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Modification of Biodegradable Polymer Nanofibers for Cartilage Tissue Engineering Applications: A Review

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Abstract: Tissue engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physiochemical factors to improve or replace biological functions at the injured site. The designing of a biomaterial that can mimic the three-dimensional tissues *in vivo* is still challenging. Biodegradable polymers are used for the development of tissue engineering constructs in the form of sponges, films, and macroporous scaffolds, which do not influence cell fate processes such as cell differentiation, migration, and proliferation. Biodegradable polymer nanofibers fabricated by electrospinning have gathered great attention in tissue engineering applications. The electrospun materials have a nanofibrous morphology that is closest to the natural extracellular matrix (ECM). The electrospun material is composed of three-dimensional networks of nanosized fibrous materials that mimic an extracellular matrix such as collagen, elastin, and keratin. These polymers fabricated in the form of fibers in nanosize cause a more favorable microenvironment for cells. We prepared the PLGA/PPG (polylactic-co-glycolic acid/polypropylene glycol) nanofibers by electrospinning technique. PLGA (85:15)/PLGA (75:25) nanofibers are hydrophobic, which can be minimized by the addition of PPG to give better hydrophilicity for cell adhesion for tissue engineering constructs. The morphology of the electrospun fibers of the composite of PLGA and PPG was observed using scanning electron microscopy (SEM). The results proved that the small amount of polypropylene glycol polymer to the polylactic-co-glycolic acid (PLGA 85:15) drastically improves the hydrophilicity of the electrospun nanofibers. The addition of a small amount of hydrophilic polymer to the biodegradable hydrophobic polymer increases the hydrophilic property and can be used for nanofiber-based tissue engineering constructs. In addition, the biomimetic approach for tissue engineering scaffolds for cartilage repair has been discussed.

Keywords: Biodegradable polymers; Electrospinning; Polylactic-co-glycolic acid; Cartilage repair and biomimetic approach

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1. Introduction

Electrospinning is an innovative method used for producing ultrafine fibers with diameters ranging from a few

nanometers to several micrometers. It involves the application of an electric field to generate a high voltage between polymer solutions or melts and a grounded collector. This voltage creates a strong electrostatic force that overcomes the surface tension of the polymer solution, causing the formation of a thin jet of polymer solution that is subsequently stretched and solidified into fibers as it travels toward the collector. The electrospinning process offers several advantages over conventional spinning techniques [1]. Firstly, it can produce nanofibers with a large surface area-to-volume ratio, which is desirable for a wide range of applications such as biomedical engineering, filtration systems, and energy storage devices. The small diameter of these fibers can also provide mechanical properties (such as strength and flexibility) superior to those of conventional fibers [2,3]. Furthermore, electrospinning is a relatively simple and cost-effective method compared to other techniques used for producing nanofibers, such as molecular self-assembly or electron beam lithography. It does not require complex equipment or elaborate setup, making it accessible to researchers in various fields. The versatility of electrospinning enables the use of different polymers, blends, composites, or functional materials to create a wide variety of fiber structures. The process parameters, such as the polymer concentration, solvent composition, and applied electric field, can be adjusted to control the morphology, diameter, and alignment of the resulting fibers. In recent years, there has been an increasing interest in the development of novel electrospinning techniques, such as coaxial electrospinning and near-field electrospinning, which further enhance the fiber properties and allow the encapsulation of active substances or the direct writing of fibers onto specific targets. Despite its numerous advantages, electrospinning presents some challenges. The process parameters need to be carefully optimized to achieve the desired fiber morphology and prevent issues such as beading or clogging [4]. The scalability of electrospinning to large-scale production is also a current area of research and development. With continued advancements in materials and process optimization, electrospinning holds great potential for the production of functional nanofibers with tailored properties to meet the demands of modern technology and industry. This method has been widely used in the development of tissue engineering constructs and medical devices [3,5].

Tissue engineering is a rapidly growing field that focuses on regenerating functional tissues and organs to replace damaged or diseased ones [6]. One key aspect of tissue engineering is the development of scaffolds, which provide a three-dimensional (3D) structure for cells to grow and regenerate tissue [7]. **Figure 1** shows the hierarchy of tissues in the human body. The tissue engineering approach can help to repair, regenerate, or restore the injured tissue [8,9]. Electrospinning has emerged as a powerful technique in scaffold development due to its versatility and ability to create structures that closely mimic the properties of natural extracellular matrix. Through electrospinning, these fibers can be collected to form a nanofibrous scaffold with desired structural and mechanical properties. One of the major advantages of electrospinning is the ability to control the fiber diameter. The electrospun fibers can range from a few nanometers to several micrometers, which is similar to the scale of natural extracellular matrix fibers found in tissues. This fine control over fiber diameter allows for the mimicking of native tissue architecture, which is crucial for cell attachment, proliferation, and differentiation [10,11]. Electrospun scaffolds also have a high surface-to-volume ratio, which can promote cell adhesion and nutrient exchange. The interconnected porous structure of electrospun scaffolds allows for the diffusion of oxygen, nutrients, and waste products, enabling cells to thrive and function properly. Furthermore, electrospinning allows for the incorporation of bioactive molecules into the scaffold, such as growth factors, peptides, and drugs [12,13]. These bioactive molecules can be loaded into the polymer solution prior to electrospinning or post-loaded onto the electrospun fibers. This controlled release of bioactive molecules can enhance cell behavior, promote tissue regeneration, and modulate the immune response. Moreover, electrospun scaffolds can be fabricated using a variety of polymers, including natural polymers (e.g., collagen, gelatin) and

synthetic polymers (e.g., polycaprolactone, polylactic-co-glycolic acid). The choice of polymer can be tailored to the specific tissue engineering application, taking into account factors such as biocompatibility, degradation rate, and mechanical properties. It is a versatile and powerful technique in tissue engineering scaffold development. It allows for the fabrication of scaffolds with controlled fiber diameter, high surface-to-volume ratio, and incorporation of bioactive molecules [14]. These scaffolds closely mimic the native tissue architecture, promoting cell attachment, proliferation, and differentiation. Electrospun scaffolds hold great potential for various tissue engineering applications, including wound healing, bone regeneration, cartilage repair, and organ transplantation. The scaffold can act as a reservoir for the cells, drugs, or bioactive molecules for delivery into the damaged site of tissues. The scaffold should have the capacity to induce the cell fate process and produce a microenvironment for better regeneration of the damaged tissues [15]. **Figures 2** and **3** show the concept of tissue engineering scaffold development.

Electrospinning of biodegradable polymers into nanofibrous scaffolds as tissue engineering construct is a versatile technique that has gained significant attention. This process involves the formation of ultrafine fibers from a polymer solution using an electric field. Biodegradable polymers, which can naturally degrade into non-toxic byproducts, are particularly attractive in various biomedical applications due to their ability to minimize long-term foreign body reactions and avoid the need for implant removal. The electrospinning process begins with the preparation of a polymer solution, where a biodegradable polymer is dissolved in a suitable solvent. The choice of polymer and solvent is crucial, as it directly influences the properties of the resulting fibers. Some commonly used biodegradable polymers for electrospinning include polylactic acid (PLA), polyglycolic acid (PGA), polylactic-co-glycolic acid (PLGA), and polycaprolactone (PCL). Once the polymer solution is prepared, it is loaded into a syringe or reservoir connected to a metallic needle or spinneret. A high voltage is applied to the syringe, creating an electrostatic field. As the polymer solution is delivered, a jet of polymer is formed at the needle tip due to the electrostatic repulsion between the charged polymer chain and the applied electric field. The jet is then elongated and thinned by the electrostatic forces, resulting in the formation of ultrafine fibers. The collection of these fibers can be done on a stationary or rotating collector, which is usually a grounded plate or drum. The distance between the needle tip and the collector, as well as the applied voltage, can be adjusted to control the morphology and diameter of the electrospun fibers. Factors such as polymer concentration, solution viscosity, and flow rate also play a crucial role in determining the fiber properties. The unique structural characteristics of electrospun biodegradable polymer fibers, including their high surface area-to-volume ratio and fine fiber diameter attributed to their suitability for various biomedical applications. They can mimic the extracellular matrix (ECM) environment and provide a three-dimensional nanostructured scaffold for cell attachment, proliferation, and differentiation. Additionally, the fiber diameter can be tailored to match the dimensions of natural ECM fibers, enhancing cellular interactions and promoting tissue regeneration. The biodegradability of these electrospun fibers allows for the controlled release of encapsulated drugs or growth factors, making them ideal for drug delivery systems. The degradation rate can be adjusted by selecting an appropriate polymer, molecular weight, or composition, ensuring the sustained release of therapeutic agents over a desired time. Furthermore, the electrospinning technique enables the formation of composite fibers by incorporating various materials such as nanoparticles, proteins, or bioactive molecules into the polymer solution [16]. This provides an avenue for the development of multifunctional scaffolds with enhanced mechanical properties, improved surface bioactivity, and tailored release profiles [17,18].

