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A REVIEW ARTICLE ON BIOLOGICAL PROPERTIES OF NANO STRUCTURED SCAFFOLDS FOR BONE TISSUE ENGINEERING

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Abstract:

Bone tissue engineering aims to address bone defects and disorders through the development of scaffolds that mimic the extracellular matrix (ECM) of bone tissue. These scaffolds, typically nanostructured with dimensions ranging from 1 to 100 nanometers, must exhibit properties such as biodegradability, bioresorbable, and biocompatibility. They serve as supportive frameworks to stimulate bone cell activity and facilitate tissue regeneration. Various natural and synthetic polymers, as well as composite materials, are utilized in scaffold fabrication. The field of tissue engineering traces its origins to pioneering work in the 1970s and 1980s, which laid the groundwork for subsequent advancements. Institutions worldwide, particularly in the United States, Europe, and Asia, have contributed to the evolution of tissue engineering research and development. Understanding the intricate structure of bone, comprising cortical and trabecular components, informs scaffold design to replicate its mechanical and biochemical properties. Bone apatite, characterized by rod-like or plate-like microstructures, plays a crucial role in bone composition and serves as a model for scaffold biomimicry.

Key word:

Bone tissue engineering, scaffolds, extracellular matrix (ECM), nanostructured, poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), tissue regeneration, bone apatite.

INTRODUCTION

Scaffolds work as the supportive framework or structures utilized across various fields like drug delivery, tissue engineering and regenerative medicine. 3D scaffold must satisfy the properties like biodegradability, bioresorbable and/or biocompatibility. Nanostructured scaffolds are scaffolds designed with dimensions typically ranging from 1 to 100 nanometers, characterized by features at the nanometer scale. Typically, scaffolds must feature a three-dimensional porous structure which resembles the porous structure of the extracellular matrix (ECM) present in environments such as cancellous bone, with the aim of mimicking the ECM of the specific tissue being targeted.[i]Bone can naturally regenerate in small defects but struggles with larger ones, requiring a supportive framework to stimulate bone cell activity. Surgeons require readily available materials that can serve as scaffolds, acting as templates and triggering the body's regenerative processes.[ii] While preparing cellular scaffolds, the three different size scales to consider are functional tissue level (>100µm), cellular level (1-100 µm) and subcellular (<1 µm) level. Before cells come into contact with it, the scaffold's design is of utmost importance. It should have a surface that promotes cell attachment, growth, and differentiation, as well as a porous network for tissue growth.[iii] The material chosen must degrade at a rate that matches new tissue formation, be biocompatible, and have biocompatible degradation products. Once implanted, the scaffold should possess the necessary mechanical properties to offer temporary structural support until new tissue forms. Furthermore, the scaffold should be highly porous, providing a suitable path for nutrient transmission and tissue ingrowth. Various natural and synthetic polymers, such as calcium carbonate, calcium phosphate, and glasses, have been utilized in the creation of scaffolds. Composite materials, consisting of a mix of two or more exceptional materials, are commonly used in bone tissue engineering to meet the diverse scaffold needs.[iv] Numerous metabolic bone disorders, like age-related parietal bone atrophy, hyperparathyroidism localized infections, vitamin D-resistant rickets (VDRR), and Paget's disease, can lead to bone deterioration. The high number of cases related to fractures, accidents, and bone health issues in females, specifically osteoporosis, has driven various scientists and researchers to explore nanomaterials for scaffold preparation.[v]

Bones provide protection, support, and mobility to the body's organs and systems. Bone tissue engineering is a complicated and ever-changing process that starts with the movement and gathering of bone cell precursors. These cells then grow, change into specialized types, create a framework, and modify the bone structure. Bone scaffolds are usually constructed from porous materials that break down over time, giving support while damaged or unhealthy bones heal and grow back.[vi]

The early stage or beginning phase.

