A Comparison of Different Bone Substitute Materials:

"Xenograft and Allograft"

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Three ALWAYS rules

Sadegh Hasannia, 2015

A BAD clinical outcome is

Almost ALWAYS bad for the patient

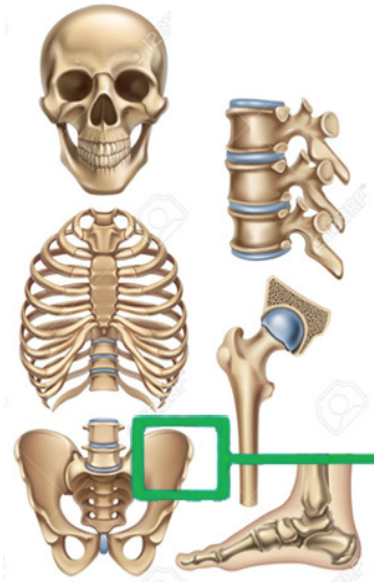
Not ALWAYS bad for the practitioner and

Because Doctors can choose another treatment plan for their patients

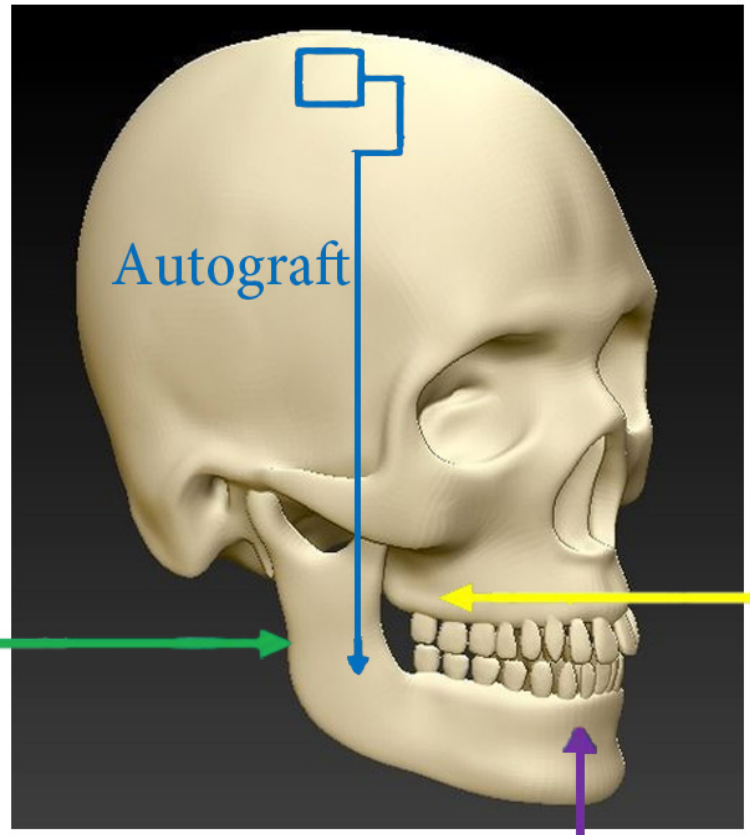
ALWAYS good for scientists

Because the scientist is looking for the biochemical, biological and pathological cause of treatment failures

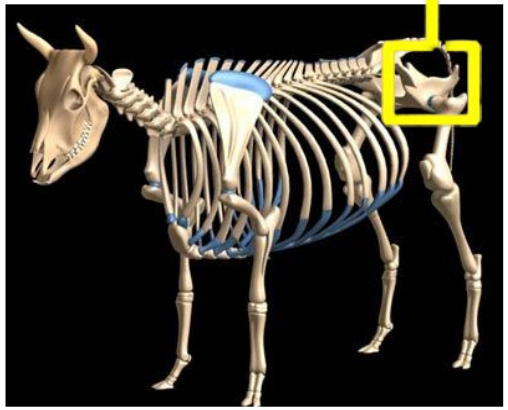
According to this rules, for Doctors to be professional, they must have a scientific view of clinical events



Allograft



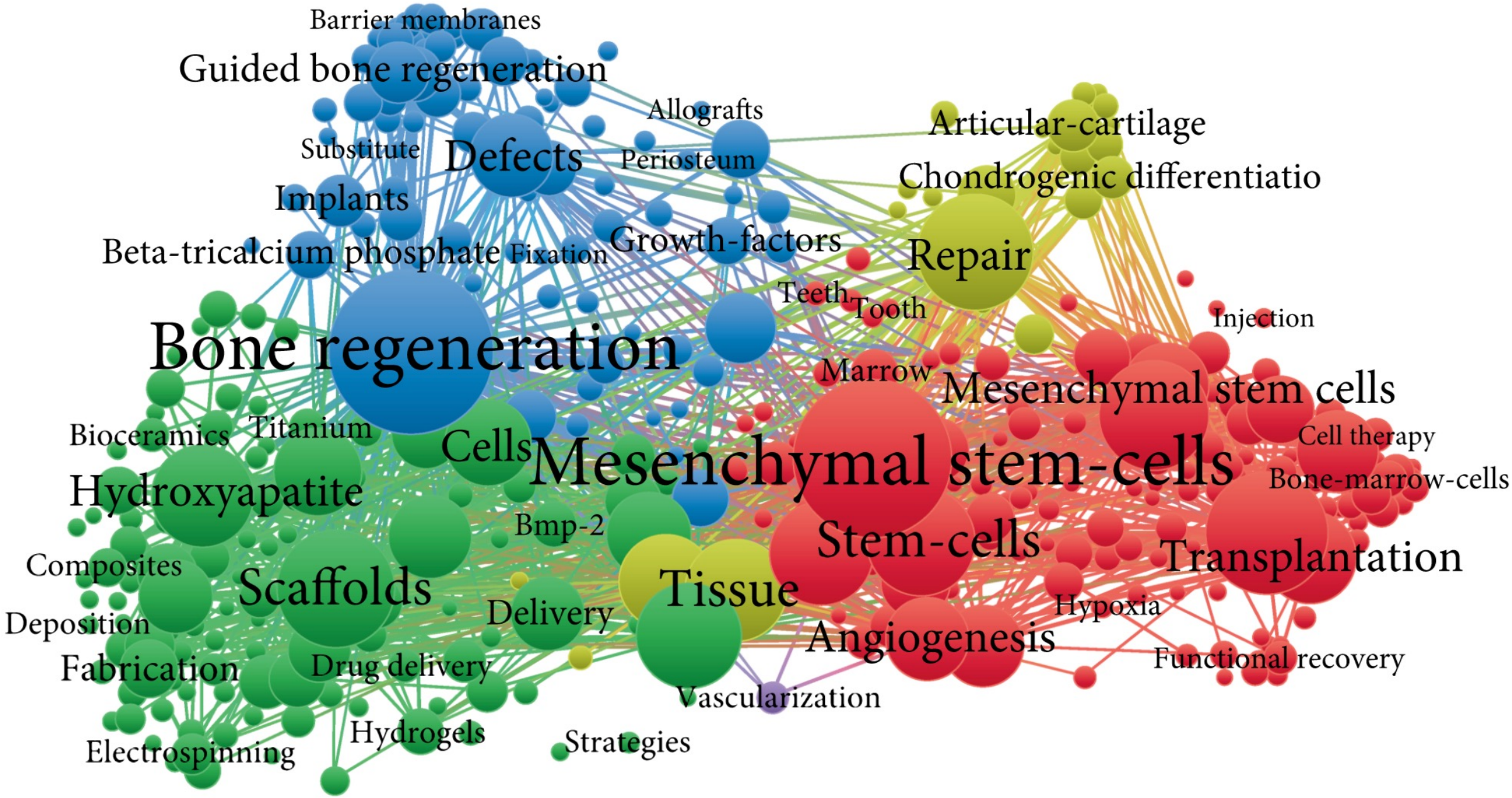
Xenograft



Synthetic

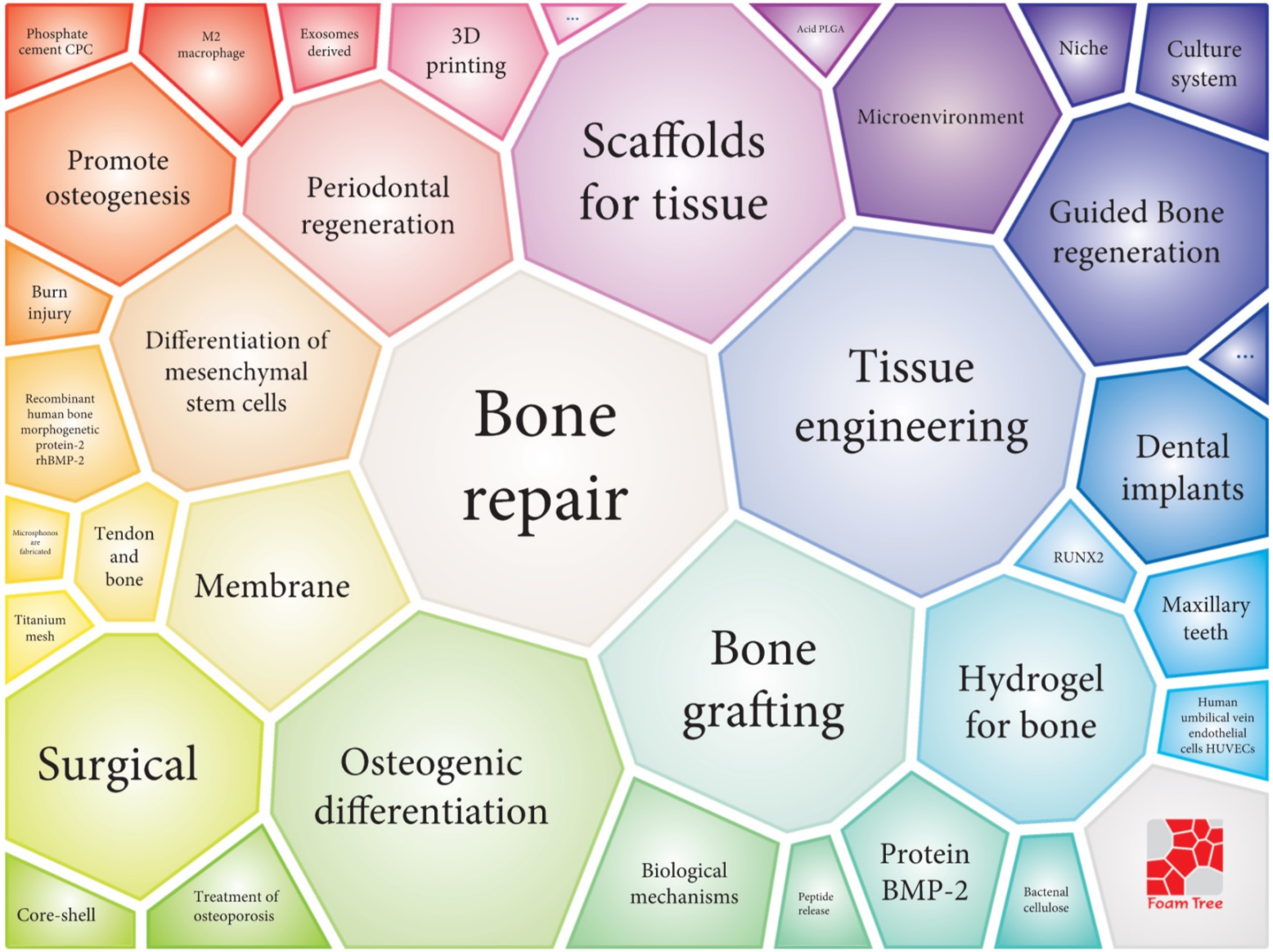
The Ideal Bone Grafts

- The ideal material to replace bone tissue should meet precise specifications, such as being:
- Biocompatible,
- Bioresorbable,
- Osteoconductive,
- Osteoinductive,
- Structurally like bone,
- Porous,
- Mechanically resistant,
- Easy to use, safe, and
- Cost-effective



Current Trends in Research on Bone Regeneration: A Bibliometric Analysis. 2020. Doi.org/10.1155/2020/8787394

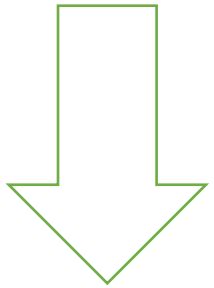
A survey of major topics in bone regeneration. The visualizations were obtained using the carrot system, based on the top-ranking results of the search.



Current Trends in Research on Bone Regeneration: A Bibliometric Analysis. 2020. Doi.org/10.1155/2020/8787394

The global dental implant market is anticipated to grow steadily

from $**3.4** billion in 2011 to $**6.4** billion in 2024



The **favorable clinical performance** of dental implants has been attributed to their firm **bone integration**

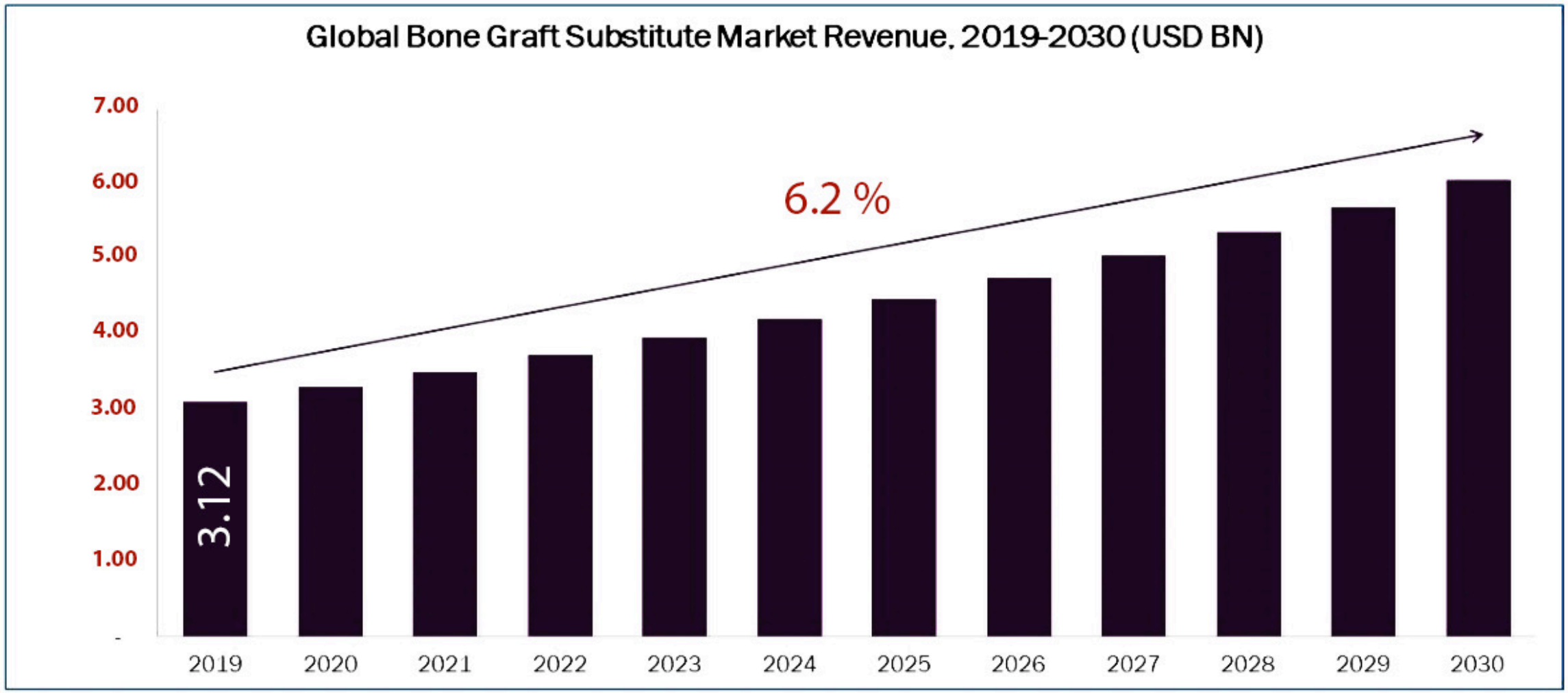
Increasing Burden of Oral Diseases and High Success Rate of Dental Biomaterials in Oral Treatment

The rapidly growing number of qualified dental professionals is expected to increase access to dental care. According to the latest statistics of the Brazilian Institute of Geography and Statistics (IBGE), Brazil has 174,000 dentists, which corresponds to 11% of the world's total. Since 2004, after the implementation of the Oral Health National Policy in Brazil, the Brazilian population has become aware of oral health and the expenditure on dental care has increased substantially.

Brazil 2020 population is estimated at 212,559,417 people at mid year according to UN data.

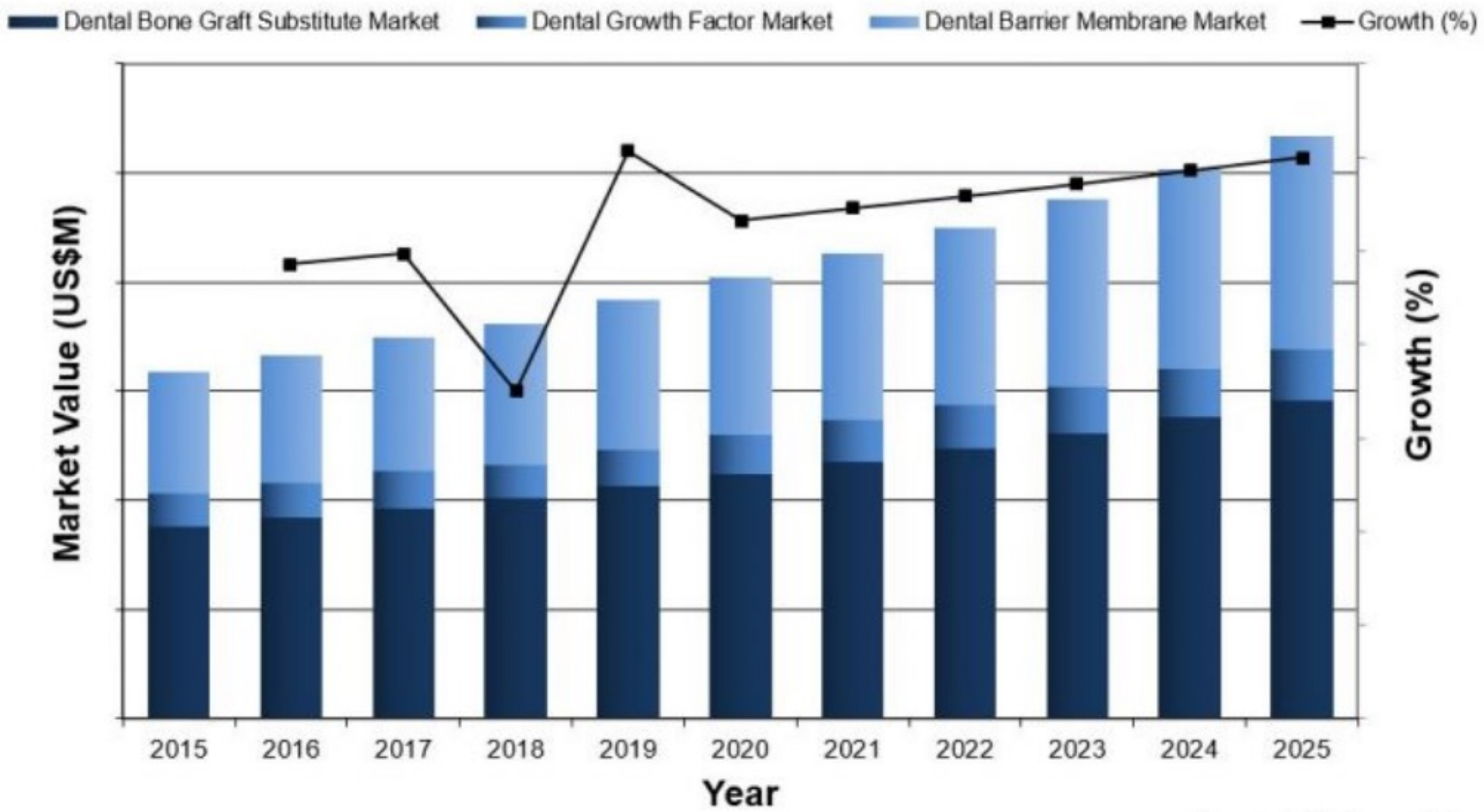
Around 120 million people in the U.S. are missing at least one tooth. Thus these all end points of demographics determines the global burden of this disease. Therefore, growing edentulous population is expected to drive the **growth of the global dental biomaterials market.**

https://www.persistencemarketresearch.com/market-research/dental-biomaterial-market.asp

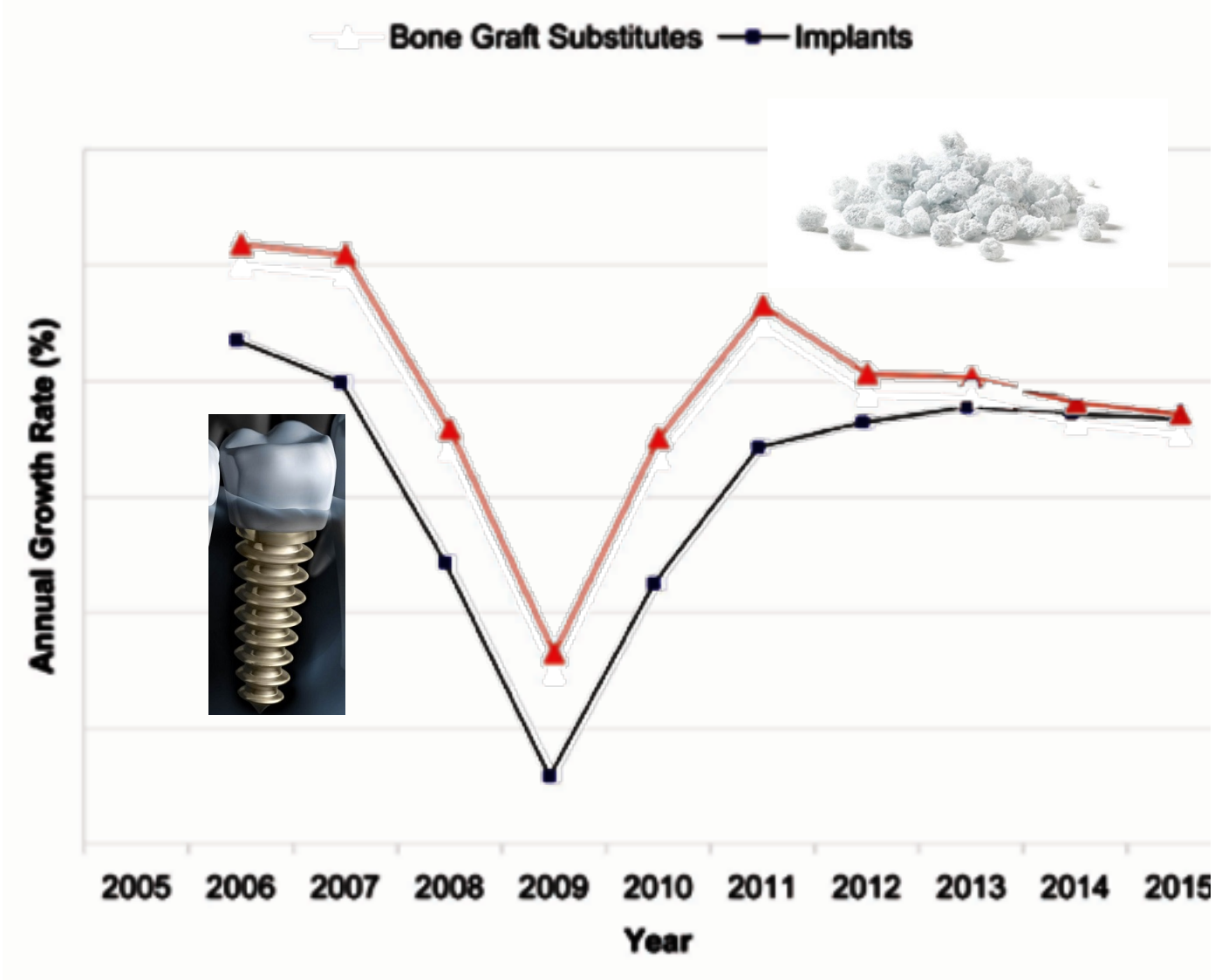


https://www.meddeviceonline.com/doc/a-regulatory-and-competitive-analysis-of-the-asia-pacific-dental-bone-graft-market-0001

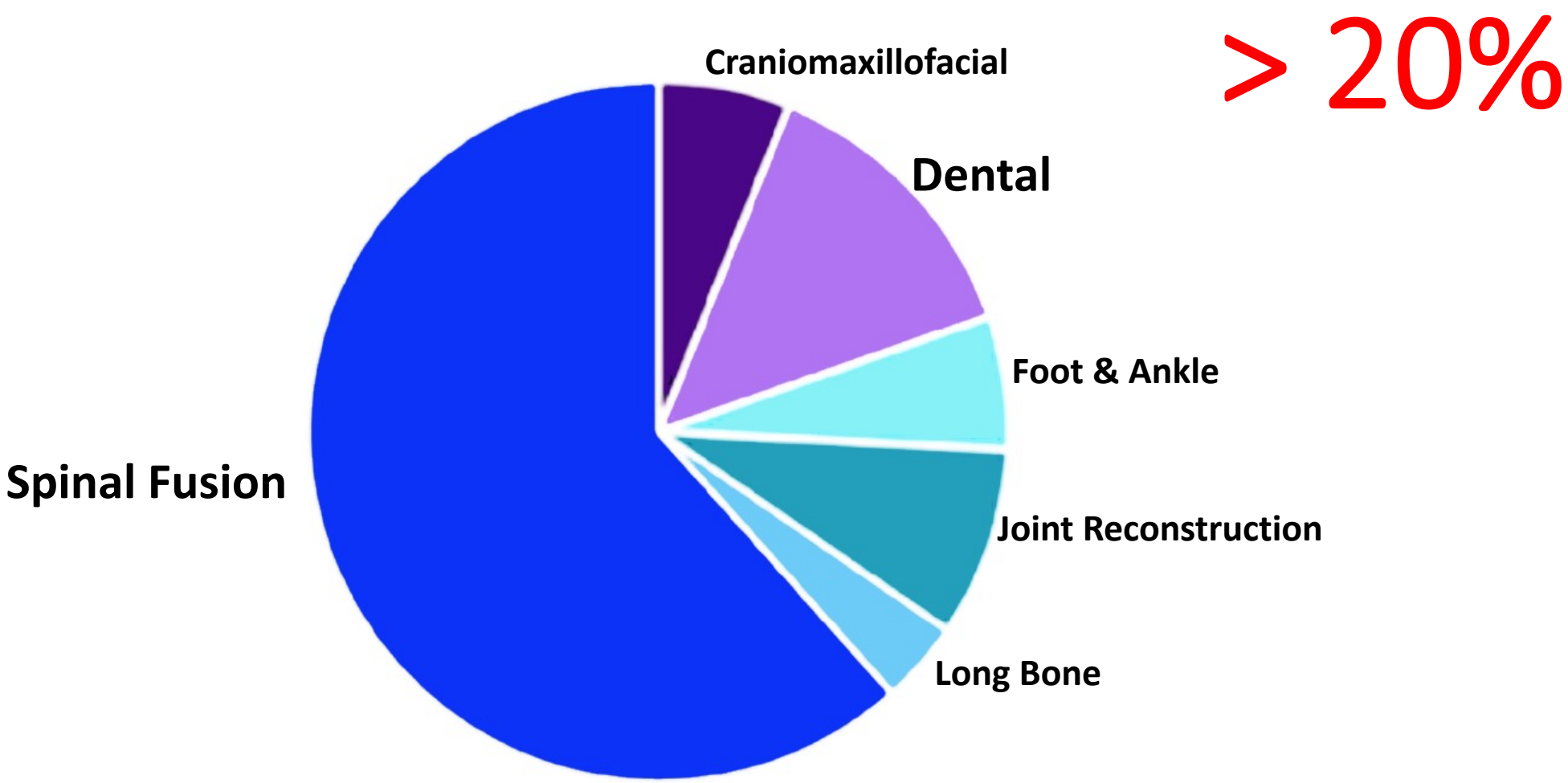
Dental Bone Graft Substitutes Market Value by Segment, U.S., 2015-2025



Source: iData Research Inc.


Bone Graft Substitutes
Implants
Annual Growth Rate (%)
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
Year

Global bone grafts market share by application, 2018



> 20%

https://www.grandviewresearch.com/industry-analysis/bone-grafts-substitutes-market

Bone grafting procedures were performed annually worldwide which is the second most frequent tissue transplantation after blood transfusion

- Figure 1 Market share of different BGS in 2012, according to Millennium Research.