Tissue engineering is a rapidly advancing field that aims to regenerate damaged or diseased tissues using a combination of cells, biomaterials, and growth factors. One area of focus in tissue engineering is the development of cartilage, a crucial tissue found in joints that provides cushioning and support. The process of cartilage tissue

engineering typically involves three main components: cells, scaffolds, and bioactive molecules [19,20]. Firstly, specialized cells called chondrocytes or mesenchymal stem cells (MSCs) are isolated from a patient's own tissues, such as bone marrow or adipose tissue. These cells have the potential to differentiate into chondrocytes, which are responsible for producing the ECM of cartilage. Next, a scaffold is used to provide structural support for the cells and mimic the natural environment of cartilage. The scaffold can be made from various materials, including natural polymers like collagen or synthetic materials like hydrogels. The scaffold should possess properties such as biocompatibility, biodegradability, and mechanical strength to support cell growth and tissue development. Additionally, bioactive molecules are added to the scaffold to enhance cell growth and tissue regeneration. Growth factors, such as transforming growth factor-beta (TGF-β) and bone morphogenetic proteins (BMPs), can stimulate cell proliferation and ECM synthesis. In addition, other molecules, such as cytokines or small molecules can be incorporated to modulate the cellular behavior and promote tissue maturation. Once the cells, scaffold, and bioactive molecules are combined, the tissue-engineered construct is cultured in a laboratory setting. During this time, the cells proliferate and deposit ECM, gradually forming functional and mature cartilage tissue. Various culture conditions, such as oxygen tension, mechanical stimulation, and nutrient supply, are optimized to promote tissue development. After an adequate period of *in vitro* culture, the tissue-engineered cartilage can be implanted into the patient. Depending on the defect size and location, different implantation techniques can be employed, such as direct injection or surgical implantation. Over time, the implanted construct integrates with the surrounding tissue and promotes tissue remodeling and regeneration. Tissue engineering of cartilage offers several advantages over traditional treatments for cartilage defects, such as arthritis or trauma. It provides a personalized approach by using the patient's cells, reducing the risk of immune rejection. It also has the potential to regenerate cartilage with native structure and function, as opposed to using artificial implants. However, there are still challenges to overcome, such as ensuring long-term durability and functionality of the engineered tissue. Tissue engineering of cartilage holds great promise for the treatment of cartilage defects. By combining cells, scaffold materials, and bioactive molecules, researchers are working towards creating functional and regenerative cartilage tissues that can improve the quality of life for patients with joint disorders. Further research and technological advancements will continue to propel the field forward, ultimately leading to more effective treatments for the regeneration of damaged tissue, either hard or soft tissue [21].

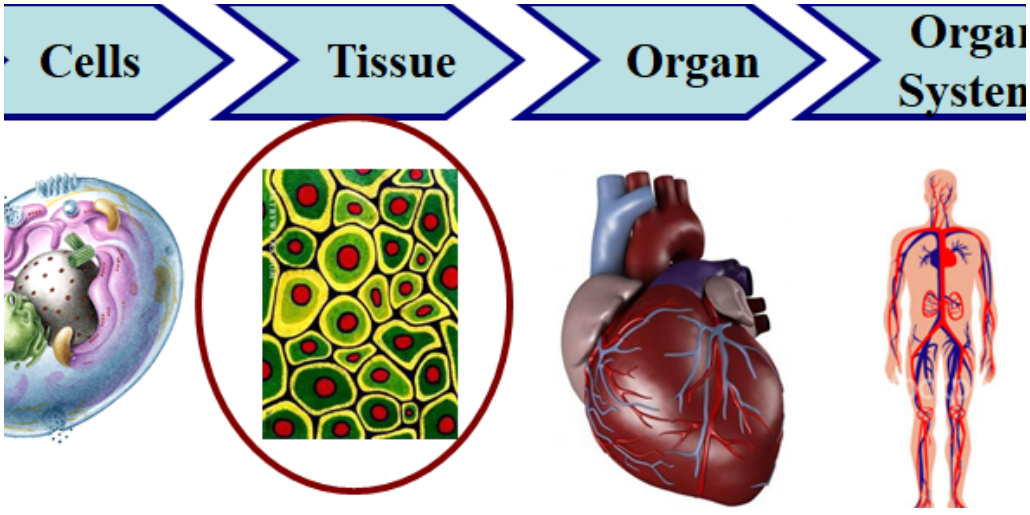

Figure 1. Hierarchical organization of human systems

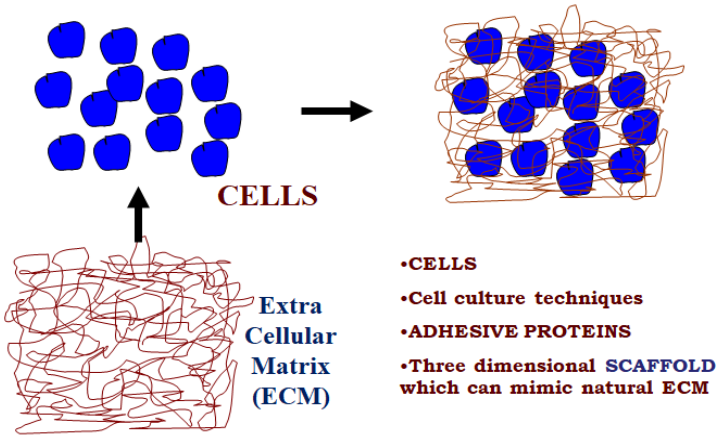

Figure 2. Concept of tissue engineering

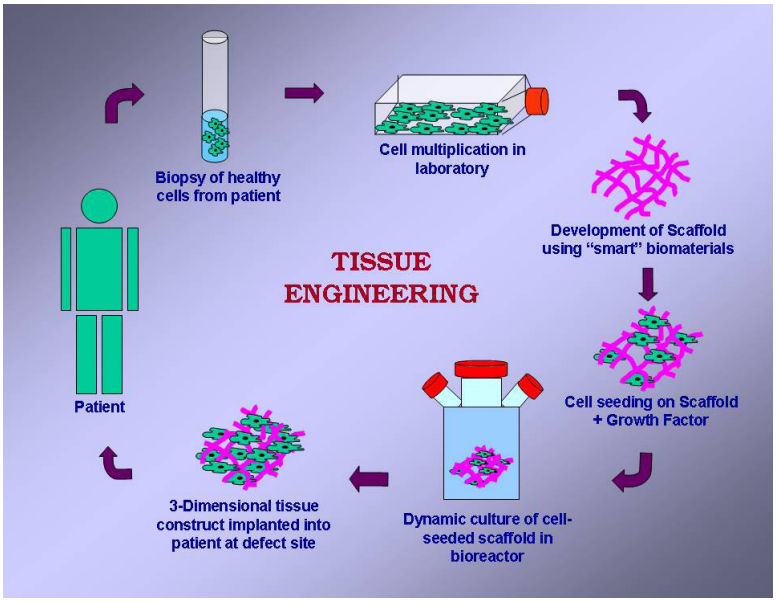

Figure 3. Fabrication of tissue engineering scaffolds (Aher *et al.*, 2015)

