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Bioinspired Electrospun Chitosan–Based Materials for Tissue Regeneration

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ABSTRACT

Chitosan (CS) is a cationic biopolymer that has shown unique opportunities in tissue engineering. The well-defined geometry, porosity, easy processability, and antimicrobial and antifungal properties are particularly beneficial for tissue regeneration. This review outlines the progress made in the preparation of bioinspired electrospun CS for tissue regeneration. The article concentrates on bioinspired electrospun CS and CS-based composites which have appeared in a good number of publications in the literature and enriched the field of tissue engineering. It also highlights the upscaling of electrospun nanofibers with recent advanced technologies. Finally, the overall summary, challenges, and future perspective in tissue regeneration based on bioinspired CS and related composite materials is presented.

1 | Introduction

The elegance and complexity of natural structures have long served as a source of inspiration for engineering functional and customized products [1–3]. Techniques such as electrospinning and additive manufacturing have enabled the translation of nature's beauty into tangible and high-performance materials. Beyond their visual appeal, natural forms offer profound insights into structural efficiency, multifunctionality, and adaptability, which can be harnessed to solve modern engineering challenges. Nature's beauty is not solely derived from its structural hierarchy, whether ordered or disordered but also from the synergistic

integration of multiple structural elements [1]. Over more than 4 billion years, nature has evolved optimized architectures that perform specific functions within the constraints of both the organism and its environment. By studying these structures, engineers can uncover sustainable, efficient solutions to complex problems.

This concept, known as biomimicry, is often described as innovation inspired by nature [1, 4]. The term originates from the Greek word biomimesis and was first introduced by Schmitt in 1969. Biomimetics involves learning from natural systems that exhibit nano, micro, and macroscale features, which collectively

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govern material properties and functionalities. This allows humans to mimic nature to exploit nanomaterials, nanodevices, and processes which provide desirable properties. In the context of tissue engineering, biomimetic design offers the opportunity to develop scaffolds with tailored mechanical properties, biocompatible surfaces, and enhanced cell-scaffold interactions. Such scaffolds can mimic the structural and physical characteristics of native tissues, creating an environment conducive to cell attachment, proliferation, and differentiation. For example, spiderweb-inspired architectures have demonstrated the ability to achieve specific mechanical properties through deliberate design [5–7]. Similarly, implants incorporating biomimetic features can serve as ideal scaffolds for tissue regeneration [8]. All these can be attained by choosing relevant fabrication techniques to afford the production of these fascinating structures.

Electrospinning has emerged as a reliable and efficient technique for producing long, continuous fibers with diameters ranging from a few nanometers to several microns [9–12]. By applying a high voltage to a polymer solution or melt, this method enables the fabrication of fibers that closely mimic the structural features found in nature. This makes electrospinning particularly well-suited for replicating the nano to microscale fibrous building blocks commonly observed in natural materials. Moreover, electrospinning offers the versatility to create a wide variety of unique fiber morphologies, including ribbon-like, helical, porous, necklace-like, bead-on-string, and hollow structures [4, 9, 10]. These diverse architectures provide opportunities to tailor material properties for specific applications, making electrospinning a powerful tool in the design of biomimetic and functional materials. With ongoing advancements in electrospinning techniques, production efficiency can be significantly improved, making it possible to achieve output levels suitable for industrial-scale applications [13–15].

Chitosan (CS) is one of the natural polymers obtained by deacetylation of natural chitin [3, 16–18]. The recent few decades have witnessed the emergence of CS as one of the fastest-growing biomaterials due to its unique features, including antibacterial, antifungal, nontoxicity, biodegradability, and gelling properties. It was first discovered in 1859 by Rouget through experimentation on the natural chitin [16, 19]. Its ability to bond with other materials such as enzymes, proteins, cell membranes, and other anionic polymers because of its cationic nature has broadened its application beyond the classic biomedical field. CS has been employed in various forms including aerogels, hydrogels, electrospun fibers, microcapsules, and nanoparticles. Amongst these forms, electrospun CS fibers have received considerable attention from researchers and industrialists because of the structure of the resultant membranes.

CS, on its own, presents significant challenges in electrospinning due to its poor spinnability [17, 20]. To overcome this, it is commonly blended with synthetic polymers to enhance the fiber formation and mechanical integrity. Achieving smooth, uniform nanofibers typically requires careful optimization of electrospinning parameters, including the polymer concentration, solvent system, and processing conditions. A wide range of synthetic polymers, such as polyvinyl alcohol (PVA) [21], polyethylene oxide (PEO) [20], polycaprolactone (PCL) [22, 23], and polyamide (PA), have been successfully blended with CS to improve its electrospinnability. For example, Biranje et al. [21] utilized PVA to produce nanofibers with diameters ranging from 80 to 300 nm.

In their study, a 2 w/v% CS solution was blended with 55 wt% PVA in varying ratios from 40:60 to 10:90 wt/wt (CS:PVA). It was observed that continuous fiber formation was only achievable when the CS content remained below 40wt%. Interestingly, increasing the CS content led to a narrower fiber diameter distribution, suggesting improved uniformity in the fiber morphology. Please note that, unless otherwise stated, polymer compositions and blend ratios in this review are reported as weight percentages (wt%) relative to total polymer mass. Polymer solution concentrations are expressed as weight/volume percentages (w/v%) and solvent compositions as volume percentages (v/v%) to ensure clarity and reproducibility. Also, the following abbreviations are used throughout this review: CS, PCL, PEO, PVA, polylactic acid [6], poly(L-lactic acid) (PLLA), gelatin (GEL), silk fibroin [24], nano-hydroxyapatite (nHAP), extracellular matrix (ECM), polydopamine (PDA), and resveratrol (RSV). Abbreviations were defined at first mention and used consistently thereafter.

2 | Background

The second largest natural source of polysaccharides derived from living things is chitin [25, 26]. Chitin can be isolated from different sources, including crabs, insects, lobsters, shrimps, and turtles. Over the past years, researchers have made significant strides in coming up with novel methods to isolate chitin for the purpose of generating CS [25–27]. In the case of mostly used sources (e.g., crustaceans) [1], there must be pretreatment with the sole purpose of removing protein from the dried shells. This can be achieved by using alkali solutions, viz. KOH, NaOH, and so on, subsequently, the deproteinized powder is treated with mild acid solution for the removal of minerals [25]. The resulting chitin is then decolorized through treatment with an oxidizing agent, for example, H₂O₂, KMnO₄, and so on, followed by washing using oxalic acid. The resulting chitin is then subjected to alkali treatment for a few hours to produce CS through drying. To produce highly pure CS, the resultant dried CS is treated with 2 v/v% acetic acid and then neutralized with NaOH. For biomedical purposes, CS is often cleaned to reach the purest form with commercially available has a deacetylation value of 80%–90%. The degree of deacetylation, which can be taken further to 95%, directly affects the molecular weight and thus the overall properties of the resultant CS.

2.1 | Electrospinning

Electrospinning is one of the effective and powerful techniques that allow the processor to produce scaffolds from different polymers [10, 11, 20, 28, 29]. The resulting materials possess remarkable properties, including large specific surface area, high porosity, and interconnective structure, making them applicable in different fields. For instance, interconnectivity can be explored for nutrient transport, cell communication, and efficient cellular responses. A classic electrospinning technique consists of four main components, viz. a high voltage supply, syringe pump, spinneret, and grounded collector were used. During the electrospinning process, the electric fields overcome the surface tension of the droplet and form a charged jet. The jet was elongated and whipped continuously due to electrostatic repulsion toward the grounded collector. During this process, the solvent is evaporated to afford solidification of the jet onto the collector, forming non-woven fibrous mats. A large number of polymers, either synthetic

or natural, semiconductors, ceramics, and the combination thereof have been used to produce electrospun nanofibers mats [28, 29]. In most cases, natural polymers are difficult to spin on their own. It is, however, mixed with the synthetic polymers to ensure their spinnability [23, 24, 29]. In some instances, post-treatment is applied to remove the synthetic polymer to afford neat, natural electrospun mats. Therefore, there are three main conditions that the processor can play around to achieve the desired morphology, viz. solution properties, processing variables, and ambient conditions. Hence, there is a need for optimization processes that one has to achieve in order to produce desirable alignment patterns [20].

The processor has the opportunity to control electrospinning parameters to afford the desired fibrous structure that can be utilized to construct bioinspired structures [28]. With such opportunities, a wide variety of structures can be produced that resemble natural objects, viz. honeycomb, spider webs, soap bubbles, lotus leaves, and silver ragwort leaves using the electrospinning technique. A detailed review on the use of electrospinning to create nanofiber scaffolds has been published [1], while advances in tissue engineering using fibrous structures have also been published by Wang et al. [30] and Tan and Zhou [31]. To the best of our knowledge, there is no review article published based on bioinspired CS via electrospinning for tissue regeneration purposes.

Significant advancements in electrospinning technology have paved the way for the development of new multifunctional materials tailored to specific applications [32–39]. Beyond improvements in collector design and spinneret configurations in conventional electrospinning, one of the major challenges has been low production rates. As a result, research efforts have intensified to enhance the scalability of electrospinning, leading to the emergence of alternative spinning methods such as centrifugal spinning [40–42] and blow spinning [43–46]. Table 1 summarizes these technologies and their respective production rates [13, 43, 47–49, 52–55, 57–59], highlighting the potential of electrospinning-based techniques to meet industrial-scale manufacturing demands.

