Functionalization of bone substitutes surfaces for orthopaedics

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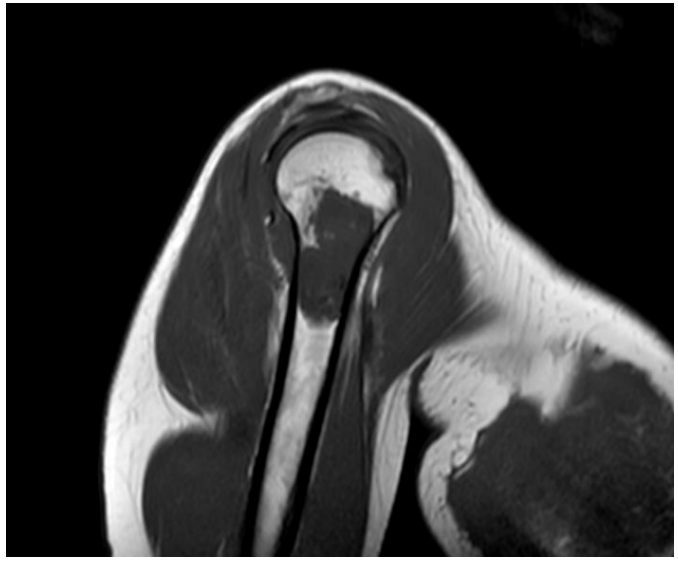
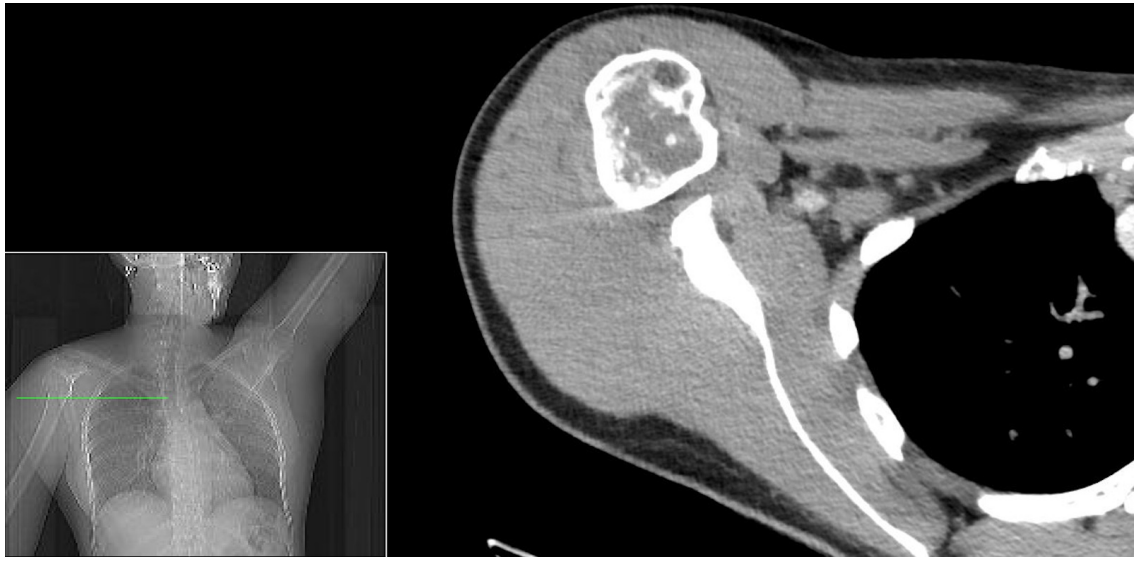
How to make a successful bone grafting?

Enchondroma of the proximal humerus.