2. Structure and function of cartilage

Figure 4 shows the structure of cartilage, which is a specialized connective tissue found in various parts of the body, such as joints, ears, nose, and the respiratory tract. It is characterized by its firm yet flexible consistency, which allows for smooth joint movement and structural support. The primary cellular component of cartilage is chondrocytes. These specialized cells are embedded within the extracellular matrix of cartilage and are responsible for producing and maintaining the cartilaginous tissue. The ECM of cartilage consists of a gel-like substance called ground substance and fibers. The ground substance is composed of proteoglycans, glycosaminoglycans (GAGs), and water, providing resilience and compressibility of the tissue. Collagen fibers, primarily type II collagen, provide tensile strength to the cartilage. There are many types of cartilage: The most common type, hyaline cartilage, is found in weight-bearing joints, the respiratory tract, and the embryonic skeleton. It has a smooth, glassy appearance and provides support, cushioning, and low-friction surfaces for joint movement. Elastic cartilage contains elastic fibers in addition to collagen fibers. It is found in the outer ear, the epiglottis, and the larynx. Elastic cartilage provides strength, flexibility, and shape maintenance.

Fibrocartilage has a dense arrangement of collagen fibers, which makes it more resistant to tension. It is found in structures like intervertebral disks, pubic symphysis, and certain tendons. Fibrocartilage provides cushioning, shock absorption, and stability to joints [22,23].

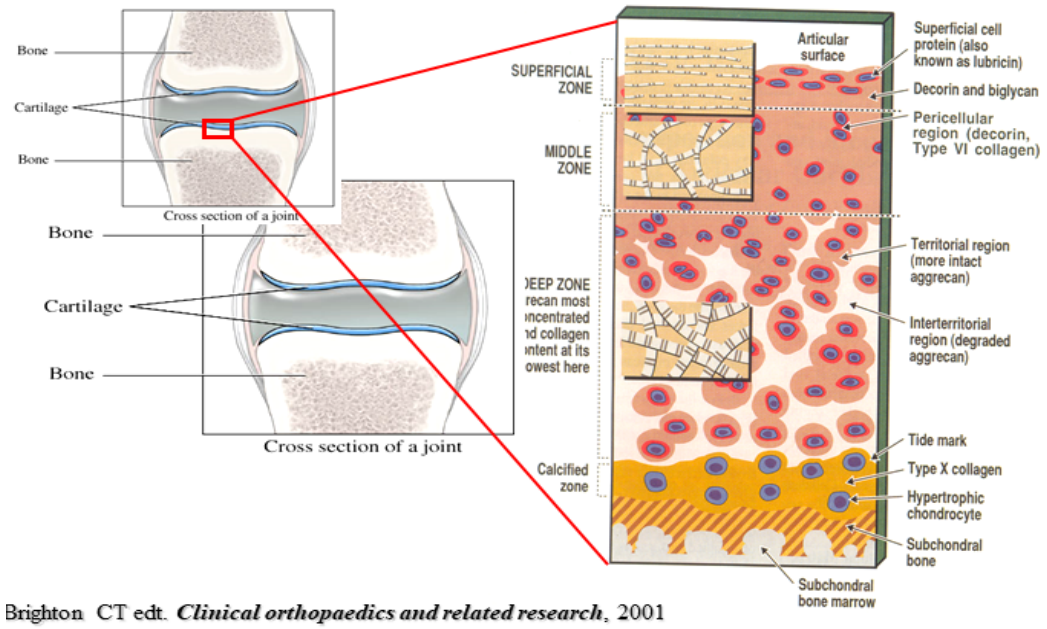

Figure 4. Structure and function of cartilage (Brighton, 2001)

Cartilage has limited regenerative capacity, which means that when it is damaged or injured, it often does not heal completely on its own. However, there are several approaches being explored for cartilage repair and regeneration. Tissue engineering approaches aim to create functional cartilage in the laboratory for transplantation. This typically involves seeding cells (such as chondrocytes or stem cells) onto biocompatible scaffolds and providing the appropriate growth factors and mechanical stimulation to promote cartilage formation [24]. This paper presents the tissue engineering of cartilage using a familiar biofabrication technique called electrospinning. The current scientific investigations reveal the tissue engineering construct via various biomimetic approaches for improving the cell fate process in the regeneration of tissues.

3. Fabrication of PLGA/PPG electrospun scaffold

A syringe pump (Harvard Apparatus, USA), a high voltage power supply (Glassman High Voltage, Inc.), and a speed-adjustable rotary mandrel coated in a copper metal sheet for fiber collection made up the customized electrospinning setup used in this study. PLGA 85:15, 24.71% (w/w) [22% (w/v)] was dissolved in a 3:1 ratio of THF:DMF (tetrahydrofuran:N,N-dimethylformamide) and mixed with different concentrations of polypropylene glycol (PPG), specifically 0.5%, 1.0%, 1.5%, and 2.0% (w/w) [0.5%, 1.0%, 1.5%, and 2.0% (w/v)] to create the polymer solution for electrospinning. Before electrospinning, polymer solutions were created using a magnetic stirrer for 24 hours. The needle gauge was 22G (internal diameter = 0.394 mm), the distance between electrodes was 22 cm, the voltage generated between electrodes was 1.2 kV/cm, and the rotary mandrel speed was set at 1.25 m/s for the electrospinning of PLGA or its blends with Pluronic® F-108 (PF-108). After that, a syringe pump was used to eject the polymer solution at a flow rate of 0.5 ml/h. A non-woven fibrous mesh was created when the polymer solution was exposed to the previously mentioned conditions, and

it was then collected on the rotating mandrel in a dry state. Following synthesis, the resulting fibrous mesh was lyophilized for 48 hours before being used in additional tests [16]. **Figure 5** shows the experimental setup for the fabrication of the nanofibrous scaffold.

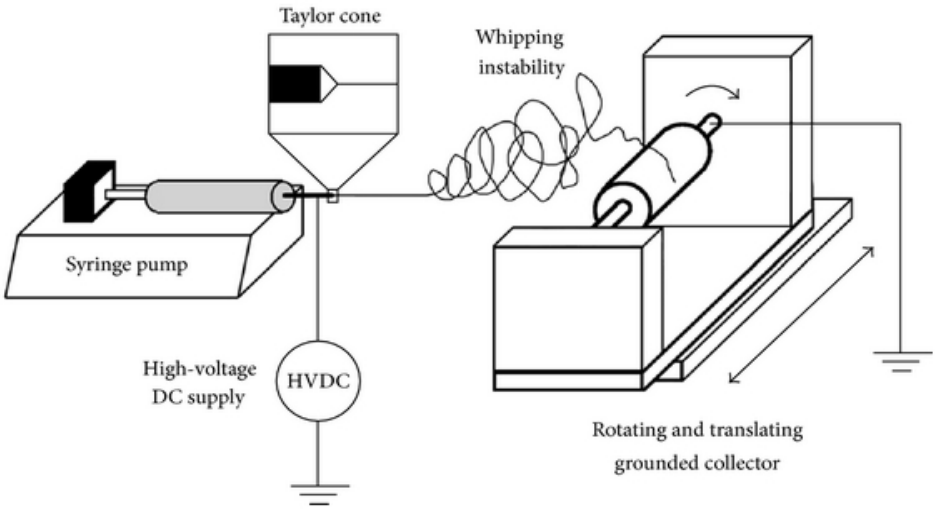

Figure 5. Electrospinning experimental setup (Liu *et al.*, 2013)

Figure 6 reveals the morphology and topography of the electrospun scaffold. The morphology and topography of an electrospun nanofibrous scaffold refer to the physical characteristics and surface features of the scaffold. The morphology of an electrospun nanofibrous scaffold refers to the overall structure and appearance of the fibers that make up the scaffold. Typically, these fibers are very thin and have a high aspect ratio, meaning they are much longer than they are wide. The size and shape of the fibers can vary depending on the electrospinning process parameters, such as the polymer solution viscosity, the applied voltage, and the distance between the spinneret and the collector. The fibers can range in diameter from a few nanometers to several micrometers. The morphology of the scaffold can be observed using various microscopic techniques, such as scanning electron microscopy (SEM).