In the early 1970s, Dr. W.T. Green, a pediatric orthopedic surgeon at Children's Hospital, conducted several experiments aimed at creating new cartilage. He did this by placing chondrocytes on bone spicules and then implanting them into hairless mice for testing.

Despite initial setbacks, Dr. W.T. Green realized that advancements in biocompatible materials could enable the creation of new tissue by placing live cells on well-designed scaffolds. Years later, a collaboration between Drs. Burke and Yannas from Massachusetts General Hospital and M.I.T. led to the development of a tissue-engineered skin substitute using aCOL(collagen) matrix to support dermal fibroblast growth. Dr. Howard Green applied sheets of keratinocytes to burn patients, and Dr. Eugene Bell used fibroblasts to seed COL (collagen) gels, calling them contracted COL (collagen) gels. These efforts laid the foundation for the field now recognized as Tissue Engineering.

In the mid-1980s, Dr. Joseph Vacanti from Children's Hospital collaborated with Dr. Robert Langer of MIT to develop planned scaffold designs for delivering cells, rather than relying on natural scaffolds with fixed physical and chemical properties that led to uncertain results when cells were seeded onto them.

To explore and understand the potential of this new field, several institutions have been set up in the United States and Europe. While most of these institutions are linked to those in the Boston area, some have developed on their own. An early example of this outside Boston was the founding of the Pittsburgh Tissue Engineering Initiative (PTEI) in the early 1990s, led by Peter Johnson.

The Cardiovascular Tissue Engineering project, led by Dr. Robert Nerem at Georgia Tech, is another notable example.

In the mid to late 1990s, tissue engineering projects like the one led by Drs. Chris Brewer and Mark Saltzman at Yale University were developing in almost every developed country worldwide. Furthermore, various privately funded endeavors in tissue engineering were also beginning to emerge around this time.

In Asia, Dr. Minoru Ueda from Nagoya University led a substantial tissue engineering initiative in Japan and was instrumental in arranging the first gathering of the Japanese Tissue Engineering Society in Nagoya in 1997. Likewise, in China, the initial tissue engineering project, supported by the Chinese government, was launched by Dr. Yi Lin Cao in Shanghai.[vii]

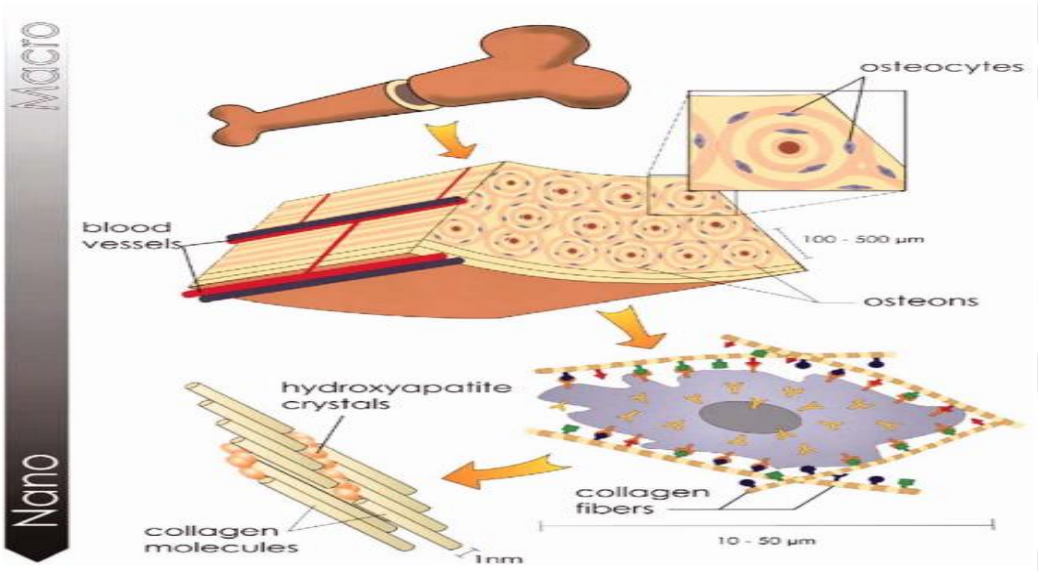


Fig No.1

BONE AND SCAFFOLD ENGINEERING:

Bone structure:

Bone is made up of different layers and structures, each serving a specific purpose. From a structural point of view, bone tissue has two primary components: a hard outer layer known as cortical bone, and an inner porous section called spongy or trabecular bone. Cortical bone is like a solid shell, while trabecular bone has a honeycomb-like structure with small beams running through it.On a very tiny scale, bone is like a mixture made up of mostly hard calcium phosphate crystals (about 70%) and a softer collagen matrix (about 20-30%), with a bit of water mixed.

Bone apatite is made up of mineral crystallites that are structurally deficient in calcium and are substituted with carbonate, forming hydroxyapatite. This type of apatite is commonly found in bones and is typically 5 × 5 × 50 nm in size, with a rod-like or plate-like microstructure. It is embedded within collagen fibers and makes up approximately half of the total volume in mature bone. The specific arrangement of this microstructure depends on age and differs among various bones and within different parts of the same bone.[viii]

Table no 1:
Mechanical properties of bone and current implant materials:

Material	***E* (GPA)**	***a* (MPa)**	***c* (%)**
Cortical bone	7-30	50-150	1-3
Cancellous bone	0.05-0.5	10-20	5-7
Co-Cr alloys	230	900-1540	10-30
Stainless steel	200	540-1000	6-70
Ti-6Al-4 V	106	900	12.5
Alumina	400	450	-0.5
Hydroxyapatite	30-100	60-190	
Polyethylene	1	30	>300

E Young's modulus, σ tensile strength (flexural strength for alumina), ε elongation at fracture[ix]

Requirements for an ideal scaffold:

The important qualities needed for a perfect scaffold in bone tissue engineering include:

1. having both large pores (greater than 100 micrometers) and small pores (less than 20 micrometers);
2. having interconnected open pores that allow new tissue to grow into them when placed inside a living organism;
3. having enough strength to bear mechanical loads and a controlled rate of degradation to transfer loads properly to nearby tissue;
4. having initial strength to handle safely during sterilization, packaging, transportation, and in vivo stresses;
5. providing a sterile environment for seeding cells onto the scaffold.

The biomechanical system of bone is complex so that the following requirements for an ideal scaffold are diverse:

- Biocompatibility is a key requirement for bone scaffolds, referring to their ability to support normal cell activity without causing any harmful effects to the surrounding tissue. An ideal bone scaffold should be osteoconductive, allowing bone cells to attach, grow, and create a matrix on its surface and within its pores. It should also have the capability to stimulate new bone formation by signalling molecules and attracting precursor cells, a process known as osteoinduction. Additionally, a perfect scaffold should promote blood vessel formation nearor within the implant shortly after it's placed, ensuring proper transport of nutrients, oxygen, and waste products.
- The mechanical characteristics of an ideal bone scaffold should align with those of the host bone to ensure effective load transfer. Bone's mechanical properties can vary significantly, ranging from cancellous to cortical bone. For instance, the Young's modulus for cortical bone falls between 15 and 20 GPa, while for cancellous bone it's between 0.1 and 2 GPa. Similarly, compressive strength can range from 100 to 200 MPa for cortical bone and from 2 to 20 MPa for cancellous bone. The wide range in mechanical properties and bone structure complexity makes designing a universally "perfect" bone scaffold challenging.

- Interconnected porosity with a minimum pore size of 100 micrometres is essential for scaffolds, as it allows for the effective diffusion of vital nutrients and oxygen, crucial for cell survival. However, optimal pore sizes for bone tissue growth are typically in the range of 200 to 350 micrometres. Recent research suggests that scaffolds with multiple scales of porosity, including both micro and macro pores, perform better than those with only large pores.

