

Nanotechnology in Tissue Engineering: Enhancing Scaffold Properties for Regenerative Medicine – A Review

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Abstract

This document explores the transformative role of nanoparticles in tissue engineering, highlighting their potential to enhance scaffold properties and support tissue regeneration. By integrating nanotechnology, researchers can develop biomaterials that mimic the extracellular matrix, providing a conducive environment for cell growth and differentiation. Key nanoparticles, such as graphene oxide and gold nanoparticles, are examined for their unique physicochemical properties, which improve mechanical strength, biocompatibility, and bioactivity of scaffolds. The document discusses various in vivo and in vitro studies that demonstrate the effectiveness of nanoengineered scaffolds in promoting cellular activities, such as adhesion, proliferation, and tissue integration. Additionally, it addresses the challenges associated with assessing the long-term safety and toxicity of these materials, emphasizing the need for comprehensive evaluations. Advanced imaging techniques, including Scanning Electron Microscopy (SEM), are highlighted as essential tools for understanding the interactions at the nanoscale. Overall, the findings underscore the potential of nanoparticles to revolutionize tissue engineering and regenerative medicine, paving the way for innovative therapeutic strategies and improved clinical outcomes. Continued research and interdisciplinary collaboration are vital for advancing this promising field.

Keywords: Nanotechnology, Tissue Engineering, Regenerative, Scaffolds, Biocompatibility, Osteogenesis, Nanomaterials, Polymer.

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1. Introduction

Tissue engineering is a field that merges engineering, materials science, and life sciences to create substitutes for damaged organs and tissues [1-3]. The key components are cells and scaffolds, instead of embedding cells directly into damaged tissues, they are placed on biomaterials. These biomaterials act as scaffolds, guiding the cells to rearrange and form functional tissues [4-6]. The scaffolds mimic the extracellular matrix (ECM) and provide a supportive environment for tissue development. Important properties of scaffolds include porosity, biodegradability, and biocompatibility [7-11]. Nanotechnology enhances tissue engineering by improving scaffold properties and providing materials that support cell growth and tissue regeneration [12-14]. Nanomedicine uses nanotechnology for targeted drug delivery, enhancing biological interactions, and controlled drug release. This allows for multifunctional capabilities, such as combining drug delivery with imaging and monitoring. Nanoparticles are often biocompatible and biodegradable, reducing toxicity and allowing precise control over drug delivery and tissue reconstruction, outperforming traditional methods [15,16].

Nanomaterials improve tissue interfaces by offering better cell adhesion, suitable mechanical properties, increased bioactivity, and controlled release of bioactive factors, leading to better tissue integration and regeneration compared to conventional polymeric biomaterials [17]. Engineered biocompatible nanomaterials, enhance orthopedic tissue repair by strengthening bone-ligament bonds and improving cell adhesion and antimicrobial properties. Three-dimensional bioprinting and high-throughput genetic screening help optimize biomaterial formulations for diagnostics and sensing [17]. Nanoparticles enhance tissue reconstruction by providing controlled and stable delivery of therapeutic agents, improving interactions with biological tissues, and protecting agents from degradation. They mimic the extracellular matrix, support cell growth, and can be tailored for targeted drug delivery, making them versatile and biocompatible carriers in tissue engineering and regenerative medicine [15,16]. The role of nanomedicine in tissue engineering has been summarized in Figure 1.

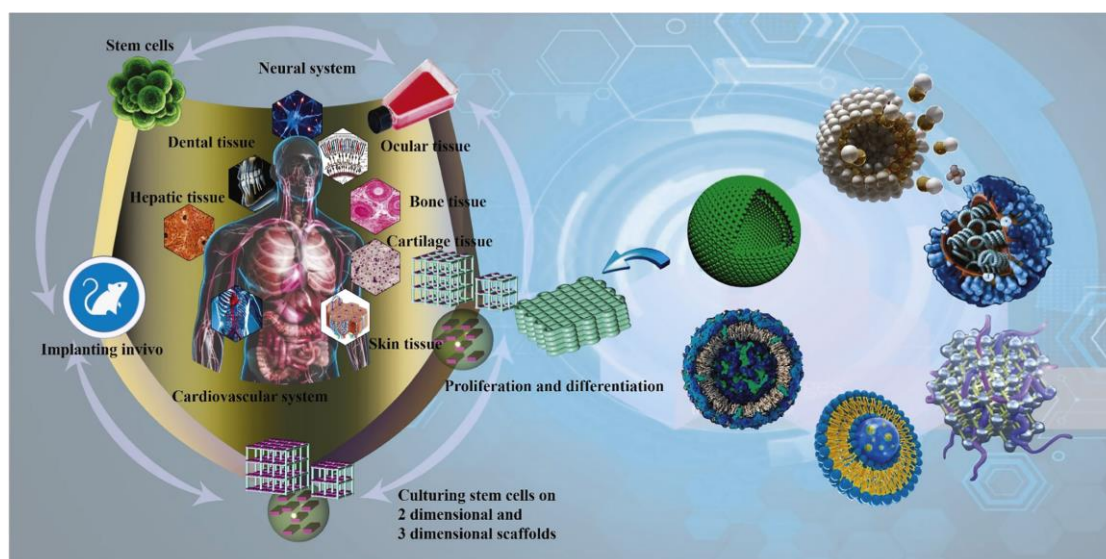


Figure 1. Tissue regeneration empowered by nanomedicines [11].

2. Common materials used

In tissue engineering, we can use the following polymers:

2.1 Poly (l-lactic acid) (PLLA)

the use of poly (L-lactic acid) in orthopedic tissue engineering, focusing on its biocompatibility, biodegradability, and mechanical properties. PLLA is biodegradable and biocompatible, minimizing adverse reactions when implanted. Its mechanical properties can be tailored for specific orthopedic applications, essential for load-bearing tissues. PLLA degrades gradually, allowing for load transfer to regenerating tissue during healing. Combining PLLA with nanomaterials like nanohydroxyapatite and magnesium oxide enhances scaffold bioactivity and mechanical properties, promoting cell adhesion, proliferation, and differentiation. PLLA's versatility in forming complex scaffold structures mimics the natural extracellular matrix, making it suitable for bone and soft tissue engineering [17-19].

2.2 Polyethylene Glycol (PEG)

PEG is widely used in biomedical applications due to its hydrophilicity, ability to increase drug solubility, drug stability, and biocompatibility [15,26] and is also used to modify the surface of nanoparticles to improve biocompatibility and reduce immunogenicity [15]. This polymer has been used as a scaffold material in eye tissue engineering. It offers properties such as transparency, mechanical stability, oxygen permeability, wettability, flexibility, and biocompatibility that make it a popular choice for applications in the eye [11,23,24,25].

Composites of PEG and graphene oxide have been developed to increase lubrication in artificial joints, reduce friction coefficients, and improve joint performance [26].

2.3 Poly vinyl alcohol (PVA)

PVA is known for its excellent film-forming, emulsifying, and adhesive properties, making it suitable for various biomedical applications [15]. This is a promising polymer in tissue engineering due to its biocompatibility and low friction coefficient. Combining with graphene oxide (GO) enhances the hydrogel's mechanical properties, making it suitable for applications like cartilage repair by improving tensile and compressive strength while maintaining high water content [26]. PVA has been blended with chitosan and CaCO₃ nanoparticles to create nanofiber mats with improved mechanical properties. These nanofibers have demonstrated enhanced cell proliferation and provided a positive environment for cell growth, showcasing the significance of polymer-nanoparticle combinations in orthopedic tissue engineering [27].

2.4 Chitosan

Chitosan is a natural biodegradable polysaccharide widely used in tissue engineering applications [26]. However, the mechanical properties of pure chitosan can limit its effectiveness in cartilage repair, prompting the development of composite materials like Graphen oxide/Chitosan with enhanced biocompatibility and mechanical strength [28-30]. Chitosan has been reinforced with various nanoparticles, such as calcium carbonate (CaCO₃), to improve the mechanical capacity of constructs used in hyaline cartilage regeneration. The incorporation of silk fibers into chitosan/glycerophosphate hydrogels has shown enhanced mechanical properties and proliferation potential for chondrocytes, emphasizing the importance of polymer selection in tissue engineering [27].

