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Nanofibers and Their Applications in Tissue Engineering

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Abstract

One of the primary challenges in the science of tissue engineering is the development of scaffolds that imitate the architecture of tissue at the nanoscale. The potential for creating scaffolds that might be able to fulfill this problem has been substantially expanded by the creation of nanofibers. Currently, electrospinning, self-assembly, and phase separation are the three methods that can be used to create nanofibers. The most extensively researched method among these is electrospinning, which has also shown the most encouraging outcomes in terms of tissue engineering applications. The potential for creating nanofibrous scaffolds has increased thanks to the availability of a variety of organic and synthetic biomaterials, particularly when using the electrospinning technique. Nanofibers were used to create three-dimensional synthetic biodegradable scaffolds that provide an ideal framework for cell adhesion, proliferation, and differentiation. Because of this, nanofibers regardless of how they were made have been used as carriers for the carefully controlled delivery of drugs, proteins, and DNA, as well as scaffolds for the engineering of musculoskeletal tissue (including bone, cartilage, ligament, and skeletal muscle), skin, vascular, and neural tissue. This article examines the usage of nanofibers in tissue engineering and drug delivery applications as well as the current methods for synthesizing them.

Introduction

One of the most promising treatments for tissue regeneration is autologous cell/tissue transplantation. However, there are drawbacks to autografts, such as morbidity at the donor site and scarce supply. Allografts are a substitute for autografts. (tissue taken from another subject of the same species). Although there is no shortage of allografts, they run the danger of transmitting illness and triggering an immunological response. The potential to overcome all the drawbacks of autologous and allogenic tissue healing makes tissue engineering an excellent strategy for the repair/regeneration of injured tissue.(Vasita & Katti, 2006)

Applying the concepts of biological, chemical, and engineering sciences with the aim of tissue regeneration, tissue engineering is an emerging subject in interdisciplinary research. To maintain, improve, or restore tissue function, tissue engineering techniques use biomaterials, cells, and factors either individually or in combination. Typically, the tissue engineering approach entails removing healthy cells from a patient, expanding them in a dish, and then recombining them. These enlarged cells are then seeded onto a three-dimensional (3D) biodegradable scaffold that offers structural support and can also serve as a reservoir for bioactive chemicals like growth factors. As time passes, the scaffold gradually deteriorates, eventually giving way to newly formed tissue derived from the seeded cells.(Nor et al., 2022)

By acting as 3D synthetic frameworks (often referred to as scaffolds, matrices, or constructions) for cellular attachment, proliferation, and growth ultimately leading to new tissue synthesis, biomaterials play a significant role in tissue engineering. Biomaterial-based 3D scaffolds can be created using a variety of innovative techniques. More recently, nanofiber-based scaffolding systems are being investigated as scaffolds for tissue engineering.(Okonogi et al., 2020)

The creation of nanofibers has expanded the possibilities for creating scaffolds that may one day resemble the nano-meter-scale structure of normal human tissue. The nanofibers' high surface area to volume ratio and their microporous shape encourage cell adhesion, proliferation, migration, and differentiation—all extremely desirable characteristics for tissue engineering applications. As a result, current research in this field is focused on creating, characterizing, and using nanofibrous structures as tissue engineering scaffolds. The nanofiber-based technologies are also being pursued for a range of other biological and non-biological applications because of their potential.

The existing methods for creating nanofibers are outlined in this article, along with how they can be used to create different kinds of tissues.(Okonogi et al., 2020)

Methods for Nanofiber Synthesis

The three methods that can currently be used to create nanofibers are electrospinning, self-assembly, and phase separation. The technology that has been the subject of the greatest research and appears to provide the best prospects for use in tissue engineering is electrospinning. There have been very few research that investigated the use of self-assembling and phase-separated nanofibers as tissue engineering scaffolds.