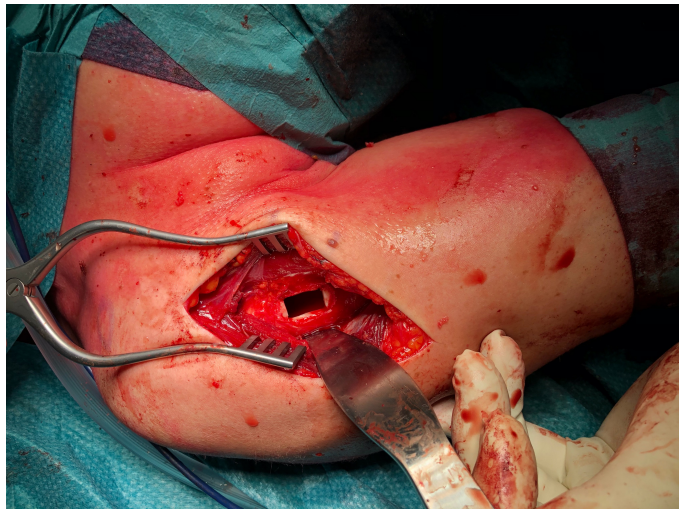


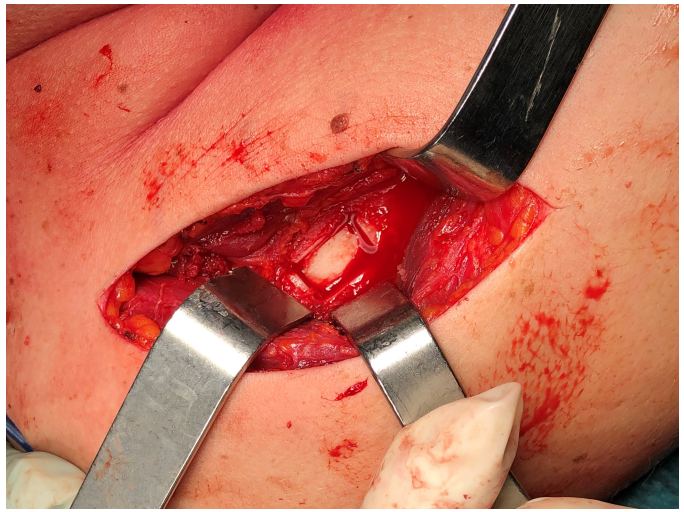
MRI of the initial situation



CT Scan of the initial situation

Intraoperative views



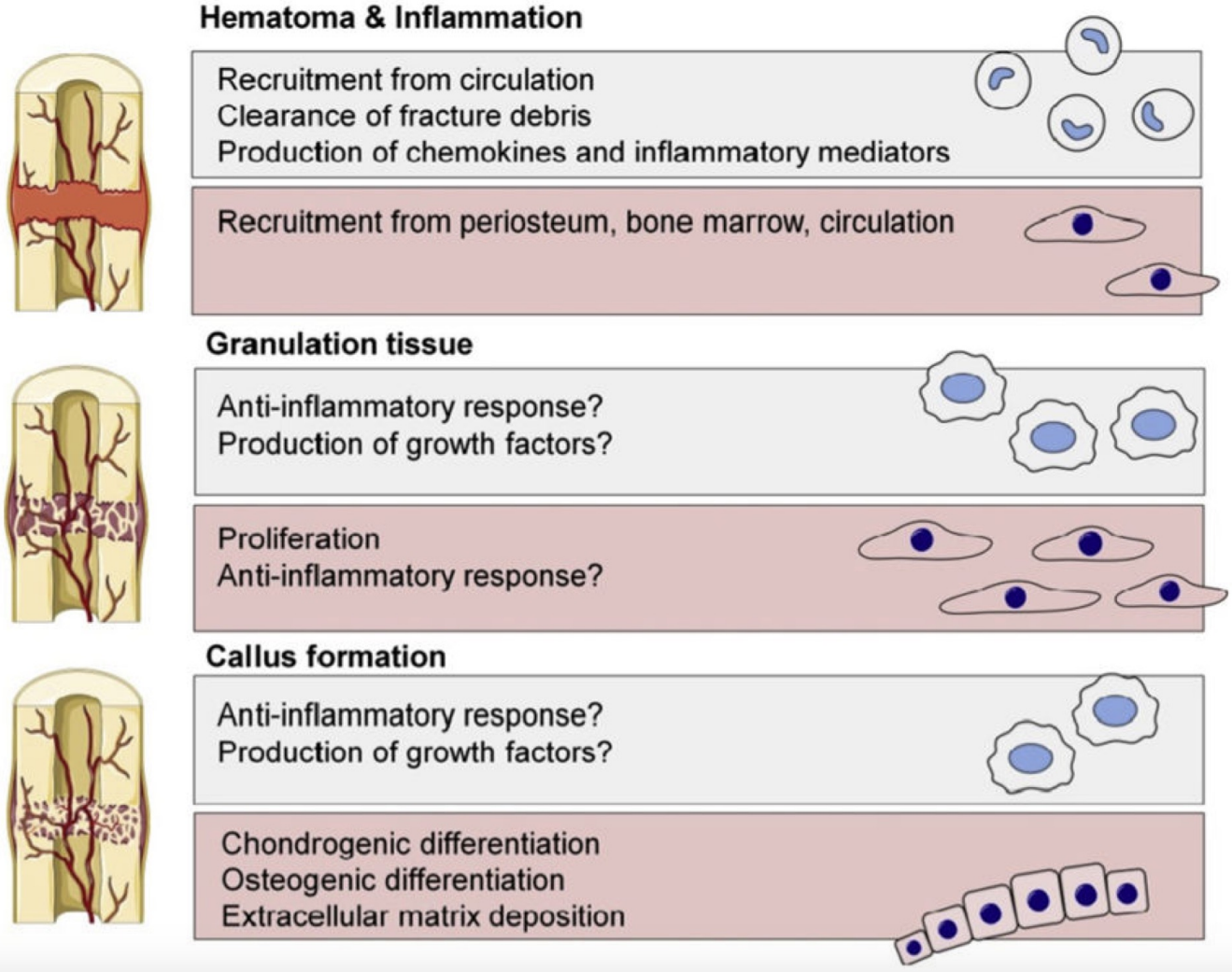


Removal of an enchondroma of the proximal humerus treated with a bone xenograft.

Good graft integration
X-Ray at 6 months follow up

Bone has a self-repair ability so that it can repair itself when the nature and extent of injury is not large, chronic, severe, and complicated. Bone healing is a **complex process of overlapping phases**, including **inflammation, repair, and remodeling** that involves the many intracellular signaling pathways which are responsible for regeneration of the new bone with the help of surrounding tissues

Despite an improving knowledge of the repair mechanisms, **malunions, delayed unions, or nonunions occur and persist in clinical cases** where the osteogenesis and subsequent bone remodeling are impaired



In order to improve and accelerate the healing process, often times it in not possible or convenient to use autologous bone, several alternatives are available in clinical practice. First of all, after the diseased bony tissue has been removed and the remaining bones fixed, the resulting defect should be reconstructed and filled. **An ideal bone graft** substitute should have certain properties, including **osteoconduction, osteoinduction, osteoincorporation, osteointegration, and promotion of osteogenesis**.

Post operative X-Ray Shatzker type VI

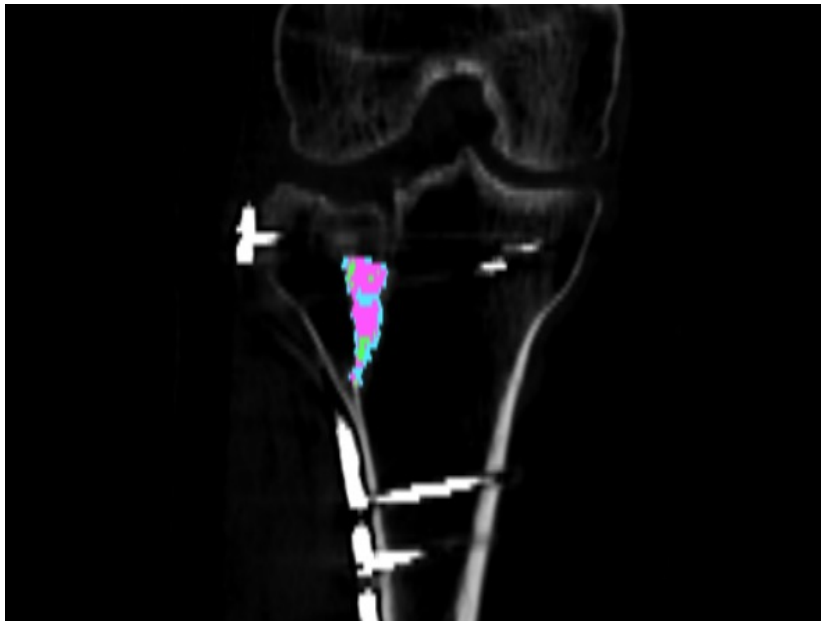
Post operative axial, coronal and sagittal CT Scan

Shatzker type 2 TIBIAL PLATEAU FRACTURE (split depression)