Unfortunately, increasing porosity can reduce mechanical properties like compressive strength and make scaffold manufacturing more complex. Scientists have explored various materials for porous scaffolds, including polymers, ceramics, composites, and metals. Dense bioceramic materials have strength comparable to cortical bone, while different polymers mimic cancellous bone. However, ceramic-polymer composites are generally weaker than bone. Porous metallic scaffolds meet bone's mechanical requirements but struggle with implant-tissue integration and raise concerns about metal ion leaching.

- Bioresorbable is a critical aspect for scaffolds used in bone tissue regeneration. An ideal scaffold should not only have mechanical properties similar to the host tissue but should also be capable of gradually breaking down in the body over time, ideally at a controlled rate. This controlled resorption allows the scaffold to create space for new bone tissue to develop. The rate of degradation should be adjusted according to the specific application, such as longer durations (around 9 months or more) for spinal fusion scaffolds or shorter periods (3-6 months) for cranio-maxillofacial applications.

Designing and manufacturing multi-scale porous scaffolds with the right composition, including targeted biomolecules, mechanical properties, and suitable bioresorbability, remain significant challenges in bone tissue engineering today.[x]

Factor affecting:

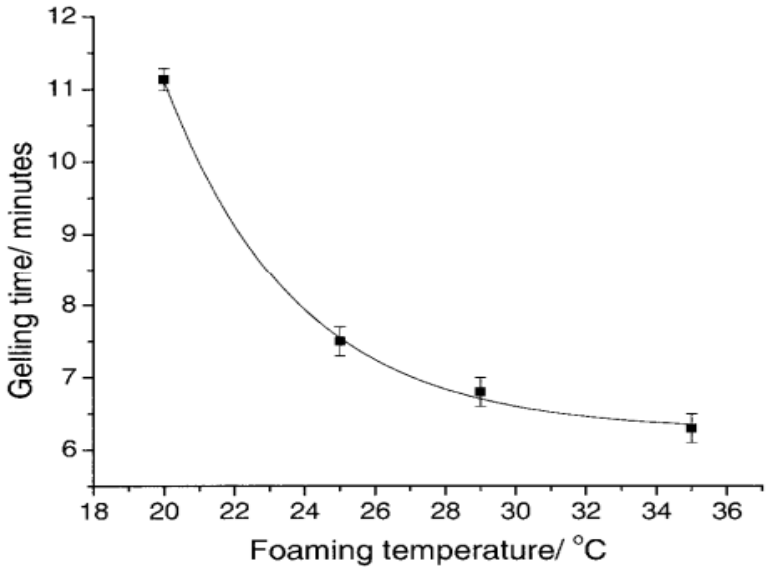
There is main two factor that affect the structure of scaffolds

1. Temperature: The production of 58S foams using the control recipe involved varying foaming temperatures between 20 and 35 °C. The gelling time decreased from 11 minutes 10 seconds to 6 minutes 20 seconds as the foaming temperature increased. This decrease in gelling time is attributed to the higher condensation rate resulting from elevated temperatures. Additionally, foam volume decreased from approximately 180 ml at 20 °C to 70 ml at 35 °C, with corresponding bulk density values increasing from 0.30 g/cm³ to 0.40 g/cm³. All foams experienced a shrinkage of 65–75% during thermal processing. Mercuryporosimeter revealed wide pore distributions in foams produced at each temperature, indicating the presence of pores exceeding 200 µm. Scaffolds foamed at lower temperatures exhibited normal pore distributions, while those foamed at higher temperatures displayed positive skewness in pore distributions, with smaller modal pore diameters. SEM micrographs depicted crack-free pore networks with spherical pores up to 600 µm and interconnected pores up to 100 µm in diameter in scaffolds foamed at 25 °C. Conversely,