There are numerous methods for creating carbon nanofibers, including chemical vapor deposition using a template, catalytic synthesis (catalytic deposition, floating catalyst method), and synthesis using microwave plasmas supported by radio frequency, but it is outside the scope of this review to describe each of these methods in detail. As a result, the discussion of carbon and alumina nanofibers is limited to how they may be used in tissue engineering.(Pathak et al., 2023)

- **Electrospinning**

An appealing method for turning polymeric biomaterials into nanofibers is electrospinning. Using a very straightforward experimental setup, this approach also provides the option to adjust the porosity of the nanofiber meshes as well as the thickness and composition of the nanofibers. The idea of electrospinning or electro-spraying has been around for more than a century, but it has only been in the last ten years that polymeric nanofibers created by electrospinning have attracted significant attention. Electrospun nanofibers are prospective candidates for tissue engineering applications due to their large surface area and high porosity, which promote favorable cell interactions.(Owida et al., 2022)

The idea of electrospinning or electro-spraying has been around for more than a century, but it has only been in the last ten years that polymeric nanofibers created by electrospinning have attracted significant attention. Electrospun nanofibers are prospective candidates for tissue engineering applications due to their large surface area and high porosity, which promote favorable cell interactions. The main causes of the charged jet's instability and eventual thinning in the air are elongation and solvent evaporation. The charged jet finally develops randomly oriented nanofibers that may be gathered on a grounded metallic collector that is spinning or stationary. (Vasita & Katti, 2006)

The process of electrospraying, in which a conducting liquid is given an electric charge to create a jet that divides into tiny particles to mimic a spray, gave rise to electrospinning. The long-chain structure of polymers prevents the splitting of the jet into particles when they are employed in place of a low-molecular-weight component for the electrospraying procedure. Instead, instability occurs and the jet thins to generate nanofibers. Therefore, the electrospinning/electrospraying method requires the use of polymers, either natural or synthetic, to create nanofibers.

Electrospinning nanofibers have been done with a variety of polymers. Nanofibers that might serve as possible scaffolds for tissue engineering applications have been created using natural polymers such as collagen, gelatin, chitosan, hyaluronic acid, and silk fiber. Recently, it has been shown that protein nanofibers may be used in tissue engineering in a promising way.Poly(lactic acid), polyurethane, poly(-caprolactone), poly(lactic-co-glycolic acid), poly(ethylene-co-vinyl acetate), and poly(l-lactide-co--caprolactone) are some of the synthetic polymers investigated for the construction of nanofibers.

System parameters and process parameters both have an impact on the electrospinning process. (1) System variables including the molecular weight and dispersion of the polymer play a role in how quickly nanofibers deteriorate. The diameter of the nanofiber is determined by system factors such as polymer solution qualities, such as viscosity, surface tension, and conductivity, which also lessen the likelihood of bead formation. (2) Fibre diameter is influenced by process variables such as orifice diameter, polymer flow velocity, and electric potential. The extent of the solvent evaporation from the nanofibers and their deposition on the metal collector is determined by process factors like the distance between the capillary and the collector, whilst the motion of the collector influences the pattern creation during fiber deposition. distinct polymeric systems have distinct systemic and process properties, and most of them are modifiable, allowing for the customization of nanofibers for certain end purposes.(Xu et al., 2020)

The charge on the polymer solution allows the electrospinning process to regulate the polymer solution's trajectory by applying an electric field. This control makes it possible to create orientated nanofibers that may be used to create scaffolds for tissue engineering. This control makes it possible to create orientated nanofibers that may be used to create scaffolds for tissue engineering. Nanofibers that are randomly oriented are created by conventional electrospinning. The idea of giving an orientation to nanofibers has been explored in recent works. By using a revolving disc with sharpened edges for the deposition of nanofibers, some recent experiments have attempted to achieve nanofiber alignment. To manufacture aligned nanofibers, the revolving disc's sharpened edge attracts ions and deposits nanofibers along its edge by focused electrostatic forces. The employment of a single sharp pin as a collecting electrode and a reduction in interelectrode distance, among other factors, might affect the alignment of nanofibers. In a different recent work, a technique for gathering electrospun nanofibers utilizing patterned electrodes was created. They proved that it is possible to create uniaxially aligned nanofibers by adding insulating gaps to the conductive collector.(Shang et al., 2006)

To gain orientation for this investigation, the scientists made use of the fact that isolated fiber segments frequently align themselves in the direction of the lowest net torque. These investigations showed that by changing the collecting electrode's design pattern, nanofiber alignment and assembly may be changed.