POST OP

FOLLOW-UP 5 MONTHS

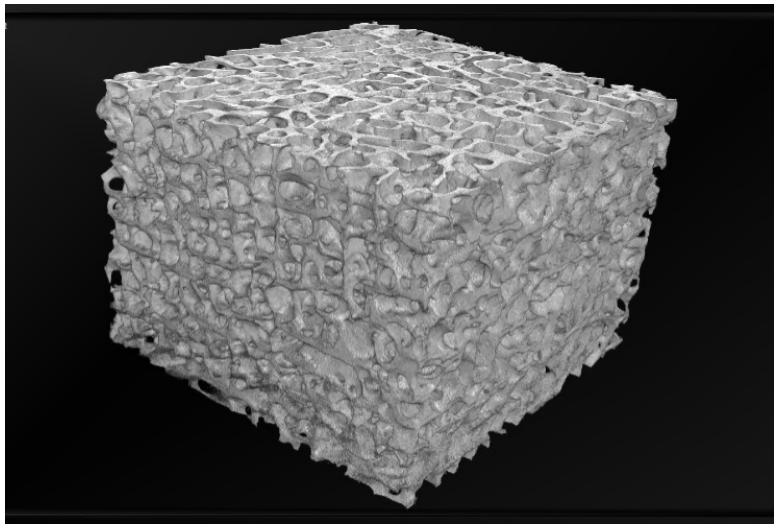
FOLLOW-UP 6 MONTHS



Showing active remodelling of the grafted bone

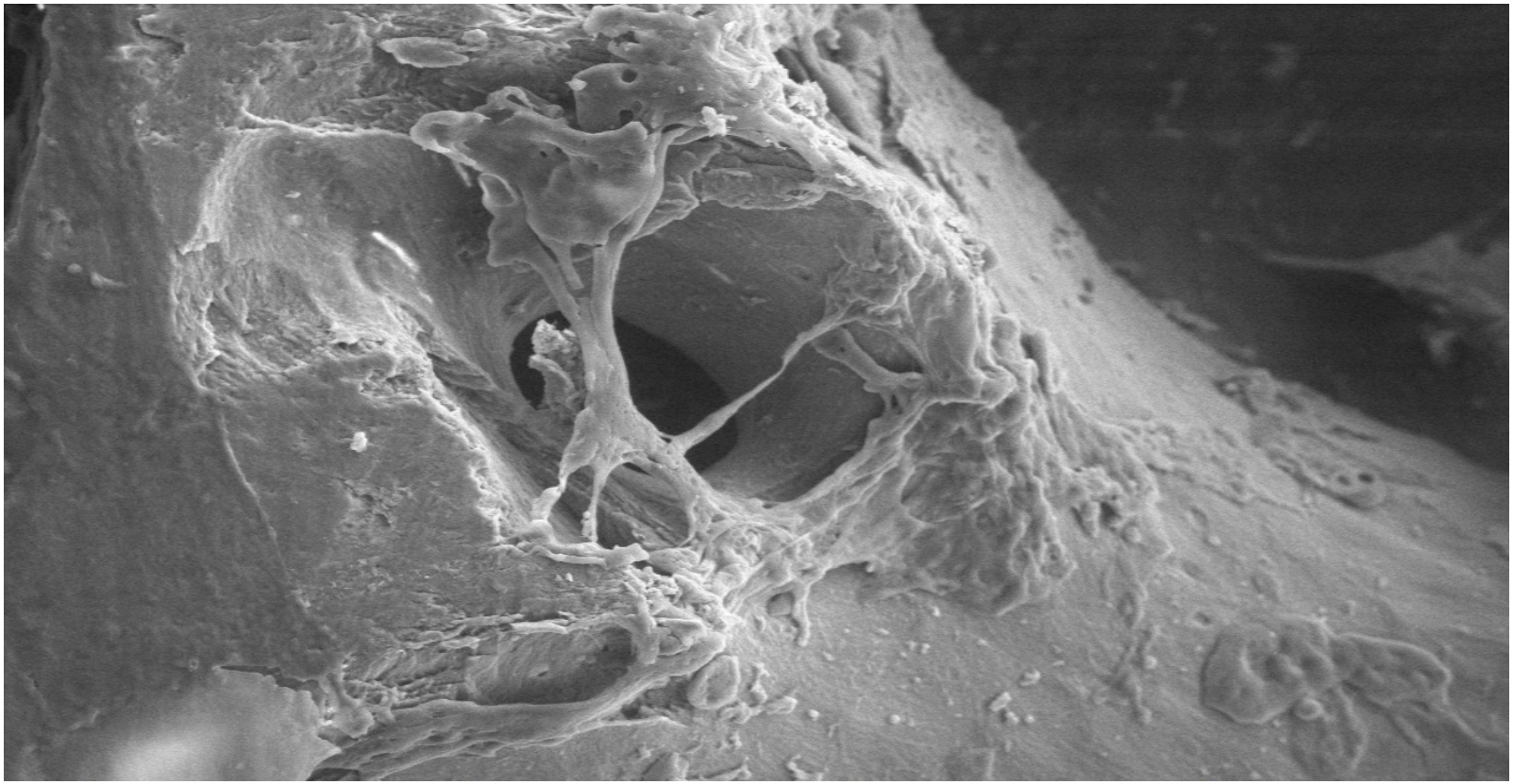
Ferracini R. et al, 2019

We used a biohybrid bone substitute composed of a bovine bone-derived matrix mechanically reinforced with poly(L-lactide-*co*-ε-caprolactone) (PLCL) and integrated with RGD-containing collagen fragments to improve the cell adhesion, the hydrophilicity of the matrix, and the overall biocompatibility .



SmartBone (SB) is a commercially available CE-labeled class III medical device, and it has a vast clinical record being commercially available as a bone substitute for bone regenerative surgery.