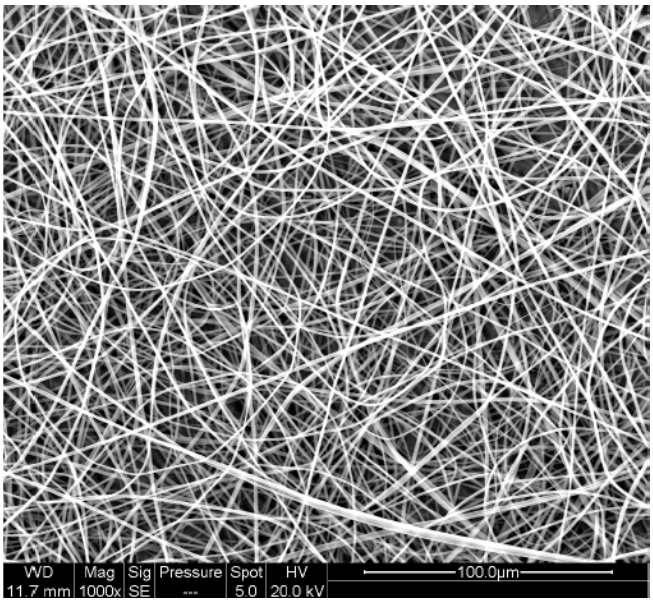

Figure 6. Electrospun PLGA nanofibrous scaffold for tissue engineering constructs

The topography of an electrospun nanofibrous scaffold refers to the surface features and characteristics of the scaffold. The topology can be smooth, rough, or have specific patterns. The topography of the scaffold is mainly determined by the arrangement and alignment of the fibers. The alignment can be random or controlled by using techniques such as multi-needle electrospinning or collector design modifications. The topography can greatly influence cell behavior, including cell adhesion, proliferation, migration, and differentiation. Therefore, controlling the topography of the scaffold is important for achieving desired cellular responses. Overall, the morphology and topography of an electrospun nanofibrous scaffold play crucial roles in its performance as a tissue engineering scaffold. By tailoring these characteristics, it is possible to create scaffolds with specific properties suitable for various tissue engineering applications [25].

4. Biomimetic approach for the development of tissue engineering construct

A biomimetic approach in tissue engineering aims to produce constructs that closely mimic the extracellular environment of the target tissue, thereby promoting better integration, functionality, and regeneration. This approach takes inspiration from the structure, composition, and mechanical properties of native tissues, and seeks to replicate them in engineered constructs [26]. Some key aspects of a biomimetic approach in tissue engineering are as follows. The first aspect is scaffold design, the scaffold serves as the structural framework for cell attachment, growth, and tissue formation. Biomimetic scaffolds aim to mimic the natural ECM of the target tissue, both in terms of composition and architecture. This includes replicating the fibrous structure, pore size and distribution, and mechanical properties of the native tissue. The second aspect is biomaterial selection, choosing the appropriate biomaterials is crucial for a biomimetic approach. Ideally, the selected biomaterials should possess biocompatibility, biodegradability, appropriate mechanical properties, and the ability to support cell adhesion, migration, and tissue formation. Natural polymers, such as collagen, chitosan, and hyaluronic acid, are often used due to their similarity to native ECM components. The third aspect is cell source. Biomimetic tissue engineering often involves using cells that closely resemble the target tissue. This can include primary cells, stem cells, or progenitor cells. The cells are seeded onto the scaffold and encouraged to differentiate and proliferate in a way that mirrors the natural tissue development and repair processes [27]. Another aspect is bioactive molecules. Growth factors, cytokines, and other bioactive molecules play a crucial role in tissue development and regeneration. In a biomimetic approach, these molecules are incorporated into the scaffold or delivered in a controlled manner to guide cellular behavior, promote tissue-specific differentiation, and facilitate the regeneration process. Mechanical stimulation is another aspect of biomimetic tissue engineering. Native tissues experience mechanical forces and stimuli that influence their development and maintenance. Biomimetic tissue engineering seeks to replicate these mechanical cues by applying appropriate mechanical stimulation to the engineered constructs. This can include the use of bioreactors, stretching devices, or other mechanical loading techniques to enhance cellular function, organization, and tissue maturation. By adopting a biomimetic approach, tissue engineering constructs can better mimic the complex microenvironment of native tissues, promoting cell viability, differentiation, and tissue regeneration. This approach holds great promise in developing more effective and clinically relevant tissue engineering strategies for a wide range of applications, including bone, cartilage, skin, and organ regeneration [28,29].

Biomimetic of cartilage tissue in tissue engineering aims to replicate the structure, composition, and mechanical properties of native cartilage. These mimetic approaches involve various strategies to create constructs that closely resemble the characteristics of natural cartilage tissue. There are physical, chemical, and biological approaches to restoring native cartilage. It has a zonal organization, with distinct regions of different cell densities and ECM compositions. In mimetics, efforts are made to create constructs with zonal organization [30].

5. Physical approach for mimetic of cartilage tissue engineering constructs

A physical approach for mimicking tissue engineering constructs of cartilage involves utilizing various physical techniques to create structures that resemble the characteristics of native cartilage tissue [31]. Electrospinning is an appropriate approach for the fabrication of tissue engineering constructs. This method can be employed to fabricate scaffolds with a fibrous structure similar to the size of collagen fibers found in native cartilage. By controlling factors such as polymer concentration, electric field strength, and collector design, the diameter and alignment of the electrospun fibers can be adjusted to mimic the organization of collagen fibers in cartilage. Electrospinning has the capacity to tailor the orientation and alignment of the electrospun nanofibers to mimic the structure of cartilage [29].

5.1. Shape and size-based mimetic

In electrospinning, the shape and size of nanofibers can be controlled through various factors and parameters. The key considerations for controlling the shape and size of nanofibers in electrospinning are as follows.

(1) Polymer solution properties: The properties of the polymer solution used in electrospinning can significantly influence the fiber morphology. Factors such as polymer concentration, viscosity, surface tension, and conductivity play a role in determining the final fiber diameter. Higher polymer concentrations generally result in thicker fibers, while lower concentrations tend to produce finer fibers.

(2) Electric field parameters: The electric field applied during electrospinning affects the stretching and elongation of the polymer solution, thus influencing the fiber diameter. Increasing the electric field strength generally leads to thinner fibers, while reducing the field strength can result in thicker fibers. The distance between the needle orifice and the collector, known as the needle-to-collector distance, also affects fiber morphology, with longer distances typically producing larger-diameter fibers.

(3) Needle and collector setup: The design and configuration of the electrospinning setup can influence the shape and size of the nanofibers. Factors such as the size and shape of the spinneret needle, its tip shape, and the geometry of the collector can impact the fiber morphology. For example, a needle with a smaller diameter or a finer tip can produce finer fibers.