The electrospinning technology is extremely adaptable and has the benefit of generating nanofiber meshes with high porosity and surface area on a wide range of natural and manmade polymers. However, this method also has drawbacks, including a wide range of fiber thickness, unpredictable nanofiber orientation, and weak mechanical qualities of the fiber meshes. In general, electrospinning is a generally reliable and straightforward method for creating nanofibers from a wide range of polymers.(Tai et al., 2021)

- **Self-assembly**

Through cell receptors that can identify extracellular tissue markers like collagen and fibronectin, eukaryotic cells may feel their immediate surroundings. Therefore, a practical method for designing a range of tissue types would be to replicate the extracellular matrix (ECM) using biomaterials. Berndt et al. created a peptide amphiphile (PA)--b-based self-assembling system to imitate the human ECM to develop a straightforward self-assembling system that enables the production of thermally stable protein-like molecular structures. The authors created PAs that had a "peptide amphiphile" made up of an N-alpha amino group from a peptide chain connected to a dialkyl chain moiety (hydrophilic component/head group) that was hydrophobic. The collagen ligand sequence of the ECM served as the source for the peptide head groups. The synthesized PA hydrophilic head group has the same amino acid sequence as human 1 (IV) 1263–1277 collagen: Gly–Val–Lys–Gly–Asp–Lys–Gly–Asn–Pro–Gly–Trp–Pro–Gly–Ala–Pro [IV–H1]. In a different investigation, Yu et al. substituted monoalkyl chains for the dialkyl chains of the PA utilized in the earlier study. They showed that the hydrophobic interaction between the alkyl chains boosted the thermal stability of the PA when the monoalkyl chain length grew from C6 to C16. The collagen ligand sequence of the ECM served as the source for the peptide head groups. The synthesized PA hydrophilic head group has the same amino acid sequence as human 1 (IV) 1263–1277 collagen: Gly–Val–Lys–Gly–Asp–Lys–Gly–Asn–Pro–Gly–Trp–Pro–Gly–Ala–Pro [IV–H1]. In a different investigation, Yu et al. substituted monoalkyl chains for the dialkyl chains of the PA utilized in the earlier study. They showed that the hydrophobic contact between the alkyl chains enhanced the thermal stability of the PA as the length of the monoalkyl chain rose from C6 to C16. They showed that the hydrophobic contact between the alkyl chains enhanced the thermal stability of the PA as the length of the monoalkyl chain rose from C6 to C16. At the liquid-air interface, both the dialkyl and monoalkyl chain-based PAs quickly self-assembled to produce a stabilized triple-helical conformation in an aqueous solvent.(Ho et al., 2007)

Maker et al. conducted a more recent investigation in which they included bioactive sequences into the PA to test the bioactivity of the self-assembled PAs. Their findings showed that the formation of the triple-helix for such a PA (i.e., containing a bioactive sequence) produced an ordered structure of the bioactive sequence on the exterior of the triple helix, which in turn led to a favorable cell response (i.e., cell adhesion, spreading, and proliferation) because the self-assembled triple helix resembled natural ECM. According to the findings of this work and a previous study by Fields et al, these PA structures may be employed as surface coatings for biomaterials to increase biocompatibility. Stupp et al. created di- and tri-block PAs that self-assembled into a rod-like architecture based on their prior understanding of PA self-assembling systems. The scientists created a

novel method for the pH-controlled self-assembly of PAs into nanofibers by manipulating the peptide head group of the PA.(Banasiak et al., 2022)

- **Phase separation**

Ma and Zhang came up with a new method for creating nanofibrous foam materials termed thermally induced liquid-liquid phase separation to simulate the 3D collagen structure found in natural ECM. The size of the nanofibrous foams created by the phase separation process (50-500 nm) is remarkably comparable to the natural collagen found in the ECM of tissue. There are five simple stages in this method.

1. Dissolution of polymer.
2. Liquid–liquid phase separation process.
3. Polymer gelation (controls the porosity of nanoscale scaffolds at low temperatures).
4. Extraction of solvent from the gel with water.
5. Freezing and freeze-drying under vacuum.

