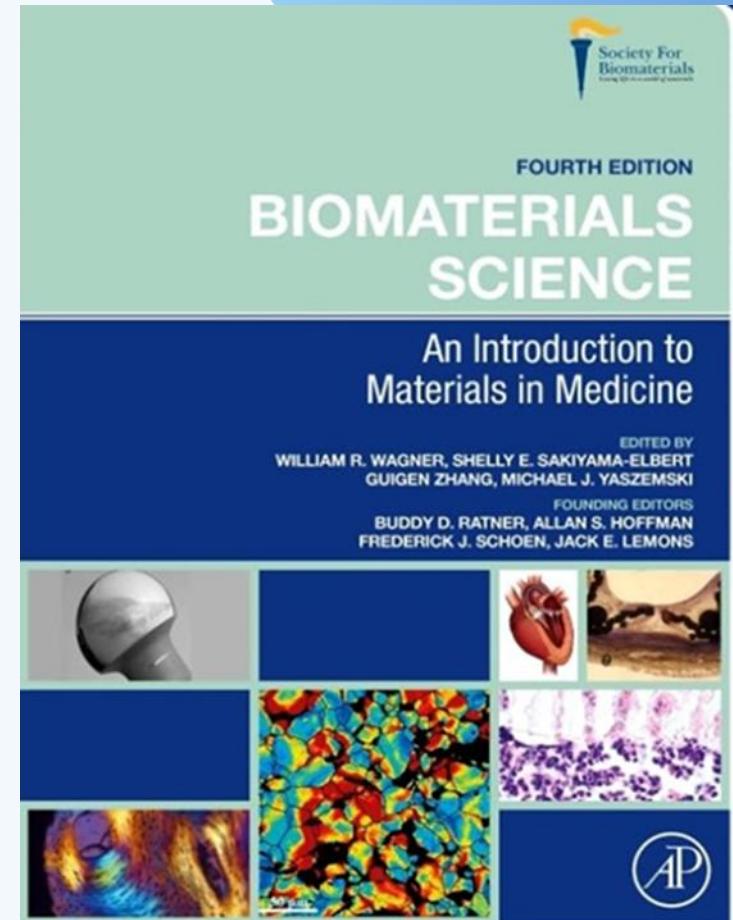


Bone Substitutes in Oral and Maxillofacial Applications; Bone Tissue Engineering

A Comprehensive Overview

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Historical Context: Early Innovations



The investigation into using biocompatible biomaterials for orthopedic and dental applications, particularly as bone graft substitutes, began as early as the 1940s.

Early studies by researchers like Blaine (1946) and Leveen & Barberio (1949) laid the groundwork for modern tissue engineering.

The 1970s: A Paradigm Shift

By the 1970s, research had matured significantly. Scientists and engineers recognized the critical importance of ***three key factors for successful bone regeneration:***

Appropriate Mechanical Properties

The graft must be strong enough to withstand physiological loads without failing, yet not so stiff that it causes stress shielding.

Inter-connected Porosity

A network of connected pores is essential for cells to migrate into the scaffold, and for nutrient and waste transport.

Promotive Microstructure

The scaffold's internal architecture must actively encourage and guide the ingrowth of new bone tissue.

Modern Focus: The Nanoscale Frontier



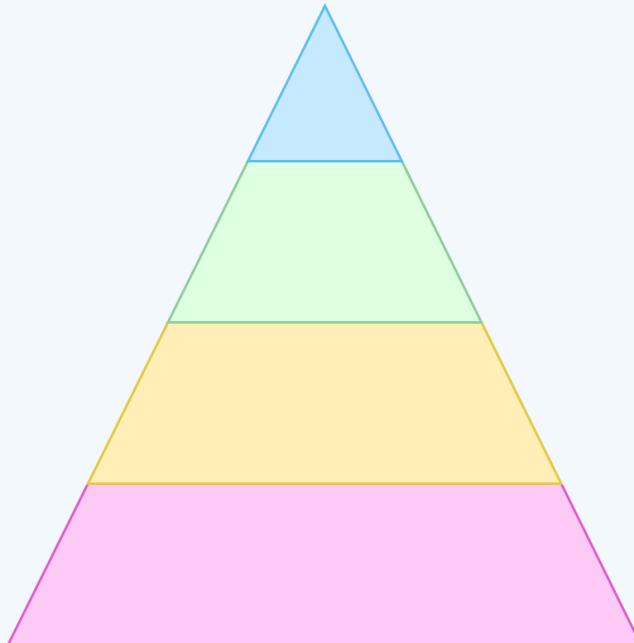
Contemporary research has further refined our understanding, highlighting the necessity for a subcellular dimension, or nanostructure, in synthetic bone grafts.



This nanoscale architecture is important for promoting the appropriate organization of bone cells, which in turn is essential for generating or regenerating bone tissue effectively.

Presentation Roadmap

We will explore the field of bone tissue engineering in a structured manner:

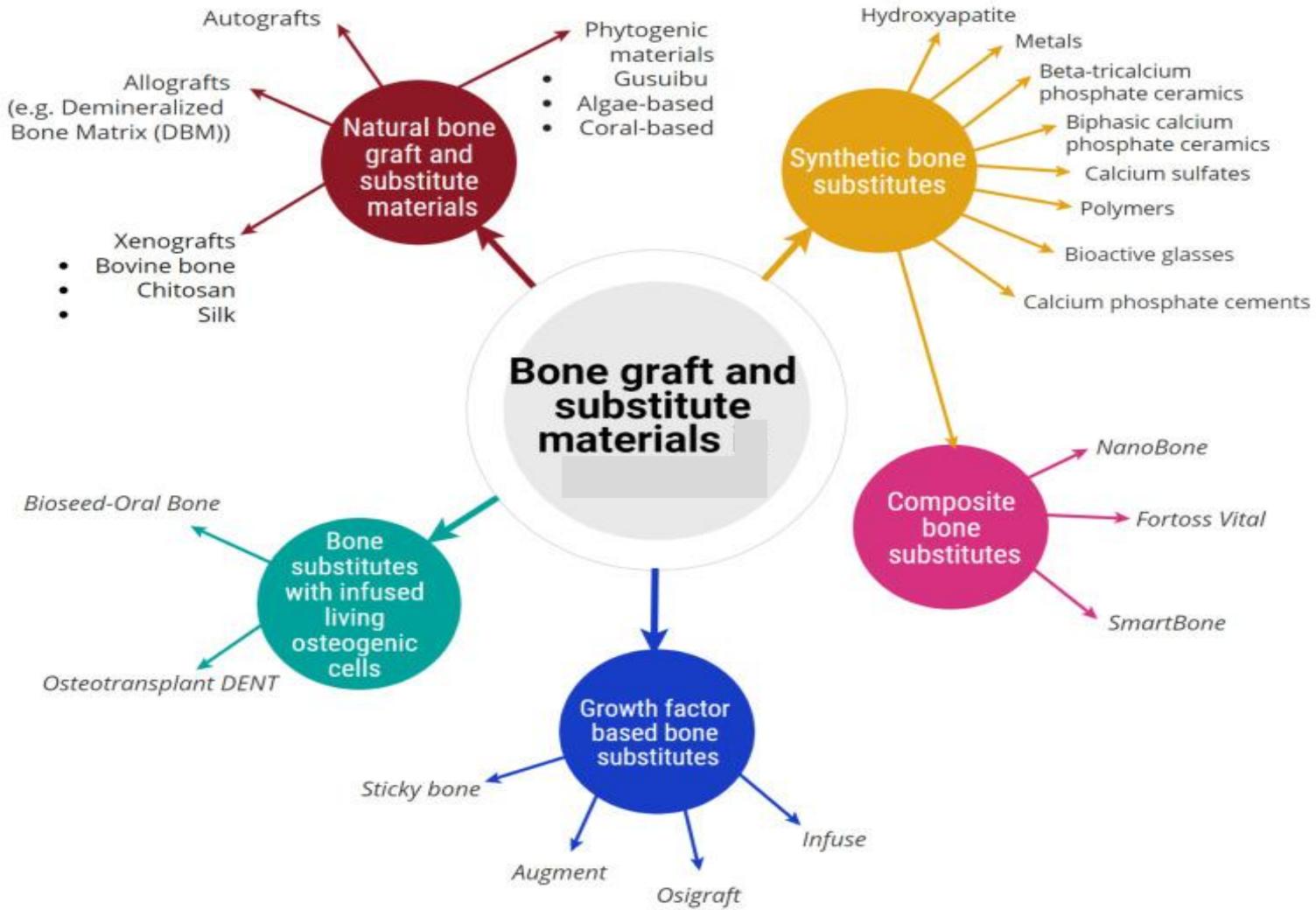


Bioreactors in Bone Tissue Engineering

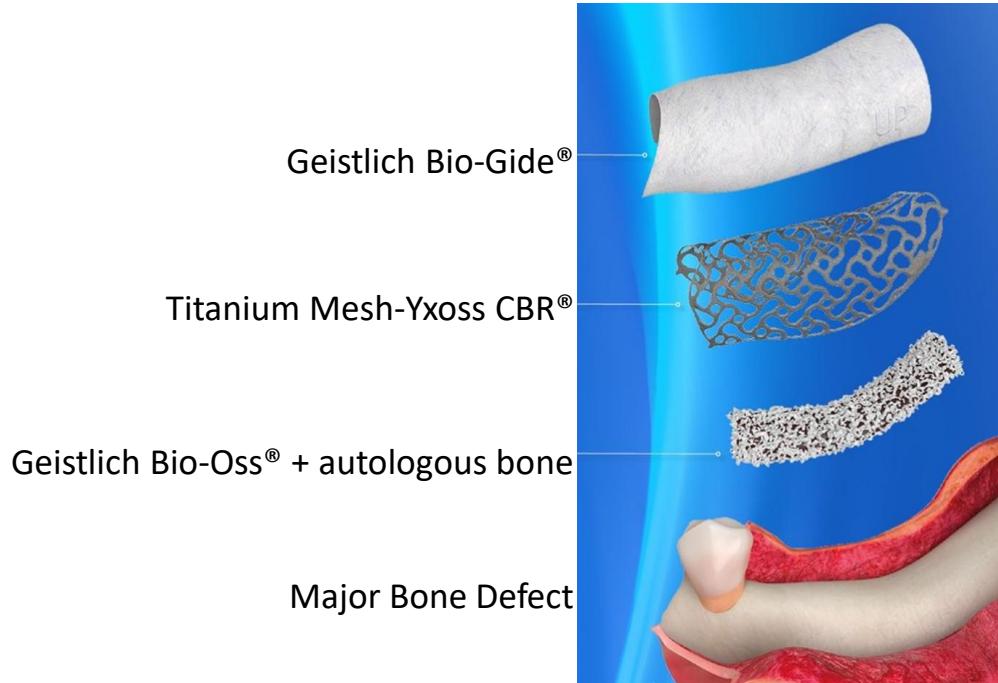
Bone Graft Substitutes (Synthetic & Natural)

Natural Bone Grafts (Autografts & Allografts)

The Biology of Bone: Structure, Cells, and Development



Major Bone Regeneration



Part 1: Bone Biology



Understanding the Blueprint for Regeneration

We begin with a ***foundational understanding of bone biology*** to establish the framework for what biomaterials aim to recreate.

The Bone Organ: A Living System

Bones are not static structures; they are ***dynamic, vascularized, and innervated organs.*** Each bone is a complex system composed of multiple components working in living bodies.



Bone Tissue

The rigid, calcified matrix that provides structural support.

Bone Marrow

The soft tissue within the bone cavity responsible for producing blood cells.

Periosteum

A dense layer of connective tissue that envelops the bones, crucial for growth and repair.

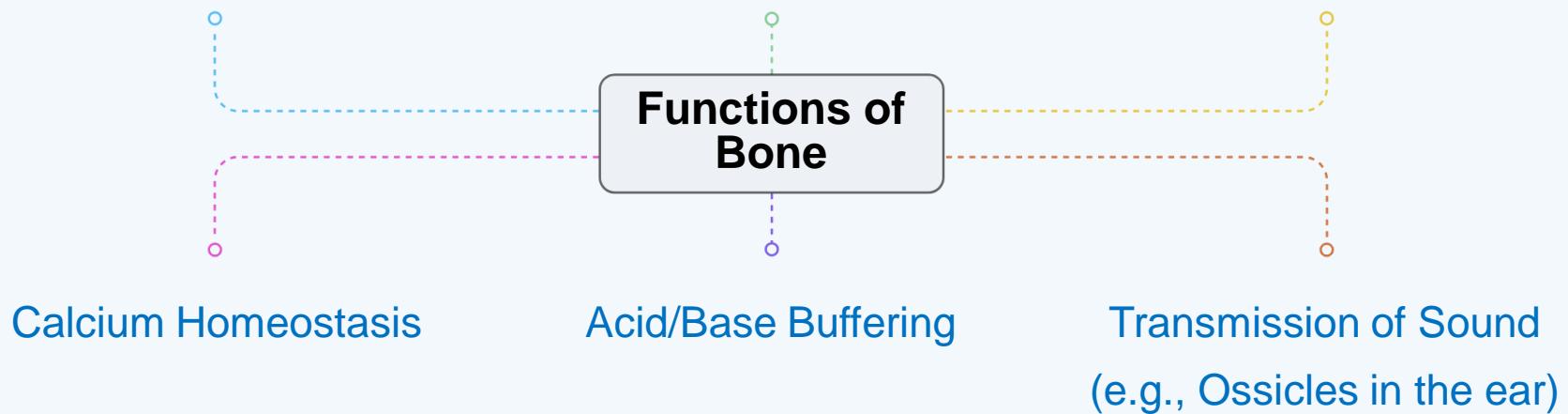
The Multifaceted Functions of Bone

Bone serves a wide range of critical physiological functions beyond simple support.

