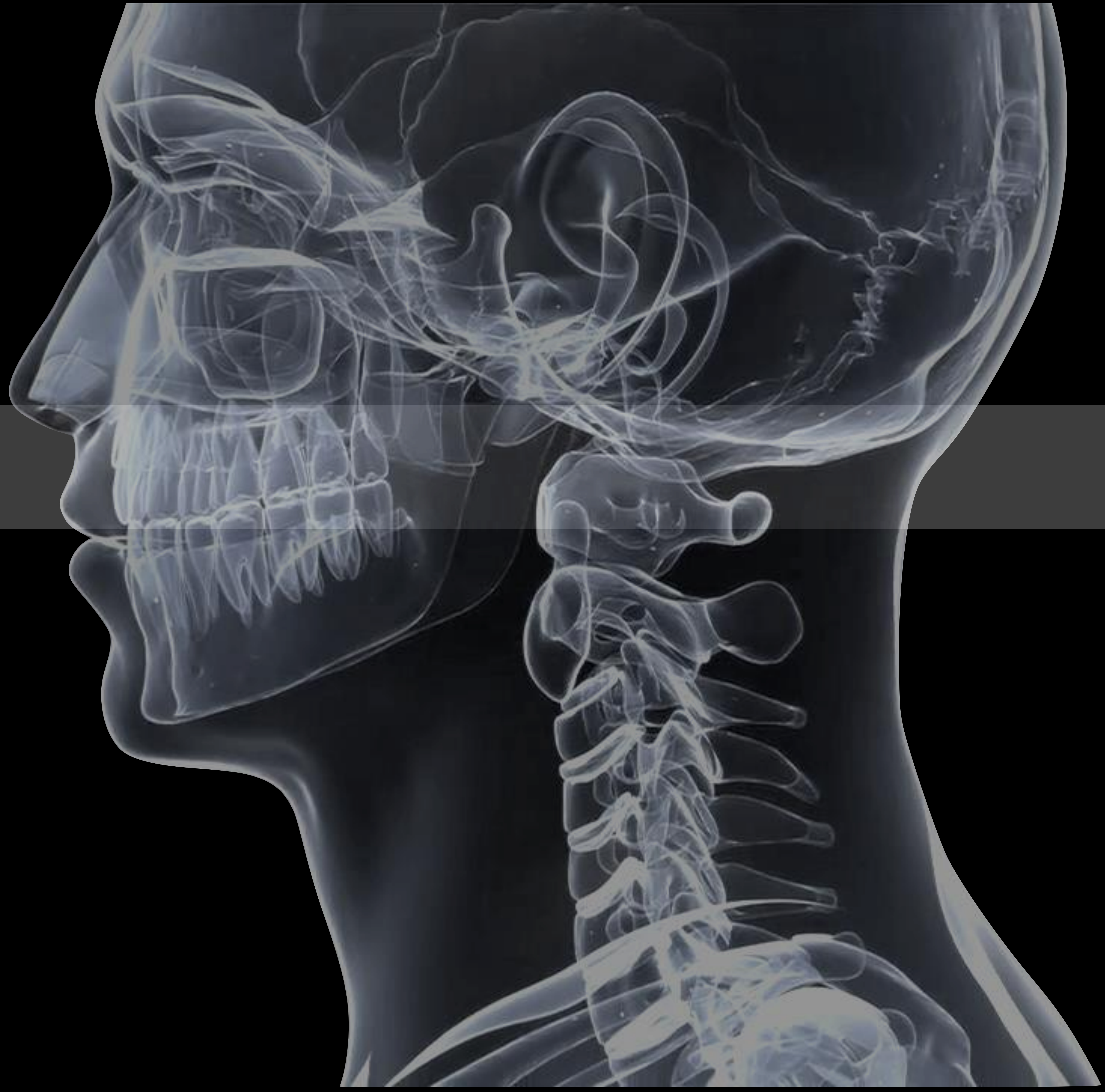
New scaffold shape with 3D printed "wings"