2.5 Poly caprolactone (PCL)

Poly Caprolactone is a biodegradable polyester polymer derived from ϵ -caprolactone monomers, widely used in tissue engineering and drug delivery applications due to its biocompatibility and biodegradability. PCL supports cell adhesion, proliferation, and differentiation, promoting tissue regeneration [27]. Its biodegradability ensures it breaks down into non-toxic byproducts, eliminating the need for removal surgeries [27]. PCL exhibits favorable mechanical properties like flexibility and toughness, making it suitable for scaffolds and implants; these properties can be enhanced with nanoparticles like CNTs or graphene [31]. PCL is versatile in processing, allowing it to be shaped into various forms such as fibers, films, and scaffolds using techniques like electrospinning, 3D printing, and solvent casting [27,32]. PCL's compatibility with other materials allows for composite structures that enhance mechanical properties and tailor degradation rates [27,31]. It is extensively used in bone regeneration, cartilage repair, wound healing, and drug delivery systems, making it a valuable biomaterial in regenerative medicine [31,32,33].

2.6 Poly (sulfobetaine methacrylate) (PSBMA)

Poly (sulfobetaine methacrylate) is a polymer notable for its biocompatibility, making it suitable for tissue engineering and regenerative medicine. PSBMA's key features include hydration lubrication, which enhances lubrication capabilities, and electrostatic interaction, allowing for the creation of composite hydrogels with improved properties [1]. In tissue engineering, PSBMA is used in hydrogel synthesis, providing mechanical support and biocompatibility, and in drug delivery systems, offering controlled and sustained drug release. Its enhanced lubrication and minimal adverse reactions make PSBMA a valuable polymer for biomedical applications, particularly in tissue engineering and regenerative medicine [26].

2.7 Poly acrylic acid (PAA)

Poly Acrylic Acid is a synthetic polymer derived from acrylic acid, known for its hydrophilicity and versatility. It efficiently absorbs and retains water, making it useful in applications like water treatment, adhesives, and biomedical uses [11,34]. PAA's biocompatibility in certain formulations allows for its incorporation into hydrogels and drug delivery systems for controlled release of therapeutic agents. Its chelating properties aid in water purification and scale prevention in industrial systems. Studies have shown that PAA-based hydrogels promote wound healing and tissue regeneration by providing a moist environment [11,34].

2.8 Alginate

Alginate, a biopolymer from brown seaweed cell walls, is primarily composed of mannuronic acid (M) and guluronic acid (G) units, forming a gel-like structure. Its biocompatibility and gel-forming properties make it highly versatile. Alginate can encapsulate drugs, providing controlled release for therapeutic applications. It serves as a scaffold material, supporting cell growth and tissue regeneration. Alginate dressings absorb exudate, maintain a moist environment, and protect against infection and is used for immobilizing cells and enzymes in various biochemical processes [15,35,36,37].

We can categorize the nanoparticles used in tissue engineering into the following groups:

2.9 Mineral nanoparticles

2.9.1 Nano-Hydroxyapatite (nHAP)

Hydroxyapatite nanoparticles closely resemble bone minerals, making them highly bioactive and ideal for bone regeneration. They promote osteo conductivity, enhancing the attachment and growth of bone cells. Incorporated into polymeric scaffolds, nHAP improves their mechanical properties and bioactivity, facilitating bone healing and promoting osteogenic differentiation of stem cells [11,17,27].

2.9.2 calcium phosphate

These nanoparticles are used to enhance the bioactivity of scaffolds and promote bone regeneration [17]. They can improve cell proliferation and differentiation, though low crystallinity and high dissolution rates may inhibit osteogenic differentiation under certain conditions [17]. Commonly used in scaffold coatings, they improve bioactivity and mechanical properties, facilitating better integration with surrounding tissues [38-42].

2.9.3 Silicate

Silicate nanoparticles are promising materials for bone regeneration due to their ability to induce osteogenic differentiation and enhance mechanical properties. When incorporated into hydrogels and various scaffold designs, they improve bioactivity and overall scaffold performance. Their use in bone tissue engineering applications makes them suitable for promoting better integration with surrounding tissues [11].

2.9.4 Black phosphorus (BP)

Black Phosphorus (BP) is a novel 2D nanomaterial discovered in 2014, known for its excellent biodegradability and biocompatibility, making it ideal for biomedical applications. BP has significant potential in optoelectronics, biomedicine, photothermal and photodynamic therapies, drug delivery, and theranostics. In tissue regeneration, BP promotes mineralization and osteogenesis, making it effective for bone tissue repair. BP also exhibits antibacterial activity by damaging cell membranes and inducing ROS production. Incorporating BP into hydrogel platforms enhances functionalities like mineralization and bone formation, offering innovative solutions in regenerative medicine [26]. Due to the photothermal effect of BP, osteosarcoma can be effectively eliminated without recurrence. Moreover, as illustrated in Figure 2, the release of PO_4^{3-} that conjugated Ca^{2+} to form new calcium phosphate nanoparticles promoted biomineralization and osteogenesis [26] and provide a comprehensive overview of the role of bioactive glass in tissue engineering, particularly in bone regeneration, by illustrating its mechanisms of action, applications, and interactions with biological systems.

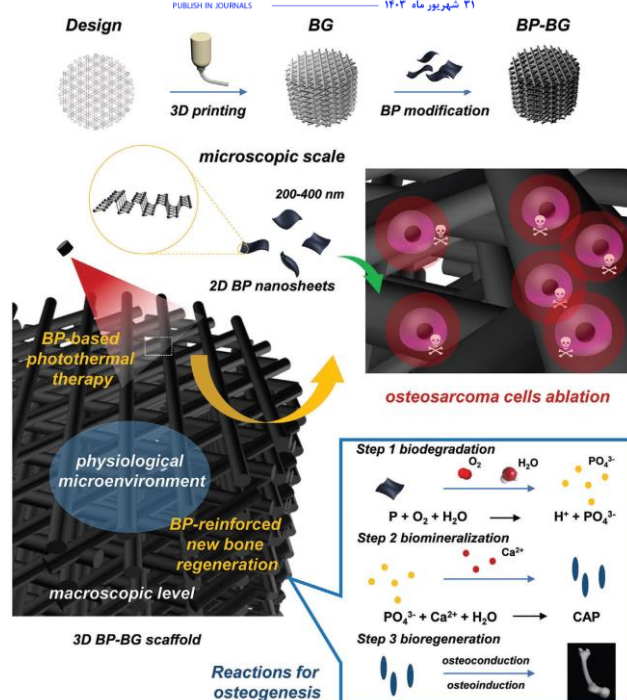


Figure 2. Schematic illustration of the fabrication process for BP–BG(bioactive glass) scaffold and the stepwise therapeutic strategy for the elimination of osteosarcoma followed by osteogenesis by BP–BG [94].

2.10 Carbon nanoparticles

2.10.1 Graphene oxide (GO)

Graphene oxide is derived from graphene through the oxidation of graphite, introducing functional groups like hydroxyl, epoxy, and carboxyl, which make it hydrophilic and dispersible in water. Its functional groups enhance chemical reactivity, allowing for composite formation and further modifications. GO is utilized in biomedical applications (drug delivery, tissue engineering, biosensing), electronics (conductive inks, sensors, flexible devices), composites (as a polymer reinforcing agent), and energy storage (supercapacitors, batteries) due to its unique properties and versatility [43,44]. GO nanoparticles have been extensively utilized in tissue engineering due to their unique physicochemical properties, such as high surface area and excellent mechanical strength [27,45].

When incorporated into composite hydrogels, GO enhances the mechanical properties and biocompatibility of the scaffolds, making them suitable for applications like cartilage repair [26].