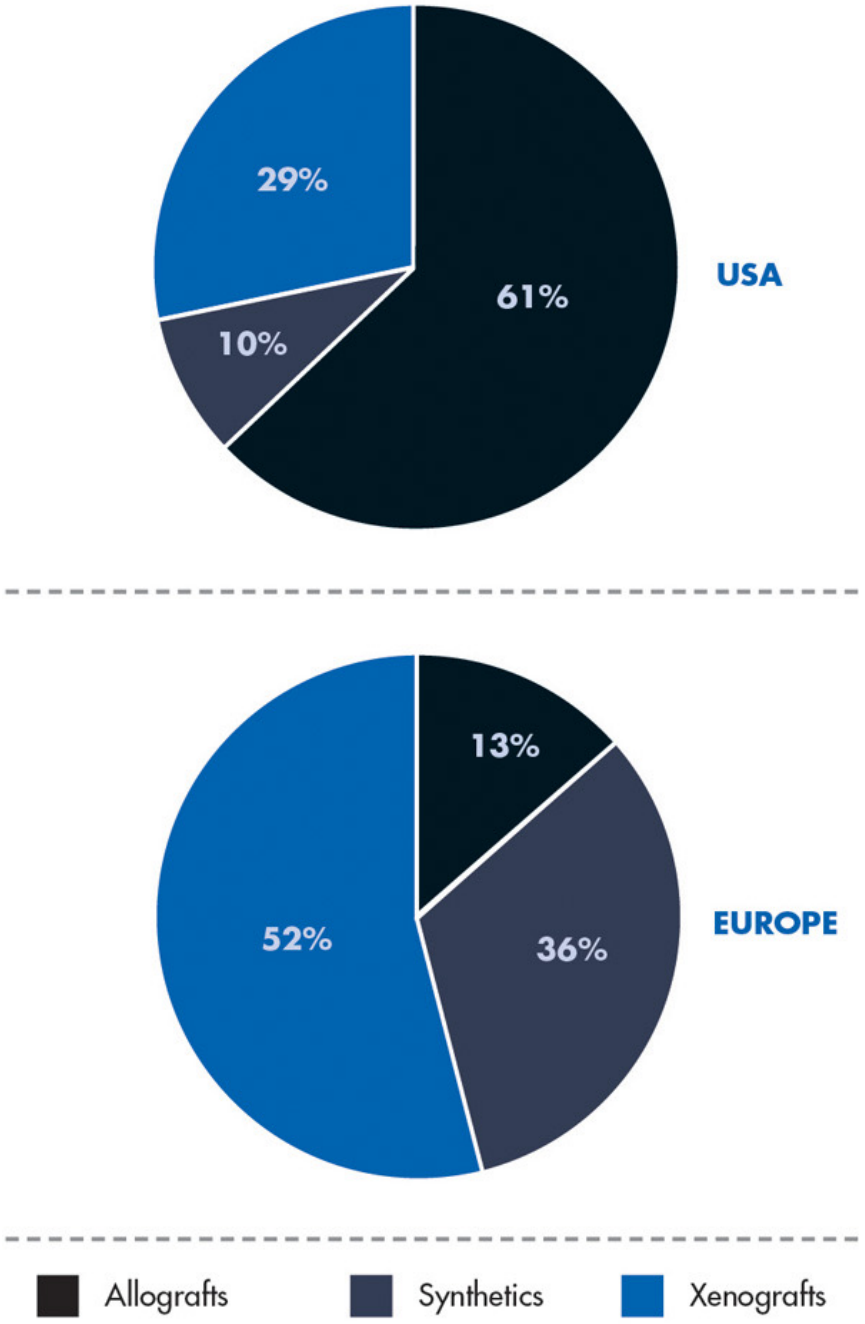
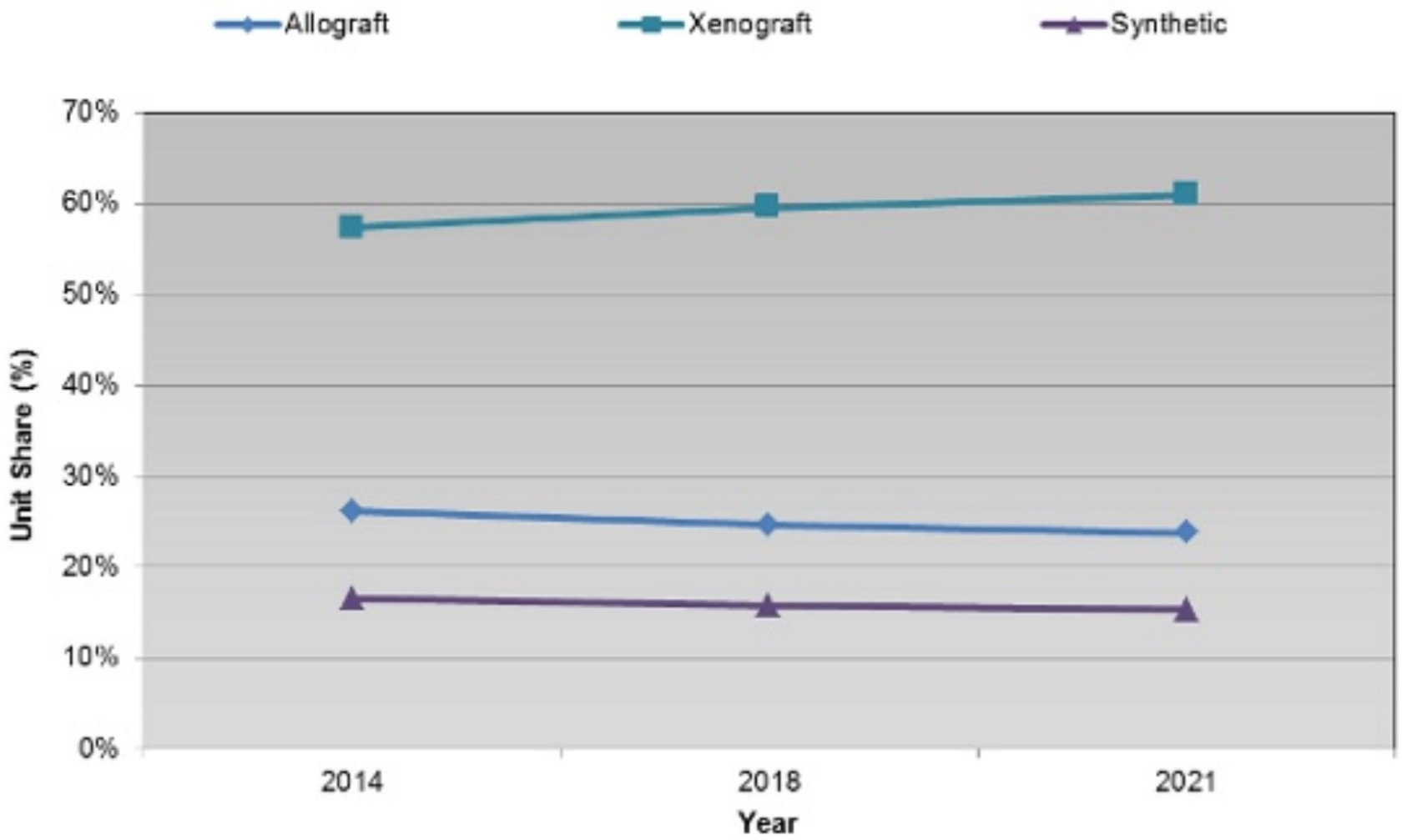
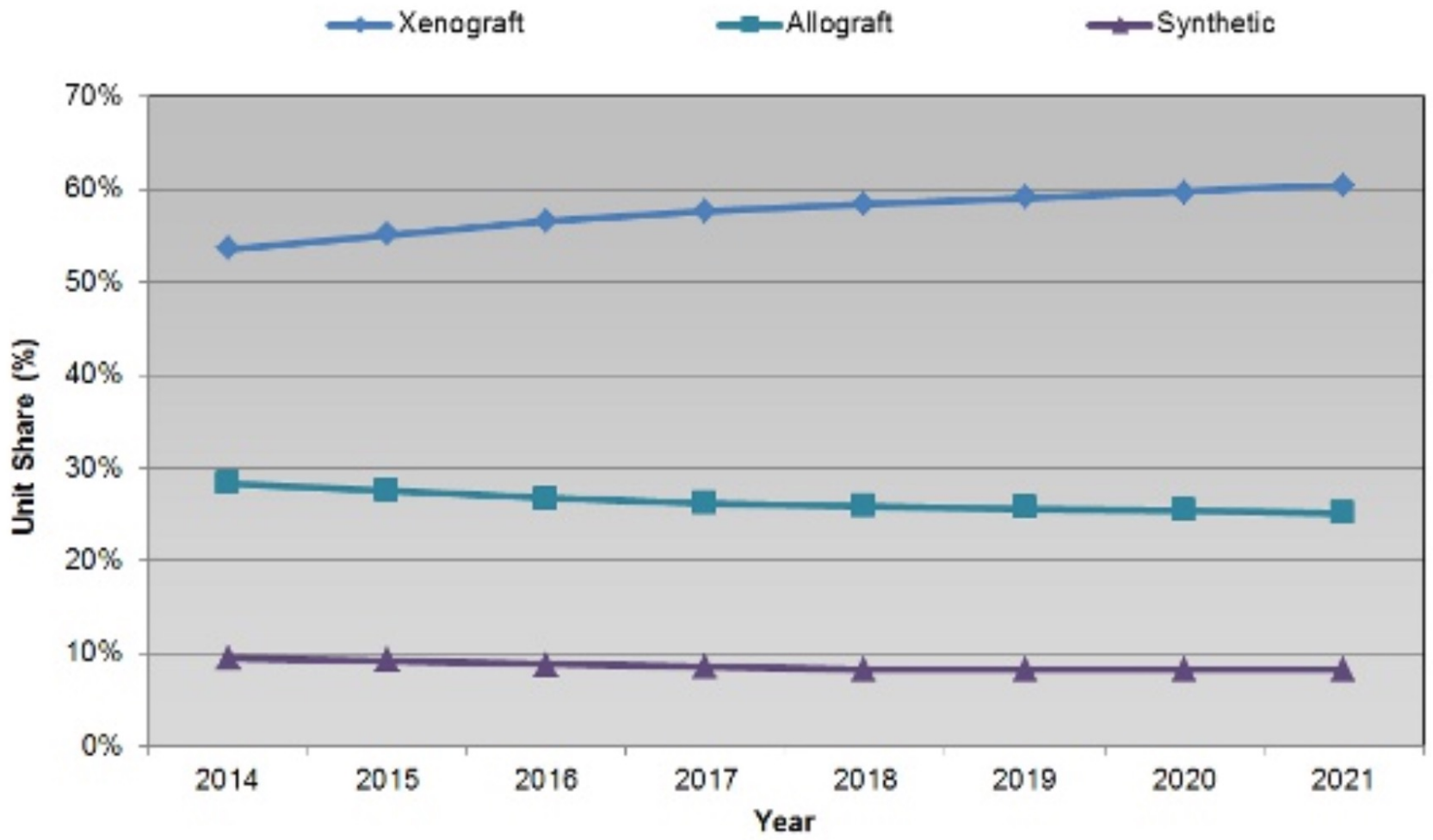


Chart 1: South Korea DBGS Unit Share by Material Type (2014/2018/2021)



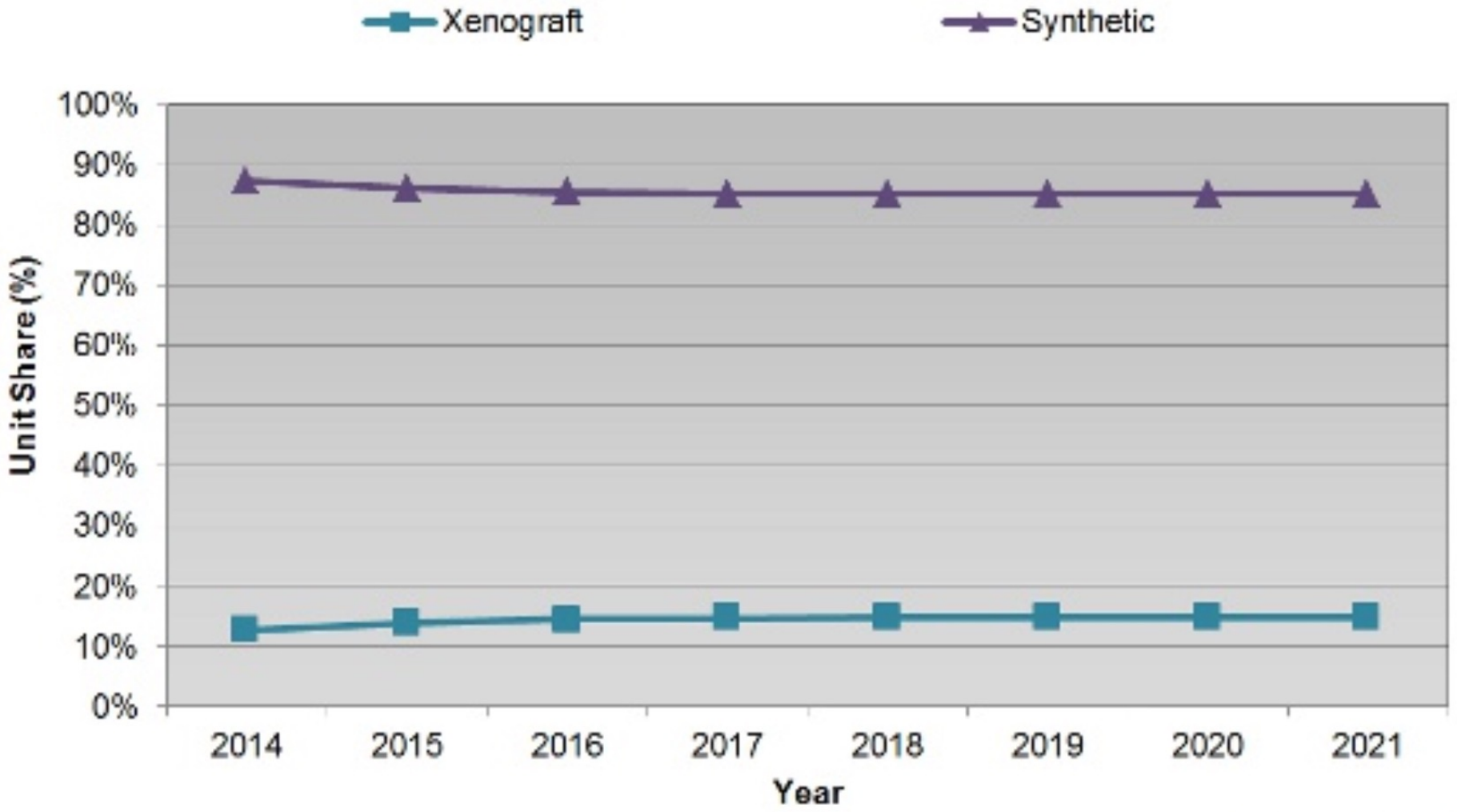
Source: iData Research Inc.

Chart 3: Allograft, Synthetic, and Xenograft Unit Share Analysis China (2011-2021)



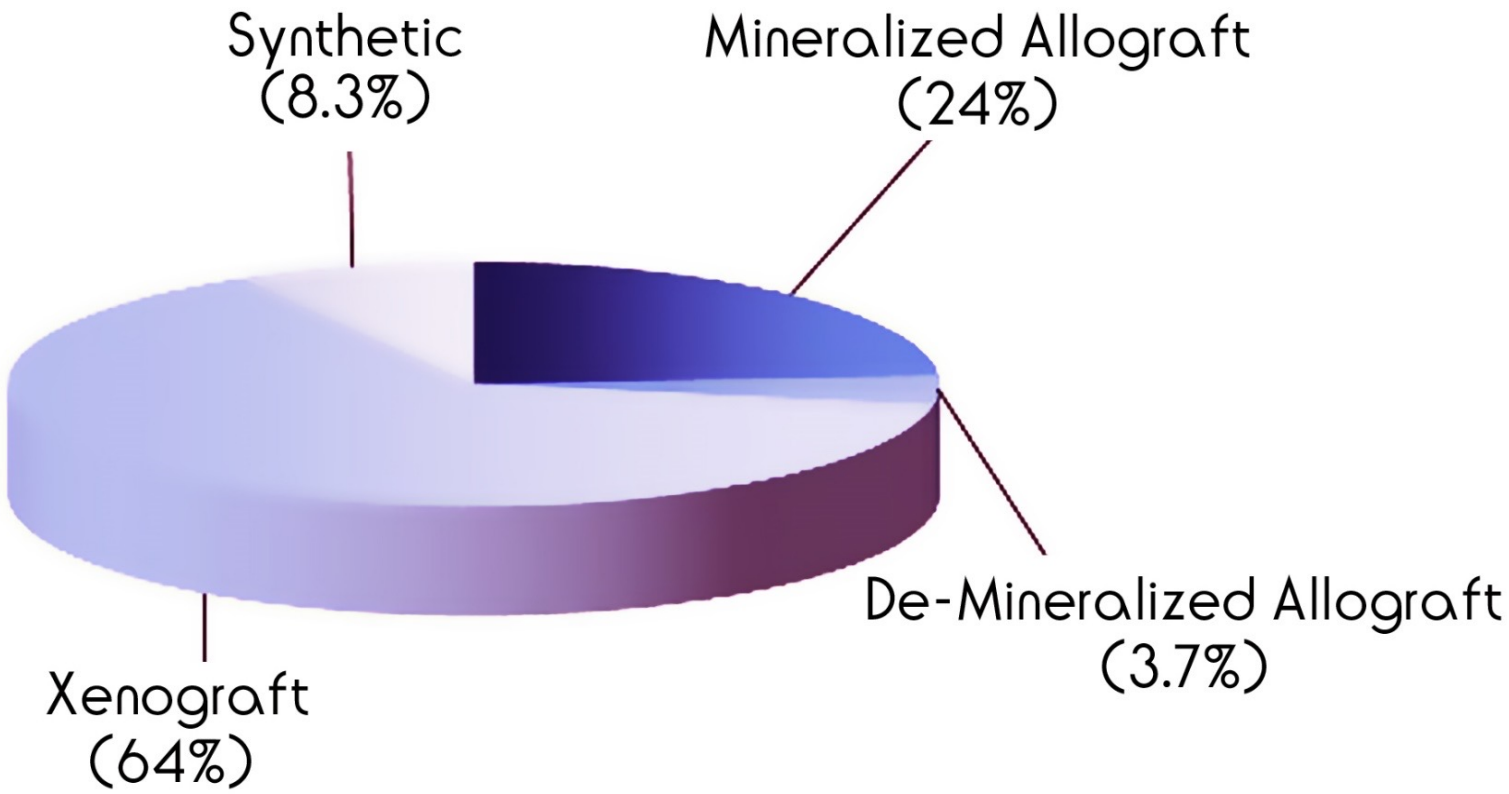
Source: iData Research Inc.

Chart 4: Xenograft and Synthetic Unit Share Analysis Japanese DBGS Market (2014-2021)



Source: IData Research Inc.

Chart 2: Expected Unit Share of DBGS Market in Australia 2021

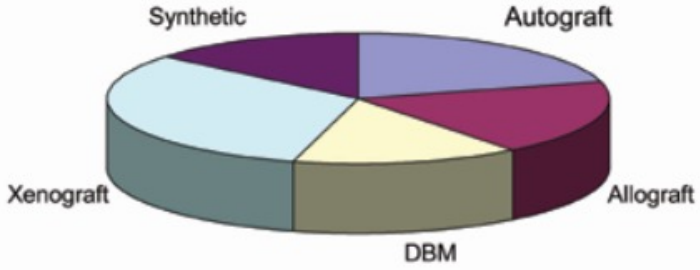


Source: iData Research Inc. 2015

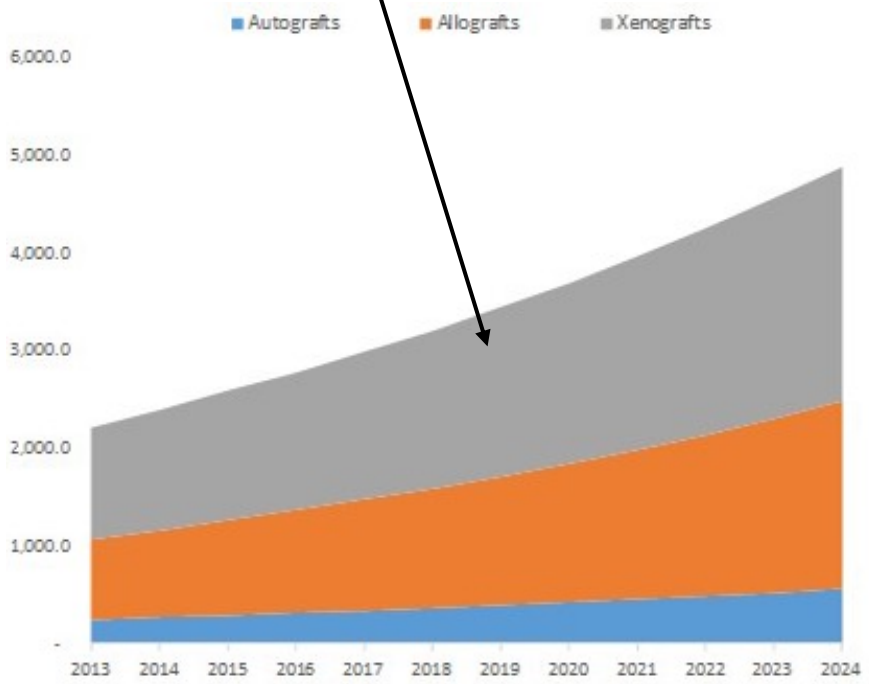
Dental Bone Graft Substitute by Material Type in USA

Since 2013 و Using xenograft in the North American market has increased compared to the allograft

2009



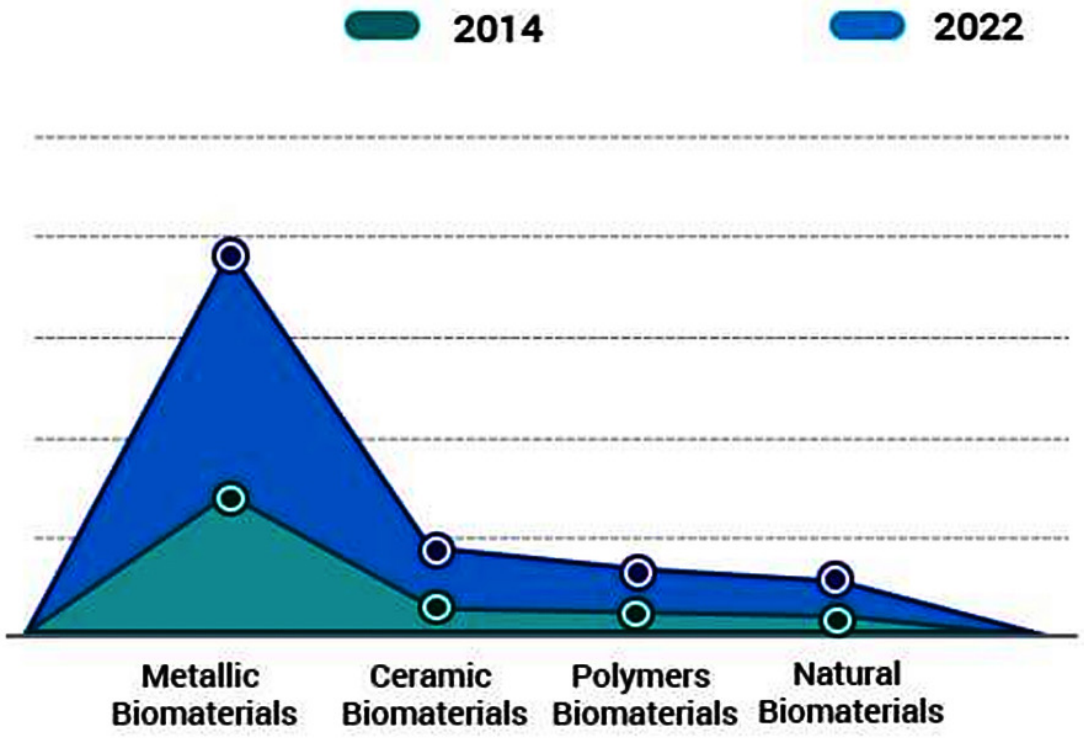
Source: iData Research Inc



2013-2024

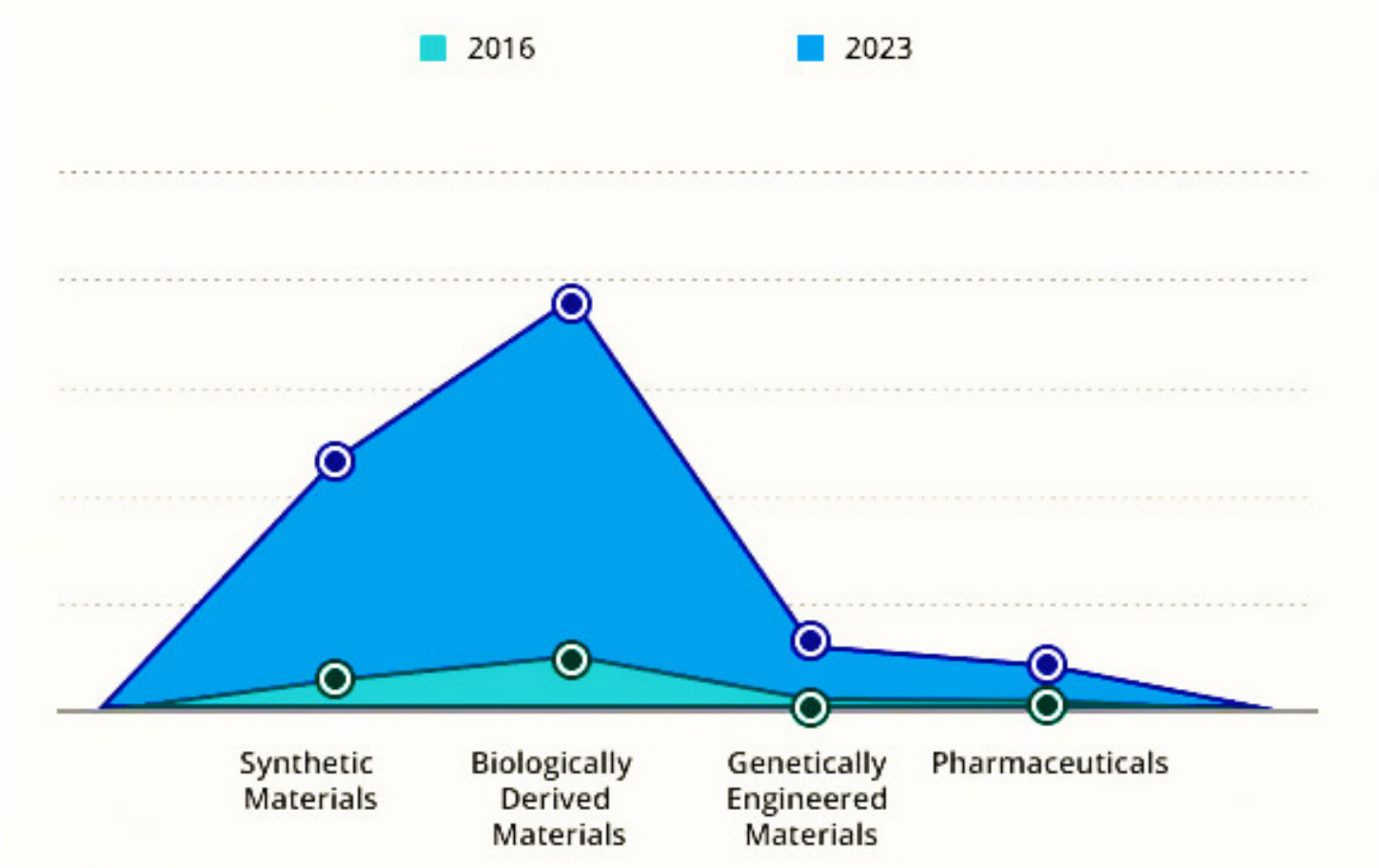
SEGMENT OVERVIEW	
Product	Allografts expected to witness fastest growth due to presence of benefits such as short healing times and low material costs.
Application	Implants for soft tissue reconstruction that enhances aesthetic appearance as well as organ functioning attributed for the largest share.
Mode of administration	Emergence of minimally invasive form of grafting surgical procedures is expected to boost the growth of surgical bioimplants.
Region	North America accounted for the largest share in this market owing to presence of key participants operating in this region

Global Implant Market by Material



METALLIC BIOMATERIALS segment holds a dominant position in 2014 and would continue to maintain the lead over the forecast period

Global Regenerative Medicine Market by Material

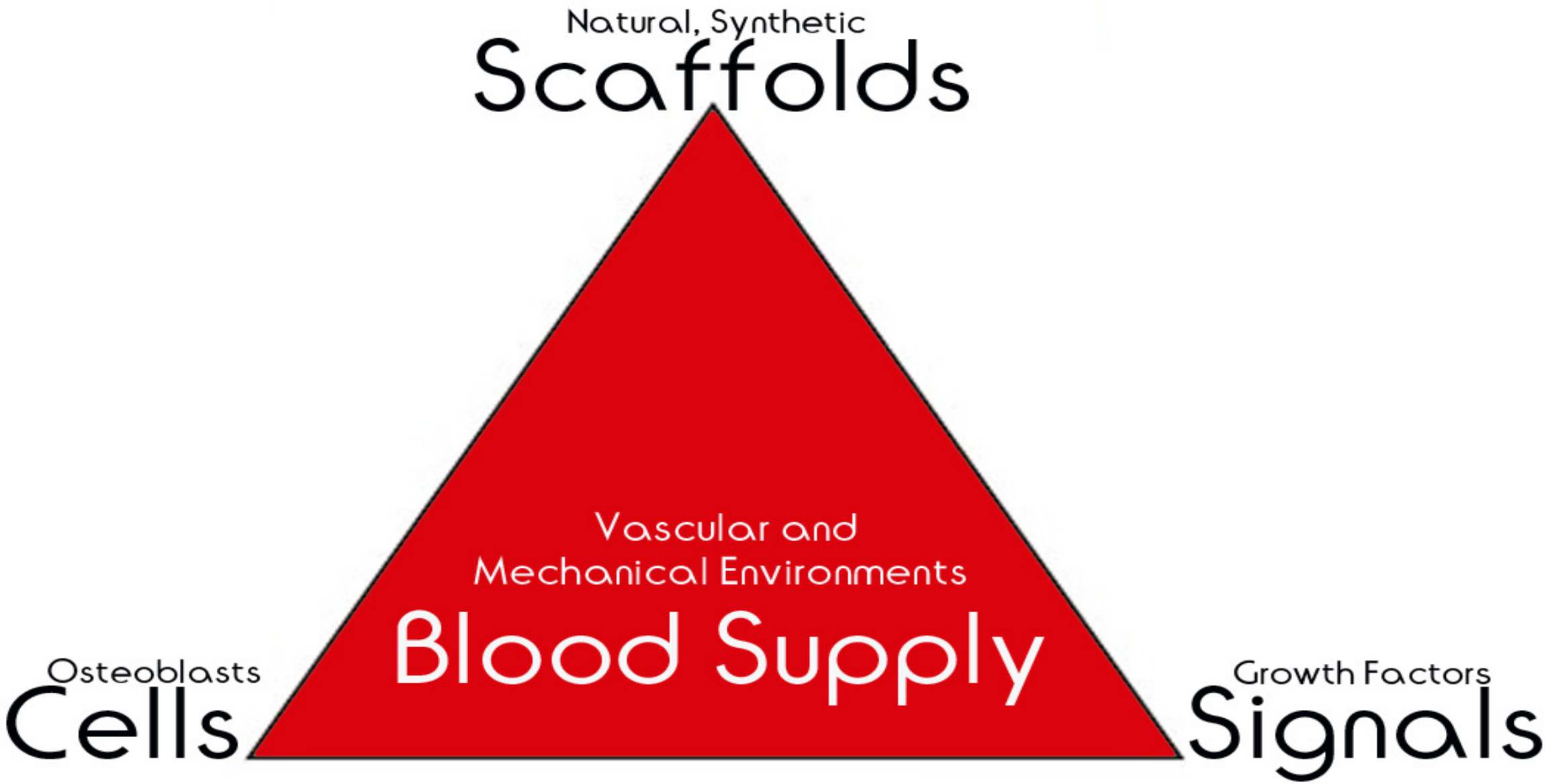


The Pittsburgh Tissue Engineering Initiative Definition

- Tissue engineering is the development and manipulation of laboratory grown molecules, cells, tissues, or organs to replace or support the function of defective or injured body parts. Although cells have been cultured, or grown, outside the body for many years, the possibility of growing complex, three-dimensional tissues literally replicating the design and function of human tissue is a recent development.