Mechanical Support for
Muscles

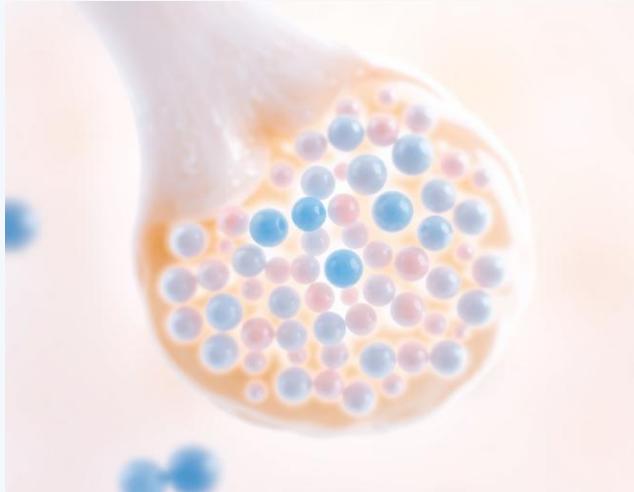
Protection of Internal
Organs

Production of Blood
(Hematopoiesis)



Focus on Bone Tissue

Bone tissue is the rigid, calcified portion of the bone organ.



It is the primary material responsible for the mechanical and structural functions of the skeleton.

Its unique composition and structure are what tissue engineers strive to replicate.

Bone Tissue Types: A Tale of Two Structures

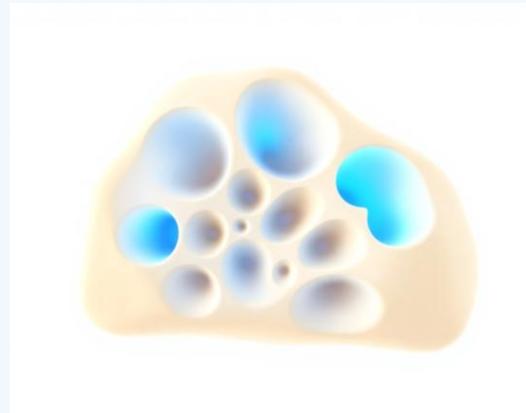
Bone tissue is broadly classified into two main types of structures, each with distinct structures, properties, and functions.

Cortical Bone



Dense and compact, found on the outer surface of bones.

Trabecular Bone



Porous and sponge-like, found on the interior, adjacent to the marrow cavity.

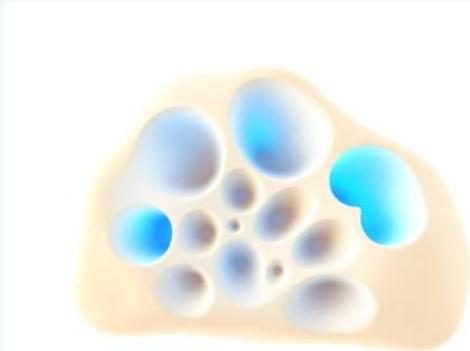
Cortical Bone: The Structural Powerhouse

- **Location:** Forms the dense, protective outer layer of bones.
- **Composition:** Highly mineralized, accounting for 80-90% of its volume.
- **Mass:** Constitutes approximately 80% of the total bone mass in the adult skeleton.
- **Primary Function:** Its high density and strength make it ideal for providing mechanical support and protection.
- **Adaptation:** Its thickness and density can be influenced by mechanical loading, though other variables also play a role.

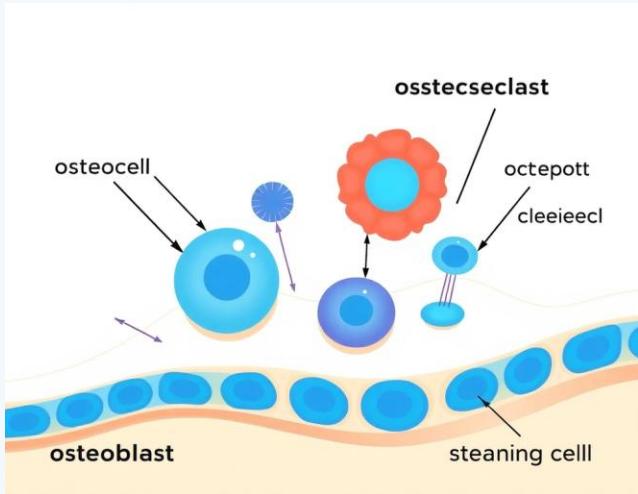


Trabecular Bone: The Metabolic Hub

- **Location:** Found on the interior of bones, particularly at the ends of long bones and within vertebrae.
- **Structure:** Characterized by a high porosity of approximately 80%, giving it a sponge-like appearance.
- **Mechanical Properties:** Exhibits less than 10% of the compressive strength and less than 5% of the compressive modulus of cortical bone.
- **Surface Area:** Despite lower density, it has a much higher surface area than cortical bone.
- **Primary Function:** More critical for metabolic functions like calcium homeostasis and acid/base regulation due to its high surface area and proximity to bone marrow.



Part 2: The Cells of Bone



The Architects and Laborers of the Skeleton

Several distinct cell types are involved in the continuous process of bone formation and remodeling.

Each has a specialized role.

The Cellular Cast

1

Osteoblasts

The '**builders**', responsible for synthesizing new bone matrix.

2

Bone Lining Cells

Quiescent cells that cover bone surfaces and regulate remodeling.

3

Osteocytes

The 'supervisors' embedded within the bone, sensing mechanical loads and orchestrating cell activity.

4

Osteoclasts

The '**demolition crew**', responsible for resorbing old or damaged bone.

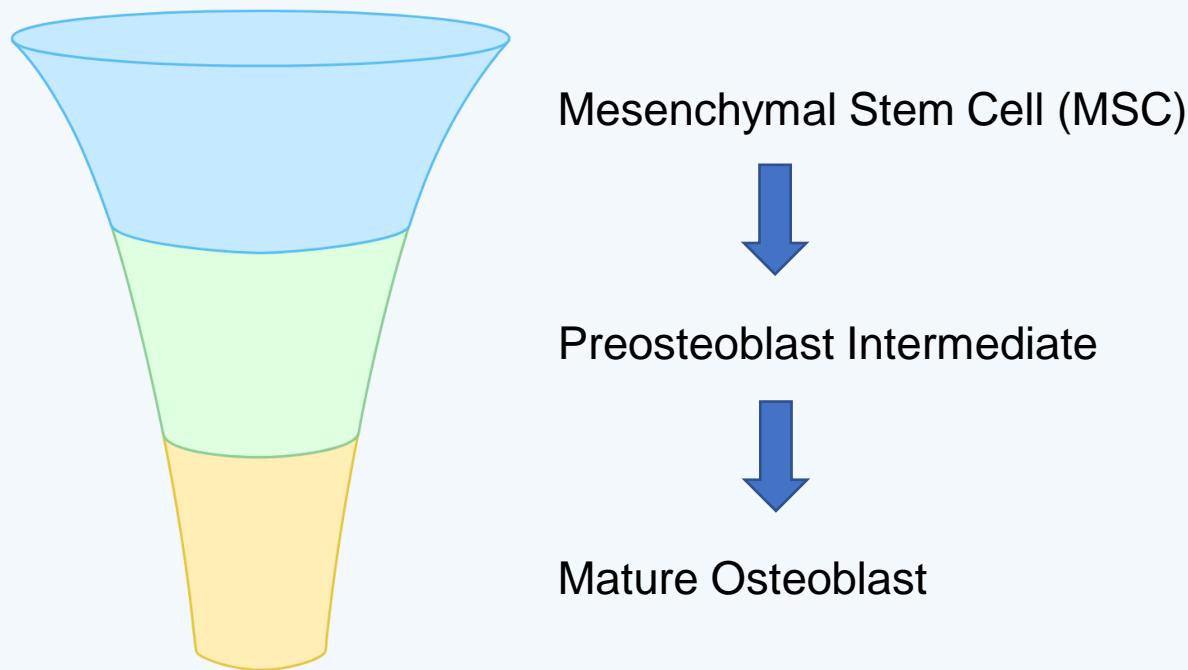
1. Osteoblasts: The Workhorses of Bone Formation

Osteoblasts are the primary cells responsible for **synthesizing** and **depositing new bone tissue**. They are found at the surface of developing bone.

- **Origin:** Fully differentiated cells derived from mesenchymal stem cells (MSCs).
- **Morphology:** Exhibit a characteristic cuboidal shape when active.
- **Location:** Positioned on the surface of the bone matrix.
- **Function:** Actively secrete the organic component of bone, known as the osteoid matrix.

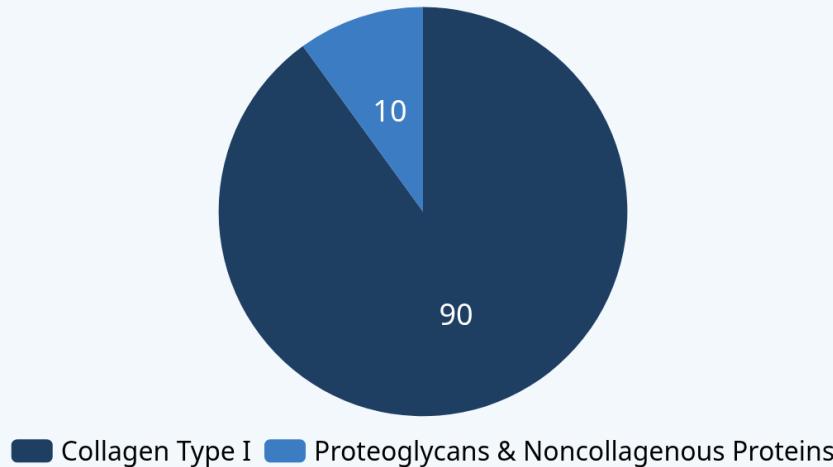
The Osteoblast Lineage

The journey from a multipotent stem cell to a specialized bone-forming cell is a regulated process.



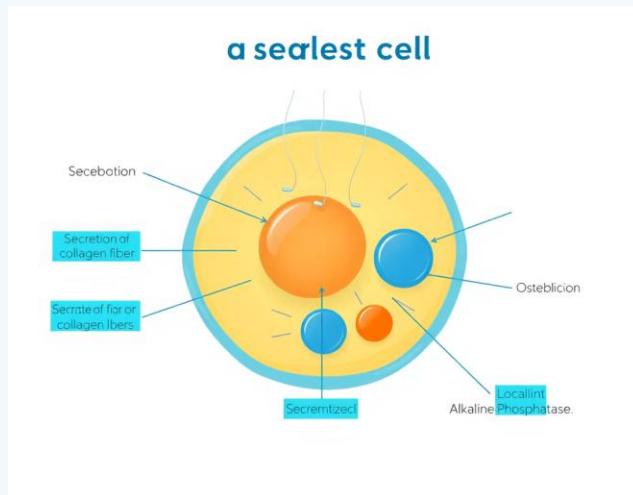
The Osteoid Matrix: Blueprint of Bone

The unmineralized osteoid matrix secreted by osteoblasts forms the organic scaffold of bone.



The noncollagenous proteins include osteopontin, osteocalcin, and osteonectin, which play roles in mineralization and cell binding.

Identifying the Osteoblast Phenotype



The distinct characteristics, or phenotype, of an active osteoblast are defined by its function and molecular markers.

- Active production of the osteoid matrix.
- The presence of a key membrane protein: Alkaline Phosphatase (ALP). ALP is crucial for the subsequent mineralization process.

The Fate of an Osteoblast

Once an osteoblast has completed its task of laying down osteoid, it faces one of three fates as the matrix around it begins to calcify:

Become an Osteocyte

Approximately 20% of osteoblasts become entrapped within the newly mineralized matrix and differentiate into osteocytes.

Become a Bone Lining Cell

A portion of the remaining osteoblasts flatten and become quiescent bone lining cells on the bone surface.

Undergo Apoptosis

The majority of osteoblasts that are not incorporated into the matrix or converted to lining cells undergo programmed cell death.

2. Bone Lining Cells: The Silent Sentinels

These cells form a thin, protective layer on the surface of resting bone. Initially thought to be precursors to osteoblasts, they are now understood to be derived from them.

Morphology

Unlike the cuboidal osteoblasts, bone lining cells have a long, slender, and flat morphology, perfectly suited to cover a surface.

Origin

Current opinion holds that they are osteoblasts that have completed their matrix-secreting function and did not undergo apoptosis or become osteocytes.

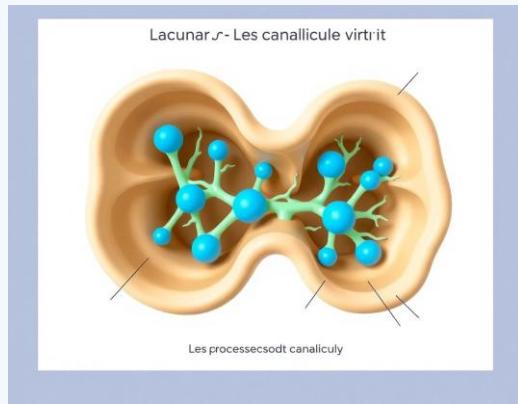
3.Osteocytes: The Master Regulators

Osteocytes are terminally differentiated cells that were once mature osteoblasts. They become encased within the calcified matrix they helped create.