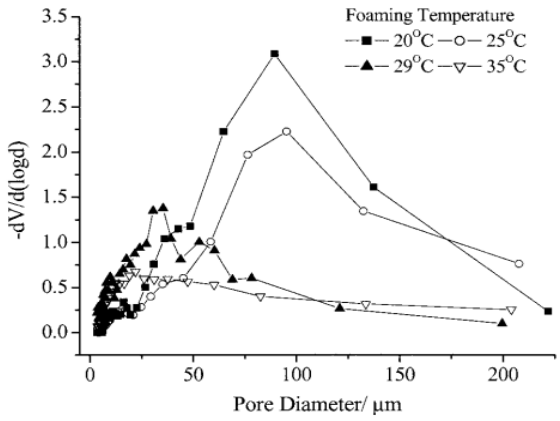
scaffolds foamed at 35 °C exhibited small isolated pores, extensive cracking, and fragility. These interconnected pores are crucial for vascularization and tissue ingrowth.

2. Water:

Increasing the water content initially led to a proportional increase in foam volume up to 170 ml at 3.5 ml of water, resulting in larger pore sizes. However, beyond 3.5 ml, the foam volume increased significantly, reaching 400 ml at 5 ml of water, equivalent to eight times the original sol volume. This caused the formation



of very large air bubbles and enhanced interconnectivity within the foam. Nonetheless, the thin struts (cell walls) between the pores were unable to support the foam's weight upon gelation,leading to its collapse. The maximumfoam volume achievable from 50 ml of 58S sol was 170 ml, as indicated by the foam survival limit line.



MATERIALS &METHOD:

1. **Natural Polymers scaffolds:**

Bone tissue engineering has been centered on developing 3D scaffolds that can imitate the extracellular matrix (ECM), aiding in new bone formation while degrading as new bone forms. Natural polymers offer appealing characteristics for constructing these 3D scaffolds, such as biocompatibility and biodegradability. Controlling porosity, charge, and mechanical strength is possible by adjusting polymer concentrations, polymerization conditions, or introducing various functional groups. Bioactivity can also be managed by adding chemicals, proteins, peptides, or cells. The primary natural polymers studied for bone engineering include

collagen/gelatin,CS(Chitosan), silk, alginate, hyaluronic acid, and peptides. Recent studies explore how these natural polymers, when used as 3D scaffolds, are modified to enhance their ability to promote bone regeneration and improve their osteogenic properties.[xi]

2. **Synthetic polymer scaffolds:**

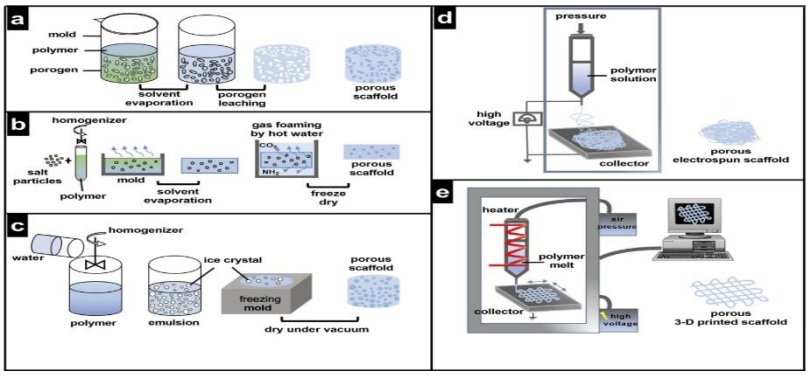
The primary consideration for any biomaterial is its biocompatibility. Many synthetic polymers have been identified as bio-compatible and are approved by the FDA for specific uses within the body. These polymers have a well-established presence in tissue engineering (TE). For instance, poly (ethylene glycol) (PEG), or poly (ethylene oxide) (PEO) at elevated molecular weights, is an exceptionally hydrophilic polymer. It demonstrates outstanding solubility in various solvents and high mobility in solution. PEG is widely utilized in tissue engineering, especially as a component of hydrogels due to its capacity to absorb water.The fundamental strategy in tissue engineering (TE) involves using scaffold materials that are not permanent but gradually get replaced by the natural extracellular matrix. The goal is to introduce a scaffold that remains stable long enough to support new tissue formation but eventually breaks down and gets replaced by this newly formed tissue. Among the most commonly utilized synthetic degradable polymers are poly (alpha-hydroxy acids) such as poly (lactic acid) (PLA), poly (glycolic acid) (PGA), poly(caprolactone) (PCL), and their copolymers.[xii]