NIH Definition of Tissue Engineering

- Tissue engineering is an emerging multidisciplinary field involving biology, medicine, and engineering that is likely to revolutionize the ways we improve the health and quality of life for millions of people worldwide by restoring, maintaining, or enhancing tissue and organ function.


Natural, Synthetic
Scaffolds
Vascular and
Mechanical Environments
Blood Supply
Osteoblasts
Cells
Growth Factors
Signals

Clinical Outcome “Triangle”

Osteoconduction

Osteoblasts and osteoclasts can attach, migrate, grow and/or divide.

- Autograft
- Allograft
- Xenograft
- Growth factors plus scaffold
- Synthetics and other additives

Osteoinduction

Osteoinduction refers to the ability of the graft to send a signal to attract, proliferate, and differentiate early-lineage cells (e.g., mesenchymal stem cells or osteoprogenitor cells) into bone-forming cells, resulting in the formation of healthy mineralized bone.

Osteogenesis

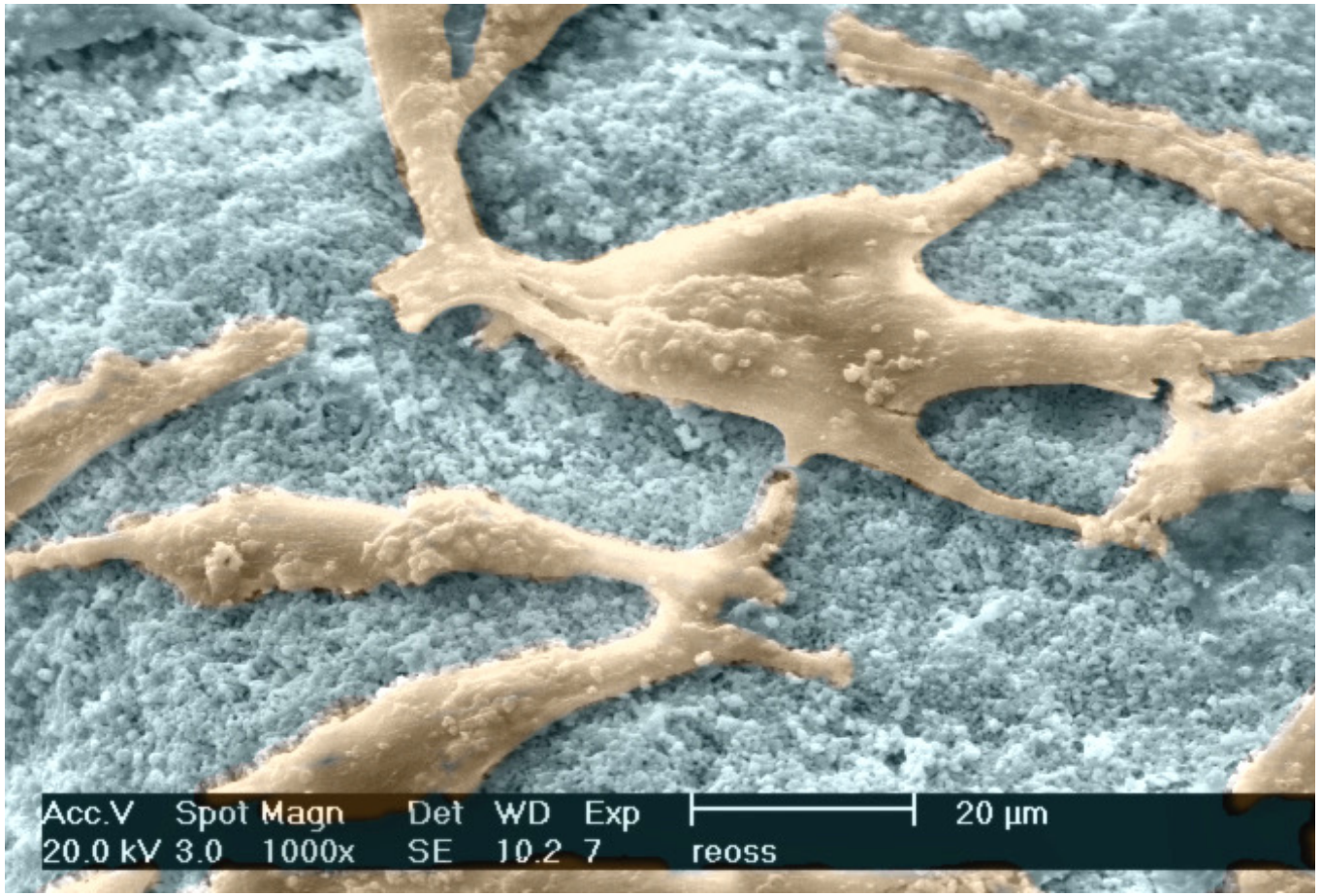
- Autograft
- Bone marrow aspirate

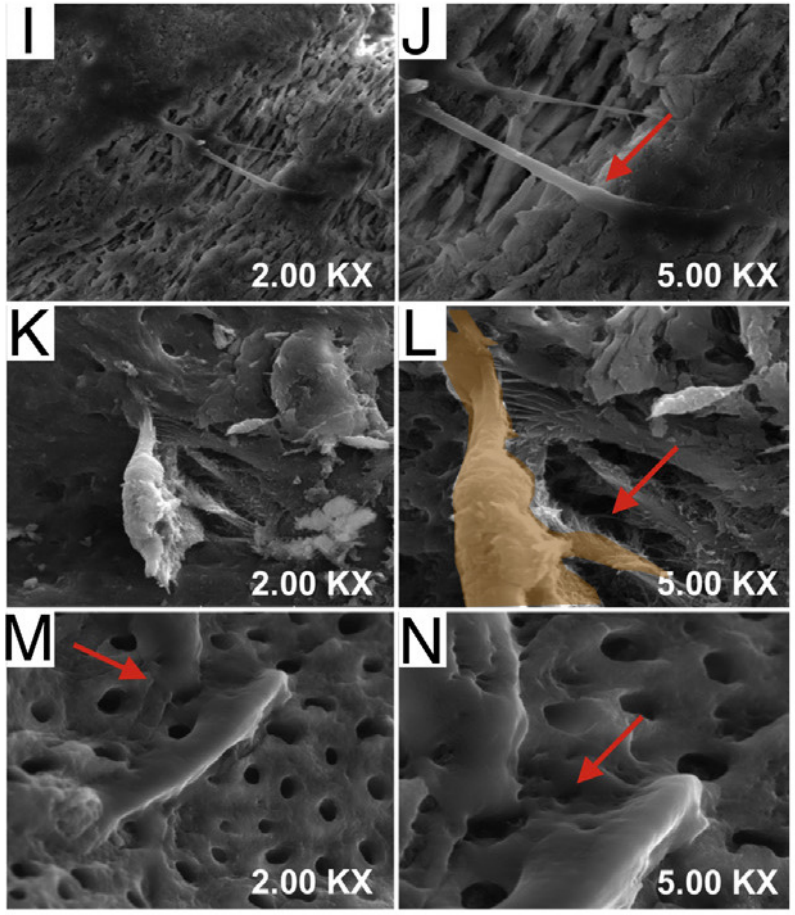
Osteogenesis is the development and formation of bone
Osteogenesis occurs when stem cells must exist

Osteo-promotion

Involves enhancement of osteoinduction without possession of osteoinductive properties. For example, enamel matrix derivative enhances the osteoinductive effect of demineralized freeze-dried bone allograft (DFDBA), but will not stimulate bone growth alone

Osteoconductivity





rh**PDGF**-BB.

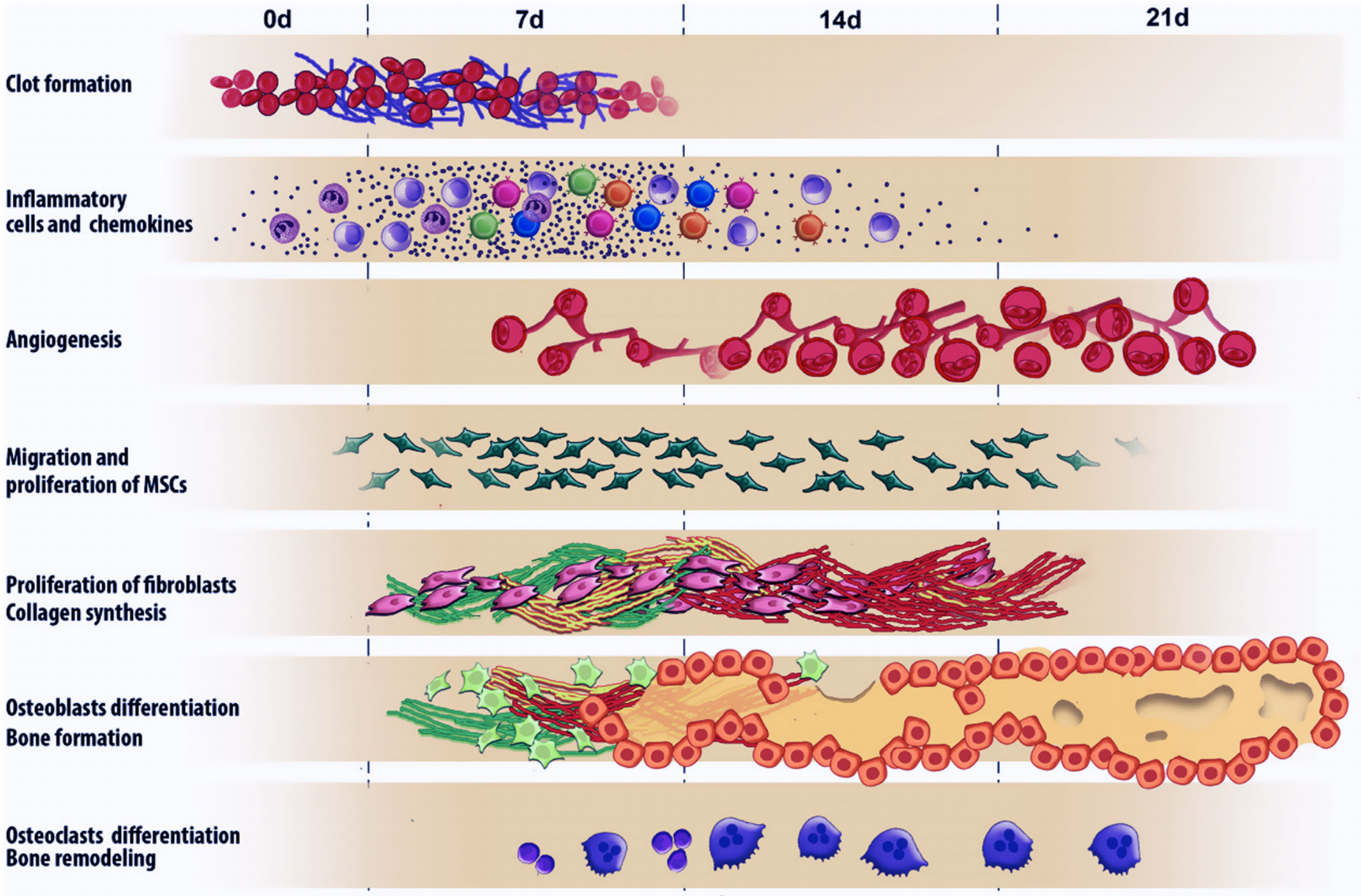
rh**BMP-2**

rhBMP-2

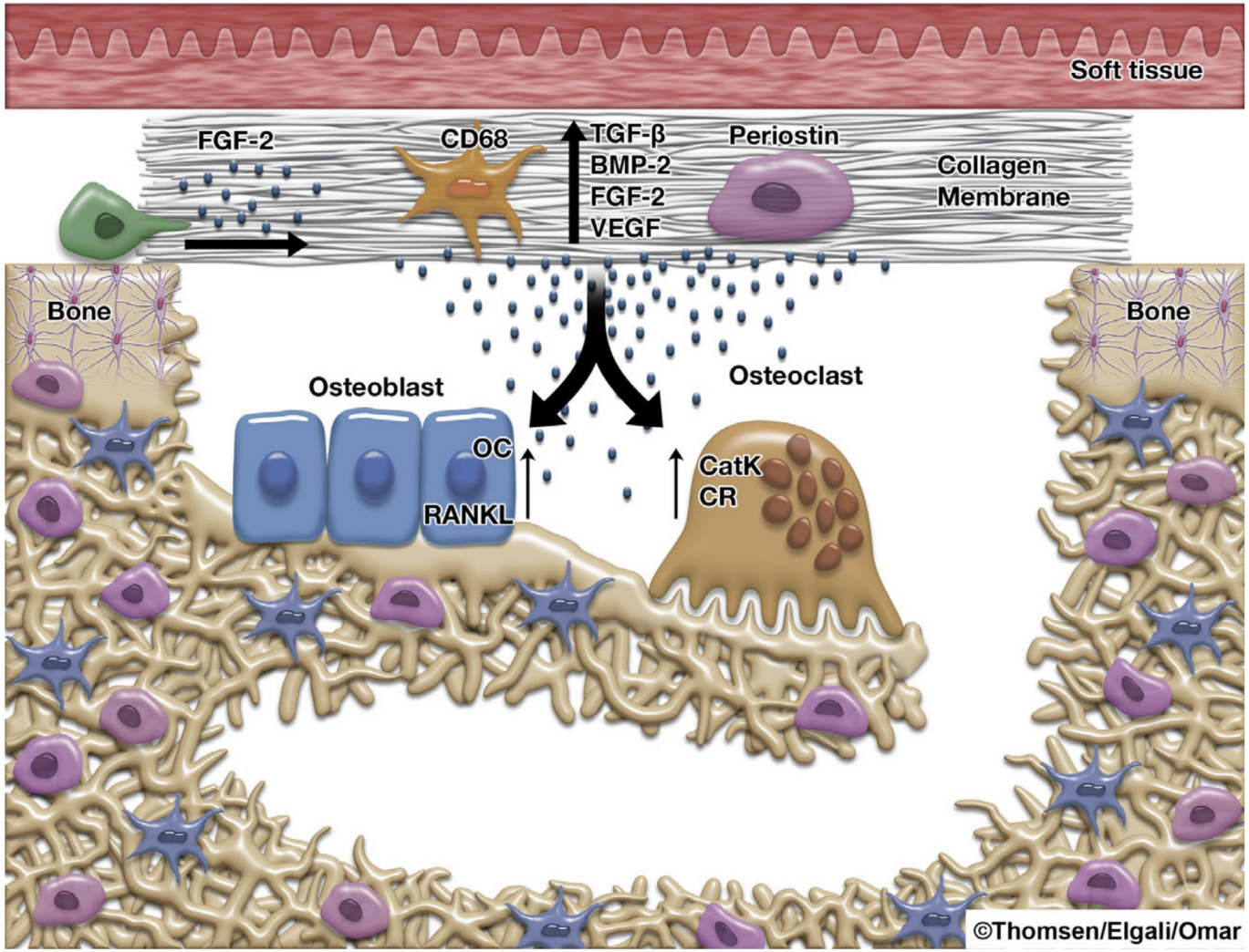
TOTAL GRAFT VOLUME	2.8 cc	5.6 cc	8.0 cc	8.0 cc
ORDER NUMBER	7510200 SMALL KIT	7510400 MEDIUM KIT	7510600 LARGE KIT	7510800 LARGE KIT II
Medtronic	INFUSE		Sm 7510200	$3600
			Med 7510400	$4692
			Large 7510600	$5202
			Large II 7510800	$5202
Stryker	OP-1 Putty		300-50 (ASP)	$4828

F
maxillofacial applications; while only the large II kit is approved for orthopaedic trauma applications

Source	rhPDGF-BB combined with a b-TCP scaffold: recombinant DNA technology	rhBMP combined with a collagen sponge scaffold: recombinant DNA technology
Dose	Uniform	Uniform
Purity	Validated	Validated
Potency	Consistent	Consistent
Protein composition	Assured	Assured
Protein content	Single protein	Single protein
Therapeutic concentration needed for bone repair (mg/mL)	0.3	1.5
Mechanism of action	Chemotaxis Mitogenesis Angiogenesis	Induction
Risk of ectopic bone formation	No	Yes


0d
7d
14d
21d
Clot formation
Inflammatory cells and chemokines
Angiogenesis
Migration and proliferation of MSCs
Proliferation of fibroblasts
Collagen synthesis
Osteoblasts differentiation
Bone formation
Osteoclasts differentiation
Bone remodeling

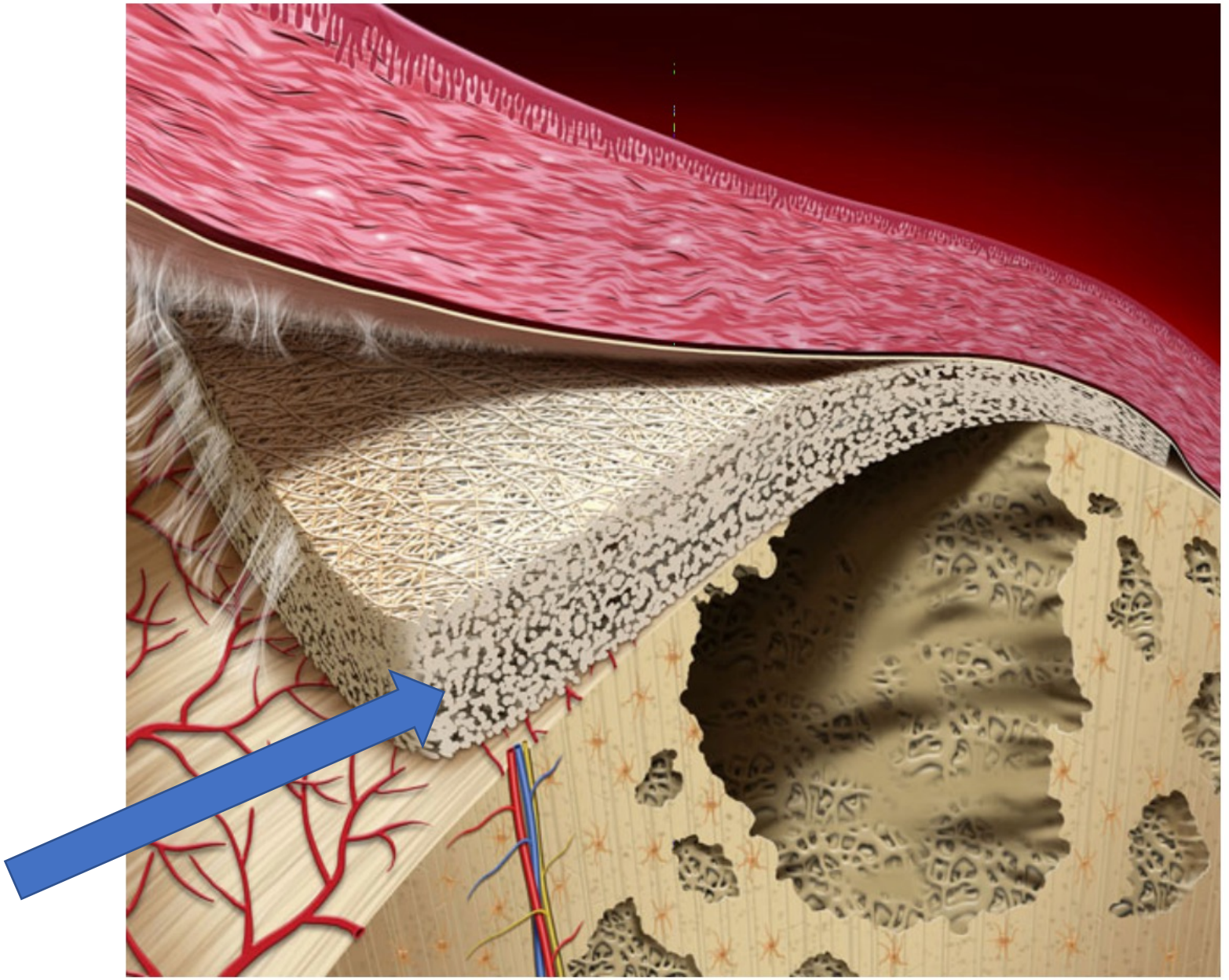
Cellular and Molecular Cascades During Guided Bone Regeneration



Guided bone regeneration: materials and biological mechanisms revisited. Eur J Oral Sci 2017; 125: 315–337. DOI: 10.1111/eos.12364

Guided Bone Regeneration has been defined as:

principle of GBR using barrier membranes, either resorbable, to exclude certain cell types such as rapidly proliferating epithelium and connective tissue, thus promoting the growth of slower-growing cells capable of forming bone. GBR is often combined with bone grafting procedures



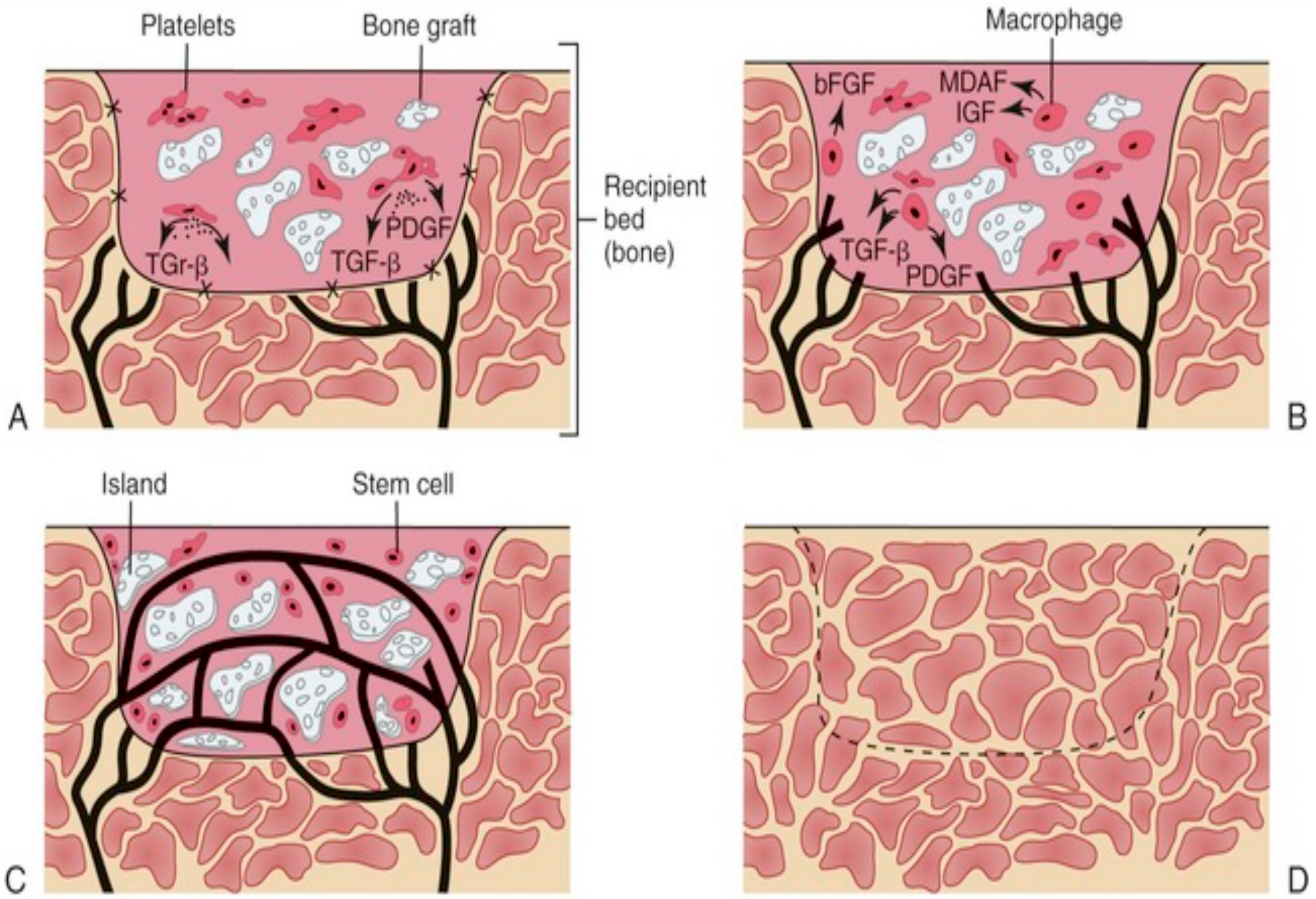
LANEY WR. Glossary of oral and maxillofacial implants. Berlin: Quintessence Publishing Co Ltd, 2007; 1–212

The healing stages of an bone graft placed into its recipient bed

- **D, By 4 months, bone graft integration generally has been completed. Remodeling will occur throughout the life of bone, however at this stage the grafted site will be ready to receive a dental implant.**

- [illegible] many growth factors such as platelet derived growth factor (PDGF) and transforming growth factor-beta 1 ($TGF-\beta_1$). The released PDGF initiates capillary ingrowth and $TGF-\beta_1$ stimulates endosteal osteoblasts and hematopoietic stem cells to initiate mitosis and increase their production of osteoid.

We only need about 30 to 50% of new bone formation in 3 to 4 months after implantation or bone reconstruction



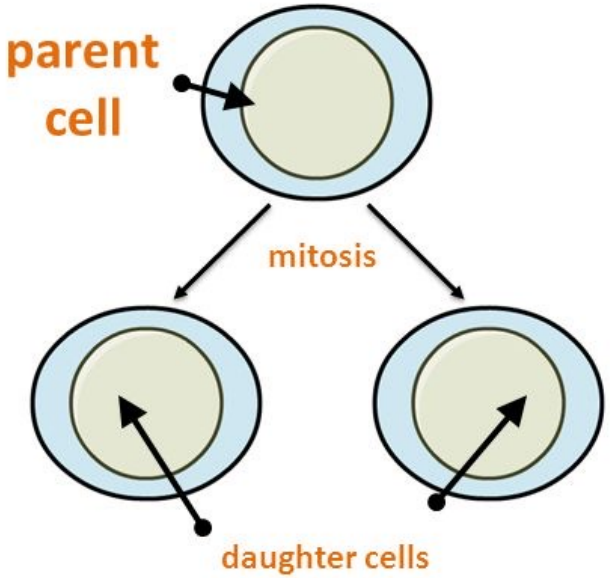
Human cells doubling time is about 1 day (24h)

Divide and grow

Most animals and plants start off life as just a single cell, but grow to become adults containing billions and billions of cells.
How does one cell become billions and billions of cells? The type of cell division that makes animals and plants grow is called **mitosis**.

In mitosis, a **parent** cell divides into two identical **daughter** cells. These daughter cells divide in two, and so on.

Mitosis is also the way in which old and damaged cells are replaced.



Human cells doubling time is about 1 day (24h)

Doubling time is defined as the average duration of **cell** growth and division as reflected by the **cell** cycle "clock"

To evaluate the number of proliferated cells, we use the following formula

$$N_t = N_0 \times 2^t$$

N_t	**Number of cells at time t** cell number after a certain day after surgery
N_0	**Number of cells initially** Number of stem cells in defect site after elimination of inflammation phase (about 4th day after surgery)
t	**Time (days)** Number of days after surgery

Efficiency of cell proliferation=100%

We use this mathematical equation to estimate the number of cells at 100%

for example: =>. $2_{cells} * 2^{10\ days} = 1024$ cells

Human cells doubling time is about 1 day (24h)

We don’t have never 100% efficiency of cell proliferation in any tissue of the human body.

Many cells die from programable cell death (apoptosis). Because that humans and animals have a very accurate system to eliminate cells that have errors in their genetic information.

That's why in every tissue, cell proliferation to occur more, the more likely that genetic errors occur. That's why leukemia, lymphoma, esophageal and gastric cancer is more likely to other cancers in human body. Because these tissues are mostly renewed.

Human cells doubling time is about 1 day (24h)

At best, cell proliferation in the human body under repair and reconstruction of tissue conditions is about 50 to 70 percent. So, we must use another formula to estimate the real number of amplified cells.

In this formula, we must apply the efficiency of cell proliferation in estimating cell population.

$$N_t = N_0 (1+E)^t$$

Efficiency of cell proliferation → **E≤1**

1f we have 2 cells in first healing time we will have:

$$1\times(1+0.7)^{10} = 201 \text{ cells}$$

Human cells doubling time is about 1 day (24h)

$N_{10} = N_2 \times 2^{10}$ Start with 2 cells/**100%** 1024 cells

$N_{10} = N_2(1+0.7)^{10}$ Start with 2 cells/**70%** 403 cells

$N_{10} = N_2(1+0.5)^{10}$ Start with 2 cells/**50%** 115 cells

Now, do you think, how many stem cells we will need at the site of the bone defect before dental implantation procedure?

Cell Number Estimation

Day	Number of cells	100%	70%	50%	20%
0	1	1	1	1	1
1	1	2	2	2	1
2	1	4	3	2	1
3	1	8	5	3	2
4	1	16	8	5	2
5	1	32	14	8	2
6	1	64	24	11	3
7	1	128	41	17	4
14	1	16384	1684	292	13
21	1	2097152	69092	4988	46
14	100	1638400	168378	29193	1284
14	500	8192000	841889	145965	6420
14	1000	16384000	1683778	291929	12839
21	100	209715200	6909193	498789	4601
21	500	1048576000	34545967	2493943	23003
21	1000	2097152000	69091934	4987885	46005

Biomaterials

Devices for biomedical use designed to interact with biological systems.

- Biomaterials are divided into four types:
  - Polymers
  - Metals
  - Ceramics
  - Natural materials.

*

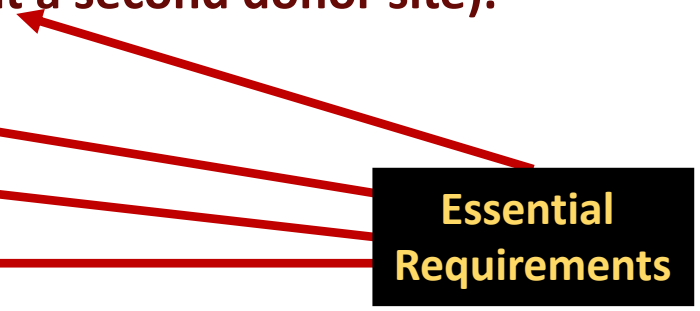
Biomaterial properties

Several properties can be considered in biomaterials

- Chemical Composition
- Physical Properties
- Morphology
- Absorption process
- Timing

Ideal Bone Graft Materials

- **Should be readily available (not require surgical intervention at a second donor site).**
- **Should not elicit immunological responses.**
- **Should provide (elicit, create) osteoconduction**
- **Should enhance revascularization.**
- Should be highly osteoinductive.
- Should provide rapid osteogenesis.
- Should provide for the formation of new attachment in periodontal lesions.
- Should not impede (slow, stope, prevent) bone growth.



Autogenous Bone: Is It Still the Gold Standard?

- Several advantages over other augmentation techniques: Including short healing times, favorable bone quality, lower material costs, no risk of disease transmission or antigenicity, and predictability in the repair of larger defects or greater atrophy. Denser cortical bone grafts exhibit minimal resorption on incorporation, making them ideal for site development.
- The obvious disadvantage: Morbidity from bone harvest.