Figure 1 illustrates the evolution of electrospinning technologies [13, 43, 47, 48, 52, 53, 55, 58, 59]. These innovations have enabled the fabrication of multifunctional nanofibers suitable for diverse applications. For example, solution blow spinning has been used to produce doxycycline-loaded nanofibers for biomedical use. Such studies underscore the feasibility of scaling up electrospun nanofibers for biomedical applications, particularly in tissue regeneration. It's worth noting that the choice of electrospinning technique should be guided by the intended application. For example, blow-spinning, being more chaotic and less controlled, may be suitable in cases where fiber uniformity is not a critical requirement. On the other hand, classical electrospinning offers greater control over the fiber morphology, making it ideal for applications such as tissue regeneration and other areas where precision is essential. Extensive reviews have been conducted on the upscaling potential and production rate improvements of electrospinning technologies [51, 56, 60, 61]. These studies provide valuable insights into how the process can be adapted for industrial-scale applications while maintaining the fiber quality and consistency. Interested readers are encouraged to consult the referenced studies for a deeper understanding of the

advancements, challenges, and strategies involved in scaling up electrospinning systems.

2.2 | Electrospinning CS

One of the primary challenges in electrospinning CS lies in its inherent cationic nature, which significantly affects its electrospinnability [23]. Table 2 presents an overview of selected studies on various efforts to electrospin CS and its composites [62–69, 72].

CS's high charge density in acidic solutions leads to strong intermolecular repulsion and high viscosity, making it difficult to form uniform and continuous nanofibers under standard electrospinning conditions. Additionally, its limited solubility in common organic solvents further complicates the process. To overcome these limitations, researchers have explored various strategies, such as blending CS with more spinnable polymers such as poly(ethylene oxide) (PEO) [65], polyamide, or poly(vinyl alcohol) (PVA), optimizing solvent systems, and adjusting electrospinning parameters, including voltage, flow rate, and collector distance [23]. These approaches help stabilize the jet formation and improve the fiber morphology, enabling the successful fabrication of CS-based nanofibrous scaffolds for biomedical applications. Singh et al. [73] investigated the electrospinnability of CS by blending it with PEO, a well-known spinnable polymer, using acetic acid as the solvent. They observed that increasing the PEO content from 10 to 50 wt% enhanced the solution's viscosity, while higher concentrations of acetic acid reduced the surface tension, both of which are critical parameters for successful fiber formation. The optimal conditions for producing bead-free nanofibers were achieved with a solution containing 50 wt% PEO and 85 v/v% acetic acid, along with CS concentrations of 0.2 w/v% and 5 w/v%. Under these conditions, nanofibers with an average diameter of ~149 nm were obtained at an applied voltage of ~23 kV. The smallest fiber diameter, around 110 nm, was achieved when the tip-to-collector distance was set at 15 cm. The optimal feed rate was determined to be 0.4 mL/h. In summary, the ideal electrospinning parameters for producing smooth and uniform CS nanofibers were a 50:50 CS/PEO blend dissolved in 90 v/v% acetic acid, electrospun at 20 kV, with a 15 cm needle-to-collector distance, a 0.2 mm inner diameter needle, and a feed rate of 0.4 mL/h. The resulting nanofibers supported the proliferation and differentiation of MG-63 osteoblast-like cells, indicating their potential for tissue engineering applications.

In addition to synthetic polymers, several natural polymers have been blended with CS to facilitate the production of composite nanofibers (Table 2). These include biopolymers such as GEL, collagen, hyaluronic acid, and silk fibroin. Typically, each polymer is dissolved separately in a compatible solvent, and the resulting solutions are then combined to form a homogeneous spinning solution. The spinnability of these blends largely depends on the molecular interactions between CS and the copolymers. A wide range of solvents, spanning from acid-based to organic solvents, has been employed to electrospin CS and its composites [62].

However, it is worth noting that only a limited number of studies have successfully reported the electrospinning of neat CS without the aid of a supporting polymer, highlighting the challenges associated with its standalone processability. Homayoni et al. [62]

TABLE 1 | Summary of electrospinning technologies and their production rates, highlighting the potential of these techniques to meet industrial-scale manufacturing demands.

Technique	Production rate	Dimensions	Advantages	Limitations	References
Classic spinning	1–100 mg/h	1–1000 nm	• Produces highly uniform fibers • Enables precise control and easy fine-tuning of porosity • Compatible with a wide range of materials; from pure polymers to complex composites • Supports various solvent systems, including conventional solvents, ionic liquids, and deep eutectic solvents • Accommodates different types of collectors such as flat plates, rotating drums, disks, and so on	• High humidity and elevated temperatures can negatively impact fiber formation, requiring careful optimization to achieve the desired morphology • Current production capacity is limited, which may constrain scalability • Needle clogging often occurs, which disrupts continuous fiber production and leads to poor nanofiber quality	[47]
Coaxial and multiaxial	1–100 mg/h	1–1000 nm	• Enables the production of complex core–shell structures, which help protect active substances within the sheath layer • Allows for the formation of hollow fibers by removing the core material postprocessing • Facilitates the spinning of substances that are otherwise difficult to process by incorporating them as core materials	• The complexity of producing core–shell structures largely stems from the need to optimize process parameters to achieve the desired outcomes • Needle clogging is also a challenge, especially during the optimization phase, as it increases maintenance efforts and can disrupt workflow	[33, 35]
Multineedle spinning	1–2 g/h	10–1000 nm	• High throughput when compared to single needle-based electrospinning	• Electrostatic interactions between multiple jets (needles) can complicate the operation of this technique, making it challenging to produce fibers with the desired characteristics • Needle clogging is another common issue that can disrupt the process and affect consistency	[12, 34, 36]
Blow spinning to Electro-assisted blow spinning	0.5–400 g/h	100 nm–5 μ m	• Supports high production rates, making it suitable for industrial-scale manufacturing • Allows the use of multiple needles, enhancing the throughput and efficiency	• Vacuum collectors are preferred to minimize material waste during the process • The resulting fiber topologies can vary, leading to inconsistencies in the structure • The technique is limited by a narrow selection of compatible solvents • There are constraints in effectively encapsulating active substances within the fibers	[43, 45, 47, 48]
Centrifugal spinning to Electrocentrifugal spinning	5–30 g/h	200 nm–10 μ m	• Capable of producing uniform fibers with significantly higher throughput compared to classical electrospinning techniques • Effectively processes highly concentrated polymer solutions • Offers high fiber production rates, suitable for scalable applications • Enables the fabrication of core–shell structures for advanced material functionalities	• The technique involves complex needle and collector setups, which can complicate the system design and operation • Incorporating additional substances into the resulting fibers can be challenging, limiting material versatility • Needle clogging remains a persistent issue, increasing maintenance demands and potentially disrupting the process	[41, 49, 50]
3D electrospinning	—	—	• Enables the production of 3D structures with customizable morphology, patterns, and both physical and chemical properties • Capable of fabricating complex architectures without the need for postprocessing steps • Generates minimal waste, contributing to a more sustainable manufacturing process	• Often require highly conductive materials and thus limit the range of materials that can be effectively processed using this technique • Scaling up the process is challenging, as increasing the height of the construct often reduces precision, thereby affecting the overall quality and consistency of the output	[51]

(Continues)

TABLE 1 | (Continued)

Technique	Production rate	Dimensions	Advantages	Limitations	References
Bubble spinning	7.5 g/h	—	• Suitable for mass production applications • Operates at lower working voltages compared to other high-throughput techniques	• The use of toxic solvents makes the process less environmentally friendly and poses safety concerns • The technique involves complex optimization, as multiple parameters influence the outcome, including ambient conditions, polymer solution properties, solvent volatility, bubble formation rate, as well as the pressure differential between submerged bubbles and the external environment, which affects surface tension dynamics	[52]
Corona electrospinning	—	100–700 nm	• Offers high throughput, making it suitable for industrial-scale production • Supports the use of volatile solvents, enhancing their applicability in biomedical fields	• The optimization process is complex, requiring careful adjustment of rotation speed to prevent overflow during operation • A high-voltage power supply, often reaching up to 100 kV, is typically required, which can increase operational costs and pose safety risks for operators • There is limited literature available on this technology, particularly regarding its application to challenging polymers that are difficult to spin (e.g., CS and its derivatives)	[53, 54]
Roller and wire needleless spinning	—	—	• Achieves high production rates without the complication of needle blockage • Capable of processing highly concentrated polymer solutions, contributing to efficient and large-scale fiber production	• The resulting fibers tend to have larger diameters compared to those produced by classical electrospinning techniques • The optimization process is complex due to the formation of Taylor cones from a free surface, requiring precise control of multiple parameters	[51]
Stainless-steel disc	250–450 g/h	2–10 μ m	• Capable of producing large quantities of fibers, making it suitable for scalable applications • Supports the use of volatile solvents, which enhances their feasibility for biomedical applications • Enables the production of fragmented fibers, useful for specific functional or structural requirements	• The process requires careful optimization, particularly with high rotational speeds and elevated voltage levels to ensure stable operation • This technique is not suitable for producing complex structures, limiting its versatility in advanced applications	[55, 56]
Ultrasound-enhanced electrospinning (USES)	—	300–600 nm	• Offers high throughput, making it suitable for industrial-scale production • Capable of producing layered fibers, which can be beneficial for multifunctional or composite applications	• Marked heterogeneity in the fiber diameter • Complex process to optimize morphology and throughput	[38, 57]

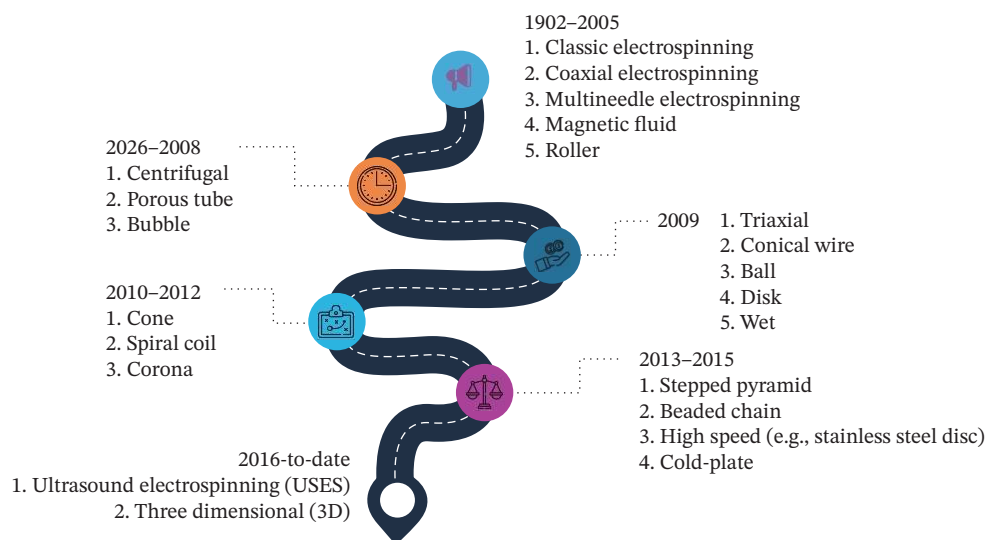


FIGURE 1 | Progression of electrospinning methods through time.