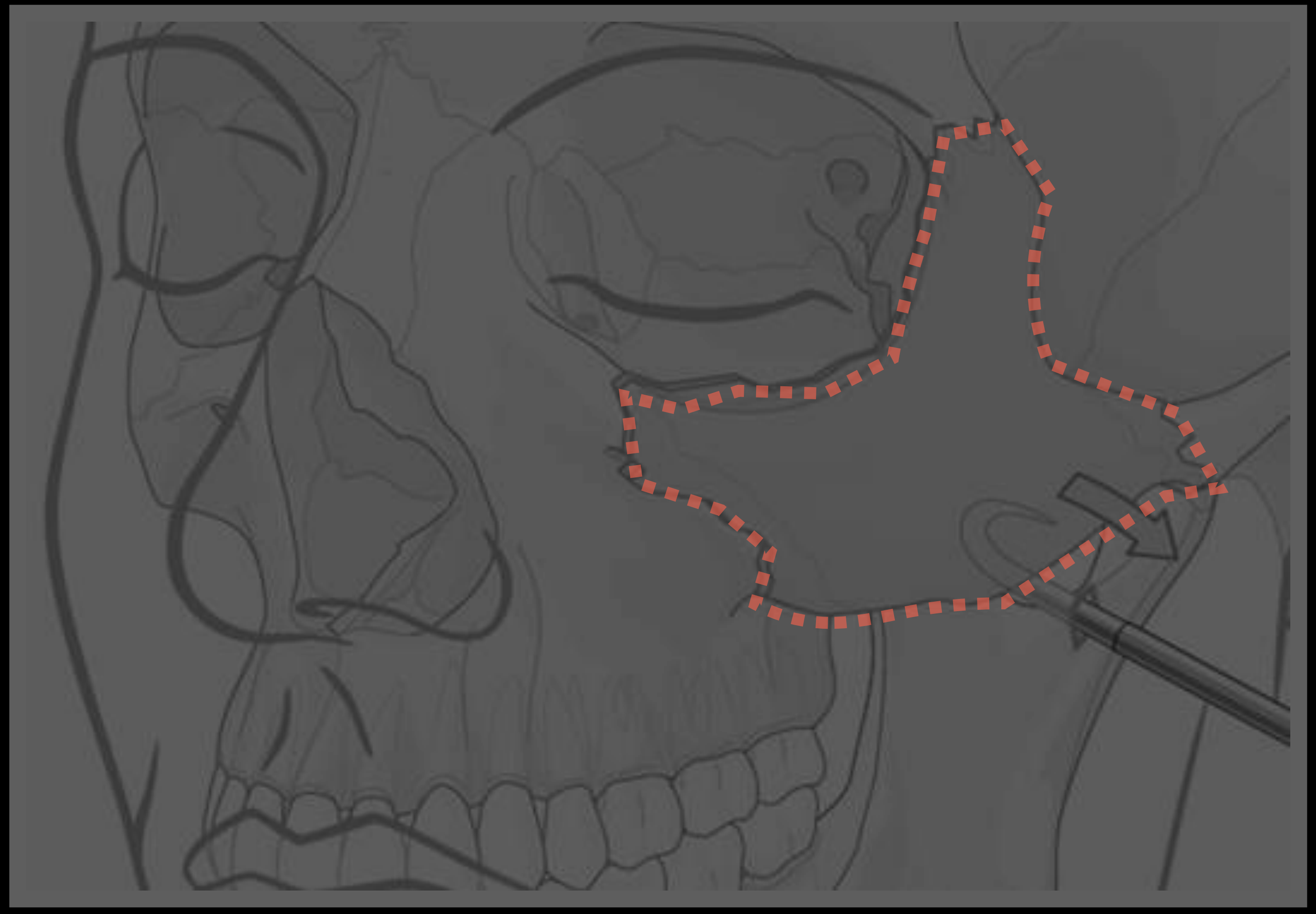


Bioengineered scaffolds in head and neck surgery

- Background - literature review
- Research updates
- Ancillary projects



CLINICAL SCENARIOS



TRAUMA

BONE-INCLUDING FREE FLAP RECONSTRUCTION

BONE AUGMENTATION

ANCILLARY PROJECTS

DEVELOPEMENT A PRECLINICAL
IRRADIATED BONE AND OSTEORADIONECROSIS MODEL

ORIGINAL ARTICLE OPEN ACCESS

Head&Neck, 2024

Development of a Preclinical Double Model of Mandibular Irradiated Bone and Osteoradionecrosis in New Zealand Rabbits