(4) Processing parameters: Additional processing parameters, such as the flow rate of the polymer solution, the spinning time, and the rotational speed of the collector, can also affect the fiber diameter and shape. Higher flow rates generally result in thicker fibers, while lower flow rates produce finer fibers. Adjusting the spinning time and rotational speed can allow for control over the alignment and organization of the fibers.

(5) Additives and blends: Incorporating additives, such as surfactants or plasticizers, into the polymer solution can modify the solution's properties and influence the fiber morphology. Additives can help reduce the surface tension of the solution, improve its conductivity, or enhance its spinnability, resulting in different fiber shapes and sizes. Blending different polymers can also allow for control over fiber morphology and properties. By carefully adjusting these factors and parameters, researchers can achieve control over the shape and size of nanofibers in electrospinning, enabling the production of tailored scaffolds for various tissue engineering applications [32,33].

5.2. Alignment of fibers

Controlling the alignment of fibers in electrospinning is important for mimicking the organized structure of native tissues and enhancing the functionality of tissue engineering constructs [32,33]. The following are some strategies for achieving fiber alignment in electrospinning.

(1) Electrospinning setup: The configuration of the electrospinning setup can influence fiber alignment. Using a rotating collector, such as a drum or mandrel, can promote fiber alignment along the axis of rotation. As the fibers are deposited on the rotating collector, the centrifugal force can align the fibers in the same direction [34].

(2) External forces: Applying external forces during electrospinning can help align the fibers. For example, a uniaxial tensile force can be applied to the polymer solution or the collected fibers to encourage alignment. This can be achieved by attaching one end of the fiber to a fixed point and applying a controlled tensile force to the other end.

(3) Template-assisted electrospinning: Templates or substrates with predefined patterns or grooves can be used to guide fiber alignment. The polymer solution is electrospun onto these templates, and the resulting fibers conform to the pattern, leading to aligned fibers. After electrospinning, the fibers can be transferred from the template or used as a template itself for further processing.

(4) Magnetic field alignment: Incorporating magnetic nanoparticles or using magnetic fields during electrospinning can enable the alignment of the fibers. Magnetic nanoparticles are added to the polymer solution, and an external magnetic field is applied during electrospinning, guiding the alignment of the nanoparticles and, consequently, the fibers.

(5) Electric field alignment: In addition to the electric field used for electrospinning, an additional electric field can be applied to align the fibers. By introducing a secondary electric field perpendicular to the primary field, the charged fibers experience lateral forces that align them in the desired direction [35].

(6) Coaxial electrospinning: Coaxial electrospinning involves using a coaxial needle setup with a core-shell structure. The polymer solution is electrospun through the core needle, while a sheath fluid is electrospun through the outer shell. The sheath fluid can act as a guiding medium, controlling the alignment of the core fibers. By implementing these strategies, researchers can achieve fiber alignment in electrospinning, leading to the fabrication of tissue engineering constructs with enhanced structural organization and functional properties [36,37].

5.3. Architecture

Controlling the architecture of fibers in electrospinning refers to manipulating their spatial arrangement and organization to create desired structures and patterns. The approaches for controlling the architecture of fibers in electrospinning are as follows.

(1) Collector design: The design of the collector can influence the architecture of the electrospun fibers. By using collectors with specific geometries, such as rotating drums, mandrels, or patterned substrates, different fiber architectures can be achieved. For example, using a collector with grooves or ridges can result in fibers arranged in specific patterns or orientations [38].

(2) Electrospinning technique variations: Various electrospinning techniques can be employed to control the architecture of the fibers. These include multi-jet electrospinning, needleless electrospinning, and near-field electrospinning. Each technique offers unique possibilities for creating specific fiber architectures, such as aligned, random, or patterned structures.

(3) Template-assisted electrospinning: Templates or sacrificial materials with predetermined shapes or patterns can be used to guide the architecture of the fibers. The polymer solution is electrospun onto the template, conforming to its shape, resulting in fibers with corresponding architectural features. Once the electrospinning is complete, the template can be removed, leaving behind the desired fiber architecture.

(4) Coaxial electrospinning: Coaxial electrospinning can be employed to create fibers with core-shell architectures. By using a coaxial needle setup, a different polymer solution or functional material can be electrospun as the core, surrounded by another polymer solution as the shell. This allows for the creation of fibers with distinct layers or encapsulation capabilities [37].

(5) Blend electrospinning: Blending different polymer solutions or incorporating functional additives can influence the architecture of the electrospun fibers. By blending polymers with different properties, such as varying viscosities or solubilities, fibers with heterogeneous architectures can be obtained. Additionally, incorporating functional materials, such as nanoparticles or bioactive molecules, into the polymer solution can create fibers with specific architectural features and functionalities.

(6) Post-electrospinning processing: After electrospinning, post-processing techniques can be used to further control the architecture of the fibers. These techniques may include heat treatment, stretching, ultraviolet crosslinking, or chemical treatments. These processes can modify the morphology, alignment, or structure of the electrospun fibers to achieve the desired architectural characteristics. By leveraging these techniques, researchers can have greater control over the architecture of electrospun fibers, enabling the fabrication of the right scaffolds [39,40].

6. Chemical approach for mimetic of cartilage tissue engineering constructs

Chemical mimetic in tissue engineering constructs of cartilage aims to replicate the biochemical composition and signaling cues found in native cartilage tissue. These mimetic approaches involve incorporating certain chemical factors into the constructs to enhance chondrogenesis and promote the formation of cartilage-like ECM. The following are some key aspects of chemical mimetics in cartilage tissue engineering.

(1) Growth factors and cytokines: Growth factors and cytokines play crucial roles in regulating cellular processes and tissue development. In cartilage tissue engineering, growth factors such as transforming growth factor-beta (TGF-β), insulin-like growth factor (IGF), and bone morphogenetic proteins (BMPs) are commonly used to stimulate chondrocyte proliferation, differentiation, and ECM synthesis. These factors can be incorporated into the scaffolds or delivered through controlled release systems to mimic the natural signaling environment of cartilage.

(2) ECM components: The ECM of cartilage contains various components that provide structural support and signaling cues for chondrogenesis. Mimicking the ECM composition is vital in cartilage tissue engineering. Chondroitin sulfate, hyaluronic acid, collagen, and other cartilage-specific matrix molecules can be incorporated into the scaffold materials to replicate the biochemical environment. Synthetic ECM analogs, such as peptide-based hydrogels or self-assembling peptides, can also be used to mimic the cartilage ECM.

(3) Small molecule modulators: Small molecules can be used as mimetics to regulate specific biological processes in cartilage tissue engineering. These molecules can modulate key signaling pathways and cellular activities involved in chondrogenesis. For example, small molecule agonists or antagonists of specific receptors or transcription factors, such as the Wnt/β-catenin pathway or the Sox9 transcription factor, can be used to promote or inhibit chondrocyte differentiation, respectively.

(4) Mechanical loading mimetics: Mimicking the mechanical cues experienced by cartilage tissue can be achieved through chemical means. Certain compounds, such as calcium channel agonists or cyclic adenosine monophosphate (cAMP) inducers, can be used to mimic the mechanical strain and stimulate chondrogenic responses. These chemicals activate intracellular signaling pathways that regulate gene expression and ECM synthesis in response to mechanical loading.

(5) Oxygen mimetics: Oxygen tension plays a critical role in cartilage physiology and chondrogenesis. In mimetics, hypoxia-mimicking strategies can be employed to create a low-oxygen environment similar to native cartilage [41].

6.1. Matching chemical content in chemical mimetic of electrospun nanofiber

When designing chemical mimetics in electrospun nanofibers, the goal is to match the chemical content to replicate the desired properties or functions of a target material or biological structure. There are some factors to consider for matching chemical content in the mimetic.