- **Phenotypic Shift:** During transformation, they cease production of proteins like type I collagen, alkaline phosphatase, and osteocalcin.
- **Network Formation:** They create a vast, interconnected network by extending many long processes to adjacent osteocytes.
- **Communication Hub:** This network is used for nutrient/waste transfer and cell-to-cell communication via gap junctions.

The Lacunar-Canalicular Network

This intricate network is the life-support and communication system for osteocytes embedded deep within the hard bone matrix.



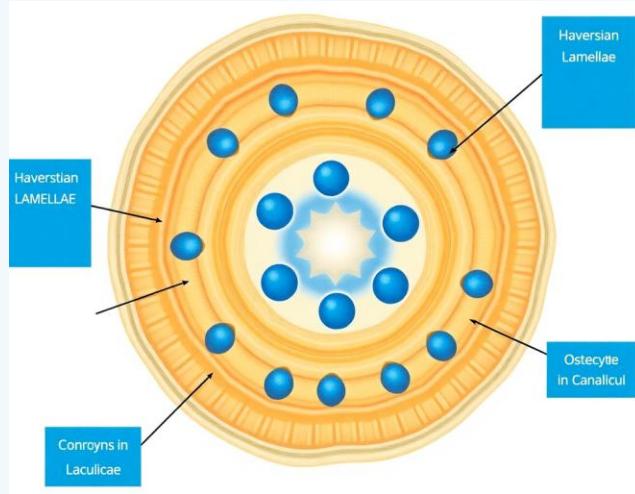
Lacuna

The small cavity or 'lake' where the main body of the osteocyte resides.

Canaliculi

The tiny channels or 'canals' through which the osteocyte's processes extend to connect with other cells and blood vessels.

The Osteon: A Functional Unit



The fundamental functional unit of compact bone is the osteon (or Haversian system). It is a complex, concentric arrangement of the lacunar-canalicular network around a central canal.

- **Central Element:** The Haversian canal, which contains blood vessels and nerves.
- **Concentric Lamellae:** Layers of mineralized matrix arranged around the canal.
- **Embedded Osteocytes:** Osteocytes are situated in lacunae at the boundaries of the lamellae.

Osteocytes as Mechanosensors

Osteocytes are widely implicated as the primary mechanosensors in bone, detecting mechanical forces and translating them into biochemical signals (a process called mechanotransduction).

Mechanism of Action

When bone is loaded (e.g., during exercise), it creates pressure gradients between lacunae. This causes interstitial fluid to flow within the canaliculi, creating shear stress on the osteocyte processes. This mechanical stimulus is believed to initiate the signaling cascade.

Outcome

The mechanotransduction in osteocytes contributes to the recruitment of osteoblasts (to build more bone in response to high load) or osteoclasts (to remove bone in response to disuse).

4. Osteoclasts: *The Bone Resorbers*

Osteoclasts are responsible for the **breakdown** and **resorption of bone matrix**. This process is essential for remodeling, repair, and calcium release.

- **Origin:** Unlike the other bone cells, osteoclasts are derived from hematopoietic stem cells (the same lineage as blood cells), not mesenchymal stem cells.
- **Structure:** They are large, multinucleated cells, formed by the fusion of several precursor cells.
- **Activation:** Their differentiation is induced in response to signaling from the osteocyte-bone lining cell network.

The Process of Bone Resorption

Bone resorption is a two-step process orchestrated by the osteoclast.

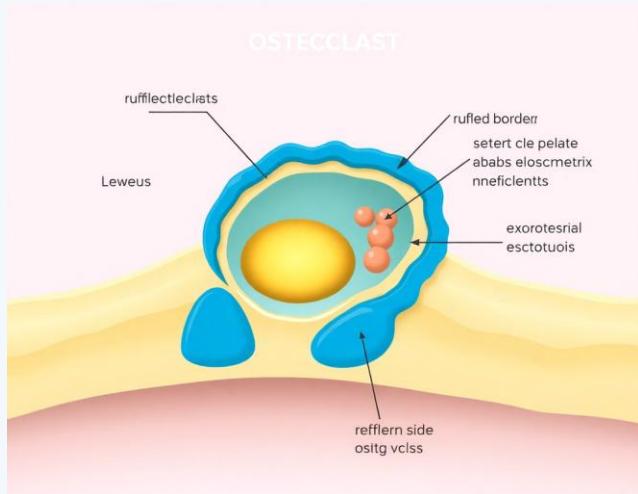
Step 1: Mineral Dissolution

The osteoclast attaches to the bone surface, forming a sealed compartment. It then secretes hydrochloric acid (HCl) into this space, which dissolves the mineralized portion (calcium phosphate) of the matrix.

Step 2: Matrix Degradation

After the mineral is removed, the osteoclast releases proteolytic enzymes (like cathepsin K) that digest the organic protein portion of the matrix, primarily collagen.

Waste Removal by Osteoclasts



The resulting matrix fragments and ions (calcium, phosphate) created from the dissolution are not simply released. They are taken up by the osteoclast through endocytosis.

These materials are transported through the osteoclast in vesicles and then emptied into the extracellular space on the basolateral side (the side away from the bone), allowing them to enter the bloodstream.

Summary of Bone Cell Lineages and Functions

Cell Type	Origin	Primary Function
Osteoblast	Mesenchymal Stem Cell	Bone Formation (Synthesizes osteoid)
Osteocyte	Osteoblast	Mechanosensing and Regulation
Bone Lining Cell	Osteoblast	Surface Maintenance and Remodeling Initiation
Osteoclast	Hematopoietic Stem Cell	Bone Resorption (Breaks down matrix)

Geistlich Bone Substitutes

a stable scaffold for new bone formation.



Geistlich Bio-Oss®



Geistlich Bio-Oss® Collagen



Geistlich Bio-Oss® Pen



Geistlich Membranes

for graft protection[®] and support of the final outcome.



Geistlich Bio-Gide®



Geistlich Bio-Gide® Compressed



Geistlich Bio-Gide® Perio



Geistlich Bio-Gide® Shape



Geistlich Matrices

as the alternatives to autologous grafts.



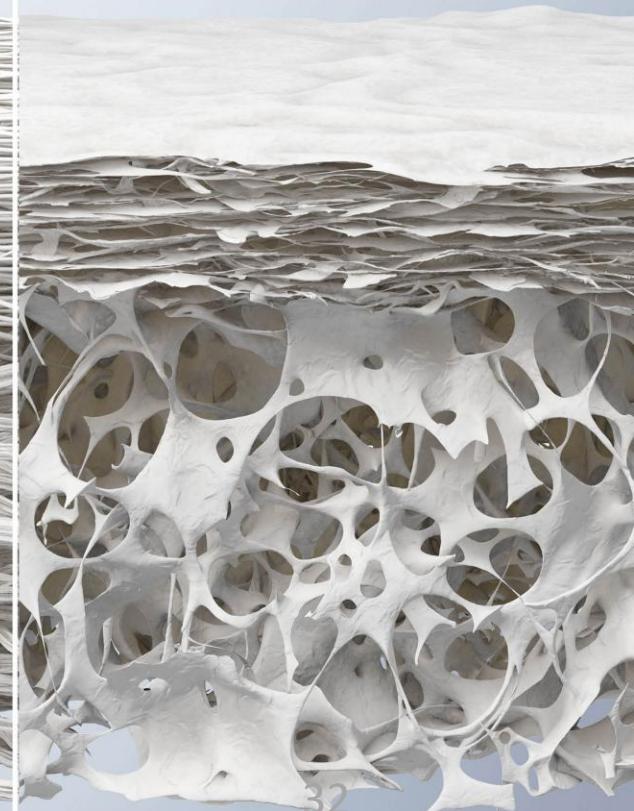
Geistlich Mucograft®



Geistlich Mucograft® Seal



Geistlich Fibro-Gide®



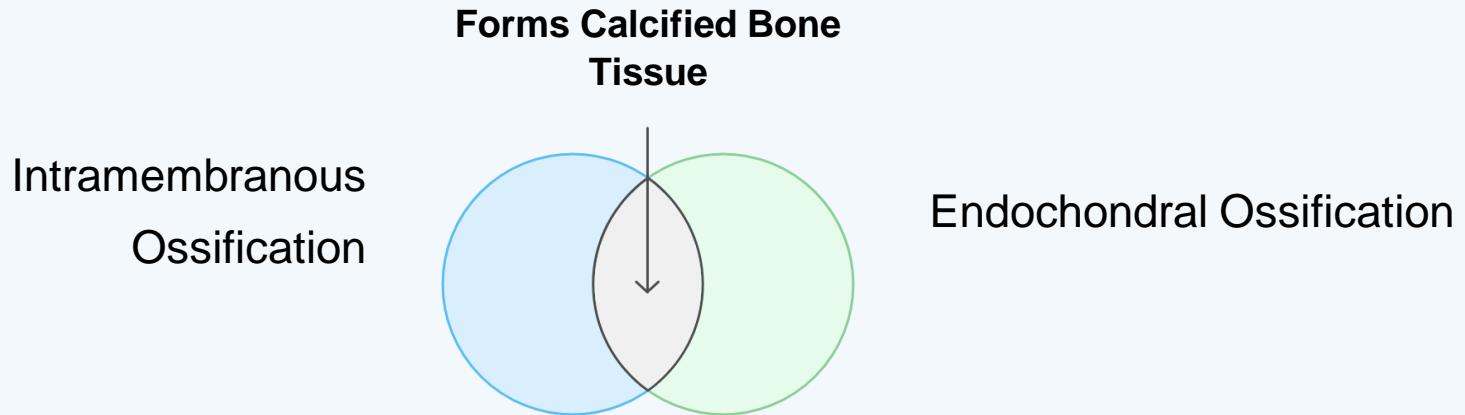
Part 3: Bone Tissue Development



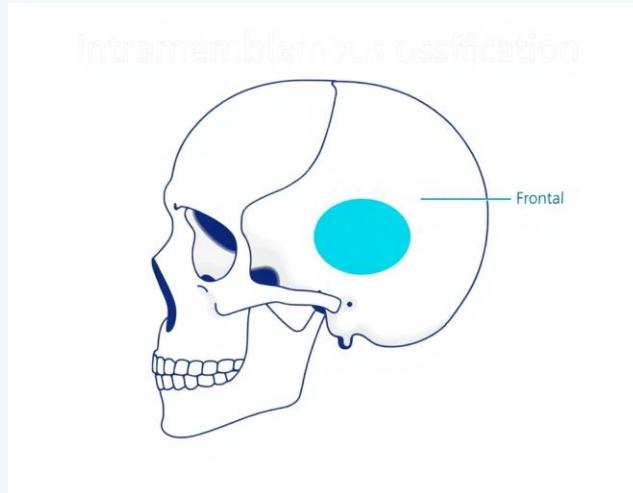
From Blueprint to Structure

Ossification: The Process of Bone Formation

Calcified bone tissue is formed by two distinct modes of ossification (calcification). The method used depends on the type of bone being formed.



Mode 1: Intramembranous Ossification



This method is involved in the formation of flat and irregularly shaped bones, such as the bones of the cranium.

It is characterized by the direct formation of bone without a pre-existing cartilage model.

Mode 2: Endochondral Ossification

Hutmaefodayss



This method is involved in the formation of long bones—bones that are longer than they are wide, such as the femur, humerus, and metacarpals.

It occurs in several steps, beginning with a pre-existing cartilage template.

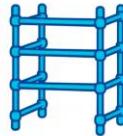
Part 4: The Bone Tissue Engineering Paradigm



Defining the Ideal Bone Graft

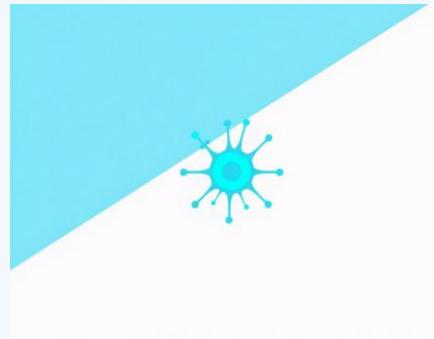
The Tissue Engineering Triad

Strategies for bone tissue engineering often focus on the synergistic combination of three key elements:



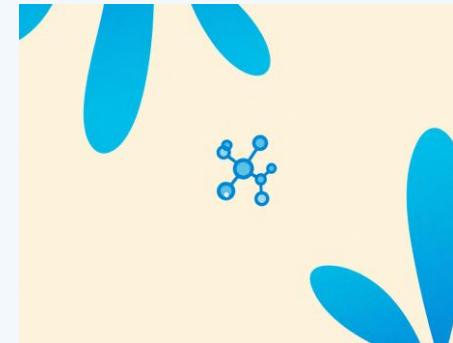
Biomaterials

Scaffolds that provide a temporary structure for tissue growth.



Stem Cells

The cellular building blocks that will form the new tissue.