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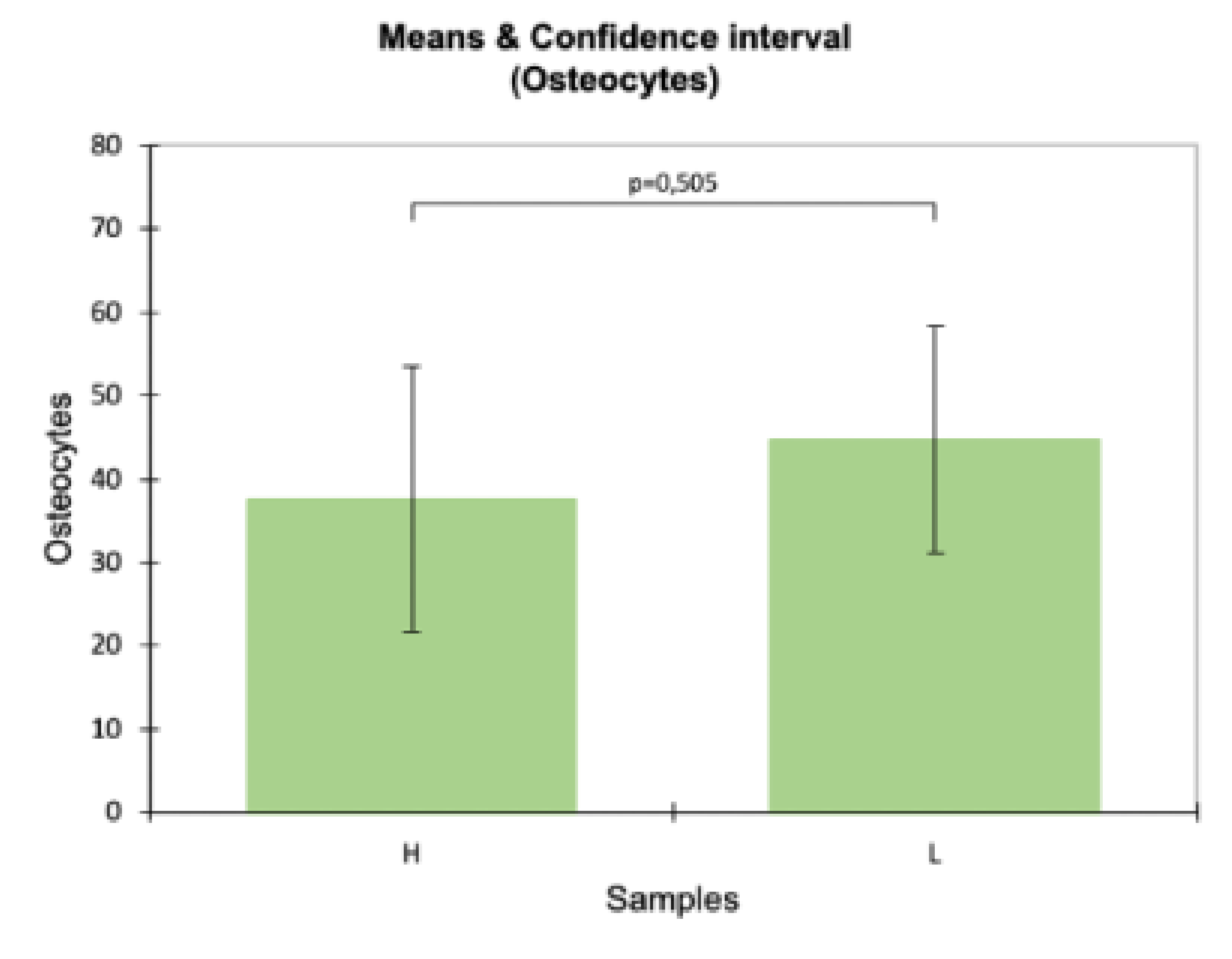