The most important process that managed the porous morphology of the nanofibrous foams was discovered to be gelation. The polymer concentration and gelation temperature had an impact on the gelation time. Low gelation temperatures resulted in the creation of nanoscale fiber networks, whereas high gelation temperatures resulted in the growth of crystals and the nucleation of platelet-like structures. Increased cooling rates, which created homogenous nanofibers, overcame the restriction of platelet-like structure development. However, neither the gelation condition nor the polymer concentration had a significant impact on the average fiber diameter. Process variables including polymer concentration were shown to significantly affect the characteristics of nanofibers. Young's modulus and tensile strength rose as polymer concentration increased and porosity decreased. The shape of the nanofibrous scaffolds was also affected by additional process variables, including type of polymer, type of solvent, and heat treatment. The phase separation technique resulted in the formation of a 3D porous continuous fibrous network with a high porosity of around 98% inside material blocks. By including porogens like sugar and salt in the mold along with the polymer solution during phase separation, the authors added macroporosity to the scaffold. To enhance mass transfer, cell distribution, and tissue organization, macroporosity was added. As a result, the scaffolds made using this approach have three layers of architecture: macroporous (100 m), where the pore size and shape are regulated by porogen; interfiber distance; and fiber diameter. The interaction of these nanofibrous scaffolds with osteoblastic cells was next investigated by the scientists. The outcomes showed that the nanofibers enhanced fibronectin and collagen protein adsorption, which are qualities required for cell and ECM interaction. The scientists credited the geometry of these 3D macroporous scaffolds for enhanced cell adhesion and dissemination.(Vasita & Katti, 2006)(Pathak et al., 2023)

Comparing the phase separation method to the previously stated electrospinning and self-assembly procedures, the phase separation process has the benefit of being a reasonably straightforward approach with extremely low equipment needs. A mold can be used to directly construct the scaffold for the appropriate anatomical form of a body component. Another benefit is the coexistence of nano and macro architecture, which can improve tissue architecture and cell dispersion at the macroporosity level as well as cell responsiveness at the nanofiber level.(Hajiali et al., 2011)

- **Natural polymeric materials for nanofibers**

The benefit of being extremely close to, and frequently identical to, macromolecular components found in the human body is provided by natural polymers. The biological environment is therefore set up to recognize and interact favorably with natural polymers. Collagen, hyaluronic acid, gelatin, chitosan, elastin, silk, and wheat protein are a few examples of natural polymers utilized as biomaterials.

The most well-liked and researched natural biomaterial is collagen (Shields et al 2004). It has been shown that collagen nanofibers are compatible with a variety of cell types, including myoblasts and chondrocytes. Additionally, collagen type II scaffolds have high mechanical qualities due to cross-linking, which makes them an ideal habitat for cell development. Type I collagen nanofibers (made by electrospinning) were blended with poly(ethylene oxide) (PEO) in a study by Huang et al. Their findings showed that a considerable improvement in the mechanical strength of the nanofiber system was caused by a large number of intermolecular contacts between collagen and PEO. (Delaine-Smith et al., 2021)

This research demonstrated collagen's prospective function in tissue engineering. Another natural biomaterial that has been utilized to create nanofibers is chitosan. Using the electrospinning approach, nonwoven or aligned chitosan/PEO (90:10) nanofibers have been created. These nanofibers had structural integrity in water, and experiments on them in cells showed that human osteoblasts and chondrocytes attached to them more readily. Additionally, the cells were viable and retained their typical shape on these nanofibers, demonstrating high cytocompatibility. Consequently, chitosan nanofibers may be a suitable choice for use as a scaffolding material in tissue engineering.

Hyaluronic acid has been utilized as a biomaterial and is a naturally occurring part of the ECM of tissue. Hyaluronic acid nanofibers were created utilizing the electrospinning and electroblowing techniques, which include blowing hot air while electrospinning. The authors noted that consistent manufacture of high-quality nonwoven nanofibers is impossible using hyaluronic acid electrospinning. They, therefore, used a novel electroblotting method that combined electrospinning and airflow. In this work, the scientists created hyaluronic acid nanofibers by electrospinning and blowing air at 57 °C with a flow rate of 70 ft/hour.(Shang et al., 2006)

Gelatin is a different natural biomaterial that has undergone extensive research. created fibrous scaffolds made of gelatin/PCL composite utilizing the electrospinning method. According to their research, as compared to PCL or gelatin alone, composite nanofibers exhibit better mechanical strength and wettability. Additionally, the gelatin-PCL nanofibrous scaffold demonstrated strong bone marrow stromal cell adhesion, proliferation, and migration. To enhance the mechanical characteristics of natural biomaterials for tissue engineering applications, composite nanofibers of natural and synthetic materials may be a useful solution.