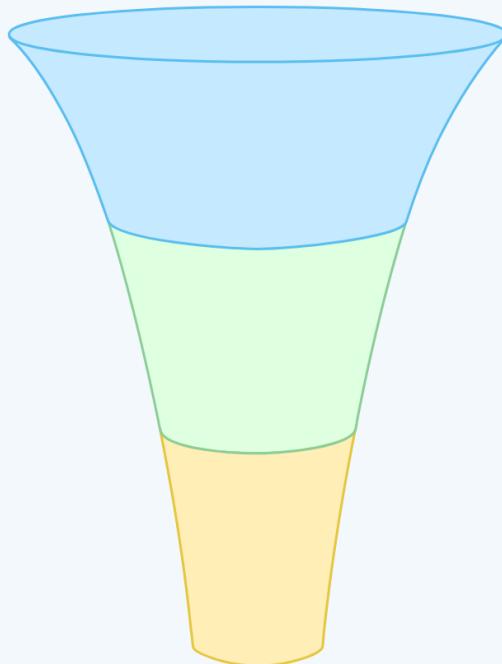


Soluble Factors

Biochemical signals that direct cell behavior and differentiation.

The Biomaterial Scaffold Approach

A common tissue-engineering strategy involves fabricating biomaterials into porous scaffolds. These scaffolds are designed to facilitate cell growth and guide the eventual repair, restoration, or regeneration of the target tissue. Their application can range in complexity.



Level 3: Develop a fully functional replacement tissue *in vitro* for implantation.

Level 2: Culture the scaffold seeded with cells *in vitro* before implantation.

Level 1: Use the biomaterial scaffold alone *in vivo*, relying on host cells to populate it.

The Paradigm for a Successful Bone Graft

Applying these principles, a paradigm emerges for the ideal bone graft or construct.

It should possess five key characteristics to successfully integrate and restore function.



Property 1: Osteoconduction

Definition

Osteoconduction refers to the ability of a scaffold or implant to serve as a passive scaffold that promotes the attachment, migration, and proliferation of osteoblasts and their progenitors on its surface and throughout its interior.

Analogy

Think of it as providing the 'trellis' upon which new bone 'vines' can grow. The trellis itself doesn't create the vine, but it provides the necessary support and framework.

Property 2: Osteoinduction

Definition

Osteoinduction is the active process of stimulating undifferentiated mesenchymal stem cells to differentiate down an osteoblastic lineage, ultimately leading to the formation of mineralized tissue. It can also refer to promoting the maturation of early osteoblasts into mature osteocytes.

Analogy

This is like providing the 'seeds' or 'fertilizer' that causes new bone to grow, even in a location where it wouldn't normally form (e.g., in muscle tissue).

Property 3: Osteogenicity

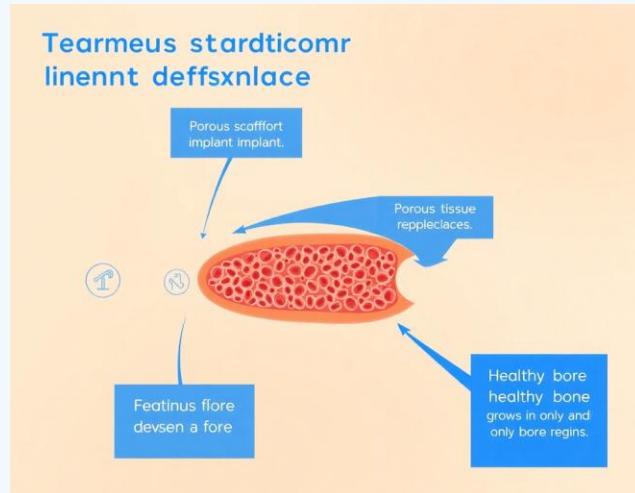
Definition

Osteogenicity refers to the ability of a scaffold or implant to form new bone de novo, entirely from cells contained within the graft itself, without relying on the invasion of host cells. For a scaffold to be osteogenic, it must be seeded with living, bone-forming cells prior to implantation.

Analogy

This is like transplanting a 'fully grown plant' with its own soil and roots. The plant can continue to grow on its own, independent of the surrounding environment.

Property 4: Resorbability / Degradability



The necessity of a bone tissue-engineering construct to be degradable arises from the fact that bone is a living tissue that is constantly remodeling.

A permanent, non-resorbable implant would act as a foreign body, impeding the natural remodeling process and preventing the bone from returning to its normal function and mechanical integrity.

Property 5: Mechanical Properties



The graft, scaffold, or implant should have mechanical properties that closely match those of the native bone tissue at the implant site.

This is crucial for proper load transfer and to avoid complications.

Target Mechanical Properties

The required mechanical properties depend heavily on the location of the implant.

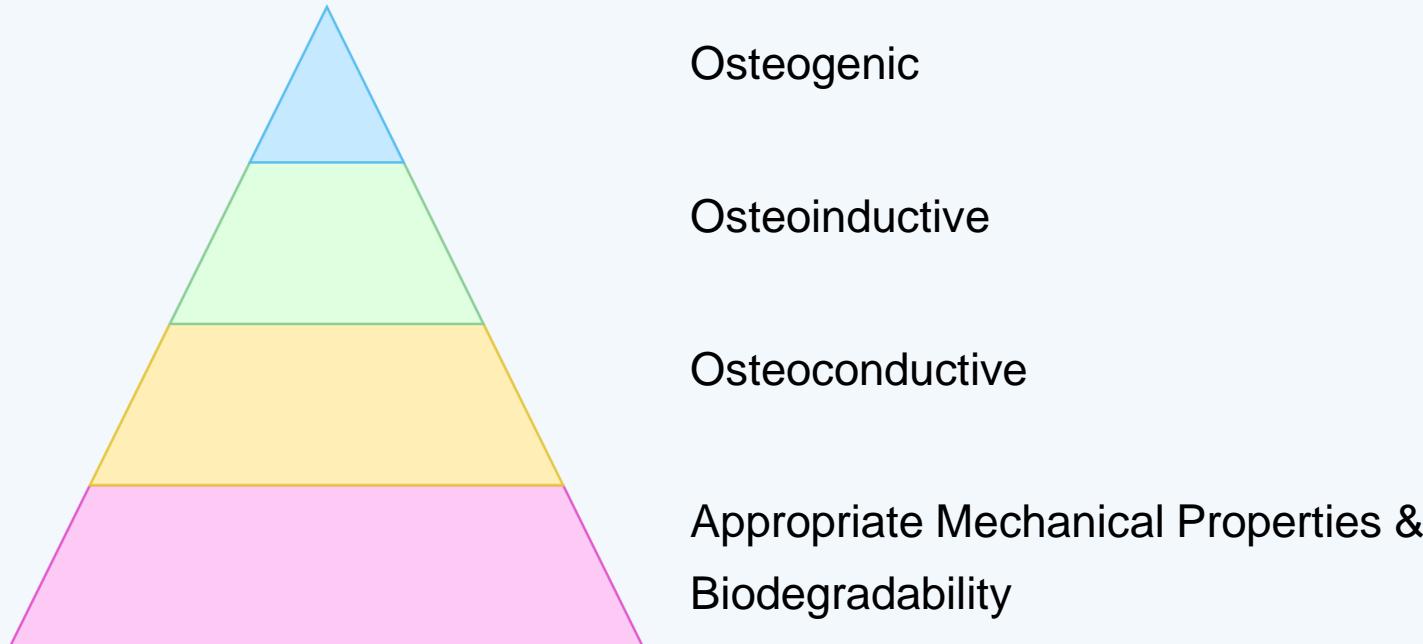
Trabecular Bone

- Compressive Strength: 4 - 12 MPa
- Compressive Modulus: 100 - 500 MPa

Cortical Bone

- Compressive Strength: 130 - 180 MPa
- Compressive Modulus: 12 - 18 GPa

Summary: The Ideal Bone Graft



*An ideal graft combines all these properties
to promote healing and restore the native tissue.*

Part 5: Bone Grafts and Substitutes



Bone graft

From the Gold Standard to Synthetic Solutions

Bone Grafts: The Natural Approach



Bone grafts are pieces of bone that are harvested and placed at a site of bone injury or defect to facilitate healing.

They are sourced either from the patient themselves or from a donor.

Autograft: The "Gold Standard"

An autograft is a section or fragment of bone removed from one site on a patient (typically the iliac crest of the pelvis) and implanted at another site on the same patient.

It is considered the '**gold standard**' because it fulfills all elements of the bone tissue-engineering paradigm.

- Osteoconductive: Provides a natural scaffold.
- Osteoinductive: Contains native growth factors.
- Osteogenic: Contains living bone-forming cells.
- Biocompatible: No risk of immune rejection.

Defect selection

The predictability of regeneration is generally thought to increase as the number of remaining bony walls increase:



THREE
WALL
DEFECTS

TWO
WALL
DEFECTS

ONE
WALL
DEFECTS

The decision to use autogenous

- consideration of the donor site,
- procurement technique and
- handling or processing of the harvested material.

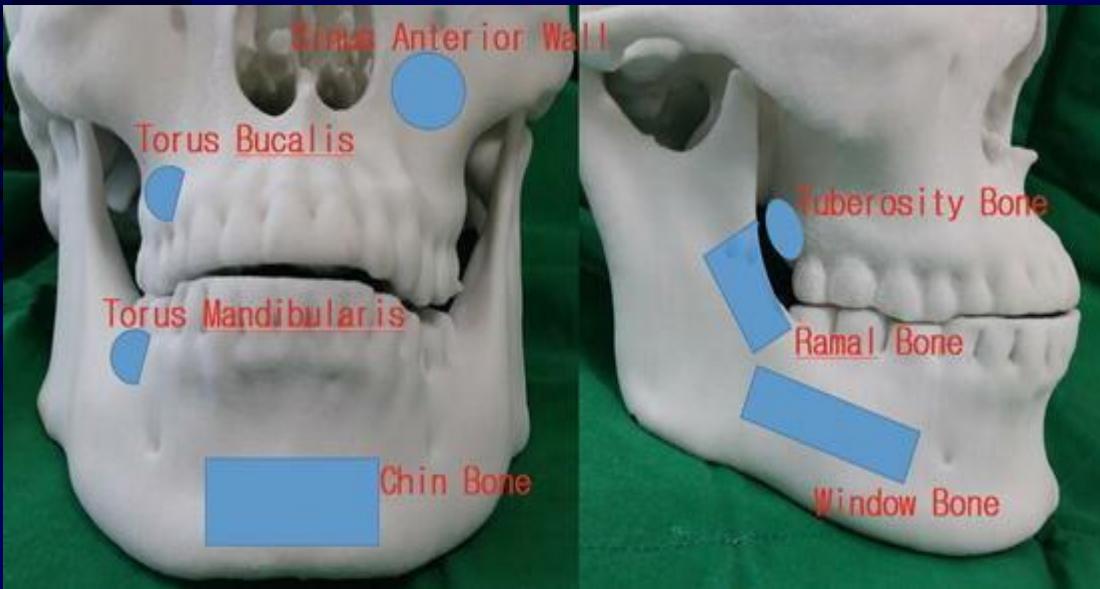
For example, extraoral donor sites permit the harvesting of larger amounts of graft material; however, the scheduling and cost and a second surgical procedure often affects the decision-making process.

CLASSIFICATION

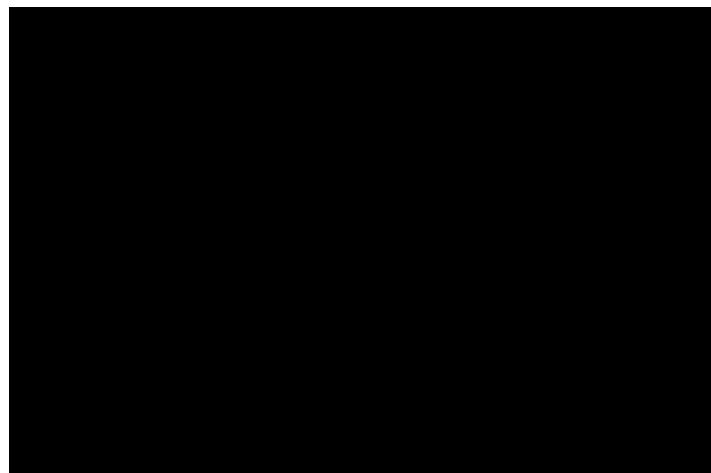
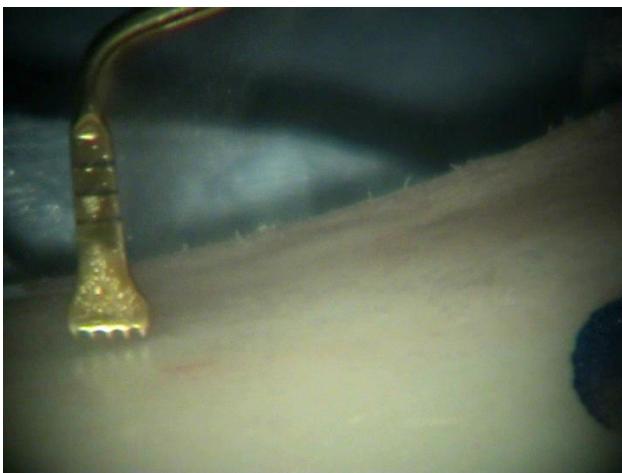
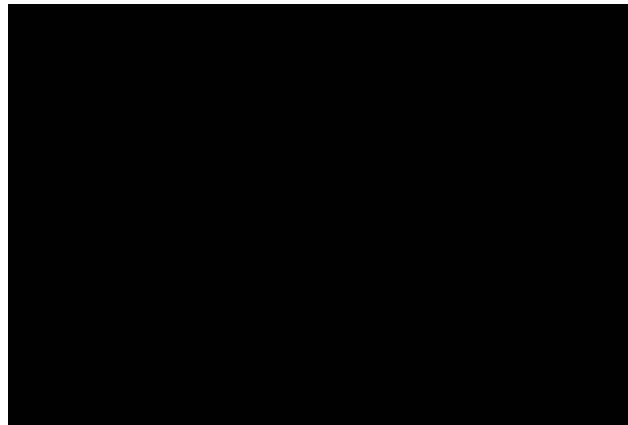
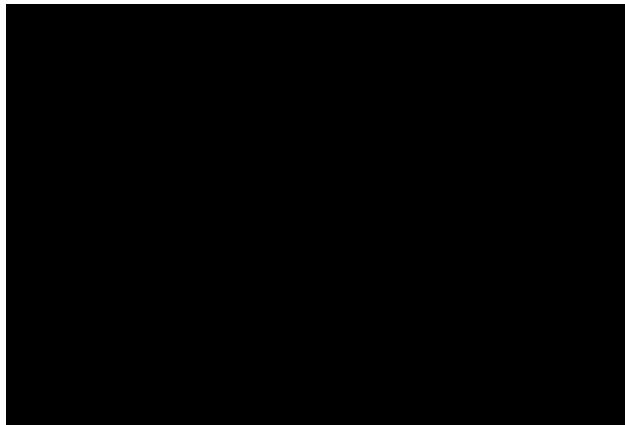
*A. INTRAORAL AUTOGENOUS
BONE GRAFTS*