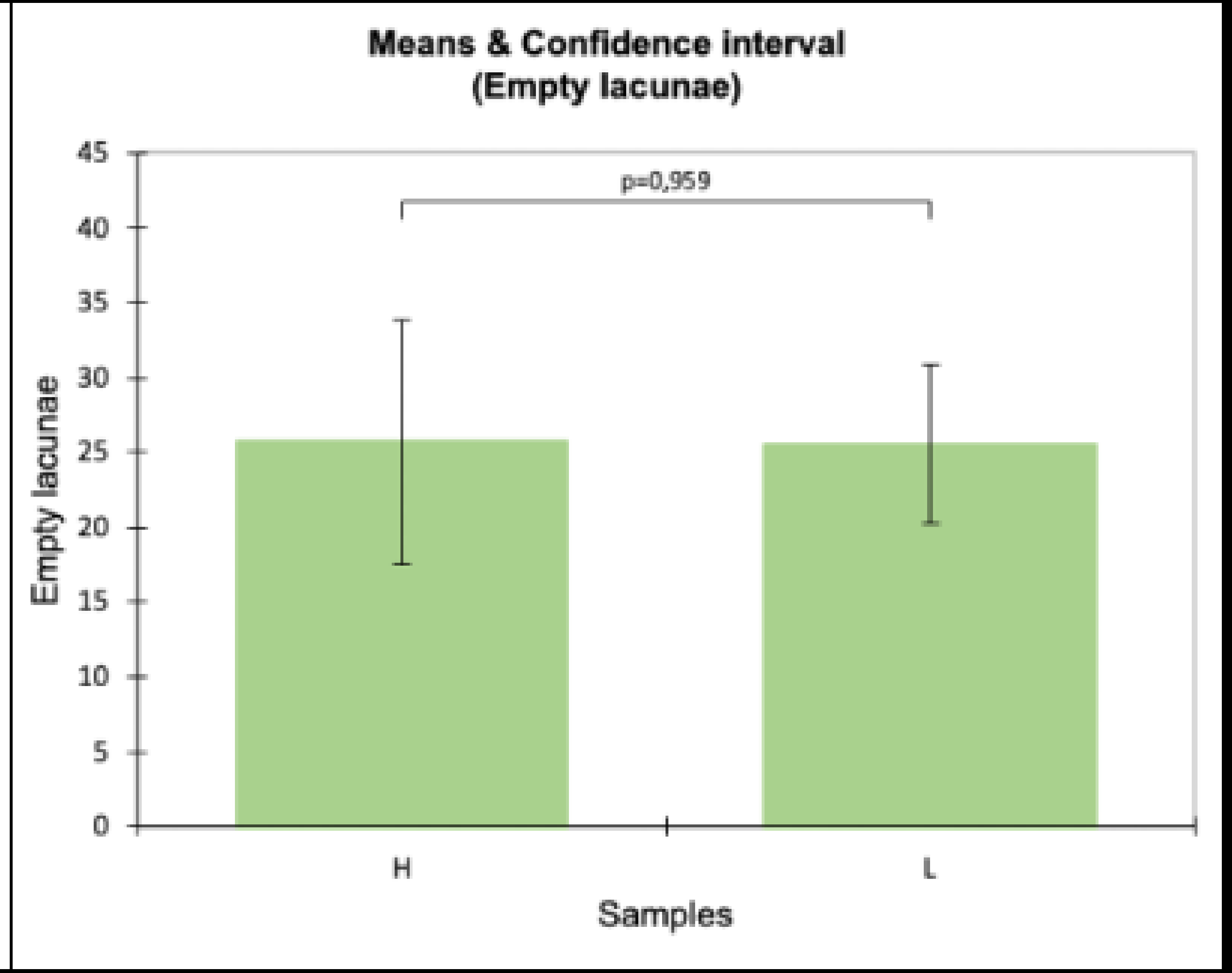

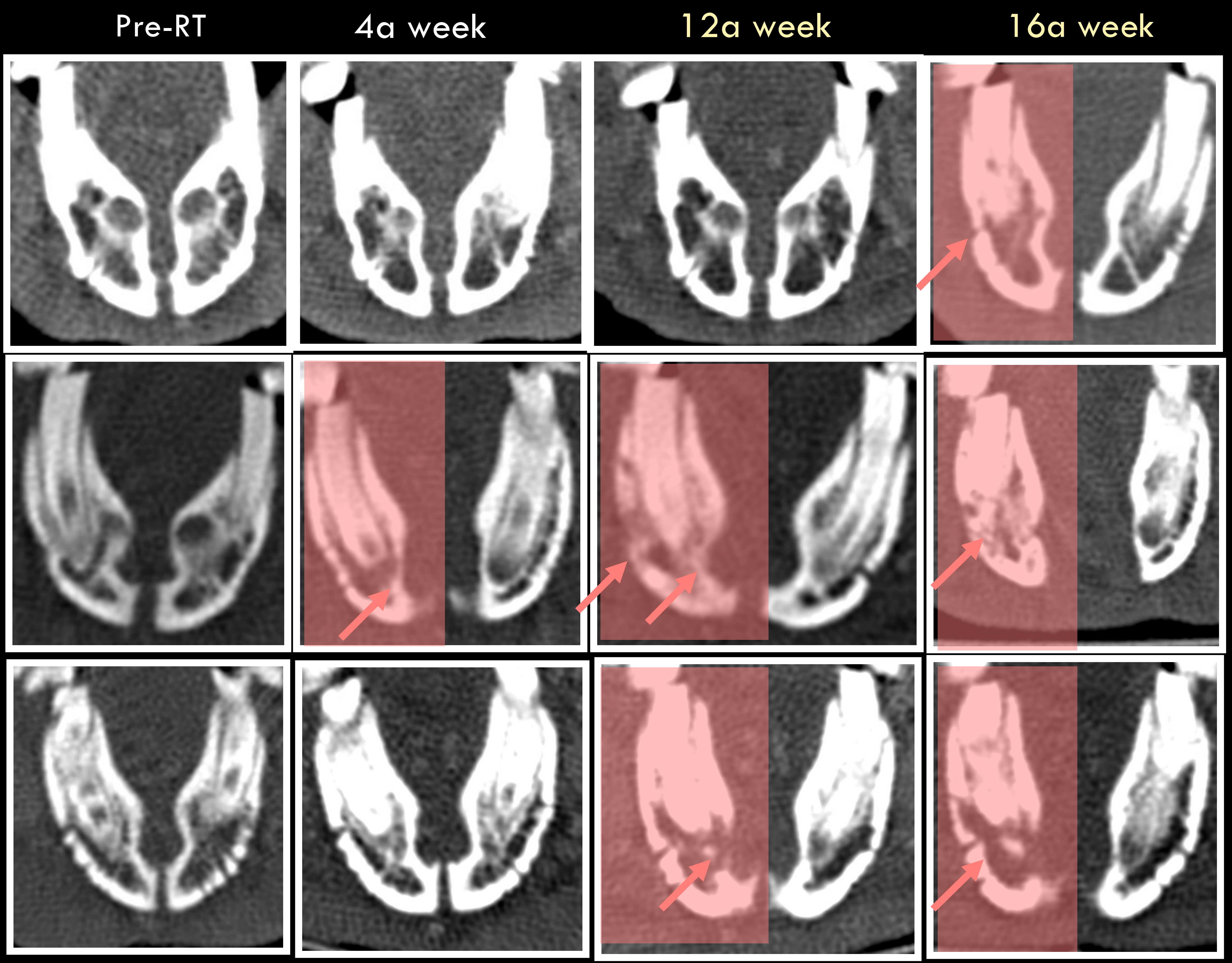

Bioengineered scaffolds in head and neck surgery: optimization of bone regeneration devices and development of local release systems

THANK YOU!

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Alessandra Ruaro MD

Tutor: Prof. Luciana Sartore