- However, approaches to minimize morbidity have been addressed including the use of preemptive analgesia, long-acting anesthesia, and harvesting techniques such as piezoelectric surgery. There are also donor sites associated with a lower incidence of complications (proximal tibia and mandibular ramus) that can be procured in the office setting. In the treatment of more demanding reconstructions, the benefits of autograft often outweigh the risks of complications. Iliac bone grafts are reserved for the reconstruction of larger defects and severe atrophy.

Autogenous Bone: Is It Still the Gold Standard?

- Meta-analyses comparing bone graft materials via histo-morphometrical evaluation of human bone biopsies from sinus augmentation demonstrated that compared with bone substitutes, autogenous bone enabled faster initial bone formation, but the **final amount of bone formation did not differ from that observed with bone substitutes**. A combination of autogenous bone with a xeno-bone substitute led to the greatest final amount of bone formation within the sinus cavity.

International Orthopaedics. https://doi.org/10.1007/s00264-020-04483-3

Autogenous Bone: Is It Still the Gold Standard?

- The bone substitute particles yielded a larger bone volume than autogenous bone chips in severe conditions, such as peri-implant bone defects.
- Moreover, a meta-analysis did not detect superiority of autogenous bone over bone substitutes in the clinical outcomes of maxillary sinus augmentation and alveolar ridge augmentation.
- These observations support the following conclusions:
  (1) **although autogenous bone may have higher bone formation capability than bone substitutes, the actual benefit is limited to favorable recipient conditions**; and (2) **bone substitutes not only reduce or eliminate the risk of donor site morbidity endemic to autogenous bone but also have a theoretical advantage in augmentation under severe recipient conditions.**

Autogenous Bone: Is It Still the Gold Standard?

- Several studies have demonstrated that most endogenous cells (probably osteocytes, osteoblasts, and mesenchymal stem cells) on or within autogenous bone undergo apoptosis or necrosis during bone grafting.
- Flow cytometry analysis demonstarted that the proportion of **viable** and **apoptotic** cells in bone chips collected from maxillary bone was <5% and >95%, respectively, regardless of the type of instrument, such as piezoelectric devices, scrapers, and rotary mills, used to collect the graft.

Autogenous Bone: Is It Still the Gold Standard?

- Moreover, 80% of osteocyte lacunae within a bone block showed debris or were empty at the end of grafting surgery.
- Histological examination after maxillary sinus augmentation in humans using calvarial or iliac autogenous bone particles demonstrated that the proportion of nonvital bone was 20%–25% after 5 months of healing

Allograft

An **allograft** is a bone, ligament, cartilage, tendon, section of skin or placental **tissue** that is transplanted from one person or cadaver to another. It is also referred to as "donated **tissue**"

Allograft

- **Many factors contribute to the high quality of MB and DBM, including:**
- Quality tissue
- Careful processing
- A good carrier
- Quality control
- Scientific evidence

Allograft

Allograft	
Fresh	- Highest risk of disease transmission and immunogenicity - BMP preserved and therefore osteoinductive
Fresh frozen	- Less immunogenicity than fresh - BMP preserved and therefore osteoinductive
Freeze dried	- Least immunogenic - Least structural integrity - BMP depleted (purely osteoconductive) - Lowest likelihood of viral transmission
DBM	- Osteoinductive and osteoconductive - Contains: collagen, bone morphogenetic proteins, transforming growth factor-beta, residual calcium - Does NOT contain mesenchymal precursor cells

Allograft

- **Standards:** the guidelines for screening and testing of tissue donors set forth
- ISO 13485 and GMP
- American Association of Tissue Banks (AATB)
- Food and Drug Administration (FDA)

- The AATB and FDA set only minimal guidelines to ensure safety of tissue, and donors must pass through an extensive quality assurance process

Allograft

https://www.cdc.gov/transplantsafety/protecting-patient/screening-testing.html

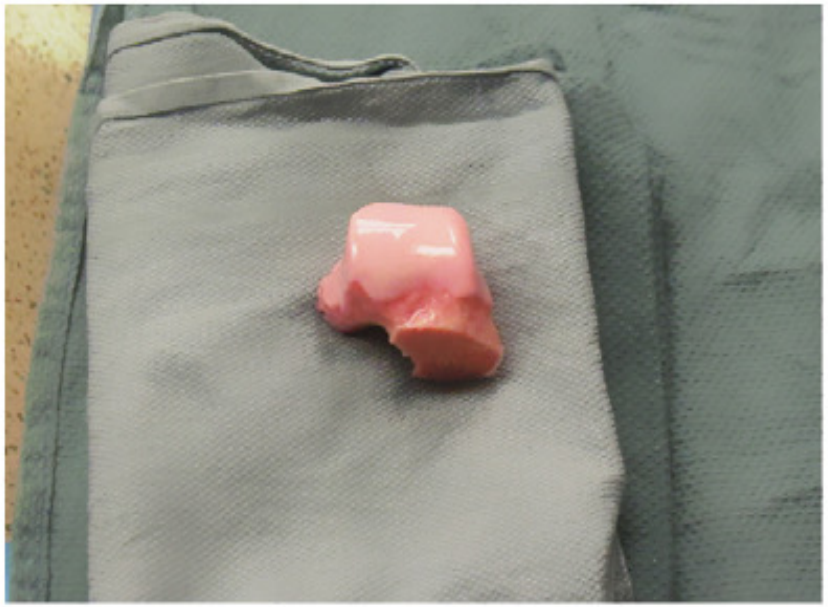
Donor Screening and Testing

Human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, cytomegalovirus (CMV), Epstein Barr Virus (EBV), and toxoplasmosis (deceased donors only)

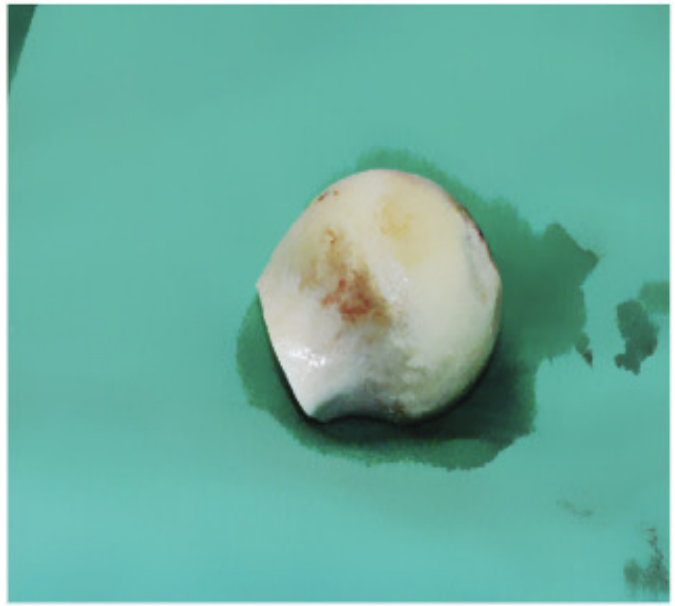
Living potential kidney donors at increased risk for tuberculosis are also tested for this infection

Risks & Complications

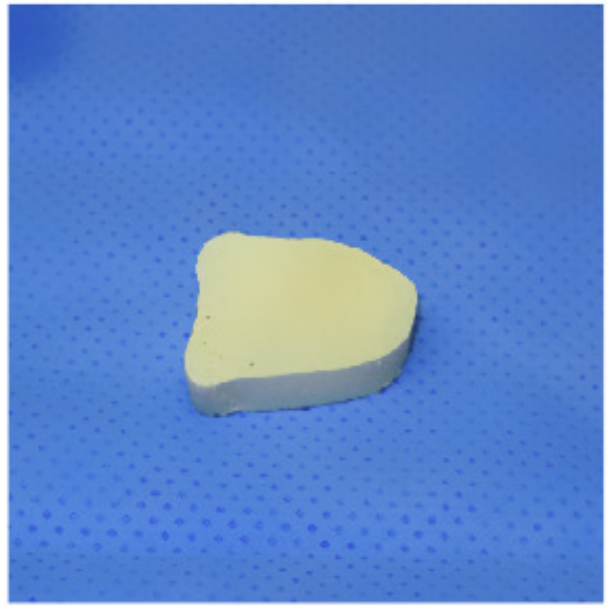
- Disease Transmission with Allograft
  - hepatitis B
    - risk of hepatitis B disease transmission in musculoskeletal fresh-frozen allograft transplantation is 1 in 63,000
  - hepatitis C
    - risk of hepatitis C disease transmission in musculoskeletal fresh-frozen allograft transplantation is 1 in 100,000
  - HIV
    - risk of transmission of HIV in fresh-frozen allograft bone is 1 in 1,000,000
  - allografts are tested for HIV, HBV, HCV, HTLV-1, and syphilis
- Serous wound drainage
  - calcium sulfate bone graft substitute associated with increased serous wound drainage



Fresh (talus) allograft

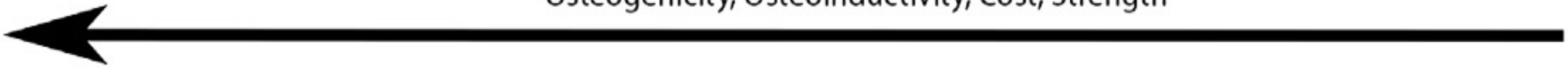


Fresh-frozen (femoral head) allograft

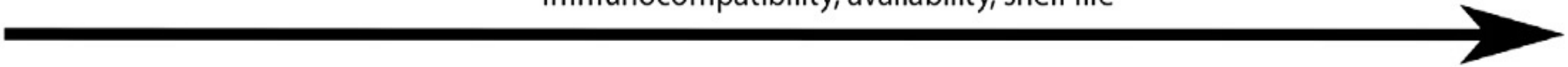


Freeze-dried (calcaneal) allograft

Osteogenicity, Osteoinductivity, Cost, Strength



Immunocompatibility, availability, shelf life



Allograft

- DBM grafts are produced from both cancellous and cortical bone which are subjected to controlled cleaning processes (including hydrogen peroxide and ethanol), followed by demineralization with hydrochloric acid.
- Aseptic processes have been designed to preserve the biological integrity of the tissue
- Each donor lot of DBM is verified for QC essential test (Immune response (cell-based test), LAL test, DNA contents, Bioburden test, Lipid and calcium content) prior to distribution

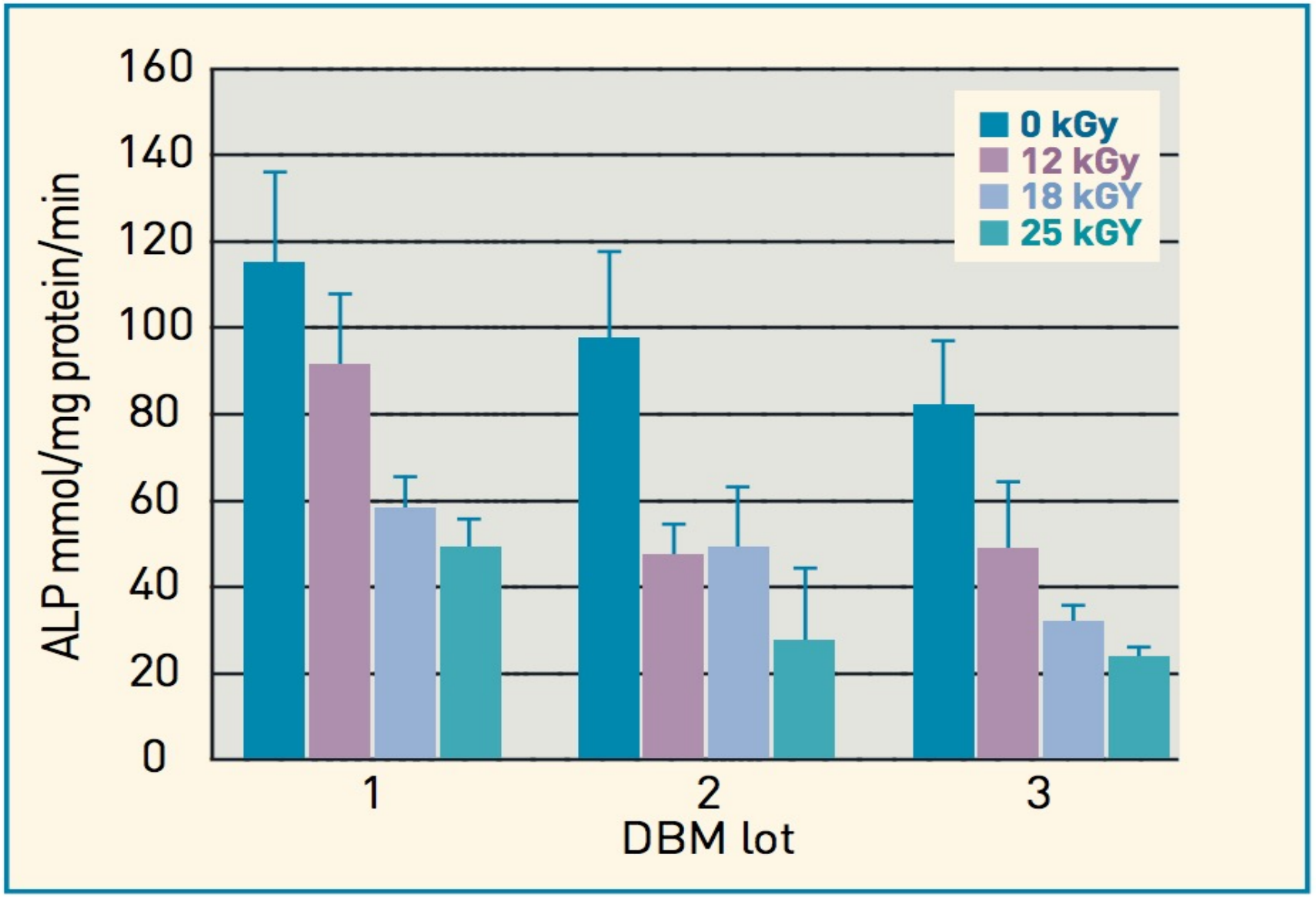
Allograft

- **Standards:** the guidelines for screening and testing of tissue donors set forth
- in ISO Class 4 (certified) clean rooms (Aseptic) to prevent any environmental contamination of the tissue and thus eliminates the need for terminal sterilization by gamma radiation, which has been shown to compromise the biological and biomechanical integrity of allograft tissue

I have a important question for you

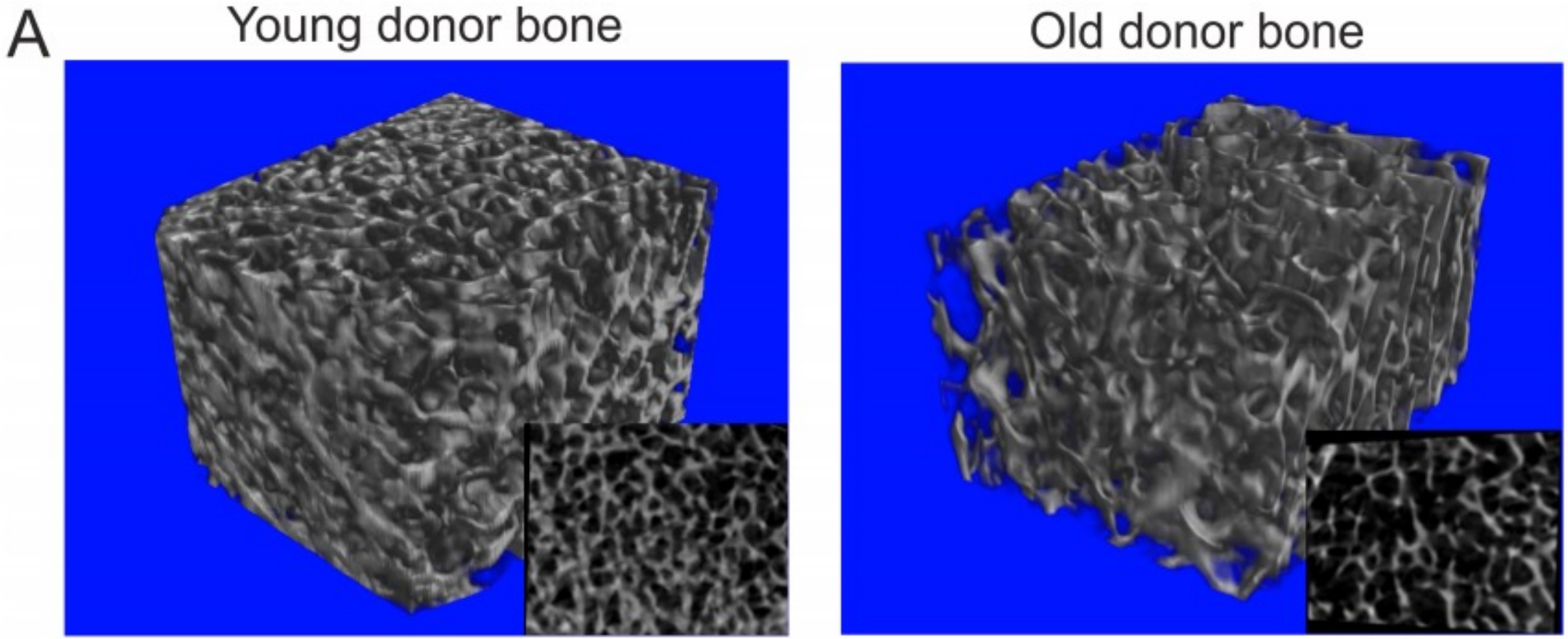
- Contrary to the claims of the manufacturer of these materials, why allograft biomaterial don't have any osteoinductive properties.

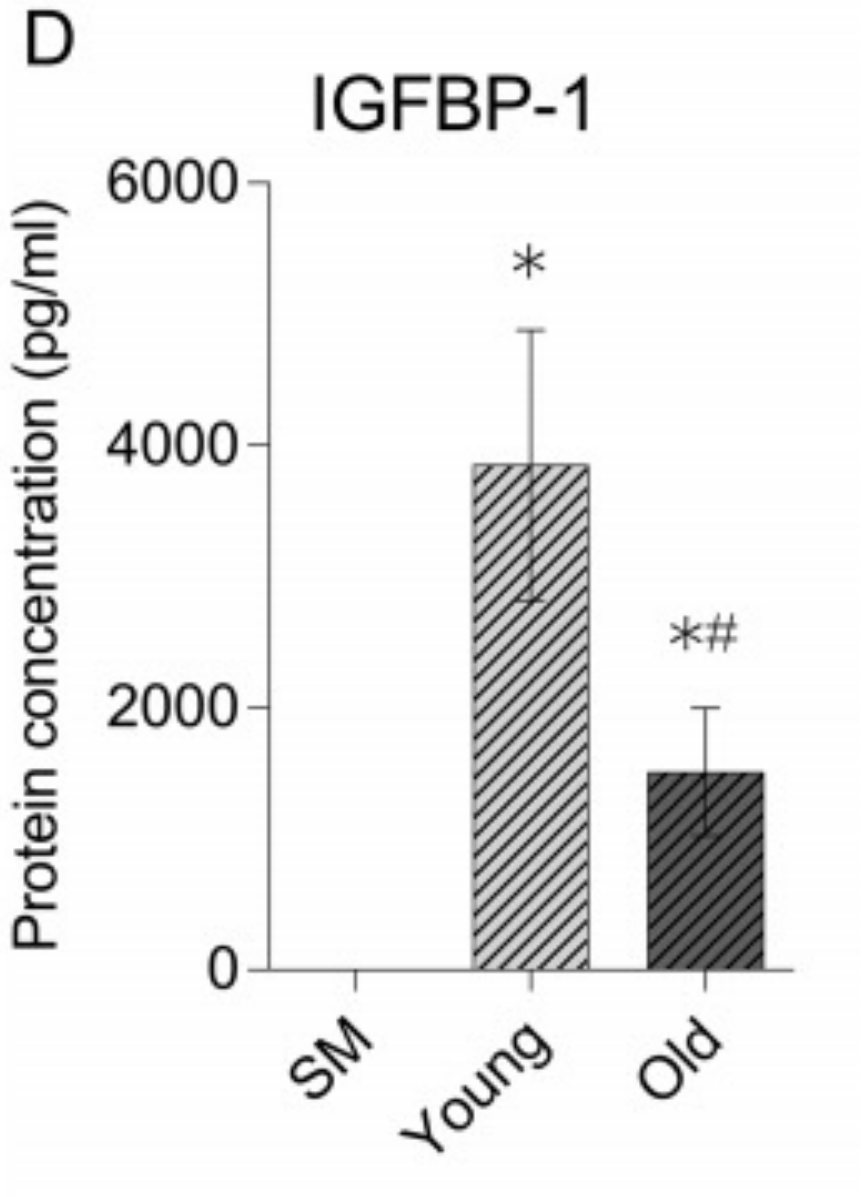
	Growth Factor (GF)	Half-Life	
		Soluble GF	Immobilized GF in Fibrin Clot
1	PDGF	~ 12 h	$K_d = \sim 10^{-9}\text{-}10^{-10}$ Sustained release for up to 10 days
2	VEGF	< 30 min	
3	EGF	~ 40 min	
4	FGF	~ 9 h	
5	BMP-2	~ 7 min	
6	BMP-7	< 30 min	
7	IGF	12 h	
8	TGF-B	22 hours	

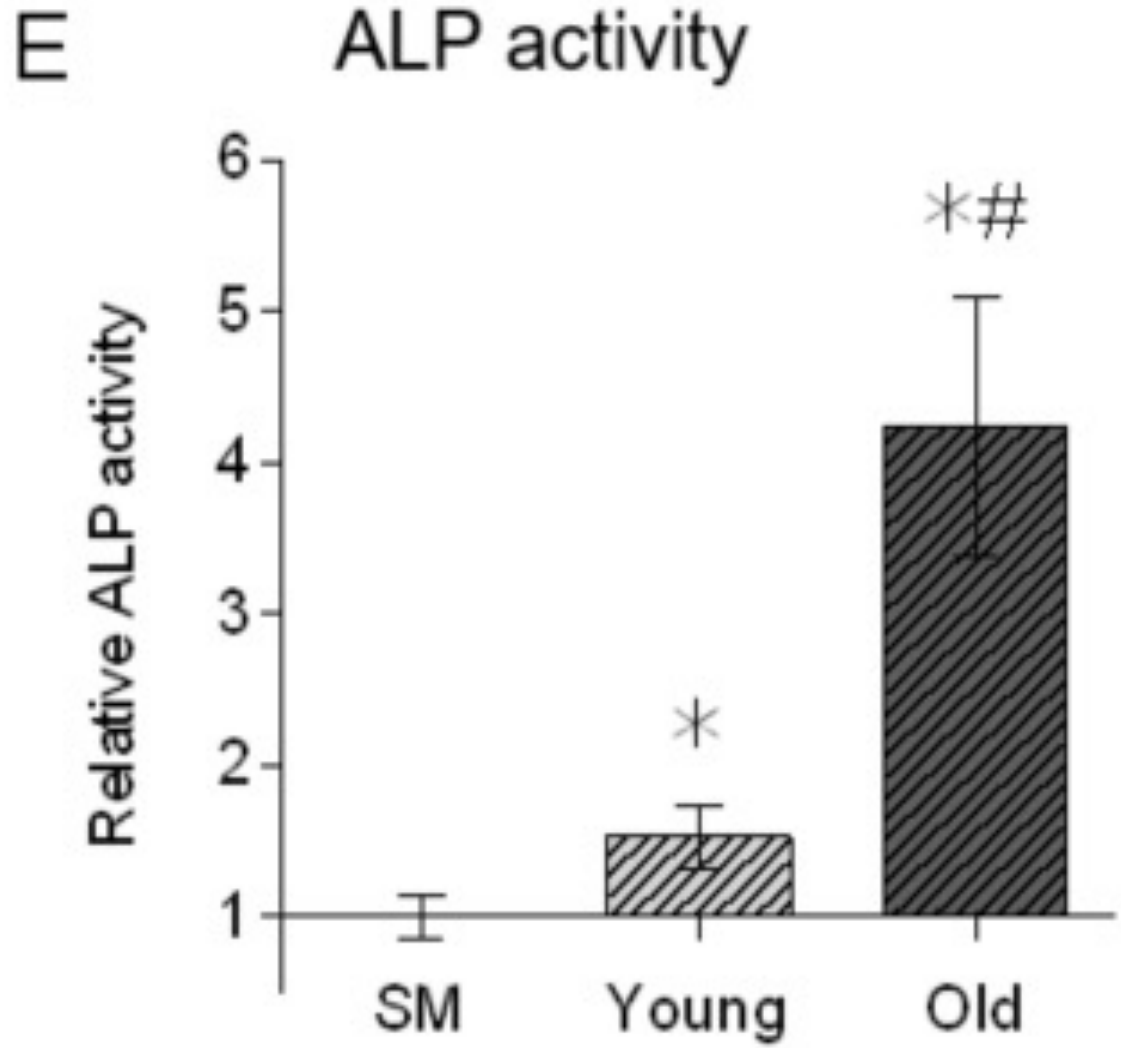


A **lot number** is an identification **number** assigned to a particular quantity or **lot** of material from a single manufacturer. **Lot numbers** can typically be found on the outside of packaging.

Young and Old Donor Bone




D
IGFBP-1
Protein concentration (pg/ml)
6000
4000
2000
0
*
*#
SM
Young
Old


E
ALP activity
Relative ALP activity
6
5
4
3
2
1
*
*#
SM
Young
Old

FDBA

Freeze Dried Bone Allograft

- It is a human bone, harvested from fresh cadavers
- It is then sterilized, freezed and dried
- It works primarily through conduction, thus over a period, it will resorb, and bone graft is replaced
- Used in sinus bone grafting procedures

Processing: Bone is washed in distilled water and ground to particle size of 500 mic to 5mm.It is then immersed in nitrogen then freeze dried, and ground to small particles(250 – 1500 mic)

DFDBA

Demineralized Freeze-Dried Bone Allograft

- Created by removing the Ca and Po4 salts.

- Processing : similar initial steps as FDBA but an additional step of demineralizing the ground bone powder in 0.6N HCL or nitric acid for 6-16 hrs.

- Freeze drying destroys all cells and the graft is rendered nonviable. It has the advantages of:
    - Decreasing antigenicity
    - Facilitating long term storage

Xenograft

A graft taken from a donor of another species i.e. bovine, porcine etc

For the bone graft to be successful:

Osteoblasts must be present at the site
Blood supply must be sufficient for nourishment
The graft must be stabilized during healing
The soft tissue must not be under tension

Xenograft

ISO 22442: Medical devices utilizing animal tissues and their derivatives

Part 1: Application of risk management

Part 2: Controls on sourcing, collection and handling

Part 3: Validation of the elimination and/or inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents

ISO 10993: Biological evaluation of medical devices

Part 1: Evaluation and testing within a risk management process

Part 2: Animal welfare requirements

Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity

Part 5: Tests for in vitro cytotoxicity.

Part 6: Tests for local effects after implantation

Part 10: Tests for irritation and skin sensitization

Part 11: Tests for systemic toxicity

Part 18: Chemical characterization of medical device materials within a risk management process

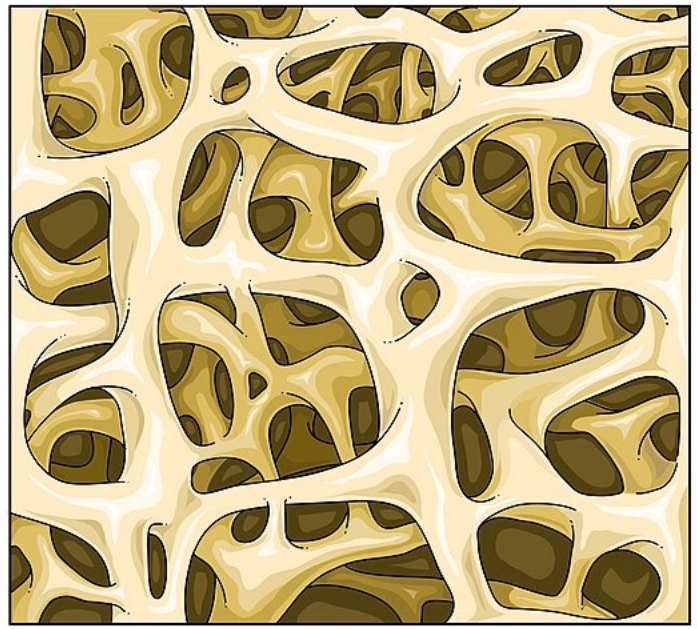
Part 19: Physico-chemical, morphological and topographical characterization of materials

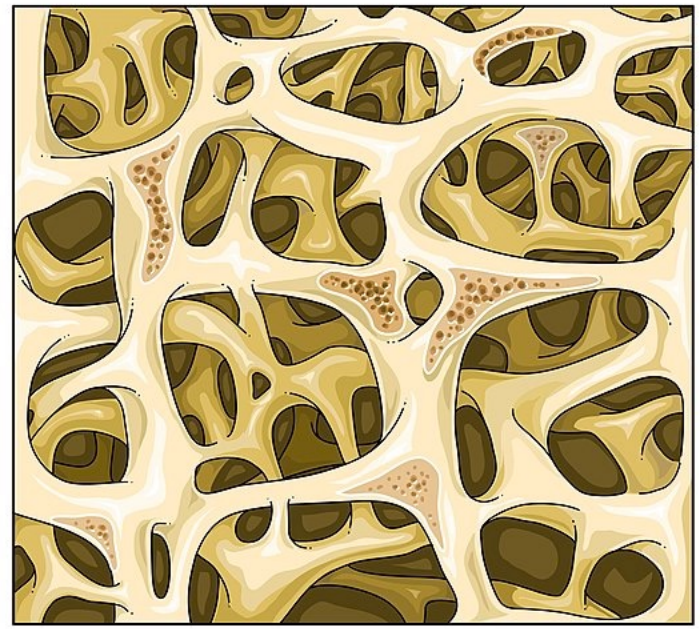
Part 20: Principles and methods for immunotoxicology testing of medical devices

Xenograft

- Bovine bone is deproteinized by heating to eliminate the risk of allergic reactions and disease transmission.
- The removal of all proteins transforms it into biologically derived hydroxyapatite ceramic.
- It is characterized by well-preserved 3D natural bone structure similar to human bone.
- The trabecular architecture with interconnecting pores allows for optimal in-growth of new vascularity.