*B. EXTRAORAL AUTOGENOUS
BONE GRAFTS*

INTRAORAL AUTOGENOUS BONE GRAFTS



- ramus,
- extraction sites,
- edentulous ridges,
- symphysis



EXTRAORAL AUTOGENOUS BONE GRAFTS

- *Iliac crest cortico-cancellous bone graft,*
- *Cranial bone graft,*

ADVANTAGES;

*More amount of graft can be taken,(indications for ridge augmentation and sinus elevations)

DISADVANTAGES;

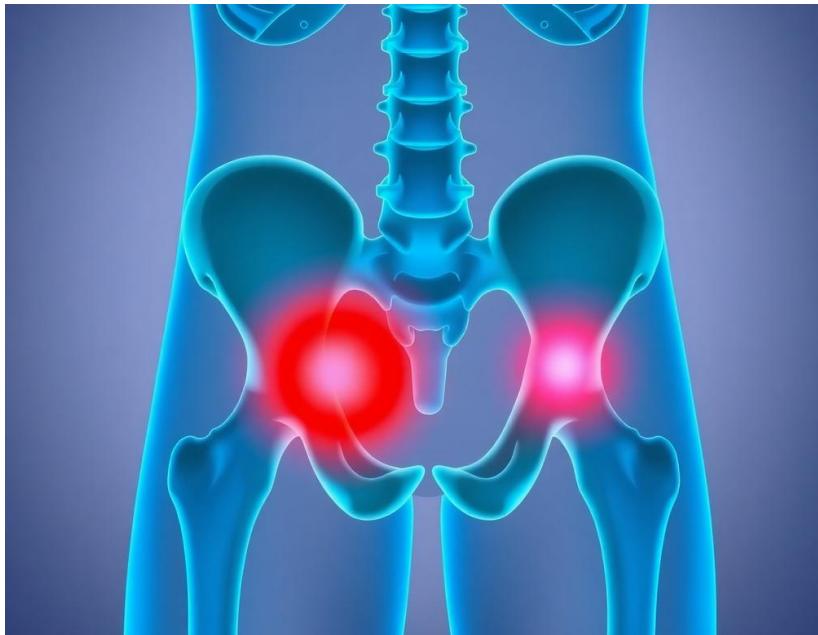
*Hospitalisation needed,

*Post operatuar pain and

*Difficulties in walking(in iliac crest),

*High costs of treatment,

Limitations of Autogenous Grafts



Key Drawbacks

- Limited quantity of available bone for harvest.
- Significant donor-site morbidity, with rates reported as high as 25%.
- Complications include persistent pain, infections, and hematomas.
- Increased anesthesia time and operative blood loss.

Drawbacks of the Autograft

Despite its effectiveness, the autograft is not without significant disadvantages, which drive the search for alternatives.

Donor Site Morbidity

The harvest site can suffer from necrosis, infection, and chronic pain, which may be worse for the patient than the implant site injury.

Limited Availability

The amount of bone that can be safely harvested is finite. This is a major issue for large defects or in certain patient populations.

Problematic Patient Populations

Availability and quality of bone are reduced in osteoporotic, pediatric, or bone cancer patients, who are often in greatest need.

Types of Autogenous Grafts

Autogenous grafts are categorized based on their structure and vascularity, each with distinct biologic activities and mechanical properties.

Property	Cancellous	Nonvascularized Cortical	Vascularized Cortical
Osteoconduction	++++	+	+
Osteoinduction	++	+/-	+/-
Osteoprogenitor cells	+++	-	+
Immediate strength	-	+++	+++
Strength at 6 months	--	++	+++
Strength at 1 year	---	+++	++++

Introduction to Bone Graft Alternatives

The search for alternatives to autogenous bone graft is driven by the need to increase the quantity of available material and decrease the morbidity of the grafting process. This section will discuss FDA-approved substitutes.

Grafting Material	Osteo-conduction	Osteo-induction	Osteoprogenitor Cells	Immuno-genicity	Donor-Site Morbidity	Immediate Torque Strength
Cancellous autograft	++++	++	+++	-	+	-
Cortical autograft	+	+/-	+/-	-	+	++
Fresh allograft	+	+/-	-	++	-	++
Frozen allograft	+	+/-	-	+	-	++
Freeze-dried allograft	+	+/-	-	+/-	-	+
Ceramics	+	-	-	-	-	+/-
Demineralized bone matrix	+	++	-	-	-	-
Bone marrow	-	+/-	++	-	-	-

Allografts: An Overview

Allografts, sourced from cadaveric donors, offer an alternative that eliminates donor-site morbidity and provides a potentially unlimited supply. They are typically processed to reduce immunogenicity.

Fresh

Requires no preservation but offers little time for disease testing. Evokes an intense immune response and has limited applications, such as joint resurfacing.

Frozen

Maintained at < -60°C to diminish enzymatic degradation. This reduces immunogenicity without altering biomechanical properties. Osteoprogenitor cells are destroyed.

Freeze-dried

Lyophilization removes water, allowing room temperature storage. Further decreases antigenicity but can alter biomechanics, reducing hoop and compressive strength on rehydration.

Properties of Allografts

Regardless of the preservation method, allografts share common biological properties:

- ****Osteoprogenitor Cells:**** Destroyed during processing.
- ****Osteoconductive Properties:**** Largely retained due to their cancellous and cortical structure.
- ****Osteoinductive Properties:**** Limited, as the deeply bound growth factors may only be partially retained.
- ****Cartilage Viability:**** For osteochondral allografts, frozen cartilage viability is controversial, with studies showing values from 20% to 70%.



Clinical Applications of Allografts

Non-Structural Uses

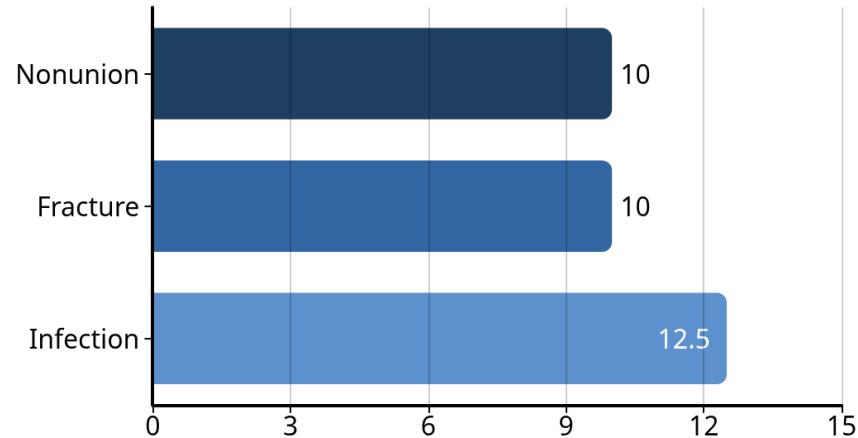
- Reconstructing defects after curettage of benign neoplasms.
- Filling periarticular bone cysts at the time of arthroplasty.
- Morcellated chips used as a filler or extender, often mixed with autogenous tissue or bone marrow to enhance healing properties.

Structural Uses

- Intercalary segments for diaphyseal defects of long bones.
- Arthrodeses of the ankle, hip, and spine.
- Large segments to replace acetabular, femoral, and tibial defects.
- Osteochondral allografts to replace resected bone and provide a biologic joint surface.

Complications and Concerns with Allografts

The use of allografts, particularly large structural segments, is associated with notable complications.



A primary concern remains the transmission of infection, most notably hepatitis and HIV/AIDS. This risk has led to stringent regulation and screening protocols.

Allograft Safety and Regulation

To mitigate the risk of disease transmission, strict standards have been implemented by regulatory bodies.

Governing Standards

- **American Association of Tissue Banks (AATB):** Represents most US tissue banks, establishing comprehensive standards for compliance.
- **Food and Drug Administration (FDA):** As of December 1993, mandated that all national tissue banks comply with governmental regulations paralleling AATB standards.
- **Key Requirements:** These include rigorous donor screening, repeated infectious disease testing, specific labeling, long-term graft tracking, and facility inspections.

Demineralized Bone Matrix (DBM)

DBM is produced by acid extraction of allograft bone, a process that removes the mineral component but leaves the collagen matrix, non-collagenous proteins, and crucial bone growth factors, most notably Bone Morphogenic Protein (BMP).

Properties

- **Primary Feature:** Osteoinductive
- **Secondary Feature:** Osteoconductive
- **Strength:** Offers no structural strength.
- **Forms:** Available as powder, granules, chips, or a moldable gel (e.g., Grafton).

Processing Concerns

The amount of active BMP in DBM is far lower than in recombinant BMP. Furthermore, FDA-required sterilization processes (like gamma radiation) may decrease the viability of the available BMP. Some proprietary processing techniques aim to protect these growth factors.

Clinical Application of DBM

Due to its lack of mechanical strength, DBM has proven most useful as an osteoinductive supplement within well-supported, stable skeletal defects, often in conjunction with internal fixation.



Case Example: Comminuted Femoral Fracture

- **A:** A preoperative radiograph shows a grade II comminuted left femoral fracture.
- **B:** Postoperatively, the fracture is stabilized with a supracondylar plate. A composite graft of DBM gel (Grafton), allograft chips, and autogenous bone marrow has been placed in the defect.

Summary of Alternative Grafts

Each bone graft alternative possesses a unique profile of properties, dictating its specific clinical applications. No single substitute perfectly replicates autogenous bone.

Allografts

Provide structure and osteoconduction, but limited osteoinduction and no osteogenic cells. Risk of disease transmission, though low with modern screening.

Ceramics

Exclusively osteoconductive. Excellent in compression but brittle. Best as a filler or expander in protected environments.

Demineralized Bone Matrix (DBM)

Osteoinductive and osteoconductive, but provides no structural strength. Best used as an adjunct to stimulate bone formation.

Bone Marrow

Solely osteogenic. Provides viable cells to kick-start healing but has no structure. Strictly an adjunct to other materials.

Allograft: The Donor-Based Alternative

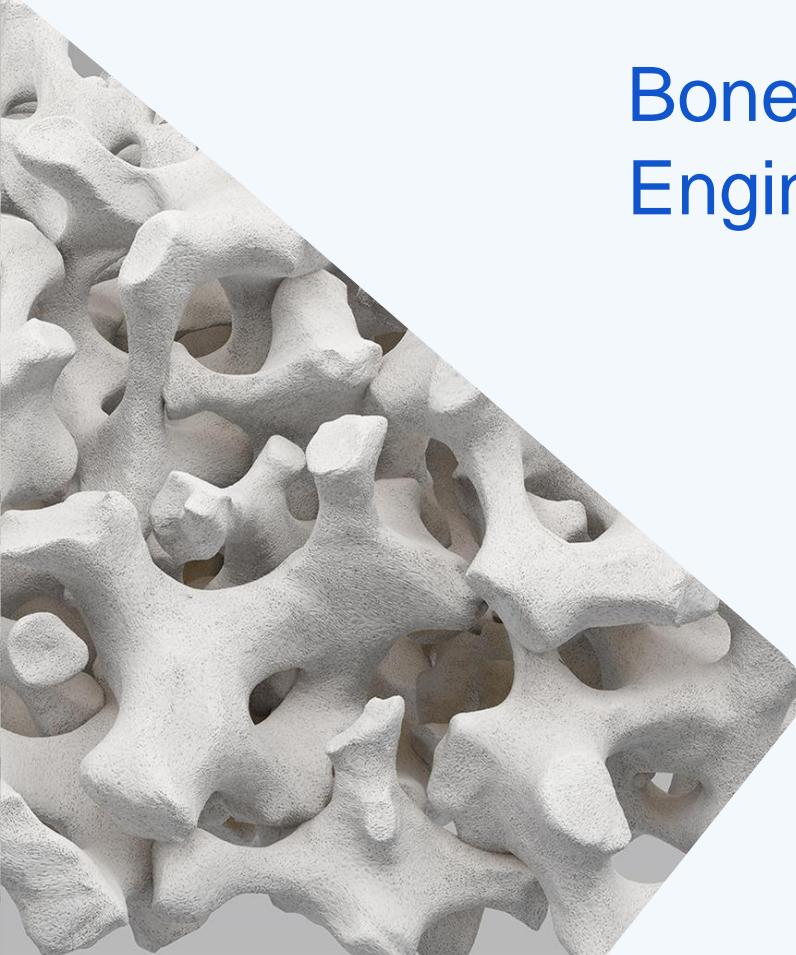
Allografts are bone fragments obtained from donors (typically cadavers). They undergo processing (freezing, irradiation, lyophilization) to reduce the risk of disease transmission and immunogenicity.