3. **Composite scaffolds**:

In recent times, there has been a development of new polymers for tissue engineering applications. Despite significant advancements in the past three decades, bioactive bio ceramics like glasses and glass-ceramics were not widely considered for tissue engineering until recently. Some bioactive glasses have demonstrated potential for bone tissue engineering, leading to the creation of tissue engineering scaffolds solely composed of bio ceramics.Compared to the strengths of metals and ceramics used in medical applications, biodegradable polymers already have lower strengths. When pores are introduced into polymers to form tissue engineering scaffolds, their strengths decrease further, as materials with higher porosity tend to have lower strength.On the contrary, polymers such as PLA and PCL are not naturally conducive to bone growth (non-osteoconductive). To improve scaffolds for bone tissue engineering, a composite approach can be adopted. Thisinvolves creating polymer-based scaffolds that incorporate bioactive bio ceramics, which can enhance the scaffold's osteoconductive properties.Particulate forms of hydroxyapatite (HA), tricalcium phosphate (TCP), and some bioactive glasses can be incorporated into composite scaffolds to achieve osteoconductivity.[xiii]

4. **Porous scaffolds:**

Bone tissue engineering necessitates a well-designed architecture for the porous scaffold. Adequate porosity with suitable sizes and interconnected pores creates an environment that encourages cell infiltration, migration, vascularization, nutrient and oxygen flow, and waste removal, all while being able to endure external stresses. The distribution and geometry of pores strongly impact cell penetration, growth, and differentiation, as well as the scaffold's degradation rate. This rate must align with tissue maturation and regeneration after transplantation in vivo. Materials with ultra-high molecular weight that don't degrade in the body have limited utility as bone graft materials. Degradation products should be non-toxic and not provoke an inflammatory response. Thus, the scaffold's physical and chemical surface properties are crucial for fostering cell attachment, infiltration, growth, proliferation, and migration.



(FIG NO 2)

Various porous scaffold fabrication technique**:**

a) Pyrogen leaching

b) Gas foaming

c) Freeze drying

d) Solution electrospinning

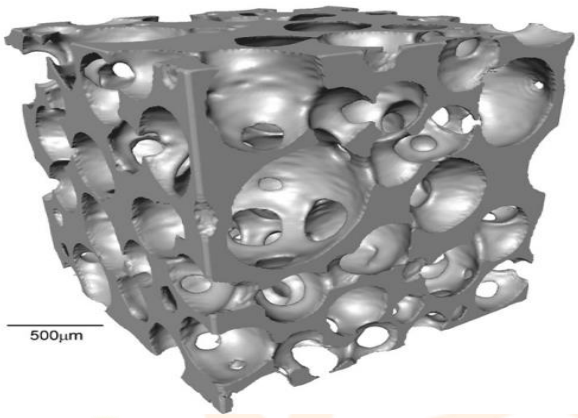
e) Melt electro writing& 3d printing [xiv]