2.10.2 Carbon nanotube (CNT)

Carbon Nanotubes are cylindrical carbon structures, either single-walled (SWCNTs) or multi-walled (MWCNTs), known for their exceptional mechanical strength and electrical conductivity. They enhance the mechanical properties and structural integrity of scaffolds in tissue engineering, particularly neural tissue, where their conductivity aids signal transmission for cell communication. CNTs support cell growth and proliferation, making them valuable for tissue repair and regeneration. Applications include neural axon regeneration and the development of composites with polymers like PLA and PCL. Their biocompatibility and electrical properties also enable their use in electrochemical sensors for biological detection and monitoring [11,27,31,32].

2.11 Metal nanoparticles

2.11.1 Gold nanoparticles (AuNPs)

Gold nanoparticles are biocompatible and easily functionalized, making them suitable for imaging and drug delivery. Their unique optical properties and ability to attach biomolecules enhance targeting specific cells or tissues. In tissue engineering, gold nanoparticles promote cellular interactions, tissue regeneration, and therapeutic agent delivery [11,15]. AuNPs are utilized in orthopedic tissue engineering for their surface conjugation and conducting properties. These nanoparticles play a crucial role in enhancing the antimicrobial properties of scaffolds, thereby reducing the risk of infections during tissue regeneration processes [27].

2.11.2 Silver nanoparticles (AgNPs)

Silver nanoparticles are valued for their antimicrobial properties, making them beneficial in wound healing, infection control, and drug delivery systems due to their controlled release capabilities [15]. They are incorporated into tissue engineering scaffolds to prevent infections during tissue regeneration, ensuring a sterile environment for cell growth and tissue repair [11]. In orthopedic implants, silver nanoparticles help reduce bacterial contamination, enhancing the success of implantation procedures [27]. Figure 3 shows a scheme emphasizing the steps involved in the mechanism of formation of AgNPs: reduction, nucleation and growth [93].

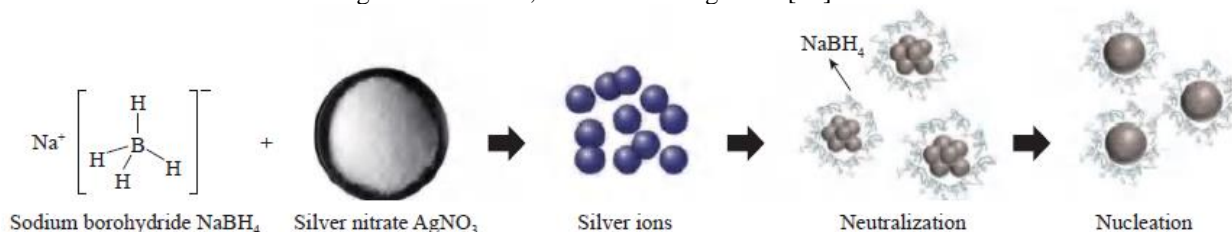


Figure 3. Schematic representation of synthesis of AgNPs by redox using sodium borohydride as a reducing agent and silver nitrate as a precursor agent [15].

2.11.3 Nano titanium oxide (TiO_2)

Nano titanium oxide (TiO_2) particles, sized between 1-100 nanometers, exhibit unique properties due to their high surface area to volume ratio, enhancing reactivity and performance in various applications. They are widely used in photocatalytic applications for environmental remediation, self-cleaning surfaces, and air purification systems. In sunscreens, nano titanium oxide effectively absorbs and scatters UV radiation, providing protection against harmful UV rays. Their antimicrobial properties make them valuable in coatings for medical devices, textiles, and other surfaces. In renewable energy, nano titanium oxide improves the efficiency of dye-sensitized solar cells by enhancing light absorption and electron transfer. Despite their benefits, potential toxicity and environmental impact are concerns, prompting ongoing studies to ensure safe use [27].

2.12 Magnetic nanoparticles (MNPs)

Magnetic nanoparticles are small particles, typically 20-300 nanometers, with unique magnetic properties suitable for various applications like tissue engineering, drug delivery, and biomedical imaging. They can be manipulated by external magnetic fields, aiding in targeted therapies and controlled drug delivery. MNPs, especially those modified with biocompatible materials like polyethylene glycol, are generally safe for biological use due to low cytotoxicity. They are used in tissue engineering to enhance cell patterning and organization, in mechanotransduction to stimulate cells mechanically, and in gene delivery systems to improve genetic material uptake. However, challenges such as improving synthesis, functionalization, and developing effective delivery systems remain [27].

2.13 Polymer nanoparticles

2.13.1 Poly(lactic-co-glycolic) acid nanoparticle (PLGA NPs)

PLGA is a biodegradable and biocompatible polymer used extensively in medical and pharmaceutical applications. It is a copolymer made from lactic acid and glycolic acid. PLGA is widely used for drug delivery, encapsulating therapeutic agents like proteins, peptides, and small molecules, protecting them from degradation, and controlling their release in the body. In tissue engineering, PLGA creates scaffolds that support cell growth and tissue regeneration, delivering growth factors like VEGF and bFGF to enhance tissue regeneration in models such as bladder and cartilage. It is also used in surgical implants that dissolve in the body, eliminating the need for surgical removal. PLGA's versatility and favorable properties make it a popular choice in drug delivery and regenerative medicine [15,20,21,22]. Figure 4 shows the general scheme of PLGA NPs synthesis, following the methodology described.

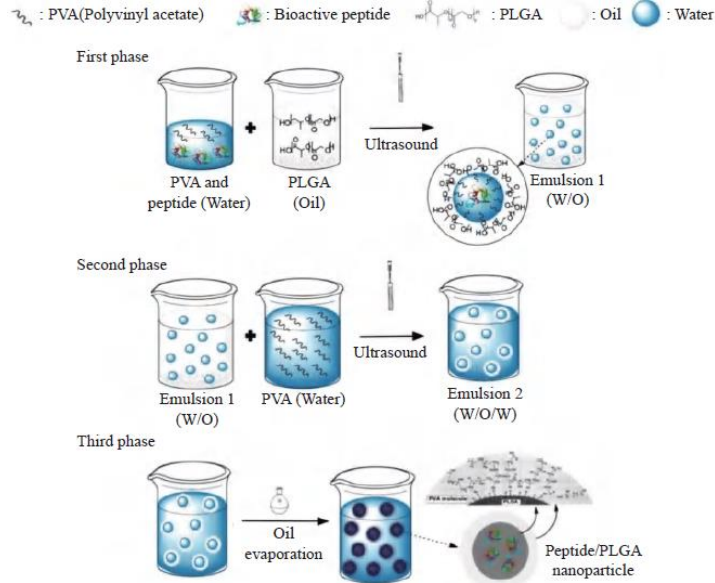


Figure 4. Schematics of PLGA NP synthesis. The first step represents the Emulsion 1 (water-in-oil) formation, which is used in the second step to prepare Emulsion 2 (water-in-oil-in-water). The third step represents oil evaporation to achieve the encapsulation of the peptide/drug.

Table 1. Optimal percentage of using the nanoparticles.

num	Nanoparticle	Optimal percentage (%)	Refrence
1	nHPA	5-30	[17]
2	Calcium phosphate	10-30	[17]
3	Graphene oxide (GO)	0.1-5	[17]
4	Silver nanoparticles (AgNPs)	0.1-1	[17]
5	Gold nanoparticles (AuNPs)	0.1-1	[27]
6	Titanium oxide (TiO ₂)	1-5	[27]
7	Silicate	1-3	[27]
8	Carbon nanotube (CNT)	0.1-1	[27]
9	Magnetic nanoparticles (MNPs)	2-5	[27]
10	Poly(lactic-co-glycolic) acid nanoparticle (PLGA NPs)	1-20	[17]

3. Manufacturing methods

The methods used to engineer body tissues involve a combination of advanced fabrication techniques, biomaterials, and biological processes. Here are some of the key methods employed in tissue engineering:

3.1 Electrospinning

Electrospinning is a versatile technique used in tissue engineering to create scaffolds with nanoscale fibers, offering control over spatial geometry and biological complexity [17]. The process involves applying an electric field to a polymer solution, forming ultrafine fibers collected on a grounded target to create a nonwoven mat or membrane. Key points include precise control over fiber diameter, alignment, and porosity, which are essential for mimicking native tissue structures [17]. Electrospun scaffolds have a high surface area-to-volume ratio, excellent mechanical properties, and can replicate the extracellular matrix, promoting cell adhesion, proliferation, and differentiation. Common polymers used include silk fibroin, polycaprolactone (PCL), and poly (lactic-co-glycolic acid) (PLGA).