Disadvantages

Advantages

- Readily available in various shapes and sizes.
- No donor site morbidity for the patient.

- Sterilization reduces bioactivity (less osteoconductive/inductive).
- Not osteogenic (no living cells).
- Reduced mechanical integrity (if lyophilized).
- Risk of immune response, requiring immunosuppressants.



Bone Graft Substitutes: Engineering a Solution

The complications arising from autografts and allografts have fueled the search for synthetic or processed bone graft substitutes.

These can be classified based on their primary material component: biomaterials, cells, or growth factors.

Substitute Type 1: Allograft-Based (DBM)

Demineralized Bone Matrix (DBM) is created from allograft bone that has been thoroughly sterilized, decellularized, and, most importantly, demineralized. The acid extraction process removes the mineral component, exposing the underlying collagen matrix and entrapped growth factors.

Hypothesis

Demineralization is thought to improve osteoinduction by making soluble factors (like BMPs) more accessible than they would be in mineralized bone.

DBM: Formulations and Properties

DBM is often sold as a putty, gel, or strip, mixed with carriers like glycerol or hyaluronic acid to improve handling characteristics for surgeons.

Performance Profile

- Osteoinductive: Yes (variable, depending on processing).
- Osteoconductive: Limited.
- Osteogenic: No (acellular).
- Mechanical Properties: Negligible; serves as a biological filler, not structural support.

Substitute Type 2: Natural Polymer-Based

Natural polymers are gaining interest for their inherent biocompatibility. The most prevalent natural polymer used for bone graft substitutes is collagen, the main protein in bone itself.

Collagen

Forms the basis for many commercial products, often used as a microfiber matrix or sponge.

Fibrin

A protein involved in blood clotting, can be formed into gels and foams as a temporary scaffold.

Chitosan

A polymer derived from crustacean shells, being investigated for its biocompatible and biodegradable properties.

Natural Polymer Grafts: Performance

These structures, typically fibers or foams, provide excellent osteoconduction. However, they generally lack inherent osteoinductivity and osteogenicity. Their mechanical properties are also typically much lower than those of native bone.

Clinical Application

A common strategy is to soak the collagen sponge or matrix with bone marrow aspirate from the patient just before implantation. This adds progenitor cells, making the composite construct osteogenic.

Substitute Type 3: Synthetic Polymer-Based

The use of synthetic polymers offers significant advantages for tissue engineering, providing precise control over properties that is difficult to achieve with natural materials.

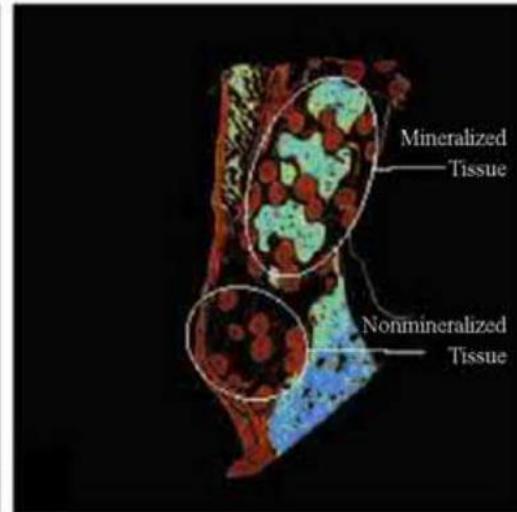
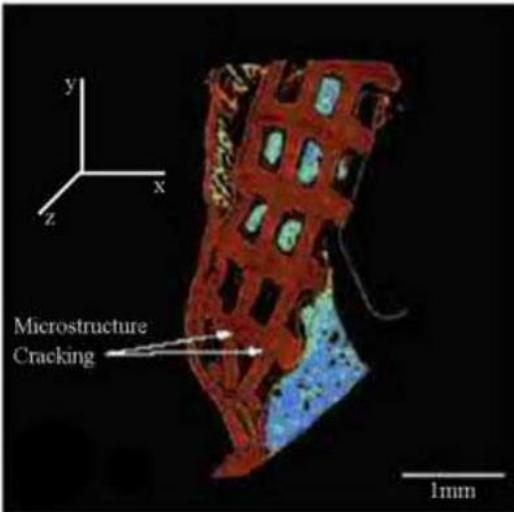
- Control over surface chemistry.
- Tunable degradation kinetics.
- Precise control over geometry and architecture (e.g., via 3D printing).
- Batch-to-batch consistency.

FDA-Approved Synthetic Polymers for Bone

A limited number of synthetic polymers are FDA-approved for non-life-threatening applications like bone graft substitutes.

The most common are the poly(α -hydroxy esters):

- *Poly(lactic acid) / Poly(lactide) (PLA)*
- *Poly(glycolic acid) / Poly(glycolide) (PGA)*
- *Poly(lactic-co-glycolic acid) (PLGA) - A copolymer of PLA and PGA*
- *Poly(caprolactone) (PCL)*



Case Study: PCL Scaffold In Vivo

Micro-CT images of a synthetic bone graft made of polycaprolactone (PCL) after 3 months in a pig model.

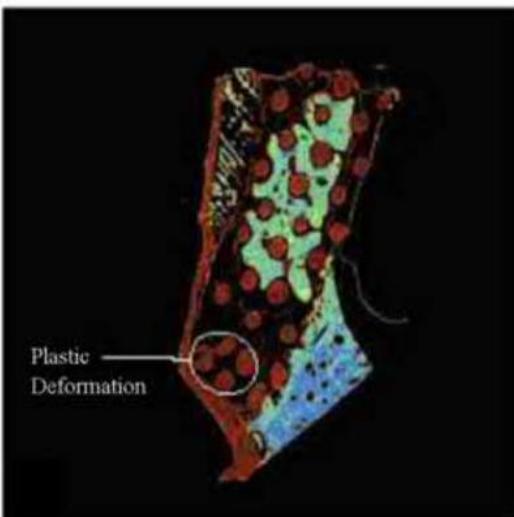


Figure 2.6.6.2:

Red = PCL scaffold.

Blue-green = new mineralized tissue.

Black = nonmineralized tissue. Native bone is in the lower right.

Analysis of PCL Scaffold Images

The images from Figure 2.6.6.2 reveal several key points:

- **Successful Ingrowth:** New mineralized tissue (blue-green) is forming within the pores of the PCL scaffold (red).
- **Osteoconduction:** The scaffold is successfully guiding bone formation into the defect space.
- **Material Degradation:** The authors note the PCL rods have shrunk from 500 to 300 μm in diameter over 3 months.
- **Mechanical Issues:** Microstructure cracking and plastic deformation are visible, indicating the scaffold may have experienced excessive loading or that its mechanical integrity was compromised during degradation.

Fabrication: Porogen Leaching

An early technique for creating porous polymer foams.

A polymer is dissolved in a solvent and mixed with a porogen (a placeholder material like salt, sugar, or gelatin).

After the solvent evaporates, the solid construct is immersed in water to dissolve (leach out) the porogen, leaving behind a porous structure.

Pros

- Can produce highly porous scaffolds.

Cons

- Poor interconnectivity between pores.
- Mechanical integrity decreases sharply with increased porosity.
- Use of organic solvents.

Fabrication: Sintered Microspheres

This technique involves fusing small polymer spheres (microspheres) together by heating them above their glass transition temperature.

The polymer chains at the surface of adjacent spheres entangle, forming a solid, interconnected scaffold upon cooling.

Pros

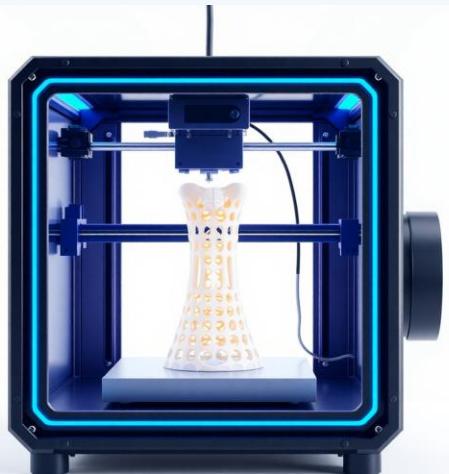
- 100% pore interconnectivity.
- Good reproducibility and control.

Cons

- Maximum porosity is limited to ~45% (based on sphere packing).

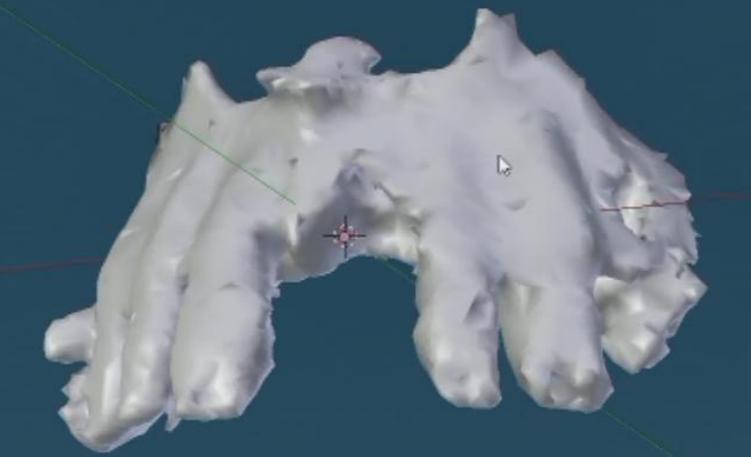
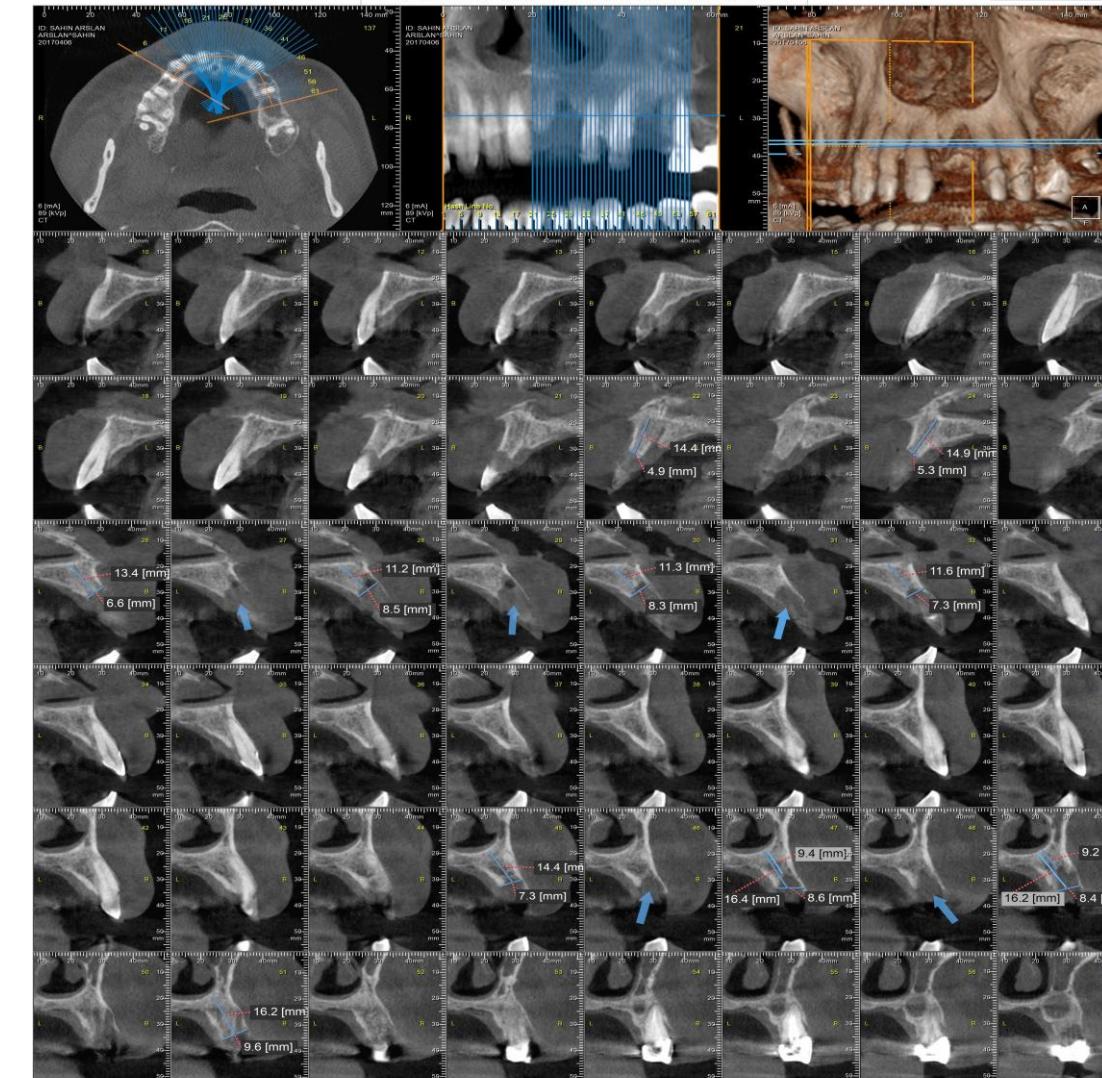
Fabrication: Solid Freeform Fabrication (3D Printing)

Also known as additive manufacturing, this uses computer-aided design (CAD) to build scaffolds layer-by-layer. Techniques include stereolithography, selective laser sintering (SLS), and fused deposition modeling (FDM/3D printing).