were among the first to successfully electrospin neat CS using a less toxic solvent, acetic acid, marking a significant improvement over earlier studies that employed highly toxic acids [74]. Despite these advancements, the concentrations of acetic acid used in such processes often remain high (≥ 70 v/v%), which continue to pose challenges for broader adoption due to safety and environmental concerns [62, 75]. Several review articles have explored the challenges and proposed solutions for improving the electrospinnability of CS, offering valuable insights for interested researchers [76]. Notably, the use of high-molecular-weight supporting polymers has proved effective, allowing for minimal polymer content while maintaining fiber integrity. For instance, Zhang et al. [77] successfully fabricated CS-based fibers using only 5 wt% of ultrahigh-molecular-weight PEO. Such innovations are crucial for scaling up production and advancing practical applications, particularly in the field of tissue engineering.

The advancement of electrospinning techniques has marked a significant breakthrough not only by increasing production rates but also by improving the spinnability of polymers that are traditionally difficult to electrospin [78]. One such innovation is coaxial electrospinning, which has been shown to enhance the electrospinnability of CS [76, 78, 79]. Pakravan et al. [78] successfully fabricated hollow CS fibers using this method. In their approach, both PEO and CS were dissolved in a common solvent, acetic acid. PEO was used as the core material and CS as the shell. After electrospinning, the PEO core was removed, resulting in the formation of hollow CS fibers.

Several studies have demonstrated the feasibility of scaling up CS-based nanofibers using advanced electrospinning techniques [58, 59, 70, 71, 80]. For instance, Bienek et al. [70] employed a blow-spinning method to fabricate biomimetic three dimensional (3D) nanofibrous scaffolds for tissue engineering applications. They successfully spun up to 10 wt% CS and poly(DL-lactide-co-glycolide) with and without polyethylene glycol. The resulting fibers had an average diameter ranging from 981 to 1139 nm, which supported appropriate cell attachment and growth. A PVA/CS hydrogel was successfully fabricated using the blow-spinning technique [71]. The resulting material combined the structural features of a nanofibrous scaffold with the functional

properties of a hydrogel dressing, making it highly suitable for tissue engineering applications. Upon exposure to water, the material transformed into a hydrogel, exhibiting water absorption capacities ranging from 459% to 680% absorption, depending on the concentration of the cross-linking agent used. Core-shell nanofibers composed of nylon-6 as the core and a CS/PVA blend as the shell were successfully fabricated using coaxial solution blow spinning [80]. In this process, PVA and CS were mixed using formic acid as the solvent, while nylon-6 was dissolved separately in formic acid. The collector was positioned 60 cm from the nozzle, and a drawing gas pressure of 0.15 MPa was applied. This setup enabled the formation of core-shell fibers with average diameters ranging from 80 to 650 nm. One of the key advantages of blow spinning is its high production rate, achieved without the need for an applied voltage. This eliminates fiber repulsion, allowing for the use of multiple needles and further enhancing the production efficiency. These studies demonstrate that CS-based nanofibers can be produced at a scalable level suitable for tissue engineering applications. Moreover, it can be argued that these techniques have the potential to be further refined to fabricate structures beyond conventional 3D nonwoven architectures.

Another promising technique for scaling up nanofiber production is centrifugal spinning, which offers a relatively low-cost alternative to traditional electrospinning methods [81]. This technique utilizes the centrifugal force generated by high-speed rotation to stretch polymer jets and form smooth fibers. Cremar et al. [81] demonstrated the feasibility of producing CS-based nanofibers using this method. Optimal spinning conditions were achieved with a 7–9 w/v% CS solution in a trifluoroacetic acid (TFA) and dichloromethane (DCM) solvent system, spun at 8000 rpm, resulting in fibers with average diameters ranging from 946 to 1421 nm. A particularly noteworthy advantage of centrifugal spinning is its ability to process polymers like CS, which are typically difficult to spin, without the need for sacrificial polymers. However, a key limitation of this technique lies in the fiber quality, which can be inconsistent. To address this, further innovations have led to the development of electro-centrifugal spinning, a hybrid approach aimed at improving both production rates and fiber uniformity.

TABLE 2 | Overview of selected studies on electrospinning CS and its composites.

Formulation	Technique	Solvent	Optimal electrospinning conditions	Diameter of the fibers	Verdict	References
CS	Classic	90 v/v% acetic acid	CS Mw = 2.94×10^5 g/mol, and DD = 75%–85% Inner needle 0.7 mm, tip-to-collector = 16 cm, voltage = 17 kV, and feed rate 8×10 –2 mg/h	140 nm	Hydrolyzing CS in acetic reduced both molecular weight and viscosity which improved the spinnability of neat CS	[62]
CS		TFA	22 kV 0.4 Mlh-1, Needle diameter 0.8 mm TTC = 10 cm Conc = 6wt%	137–164 nm	The diameters of the fibers were dependent on the speed of drum as the collection mode	[63]
CS		7/3 v/v TFA/DCM	25 kV 15 cm	150 ± 10 nm	Electrospun nanofibers were stabilized through reversible acylation, after which they demonstrated excellent biocompatibility with bone cells	[64]
CS/PEO/bioactive doped with cerium		50 v/v% acetic acid	Feed rate 0.2 mL/h 17 kV 23 cm	80–140 nm	The diameters of the fibers was dependent on the CS content, with higher content leading to finer fibers.	[65]
CS		TFA/DCM 7:3 v/v	370 kDa deacetylation degree 85% 23 kV 1 mL/h 15 cm	—	The nanofibers were neutralized with NaOH to facilitate the grafting of β -Cyclodextrin, which did not cause any morphological changes and enhanced their hydrophobic nature, making them suitable for drug attachment and delivery for tissue regenerative medicine.	[66]
CS/gelatin	—	TFA/DCM 7:3 v/v	27 kV 0.5 mL/h 10 cm	180–196 nm	A nanofibrous structured scaffold was obtained, with the fiber diameter varying according to the ratio of CS to gelatin. The scaffold exhibited mechanical properties and porosity suitable for tissue attachment and spreading.	[67]
PCL/CS	Coaxial	TFA/acetic acid 9:1 v v for CS and DMF for PLA	25 kV 13 cm Core: 0.4 mL/h Shell: 1.3 mL/h	671 ± 172 nm	Both sharp and skewed boundaries were observed between the core and shell structures. In the case of sharp boundaries, the inner core had an average diameter of approximately 115 nm, while the shell measured around 251 nm. For skewed boundaries, the core diameter ranged from 372 to 388 nm, with the shell diameter varying between 572 and 633 nm	[39]

(Continues)

TABLE 2 | (Continued)

Formulation	Technique	Solvent	Optimal electrospinning conditions	Diameter of the fibers	Verdict	References
CS/PEO/PCL	Coaxial	PEO and CS core: 50/50 v/v% acetic acid and H ₂ O PCL shell: Chloroform/DMF 9:1	0.5 mL/h 18 kV 12 cm	~ 370 nm	A shell thickness of 70 nm was achieved around a core with a diameter of 350 nm	[68]
Hydroxyapatite (HAP)/gelatin-CS core-shell/PEO	Coaxial	20 v/v% acetic acid	CS 85% deacetylation Core 0.4 mL/h Shell 0.3 mL/h 20 kV 12 cm 40% RH 25°C Rotating drum, 100 rpm	100–200 nm	The resulting nanofibers exhibited an average diameter ranging from 100 to 200 nm, forming a three-dimensional nanofibrous structure. Each fiber featured a core diameter of approximately 100 nm and a shell thickness of around 150 nm. Importantly, the nanofibrous architecture was preserved after crosslinking, enabling effective deposition of hydroxyapatite (HAP). HAP particles, roughly 100 nm in size, were observed to coat the nanofibers, enhancing biocompatibility and making the material a promising candidate for supporting osteoblast cell growth in bone tissue engineering applications	[69]
CS/poly(DL-lactide-co-glycolide)	Blow spinning	99.5 v/v% ethyl acetate	8 mL/h 20 cm Inlet air pressure 103 .42 kPa and Flow rate 31.15 L/min	625–830 nm	The average fiber diameter decreased with increasing CS concentration in the absence of polyethylene glycol. However, in the presence of polyethylene glycol, no significant change in average diameter was observed	[70]
PVS/CS	Blow spinning	Formic acid	16 mL/h Air pressure 0.5 MPa 60 cm	437–475 nm	The average fiber diameter decreased with increasing concentrations of the crosslinking agent	[71]
PEO/CS	Blow spinning	Acetic acid	50 µL/min Internal pressure = 10–14 psi 20 cm 500 rpm	150–1200 nm	A concentration of 10% PEO and above resulted in the formation of ultrafine fibrous structures	[59]

2.3 | Bioinspired CS Nanofibers for Tissue Regeneration

Electrospinning offers the opportunity to produce nanofibers with different patterns depending on the anticipated biomedical application. For instance, electrospun core shell nanofiber can be used for loading suitable drugs to afford control release toward various applications [24]. Biomimetic substitutes have been produced to mimic natural tissues in order to repair damaged or destroyed tissues, such as skin, bone, articular cartilage, and nerves, with the main aim of improving functional outcomes. The nanofibrous nature of electrospun nanofibers offers advantageous properties including a large surface area that afford sufficient oxygen permeability for skin tissue regeneration. The nanofibers mimic the skin ECM from morphology and composition perspectives, making them ideal for skin regeneration [24, 82, 83]. This indicates that by gaining insights into the biological and structural features of tissues, it can aid in choosing suitable materials that best reconstruct affected tissues. For instance, for cartilage regeneration with hierarchical organization, nanostructured ECM components require some designing to come up with adequate materials with appropriate features [83, 84]. Therefore, such designs for cartilage regeneration require the following aspects: they must (1) mimic the nanofibrous structured ECM, (2) be highly porous to sufficiently allow cell ingrowth, (3) allow for efficient mass transport of nutrients, oxygen, growth factors, and waste products, and (4) be capable of withstanding mechanical stresses during the tissue regeneration processes [83, 84].