(1) Polymer selection: The choice of polymer used in electrospinning plays a crucial role in determining the chemical composition of the nanofibers. Different polymers offer distinct properties and characteristics. For example, polylactic-co-glycolic acid (PLGA) is commonly used for tissue engineering applications due to its biocompatibility and biodegradability. Other polymers, such as polyethylene oxide (PEO), polycaprolactone (PCL), or polyvinyl alcohol (PVA), may be preferred for specific purposes [42].

(2) Incorporation of biomolecules: Mimicking the chemical content of natural tissues often involves the incorporation of specific biomolecules, such as growth factors, cytokines, or extracellular matrix components. These molecules can be added to the polymer solution before electrospinning or incorporated into the nanofibers through post-electrospinning modification techniques [43].

(3) Controlled release systems: To mimic the release of specific chemicals or drugs from the nanofibers, controlled release systems can be incorporated. This can involve the use of drug-loaded nanoparticles, microparticles, or encapsulation of the active compounds within the nanofiber matrices. The choice of release system will depend on the desired release kinetics and stability of the incorporated chemicals [44,45].

(4) Surface functionalization: The chemical content of the nanofiber surface can be modified through surface functionalization techniques. This may involve grafting specific functional groups, such as –COOH or $–NH_2$, onto the nanofiber surface to enable further chemical reactions or to provide specific functionalities [46].

(5) Nanoparticle or nanocomposite incorporation: To introduce specific chemical content, nanoparticles or nanocomposites can be incorporated into the nanofibers. These nanoparticles can provide additional properties, such as enhanced mechanical strength, conductivity, or targeted drug delivery capabilities. Examples include incorporating silver nanoparticles for antimicrobial activity or magnetic nanoparticles for targeted drug delivery.

Matching the chemical content in chemical mimetics of electrospun nanofibers involves a careful selection of polymers, incorporation of biomolecules, controlled release systems, surface functionalization, and nanoparticle incorporation. These strategies allow for the replication of desired chemical properties and functions, making electrospun nanofibers versatile and customizable [47].

6.2. Manipulation of chemical composition in chemical mimetic of electrospun nanofiber

The chemical composition of electrospun nanofibers can be manipulated through various techniques to achieve desired properties and functions. Some methods to manipulate the chemical composition in chemical mimetics of electrospun nanofibers are as follows.

(1) Polymer blending: By blending different polymers with complementary properties, nanofibers with unique compositions and properties can be produced. For example, blending a biodegradable polymer like PLGA with a conductive polymer like polyaniline can result in nanofibers with combined biodegradability and electrical conductivity [48,49].

(2) Multi-layer electrospinning: In multi-layer electrospinning, multiple layers of different polymers or polymer solutions are sequentially deposited during the electrospinning process. This technique enables the creation of nanofibers with layered structures and varying chemical compositions. Each layer can serve a specific purpose or provide different functionalities.

(3) Surface modification: Surface modification techniques, such as plasma treatment or chemical grafting, can be used to introduce specific functional groups or chemical moieties onto the surface of electrospun nanofibers. This allows for the manipulation of the surface chemistry and composition, enabling interactions with biological molecules or targeted drug delivery [50].

(4) Encapsulation of additives: Additives, such as nanoparticles, drugs, or biomolecules, can be encapsulated within the electrospun nanofibers to alter their chemical composition. By incorporating these additives during the electrospinning process, desired properties or functions can be introduced to the nanofibers.

(5) Chemical doping: Chemical doping involves introducing small amounts of a dopant material into the polymer solution before electrospinning. The dopant can modify the chemical composition and enhance specific properties, such as conductivity or mechanical strength. These methods provide ways to manipulate the chemical composition of electrospun nanofibers, allowing for customization and tailoring of their properties and functions. Depending on the desired outcome, one or a combination of these techniques can be employed to achieve the desired chemical composition in the nanofibers [51].

6.3. Surface mimetics

Surface mimetics in chemical mimetics of electrospun nanofibers involves modifying the surface properties to replicate or mimic specific features found in natural surfaces. The following are some techniques for incorporating surface mimetics into electrospun nanofibers.

(1) Surface texturing: Electrospun nanofibers can be designed to mimic the surface texture of natural structures. By controlling the parameters during electrospinning, such as the collector design or the addition of sacrificial templates, nanofibers with surface features like ridges, grooves, or pores that resemble natural surfaces can be created.

(2) Surface functionalization: Surface functionalization involves attaching specific functional groups or molecules onto the surface of electrospun nanofibers. This can be achieved through techniques such as chemical grafting, physical adsorption, or covalent bonding. By introducing functional groups like –COOH, $–NH_2$, or –OH, the surface chemistry found in natural materials can be mimicked.

(3) Biomineralization: Mimicking the mineralization found in natural structures, such as bone or teeth, can be achieved by promoting the deposition of minerals onto the surface of electrospun nanofibers. This can be done by incorporating mineral precursors into the electrospinning solution or through post-electrospinning mineralization processes. The resulting mineralized surface mimics the composition and structure of natural mineralized tissues [52].

(4) Biomolecule immobilization: To replicate the interactions between natural surfaces and biomolecules, specific biomolecules can be immobilized onto the surface of electrospun nanofibers. This can be done through techniques like physical adsorption, covalent bonding, or layer-by-layer assembly. By immobilizing proteins, growth factors, or other bioactive molecules, nanofiber surfaces that mimic the bioactivity and signaling capabilities of natural surfaces can be created.

(5) Surface patterning: Patterning techniques, such as microcontact printing or photolithography, can be used to create specific patterns or motifs on the surface of electrospun nanofibers. This allows for the replication of surface features found in natural structures, including directional cues, topographical patterns, or microscale designs. By incorporating surface mimetics into electrospun nanofibers, you

can replicate or mimic the surface properties and functionalities of natural materials. These approaches enable the development of biomimetic materials with tailored surface characteristics, which have applications in tissue engineering, drug delivery, biosensing, and other fields [53].

(6) Surface functionality: Tailoring the surface functionality of electrospun fibers is crucial for many applications, as it can influence cell-material interactions, protein adsorption, and overall biomaterial performance. There are some strategies for achieving surface functionality in electrospun fibers. The first strategy is surface modification. After electrospinning, the fibers can undergo various surface modification techniques to introduce functional groups or specific chemical moieties. Common methods include plasma treatment, ultraviolet/ozone treatment, and chemical grafting. These techniques can alter the surface chemistry of the fibers, enabling the attachment of bioactive molecules, such as peptides or growth factors, or providing functional groups for subsequent reactions. Secondly, electrospun fibers can be functionalized by immobilizing biomolecules directly onto their surfaces. This can be achieved through physical adsorption, covalent binding, or layer-by-layer assembly. Biomolecules, including peptides, proteins, enzymes, or DNA, can be attached to the fiber surface to impart specific functionalities, such as cell adhesion, bioactive signaling, or antimicrobial properties. By employing these strategies, the surface functionality of electrospun fibers can be tailored to meet specific requirements, enabling enhanced biocompatibility, controlled drug delivery, cell adhesion, and other desirable properties for various biomedical applications [46].

Figure 7 reveals the surface functionalization of PLGA nanofibers with sodium hydroxide (NaOH) treatment to activate the carboxyl/hydroxyl group of the PLGA. By grafting –COOH or $–NH_2$ groups onto the electrospun PLGA fibers, the functional groups that can enable further chemical reactions can be introduced, such as covalent coupling with biomolecules or attachment to other surfaces, for enhanced bioactivity, cell adhesion, or drug delivery capabilities. The grafting of $–NH_2$ on the PLGA nanofibers is also known as aminolysis and can be carried out with PLGA fibers treated with ethylenediamine (ED), N-aminoethyl-1,3-propanediamine (AEPDA). These amine-functionalized fibers can provide opportunities for bioconjugation and customization of the fiber surface for specific applications in tissue engineering or drug delivery [46].