5.BIOGLASS SCAFFOLD

One of the most favoured materials for repairing bone defects is synthetic hydroxyapatite (sHA, Ca10(PO4)6OH2) due to its similarity to bone mineral, making it bioactive and conducive to bone growth (osteoconductive). Porous versions of hydroxyapatite, like ApaPore (Apatech Ltd., Elstree, UK), are available commercially but are primarily used for bone augmentation rather than regeneration because they resorb very slowly. Although the resorption rates can be enhanced by incorporating silicon or carbonate substitutes, they still remain relatively sluggish. An alternative to synthetic hydroxyapatite is bioactive glass, which possesses characteristics that meet the necessary criteria for scaffold materials. Bioactive glasses bond to bone more rapidly than other bioactive ceramics as they form a similar carbonated apatite layer on their surface when in contact with physiological fluids. They also exhibit osteoinductive properties, stimulating new bone growth by dissolving in the body. This osteogenic behavior is believed to stem from the release of active ions that trigger genes associated with bone formation. The pioneering bioactive glass, Bioglass, was developed by Hench in 1971 and is commercially available under various trade names such as Perioglass and Novabone. However, the composition of this glass prevents it from being fabricated into scaffolds as it crystallizes upon sintering, forming a glass-ceramic. Consequently, while these glasses demonstrate excellent bioactive characteristics, they are not available in scaffold form.[ii]

6.Ceraminc scaffold:

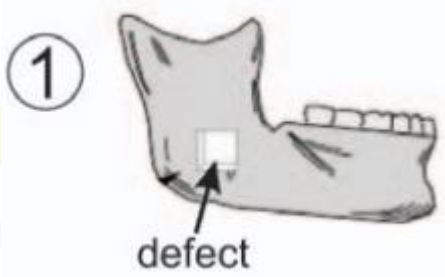
In the past forty years, ceramics, known as Bioceramics, have been extensively utilized in medical applications for reconstructing damaged body parts and skeletal repair. Bioceramics are divided into two main categories: bioinert or bioactive, with bioactive ceramics further classified as resorbable or non-resorbable. These materials are chosen for their chemical properties and similarity in crystallinity to bone mineral components, resulting in excellent biocompatibility and bioactivity.The inorganic part of bone contains substances like hydroxyapatite (HA) and calcium phosphates, which facilitate bone tissue formation on their surfaces. While these materials are excellent for implants, they do face challenges regarding mechanical properties such as fracture and fatigue.Common ceramic materials used for bone repair or regeneration include Bioglass, calcium phosphates, and ceramic scaffolds derived from corals. Various studies have examined these materials both in laboratory settings (in vitro) and in living organisms (in vivo) to evaluate their potential for promoting bone growth.xi



(Fig NO 3)

Bone regeneration method:

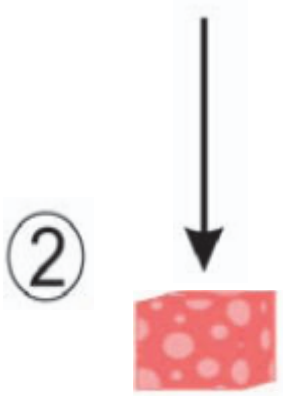
In Situ Bone regeneration method:In bone tissue engineering, creating a scaffold-tissue composite is crucial for restoring function as the body remodels bone based on its local stress patterns. Another approach is in situ bone regeneration, where a load-bearing scaffold is implanted directly into the defect, stimulating bone growth without prior cell addition. This scaffold should dissolvesynchronously with new bone formation, aiding in the restoration of function. Below is a diagram depicting this simplified process in jawbone regeneration.



(Fig NO 4)

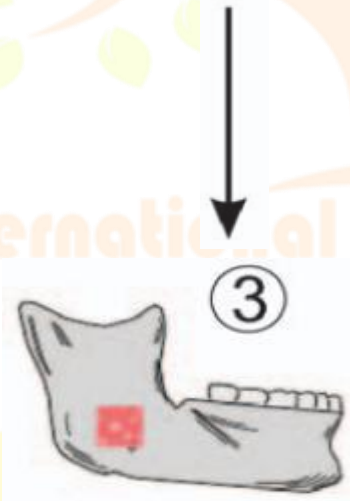
Stage 1:

Bone defects arise from accidents (trauma), illnesses (disease), and natural breakdown (degeneration), necessitating reconstruction to restore bone structure and function. These defects can range from fractures and infections to conditions like osteoporosis, requiring medical intervention such as bone grafts or implants for repair.



