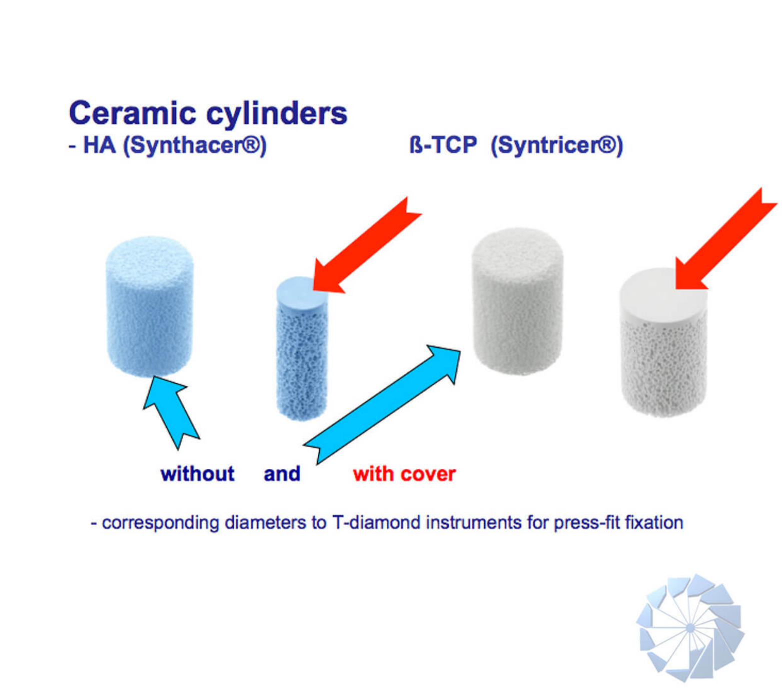
DEPARTMENT OF PHYSICS AND NANOTECHNOLOGY
SRM INSTITUTE OF SCIENCE AND TECHNOLOGY

18NTO408T- INDUSTRIAL NANOTECHNOLOGY

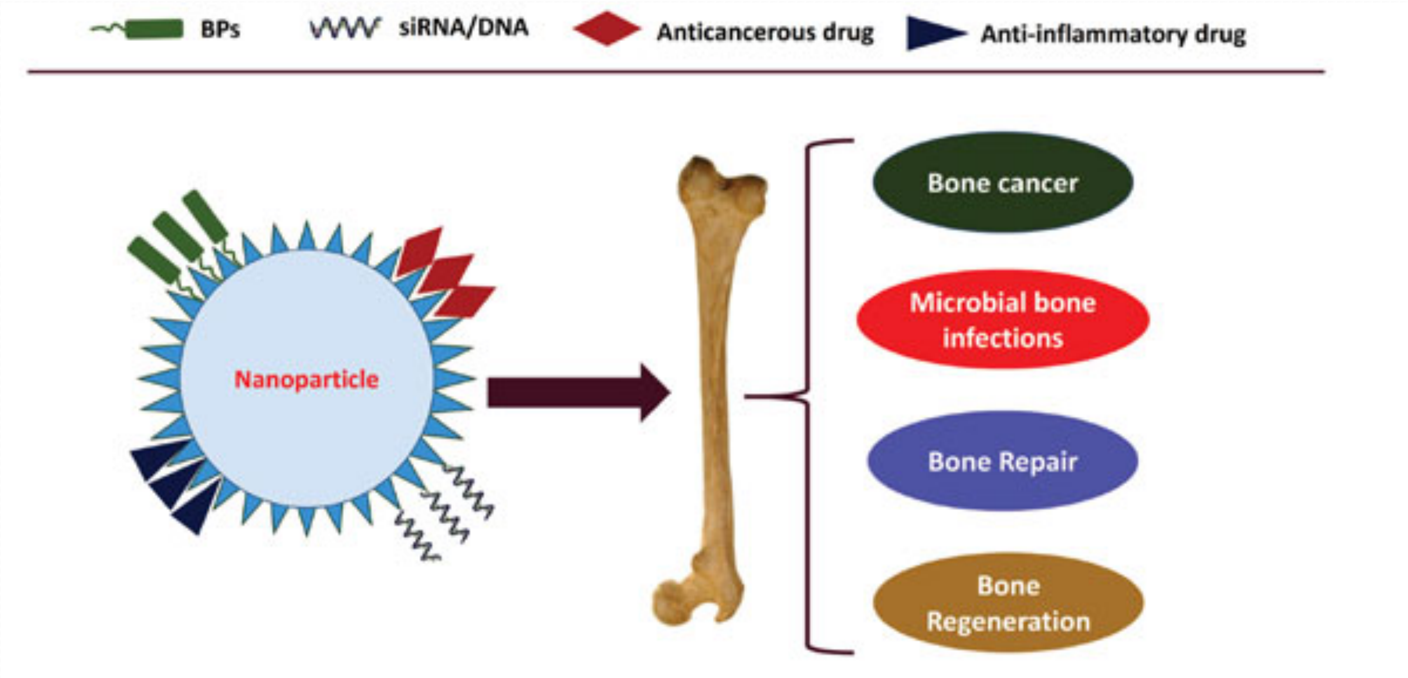
Nanoparticles in bone substitutes



In the last decade, significant growth in the fields of polymer sciences, nanotechnology, and biotechnology has resulted in the development of new nano-biomaterials.