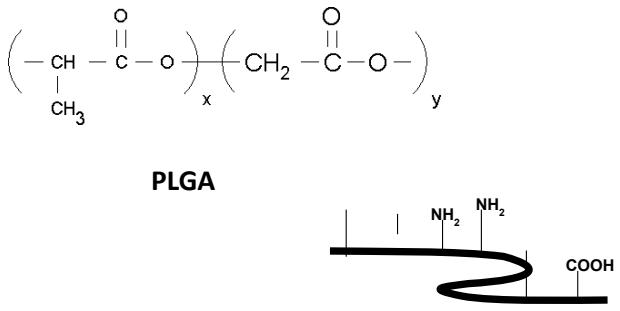

Figure 7. Surface functionalization of PLGA nanofibers

The toluidine absorbance assay is a commonly used method for quantifying the sulfated glycosaminoglycan (GAG) or carboxyl group content in biological samples or biomaterial after functionalization. The toluidine absorbance assay is widely used in various fields, including cartilage tissue engineering, osteoarthritis research, and glycosaminoglycan analysis. This assay was used to evaluate the carboxyl group in PLGA nanofibers after being treated with NaOH solutions. The prolonged treatment of fibers with NaOH affects the fibers' morphology and topography. **Figure 8** shows the toluidine absorbance assay for the quantified amount of carboxyl group on the scaffolds.

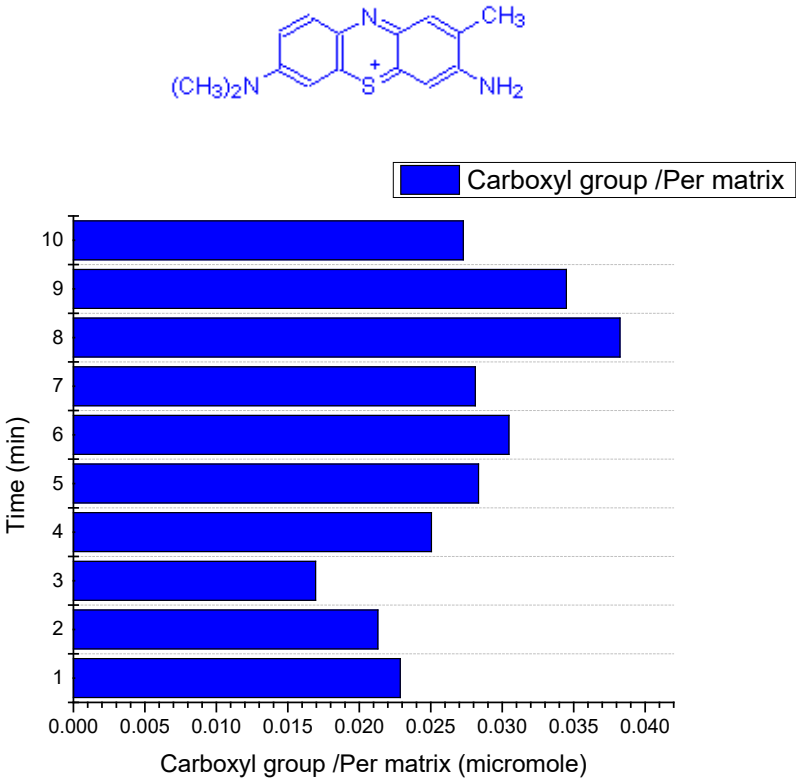

Figure 8. Toluidine absorbance assay

7. Biological approach for mimetic of cartilage tissue engineering constructs

Biological mimetic plays a crucial role in tissue engineering electrospun scaffolds as it aims to replicate the structural and functional properties of natural tissues. Some examples of biological mimetics used in tissue engineering electrospun scaffolds are as follows.

(1) ECM mimetics: The ECM provides a supportive microenvironment for cells in natural tissues. Electrospun scaffolds can be designed to mimic the composition and structure of the ECM by incorporating natural or synthetic polymers that resemble ECM components. For example, collagen, elastin, or hyaluronic acid can be electrospun to create scaffolds that mimic the ECM of specific tissues.

(2) Nanofiber alignment: Natural tissues often exhibit anisotropic properties due to the aligned arrangement of their structural components. Electrospinning techniques can be used to align the nanofibers in a specific direction, mimicking the organization found in native tissues. This aligned nanofiber structure can guide cell orientation, promote tissue alignment, and enhance mechanical properties.

(3) Growth factor mimetics: Growth factors play a crucial role in tissue development and regeneration. Electrospun scaffolds can be designed to incorporate growth factors or their mimetics, such as peptide sequences, that can guide cellular behavior and promote tissue regeneration in a controlled manner. Controlled release systems can also be incorporated to provide sustained delivery of growth factors.

(4) Cell-adhesive mimetics: Cell-adhesive molecules found in the ECM, such as fibronectin or laminin, can be incorporated into electrospun scaffolds to create cell-friendly environments. This can be achieved through surface functionalization or by blending the polymer solution with cell-adhesive peptides. Mimicking cell-matrix interactions can enhance cell adhesion, migration, and overall tissue integration.

(5) Vascular network mimetics: Vascularization is critical for the survival and function of engineered tissues. Electrospun scaffolds can be designed to mimic the architecture of blood vessels by

incorporating sacrificial templates or creating interconnected channels. These scaffolds can provide a framework for the formation of a functional vascular network, enabling nutrient and oxygen delivery to cells within the engineered tissue.

(6) Mechanical mimetics: Mimicking the mechanical properties of native tissues is essential for successful tissue engineering. Electrospun scaffolds can be tailored to match the mechanical properties of specific tissues by selecting appropriate polymer compositions, adjusting fiber diameter and density, or incorporating reinforcing materials like nanoparticles or nanofibers. By incorporating these biological mimetics into tissue engineering electrospun scaffolds, researchers can create novel tissue engineering scaffolds [54].

7.1. Protein adsorption (adhesive protein)

Electrospun nanofibrous tissue engineering scaffolds have the capability to mimic adhesive proteins found in the extracellular matrix of natural tissues. Electrospun nanofibrous scaffolds can exhibit adhesive protein capabilities through the methods below.

(1) Surface functionalization: Electrospun nanofibers can be surface-functionalized with adhesive proteins, such as fibronectin, laminin, or collagen. This can be achieved through various techniques, including physical adsorption, covalent bonding, or layer-by-layer assembly. The immobilization of these adhesive proteins on the nanofiber surface enhances cell attachment and promotes cellular interactions, mimicking the adhesive properties of natural tissues.

(2) Peptide incorporation: Synthetic peptides derived from adhesive proteins, such as the cell-binding domains of fibronectin (e.g., arginine-glycine-aspartate peptide [RGD]), can be incorporated into the polymer solution before electrospinning. These peptides provide specific sites for cell adhesion and can enhance the adhesive capabilities of the nanofibers.

(3) Bioactive molecule release: Electrospun scaffolds can also be designed to incorporate bioactive molecules, including adhesive proteins or their fragments, for controlled release. This can be achieved by encapsulating the bioactive molecules within nanoparticles or microparticles, which are then dispersed within the nanofiber matrix. Controlled release of these molecules from the scaffolds can promote cell adhesion, migration, and tissue integration.

(4) Nanofiber alignment: Electrospinning techniques can be used to align nanofibers in a specific direction, creating a biomimetic topography that enhances cell adhesion. The aligned nanofibers provide guidance cues for cell attachment and alignment, replicating the natural alignment of cells in tissues.

(5) Cell-adhesive polymer blending: Electrospinning allows for the blending of different polymers with cell-adhesive properties. By incorporating polymers with inherent adhesive capabilities, such as gelatin or chitosan, into the electrospun nanofibers, the scaffold's adhesive capabilities can be enhanced.