The in vitro cytocompatibility of silk nanofibers with keratinocytes and fibroblasts has been documented, making silk fibroin another viable natural biomaterial for nano-fibrous scaffolds. It is a good potential material for scaffolding technology because of its high porosity, cytocompatibility, and fiber diameter. Recent research looked at the potential use of electrospun protein fibers as tissue engineering scaffolds. Human tropoelastin was created by the authors for electrospinning. According to the study's findings, when compared to nanofibers made of collagen or elastin, tropoelastin nanofibers implanted with human embryonic palatal mesenchymal cells successfully promoted cell adhesion and growth. Another recent study looked at the potential of employing the plant protein wheat gluten as a novel material for electrospinning nanofibers that may be applied to tissue engineering.

Consequently, a wide range of natural polymers have been investigated for the manufacture of nanofibers as scaffolds for tissue engineering in light of this research.(Xu et al., 2020)

Synthetic polymeric materials for nanofibers

The biggest class of biomaterials is made up of synthetic polymers. The creation of nanofibers has been done using a wide range of synthetic polymers. These include PCL for neural and cartilage tissue engineering, PLA (poly(ethylene terephthalate), and several copolymeric substances, such as PLLA-CL as a biomimetic ECM for smooth muscle and endothelial cells. PEVA nanofibers for controlled drug delivery, PLGA-poly(ethylene glycol) (PLGA-PEG) block copolymeric nanofibrous scaffolds produced via electro-spinning as a matrix for DNA delivery, and PLGA, one of the most frequently used polymers to fabricate nanofibers for bone and

cartilage tissue engineering and controlled drug delivery. Nanofibers made of carbon and alumina have recently been investigated as potential biomaterials for use in orthopedic and dental implants. Due to the electrospinning technique's ease of use with synthetic polymers, a wide range of synthetic polymers have therefore been investigated for nanofiber fabrication.(Rodríguez-Lorenzo et al., 2011)(Vasita & Katti, 2006)

Applications of nanofibers in tissue engineering

There have been several ways described in the past for creating scaffolds for tissue engineering. Nanofibrous systems, on the other hand, have been created and are being investigated as prospective scaffolds for tissue engineering. They can improve cell adhesion because of their large surface area and porosity, and they offer good micro/nano environments for cells to develop and carry out their normal tasks due to the resemblance of their 3D design to natural ECM. As a result, the use of nanofibrous systems as scaffolds for tissue engineering applications has been vigorously pursued.@(Xu et al., 2020)(Kim et al., 2015)

- **Nanofibers for musculoskeletal tissue engineering**

HA, chitosan, PLGA, carbon, and aluminum nanofibers are only a few of the natural and manmade materials that have been investigated as nanofibrous scaffolding materials for bone, cartilage, ligament, and skeletal muscle tissue engineering. Though nanofibers have been investigated as tissue scaffolds for a variety of tissue types, musculoskeletal tissue has likely received the most attention.(Qian et al., 2014)

- **Nanofibers for bone tissue engineering**

Based on the physical characteristics of bone tissue, including its tensile strength, pore size, porosity, hardness, and overall 3D architecture, scaffolds are designed for bone tissue creation. Scaffolds for bone tissue engineering are favored for better cell/tissue in-growth and subsequently improved bone regeneration, with pore sizes in the range of 100-350 m and porosity of more than 90%. (Stutz et al., 2020)

- **Nanofibers for cartilage tissue engineering**

Due to the scarce chondrocyte supply and the complete lack of progenitor cells at the site to facilitate the healing process, articular cartilage tissue has a restricted capability for repair. The chondrocytes that are accessible for repair are entrenched in the articular surface's strong extracellular matrix, which inhibits their mobility and, in turn, reduces their ability to aid in the healing of wounds. Additionally, the fact that articular cartilage lacks blood vessels makes it less able to repair on its own. Several surgical procedures have been devised to address this issue, although they have met with varying degrees of success. Tissue engineering is therefore a promising strategy for regenerating cartilage tissue. By electrospinning, Li et al. created PCL-based nanofibrous scaffolds. Fetal bovine chondrocytes (FBC) were then seeded onto these scaffolds, and their capacity to keep chondrocytes in a mature, functioning condition was examined. Their findings showed that FBCs seeded on PCL nanofibers may preserve their chondrocytic phenotype by expressing extracellular matrix genes unique to cartilage, such as aggrecan, collagen type II and IX, and cartilage oligomeric matrix protein. In addition, FBCs on the nanofibrous scaffold took on a spindle or spherical form as opposed to the flat, widely dispersed shape they had when grown on tissue culture polystyrene. These findings showed that the architecture of the scaffold and the nature of the culture media influence the bioactivity of FBCs. In light of this, it appears that PCL nanofibers have the potential to be further investigated as scaffolds for cartilage tissue engineering.(Vasita & Katti, 2006)