There is growing interest **in combining bone xenografts with cellular components, e.g., mesenchymal stem cells (MSCs) to promote osteoinductivity and osteoconductivity and significantly improve bone regeneration**



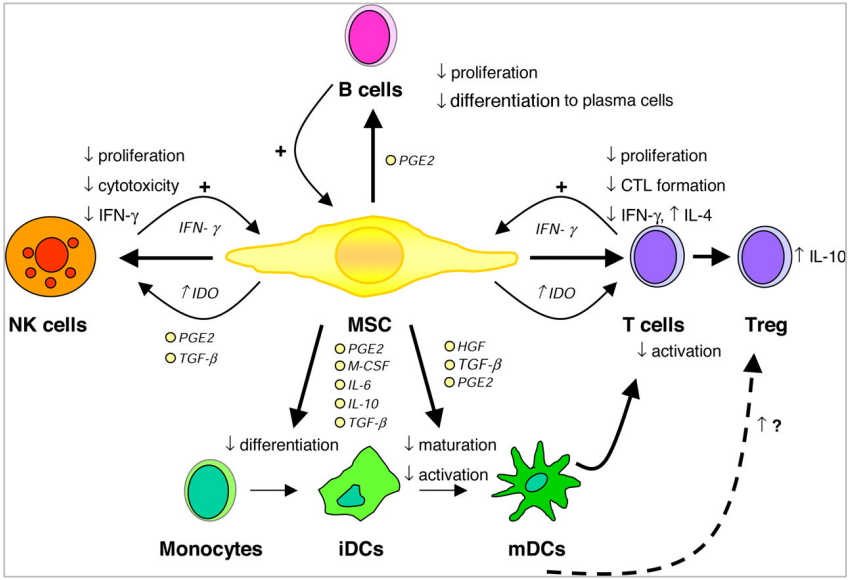
Magnification 500X

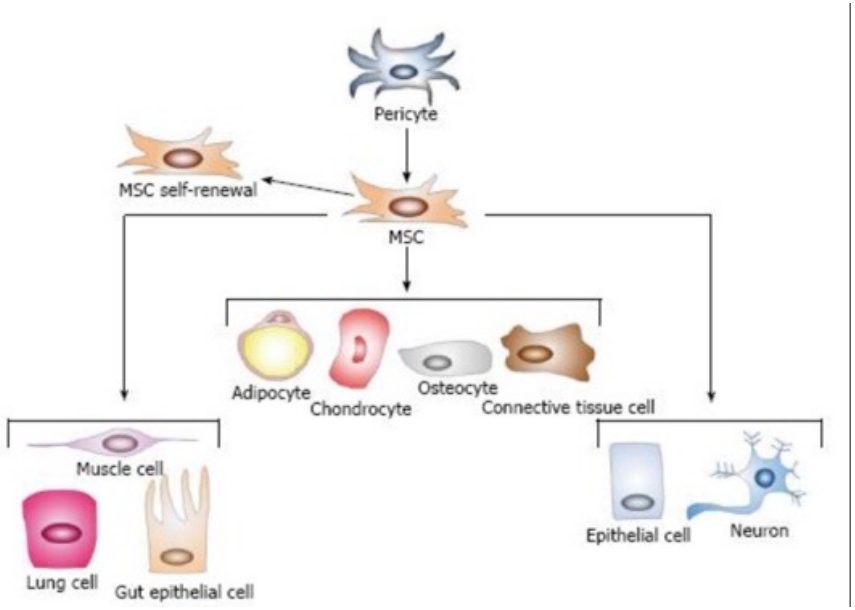
Scanning electron-microscope of adherent differentiating ASCs on SmartBone

* Roato I. et al.. *Stem Cells International, 2018*.

Mechanisms of MSC Action in Bone Healing

- They are able to differentiate into at least 3 cell lineages in vitro, including chondroblasts, osteoblasts, and adipocytes
- Immunomodulatory Effects
- Angiogenesis
- Homing and Recruitment to Bone Defects
- Induction of differentiation of host cells





Advantages of Adipose as a source of MSCs

- 1,000 times more stem cells in 1 g of fat than in 1 g of bone marrow
- Easier harvest (liposuction)
- The harvesting is much less painful compared to bone marrow extraction
- Low risk of infection
- Higher cell viability

Disadvantages of Adipose derived MSCs

- MSCs cannot be extracted from cases of severe serous fat atrophy
- High tendency to differentiate into adipocytes spontaneously if MSCs are not embedded in a “bone environment”
- The harvesting and preparation procedure is longer than with bone marrow MSCs

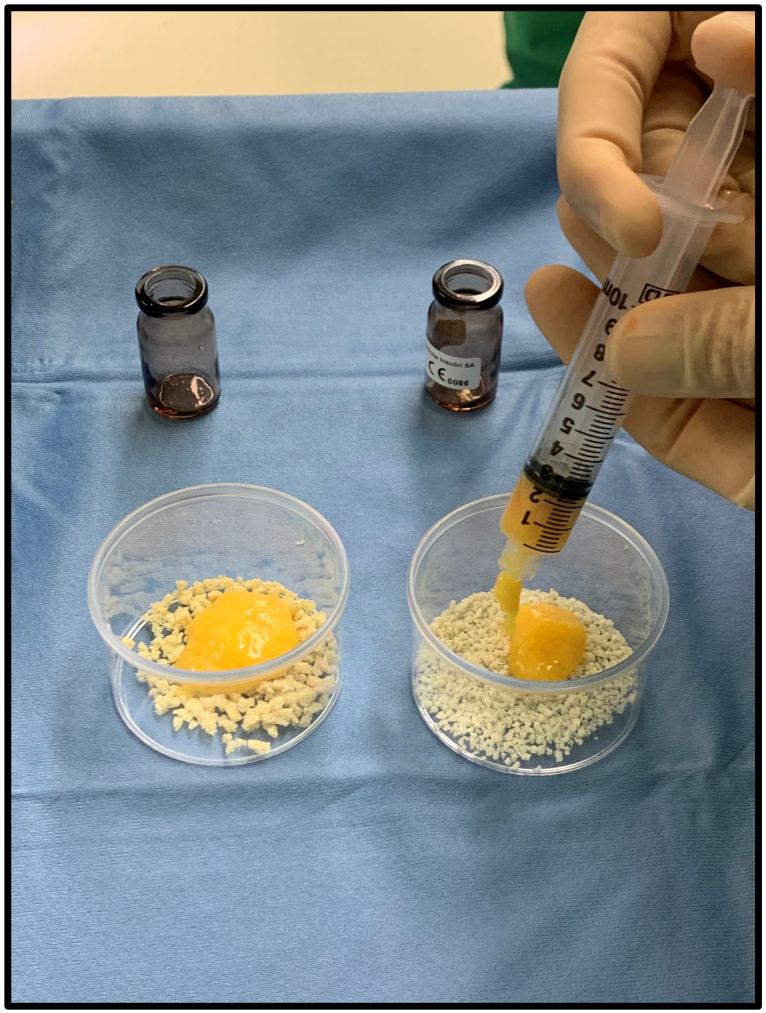
Delivery Methods of MSCs for Bone Repair

- Systemic Injection Approach
- Direct Local Injection Approach
- Tissue Engineering Approaches