2.4 | Random Nanofibrous Scaffolds

Electrospun fibers with a random orientation usually possess enhanced mechanical properties and provide isotropy, making them suitable for various regenerative medicine purposes, including wound healing and tissue reconstruction. Gomes et al. [85] investigated random nanofibrous scaffolds made of either PCL, CS, or GEL for their potential in skin tissue regeneration. Their findings revealed that GEL-based scaffolds demonstrated superior performance in *in vitro* assays, with enhanced cell adhesion and proliferation compared to those of the other formulations. While both CS- and PCL-based scaffolds supported deep cell migration, the CS- and GEL-based scaffolds showed better integration with wound sites in *in vivo* studies. Notably, CS-based scaffolds outperformed the others in minimizing wound contraction, thereby effectively promoting neodermis formation and reepithelialization. In another study, these researchers used PEO to enhance the electrospinnability of CS. This helped mitigate the individual limitations of each component, resulting in more effective composite scaffolds with varied performance outcomes [86]. Their different scaffolds demonstrated hydrophilic characteristics and acceptable levels of porosity. Notably, the highest tensile modulus was observed in the CS/PCL and CS/PCL/GEL blends. In terms of cell adhesion, the ranking followed the order PCL/GEL > CS/GEL > CS/PCL/GEL > CS/PCL. Overall, all the blended scaffolds possessed properties suitable for skin tissue regeneration, with the CS/PCL/GEL composite showing superior physical characteristics compared to the others. Gautam [87] enhanced the CS/PCL/GEL scaffold by incorporating type-I collagen, aiming to improve its overall performance. Collagen I, a key component of both skin and bone, contains specific cell-binding motifs such as Arg-Gly-Asp (RGD) and

Gly-Phe-Hyp-Gly-Glu-Arg (GFOGER), which play crucial roles in facilitating cell attachment. The modified scaffold demonstrated excellent biocompatibility, as evidenced by the viability of cells incubated with it. Both human osteoblasts and mouse fibroblasts maintained their characteristic morphologies throughout the incubation period and exhibited improved adhesion. These findings suggest that the collagen-enriched scaffold is well suited for applications in bone and skin tissue regeneration.

Elsewhere, a mesoporous CS nanofibrous scaffold was fabricated using PEO to enhance the electrospinnability of CS, followed by the removal of PEO to create mesoporous structures [88]. The scaffold was subsequently loaded with norfloxacin and functionalized with an antifungal aldehyde, as illustrated in Figure 2. The resulting fibers had an average diameter of ~170 nm and exhibited strong antibacterial and antifungal activity, including efficacy against yeast, due to the combined presence of norfloxacin and the antifungal aldehyde. *In vitro* studies using normal human dermal fibroblasts demonstrated cell viability exceeding 85%, with no signs of cytotoxicity. Furthermore, *in vivo* evaluations revealed no abnormalities in the rats' posture, movement, feeding, or hydration during the observation period. The absence of weight gain suggested a lack of an inflammatory response. Notably, the treated wounds in rats showed enhanced healing and improved skin quality.

Dopamine is a key component of mussel foot proteins and plays a vital role in both adhesion and cohesion mechanisms in mussels [89–92]. Inspired by this natural adhesion strategy, researchers have developed new mussel-inspired biomaterials characterized by low toxicity and improved hemostatic properties. In this context, the modification of CS with PDA has emerged as a promising approach to enhance its biocompatibility and hemostatic performance. Dimassi et al. [89] investigated the influence of treatment duration on the PDA coating of nanofibrous CS scaffolds by immersing them in a dopamine hydrochloride solution, aiming to enhance their suitability for bone regeneration.

A treatment time of 2 h was found to be sufficient to produce scaffolds that remained stable in phosphate-buffered saline (PBS, pH 7.4) and exhibited improved cytocompatibility with preosteoblast MC3T3-E1 cells. Additionally, these scaffolds facilitated biomineralization when immersed in 1.5 times the concentrated simulated body fluid (1.5x SBF). After 6 days of immersion, spherical mineral nodules, primarily composed of hydroxyapatite, were observed. This biomimetic deposition of hydroxyapatite onto PDA-functionalized fibers highlights the potential of the resulting scaffold for applications in bone tissue engineering.

Yu et al. [93] evaluated collagen/CS and CS/silk fibroin nanofibrous scaffolds as potential biomaterials for skin repair. Their findings showed that both fibroblasts and keratinocytes exhibited robust proliferation on and within the nanofibrous membranes, demonstrating good biocompatibility. Moreover, the scaffolds significantly enhanced wound healing compared to conventional gauze dressings. In addition to CS being a preferred biomaterial for tissue regeneration, its properties can be further enhanced through blending with other polymers and functionalization strategies, thereby broadening its applications in tissue engineering. PCL is the most used supporting synthetic polymer for the enhancement of CS electrospinnability. In addition, this polymer is biodegradable and biocompatible but suffers from

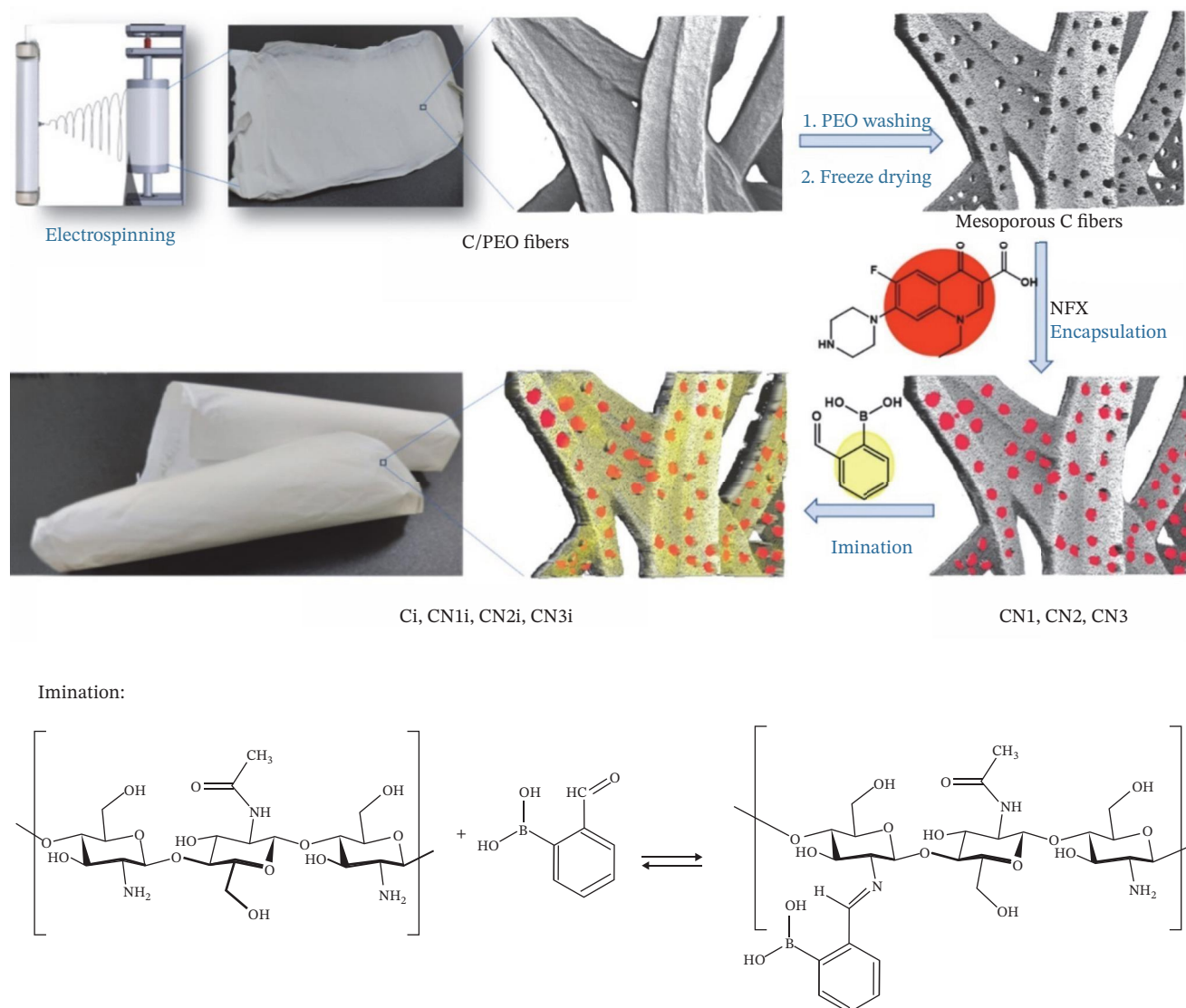


FIGURE 2 | Schematic illustration of a mesoporous CS nanofibrous scaffold functionalized with antibacterial and antifungal agents to enhance wound healing efficacy. Polyethylene oxide (PEO) was incorporated to facilitate electrospinning and subsequently removed to create mesoporous fibers, enabling efficient absorption of the bioactive compounds. Reproduced with permission from [88].

hydrophobicity, which negatively affects the cell attachment. Hence, the blending with CS makes it suitable for tissue regeneration applications. Nonetheless, some of the natural polymers were used to further improve the overall performance of the resultant biomimetic scaffolds.