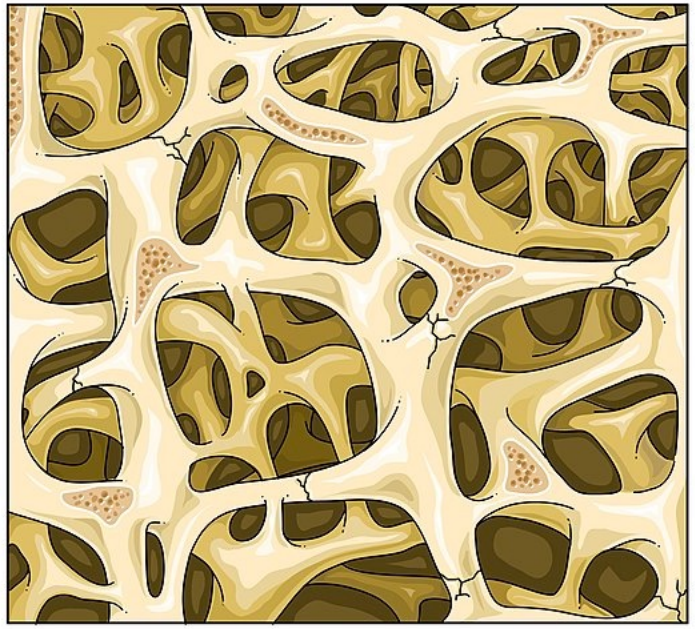
Normal trabecular bone

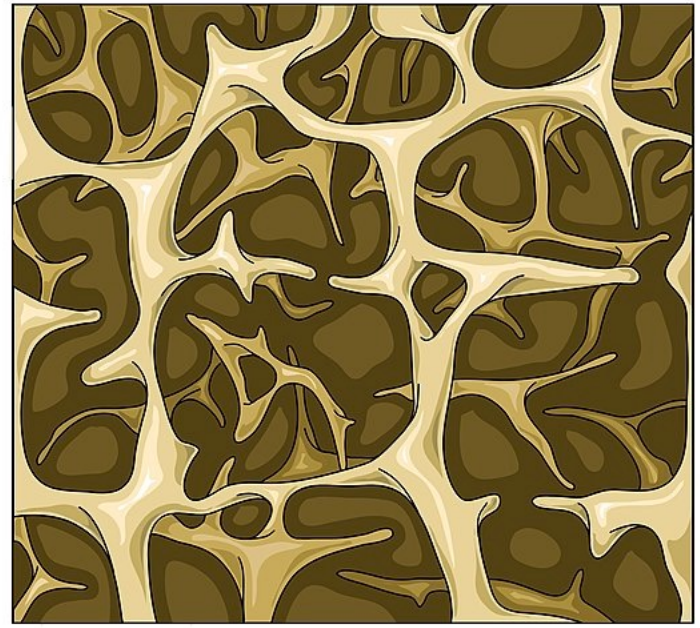




Trabecular bone with resorption areas

Trabecular bone with microcracks

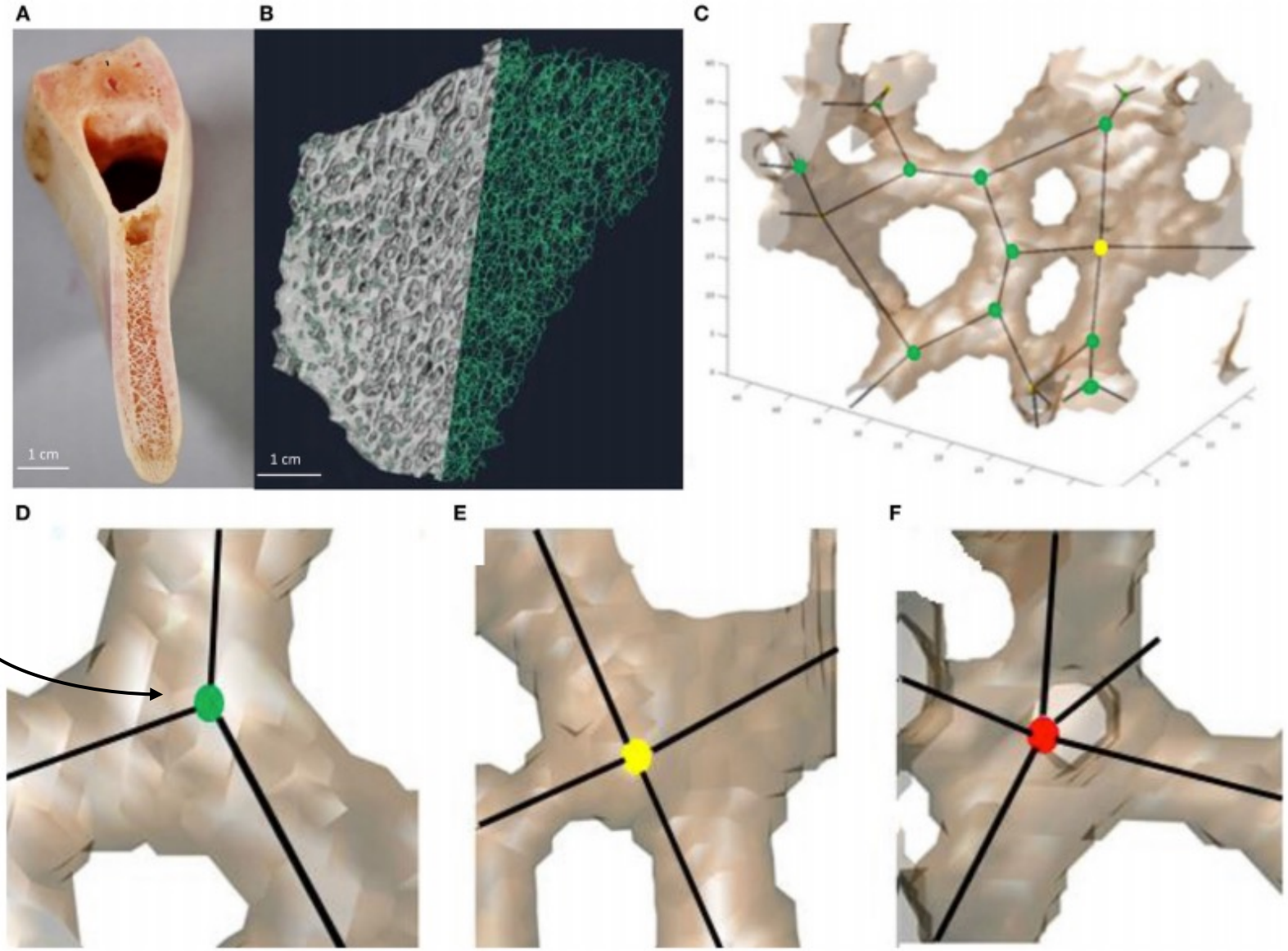


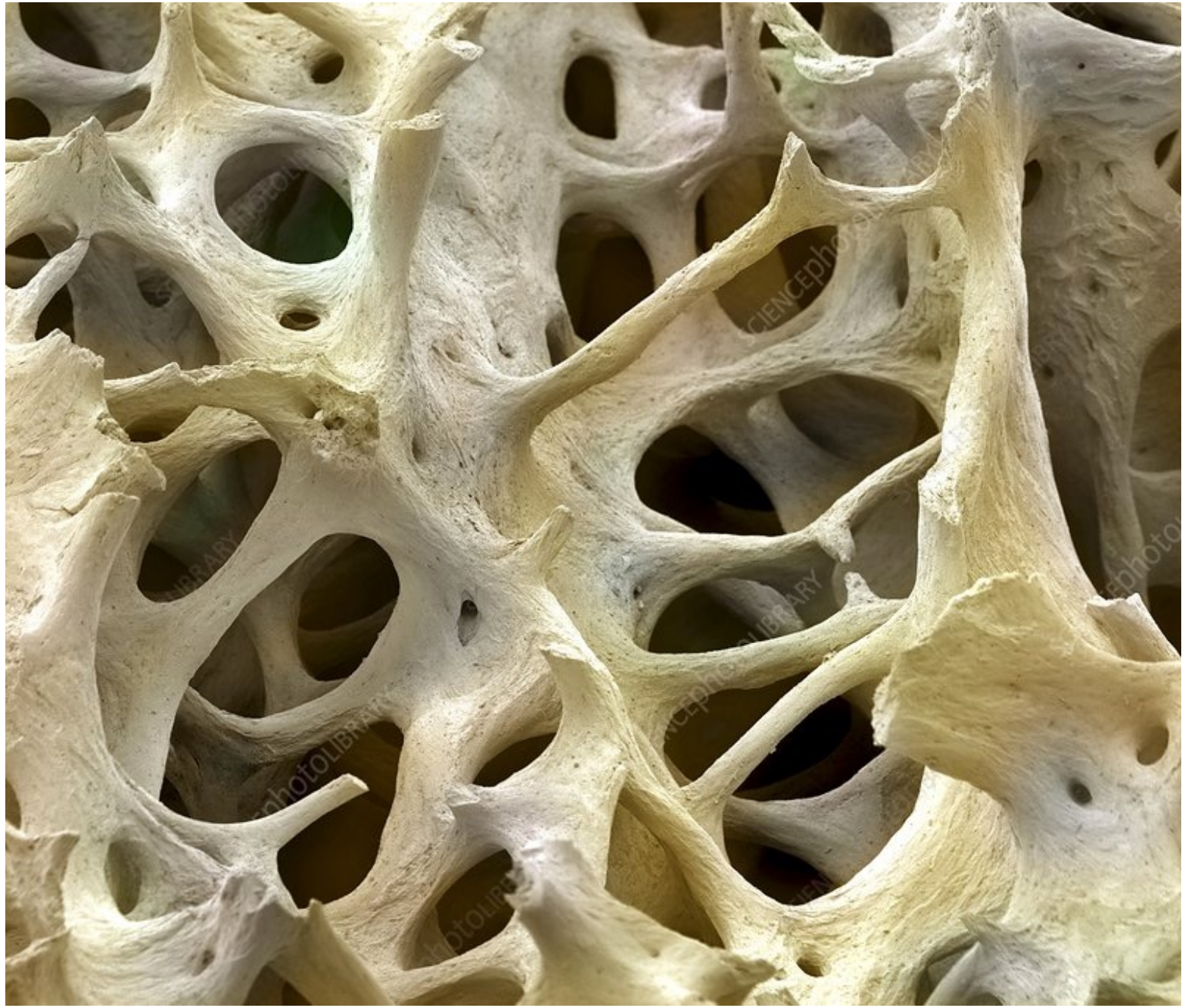


Osteoporotic trabecular bone

Workflow of bone analysis using inter-trabecular angle application

ITA

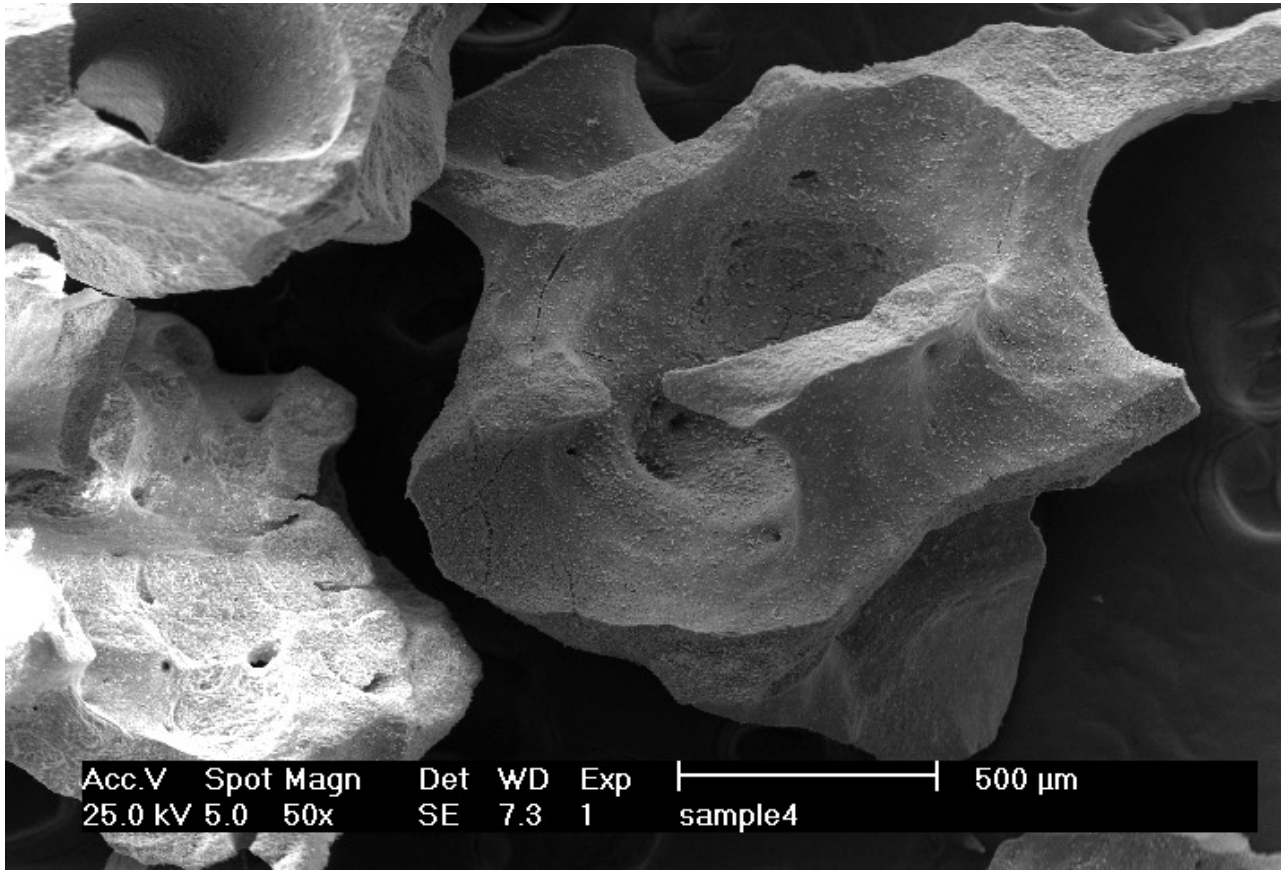


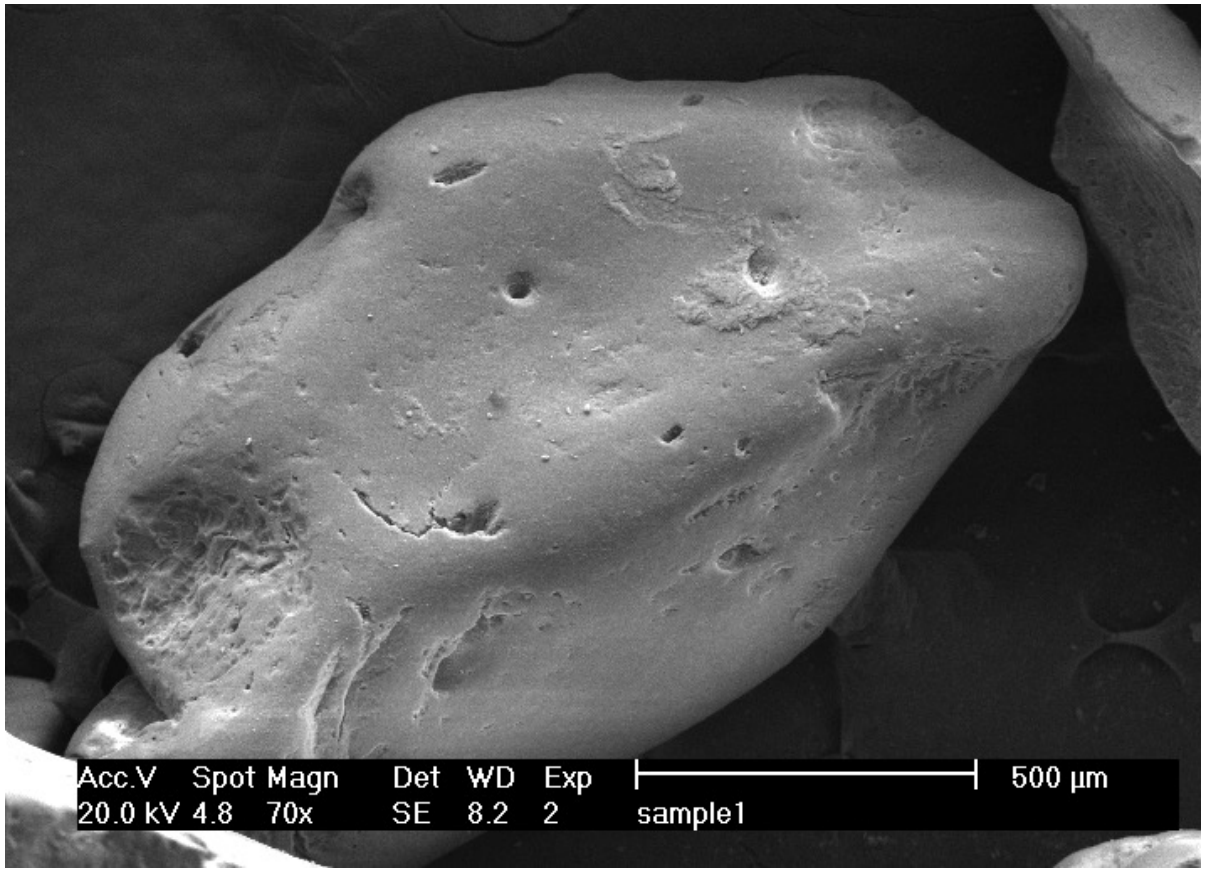


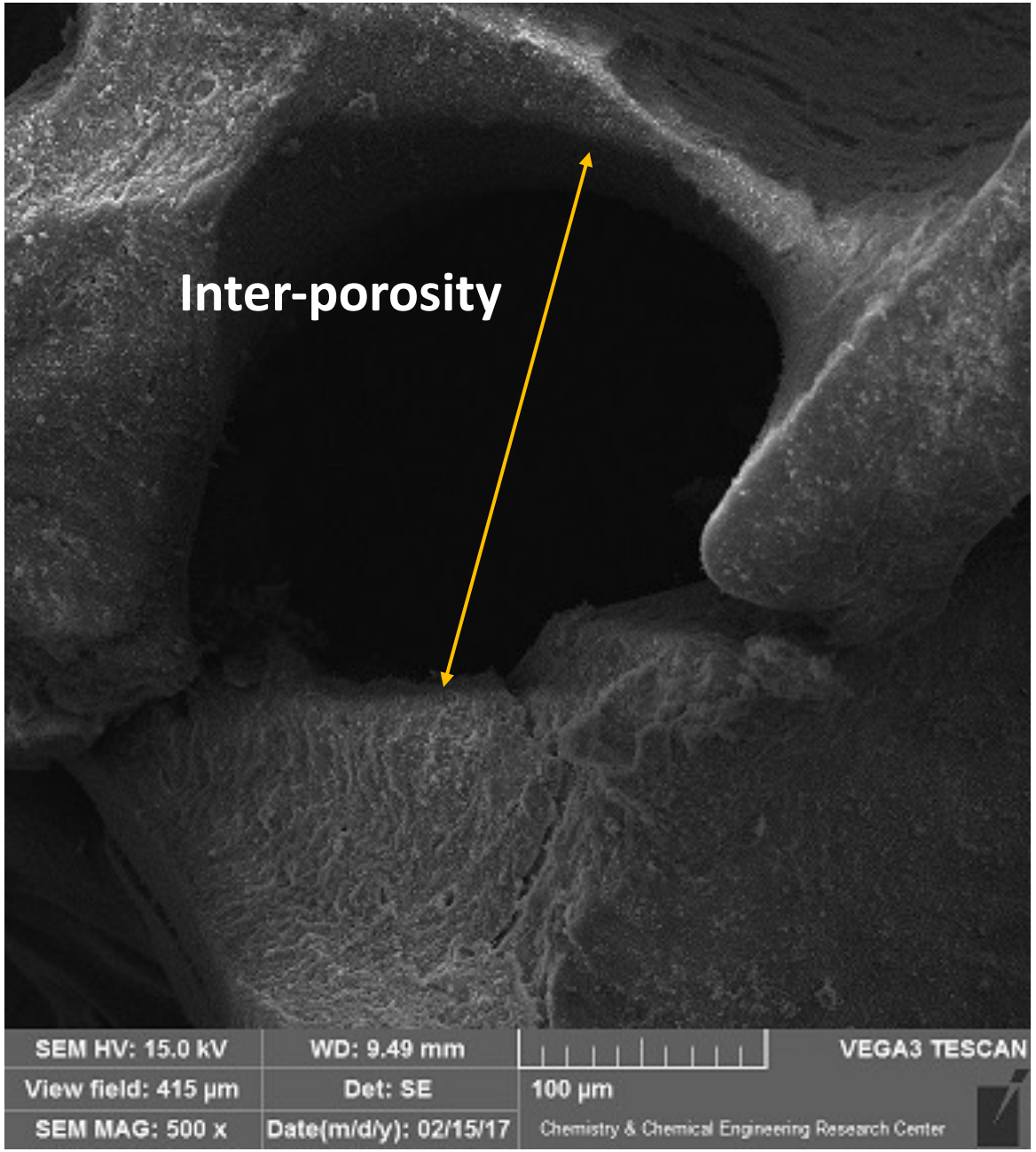
https://www.sciencephoto.com/media/726906/view/bone-tissue-sem

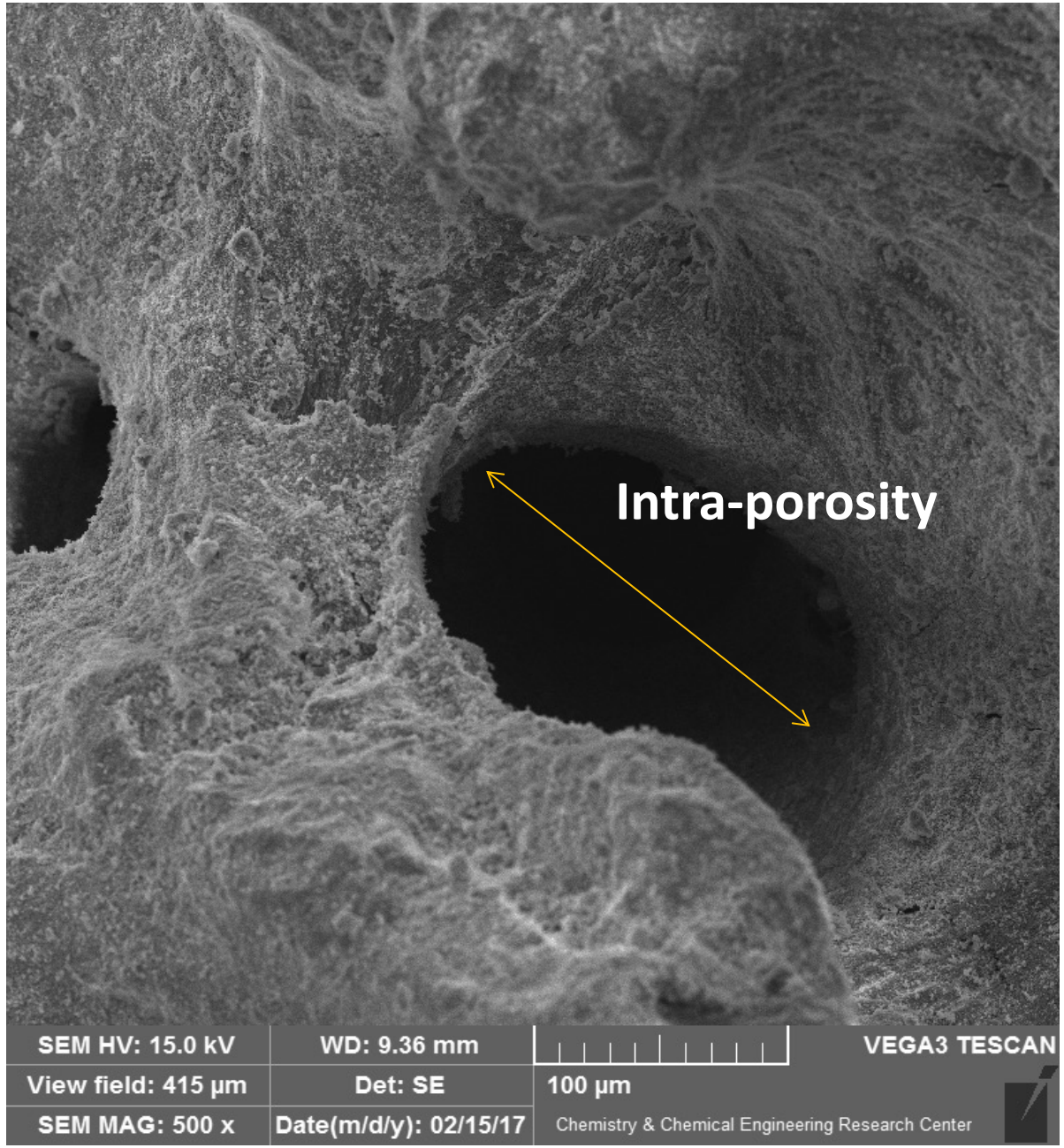
Xenograft Characterization

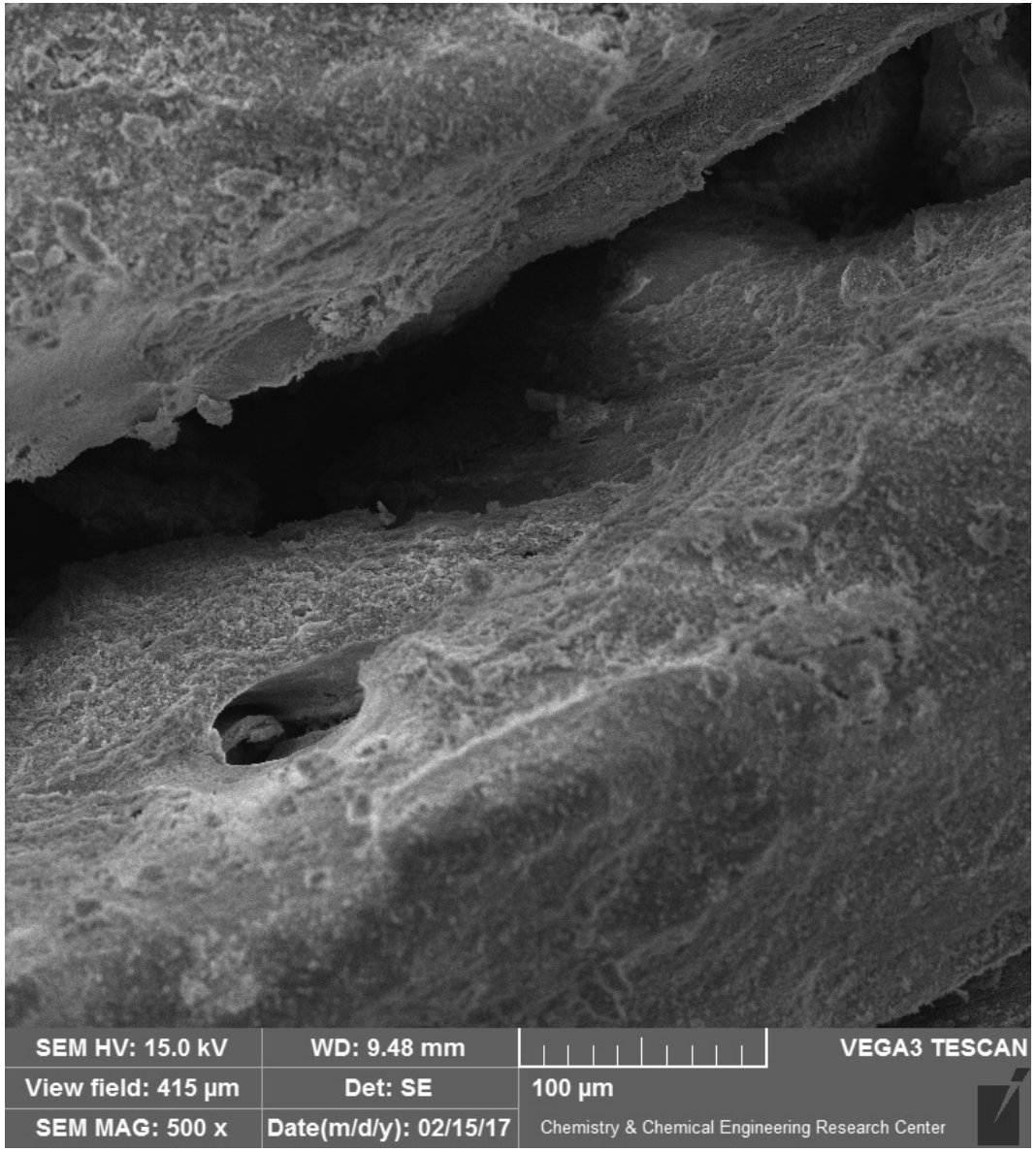
- **1- Cancellous bone feature (macroscopic and Microscopic)**

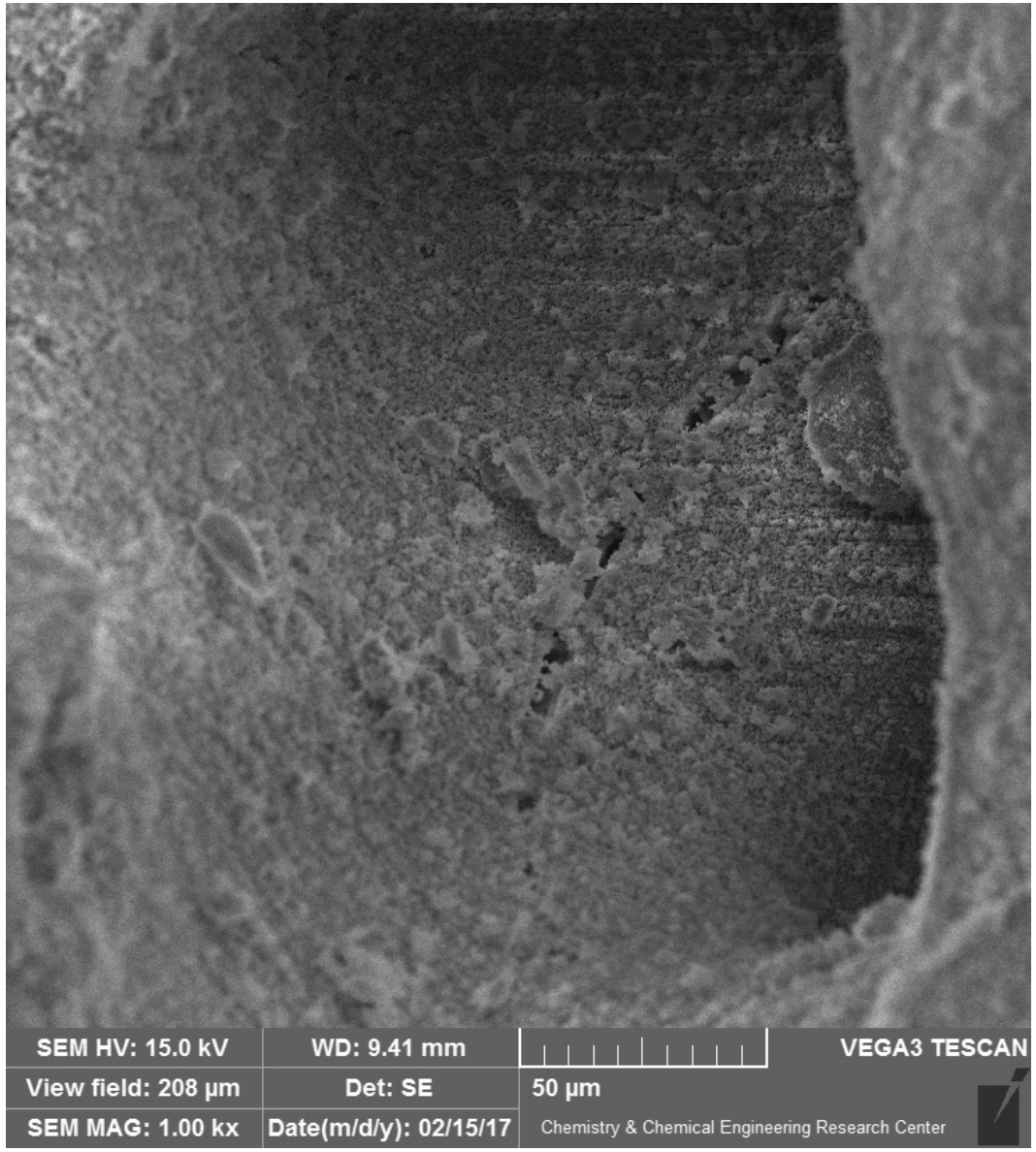





Inter-porosity
SEM HV: 15.0 kV
WD: 9.49 mm
VEGA3 TESCAN
View field: 415 µm
Det: SE
100 µm
SEM MAG: 500 x
Date(m/d/y): 02/15/17
Chemistry & Chemical Engineering Research Center