    MSC Delivery within Injectable or Prefabricated Scaffolds

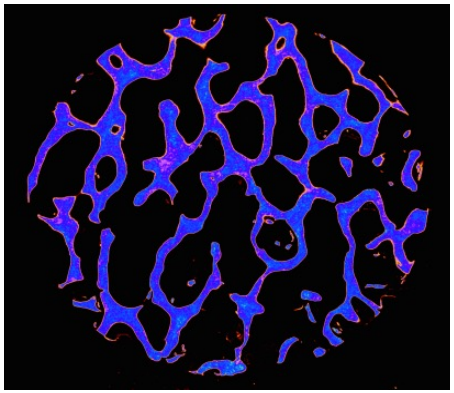
    MSC extracts delivery

    Molecular engineering

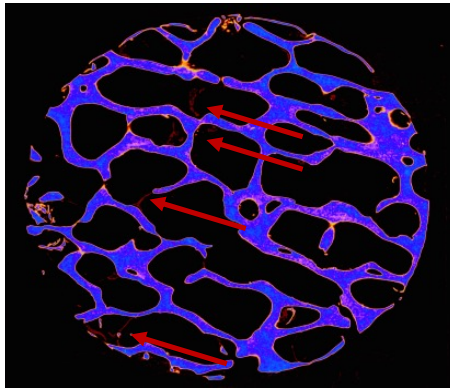


Adipose-derived Stromal Vascular Fraction & SmartBone were investigated as an alternative source for bone regeneration

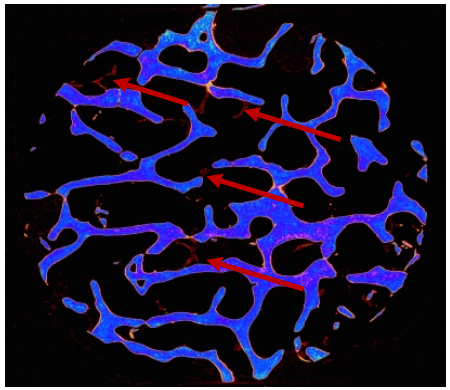
CTRL

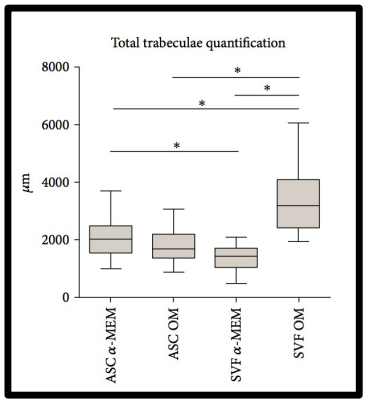


ASC



SVF

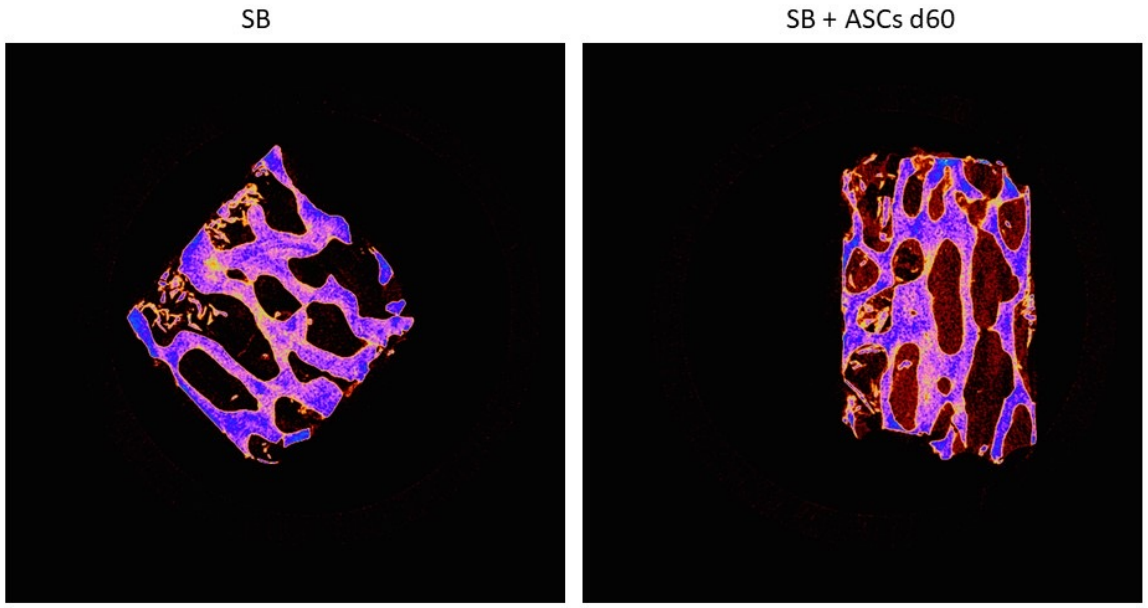




ASCs and Stromal Vascular Fraction cultured on SmartBone, promote **newly formed trabeculae in vitro.**

* Roato I. et al.. *Stem Cells International*, *2018*.

Newly formed trabeculae are visible in 3D microCT at 60 days from seeding in vitro.