The electrospinning setup typically consists of a syringe, a high-voltage power supply, a metallic needle, and a grounded collector. The polymer solution, loaded into the syringe, is dispensed through the needle under high voltage, forming a jet that solidifies into nanofibers as it travels towards the collector. These nanofibers, with diameters ranging from tens to hundreds of nanometers, mimic the natural extracellular matrix [15]. The high surface area, porosity, and

ability to functionalize with bioactive molecules make electrospun nanofibers ideal for tissue engineering applications, such as bone-cartilage, bone-tendon, and bone-ligament interfaces.

Electrospinning allows to produce nanofibers with controlled alignment, composition, and mechanical properties, crucial for scaffold design in tissue regeneration. The technique is also used in drug delivery, where fibers can be loaded with drugs for controlled release, and in filtration processes due to the high porosity and small pore size of electrospun membranes. Studies have demonstrated that electrospun scaffolds enhance cell adhesion, proliferation, and differentiation, highlighting their effectiveness in promoting tissue regeneration. Overall, electrospinning is a valuable method for producing nanofibrous scaffolds that closely resemble the native extracellular matrix, supporting cell interactions and tissue regeneration [15].

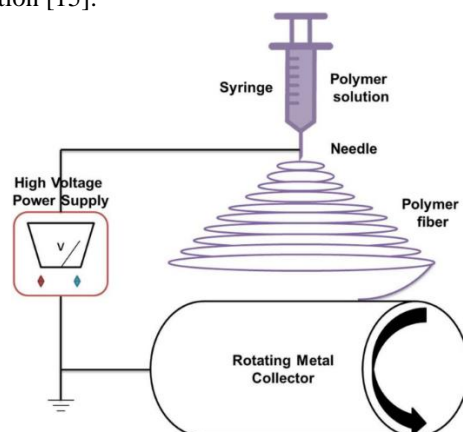


Figure 5. Schematic of electrospinning [109].

3.2 3D bio printing

3D bioprinting is an advanced technology that enables the precise layer-by-layer deposition of biomaterials, cells, and growth factors to create complex three-dimensional structures that mimic native tissues [46,47,48,49]. The process involves controlled deposition of biomaterials and bioinks to build intricate structures, allowing for the precise positioning of cells to create tissues with specific cell arrangements [46]. This technology offers significant advantages, including the ability to create personalized tissues tailored to individual patient needs, fabricate tissues with complex geometries, and maintain high cell viability during the printing process [46].

3D bioprinters can fabricate gradients in the x, y, and z directions, enhancing the biomimicry of native tissues [46,47]. The use of a dual nozzle syringe allows for simultaneous printing of multiple biomaterials, enhancing the versatility of the process [46,47]. Applications include tissue engineering for bone-cartilage, bone-tendon, and bone-ligament interfaces, as well as regenerative medicine for functional tissue development and transplantation.

Future advancements in 3D bioprinting technology are expected to revolutionize regenerative medicine and personalized healthcare, with ongoing research focusing on optimizing printing techniques, biomaterials, and cell sources for enhanced tissue regeneration and repair. Overall, 3D bioprinting offers an improved strategy for engineering interface tissues, advancing the field of tissue engineering [47,49].

The technology involves using a 3D bioprinter that deposits bio inks containing living cells and biomaterials layer by layer according to a digital model. Biomaterials such as hydrogels, bio inks, and polymers serve as the "ink," providing a scaffold for cell growth and tissue formation. Cells, including stem cells or patient-specific cells, are incorporated into the bioinks to ensure the printed tissues are functional and biocompatible.

3D bioprinting is utilized to create tissue constructs for regenerative medicine, enabling the development of customized implants and prosthetics, and holds potential for bioprinting organs like kidneys, liver, and heart tissues to address the organ shortage crisis. Bio printed tissues can also be used to study diseases, test drug efficacy, and personalize treatments. Advantages of 3D bioprinting include precision in cell and biomaterial placement, customization for patient-specific needs, and acceleration of tissue engineering and organ fabrication processes.

Challenges include creating functional blood vessel networks within bioprinted tissues and ensuring proper integration with the host's body upon transplantation. By providing a conducive environment for cell growth and differentiation, 3D bioprinting has the potential to effectively regenerate damaged tissues and organs [11, 53-57, 62] Figure 7.

3.3 Phase separation

Phase separation is a process where a homogeneous solution separates into two distinct phases, typically a polymer-rich phase and a polymer-poor phase. In tissue engineering, it is used to create scaffolds by dissolving polymers in a solvent and inducing phase separation to form porous structures. This method allows for the creation of scaffolds with interconnected pores, promoting nutrient diffusion and cell infiltration for tissue regeneration. The process can be tailored to control porosity and surface properties, influencing cell attachment and proliferation. Challenges include achieving precise control and uniformity in scaffold properties. Ongoing research aims to develop advanced phase separation techniques for improved tissue regeneration and biomimetic scaffold design [17].

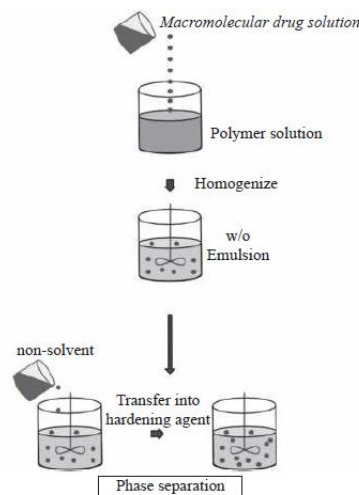


Figure 6. Nano/microencapsulation methods: polymer phase separation . The aqueous solution is dispersed in the polymeric solution through a w/o ultrasound emulsion [95].

3.4 Salt leaching

Salt leaching is a process used in tissue engineering to create porous scaffolds by dissolving salt particles from a material, leaving behind interconnected pores [11]. This technique involves mixing salt particles with scaffold materials, solidifying the composite, and then immersing it in a solvent to remove the salt, creating a porous structure [11]. Commonly used salts include sodium chloride and ammonium bicarbonate [11]. The process enhances cell infiltration, nutrient transport, and waste removal, improving the scaffold's biocompatibility and mechanical properties [11]. Salt leaching is widely used to fabricate scaffolds for bone, cartilage, and skin regeneration [11]. However, challenges include controlling pore size distribution and ensuring uniform porosity [11]. Advanced techniques are being explored to optimize scaffold properties and functionality for improved tissue regeneration [17] Figure 7.

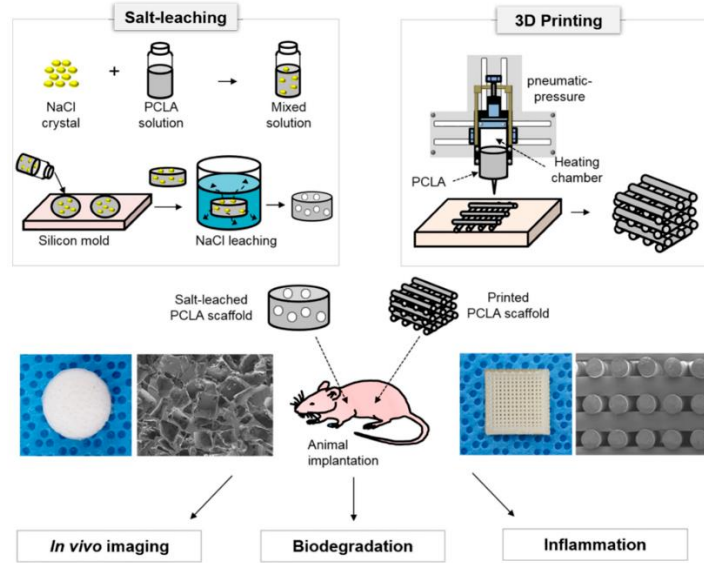


Figure 7. Schematic of salt leaching and 3D printing [110].