Junka et al. [94] developed biomimetic nanofibrous scaffolds by blending PCL with CS. The resulting scaffold was further functionalized by exploiting the amine groups of CS to conjugate laminin via a carbodiimide-mediated reaction. This crosslinking approach significantly improved the mechanical properties of the scaffold and enhanced its hydrophilicity compared to neat PCL, promoting favorable attachment and proliferation of Schwann cells (hSCs). The observed increase in cell growth rates relative to pure PCL scaffolds demonstrated the potential of this material for nerve regeneration applications.

Bone tissue regeneration using randomly electrospun nanofibrous membranes has been widely reported in the literature [95–98]. Mineralized bone matrix has four main active cells,

that is, osteoblasts, osteoclasts, osteocytes, and bone lining cells. These cells are essential for regulating bone remodeling, that is, Bone formation and resorption. The main focus in bone tissue engineering is based on designing scaffolds that can mimic the ECM, meanwhile providing mechanical assistance and helping in the formation of new bone. Therefore, the resultant scaffold is supposed to have osteoconductive, osteoinductive, and osteogenic properties to facilitate the adhesion, survival, and migration of osteogenic cells. As mentioned earlier, bone tissue engineering requires uniform fibers to improve cell contact, cell growth, communication, and differentiation. Beyond the structural need, the choice of the materials to develop such a product is also essentially crucial for bone tissue regeneration.

Therefore, the selection of materials blended with CS is typically guided by their ability to support the regeneration of damaged tissues [23]. Biodegradable synthetic polymers are often incorporated to provide mechanical strength, while fillers such as hydroxyapatite are added to enhance mechanical properties and facilitate cell attachment, organization, and growth [23].

The ECM of bone is primarily composed of collagen proteins interlinked with various polysaccharides, which contribute to elasticity, fracture resistance, and cell proliferation. Inorganic components like hydroxyapatite crystals, deposited on the collagen matrix, impart strength and toughness to the bone while offering chemical cues that promote the differentiation of cells into the bone tissue. Electrospinning enables the fabrication of nanofibrous membranes that closely mimic the structure of the natural ECM. With the appropriate combination of materials, these membranes can effectively support tissue regeneration. For example, Gautam et al. [99] developed a composite scaffold using PCL, GEL, and CS, reinforced with nHAP, for bone tissue engineering. Each component played a crucial role in enhancing the regenerative potential of the scaffold. The resulting biomimetic membrane demonstrated excellent biocompatibility, supporting high cell viability and proliferation. Moreover, it facilitated strong cell adhesion and spreading, with osteoblasts adopting a characteristic polygonal morphology, indicative of their differentiation and suitability for bone tissue regeneration. These results were attributed to both the material composition and the electrospinning technique used in scaffold fabrication. In a related study by Toullec et al. [100], CS was blended with curdlan (1,3- β -glucan), a polysaccharide known for its ability to promote bone regeneration by supporting mesenchymal stem cell (MSC) adhesion and facilitating their differentiation into osteoblasts. The resulting electrospun fibers had an average diameter of 205 ± 40 nm, which falls within the typical range of ECM fibrils (10–300 nm). To address the high protonation associated with CS, the fibers were stabilized to neutralize the membrane. Both neat CS fibers and curdlan-modified fibers exhibited swelling ratios of 300%–350% within 2 h in PBS. However, the curdlan-modified CS fibers showed slower enzymatic degradation compared to the neat CS membranes. Importantly, the curdlan-enhanced nanofibrous membranes demonstrated nontoxic behavior and improved cell migration, attributed to the bioactive properties of curdlan. Since mechanical performance is critical for scaffolds intended for tissue regeneration, many researchers incorporate fillers into nanofibrous scaffolds to ensure that the resulting materials possess adequate mechanical strength [96]. However, there is typically an optimal filler concentration beyond which mechanical properties begin to deteriorate. Govindasamy et al. [96] reported that incorporating 5 wt% halloysite into a CS/PEO scaffold yielded the highest mechanical performance. This improvement was attributed to strong interactions between CS/PEO and halloysite, facilitated by hydrogen bonding between hydroxyl groups on CS and the surface of halloysite. These interactions enhanced the stress transfer between the polymer matrix and the filler. However, increasing the filler content beyond 5 wt% led to filler agglomeration, which acted as stress concentrators and reduced the scaffold's mechanical integrity. Additionally, the scaffolds maintained hydrophilic characteristics and optimal pore sizes within the 0–5 wt% filler range, which supported improved cell attachment and interaction with the scaffold. Goh et al. [101] reported that coating PLA/CS (PLA/CS) nanofibers with functionalized bioglass significantly enhanced their *in vitro* bioactivity. The bioglass was doped with silver, copper, and cerium to improve its functional properties. Upon immersion in SBF, the resulting nanocomposites exhibited the formation of ruffled crystallites. These crystallites had a calcium-to-phosphorus (Ca/P) ratio of ~ 1.67 , indicating excellent *in vitro* bioactivity and suitability for bone tissue

engineering applications. Notably, only the nanocomposites containing silver-doped bioglass demonstrated antibacterial activity against *Escherichia coli* (ATCC 25922 strain).

A layer-by-layer nanofibrous mat was engineered to ensure that the final construct possessed the necessary physical and chemical properties for biomedical applications [102]. The mat featured a dual-functional design: one side composed of osteogenic silk fibroin/PCL and the other of antibacterial PCL/CS [102]. This configuration resulted in a scaffold with excellent biocompatibility, suitable hydrophilicity, robust mechanical properties, and strong antibacterial activity against *E. coli* and *S. aureus*. To enhance bone regeneration, the osteogenic side was further functionalized with a Runx2 plasmid, enabling targeted treatment of cranial defects. *In vivo* studies demonstrated that the modified mat (PCSP + Runx2) significantly increased bone volume compared to both the control (PCSP + control) and negative control groups, as illustrated in Figure 3a,b. Histological staining revealed newly formed bone tissue (highlighted in yellow) and residual scaffold material (marked in red), with the PCSP + Runx2 group showing a markedly higher quantity of new bone formation (Figure 3c,d). These outcomes were attributed to the scaffold's ability to provide an optimal microenvironment for cell adhesion and proliferation, supported by its excellent biocompatibility, favorable hydrophilicity, and mechanical integrity. In a related study, Zhu et al. [103] employed coaxial electrospinning to fabricate a nanofibrous mat designed to incorporate all essential components for bone regeneration. The researchers introduced varying concentrations of RSV into the scaffold to promote osteogenic activity. The mats demonstrated a sustained release of RSV over a 15-day period, with the release profile being directly influenced by the initial RSV loading. Among the tested concentrations, a 0.5 wt% RSV loading was found to be the most effective, offering a controlled and sustained release that significantly enhanced the osteogenic differentiation of preosteoblasts *in vitro* and promoted bone formation *in vivo*. After 12 weeks of implantation, the treated defect sites exhibited $\sim 83\%$ bone volume to total volume (BV/TV) and a bone mineral density (BMD) of around 0.70 g/cm^3 . These findings highlight the importance of selecting appropriate materials and fabrication techniques, such as coaxial electrospinning, to develop scaffolds that closely mimic the natural ECM, thereby significantly enhancing tissue regeneration in damaged areas.

It is worth noting that the choice of the electrospinning technique plays a crucial role in scaffold design [103, 104]. Coaxial electrospinning, in particular, enables the encapsulation of essential bioactive agents within a protective outer shell, allowing for controlled release during the tissue regeneration process [68, 103, 104]. By adjusting the thickness of the shell layer, the release rate of the core material can be finely tuned; thicker shells generally result in slower release, while thinner shells allow for faster diffusion [104]. Moreover, the shell material itself can be strategically selected not only for its stability but also for its ability to support cell adhesion, differentiation, and proliferation, thereby enhancing the overall effectiveness of the scaffold in promoting tissue regeneration. Ghasemvand et al. [68] developed core-shell nanofibers consisting of a core made from RSV, CS [105], and PEO and a shell composed of PCL, followed by plasma surface treatment. The fabricated fibers featured a shell thickness of ~ 70 nm and a core diameter of around 350 nm. The resulting scaffold