These are extensively explored as drug delivery carriers and as implantable devices.

At the interface of nanomaterials and biological systems, the organic and synthetic worlds have merged over the past two decades, forming a new scientific field incorporating nano-material design for biological applications



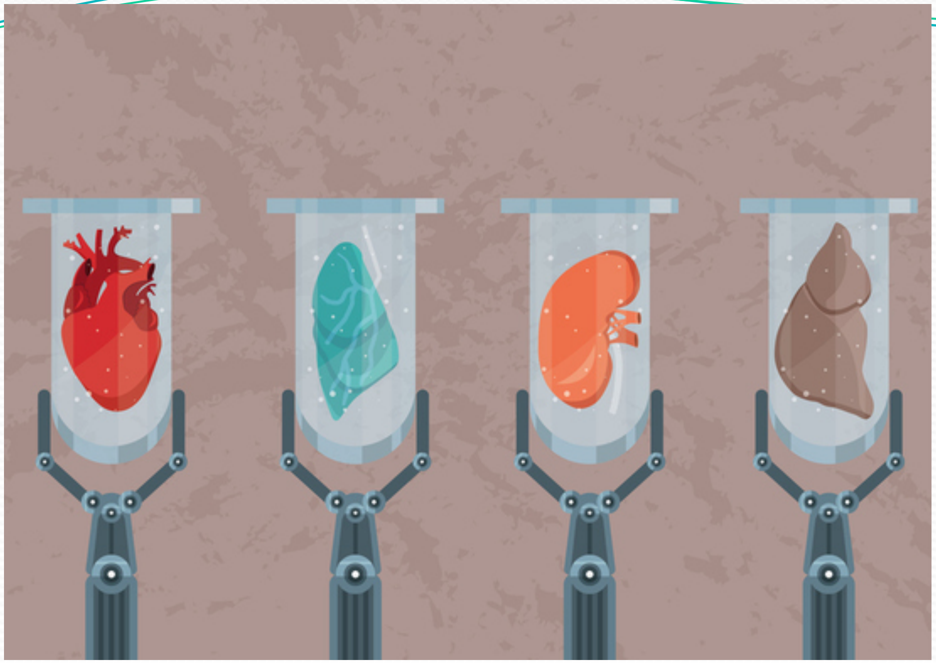
Bone undergoes self-repair of small defects due to the synergistic actions of mesenchymal cells, osteogenic cells, and cells of the immune system

This self-repaired bone contains physico-chemical and mechanical properties that recapitulate the bone which was replaced

However, larger defects are unable to undergo the same level of self-healing, and regenerative medicine approaches are paramount in addressing these clinical challenges

Tissue engineering and regenerative medicine (TERM) aims to conjugate engineering and biological properties to create functional substitutes for damaged and diseased tissues.

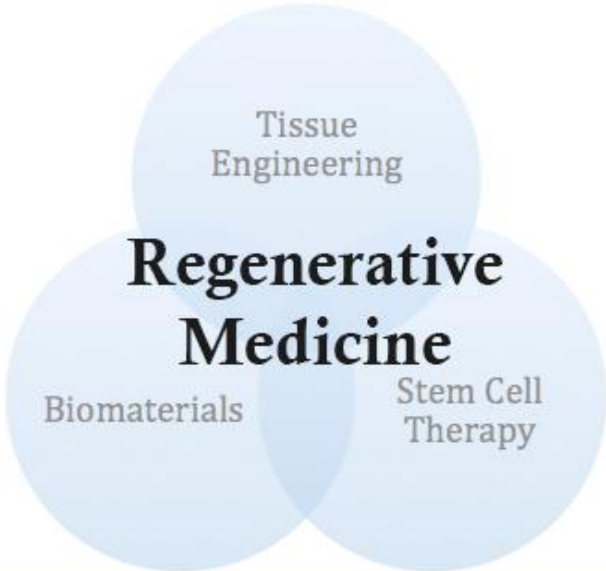
The strategy used on TERM research combines three essential elements - scaffolds, stem cells, and growth factors - to produce a tissue engineered construct