- **Nanofibers for ligament tissue engineering**

The stability and mobility of joints are controlled by bands of thick connective tissue called ligaments. Ligament tears lead to aberrant joint kinematics, frequent irreparable damage to the surrounding tissue, and tissue degenerative illnesses that do not heal spontaneously and cannot be fully treated by standard therapeutic procedures. Recently, this problem has been successfully addressed by tissue engineering techniques utilizing

nanofibers. Aligned nanofibers were investigated as scaffolds for ligament tissue creation in particular because they improved cell responsiveness.(Stastna et al., 2020)

- **Nanofibers for skeletal muscle tissue engineering**

Skeletal muscles are in charge of the body's voluntary movements and, if harmed (by disease or trauma), are difficult for adults to recover. So, although difficult, skeletal muscle tissue engineering offers an interesting alternative to surgical methods for skeletal muscle regeneration. have investigated the use of degradable polyester urethane (PEU) electrospun microfibers as scaffolds for skeletal muscle tissue engineering. The electrospun microfibers of PEU demonstrated satisfactory mechanical properties and encouraged cellular response in terms of adhesion and differentiation, according to their preliminary studies using primary human satellite cells (biopsy from a 38-year-old female), C2C12 (murine myoblast cell line), and L6 (rat myoblast cell line). The electrospun PEU microfibers have promise to be further investigated as a scaffolding method for skeletal muscle tissue engineering, according to these findings.(Stutz et al., 2020)

- **Nanofibers for skin tissue engineering**

Instead of the regeneration of new skin, skin lesions often heal via the creation of epithelialized scar tissue. The epidermis has a lower capability for healing than the dermis, yet when the epidermis has to be replaced in vast regions, regular regeneration is insufficient. The dermis also has a remarkable ability for regeneration. The absence of dermis results in the formation of scar tissue that lacks the elasticity, flexibility, and strength of the healthy dermis. As a result, scar tissue restricts motion, hurts, and is unattractive on the surface. Therefore, engineered skin tissue would be a fantastic substitute, not only for healing the lesion but also for promoting dermal regeneration. Other organic and synthetic polymers have also been investigated for skin tissue engineering, in addition to collagen, but there hasn't been much success using these materials as nanofibers. created silk fibroin nanofibers for skin tissue engineering by electrospinning for nonwoven materials. Fibroin nanofibers coated with type I collagen were discovered to increase keratinocytes/fibroblast adhesion and spreading because of their high porosity and high surface area to volume ratio. The silk fibroin nanofibers therefore have the potential to be used as a scaffold for skin tissue engineering.(Hajiali et al., 2011)

- **Nanofibers for controlled drug delivery**

Utilizing controlled delivery systems, which carry pharmaceuticals to the site of action at a rate determined by the physiological environment's requirements, can increase the therapeutic efficacy and safety of medications. A wide range of polymeric materials have been employed as delivery matrices, and the needs of the particular application govern the choice of the delivery vehicle polymer. Recently, the capacity of polymeric nanofibers to convey and encapsulate bioactive compounds for medicinal purposes has been investigated.(Vasita & Katti, 2006)

A study has shown the usage of PU nanofibrous electrospun scaffolds (nonbiodegradable polymer scaffolds) for the administration of water-insoluble medications like itraconazole and ketanserin. The water-insoluble medication was produced by the authors of the study as an amorphous nanodispersion on the nanofibrous scaffold. The nanofibrous scaffold's enormous surface area allowed for quick and effective solvent evaporation, which reduced the amount of time the drug in the mixture could crystallize and favored the creation of an amorphous dispersion. This research showed that weakly water-soluble pharmaceuticals could be released from water-insoluble polymers and that the rate of release could be controlled by adjusting the polymer's content. Another study showed the possible use of PLGA nanofibrous scaffolds for hydrophilic antibiotics with controlled release. During the electrospinning process, the scientists added Mefoxin (cefotoxin sodium) to PLGA nanofibers. Staphylococcus aureus growth was inhibited, indicating that electrospinning had no impact on the bioactivity or structure of the antibiotic medication.