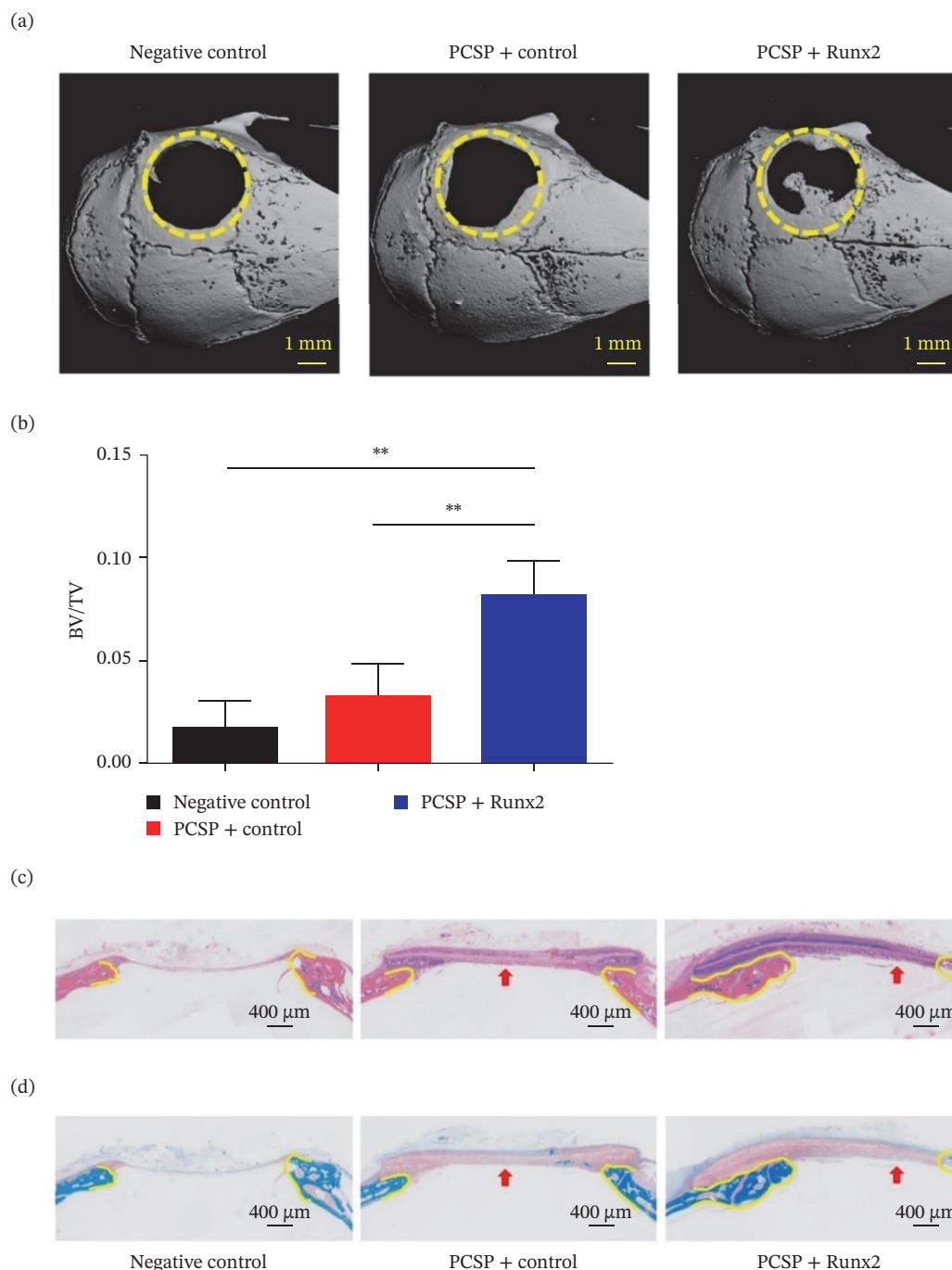


FIGURE 3 | Evaluation of mouse cranial defects 8 weeks postsurgery: (a) micro-CT imaging, (b) quantitative analysis of bone volume, (c) hematoxylin and eosin (H&E) staining, and (d) Masson's trichrome staining. Comparative analysis includes the negative control, PCSP control, and PCSP + Runx2 scaffold groups. Newly formed bone tissue is outlined in yellow, while residual scaffold material is indicated by red arrows. Reprinted with permission from [102].

exhibited a sustained and controlled release of RSV, achieving complete release over a period of 90 days. This prolonged release was attributed to the plasma-treated PCL shell, which effectively delayed the degradation of the CS and PEO cores while still allowing water to penetrate and facilitate the release of RSV. The scaffold demonstrated excellent biocompatibility with no signs of cytotoxicity. The released RSV remained within a concentration range conducive to promoting cell adhesion and proliferation. After 3 days of incubation, MSCs adhered well to the scaffold and showed robust proliferation. These findings

underscore the potential of coaxial electrospinning for the controlled delivery of bioactive compounds, making core-shell nanofiber structures highly promising for targeted drug delivery in tissue regeneration applications.

2.5 | Aligned Nanofibrous Scaffolds

The conventional electrospinning technique generally produces non-woven membranes with a random fiber orientation and poor mechanical performance. The native ECMs found in tissues,

including the sciatic nerve, heart, tendon, and blood vessels, are recognized to feature anisotropic architecture essential for tissue function. This has led to more research activities toward creating scaffolds with well-defined structures to afford the necessary morphology to mimic native ECM [106]. Numerous alignments such as axially aligned, yarns, and radially aligned demonstrated best cell morphology shaping, cell guidance, and migration and positively affect cell differentiation compared to other scaffolds either in vitro or in vivo [107]. Such alignments can be achieved through modification of conventional electrospinning techniques either the spinnerets and/or the mode of collection [106–108]. Common approaches include the use of parallel electrodes, high-speed rotating drums, or the application of external mechanical forces such as stretching or electric fields [4, 109, 110]. Achieving fiber alignment is particularly important, as it influences cell behavior, osteoblasts and other cell types tend to align and elongate along the direction of the fibers, closely mimicking the native cellular orientation found in tissues like bone. Consequently, numerous studies have explored the use of aligned nanofibers as scaffolds for tissue regeneration applications. Wang et al. [108] used a drum collector to afford aligned CS nanofibers with appropriate mechanical performance for nerve regeneration. It was demonstrated that the aligned electrospun CS nanofibers provided contact guidance to cultures of rat hSCs, leading to elongated and aligned cells along the axes of the fibers. The hSCs were attached to the random fibers with high affinity but without a specific orientation. On the other hand, oriented fibers resulted in cells aligning in the same direction due to secure adhesion to the oriented fibers. It was reported that some cells continuously extend to the adjacent fibers (Figure 4). In this study, various CS-based nerve guidance conduits were tested by implanting them into a rat sciatic nerve defect model for a period of 30 days. The tested constructs included a mesh tube with and without fiber orientation and a bilayered tube composed of an inner layer of aligned nanofibers and an outer layer of randomly oriented fibers. The results showed that both the oriented CS tube and the bilayered tube were able to maintain the internal lumen structure, thereby supporting the formation of new nerve tissue. These regenerated nerves closely resembled those in isograft controls, with axons maturing into dense bundles within the aligned and bilayered grafts. In contrast, the graft composed solely of randomly oriented nanofibers exhibited partial collapse of the lumen. In this case, the regenerated axons were scattered within the loose connective tissue, and only limited blood vessel formation was observed.

In general, the electrospinnability of CS for biomedical applications often requires the use of harsh solvents, such as TFA. Moreover, the resulting electrospun CS fibers tend to be mechanically fragile. To address these limitations, researchers have explored blending CS with synthetic polymers to enhance both mechanical integrity and electrospinnability, thereby expanding its potential in tissue engineering [20]. Several studies have demonstrated the successful fabrication of aligned nanofibrous scaffolds for a variety of regenerative applications, including bone, cartilage, and dural tissue repair, as is presented in Table 3. Nonetheless, Mokheena et al. [109] developed aligned CS-PCL nanofibers for applications in nerve regeneration. In their study, both CS and PCL were dissolved using TFA, and electrospinning was performed using either a rotating grounded drum or a pair of parallel electrodes to produce aligned and random fibers, respectively. The resulting nanofibers had average diameters of ~405 nm for random and ~408 nm for aligned configurations. Notably, the aligned fibers exhibited

enhanced mechanical properties and provided superior support for the attachment and directional growth of neuron-like PC-12 cells, along with elevated gene expression levels. Interestingly, hSCs were able to attach and proliferate across all fiber types, regardless of their alignment or surface topography.

In recent years, several studies have focused on blending polyamide-6 (PA6) with CS to enhance the electrospinnability of CS, which is otherwise challenging to process on its own [112–116]. A key advantage of this approach is the use of less toxic solvents, such as formic acid, in contrast to traditionally used solvents like TFA. For instance, Ghani et al. [112] successfully blended PA6 with CS and employed formic acid as the solvent. Electrospinning was carried out using a grounded drum collector. At a drum rotation speed of 20 rpm, the resulting scaffolds were predominantly composed of random-oriented fibers. The study found that the maximum CS content that could be electrospun was 40%, beyond which the solution became too viscous to process effectively, even with the addition of surfactants. This limitation was attributed to the increased viscosity of the polymer solution, which hindered the formation of continuous fibers. Nirmala et al. [114] successfully fabricated PA6/CS nanofibers using formic acid as the solvent and a rotating drum collector.

The resulting fibers were smooth and aligned, with diameters ranging from 200 to 400 nm. Notably, the main fibers were interspersed with ultrafine mesh-like secondary fibers measuring between 20 and 40 nm in diameter, as shown in Figure 5. This unique morphology, characterized by a high surface area-to-volume ratio and enhanced porosity, is particularly advantageous for cell attachment and proliferation, making these scaffolds highly suitable for tissue engineering applications. In a related study, Nirmala et al. [117] demonstrated that the PA6/CS blend scaffold exhibited no cytotoxic effects in vitro, confirming its biocompatibility. The scaffold's ability to support cell attachment and proliferation was attributed to its unique dispersed mesh-like morphology, which provides a favorable microenvironment for cellular interactions.