The adhesive protein capabilities of electrospun nanofibrous tissue engineering scaffolds promote cell attachment, spreading, and proliferation, which are crucial for successful tissue regeneration. These capabilities improve the integration of the scaffold with surrounding tissues and facilitate the formation of functional engineered tissues [55].

7.2. Protein delivery

Electrospun nanofibrous scaffolds can be utilized for the delivery of proteins to promote cartilage growth in tissue engineering applications. Protein delivery can be achieved using electrospun nanofibrous scaffolds for cartilage regeneration as follows.

(1) Encapsulation within nanofibers: Proteins relevant to cartilage growth, such as growth factors (e.g.,

TGF-β, BMPs) or cartilage-specific proteins (e.g., collagen type II), can be encapsulated within the electrospun nanofibers during the fabrication process. This can be achieved by incorporating the proteins into the polymer solution before electrospinning. The proteins are then distributed throughout the nanofiber matrix, allowing for sustained release over time.

(2) Coating of nanofibers: Electrospun nanofibers can be coated with protein-loaded films or layers. This can involve techniques like dip-coating or layer-by-layer assembly, where the protein solution is applied to the surface of the nanofibers. The coating acts as a reservoir for protein release, enabling controlled and localized delivery.

(3) Surface functionalization: The surface of electrospun nanofibers can be functionalized with proteins using techniques like physical adsorption or covalent bonding. The protein-coated surface provides a direct interface for interaction with cells, promoting cartilage growth. Functionalization can be achieved by incubating the nanofiber scaffold with a protein solution or by modifying the nanofiber surface with specific reactive groups for protein attachment.

(4) Micro- or nanoparticle encapsulation: Proteins can also be encapsulated within micro- or nanoparticles that are subsequently incorporated into the electrospun nanofibers. These particles can release proteins gradually as they degrade or when triggered by specific stimuli. The combination of electrospun nanofibers and protein-loaded particles allows for sustained protein release and localized delivery within the scaffold. The controlled release of proteins from electrospun nanofibrous scaffolds promotes cartilage growth by providing a bioactive environment for cells and facilitating tissue regeneration. The released proteins can stimulate cell proliferation, differentiation, and extracellular matrix production, aiding in the development of functional cartilage tissue [56].

Figure 9 shows the bovine serum albumin from PLGA/PPG electrospun nanofibrous scaffold. Pure PLGA scaffold is highly hydrophobic when compared with other scaffolds and PPG addition improves the protein adsorption and release from the scaffolds. It concludes that the hydrophobicity of PLGA can be reduced by the incorporation of PPG in the scaffolds.

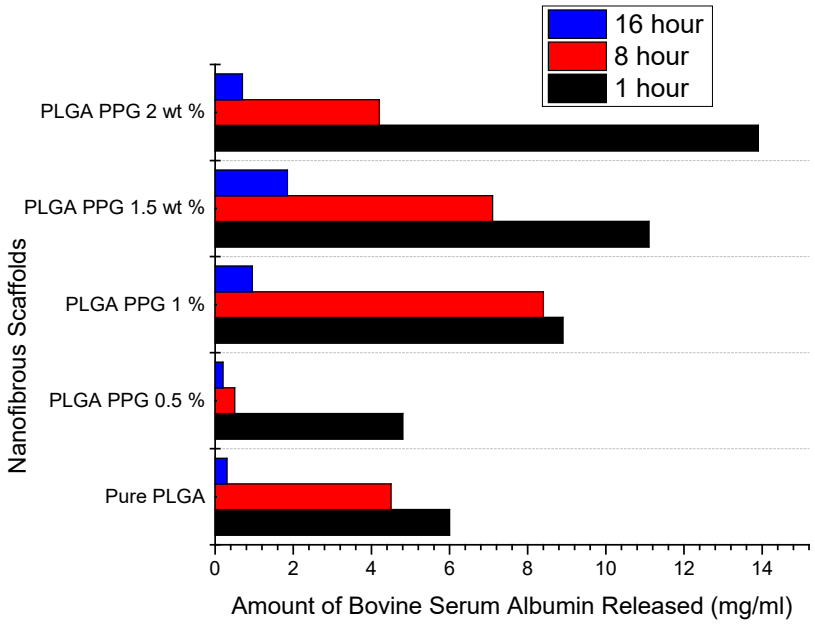

Figure 9. Protein release from PLGA/PPG electrospun nanofibrous scaffold

7.3. Cell behavior

Cells exhibit specific behaviors on electrospun nanofibrous scaffolds designed for cartilage repair. The key behaviors observed are as follows.

(1) Cell adhesion: Electrospun nanofibrous scaffolds provide a favorable surface for cell adhesion. The nanofiber structure mimics the extracellular matrix and offers physical cues for cells to attach. Cells, such as chondrocytes or mesenchymal stem cells, adhere to the nanofibers through integrin-mediated interactions, promoting cellular attachment and spreading.

(2) Cell migration: Electrospun nanofibrous scaffolds with aligned nanofiber architecture can guide cell migration. Cells tend to align and migrate along the direction of the aligned nanofibers, mimicking the natural alignment of cells in cartilage tissue. This alignment facilitates cell migration and tissue regeneration within the scaffold.

(3) Cell proliferation: Electrospun nanofibrous scaffolds can support cell proliferation. The nanofiber architecture provides a high surface area-to-volume ratio, allowing for efficient nutrient and oxygen exchange. The three-dimensional porous structure of the scaffold also allows cells to proliferate and populate throughout the scaffold, promoting tissue regeneration.

(4) ECM production: Cells cultured on electrospun nanofibrous scaffolds for cartilage repair show the ability to produce cartilage-specific ECM components, such as collagen type II and proteoglycans. The nanofiber structure promotes cell-secreted ECM deposition and organization, leading to the development of a cartilage-like matrix within the scaffold.

(5) Differentiation potential: Electrospun nanofibrous scaffolds can induce chondrogenic differentiation of stem cells. The nanofiber architecture, combined with appropriate biochemical cues such as growth factors or specific culture conditions, can drive MSCs toward a chondrogenic lineage, enabling the generation of functional cartilage tissue.

(6) Integration with surrounding tissue: Electrospun nanofibrous scaffolds can facilitate integration with surrounding tissue upon implantation. The nanofiber structure allows for cellular infiltration and vascularization, promoting the integration of the scaffold with the host tissue. This integration is crucial for the long-term stability and functionality of the repaired cartilage.

The behaviors exhibited by cells on electrospun nanofibrous scaffolds for cartilage repair are essential for successful tissue regeneration. Through adhesion, migration, proliferation, ECM production, differentiation, and integration, the cells contribute to the formation of functional cartilage tissue within the scaffold, aiding in the repair and regeneration of damaged cartilage [57].

8. Conclusion

Electrospinning is a flexible biofabrication method for developing tissue engineering scaffolds for cartilage repair. These works conclude the modification strategies for electrospun PLGA scaffolds. Tissue engineering involves using cells, engineering methods, and materials to improve or replace biological functions at an injured site. Biodegradable polymer nanofibers fabricated by electrospinning have great potential for tissue engineering applications, as they mimic the natural extracellular matrix and provide a favorable microenvironment for cells. The addition of a small amount of hydrophilic polymer to a biodegradable hydrophobic polymer can improve the hydrophilicity of electrospun nanofibers and can be used for nanofiber-based tissue engineering constructs. Physical, chemical, and biological mimicking promotes tissue engineering applications for cartilage repair. Tissue engineering offers the potential to create effective novel treatments, known as “biological substitutes,” for structural and functional ailments in human health, which have historically posed challenges

that conventional medical approaches have struggled to overcome.

Disclosure statement

The author declares no conflict of interest.

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