3.5 Lyophilization

Lyophilization, or freeze-drying, removes water from materials by freezing and sublimating the ice under vacuum conditions, bypassing the liquid phase. The process includes freezing the material, primary drying through sublimation, and secondary drying to eliminate residual water. This technique is widely used in pharmaceuticals, food preservation, and biotechnology for stabilizing sensitive substances like drugs, vaccines, and enzymes. Advantages include preserving the integrity of heat-sensitive materials and extending shelf life, but it is costly and complex due to specialized equipment and long processing times. Future advancements aim to improve efficiency and reduce costs through automation and innovative formulations. Lyophilization is particularly valuable in tissue engineering for creating porous scaffolds that support cell growth and tissue regeneration [63] Figure 8.

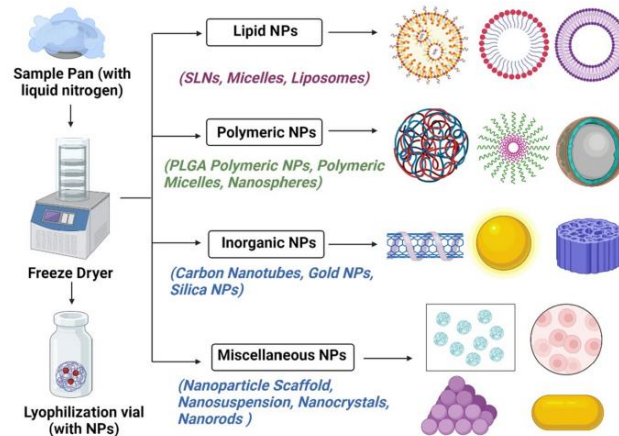


Figure 8. The structural form of various nanoparticles in lyophilization [111].

3.6 Stereolithography (SLA)

Stereolithography is an additive manufacturing technology that creates three-dimensional objects by curing liquid photopolymer resin with ultraviolet (UV) light. The process involves building objects layer by layer, where each layer is solidified by a UV laser or projector based on the design. Common applications include rapid prototyping, medical models, and customized products like dental appliances and jewelry. The technique is valued for its high precision, allowing the production of detailed and accurate parts, and its speed, which facilitates quick prototyping and manufacturing. Additionally, SLA is versatile, working with various resins that offer different properties such as transparency and flexibility [64].

3.7 Robotic dispensing

Robotic dispensing involves using robots to precisely dispense materials to create complex structures like tissue engineering scaffolds [69]. The process programs the robot to move along different planes (xy-plane), assembling 2D

fibrous structures layer-by-layer into 3D scaffolds [69]. A slurry with specific rheological properties is prepared for injection through a nozzle for precise dispensing [69]. Robotic dispensing creates bioactive scaffolds with components like bioactive glass-polycaprolactone that promote bone bioactivity [70]. The apparatus includes a syringe, needle, and force-controlled plunger for flow regulation, guided by a computer-controlled positional unit [65]. This technique enables the creation of porous scaffolds with defined pore configurations, supporting cell growth for tissue engineering [65]. Overall, robotic dispensing is crucial for fabricating advanced biomedical scaffolds [70] Figure 9.

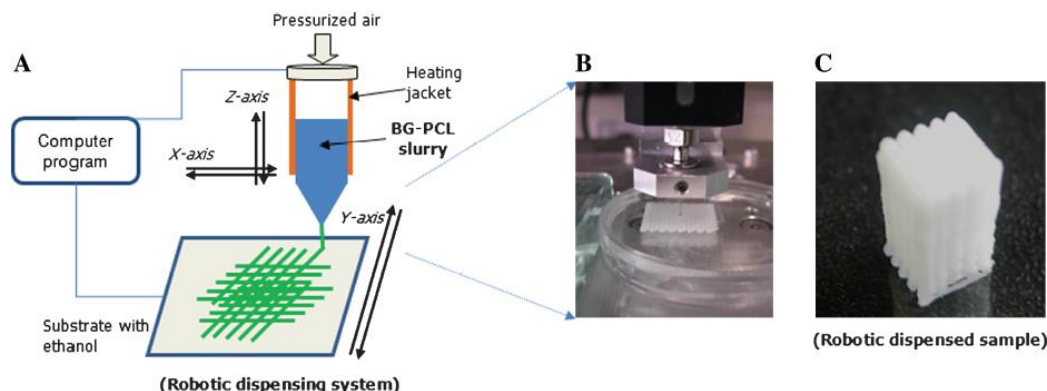


Figure 9. (A) Schematic diagram of robotic dispensing method, (B) enlargement of the dispensing nozzle and deposited fiber mesh, and (C) the constructed bioactive glass (BG)-poly(e-caprolactone) (PCL) composite three-dimensional scaffold [65].

3.8 Bio reactor system

Bioreactor systems are controlled environments for cultivating cells or tissues under specific conditions, allowing manipulation of factors like temperature, pH, and nutrient supply, crucial for cell growth and tissue development [71-73]. They enable reproducible changes in environmental factors, essential for studying their effects on tissues [71-73]. Bioreactors support the safe, reproducible production of tissue constructs necessary for regenerative medicine and transplantation [71-73]. They enhance nutrient and oxygen transfer to cells, critical for their survival and proliferation, with systems designed to improve distribution [77]. Some bioreactors apply mechanical forces, mimicking natural tissue environments to promote growth [74-76]. These systems minimize contamination risks, can be scaled up for clinical applications, and include various designs like fixed bed, fluidized bed, and well-mixed systems, tailored for specific tissue engineering needs [77,66]. In summary, bioreactor systems are crucial for controlled cell cultivation, nutrient transfer, and producing complex tissue constructs for medical use [66].

3.9 Self assemble

Self-assembly is a process where molecules spontaneously organize into structured arrangements without external guidance, significant in materials science for creating hydrogels used in drug delivery and tissue engineering [67]. Self-assembling systems involve carefully designed molecules, like peptide oligomers, forming stable structures through various interactions such as hydrogen bonding and ionic interactions [78-83]. These systems respond to stimuli like temperature and pH, transitioning between sol and gel states [78-83]. Hydrogels can encapsulate and release therapeutic agents in a controlled manner, exemplified by PEG/PLGA block copolymer hydrogels sustaining the release of proteins like insulin over extended periods [82,83]. They serve as scaffolds for tissue regeneration, supporting cell growth, and leading to cartilage regeneration with high mechanical strength [78,79,84]. Functionalized hydrogels enhance wound healing by incorporating bioactive molecules like epidermal growth factor (EGF) [85,86]. Peptides like RAD-16 form hydrogels suitable for neural repair by supporting neuronal attachment and differentiation [78,84-86]. Despite their potential, self-assembled hydrogels can trigger unwanted immune responses in vivo, posing a challenge [81-83]. In summary, self-assembly is vital for creating versatile hydrogels with applications in drug delivery and tissue engineering, driven by their responsiveness and biological support [67].

3.10 Cell spraying

The cell spraying method is an innovative technique in tissue engineering for creating three-dimensional (3D) structures that mimic natural tissues. This technique involves depositing various cell types and extracellular matrix components in a 3D configuration without significantly damaging the cells, allowing precise control over their distribution [87]. It has been used successfully for spraying keratinocytes for skin healing and chondrocytes for cartilage regeneration, helping reconstruct biological tissues [88,89]. The process involves spraying a sodium alginate solution containing cells onto a surface using an airbrush system, with adjustable spraying pressure to optimize cell viability and distribution, followed by solidifying the gel in a calcium chloride solution [68]. The method maintains

high cell viability, with over 80% of sprayed cells remaining active, which is crucial for the functionality of engineered tissues [90]. Additionally, the sprayed biomaterials exhibit enhanced mechanical properties, important for applications like cartilage tissue engineering [91,92]. The method's potential for layering different cell types and materials offers promise for developing complex, functional tissue constructs for regenerative medicine [68]. In summary, the cell spraying method is a versatile and effective approach in tissue engineering, facilitating the creation of complex tissue structures while preserving cell viability and enhancing mechanical properties [68].