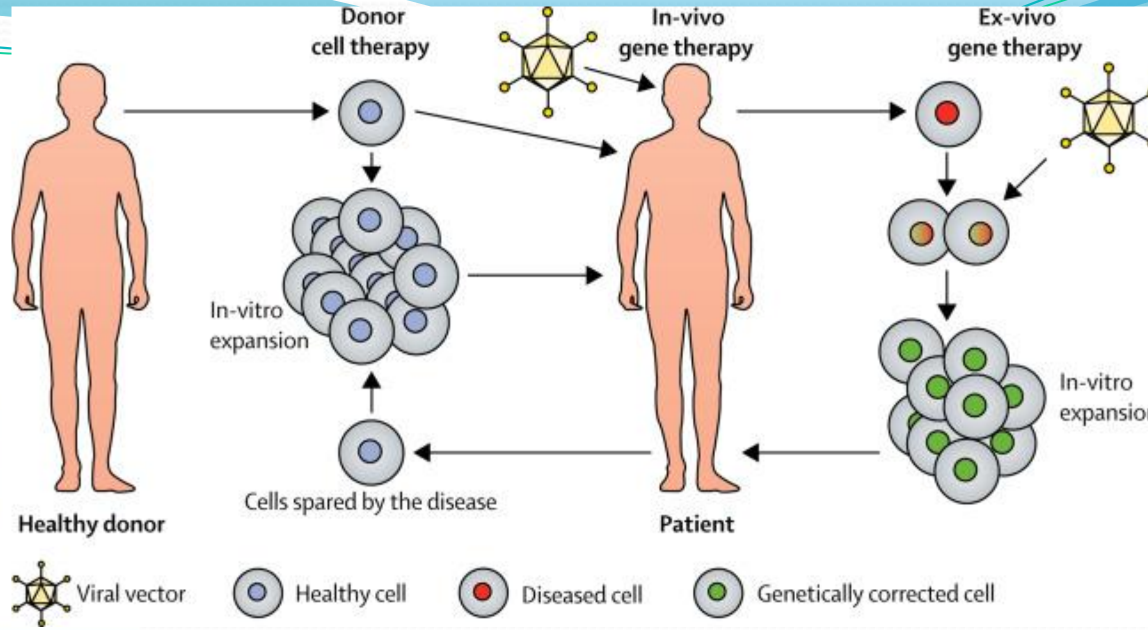


TISSUE ENGINEERING

Tissue engineering is a process wherein fibroblasts are extracted from a patient and then used to artificially grow cells and tissues in a lab setting using the principles of the body's own tissue growth.
This is done using "tissue scaffolds" which encourage cells to develop in a certain way, such as influencing them to grow into bio-artificial bone marrow when implanted under a patient's skin. Using tissue engineering, scientists have been able to create a wide variety of artificial organs including bladders, pancreases, livers, and even whole bones.

REGENERATIVE MEDICINE





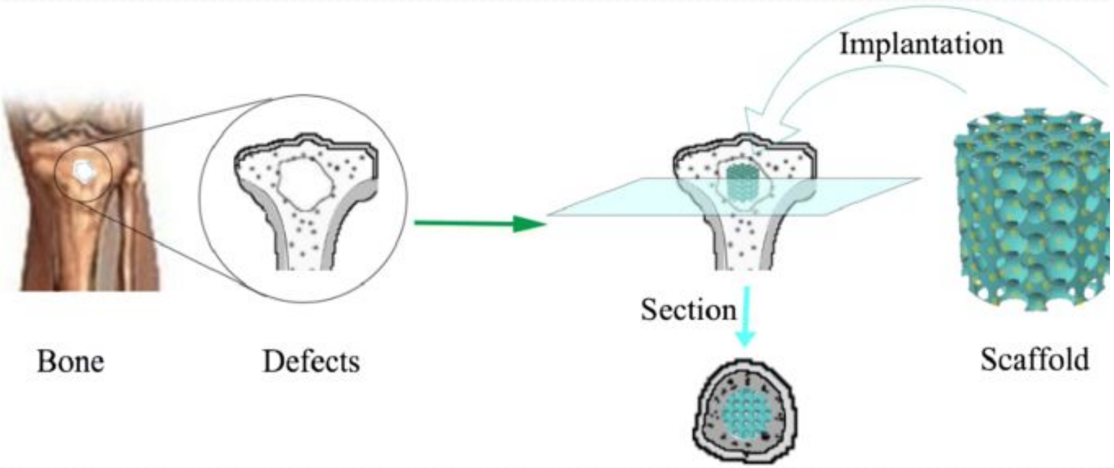
Regenerative medicine, which RegenerVate specializes in, is more focused on using stem cell regenerative therapy to kick start the patient's body's healing processes with their own stem cells, rather than utilizing lab-grown replacements and tissue scaffolds.
This natural process is less about replacing non-functioning tissue by growing new cells, and more about stimulating the body to replace them itself using stem cell therapy and stem cell treatments