The aforementioned investigations demonstrate that nanofibrous scaffolds are effective drug transporters for both hydrophilic and hydrophobic medicines and that the drug release rate can be customized by modifying the shape, porosity, and content of the nanofibrous scaffold.(Xu et al., 2020)

- **Nanofibers for DNA, protein, and enzyme delivery**

The choice of the best gene delivery method to satisfy the requirements of a certain therapeutic application might be difficult given the availability of several gene delivery technologies. Currently, therapeutic proteins are produced using viral and plasmid-based delivery systems to trigger the desired biological response. This would be particularly helpful in the field of tissue engineering, where it would be possible to trigger the creation of a desired protein (growth factor) that can speed up the regeneration of tissue. As a result, the use of gene delivery methods in the engineering of various tissues has been investigated. Cationic liposomes and condensing substances like poly(ethyl-enimine) and poly(l-lysine) are the most often employed carrier-based methods for delivering genes.(Huang, 2009)

Gene delivery scaffolds must offer structural stability, site-specific delivery, and protection of the delivered genes from the biological system until they are released. Additionally, the released DNA must maintain its structural integrity until it is incorporated into the targeted cells.

Nanofibrous scaffolds based on PLGA and PLA-PEG block copolymers were explored by Luu et al. for the transport of plasmid DNA. According to their findings, the electrospun nanofibrous scaffolds delivered the gene at the right location in a regulated way, resulting in cell transfection and the required bioactivity. When compared to naked DNA introduced directly to the culture medium, this method demonstrated greater transfection effectiveness. The nanofiber DNA system's transfection effectiveness rose as the amount of DNA used to make the scaffold was increased. The nanofibrous scaffold's structure and composition have an impact on how quickly the DNA is released. Therefore, by adjusting scaffold characteristics such as nanofiber diameter, scaffold pore size, and polymer breakdown rate, the release profile of the DNA may be adjusted. These changes make it feasible to continue DNA delivery for a longer period. The sustained/controlled delivery of undamaged DNA over many months seems to be the perfect application for this technique, in general.(Vasita & Katti, 2006)

The scientists investigated the catalytic effectiveness of alpha-chymotrypsin on electrospun polystyrene nanofibers (120 nm) in biotransformations. According to their findings, the nanofibrous enzyme system had three times greater nonaqueous activity than immobilized alpha-chymotrypsin in organic solvents, and a higher hydrolytic activity (65%) than immobilized enzyme. The authors hypothesized that covalent enzyme-nanofiber interaction would boost enzyme stability or lessen structural denaturation. They postulate that the higher activity of the enzymes coupled to the nanofibers may be caused by this greater stability. The nanofibers thus have the potential to be developed into catalytic systems for application in biotransformations.(Danwanichakul & Danwanichakul, 2014)

Researchers have created PVA electrospun nanofibers for protein delivery in a study. Proteins encoding luciferase or bovine serum albumin were added to PVA nanofibrous scaffolds. Then, using chemical vapor deposition, poly(p-xylylene) (PPX) was applied to the PVA nanofibers. Then, the bioactivity of the released proteins under physiological circumstances was examined on the coated and untreated PVA nanofibrous scaffolds. The outcomes showed that both types of nanofibers constantly released intact protein and enzymes and that their bioactivity was maintained after being released from the nanofibrous scaffolds. In contrast to the uncoated PVA nanofibers, the PPX-coated nanofibers showed much slower release rates. As a result, our study demonstrated that the nanofibrous scaffold may be a solid choice of substance for regulated enzyme/protein delivery.(Hackett et al., 2010)

The experiments mentioned above showed that nanofibers have the potential to be used as controlled delivery systems, and as a result, further in-depth research is needed to make this technology useful for patients.(Flores-Hernandez et al., 2020)

- **Application of carbon nanofibers in tissue engineering**

Carbon nanofibers have exceptional mechanical properties (three times that of bone tissue), in addition to having nanoscale fiber dimensions that are comparable to the HA and collagen fibers found in bone. This

provides a strong justification for investigating them for use in orthopedic or dental tissue engineering. Additionally, it has been demonstrated that carbon nanofibers have good conductivity, which might make them a possible option for use in brain tissue engineering. Due to their outstanding cytocompatibility characteristics and lack of difficulties brought on by leachables in the form of metal ions discharged from implants, carbon nanofiber-based implants can outperform traditional metal alloy orthopedic implants in several areas.(Panek et al., 2018)