4. Common tests taken and their results

4.1 Mechanical tests

4.1.1 Tensile modulus

The tensile modulus, also known as the elastic modulus or Young's modulus, measures a material's ability to resist deformation under tensile stress. It is defined as the ratio of tensile stress (force per unit area) to tensile strain (proportional deformation in length) in the linear elastic region of the material's stress-strain curve. A higher tensile modulus indicates that the material is stiffer, less prone to stretching, and can withstand greater forces without deforming. Conversely, a lower tensile modulus suggests greater flexibility [27].

The incorporation of graphene oxide (GO) significantly increases the tensile modulus of polycaprolactone (PCL) composites, which indicates an increase in stiffness and load-bearing capacity, for example, unmodified GO increases the Young's modulus of PCL by 48%. While GO modified with amine functionalized GO (AGO) increases it to 71% and for different formulations to 76% [11], in the field of hydrogels, such as hydroxylated boron nitride nanosheets (OH-BNNS)/PVA composite, the tensile modulus due to The interaction between PVA chains and OH-BNNS is significantly improved, this increase makes the hydrogel more suitable for applications that require mechanical strength, such as cartilage repair (Figure 10) [26]. The incorporation of nanohydroxyapatite (nHAp) into polycaprolactone (PCL) scaffolds increases the tensile modulus. In particular, the presence of nHAp helps to improve the mechanical properties by increasing the stiffness of the scaffold (Figure 11) This is important for applications in bone regeneration, as a higher tensile modulus indicates better load-bearing capacity and structural integrity of the scaffold. The mineral gradient created by the different nHAp concentration plays an important role in achieving the desired mechanical properties and makes the scaffolds more effective in mimicking the natural bone environment [17].

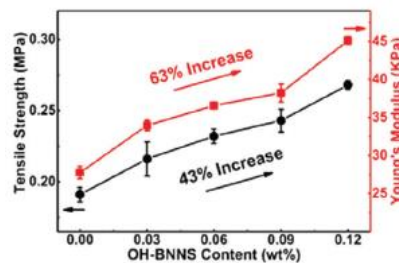


Figure 10. tensile strength of the OH-BNNS/PVA were controllably enhanced with increasing OH-BNNS content. [96]

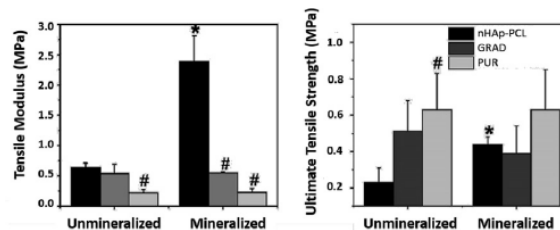


Figure 11. The tensile modulus for the mineralized nHAp-PCL fibers is significantly higher than all other fibers (*p = 0.002).

4.1.2 Stiffness

Mechanical stiffness refers to the rigidity of a material or structure and its resistance to deformation under an external force. It is influenced by factors such as material composition, structure, and geometry, and is assessed through mechanical tests like tensile, bending, and compression tests [15]. The addition of agarose gel with hydroxyapatite (AGA) in scaffolds has a notable impact on mechanical stiffness. When nHAp is combined with agarose gels, it results in a significant increase in compressive modulus, which is a measure of mechanical stiffness. The study indicates that the uniform distribution of nHAp within the agarose matrix enhances the overall mechanical properties, leading to scaffolds that can better withstand mechanical loads. This improvement in mechanical stiffness is essential for the scaffolds to support cellular activities and tissue regeneration effectively [17].

4.1.3 Elongation

Elongation refers to the extent a material can be stretched or deformed before breaking, expressed as a percentage of its original length. High elongation indicates good ductility, essential for materials that need to withstand deformation without fracturing. It is a critical measure of how much a material can deform plastically before failure, with high elongation values indicating better energy absorption and lower fracture risk. Results showed that modified GO composites had superior ductility and strength compared to unmodified GO and free polymer, enhancing their ability to elongate under stress [11].

4.1.4 Compressive strength

Compressive strength refers to a material's ability to withstand axial loads that compress or shorten it. It is crucial for materials used in load-bearing applications. The compressive modulus indicates a material's resistance to deformation under compression; higher values signify better resistance. The inclusion of OH-BNNS in PVA hydrogels enhances compressive strength, beneficial for cartilage or bone repair. Similarly, scaffolds with GO nanoparticles show improved compressive strength, making them suitable for tissue engineering. These improvements are due to enhanced mechanical properties, such as hydrogen bonding between PVA and OH-BNNS, contributing to the material's overall stability and strength [11,26].

4.2 In vivo (inside the body) and In vitro (in a laboratory environment)

both in vitro and in vivo testing are crucial for evaluating the potential of nanoparticles in tissue engineering. In vitro studies provide foundational insights into cellular interactions, while in vivo studies confirm the efficacy and safety of nanoparticles in biological environments, highlighting the need for further research to validate findings and ensure safe applications.

In vivo studies involve testing nanoengineered scaffolds in living organisms, typically animal models, to assess their integration with biological tissues and effectiveness in promoting tissue regeneration. This approach evaluates the scaffolds' performance in a real biological environment, focusing on mechanical properties and biological responses. In vivo testing provides a comprehensive understanding of material interactions with biological systems, including metabolism, immune response, and physiological effects. These studies are crucial for evaluating the real-world effectiveness and safety of medical applications like drug delivery systems and tissue engineering scaffolds. An example includes MOS₂ nanosheets used in spinal cord injury models, where in vivo experiments confirmed neuroprotection and locomotor recovery, highlighting their therapeutic potential [11,97].

In vitro studies, conducted in controlled laboratory settings using cell cultures, are crucial for evaluating biological responses of scaffolds and understanding fundamental mechanisms before in vivo applications. These studies isolate specific processes and observe treatment effects on cells or tissues without the complexities of a whole organism. In vitro testing is essential for assessing the initial efficacy and safety of new drugs or materials. The paper highlights using in vitro assays to evaluate biocompatibility of hydrogels and scaffolds, like polysaccharide-based hydrogels showing high cellular viability for skin tissue engineering [11]. In vitro studies of 2D nanomaterials, such as graphene oxide, demonstrate enhanced cell proliferation and differentiation, with insulin-loaded GO in a hydrogel promoting adipogenic differentiation [107,108]. Gold nanoparticles (GNPs) and titanium dioxide (TiO₂) nanoparticles also show promising outcomes, enhancing osteogenic and adipogenic differentiation of mesenchymal stem cells (MSCs) [27].

Nanoparticles' impact on cell viability and processes is crucial in both in vivo and in vitro studies, necessitating detailed investigations into shear stress effects, especially using animal models [17]. Assessing short-term and long-term toxicity is challenging due to complex interactions with biological systems, particularly for new materials [17]. Nanoparticle size, shape, and surface properties significantly influence their behavior, with smaller particles exhibiting different cellular uptake mechanisms [98,99]. Long-term accumulation can cause inflammatory reactions, requiring thorough safety evaluations [17]. In vivo tests show that nanoengineered scaffolds, like those with nanohydroxyapatite (nHAp), integrate well with tissues and aid tissue regeneration and mesenchymal stem cell differentiation [17]. These tests provide insights into treatment effectiveness, including bioavailability and long-term safety. In vitro studies indicate that nHAp-loaded scaffolds enhance cellular activities such as alkaline phosphatase (ALP) activity and collagen production, suggesting support for cell viability and tissue regeneration [17]. In vitro results also show

effective cellular adhesion, proliferation, and the formation of tissue-like structures, essential for tissue engineering applications [11]. Graphene oxide (GO)-based scaffolds promote wound healing and show excellent potential for skin tissue engineering [100-103], while gold nanoparticles (GNPs) enhance bone tissue regeneration, potentially replacing traditional growth factors [27].