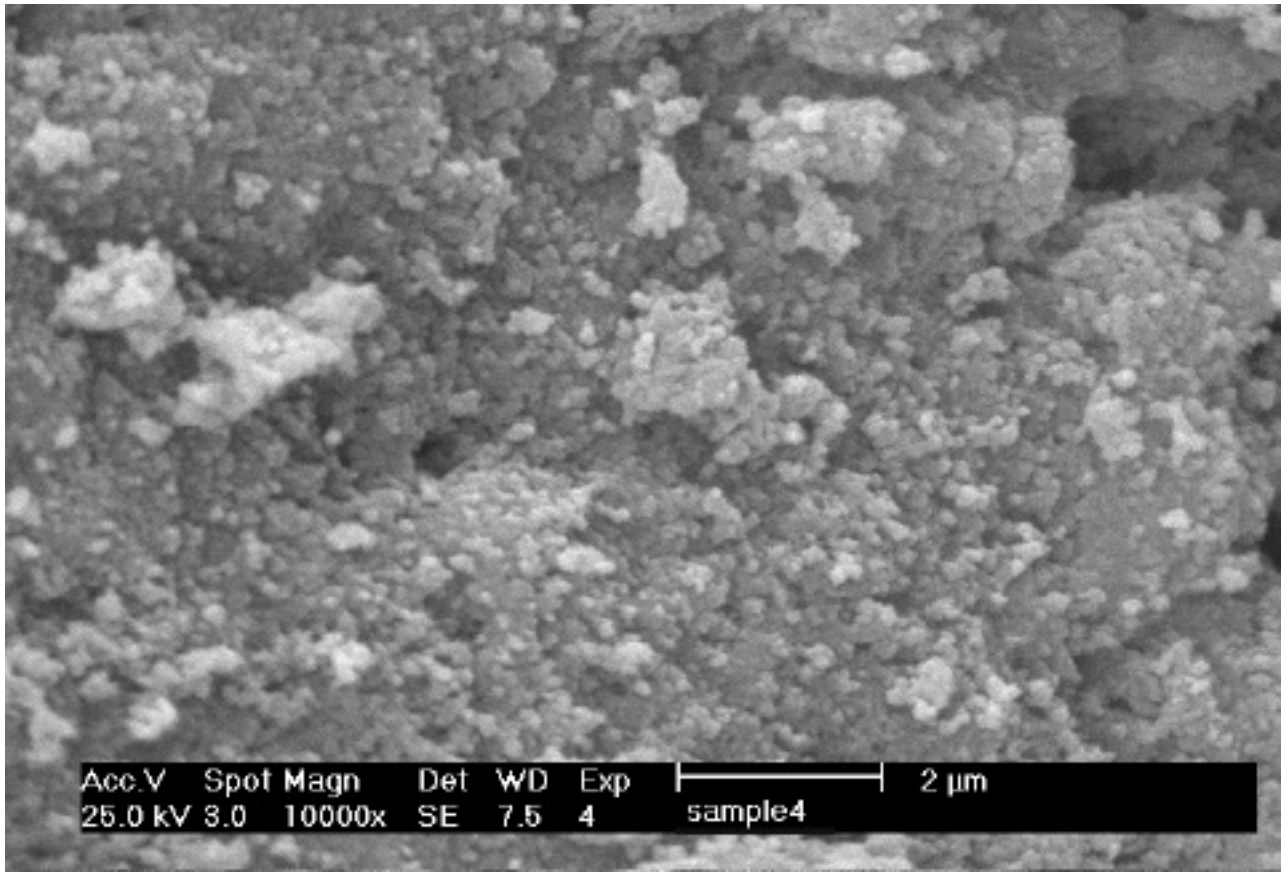

Intra-porosity
SEM HV: 15.0 kV
WD: 9.36 mm
VEGA3 TESCAN
View field: 415 µm
Det: SE
100 µm
SEM MAG: 500 x
Date(m/d/y): 02/15/17
Chemistry & Chemical Engineering Research Center

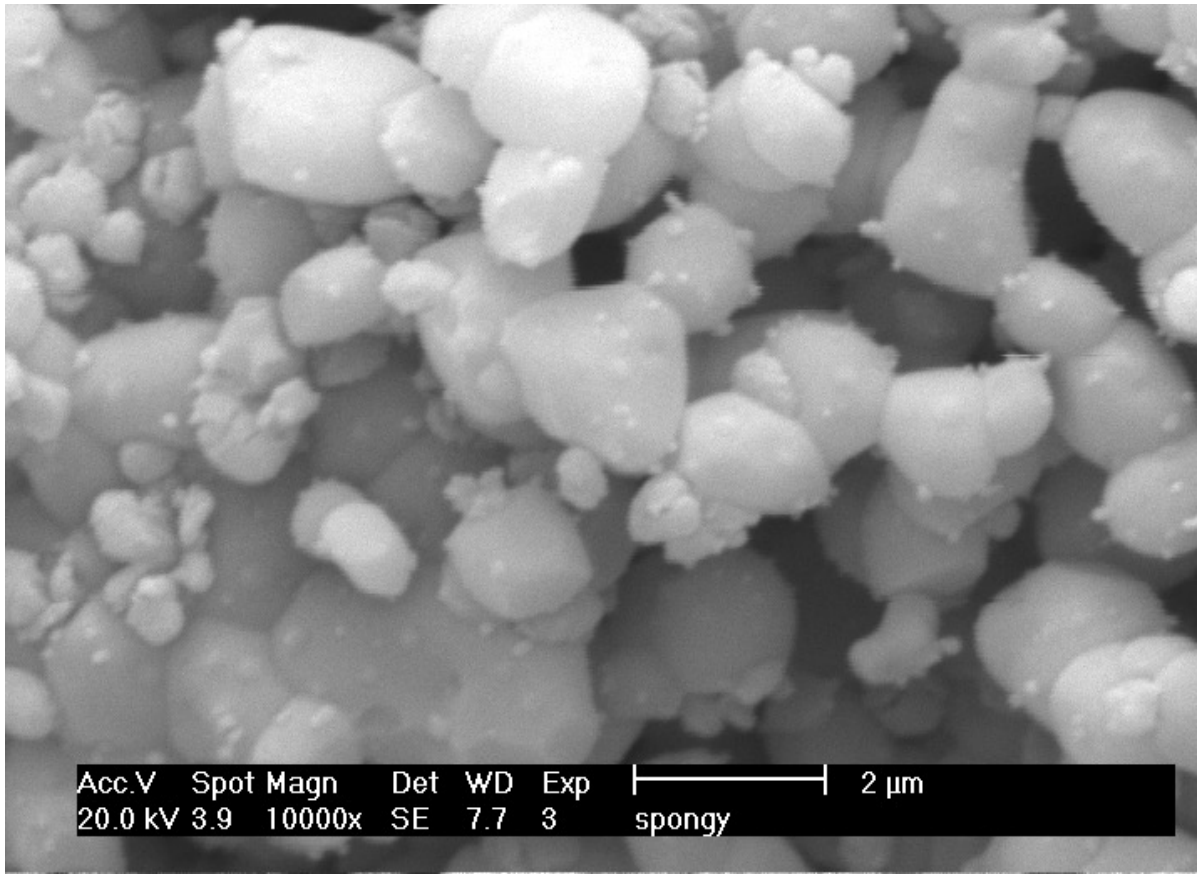

SEM HV: 15.0 kV
WD: 9.48 mm
VEGA3 TESCAN
View field: 415 µm
Det: SE
100 µm
SEM MAG: 500 x
Date(m/d/y): 02/15/17
Chemistry & Chemical Engineering Research Center

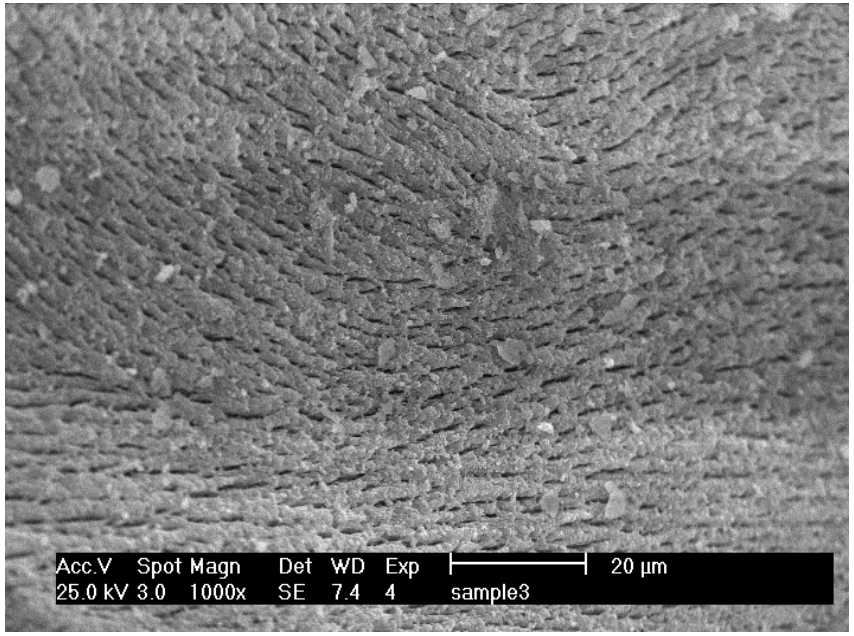

SEM HV: 15.0 kV
WD: 9.41 mm
VEGA3 TESCAN
View field: 208 µm
Det: SE
50 µm
SEM MAG: 1.00 kx
Date(m/d/y): 02/15/17
Chemistry & Chemical Engineering Research Center

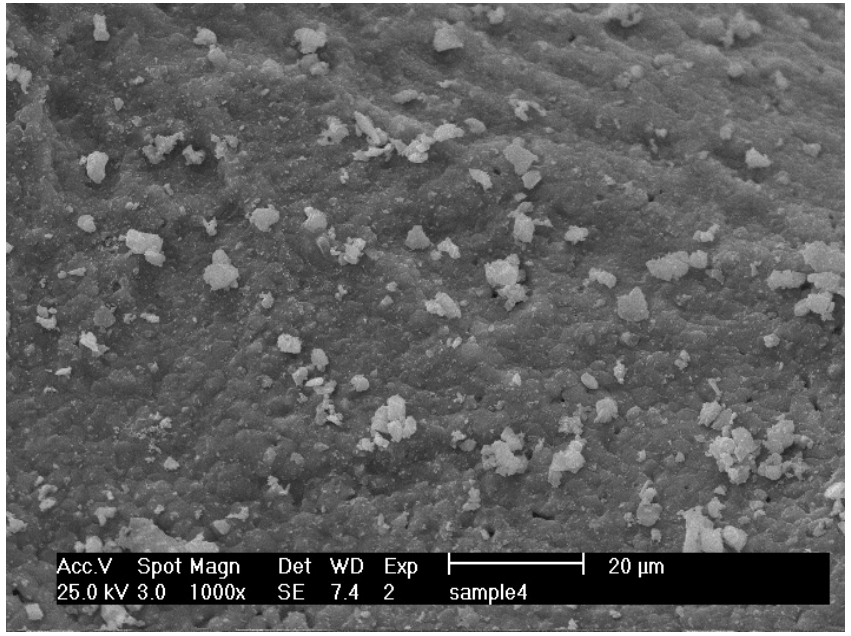
Xenograft Characterization

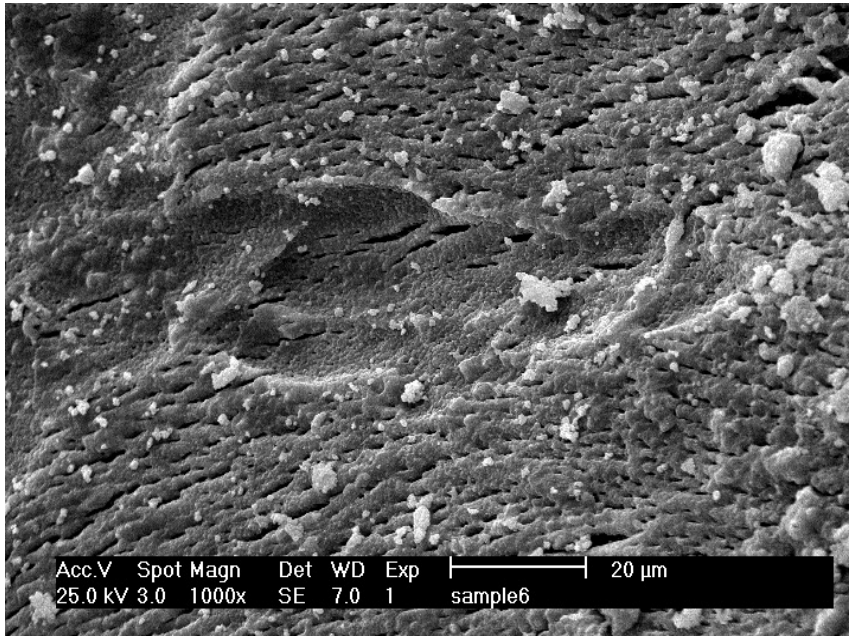
- **2- Cancellous bone feature (Microscopic)**

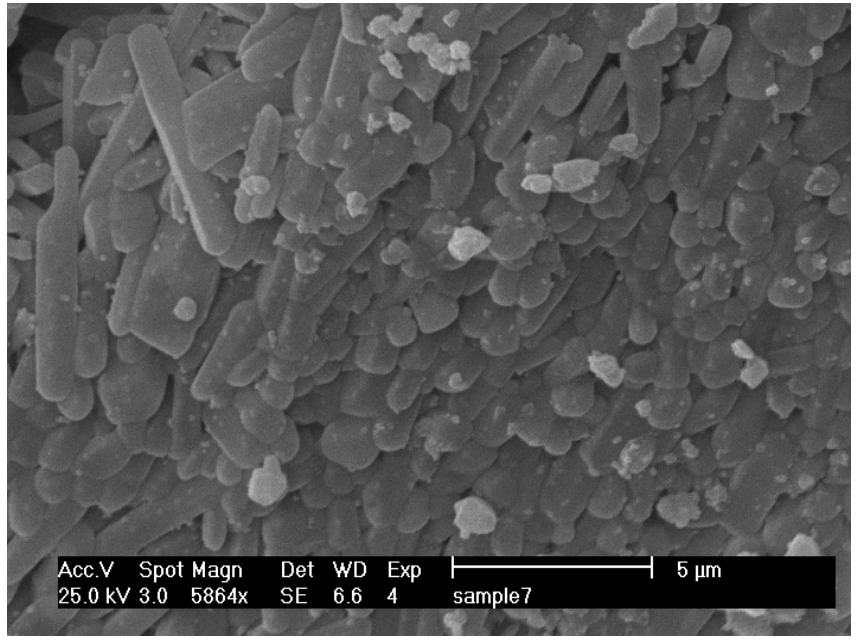





Acc.V Spot Magn Det WD Exp 20 µm
25.0 kV 3.0 1000x SE 7.4 4 sample3


Acc.V Spot Magn Det WD Exp 20 µm
25.0 kV 3.0 1000x SE 7.4 2 sample4


Acc.V Spot Magn Det WD Exp 20 µm
25.0 kV 3.0 1000x SE 7.0 1 sample6

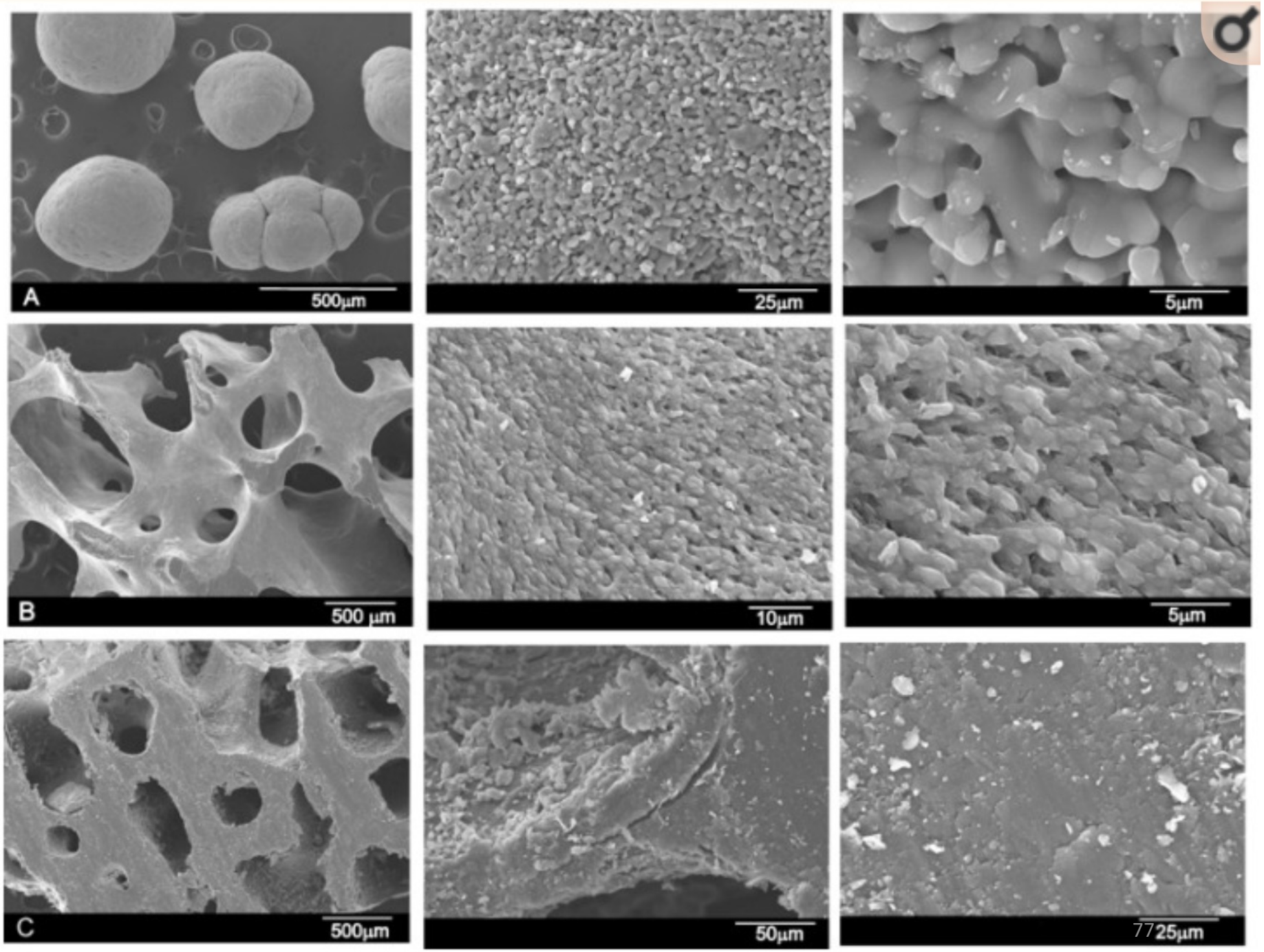

Acc.V Spot Magn Det WD Exp 5 µm
25.0 kV 3.0 5864x SE 6.6 4 sample7

SEM micrographs of the different grafting materials:

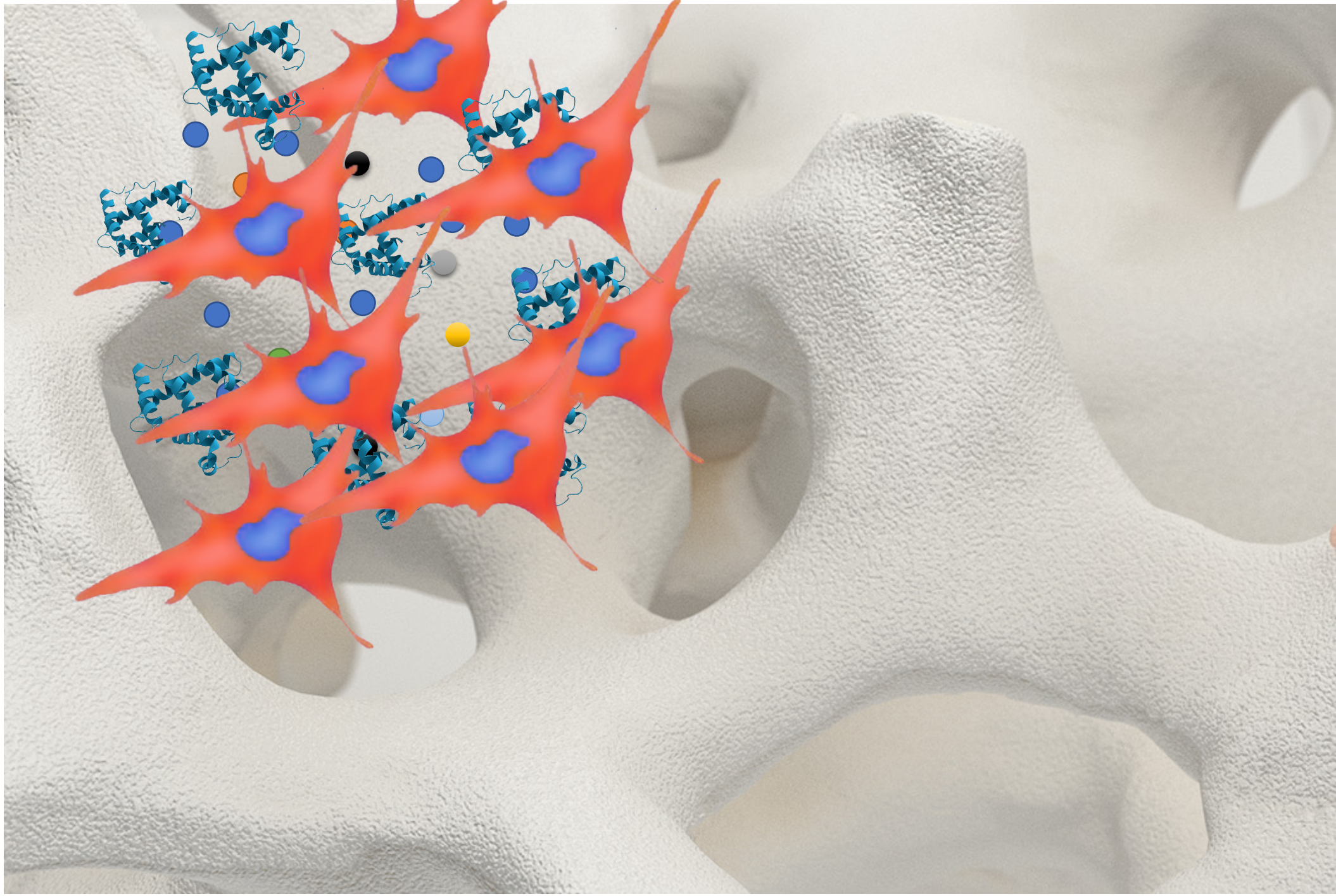
(**A**) synthetic TCP used as the control material;

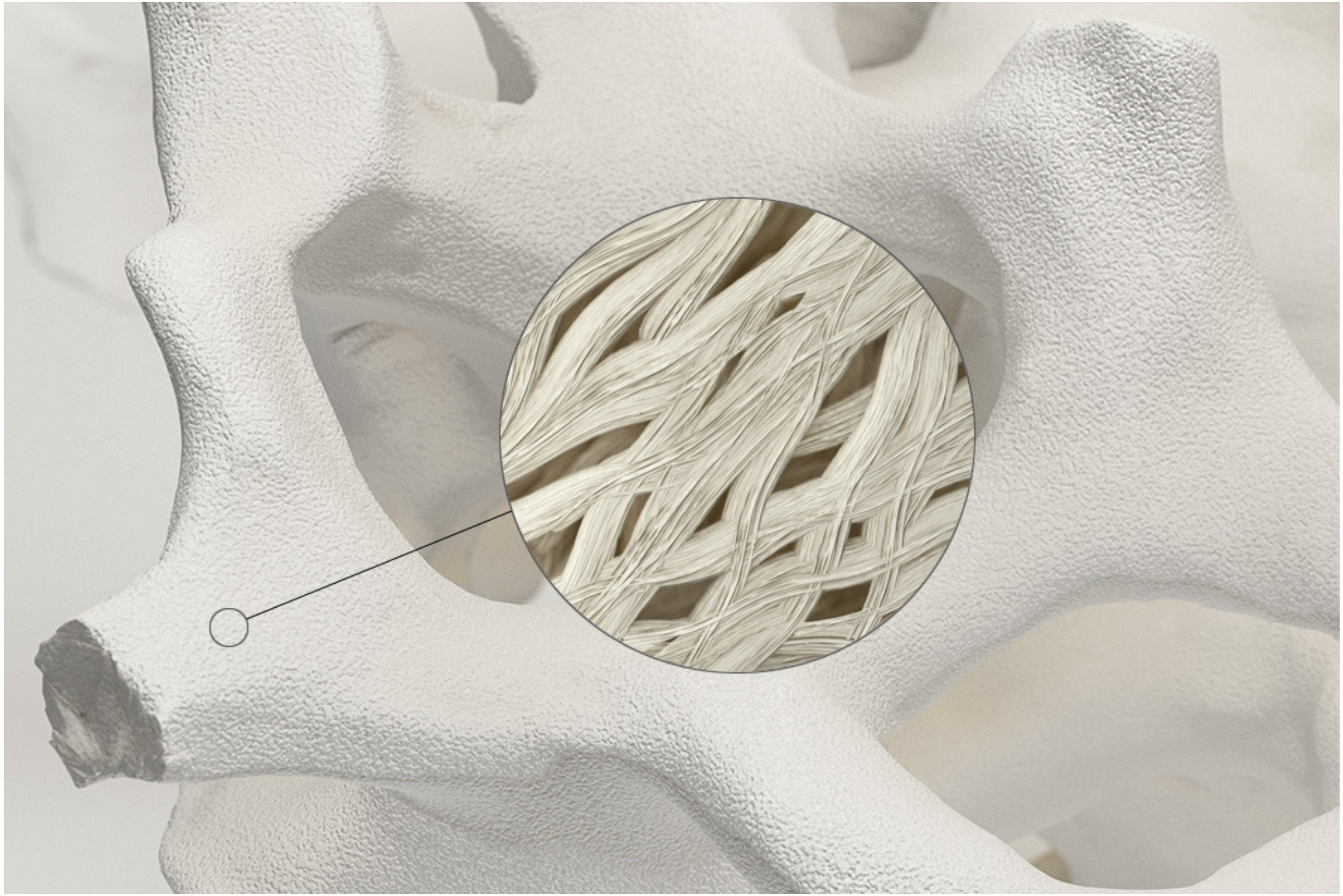
(**B**) bovine bone 1 (sintered material); and

(**C**) bovine bone 2 (material not sintered)