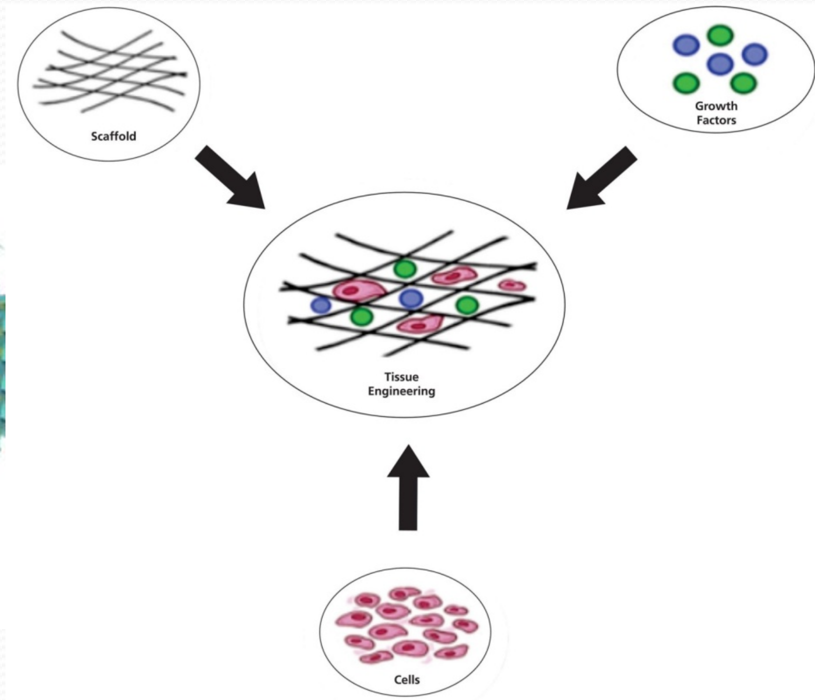
While the body naturally loses its ability to replicate cells and replace tissue as it ages, the introduction of stem cell regenerative therapy and treatment effectively "turns back the clock" on those repair functions, thus helping the body heal as it did in youth

Scaffolds provide the support for cell growth and tissue formation. For that, they are seeded with stem cells.

Growth factors are also included as they regulate the differentiation and proliferation processes.

Bone tissue regeneration is one of the greatest challenges for TERM. The anatomical complexity of bone, allied with the high mechanical stress to which it is exposed, makes it unique, and almost impossible to replicate.





Nanotechnology has made it possible to create structures within the same size as those that constitute naturally occurring bone, opening a new era for TERM.

Hence, nanoparticles (NPs) can be used to modify scaffolds properties, leading to enhanced characteristics such as superior mechanical properties and osteointegration, osteoconduction, and osteoinduction.

Moreover, NPs can be applied to deliver drugs in a controlled and dependent manner, either systemically or locally.

In another approach, NPs can be used to label cells, namely stem cells, enabling the continuous cell tracking and monitoring of its fate.

Antibodies, labeling probes, hydrophobic or hydrophilic molecules, DNA, and/or igonucleotides are some of the molecules that can be linked to NPs, allowing a tailored application for the desired purpose.

Osteoinductive growth factors, in particular recombinant human bone morphogenetic protein-2 (rhBMP-2), have demonstrated remarkable efficacy, but a number of concerns and controversies exist regarding the safety of their clinical use and high cost.

Although numerous synthetic bone graft substitutes are available, the problem of delayed and/or compromised healing remains a significant clinical challenge

The ideal biomaterials for bone regeneration should not only be biocompatible and osteoconductive but also osteoinductive.

They should be able to leverage the self-healing capabilities of the bone by

(i) providing the main structural, compositional, and biochemical cues for the formation of new tissue;

(ii) engaging the host's resident immune cells in the regenerative response;

(iii) promoting the recruitment, proliferation, and differentiation of progenitor cells; and

(iv) recovering an adequate local blood supply to support healing and remodeling

Recently, nanotechnology has become a domain with breakthrough potential to further propel the field of bone regeneration. Nanostructured biomaterials have proven superior at enhancing bone regeneration due to their unique chemical and physical properties (e.g., magnetic, electrical) that are uniquely different from their bulk counterparts

These differences stem from an ability to be engineered to precisely mimic the composition and nanoarchitecture of bone, while allowing for the recapitulation of crucial characteristics of its biochemical milieu at the nanoscale

In the rational design of regenerative nanotechnologies for bone regeneration, **four crucial elements of bone** should be considered and recapitulated as closely as possible:

(i) composition,
(ii) physical stimuli,
(iii) architecture and
(iv) biochemical cues

Mimicking Bone Composition: Bioceramics and Composite Nanostructured Biomaterials

Bioceramics

Bone is a natural nanostructured composite, consisting of approximately 60% (dry weight) mineral, mostly nano-apatite—which is a calcium phosphate (CaP) ceramic.