Advantage

Allows for the creation of scaffolds with highly complex and precisely controlled architectures, tailored to specific anatomical defects.



▼ Model Preparation

Delete Selection

Remove non-linked

Fill Mesh

Deselect all

Restart

Next

▼ Help & Tips

In order to 3D model on be more fluid at your computer you have to delete all the parts that isn't important to your goal.

You have to select the area that won't be a part of your model and then delete.

A better way is using lasso selection: you select the area where you want to separate the working model and the part that you want to delete.

After that you delete that line and place the cursor over your working model and then you use the command delete and done.

Verify if something else needs to be deleted and after you delete everything that you don't need you have to close the mesh to turn the model editable.

Demo

▼ Tailoredimplant.com



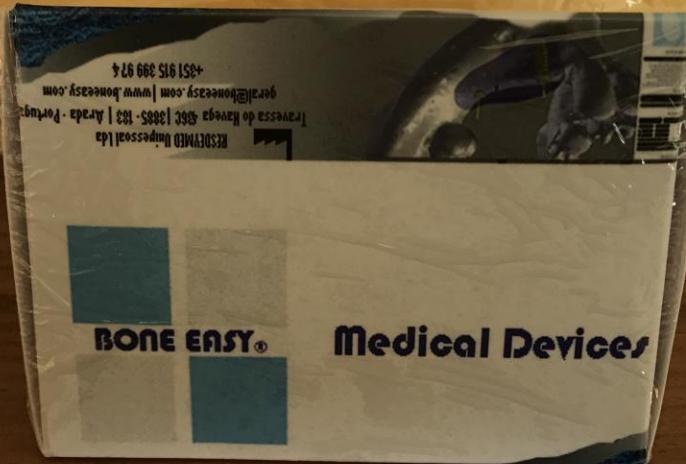
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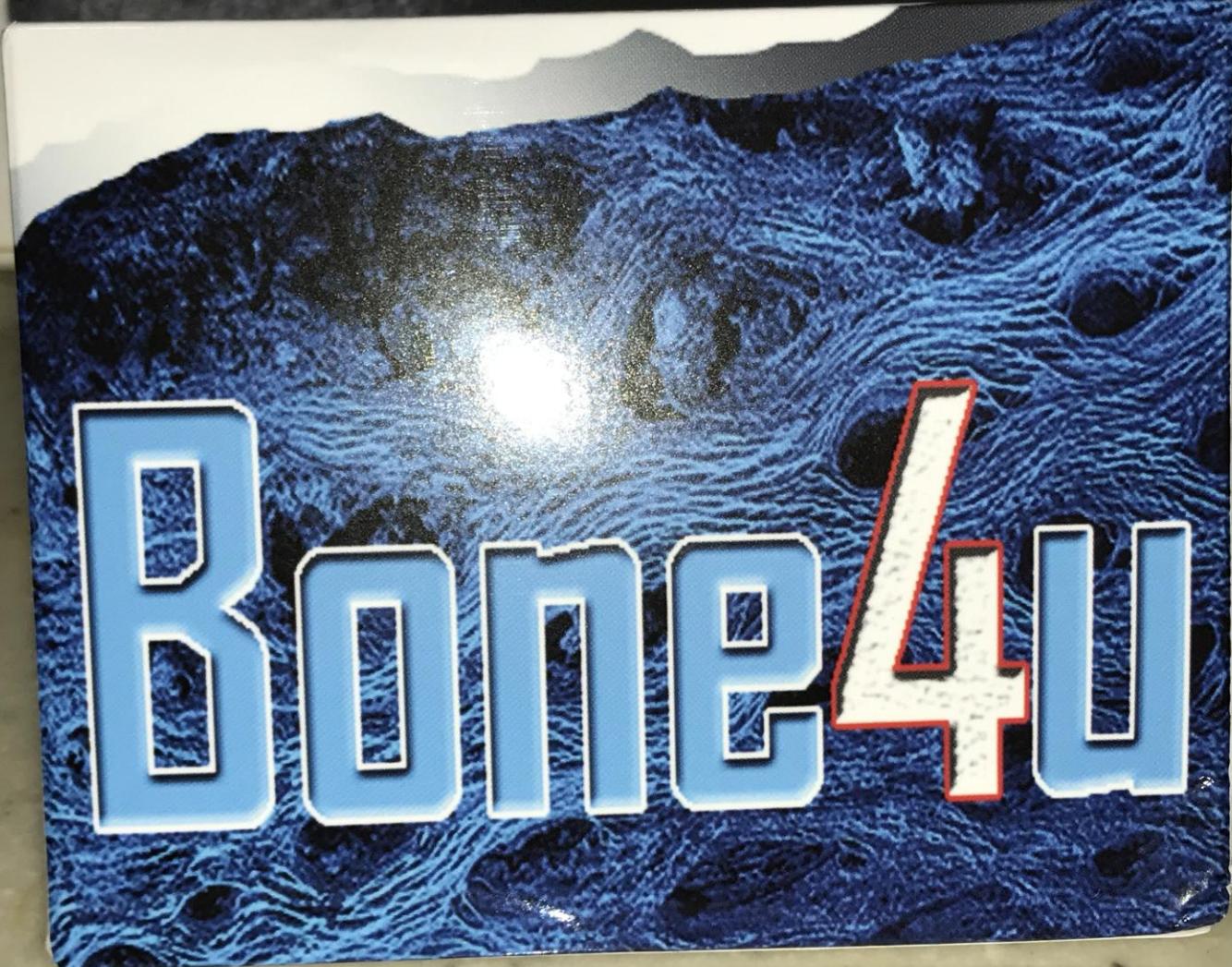
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Bone4U

Limitations of Microscale Scaffolds

While these microscale fabrication techniques can create osteoconductive scaffolds with good mechanical properties and controllable degradation, they have a key limitation:

They are inherently NOT osteoinductive or osteogenic. They act as a passive framework and require supplementation with growth factors or cells to actively promote bone formation.

The Shift to Nanoscale Scaffolds

Recognizing that the natural bone ECM is a nanofibrous structure (collagen), research has moved to examine scaffolds that mimic this subcellular dimension. These are almost invariably fiber-based, with diameters from 50 nm to several micrometers.

Potential Benefit

This subcellular dimension has shown improved osteoconductivity, and there is evidence that nanofibers may even be osteoinductive. However, this often comes at the cost of reduced compressive mechanical properties.

Next-Generation Synthetic Polymers

Research is exploring polymers beyond the standard poly(α -hydroxy esters) to address their drawbacks, such as acidic degradation by-products (which can be inflammatory) and bulk degradation (which leads to sudden mechanical failure).

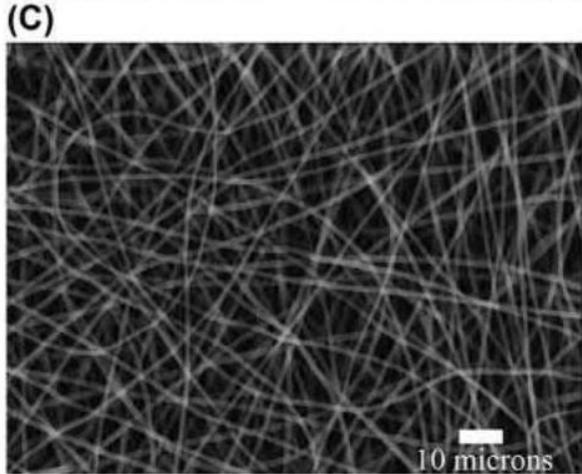
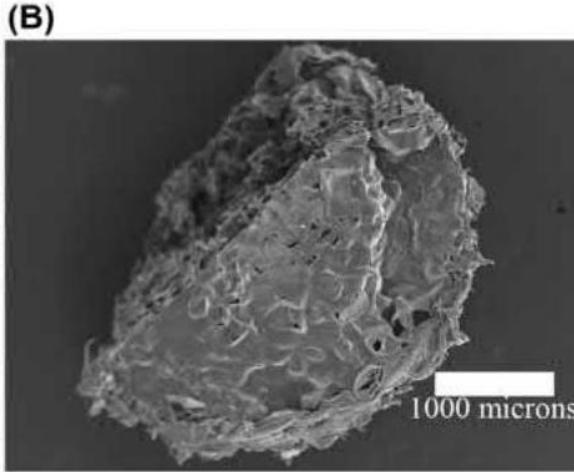
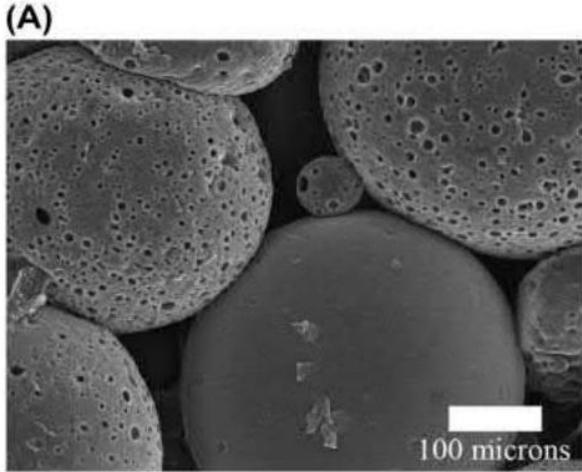
Poly[(amino acid ester)phosphazenes]

Degradate into neutral by-products (amino acids, ammonia, phosphate) and exhibit more favorable surface erosion.

Polyanhydrides

Another class of polymers that may surface erode, though less studied for porous bone scaffolds.

Polyphosphazene Scaffold Architectures



Poly[(amino acid ester)phosphazenes] are versatile and can be fabricated into various scaffold types.

Figure 2.6.6.3:

- (A) Sintered microsphere scaffold.
- (B) Salt-leached porous scaffold.
- (C) Electrospun nanofibers.

Substitute Type 4: Ceramic-Based

Ceramic-based biomaterials are widely used as bone graft substitutes due to their chemical similarity to the mineral phase of bone.

Calcium Phosphates

Mainly hydroxyapatite (HA) and tricalcium phosphate (TCP).

Calcium Sulfate

A rapidly resorbing ceramic, also known as plaster of Paris.

Bioglass

A specific glass formulation that is highly bioactive and bonds to bone.

Calcium Phosphate Substitutes

Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is the primary mineral component of natural bone, making it a logical choice for bone grafts.

These substitutes are osteoconductive and are slowly resorbed by the body as new bone remodels the area.

Limitation

Porous hydroxyapatite scaffolds often have compressive strength and moduli that fall below the range of even trabecular bone, limiting their use in load-bearing applications.

Case Study: Coral-Derived Hydroxyapatite

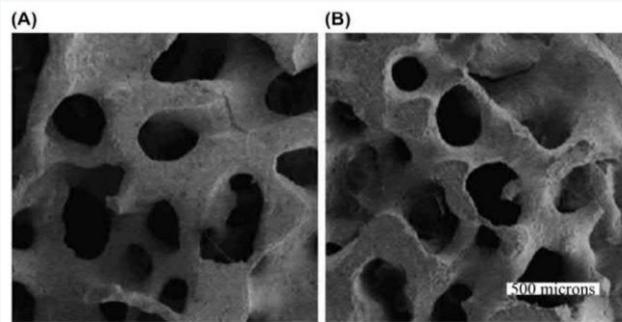


Figure 2.6.6.4:

- (A) The natural porous structure of coral.
- (B) The same structure after hydrothermal conversion to coralline hydroxyapatite.

A fascinating example is the Pro-Osteon graft substitute.

It begins as natural coral (calcium carbonate), which has a porous structure similar to trabecular bone.

The coral is then converted into hydroxyapatite via a hydrothermal process.

The Ceramic Osteoinduction Controversy

Whether hydroxyapatite-based materials are truly osteoinductive is a topic of debate. Early beliefs attributed this property to the ceramic's chemistry.

A New Theory: The Role of Microstructure

Recent evidence suggests that osteoinduction in calcium phosphates is not due to the chemistry itself, but rather a property of the material's specific surface microstructure. A study comparing two calcium phosphate structures found that the one with a subcellular microstructure was significantly more osteoinductive, even though the chemistry was similar.

Bioglass: A Uniquely Bioactive Ceramic

Developed in the late 1960s by Dr. Larry Hench, Bioglass has a specific formulation (lower SiO₂, higher Na₂O and CaO) that makes it highly reactive in the body. When implanted, it forms a hydroxyapatite-like layer on its surface, which bonds directly to living bone.

Performance

Porous scaffolds made from Bioglass fibers have been shown to be osteoconductive, potentially osteoinductive, and possess mechanical properties in the range of trabecular bone, making them a very promising option.

Substitute Type 5: Cell-Based Strategies

These strategies focus on delivering living cells to the defect site to directly stimulate bone formation. They are, by definition, osteogenic.

1 Direct Transplantation

Bone marrow aspirate is taken from the patient and placed directly into the defect. Simple, but lacks mechanical support.