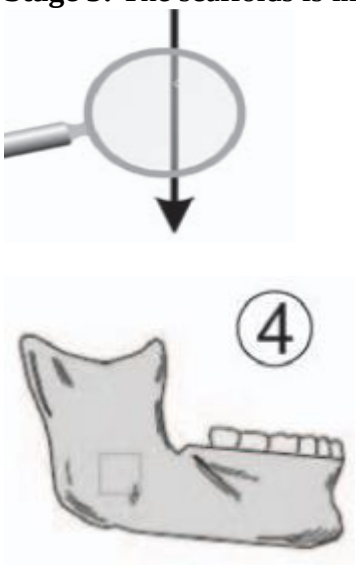
(Fig NO 5)

Stage 2: A bioactive glass scaffold with an interconnected pore structure is designed to precisely fit bone defects. This scaffold promotes cell attachment, tissue ingrowth, and mineral deposition due to its bioactivity. The interconnected pores allow for nutrient diffusion andwaste removal, aiding in bone regeneration and defect repair.



(Fig NO 6)

Stage 3: The scaffolds is implanted at the defect site where it forms a strong bond with the native bone



(Fig NO 7)

Stage 4: Over time the bioactive glass scaffolds is completely replaced by remodeled bone.[xv]

FUTURE PROSPECTIVE

The future prospects of scaffold use in bone tissue engineering (BTE) are highly promising, with ongoing research and advancements poised to address current limitations and unlock new opportunities. Here are some potential future directions:

1. **Biologically Inspired Scaffolds**: Designing scaffolds that mimic the complex hierarchical structure and composition of natural bone tissue can enhance their biological performance. Incorporating biomolecules, such as growth factors and extracellular matrix components, into scaffolds can further promote cell adhesion, proliferation, and differentiation.

2. **Smart Scaffolds**: Integration of responsive materials and stimuli-responsive elements into scaffolds can enable dynamic control over their properties and functions. Smart scaffolds capable of responding to environmental cues, such as pH, temperature, or mechanical stress, can facilitate tailored tissue regeneration and improved integration with host tissues.

3. **Bioactive Coatings and Functionalization**: Surface modification techniques can be employed to introduce bioactive coatings or functional groups onto scaffold surfaces. These modifications enhance cell-material interactions, promote osteogenic differentiation, and modulate the immune response, ultimately improving the efficacy of BTE scaffolds.

4. **Personalized and Patient-Specific Approaches**: Advances in imaging modalities, computational modeling, and additive manufacturing technologies enable the fabrication of patient-specific scaffolds tailored to individual anatomical and physiological requirements. Personalized BTE scaffolds offer improved compatibility, integration, and therapeutic outcomes.

5. **Combination Therapies**: Integrating scaffold-based approaches with other regenerative strategies, such as stem cell therapy, gene therapy, or drug delivery systems, can synergistically enhance tissue regeneration and accelerate healing processes. Combination therapies address multiple aspects of tissue regeneration, including cell recruitment, differentiation, and extracellular matrix remodeling.
6. **Biofabrication Techniques**: Continued development of biofabrication techniques, such as 3D bioprinting, enables precise spatial control over scaffold architecture and cell distribution. Advanced bioprinting methods allow the fabrication of complex scaffolds with intricate geometries, vascular networks, and heterogeneous cell populations, mimicking the native bone microenvironment.
7. **Regulatory and Clinical Translation**: Streamlining regulatory pathways and conducting robust preclinical and clinical studies are essential for the translation of scaffold-based BTE therapies into clinical practice. Establishing safety, efficacy, and long-term outcomes of scaffold implants is crucial for widespread adoption and commercialization.

Overall, the future of scaffold use in bone tissue engineering holds immense potential for addressing clinical needs, improving patient outcomes, and revolutionizing regenerative medicine approaches for bone repair and regeneration. Continued interdisciplinary collaboration and innovation will drive the development of next-generation BTE scaffolds towards clinical application and commercialization.

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