Accordingly, a number of bioceramics containing calcium and phosphorous have been proposed for bone regeneration

Of these, CaP materials most closely mimic the mineral phase of bone and have demonstrated relatively greater osteoinductivity, making CaP a common material of choice for bone grafts. A number of bioceramics have been used clinically for several decades both for load- and non-load- bearing applications

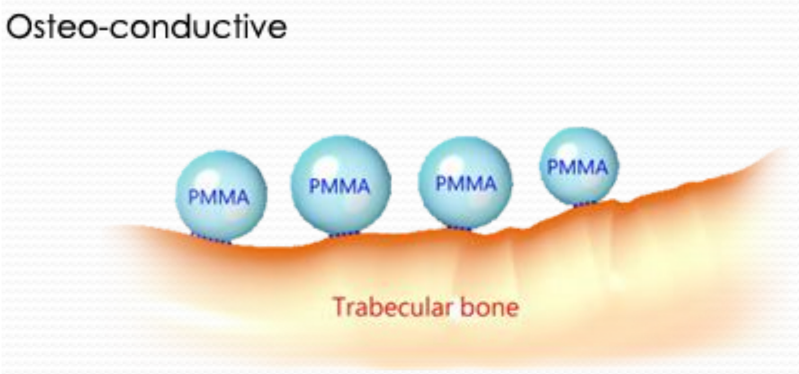
While conventional bioceramics had poor mechanical properties and unfavorable biodegradability and porosity the latest generation of bioceramics are structured at the nanoscale and have significantly improved bioactivity, biodegradation and mechanical properties

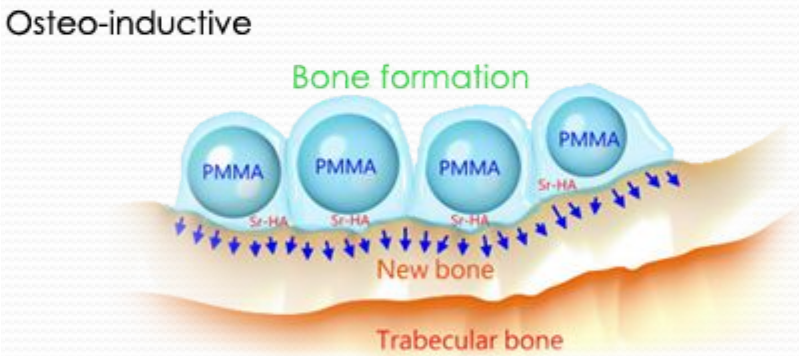
Definition of osteoinduction

- The new formation of bony tissue in a heterotopic locality. The substance inserted releases a sequence of events which leads to the formation of new bone

Definition of osteoconduction

- A " guide-line" effect; i.e. the capacity of a substance to promote the development of bony tissue





Hydroxyapatite-Based Ceramics

Among CaP ceramic phases, synthetic hydroxyapatite (HA) has been the one most extensively studied due to its biocompatibility and resemblance to the composition of natural bone mineral.

First generation materials were fabricated with stoichiometric HA [$Ca_{10}(PO_4)_6(OH)$], which has been successfully synthesized and mass produced through several synthesis strategies, including hydrothermal reactions, sol–gel syntheses, and mechanochemical syntheses

However, natural bone mineral is produced in a very dynamic environment with numerous ions present (e.g., Mg^{2+}, K^+, Na^+, CO2−332-, HPO2−442-), which frequently substitute ions in the apatite lattice. The apatite present in natural bone is calcium deficient and is characterized by a Ca/P ratio lower than the typical 1.67 of stoichiometric HA

Various substituted nanostructured HAs have been proposed, some of which have been used as tools to fine-tune or stimulate specific biological functions. For example, Mg^{2+} plays a vital role in osteogenesis and is present in young and newly formed bone.