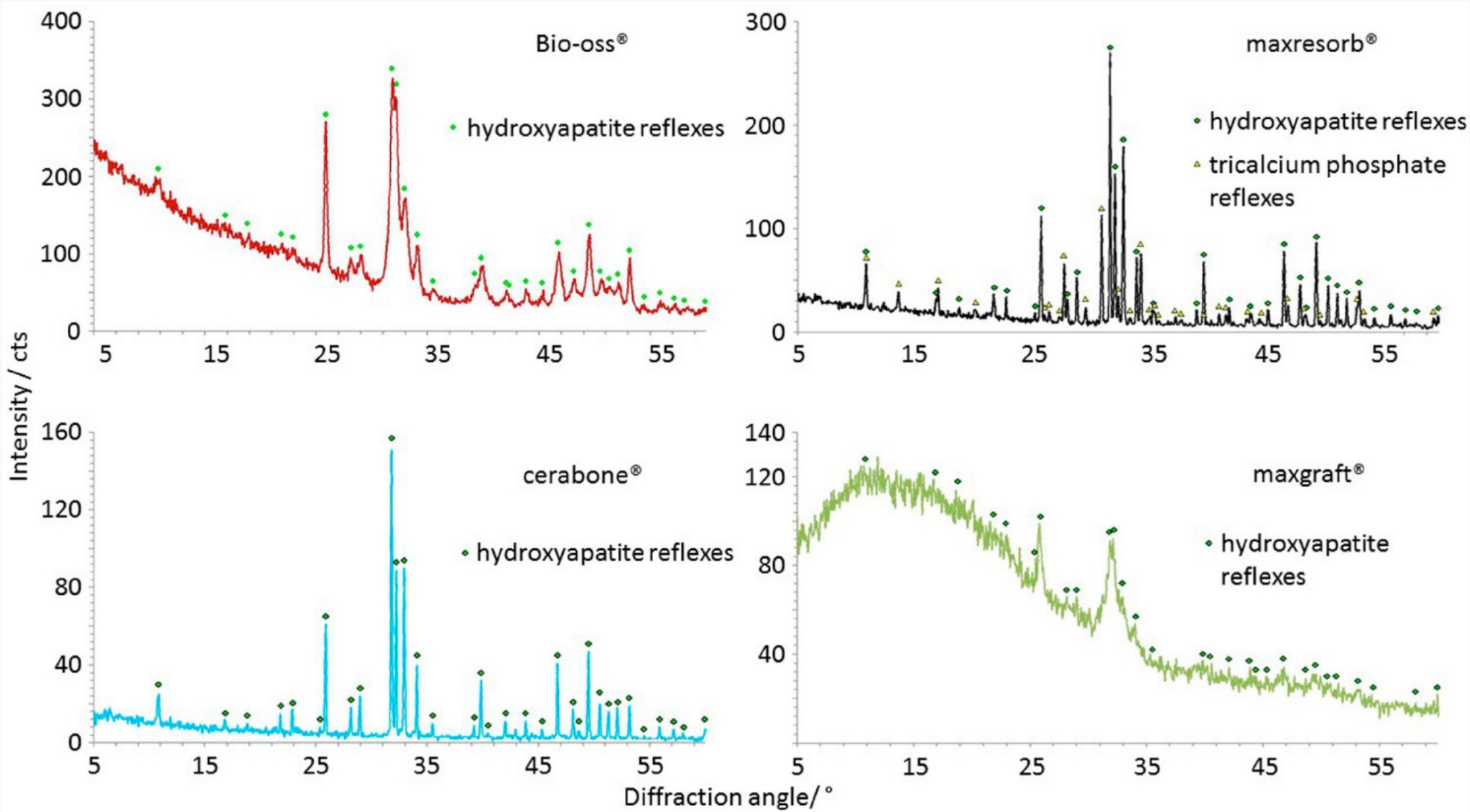
1- H_2O
2- Ions
3- Proteins
4- Cells

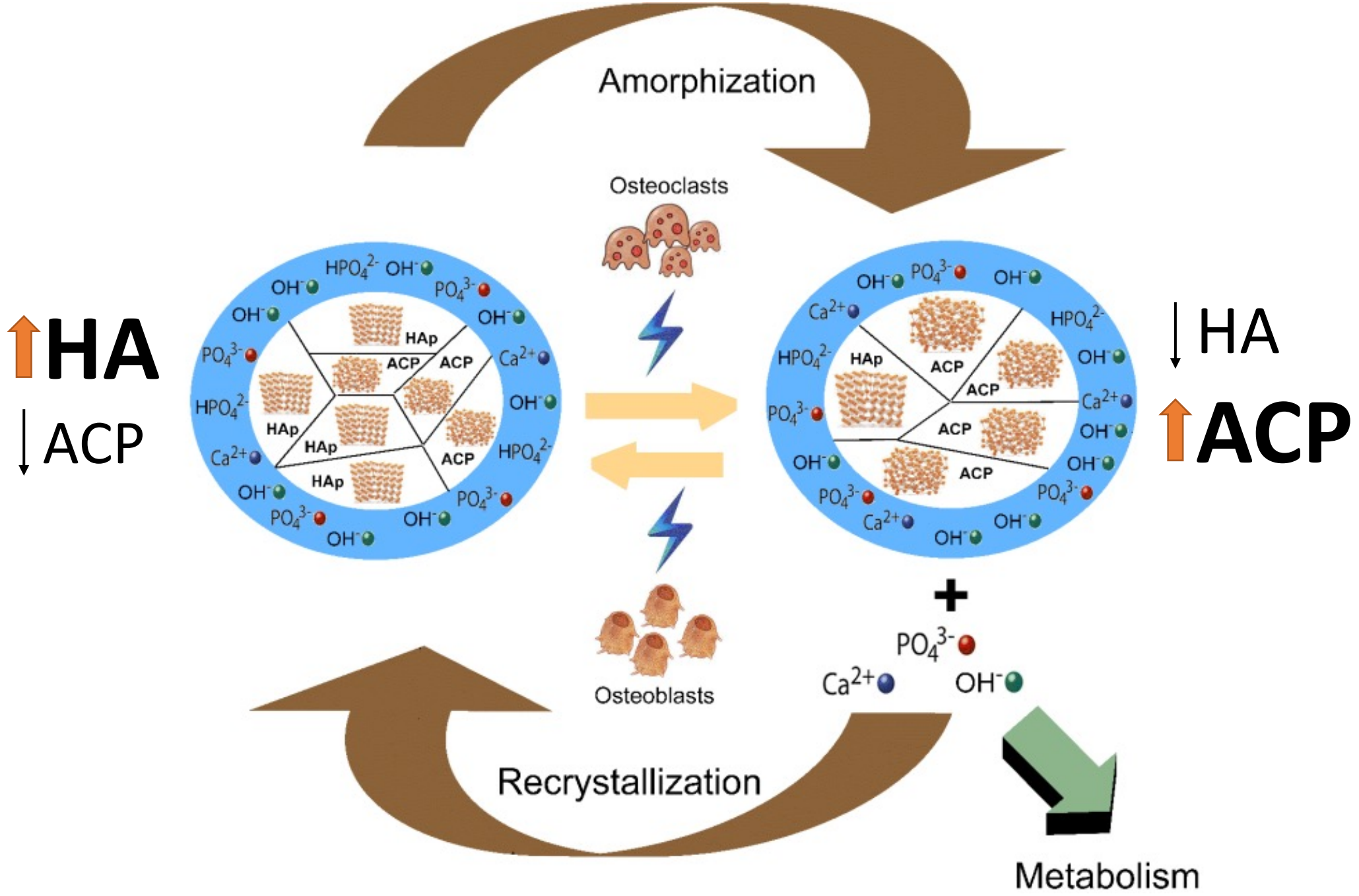


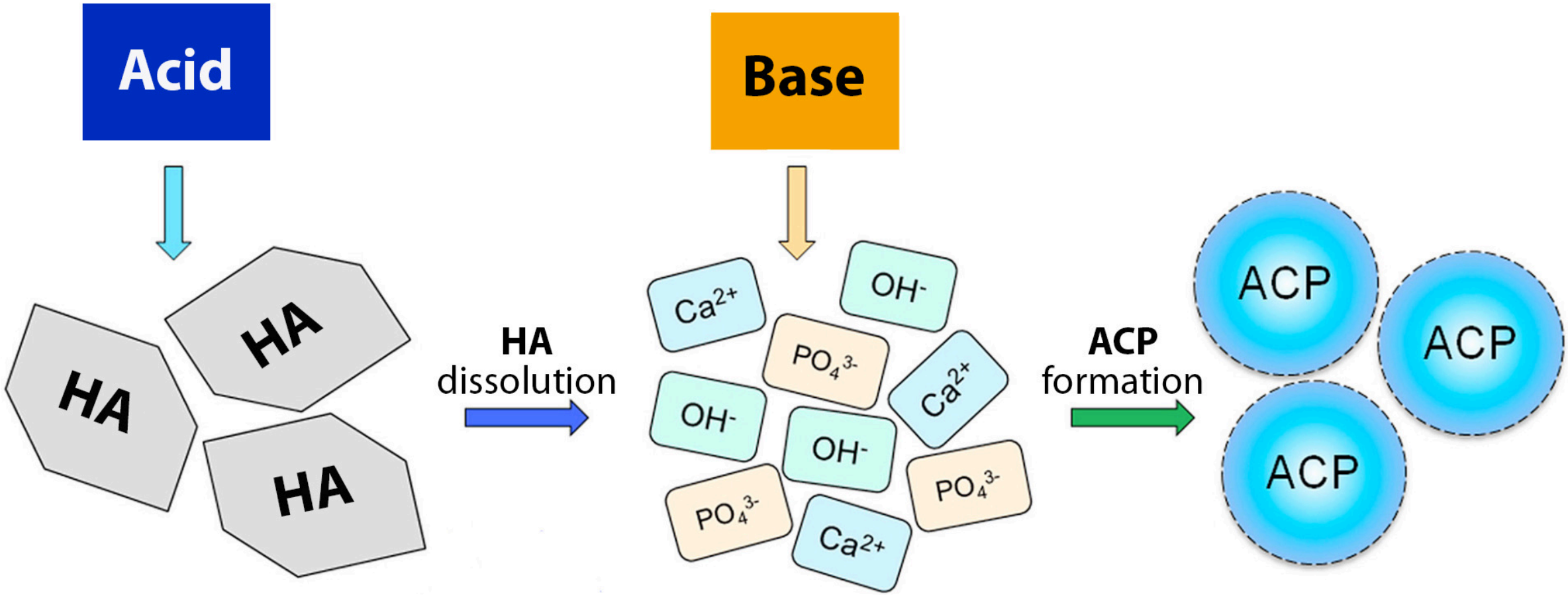


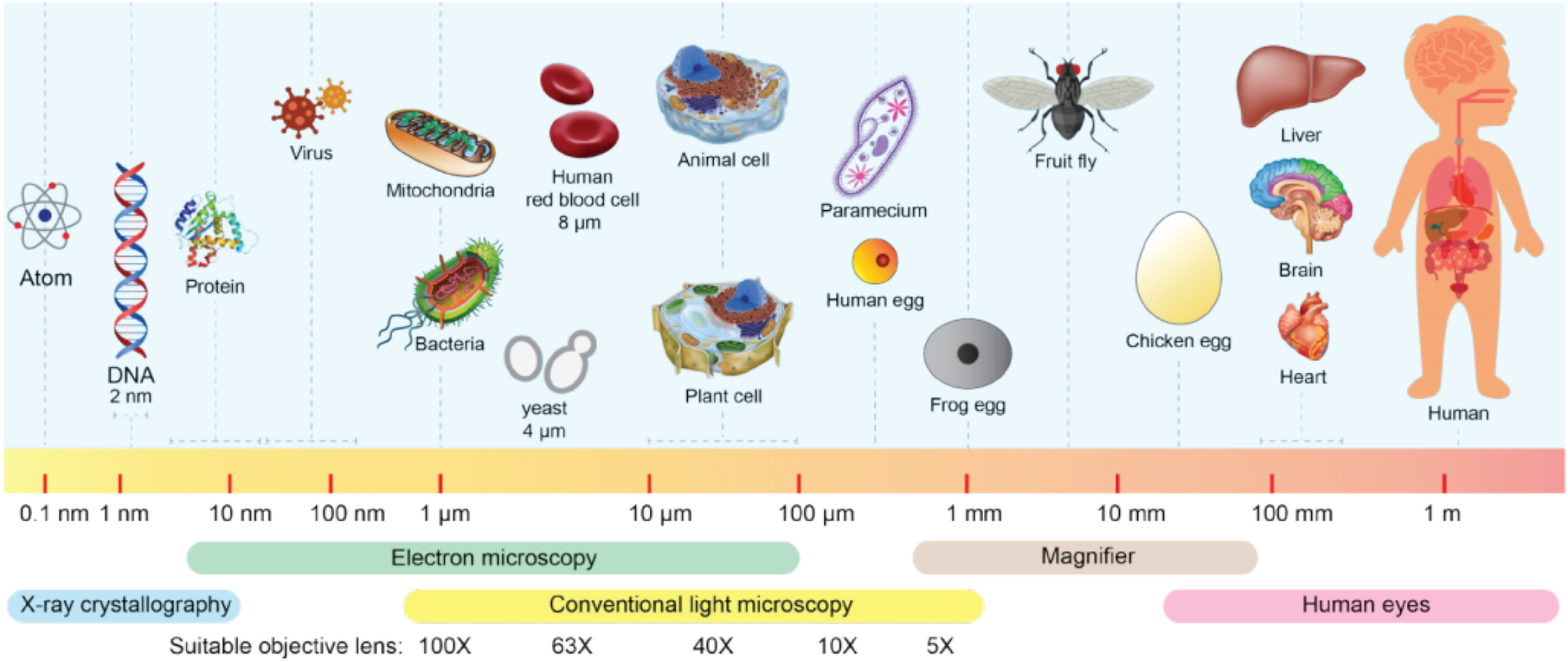
Xenograft Characterization

- **4- Calcium/Phosphate Ratio.** ⟶ **1.67**
- **5- XRD**
- **6- FTIR**
- **7- Non-genotoxic**
- **8- Non cytotoxic**
- **9- Non-Pyrogenic (LAL test)**
- **10- low Bioburden:** Bioburden is normally defined as the number of bacteria living on a surface that has not been sterilized.

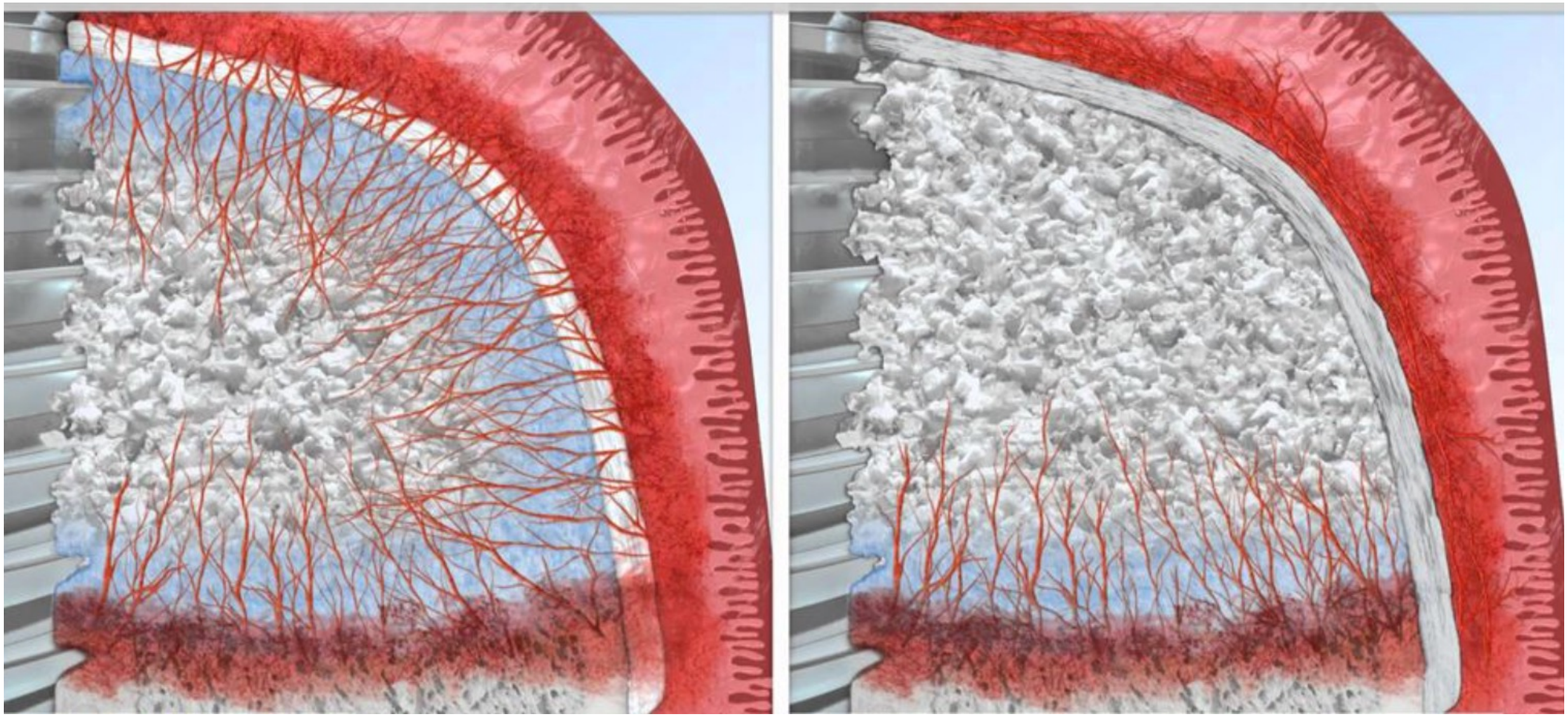

Bio-oss®
hydroxyapatite reflexes
maxresorb®
hydroxyapatite reflexes
tricalcium phosphate reflexes
cerabone®
hydroxyapatite reflexes
maxgraft®
hydroxyapatite reflexes
Intensity / cts
Diffraction angle/ °

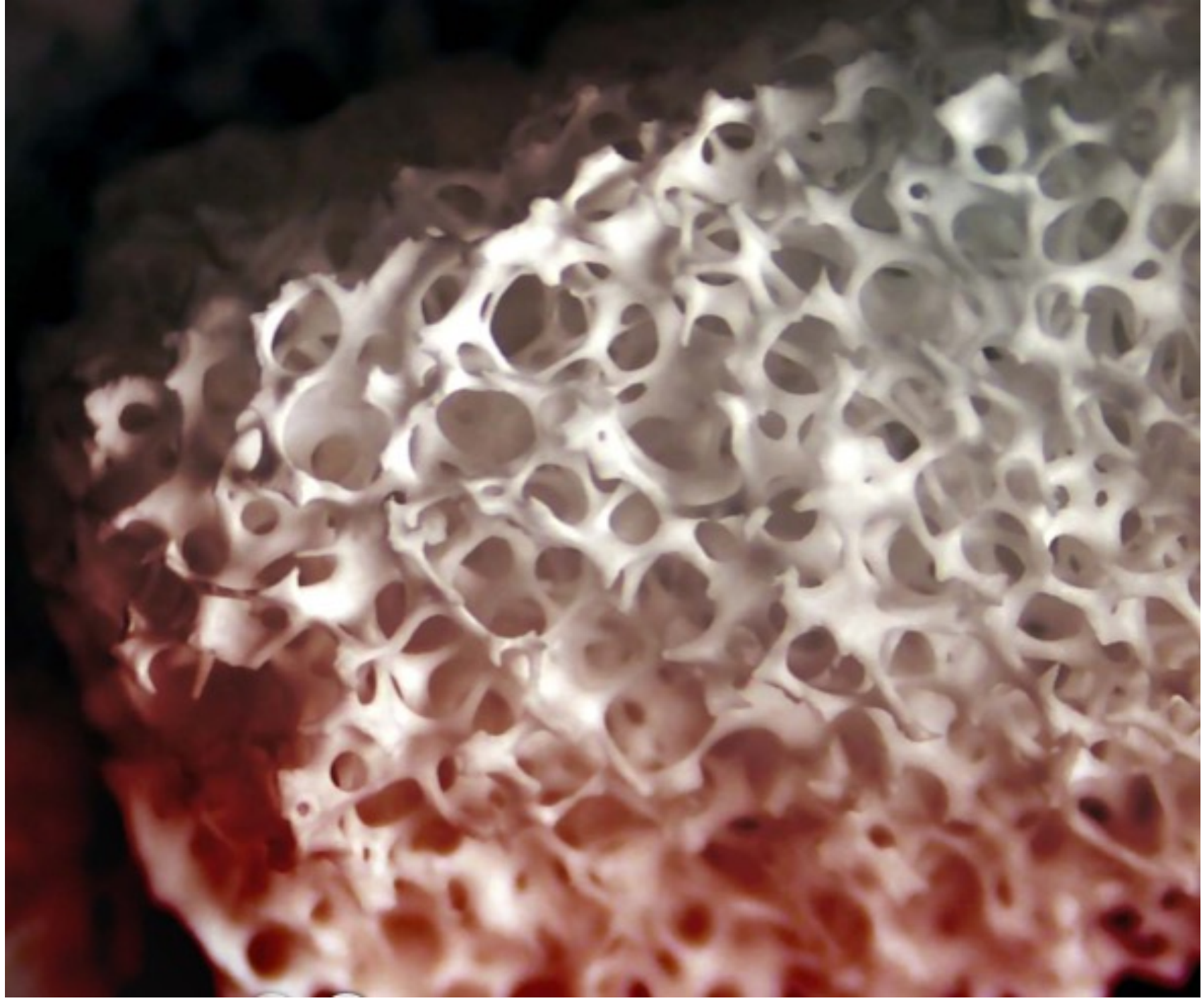

Amorphization
Osteoclasts
↑HA
↓ACP
HAp
ACP
↓HA
↑ACP
Osteoblasts
Recrystallization
Metabolism


Acid
HA
HA
HA
HA
dissolution
Base
Ca2+
OH-
PO43-
Ca2+
OH-
OH-
PO43-
PO43-
Ca2+
ACP
formation
ACP
ACP
ACP


Atom
DNA
2 nm
Protein
Virus
Mitochondria
Bacteria
Human
red blood cell
8 μm
yeast
4 μm
Animal cell
Plant cell
Paramecium
Human egg
Frog egg
Fruit fly
Chicken egg
Liver
Brain
Heart
Human
0.1 nm
1 nm
10 nm
100 nm
1 μm
10 μm
100 μm
1 mm
10 mm
100 mm
1 m
Electron microscopy
Magnifier
X-ray crystallography
Conventional light microscopy
Human eyes
Suitable objective lens: 100X 63X 40X 10X 5X


www.implantodent.ro





What does this means?

The shorter the turnover time of the cell, the more difficult to regulation. Because of this, osteoclasts may grow faster and dominate the osteoblast population.

cell type	turnover time	BNID
small intestine epithelium	2-4 days	107812, 109231
stomach	2-9 days	101940
blood Neutrophils	1-5 days	101940
white blood cells Eosinophils	2-5 days	109901, 109902
gastrointestinal colon crypt cells	3-4 days	107812
cervix	6 days	110321
lungs alveoli	8 days	101940
tongue taste buds (rat)	10 days	111427
platelets	10 days	111407,111408
bone osteoclasts	2 weeks	109906
intestine Paneth cells	20 days	107812
skin epidermis cells	10-30 days	109214, 109215
pancreas beta cells (rat)	20-50 days	109228
blood B cells (mouse)	4-7 weeks	107910
trachea	1-2 months	101940
hematopoietic stem cells	2 months	109232
sperm (male gametes)	2 months	110319, 110320
bone osteoblasts	3 months	109907
red blood cells	4 months	101706, 107875
liver hepatocyte cells	0.5-1 year	109233
fat cells	8 years	103455
cardiomyocytes	0.5-10% per year	107076, 107077, 107078
central nervous system	life time	101940
skeleton	10% per year	109908
lens cells	life time	109840
oocytes (female gametes)	life time	111451

Xenograft Characterization

- **3- Specific Surface Area** Human Cancellous Bone = ~72 m^2/gr

Manufacturers' specification	Surface Area	Cristallinity	Carbonate %	*Expected Biodegradability*
HA-1(synthetic, non-resorbable)	low	high	high	moderate-low
HA-2(synthetic, non-resorbable)	low	high	low	low
HA-3 (synthetic, resorbable)	moderate	moderate	moderate	moderate
HA-4 (synthetic, resorbable)	moderate	low	moderate	moderate
HA-5 (natural HA, resorbable)	low	high	high	moderate-low
HA-6 (inorganic bovine bone, resorbable)	high	low	high	fast

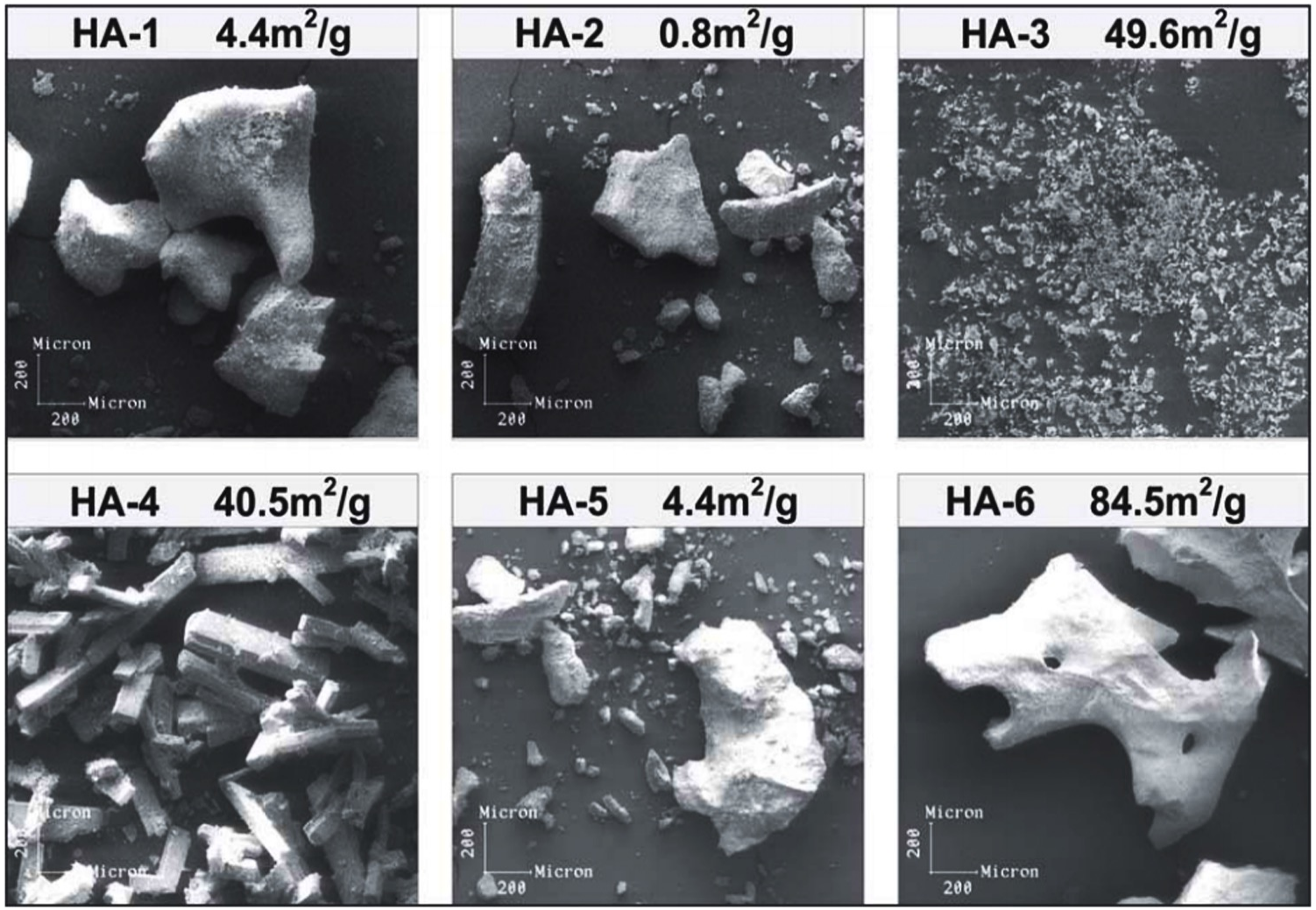
4.4 m^2/g

84.5 m^2/g

Xenograft Characterization

- **3- Specific Surface Area**

Brunauer–Emmett–Teller (**BET**) theory aims to explain the physical adsorption of gas molecules on a solid **surface** and serves as the basis for an important analysis technique for the measurement of the specific **surface area** of a material.



Specific Surface Area

Xenograft	Manufacturer	Country	Surface area (m2/gr)
BioOss®	Geistlich Pharma	Switzerland	**84**
Ocs-B®	NIBEC	South Korea	**81**
Bone+B®	Nova Teb Pars	Iran	**82**
Cerabone®	Botiss	Germany	**8**
Allograft			
enCore®	Osteogenics Biomedical	USA	**4.8**

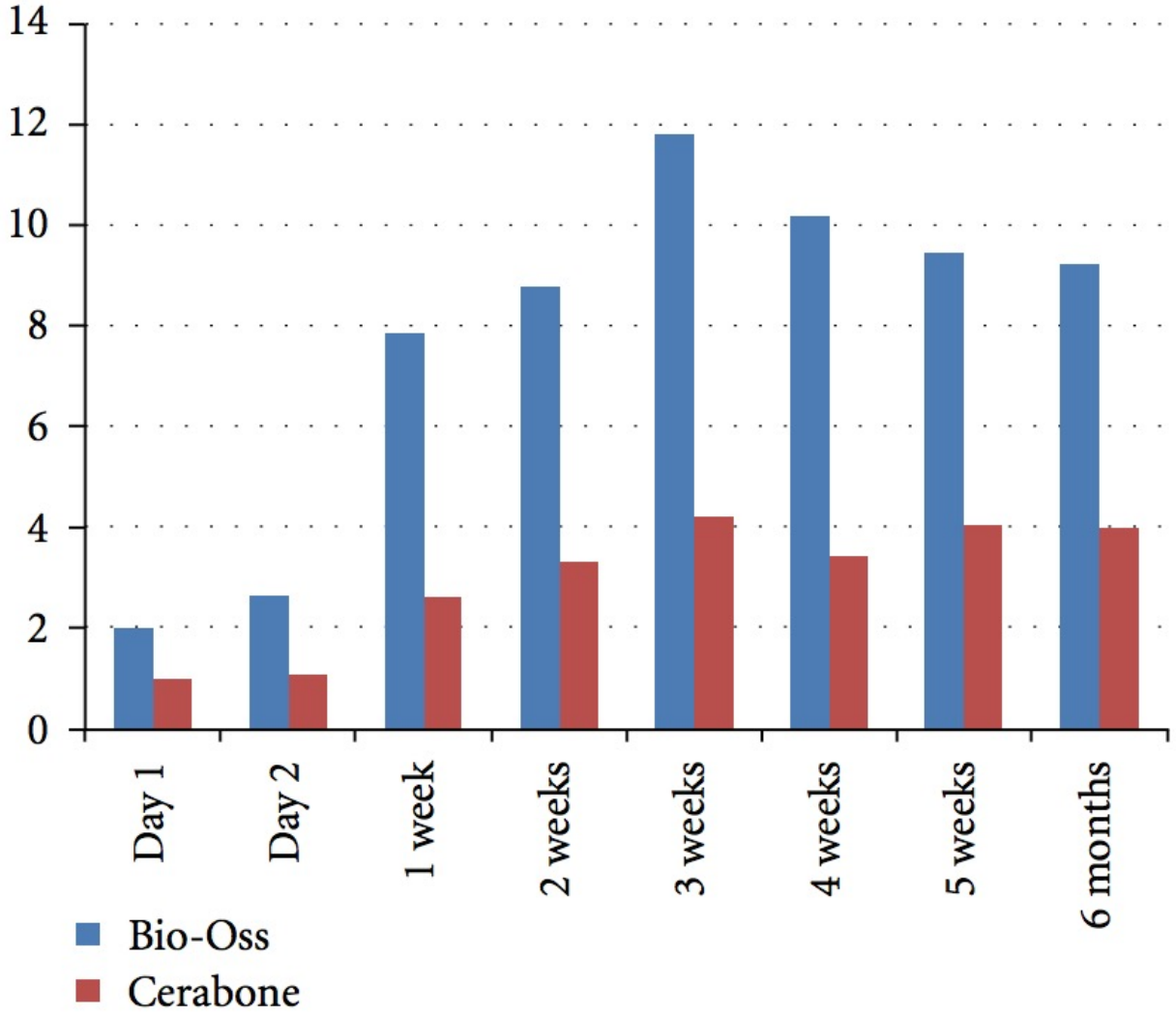
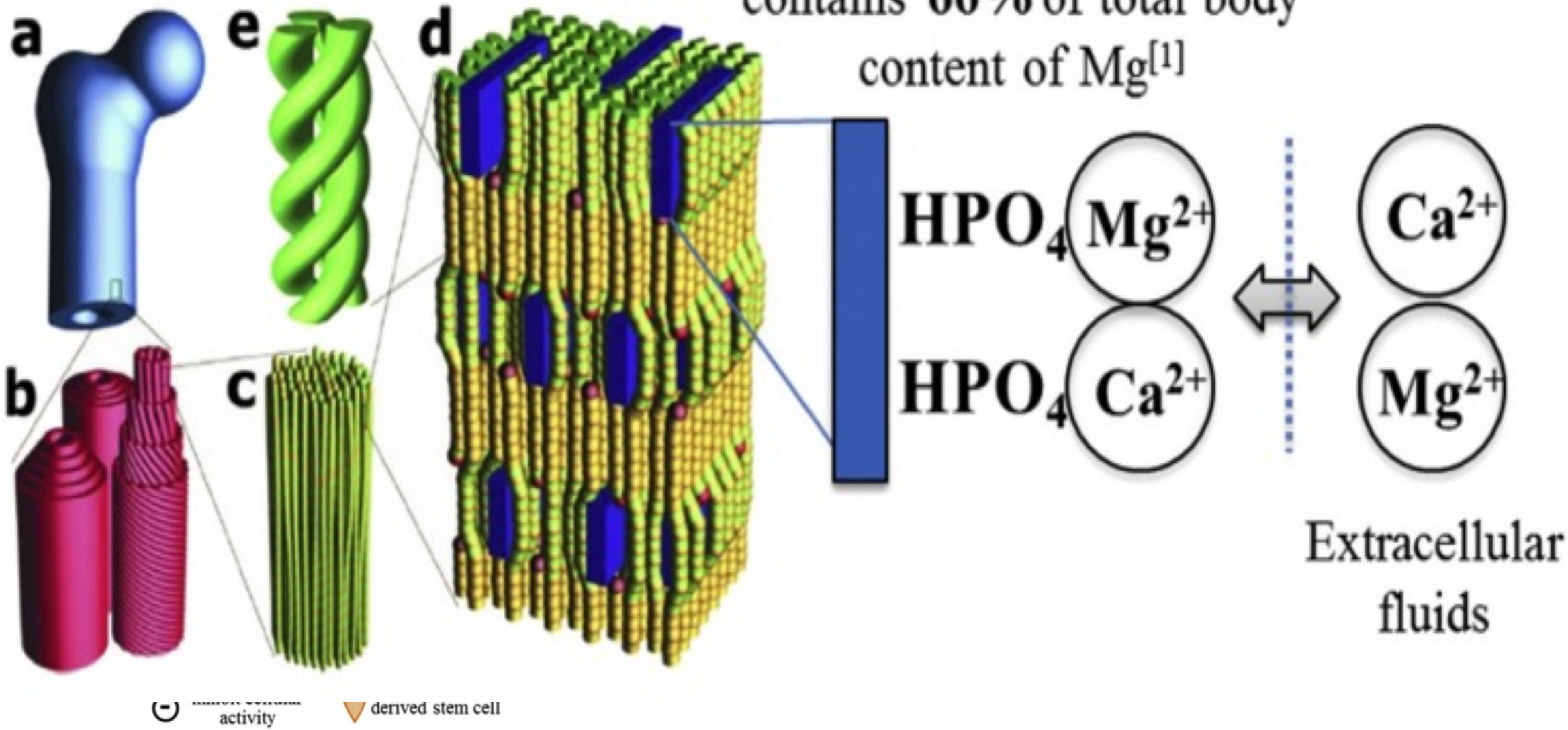


FIGURE 4: Calcium release (mg/g) at different time intervals. Release rate was almost constant after 2 months.