4.3 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) uses a focused electron beam to produce high-resolution images of a sample's surface, allowing for detailed observation of fine structures at the nanometer scale. SEM is particularly effective for studying surface topography and can be combined with Energy Dispersive Spectroscopy (EDS) for elemental analysis. This technique is essential in fields like materials science, biology, and nanotechnology for understanding material properties and interactions.

In Figure 12 show the Alginate scaffolds do not allow uniform distribution of hydroxyapatite. While agarose gels allowed uniform distribution of hydroxyapatite in micro and nano dimensions. EDS and FTIR confirm the presence of calcium and phosphorus, which are essential for bone conduction, and show that cell-loaded scaffolds exhibit higher mechanical stiffness than cell-free scaffolds. These findings emphasize the importance of scaffold design, uniformity, morphology and mineral composition for orthopedic applications. In a study, a mineralized graded scaffold formed on plasma treated PLGA and gelatin coated PCL electrospun fibers was investigated [112] in Figure 13, provides critical insights into nanoengineered biomaterials for orthopedic tissue interfaces, illustrating nanofibrous structures essential for mimicking extracellular matrix and enhancing cell attachment and proliferation. In Figure 14, shows the addition of nHAp creates specific morphologies in the bone and fibrocalcified cartilage areas, and the pore size of the bone area decreases due to the penetration of HA crystals into the collagen matrix [17]. critical structural characteristics of nanoengineered scaffolds for orthopedic tissue interfaces. a well-defined nanofibrous architecture for replicating the extracellular matrix and enhancing cell adhesion and proliferation, a rough surface texture for better cell attachment and migration, and a uniform distribution of nano-hydroxyapatite (nHAp) to enhance bioactivity and osteoconductivity [113]. Additionally, morphological features like pore size and interconnectivity are crucial for nutrient exchange and long-term performance, while the robust nanofibrous structure contributes to the mechanical strength and stability of the scaffolds in load-bearing applications [113-115].

In Figure 15, shows the presence of nHAp on PCL fibers and the smooth morphology of PUR fibers. Furthermore, when exposed to Simulated Body Fluid (SBF), a gradient in mineral content is apparent [17]. In Figure 16, PCL and PLGA is respectively chosen for the aligned and random portions of the scaffold and the transition region contained both materials [17].

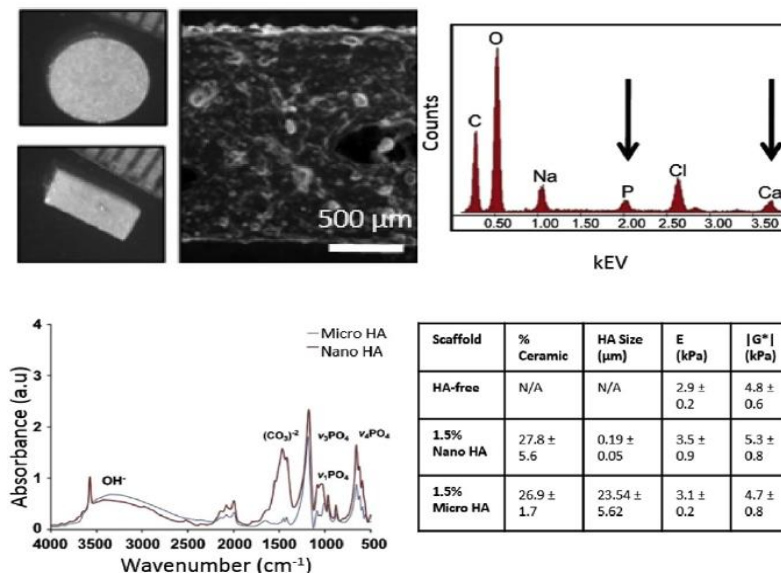


Fig 12. SEM show uniform distribution of nHAp in agarose gel and presence of calcium (Ca) and phosphorous (P) is confirmed by EDS and FTIR analysis. No significant effect of nHAp on elastic modulus and shear modulus is observed [17].

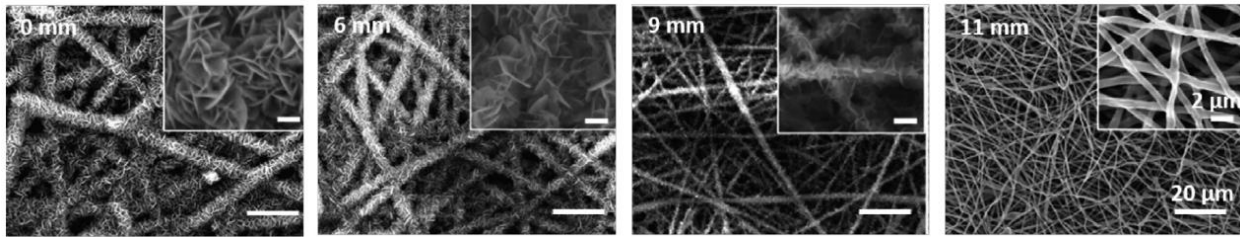


Fig 13. SEM images of calcium phosphate graded PLGA nanofibers at different distances along the scaffold [17].

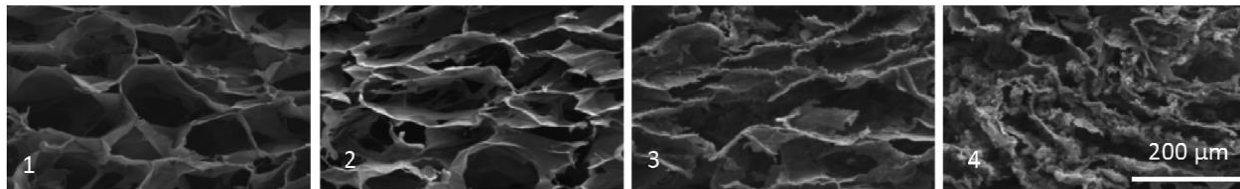


Fig 14. SEM images of different layers of the scaffold indicate a porous and interconnected network. A gradual change in pore size and mechanical stiffness was observed from the tendon layer to the bone layer [17].

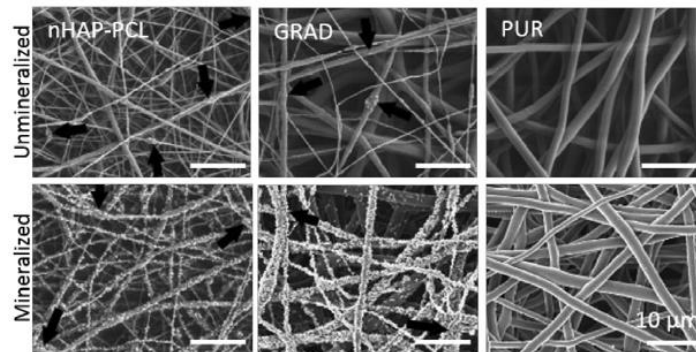


Fig 15. SEM images before and after treatment with SBF [17].