2 In Vitro Expansion

Progenitor cells from bone marrow are cultured in the lab to increase their numbers before being implanted.

3 Genetic Modification

Cells are genetically engineered (e.g., to overexpress BMP-2) to make them osteoinductive as well as osteogenic.

4 Ex Vivo Tissue Generation

The most ambitious goal: to grow a fully viable piece of bone tissue in a bioreactor outside the body for later transplantation.

Substitute Type 6: Growth Factor-Based

This strategy involves the direct delivery of potent osteoinductive proteins to the defect site to recruit host stem cells and induce them to form bone. Two factors are FDA-approved for clinical use:

rhBMP-2

Recombinant Human Bone

Morphogenetic Protein 2. Shown to be
highly osteoinductive.

rhBMP-7

Recombinant Human Bone

Morphogenetic Protein 7.

Growth Factors: Application and Limitations

Due to their soluble nature, BMPs are typically packaged with a carrier, such as a simple collagen sponge, to hold them at the surgical site. The combination of rhBMP-2 with a collagen sponge has shown remarkable effectiveness, even outperforming the autograft 'gold standard' in some spinal fusion studies.

Primary Limitation: Cost

The use of recombinant growth factors is significantly more expensive than other procedures. Consequently, their use is often reserved for high-risk cases where other options have failed or are not viable.

Substitute Type 7: Composite Strategies



The final and most advanced strategy is based on composites that combine two or more of the previously discussed elements (biomaterials, cells, factors).

The goal is to create a construct that leverages the benefits of each component to better satisfy the full bone tissue-engineering paradigm.

Summary: Properties of Bone Graft Substitutes

This table summarizes how the different non-composite strategies align with the ideal bone graft paradigm.

	Osteoconductive	Osteoinductive	Osteogenic	Mechanical Match
Allograft based (DBM)	Yes	Yes	No	No
Microscale Biomaterials	Yes	No	No	Yes
Nanoscale Biomaterials	Yes	Potentially	No	No
Ceramics	Yes	Potentially	No	Yes/No (Varies)
Cells	No	No	Yes	No
Growth Factors	No	Yes	No	No

Composite Example 1: Biomaterial + Cells

A clinically used example involves combining Grafton DBM with bone marrow aspirate from the patient. This composite is prepared ex vivo just prior to implantation.

Synergy

- Grafton DBM provides osteoconduction and osteoinduction.
- Bone marrow aspirate provides the osteogenic potential of progenitor cells.
- The resulting composite has shown performance comparable to an autograft.

Composite Example 2: Ceramic + Polymer

This involves incorporating a ceramic like calcium phosphate with a polymer scaffold. One method is to electrospin a slurry of β -tricalcium phosphate (β -TCP) particles within a dissolved polymer like PCL.

Synergy

- The PCL nanofibers provide a flexible, osteoconductive scaffold.
- The β -TCP particles on the surface may improve osteoinductivity.
- The ceramic component can also improve the mechanical properties of the otherwise weak nanofiber scaffold.

Composite Example 3: Biomaterial + Growth Factors

This concept involves loading a scaffold with growth factors. For example, a PLGA foam can be loaded with microspheres containing rhBMP-2 and rhBMP-7. The properties of the microspheres can be tuned for staged release.

Synergy

- The PLGA foam provides an osteoconductive structure.
- The release of BMPs makes the entire construct actively osteoinductive.
- Staged release (e.g., fast release of BMP-2, slow release of BMP-7) could potentially mimic natural healing cascades.

Part 6: Advanced Scaffold Concepts

Porosity, Dimension, and In Vitro Culture



The Critical Role of Porosity

An adequate pore structure is arguably one of the most critical components of a successful biomaterial scaffold. Without it, migration of cells into the scaffold is restricted, which severely limits osteoconduction, osteoinduction, and osteogenicity.

Key Functions of Porosity:

- Allows cellular migration and infiltration.
- Facilitates nutrient delivery to cells in the interior.
- Enables waste removal from the scaffold's core.
- Provides space for new tissue and vasculature to form.

Porosity in Microscale Scaffolds

For scaffolds with features much larger than a cell (e.g., from 3D printing, salt leaching), the critical design aspects are pore diameter and interconnectivity.

- **Interconnected Pores:** This is an absolute necessity to allow cells to travel from one pore to another.
- **Pore Diameter:** Research shows that pore diameters must be above 40 μm to permit cell migration. Larger pores (100-300 μm) facilitate faster migration, but smaller pores can eventually achieve the same level of cellular penetration.

Porosity in Nanoscale Scaffolds

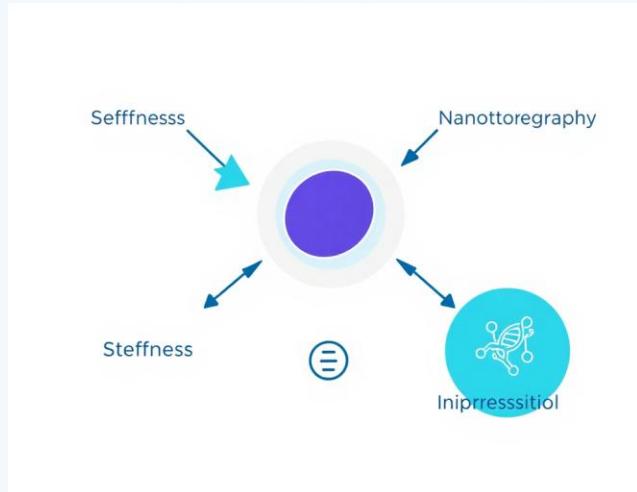
When the scaffold's characteristic dimension shrinks to the nanoscale (e.g., nanofiber mats), the rules change. Cells have been shown to invade these scaffolds despite pore diameters being much smaller than a cell body (e.g., 300 nm to 10 μm).

How is this possible?

Cells switch their mode of migration. Instead of moving through large pores, they extend thin, pseudopodia-like processes along individual fibers and engage in 'ameboidal migration', squeezing through the nanofibrous network. This suggests that for nanoscale scaffolds, high total porosity (>90%) may be more critical than large pore diameter.

The Impact of Scaffold Dimension

Recent research has demonstrated that the characteristic dimension of a scaffold is not just a passive structural feature.



It can directly affect intracellular signaling and alter gene expression, a process termed mechanotransduction.

Both material stiffness and scaffold topography (dimension) can alter the mechanical state of a cell.

Nanofibers and Osteogenesis

It has been shown that scaffolds that stimulate actomyosin contractility within mesenchymal stem cells also promote their differentiation into bone tissue. This can be achieved in two ways:

Stiffness

Culturing cells on a substrate with stiffness mimicking that of the natural osteoid matrix (~30 kPa).

Dimension

Culturing cells on nanofiber scaffolds with fiber diameters of approximately 500-1000 nm.

Both methods promote RhoA activation, which leads to increased contractility and bone differentiation.

Limitations of Static In Vitro Culture

When trying to grow tissue on a 3D scaffold in a standard petri dish (static culture), a critical limitation arises: transport. Waste efflux and nutrient influx are governed solely by passive diffusion. In large constructs, this leads to:

- A necrotic core due to lack of oxygen and nutrients.
- Accumulation of waste products (e.g., lactic acid).
- An acidic microenvironment that impedes calcification and compromises cell viability.

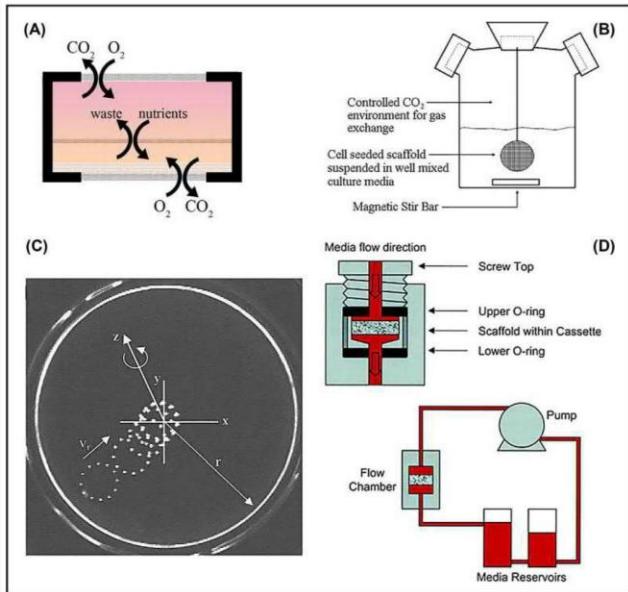
The Solution: Bioreactors

To overcome the limitations of static culture and achieve significant tissue growth in vitro, the use of bioreactors becomes necessary. A bioreactor is a device that provides a more controlled and dynamic environment for tissue culture.

Key Functions of a Bioreactor:

- Provides fluid flux to actively replenish nutrients and remove waste.
- Can provide mechanical stimulation (e.g., shear stress, compression) to encourage the development of mechanically viable tissue.

Types of Bioreactors for Bone Tissue Engineering



Several types of bioreactors have been developed, each with different mechanisms of action.

Figure 2.6.6.6: (A) Dialysis-based bioreactor. (B) Spinner flask. (C) Rotating wall vessel. (D) Perfusion bioreactor.

Bioreactor Analysis: Dialysis & Rotating Wall

Dialysis Bioreactor (A)

Uses permeable membranes for gas exchange and nutrient/waste diffusion from a fresh media reservoir. Relies on passive diffusion, limiting tissue thickness, but helps maintain gradients of cell-secreted factors.

Rotating Wall Vessel (C)

Developed by NASA to simulate microgravity. Rotation negates gravity's effect and provides a well-mixed media volume. Overcomes diffusion limits but the lack of mechanical stimulation discourages bone development.

Bioreactor Analysis: Spinner Flask & Perfusion

Spinner Flask (B)

Suspends constructs on needles in a stirred flask. Provides a well-mixed environment and mechanical stimulation through fluid shear forces. Shown to increase calcification compared to rotating wall vessels.

Perfusion Bioreactor (D)

Works by actively forcing (pumping) culture media through the porous scaffold. Provides excellent nutrient/waste exchange and controllable shear stress. Shown to significantly increase calcium production and osteogenic differentiation.

Conclusion: Progress and Persistent Challenges

Bone tissue engineering has made profound advances, with significant clinical achievements in using biodegradable scaffolds (with or without cells/factors) to heal large defects. However, despite the ground covered, unmet goals and challenges remain.

The Major Unmet Goal

No research has yet demonstrated the capability to grow a fully functional, vascularized piece of de novo bone in an in vitro setting. So far, only rudimentary calcified cell masses approaching bone tissue have been developed.

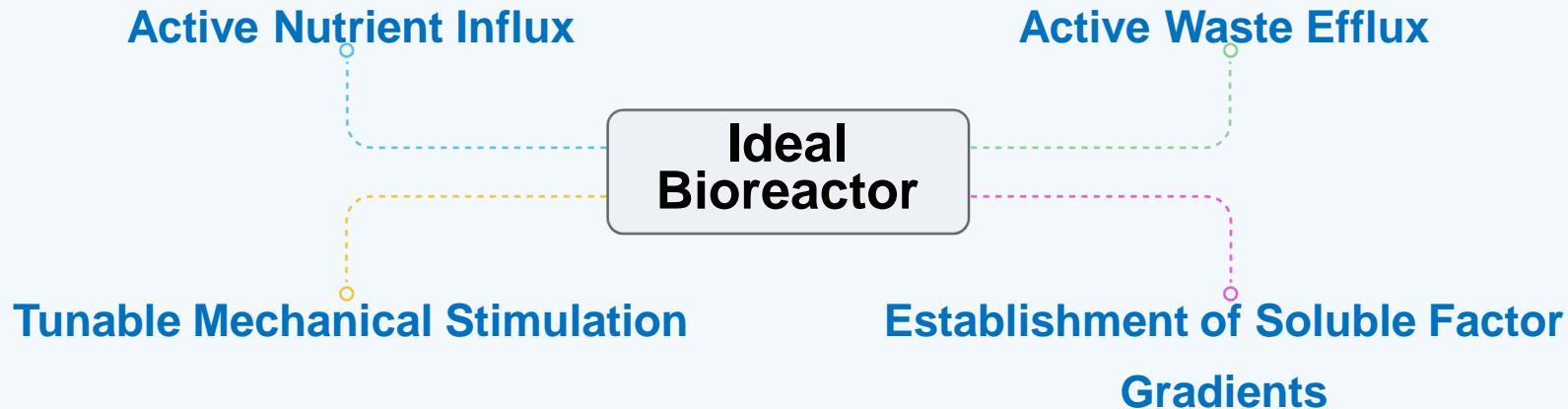
Future Directions: The Path Forward

Future researchers must consider a holistic design approach that integrates multiple factors:

- **Macroscale Design:** Flexibility to accommodate complex anatomical shapes and mechanics.
- **Biodegradability:** Ensuring the scaffold is replaced by healthy, natural bone.
- **Osteoconductivity:** Promoting rapid and thorough cell proliferation.
- **Osteoinductivity:** Actively driving the differentiation of stem cells into organized bone tissue.

The Bioreactor of the Future

The earliest, simplest dialysis bioreactor managed to get one thing right that has been lacking in more dynamic designs: the ability to maintain gradients in soluble growth factors secreted by cells. Future successful bioreactors will need to incorporate the best aspects of all existing designs.



Combining all four of these elements with an advanced bone graft substitute may finally lead to the de novo formation of hierarchical bone tissue in vitro.