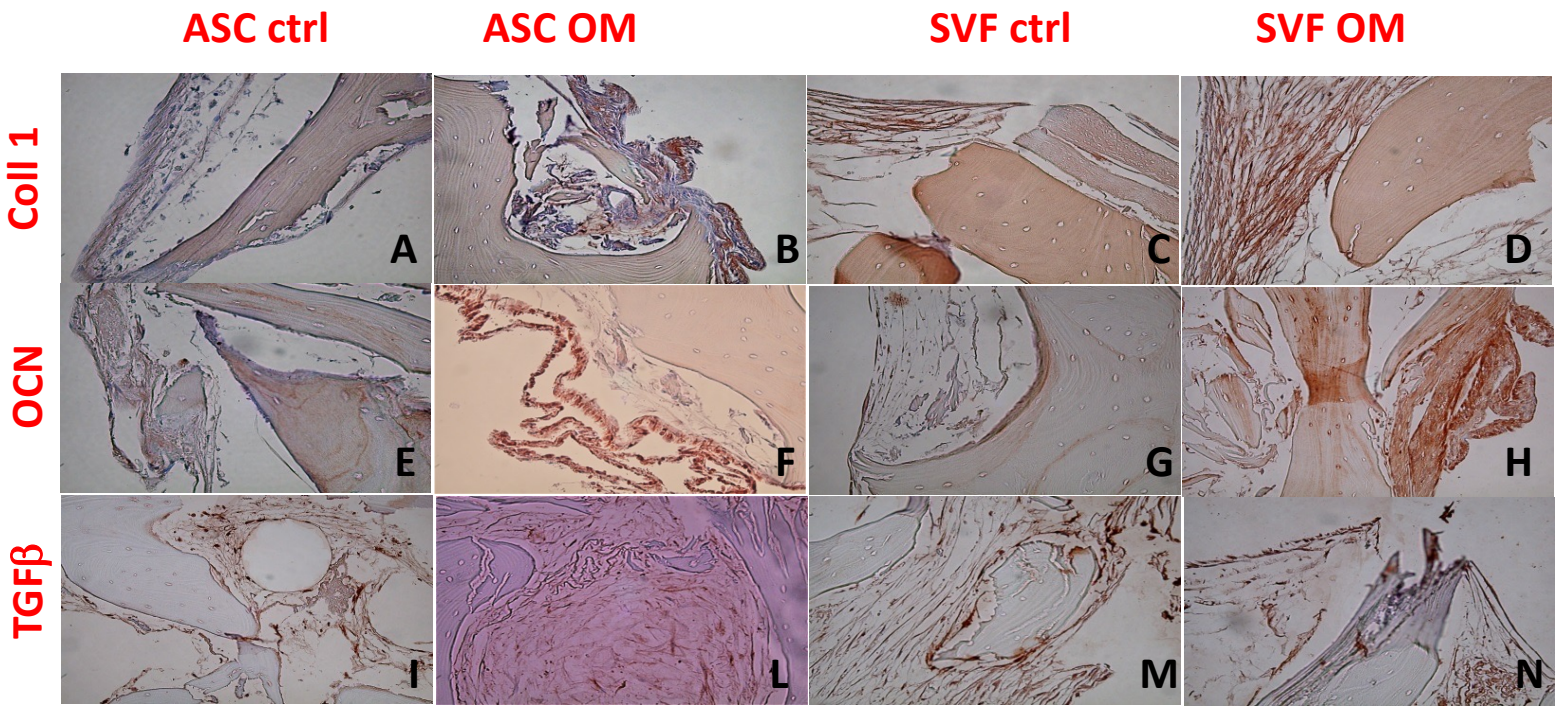


ASCs and SVF cultured on SmartBone , promote **newly formed trabeculae**

A color contrast mask was used to allow a clear identification of newly formed mineralized tissue.

* Roato I. et al.. *Stem Cells International*, *2018*.

ASCs and SVF colonization on SmartBone is documented by the presence of bone markers using immunohistochemistry



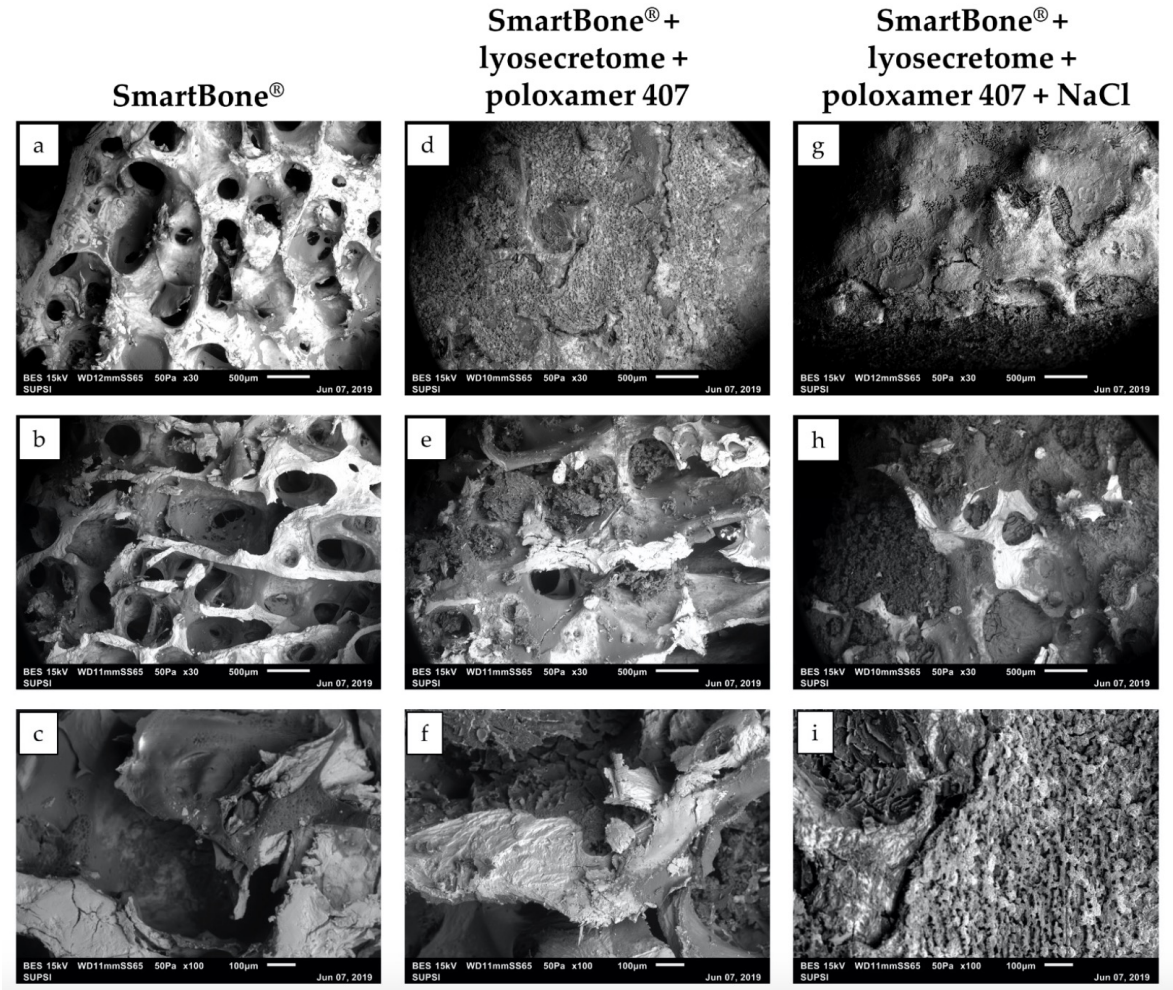
Magnification 20X

* Roato I. et al.. *Stem Cells International*, *2018*.

Can MSC extraction and secretome production represent an alternative to viable autologous MSC cells for SB enrichment?