The mechanical characteristics of carbon nanofibers include a Young's modulus of 2 TPa, which is much greater than that of bone, and a nearly equivalent tensile strength to bone. As a result, Price et al investigated the use of carbon nanofibers for bone tissue engineering. When osteoblast adhesion on carbon nanofibers was compared to that on traditional carbon fibers, it was found that the osteoblast adhesion on carbon nanofibers was higher. The authors looked at examined osteoblast adhesion on carbon nanofibers coated with PLGA to identify the characteristics that led to improved adhesion on carbon nanofibers. As compared to regular carbon fibers, their findings demonstrated that PLGA-coated carbon nanofibers had improved osteoblast adhesion.(Park et al., 2019)

Due to their electrical conductivity, carbon nanofibers were first investigated for use as electrically conductive fibers, in field emitters, nanoelectronic devices, and reinforcement. Carbon nanofibers have been investigated as viable options for brain tissue engineering more recently because of their conductivity. Researchers looked at how astrocytes—glial cells that produce scar tissue—interacted with carbon nanofibers in terms of adhesion and proliferation to ascertain if they were cytocompatible for use as brain implants. According to their research, astrocyte activities on nanoscale fibers were inhibited, which decreased the production of scar tissue. These findings led the authors to the conclusion that little glial scar tissue development and good neuronal contact are qualities that would substantially assist the effectiveness of a neural implant. (Lind et al., 2013)

Therefore, more research is required to determine the potential of carbon nanofibers for brain tissue engineering.

- **Applications of alumina nanofibers in tissue engineering**

A crucial prerequisite for dental and bone implantation is osteointegration. It has been shown that osteointegration can be improved by reducing the size of surface features.

The most extensively researched materials for both dental and orthopedic applications are alumina, titania, HA, and their composites. Alumina nanofibers may improve osteointegration, according to Price et al's hypothesis, because of the similarities between the physical shape of HA and aluminum nanofibers. They investigated how alumina nanofibers affected osteoblast cells' behavior. Their findings showed that the alumina nanofibers improved cell adherence and the production of osteoblastic phenotypic indicators including calcium and alkaline phosphates. According to the findings mentioned above, carbon and aluminum nanofibers are potentially useful materials for orthopedic and dental tissue engineering.(Pacurari et al., 2016)

Conclusion

One of the most difficult aspects of tissue engineering is replicating the ECM's architecture. The method using nanofibers has shown the most encouraging results out of all those used to manufacture ECM synthetically. The three most common methods for creating nanofibers are electrospinning, self-assembly, and phase separation. The approach that has received the most research and produced the most encouraging outcomes is electrospinning. The field of nanofiber production, particularly when utilizing the electrospinning process, has been stimulated by the availability of a wide variety of natural and synthetic biomaterials.(Kohoutek et al., 2017)

Whatever the technique of synthesis, nanofibers have produced scaffolds with a large surface area and improved porosity. It has been shown that these characteristics significantly influence cell adhesion, proliferation, and differentiation. As a result, nanofibrous matrices are being investigated as scaffolds for musculoskeletal tissue engineering (including bone, cartilage, ligament, and skeletal muscle), skin tissue engineering, neural tissue engineering, vascular tissue engineering, and controlled delivery of drugs, proteins, and DNA. All of these research findings overwhelmingly support the notion that nanofiber-based scaffolds have great promise for use in a range of tissue engineering applications.(Çavdar & Uguz, 2019)

Therefore, nanofibrous matrices are being investigated as scaffolds for musculoskeletal tissue engineering (including bone, cartilage, ligament, and skeletal muscle), skin tissue engineering, neural tissue engineering, vascular tissue engineering, and controlled delivery of drugs, proteins, and DNA. These research findings unmistakably reveal that nanofiber-based scaffolds have a great deal of promise for use in a range of tissue engineering applications.

In conclusion, the use of nanofibers in tissue engineering has shown great potential in replicating the architecture and characteristics of the extracellular matrix.(Çavdar & Uguz, 2019)

Nanofiber-based scaffolds have demonstrated significant advantages, including a large surface area for drug delivery, nutrients, and biochemical materials (Roozbahani et al., 2015).

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