Influence of Material Properties on Rate of Resorption of Two Bone Graft Materials after Sinus Lift Using Radiographic Assessment
•July 2012 International Journal of Dentistry 2012(7):737262


Hydrate layer
contains 66% of total body content of Mg[1]
a
b
c
d
e
HPO4 Mg2+
HPO4 Ca2+
Ca2+
Mg2+
Extracellular fluids
activity
derived stem cell

Excessive release of calcium ions

- Pure water-soluble calcium phosphates such as b-TCP release calcium ions into local tissues. Calcium ions control osteoblastic viability , proliferation, and differentiation via intracellular calcium signaling after influx into the cells through calcium channels
- In addition, calcium ions may induce osteoblastic apoptosis by increasing cytosolic calcium ion concentrations and triggering downstream events leading to apoptosis
- Controlling the amount of calcium ions released may facilitate or hamper the application of pure water-soluble calcium phosphates as bone substitutes apoptosis

Space-Making Capability and Volume maintenance

Basic properties of bone substitutes required for bone formation

(A) **Osteoconductive.** (and Osteoinductive)

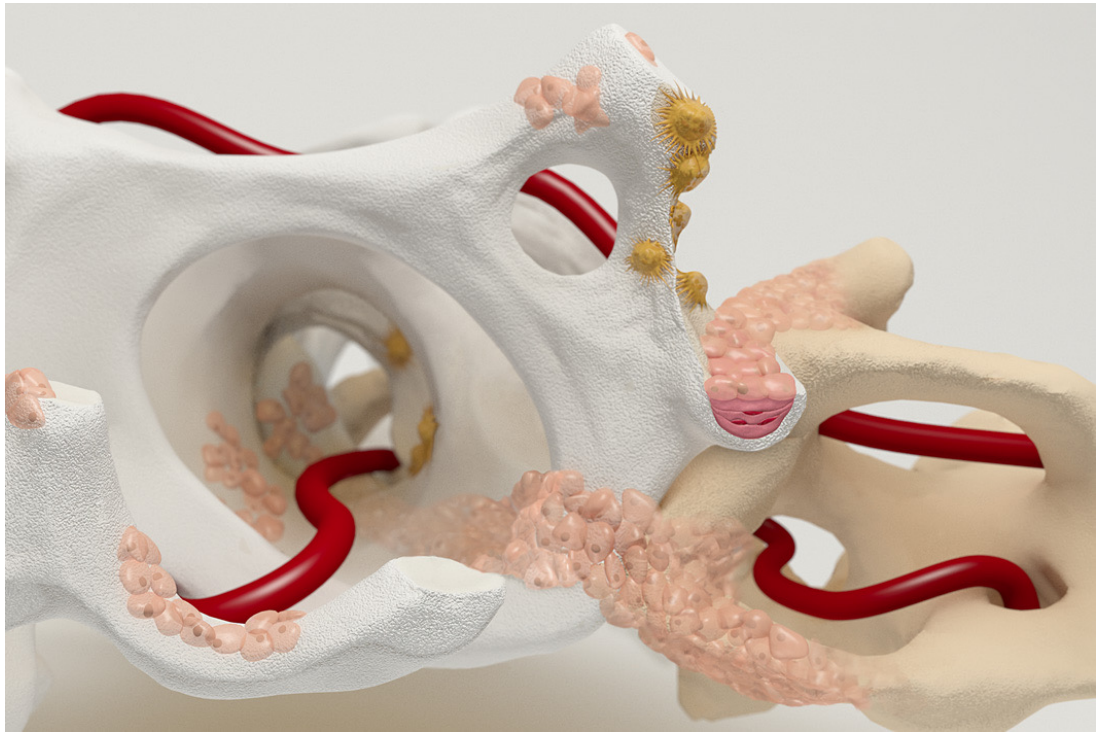
(B) **Biocompatibility.**

(C) **Space-making capability.**

(D) **Volume maintenance** by replacement with bone over time (regeneration).

Requirement for bone substitutes in implant dentistry

- Versatility of autogenous bone as a bone graft material
- Basic properties of bone substitutes required for bone formation



Factors for the space-making capability of bone substitutes

- Enzymatic or chemical dissolution
- Mechanical properties
- Particle size

Need for improved biocompatibility of currently available bone substitutes

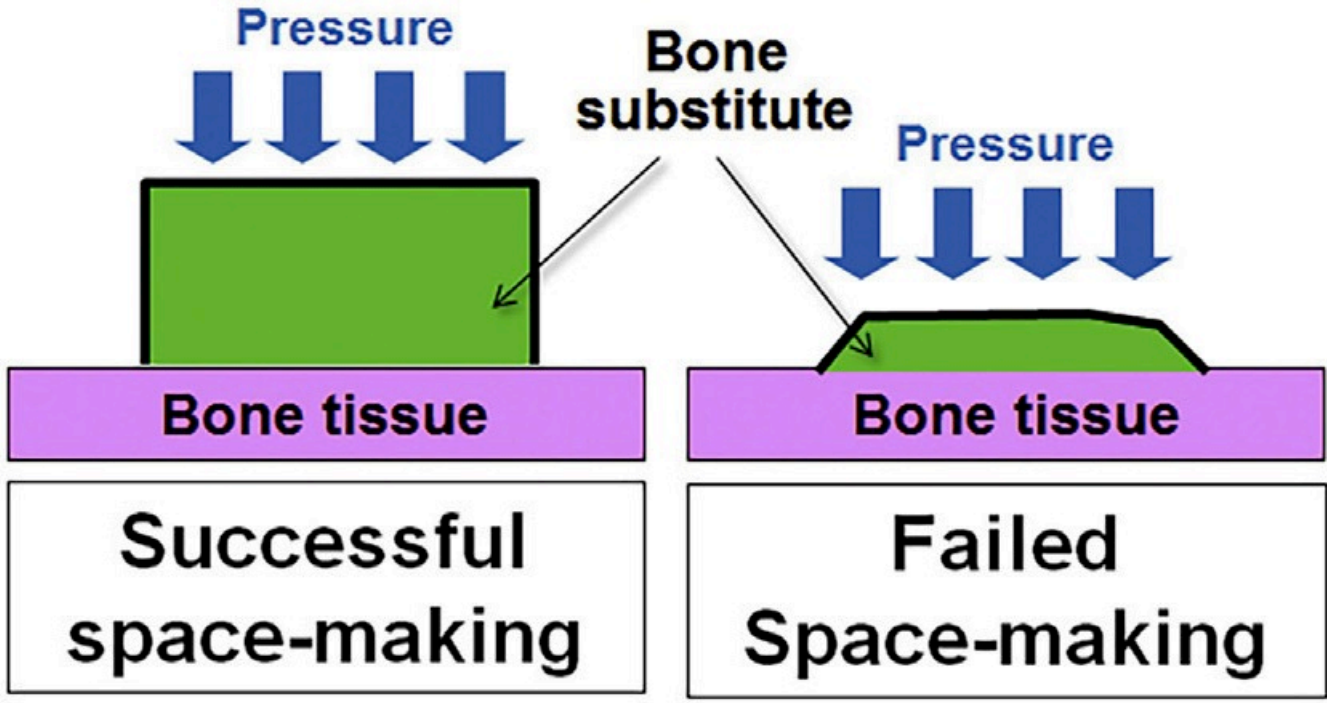
- Nature of collagen fibers
- Micro- or nanoparticulates
- Excessive release of calcium ions
- Improving the biocompatibility of current bone substitutes

Factors affecting bio-absorbability and volume maintenance of bone substitutes

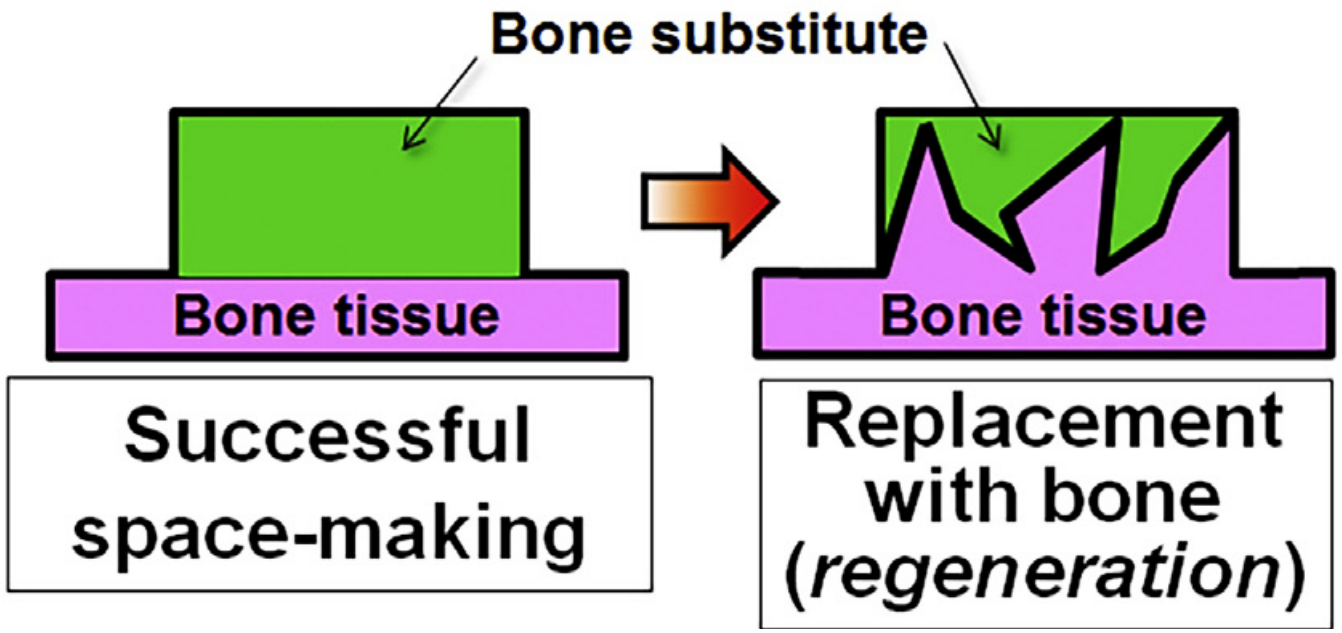
- Pore size and porosity
- Water solubility
- Integrity and crystallinity of the apatite structure
- Influence of manufacturing process on integrity and crystallinity of apatite structure
- Inverse relationship between bio-absorption and volume maintenance

International Orthopaedics. https://doi.org/10.1007/s00264-020-04483-3

Space-making capability



Volume maintenance



Mechanical properties

- Mechanical properties of bone graft materials can also affect their space-making capability
- Bone substitutes are generally used in particle, and not block, form in alveolar ridge augmentation because shapeability is required to mold three-dimensional ridges on the irregularly shaped alveolar bone
- **Mechanical resistance to compression depends more on granule size than on the actual mechanical properties of the material**

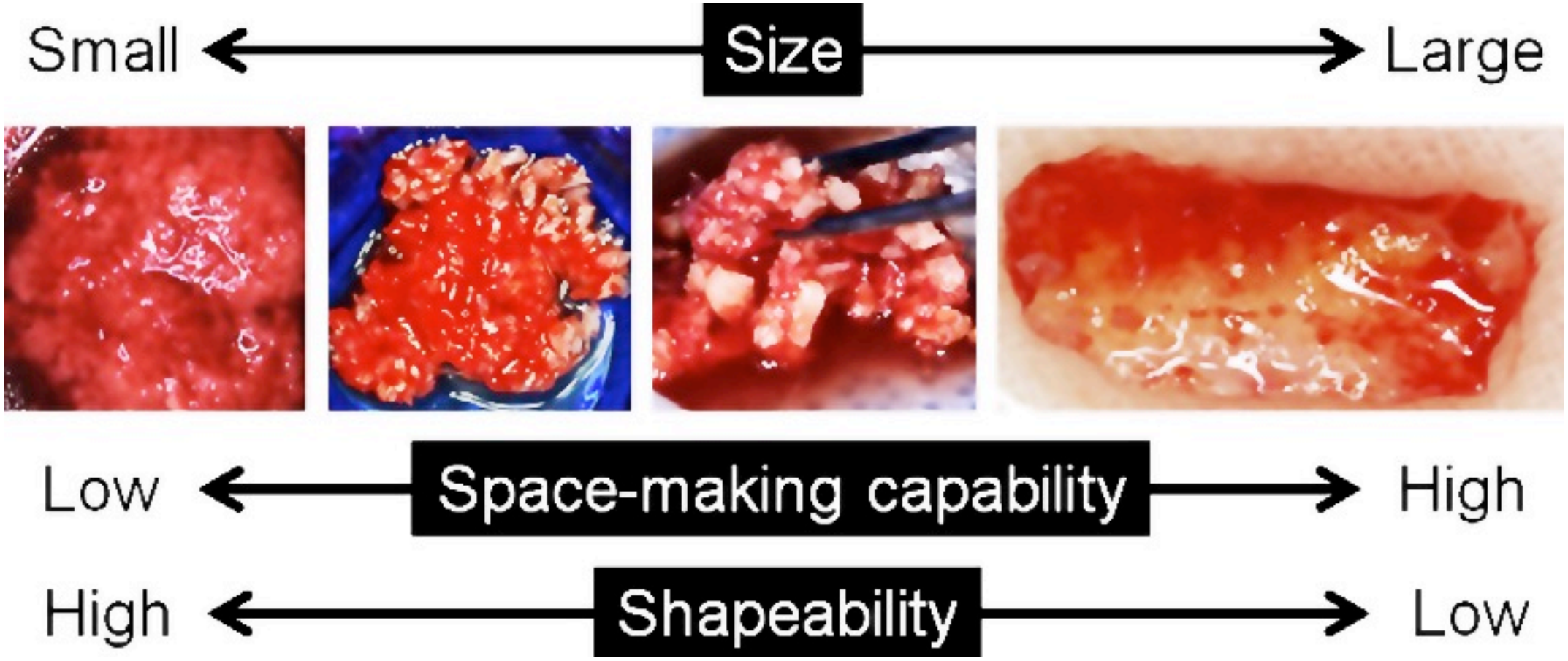
Elastic modulus of autogenous bone and currently available bone substitutes

Bone graft materials	Elastic modulus (GPa)
Autogenous bone	Edentulus jaw: 15–18 Mandibular cortical bone: 30–39
Freeze-dried bone allograft (FDBA)	Theoretical 20% reduction as compared to fresh bone
Demineralized freeze-dried bone allograft (DFDBA)	1–2.5
Chemically deproteinized bovine bone xenograft (CD-BB)	28
Thermally deproteinized bovine bone xenograft (TD-BB)	30–33
Synthetic hydroxyapatite in porosity-free bulk (HA)	80–120
β-Tricalcium phosphate in porosity-free bulk (β-TCP)	110

Particle size

- Osteoclast-like multinucleated giant cells appear to prefer small particles (<1 mm) in both autogenous bone and bone substitutes, such as bovine bone mineral
- Bovine bone mineral granules with a large size (1–2 mm) generated 1.4 times higher volume in sinus augmentation than smaller granules (0.25–1 mm)
- These observations indicate that compared with smaller particles (<1 mm), larger particles (1 mm) possess greater mechanical resistance as a lump for space-making and that the space-making capability is more important for initial bone formation than the balance between bone resorption and formation

Particle size



Biocompatibility

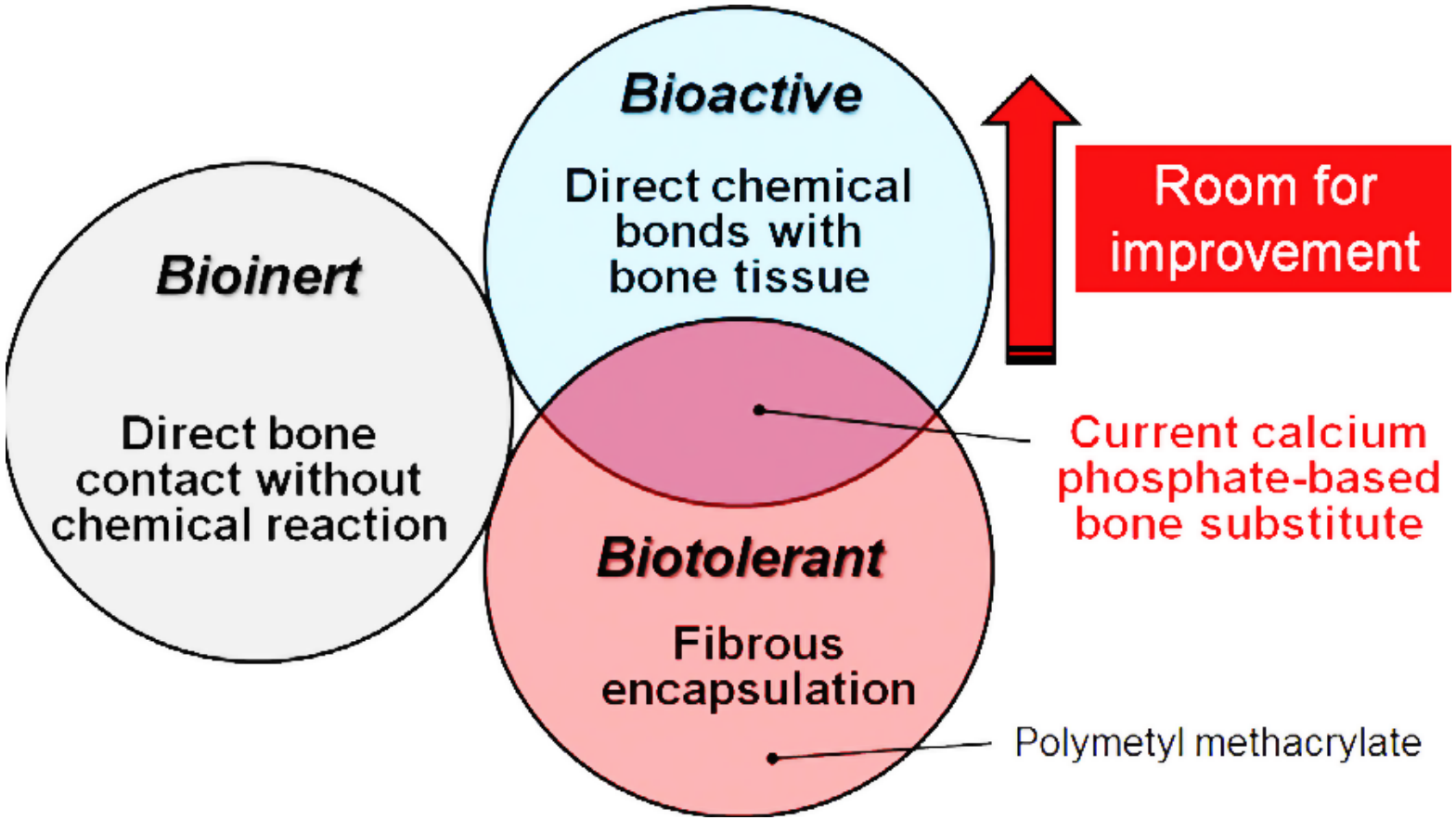
Biocompatibility is defined as “the ability of a material to perform with an appropriate host response in a specific application” Traditionally, in terms of biocompatibility, bone graft materials are classified as bio-tolerant, bioinert, or bioactive.

Bio-tolerant implant materials remain in the body with fibrous encapsulation by evoking a tissue reaction. (PMMA)

Bioinert implant materials have direct contact with the adjacent bone tissue without any chemical reaction.

Bioactive implants establish chemical bonds with adjacent bone tissue, which leads to direct deposition of bone matrix on the implant material.

Biocompatibility



Factors affecting bioabsorbability and volume maintenance of bone substitutes

Bioabsorption = Bioresorption = Biodegradation = Bioerosion

Favorable bioabsorption of bone substitutes should involve the replacement of the implanted material by newly formed bone tissue via bone remodeling, i.e., “Regeneration” and not “Reconstruction”

Bioabsorption during the bone formation phase is associated with space-making capability and biocompatibility, and is predominantly mediated by passive chemical dissolution of the bone substitute.

Maintenance of the augmented bone volume over time is important in pre-prosthodontic alveolar bone augmentation to control the three-dimensional alveolar bone morphology

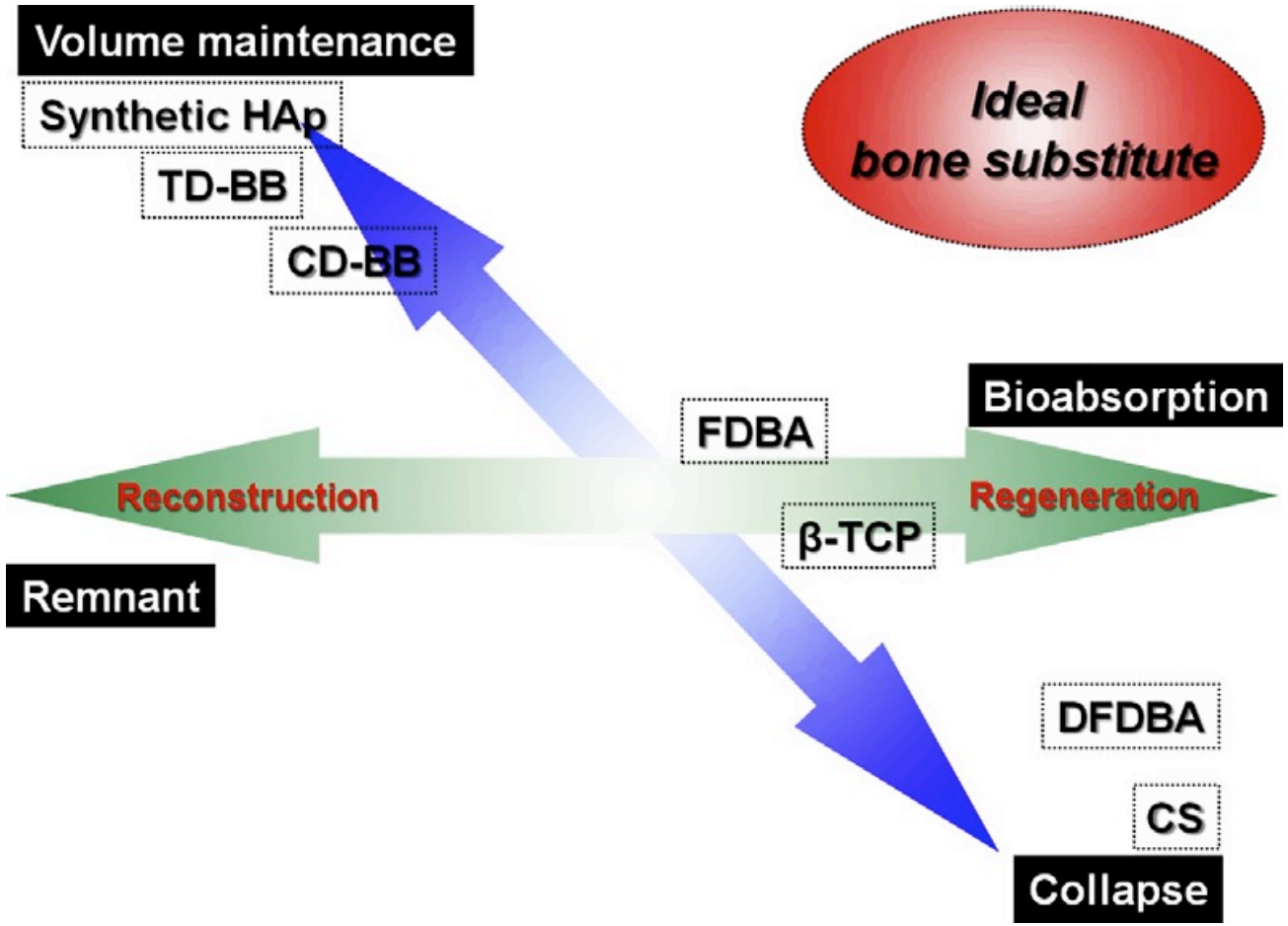
Factors affecting bioabsorbability and volume maintenance of bone substitutes

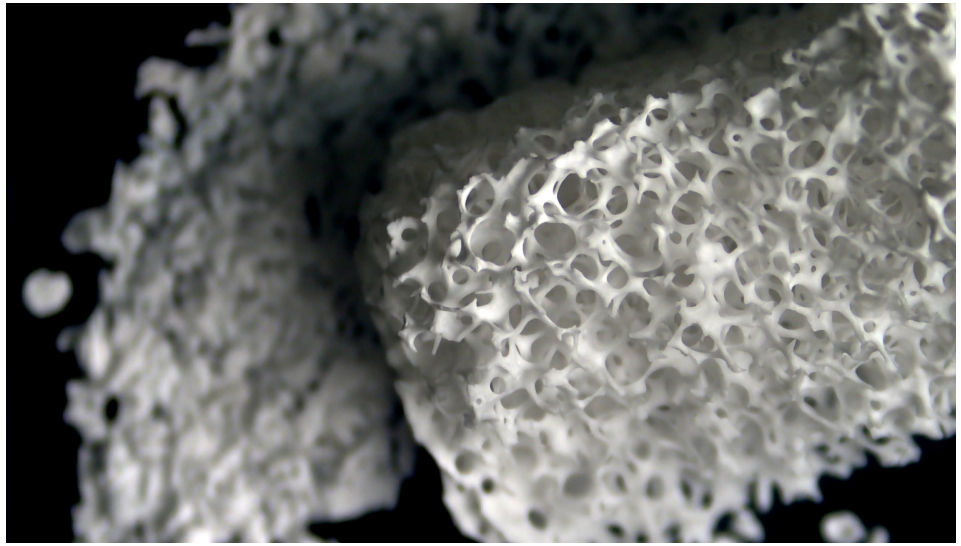
- ✓Pore size and porosity (100-300 μm) increased pore size and porosity also reduce the mechanical resistance of such materials and thus must be balanced against the space-making capability and maintenance of volume.
- ✓Water solubility (surface areas). It is critical factor determining the bioabsorption rate during the remodeling phase is the chemical composition and water solubility of the bone substitute. Osteoclasts can degrade bone substitutes in a manner similar to hydrolysis by secreting hydrogen ions
- ✓Integrity and crystallinity of the apatite structure The integrity and crystallinity of the apatite structure determine acid resistance and directly affect the bioabsorption of bone substitutes other than pure water-soluble calcium phosphate. High crystallinity decrease the surface area and then decrease wettability properties.
- ✓Inverse relationship between bioabsorption and volume maintenance

Contents of hydroxyapatite (HAp) and carbonate apatite (CAp) and extent of chemical dissolution or enzymatic and acid resistance of autogenous bone and currently available bone substitutes

Bone graft materials	HAp content (wt%)	CAp content (wt%)	Chemical or enzymatic dissolution	Acid resistance
Autogenous bone	Young cortical bone: 44 Young cancellous bone: 26 Old cancellous bone: 34	Young cortical bone: 1.4 Young cancellous bone: 0.6 Old cancellous bone: 2.4	Slightly	Low
Freeze-dried bone allograft (FDBA)	49	7.5	Slightly	Low
Demineralized freeze-dried bone allograft (DFDBA)	Unknown	Unknown (theoretically 0)	Completely	–
Chemically deproteinized bovine bone xenograft (CD-BB)	93.6	3.4	Slightly	Moderate/ high
Thermally deproteinized bovine bone xenograft (TD-BB)	≒100	0	Hardly	High
Synthetic hydroxylapatite in porosity-free bulk (HA)	≒100	0	Hardly	High
β-tricalcium phosphate in porosity-free bulk (β-TCP)	–	–	Completely	Low

Factors affecting bioabsorbability and volume maintenance of bone substitutes





THANK YOU FOR YOUR ATTENTION