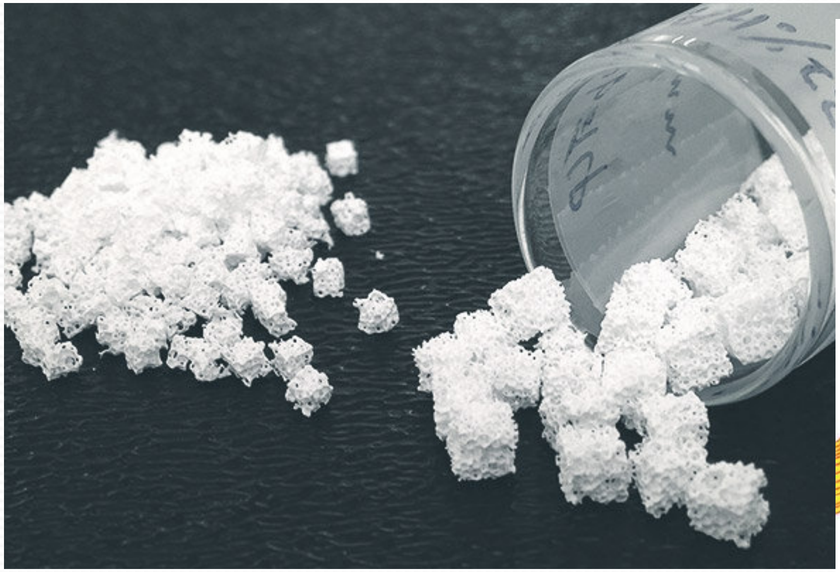
Mg-substituted HA showed enhanced cell adhesion, proliferation, and metabolic activity compared to HA

Sr acts to enhance bone formation *in vivo* by inhibiting osteoclast-mediated bone resorption while upregulating osteoblast activity which is why Sr-based drugs have been long used to treat osteoporosis (e.g., strontium renelate. Thus, Sr-doped nano-HA has also been extensively used in bone regenerative strategies al.
Similarly, substitution with Zn has been shown to enhance osteogenic activity

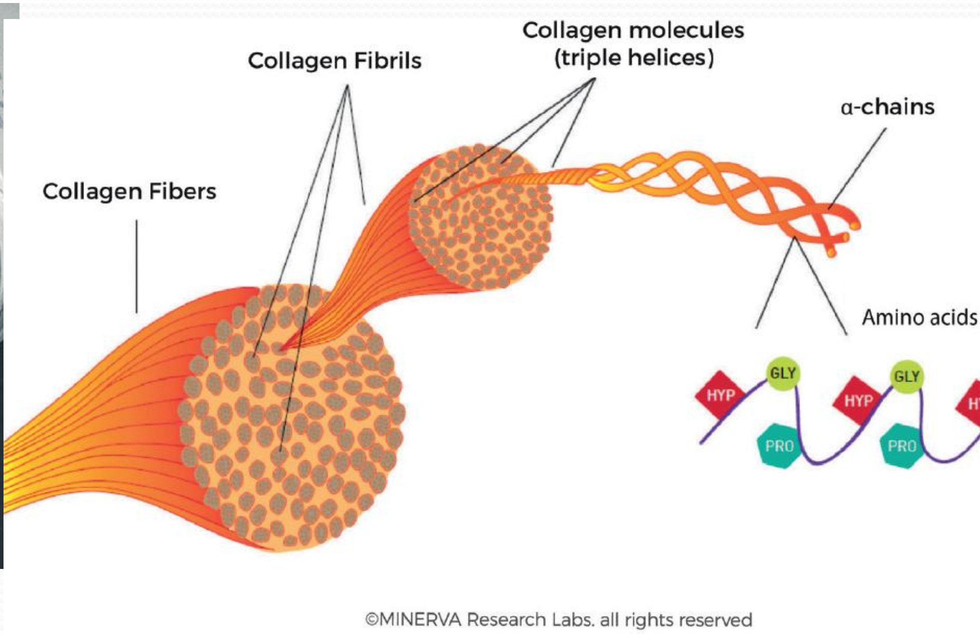
A conceptually new type of nanostructured calcium-deficient HA, by substituting it with Fe^{2+} and Fe^{3+} to endow the HA with superparamagnetic properties. This magnetic behavior may potentially be exploited for bone regeneration purposes to enhance osteogenesis

Nanostructured Composites

Biomimicry is an increasingly popular strategy in regenerative medicine, aiming to engineer materials that closely resemble the target tissue. Since bone is a natural composite—made of an inorganic component (mostly multi-substituted HA) and an organic component (mostly type I collagen)—researchers have long focused on developing nanostructured ceramic/polymer composite materials with the purpose of recreating the composition and function of natural bone. Nanostructured composites for bone regeneration leverage the osteoconductivity of synthetic CaP ceramic phases and the unique mechanical properties of polymers



HA



For example, both synthetic polymers like poly(L-lactic acid) (PLLA; poly(e-caprolactone) (poly(lactic-co-glycolic acid) (PLGA; as well as naturally occurring polymers such as gelatin silK, chitosan alginate and collagen have been combined with HA and TCP to fabricate a plethora of composite materials over the past three decades

The major drawback, common to all these approaches in the manufacturing of porous structures is the inability of conventional methods to completely control the architecture of scaffolds, such as pore size and interconnections.