- SB scaffolds were loaded with lyosecretome, a freeze-dried formulation of mesenchymal stem cell (MSC) secretome, containing proteins and extracellular vesicles (EVs).
- MSC-secretome was prepared according to GMP-compliant procedures, and it was formulated into a standardized and ready-off-the-shelf product, named Lyosecretome.
- Lyosecretome was loaded onto SmartBone scaffolds via an adsorption method since it is straightforward and cost-effective.

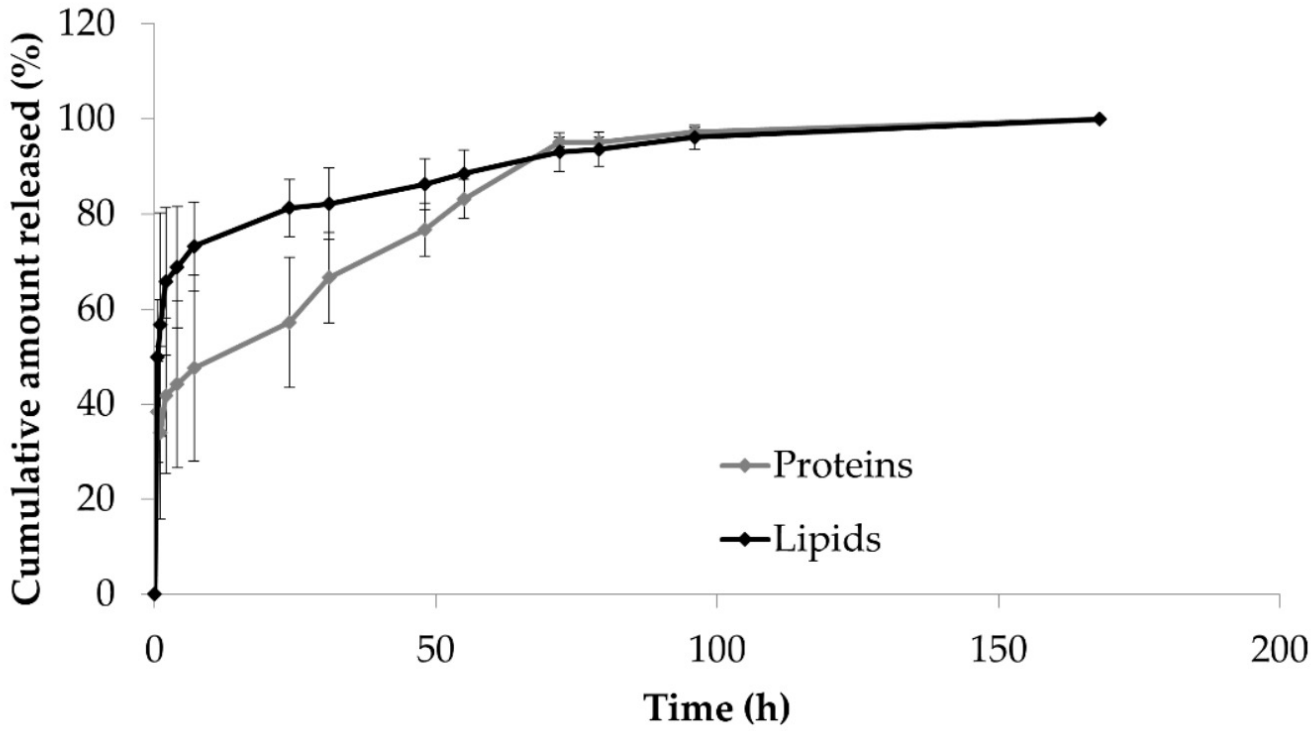
The Lyosecretome formulation was optimized to achieve proper and homogeneous loading of proteins and EVs into SmartBone



Using a formulation with poloxamer 407 and NaCl, uniform deposition of lyosecretome was observed on the surface of the support structure (Figure g), with a complete closure of the external and the central pores (Figure h,i)

Bari E. et al., Int. J. Mol. Sci. 2021

After loading, the scaffolds were characterized in terms of drug loading, and release.