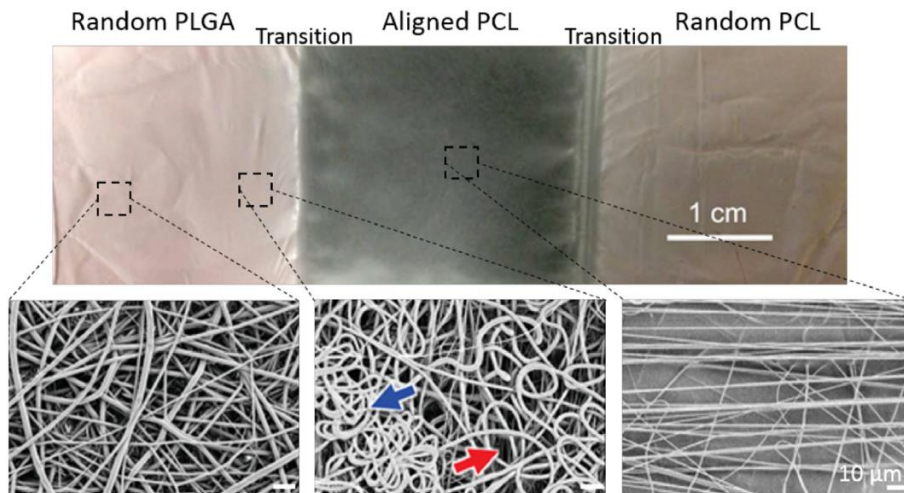


Fig 16. The electrospun scaffold consists of three regions, random PLGA fibers, aligned PCL fibers and random PCL fibers. SEM images reveal microstructure of each region [17].

The scaffold surface was further tailored with a calcium phosphate mineral phase. The BG-PCL composite scaffolds were found to induce a mineral phase rapidly and profoundly within the simple body fluid (SBF) under in vitro conditions [65]. Hence, this mineralization behavior was used to coat the surface of the scaffold. Figure 17 shows the robotic dispensed BG-PCL scaffold after the SBF treatment for 7 days. Throughout the scaffold surface calcium phosphate precipitation occurred and the 3D pore configuration was not changed significantly (Fig. 17A). A higher magnification of the surface showed a uniform thick layer of nanocrystallites (Fig. 17B). The atomic composition of the mineral phase was detected by energy dispersive spectroscopy (Fig. 17C), which shows a significant increase in

the Ca and P peaks, and the disappearance of the Si peak. The Ca=P ratio was 1.45, which was slightly lower than that for stoichiometric hydroxyapatite (Ca=P/1.67) but similar to the characteristics of a biological carbonated apatite found in bone and teeth [116,117].

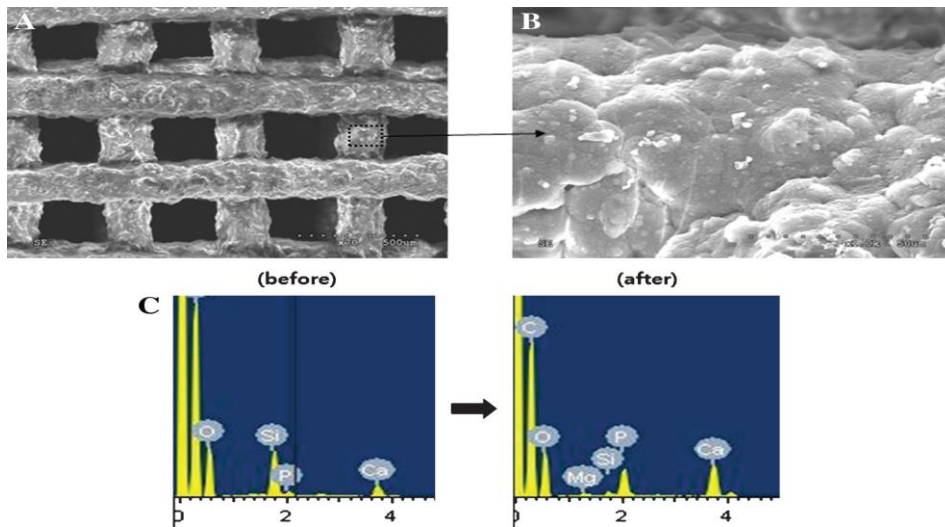


Figure 17. Morphology of the BG-PCL 3:1 composite scaffold with surface mineralization for 7 days: (A) macroscopic view and (B) magnified image showing the covered calcium phosphate minerals on the scaffold surface. (C) Energy dispersive spectroscopy atomic profile showing the increase in the Ca and P peaks with a concomitant decrease in the Si peak after mineralization. The Ca-to-P ratio was 1.45 around the mineralized phase [65].

The scaffold stems were covered almost completely with a number of cells that formed a thick cell layer (Fig. 18A, B). The cell layer appeared to secrete a fibrous extracellular matrix (Fig. 18C), and bridged some parts of the pore channels (Fig. 18D) [65].

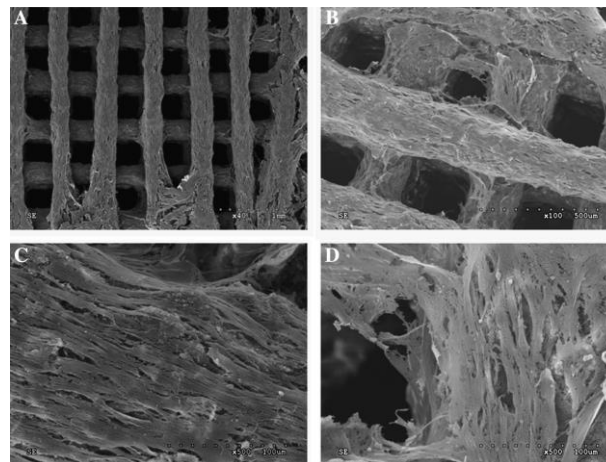


Figure 18. (A, B) Scanning electron micrographs of the cells populated on the mineralized BG-PCL scaffold with culturing for 2 weeks by the perfusion system, taken at different magnifications. Scaffold stems were completely covered with a thick layer of cells. (C) The cells secreted a fibrous extracellular matrix on the mineralized surface. (D) Some parts of the pore channels were bridged with cell layers [65].

5. Conclusion

The integration of nanotechnology into tissue engineering is a major advancement in regenerative medicine, providing innovative solutions for repairing and regenerating damaged tissues and organs. Nanomaterials like graphene oxide and carbon nanotubes improve the mechanical properties and biocompatibility of scaffolds, crucial for bone and cartilage repair. These materials not only provide structural support but also enhance cellular interactions and promote osteogenesis, essential for successful tissue regeneration. Bioactive glasses and other bioactive materials stimulate biomineralization and release ions that encourage cell proliferation and differentiation, particularly aiding bone regeneration. Some nanomaterials, such as black phosphorus, possess photothermal properties, allowing for targeted therapies that can eliminate cancer cells while promoting tissue repair.

Despite these promising applications, challenges remain in the synthesis, functionalization, and safe delivery of nanomaterials. Ensuring their safety and efficacy in clinical settings is critical, requiring rigorous testing and regulatory oversight. Future research should focus on optimizing nanocomposite designs and exploring wider biomedical applications beyond tissue engineering. The convergence of nanotechnology and tissue engineering holds significant promise for regenerative medicine, as advanced scaffolds can support tissue growth and actively participate in the healing process. Continued research in this field is expected to lead to breakthroughs that enhance patient outcomes and expand the possibilities for tissue regeneration.

Tissue engineering is a transformative approach in biomedical science, merging engineering principles with biological systems to repair or replace damaged tissues and organs. Scaffolds, often enhanced with nanotechnology, play a crucial role as templates for cell growth and organization, mimicking the natural extracellular matrix to promote more effective tissue regeneration. Materials such as poly (L-lactic acid) and polyethylene glycol are promising due to their biocompatibility and modifiability for specific uses, such as bone and eye tissue engineering. The integration of nanoparticles like hydroxyapatite and graphene oxide has further enhanced scaffold bioactivity and mechanical properties, improving tissue repair outcomes. The use of three-dimensional bioprinting and high-throughput genetic screening helps optimize biomaterial formulations, offering new diagnostic and therapeutic avenues. Nanomedicine also contributes to drug delivery systems, providing targeted and controlled release of therapeutic agents, which increases treatment efficacy and reduces side effects. Advanced imaging techniques, like Scanning Electron Microscopy (SEM), play a crucial role in studying the interactions between cells and scaffolds at the nanoscale. Overall, the convergence of nanotechnology, advanced materials, and biological science in tissue engineering offers immense potential for future medical applications, paving the way for innovative treatments and improved patient outcomes.

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