D-printing techniques have received much attention due to the capacity to fabricate specific and complex structures

Nanostructured Bio-Glasses

Bioactive glasses are mainly comprised of calcium oxide, silicate, borate, and phosphorous

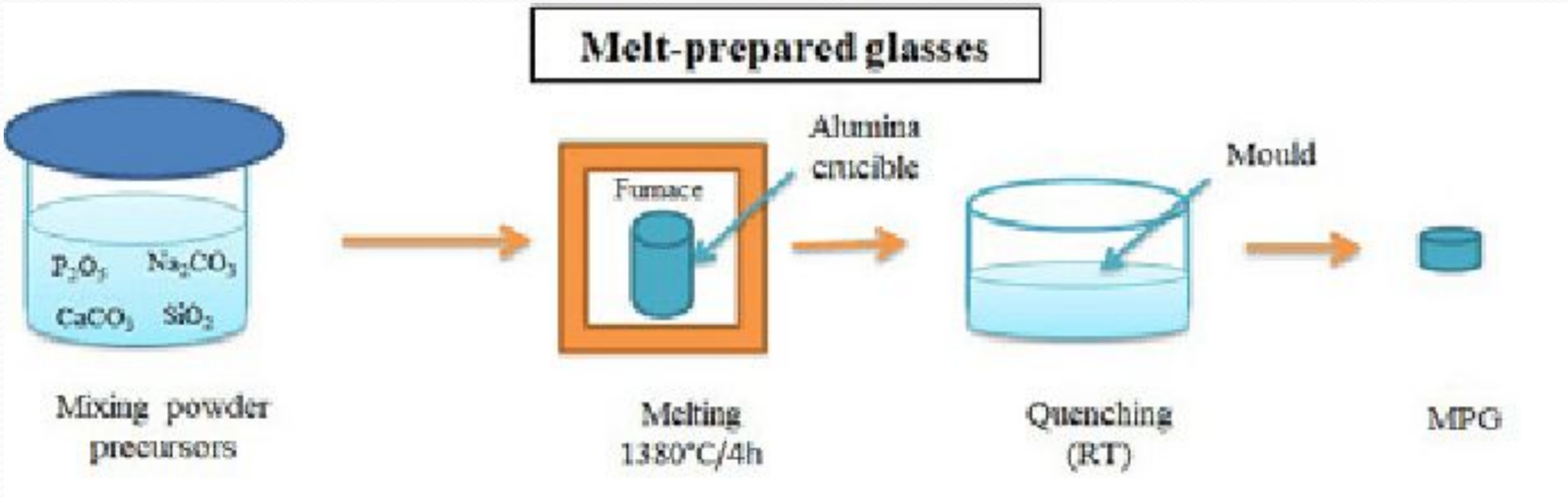
By varying the relative amounts of these components, different bioactive glasses can be manufactured and, over the past three decades, many variants have been proposed for bone regenerative applications

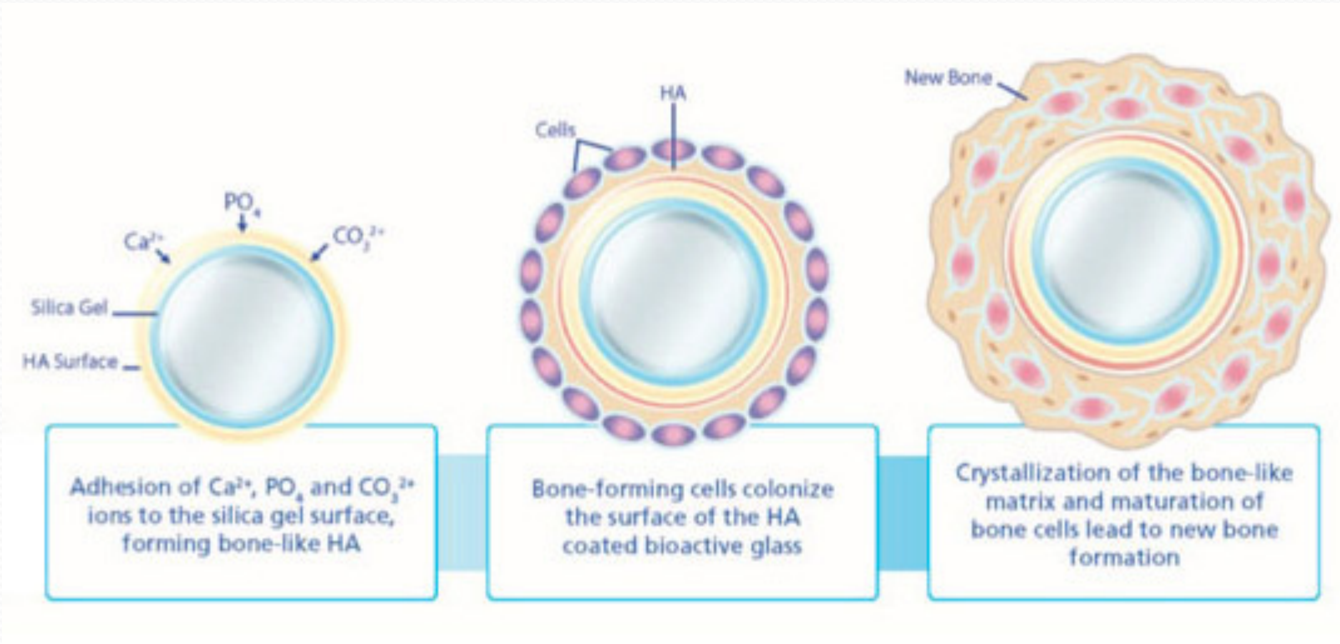
Bioglasses can be prepared by melt–quench or sol–gel process . While the first generations of bioglasses were solid or macroporous, the latest nanostructured versions, synthesized through the sol–gel approach, have unique nanostructural features, including improved nanotextural properties, highly ordered structure, and controlled pore size and pore interconnectivity