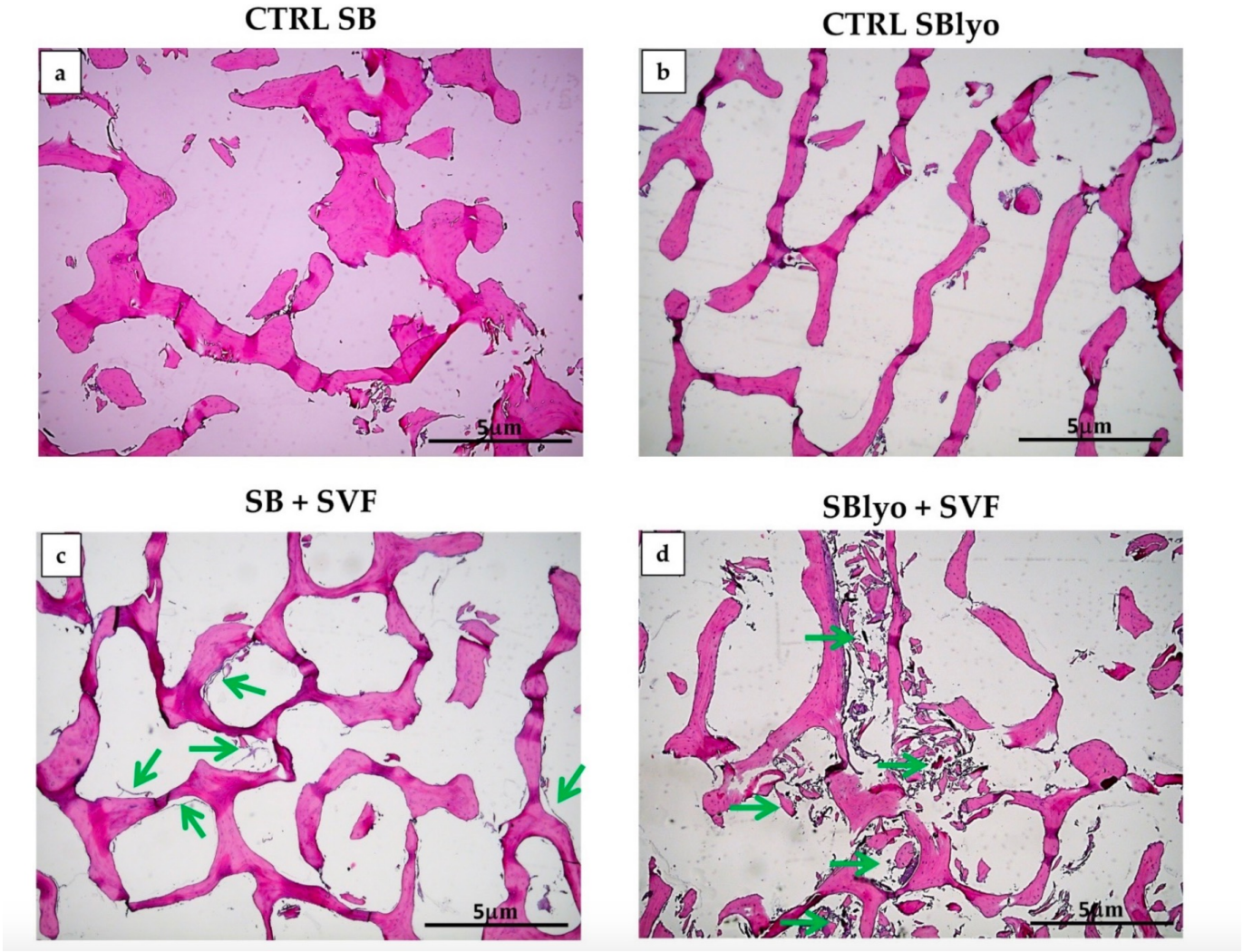


It is worth noting that the release of proteins is slower than the release of lipids.
This may result from the high affinity of proteins for the bone-derived matrix and the RGD-containing collagen fragments of SB scaffolds.

Bari E. et al., 2021

Protein and lipid profile: the cumulative release of SBlyo scaffolds showed an initial burst release, within 30 min, of 39% of proteins and 50% of lipids. Then proteins were gradually released from SB, reaching 95% after 72h.

After loading, the scaffolds were characterized in terms of morphology



After 60 days, new bone tissue was evident in both SB and SB-Lyosecretome (Figure c,d).

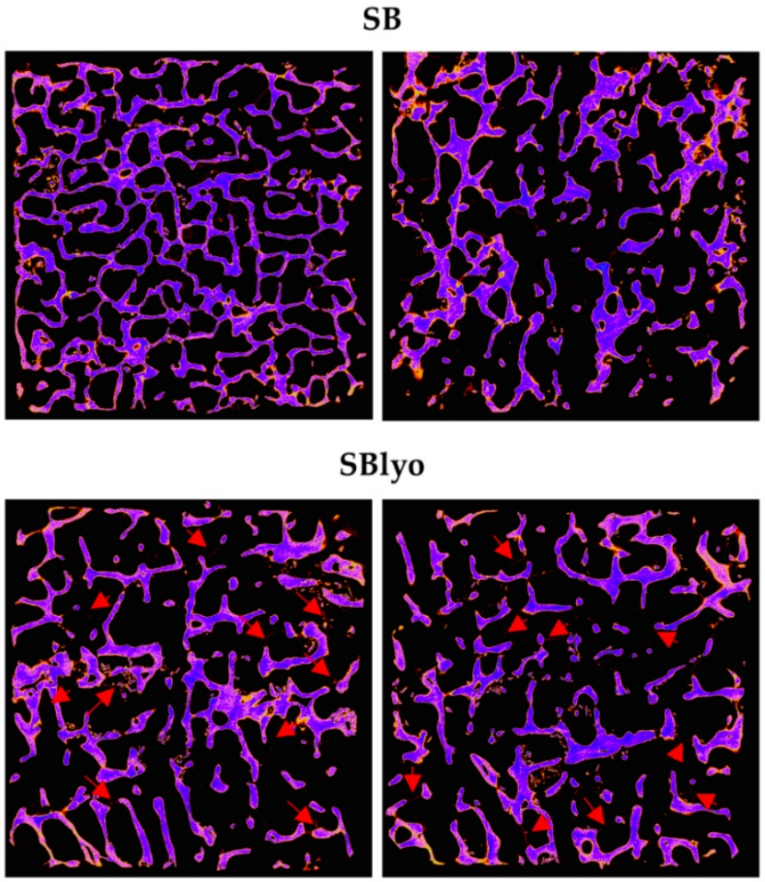
In all OCN, and COLL-1, RNA was extracted from cells grown on SB and Sblyo.

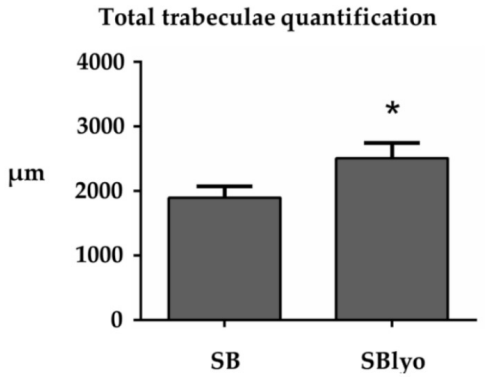
However though the neo-tissue formation area was detectable on both SBs, it was more marked on SBlyo than SB, as shown in Figure c,d, respectively.

Bari E. et al., 2021

Trabeculae quantification with or without Lyosecretomes.

The neo-trabeculae are visible as red filaments (indicated by the the red arrows). They show a significant difference between SB and SBlyo ($p < 0.05$).





After 60 days, SB and SBlyo were collected, washed with PBS to remove the re- maining medium, and fixed in a neutral buffer containing 4% *v/v* formaldehyde. Samples were analyzed using high-resolution X-ray microtomography (SkyScan) to study the structure and quantify the newly formed tissue after SVF colonization

Bari E. et al., 2021

Conclusions

- SB represents a tool for bone reconstruction in bone loss and fracture reconstructive surgery

- MSCs from adipose origin are a potential source of osteoblast precursor and can participate to the process of fracture healing, inhibition of inflammation and angiogenesis promotion.

- Adipose derived MSC loaded SB has a potential for novel in vivo studies on bone suppementation

Conclusions

- Lyosecretome from AD MSC can promote a bone-forming mechanism when loaded on SB.
- Overall, our results confirm the role of MSC-secretome as an intercellular messenger able to orchestrate bone regeneration.
- This strategy has the advantage of using a standardized and ready off-the-shelf MSC-secretome powder, with a controlled release reproducible bioactive bone graft.

Open question

Should we start a clinical trial using SB loaded with Lyosecretome or with SB loaded with autologous MSC from adipose tissue?

Cells or lyosecretomes?

Advantages of cell extracts:

- Standardized
- Easy marketing
- Standard response
- Reduce surgical trauma

Advantages of autograft MSC:

- Easier to obtain
- Readily available in the OR
- Fresh material and selected ratio scaffold/cells
- No further studies are necessary before starting a human investigation

Acknowledgements

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Disclosures

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Thanks for your attention