The alignment of CS electrospun nanofibers further improves their overall mechanical performance that affords their application in bone tissue engineering [107]. The aligned and uniform electrospun CS nanofibers with high porosity and high surface area to volume create a conducive environment for bone repair. These systems support oxygen permeability and nutrient transport for promoting cell adhesion, proliferation, and differentiation. In addition, the incorporation of synthetic polymers and/or fillers can further enhance not only the mechanical performance but also the overall properties of the scaffolds, improving their suitability for the intended tissue regeneration. Liverani et al. [23] investigated the influence of nano- and micro-sized bioactive glass (BG) particles on the mechanical properties of PCL/CS scaffolds. Their findings showed that the incorporation of either n-BG or m-BG led to a decrease in both tensile modulus and tensile strength. However, only the scaffolds containing n-BG exhibited an increase in tensile strain, while those with m-BG showed no significant change compared to the PCL/CS scaffold. Furthermore, acellular bioactivity tests revealed the formation of hydroxycarbonate apatite on both BG-containing composites, whereas no mineral deposition was detected on the PCL/CS scaffold after immersion in SBF. These findings indicate that even though the fillers adversely affected certain mechanical

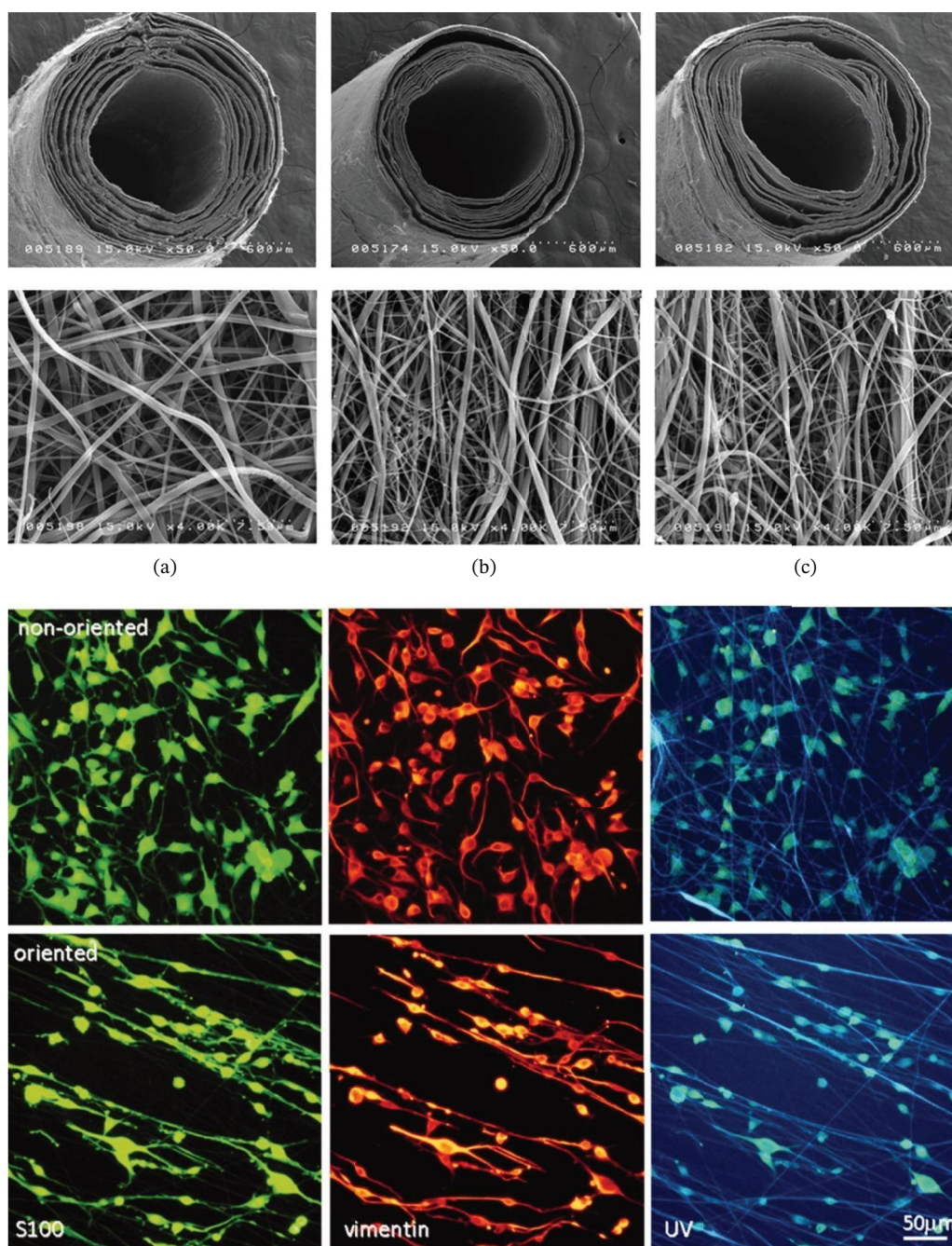


FIGURE 4 | SEM micrographs of electrospun CS nanofiber mesh tubes with and without orientation, as well as bilayered CS mesh tube. (a) Nonoriented CS mesh tube, (b) oriented CS mesh tube, and (c) bilayered CS mesh tube. The upper stand is SEM micrographs of each CS nanofiber mesh tube, and the lower stand is magnification of the inner surface of each tube. Immunocytochemistry of the immortalized Schwann cell line, IMS32 cultured for 4 days on coverslips. The upper and lower lines are IMS32 cultured on the nonoriented and oriented CS nanofiber mesh sheet, respectively. Although Schwann cells cultured on randomly arranged CS nanofibers show no specific orientation, they do align in one same direction on the oriented fiber. The scanning electron micrograph of Schwann cells cultured on the oriented CS nanofiber mesh sheet. Individual Schwann cells and their processes are capable to attach to single CS oriented fibers, although these cells sometimes continuously extend their processes to adjacent fibers. Reproduced with permission from [108].

properties, the overall performance of the composites was adequate for use in bone tissue engineering.

2.6 | Gradient

Due to the difficulty in incorporating a biochemical cue into the physical cue provided by nanofibers, generating gradients of

bioactive molecules on nanofiber mats is often implemented. Du et al. [118] developed vertically graded, heparinized nanofibrous mats designed to biomimetically replicate the vascular ECM, addressing common challenges in tissue-engineered blood vessels, such as thrombosis and progressive intimal hyperplasia following implantation. In their approach, CS was selected for its ability to be functionalized with proteins or peptides, enhancing

TABLE 3 | summary of studies on CS-based electrospun nanofibers their mechanical properties, porosity, contact angle, and their applications.

Sample	Spinning technique	Collector	Porosity	Mechanical Properties	Contact angle (°)	Comments	Application	References
PCL-CS	Conventional	Drum	—	$\sigma = 2.6 \pm 0.5$ MPa (aligned) $E = 22.4 \pm 1.1$ MPa (aligned) $\epsilon = 84.5 \pm 7.0\%$ (aligned) $\sigma = 1.8 \pm 0.3$ MPa (random) $E = 12.4 \pm 1.4$ MPa (random) $\epsilon = 31.1 \pm 7.5\%$ (random)	—	- Aligned fibers supported the attachment of both Schwann cells and PC-12 cells, promoting enhanced neurite outgrowth along the direction of fiber alignment and resulting in high gene expression - Aligned nanofiber exhibited superior mechanical properties when compared to random ones	Nerve regeneration	[107]
CS-PEO-halloysite nanotubes (HAL)	Conventional	Aluminium	31%–62%	$\sigma = 9.2$ – 26.5 MPa $E = 0.62$ – 1.25 MPa $\epsilon = 1.5$ – 3.9%	30.8–94.2	- The average fiber diameter and scaffold porosity decreased with increasing halloysite (HAL) content - The amount of HAL played crucial role in the mechanical properties, porosity, and hydrophilicity of the nanofiber. 5% was found to be optimal content to achieve favorable properties for tissue engineering - The scaffolds supported strong cell adhesion and effective cell–scaffold interactions	Bone regeneration	[96]
PCL-gelatin-CS-nanohydroxyapatite	Conventional	Aluminium plate	89%	—	—	Human osteoblasts exhibited significantly enhanced viability, attachment, and growth on electrospun fibers modified with nano-hydroxyapatite (nHAP) compared to those on unmodified fibers	Bone regeneration	[99]
PCL-CS-fish skin (gelatin)	Conventional	Aluminium plate	$90 \pm 4.0\%$	$\sigma = 3.8 \pm 0.5$ MPa $E = 48 \pm 10$ MPa $\epsilon = 18 \pm 3.0\%$	0	The scaffolds exhibited outstanding mechanical strength and porosity, which facilitated excellent cell attachment efficiency	Skin regeneration	[86]
Gelatin-CS/PCL/hydroxyapatite	Co-electrospinning	Aluminium plate	—	—	—	The scaffold facilitated cell adhesion and proliferation, suggesting its potential suitability for bone tissue engineering applications	Bone regeneration	[22]
SF/CS/nHAP@bone morphogenetic protein-2 (BMP-2)	Coaxial electrospinning	Aluminium	—	—	—	- The release of BMP-2 reached up to 80% within 2 weeks, with the release rate being dependent on the thickness of the shell layer - The presence of BMP-2 promoted osteogenesis and ectopic bone formation in human bone marrow-derived mesenchymal stem cells (hMSCs)	Bone tissue regeneration	[104]
CS/simvastatin	Conventional	Aluminium	—	—	—	- Histological and micro-CT analyses confirmed bone formation within calvarial defects within 4 weeks - Newly formed bony bridges were observed histologically, consolidating the defects, along with partial radiographic coverage of the defect areas within 8 weeks	Bone tissue regeneration	[97]

(Continues)

TABLE 3 | (Continued)

Sample	Spinning technique	Collector	Porosity	Mechanical Properties	Contact angle (°)	Comments	Application	References
CS/silver ion-loaded calcium phosphate (Ag-CaP)	Conventional	Aluminium	—	—	—	• The physicochemical properties of the scaffolds closely resembled those of the natural extracellular matrix (ECM) • The incorporation of calcium phosphate [46] enhanced the mineralization behavior of the mats • A controlled release of silver (Ag) ions was achieved, which is essential for sustained antibacterial activity	Bone tissue regeneration	[98]
CS/fucoidan (Fu)/ Copper Sulfide (CuS) NPs	Conventional	Aluminium	—	—	—	• The incorporation of fucoidan (Fu) promoted osteoinductive activity without any signs of cytotoxicity • Crosslinking the mats with genipin improved their resistance to acidic environments and significantly reduced their swelling rate in water. Moreover, the crosslinking process increased the rigidity of the fibers • The addition of copper nanoparticles enhanced the antibacterial activity, while the controlled release of copper ions supported the formation of endothelial cells	—	[111]
PCI/CS/bioactive glass (BG)	—	—	—	$\sigma = 7.0 \pm 2.0$ MPa nano-BG $E = 0.27 \pm 0.04$ MPa nano-BG $\varepsilon = 45 \pm 12\%$ nano-BG $\sigma = 9.2\text{--}26.5$ MPa micro-BG $E = 0.62\text{--}1.25$ MPa micro-BG $\varepsilon = 1.5\text{--}3.9\%$ micro-BG	—	• The incorporation of micro-scale bioactive glass (BG) particles resulted in nanofibers with an average diameter of 95 ± 105 nm • The use of nano-scale BG particles produced finer nanofibers with an average diameter of 74 ± 41 nm • Electrospun nanofibers containing both micro and nano BG demonstrated the formation of biomimetic hydroxycarbonate apatite (bHCA) within 1 day, highlighting their potential for bone tissue engineering applications	Bone regeneration	[23]
PEO/CS/Ce-doped-BG	Co-electrospinning	Rotating drum 500 rpm	—	—	56–73	• An increase in bioglass (BG) content enhanced cytocompatibility and promoted improved cell adhesion and spreading	Soft tissue regeneration	[65]

Note: σ = tensile strength; E, modulus; ε = elongation-at-break.