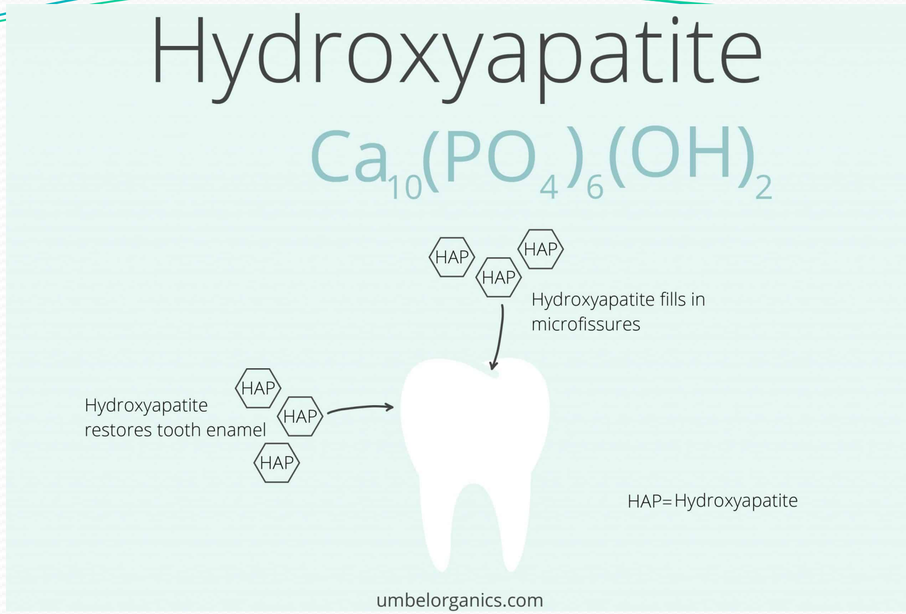
Such nano-features greatly enhance osseointegration compared to first generation bulk bioglasses. The graft-bone integration begins with the solubilization of surface ions resulting in a silica gel layer. A nanostructured calcium phosphate phase (i.e., hydroxyapatite) starts to nucleate on this layer, activating local osteoblasts to form new bone

This mechanism contributes to the nano-bioglass degradation, while promoting bone formation. Even their degradation depends on their composition and nanostructure and can be tailored from days to months; for example, borate-based bioglasses have been shown to degrade much faster than silicate varieties

Recent studies showed that increasing the surface area and porosity of nanostructured bioglasses can greatly accelerate their biodegradation, as well as biointegration


Melt-prepared glasses
P_2O_5
Na_2CO_3
$CaCO_3$
SiO_2
Furnace
Alumina crucible
Mould
Mixing powder precursors
Melting 1380°C/4h
Quenching (RT)
MPG


Ca²⁺
PO₄
CO₃²⁺
Silica Gel
HA Surface
Cells
HA
New Bone
Adhesion of Ca²⁺, PO₄ and CO₃²⁺ ions to the silica gel surface, forming bone-like HA
Bone-forming cells colonize the surface of the HA coated bioactive glass
Crystallization of the bone-like matrix and maturation of bone cells lead to new bone formation


Hydroxyapatite
$Ca_{10}(PO_4)_6(OH)_2$
HAP
HAP
HAP
Hydroxyapatite fills in microfissures
Hydroxyapatite restores tooth enamel
HAP
HAP
HAP
HAP=Hydroxyapatite
umbelorganics.com