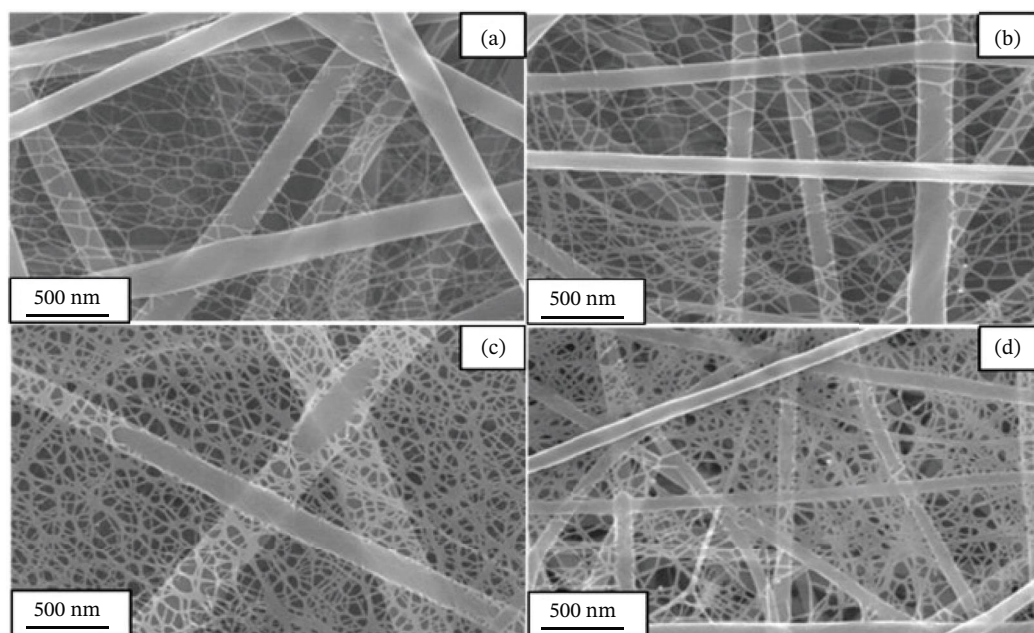


FIGURE 5 | High surface area-to-volume ratio membrane composed of primary large fibers interlaced with secondary ultrafine fibers. Reprinted with permission from [114]. With different wt.% of chitosan (a) 0, (b) 1, (c) 1.5, and (d) 2 wt.%.

the bioactivity of the scaffold. However, due to CS's inherent poor mechanical properties, it was blended with PCL to improve the structural integrity and make the material more suitable for tissue regeneration. To fabricate the gradient structure, the authors employed a rotating drum collector, sequentially coelectrospinning neat PCL and PCL/CS solutions onto the collector. This process resulted in a vertically graded fiber mat, as illustrated in Figure 6. The study found that cell proliferation was significantly more rapid on the gradient CS/PCL scaffold loaded with vascular endothelial growth factor (VEGF) compared to a uniform CS/PCL scaffold with VEGF, particularly within the first 24 h. The gradient CS/PCL scaffold demonstrated excellent support for the formation of both endothelial and smooth muscle layers, closely mimicking the natural architecture of the vascular wall. The rapid proliferation of endothelial cells is especially promising as it may help prevent restenosis by reducing the risk of thrombus formation and stromal cell overgrowth.

The use of multiple spinnerets and integration of electrospinning with 3D printing present some recent approaches for fabricating gradient structures and complex, multifunctional scaffolds [119]. The multiple spinnerets method involves simultaneously ejecting different polymer solutions to produce distinct fiber types that are deposited in overlapping patterns. These techniques enable the creation of scaffolds with tailored properties and spatially controlled architectures that closely mimic the ECM. However, despite these promising advancements, several challenges remain. The increased technical complexity of combining these fabrication techniques can impact the product quality, production efficiency, and cost-effectiveness. Moreover, achieving precise control over fiber alignment, composition, and gradient transitions remains a significant hurdle. Therefore, continued research and development are essential to refine these hybrid fabrication methods. The goal is to enable the precise engineering of ECM-like structures in a highly efficient, reproducible, and scalable manner, ultimately advancing the field of tissue engineering and regenerative medicine.

2.7 | 3D Nanofibers

Porous scaffolds that can provide a suitable environment for tissue regeneration are often difficult to produce using the electrospinning technique alone [82, 120, 121]. In order to overcome such challenges, electrospinning and freeze-drying are combined to afford 3D porous structures that can further be modified to produce multifunctional materials. Such structures are essential for supporting cell attachment and growth. Moreover, their high porosity allows for effective cell infiltration throughout the scaffold [82]. Deng et al. [120] prepared a 3D porous scaffold from electrospun CS nanofibers (Figure 7). The porous structures were prepared by homogenizing electrospun nanofibers to obtain shorter fibers, followed by freeze-drying to afford high porosity and interconnected structures, as presented in Figure 7. Due to the high porosity, the cells spread and migrate into the structure as they infiltrate into the inner part of the scaffold from 3 to 7 days. Results from RT-PCR demonstrated that the structure could support cell proliferation, attachment, and migration because the expressions of ECM-associated proteins were unregulated and thus show potential for tissue engineering applications.

3D nanofibrous structures, including monolayer and trilayer constructs, were developed as biomimetic models to serve as substitutes for damaged basement membranes (BMs) [122]. These constructs were composed of PCL, cellulose acetate, and CS. The trilayered structure, fabricated through sequential coelectrospinning, consisted of a microfibrillar outer layer and a central nanofibrous layer arranged in a PCL–PCL/CA/CS–PCL configuration. The trilayered constructs demonstrated effective spatial segregation of keratocytes and fibroblasts within the central fibrous barrier, along with enhanced cell adhesion and proliferation compared to the monolayer design. This is attributed to the unique topography of the interwoven network, which features high porosity and mechanical properties closely resembling those of natural BMs.

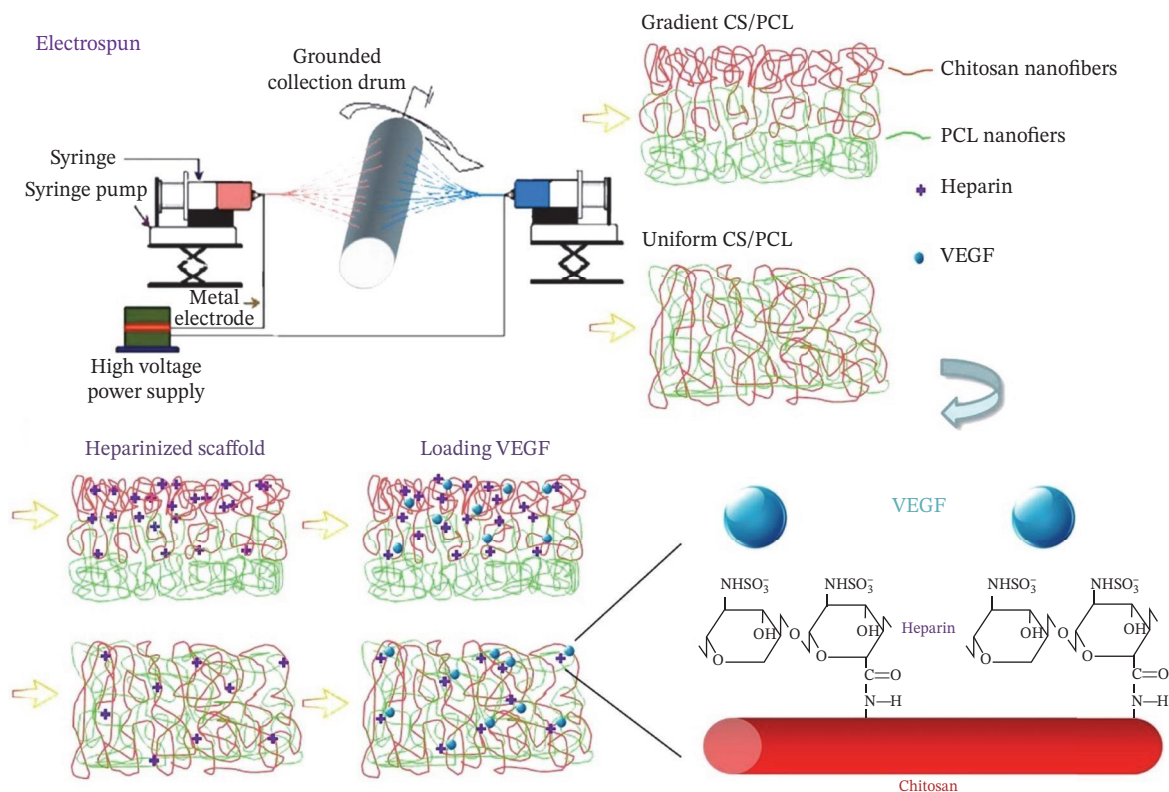


FIGURE 6 | Schematic illustration of gradient nanofibrous scaffold for vascular tissue regeneration. Reprinted with permission from [118].

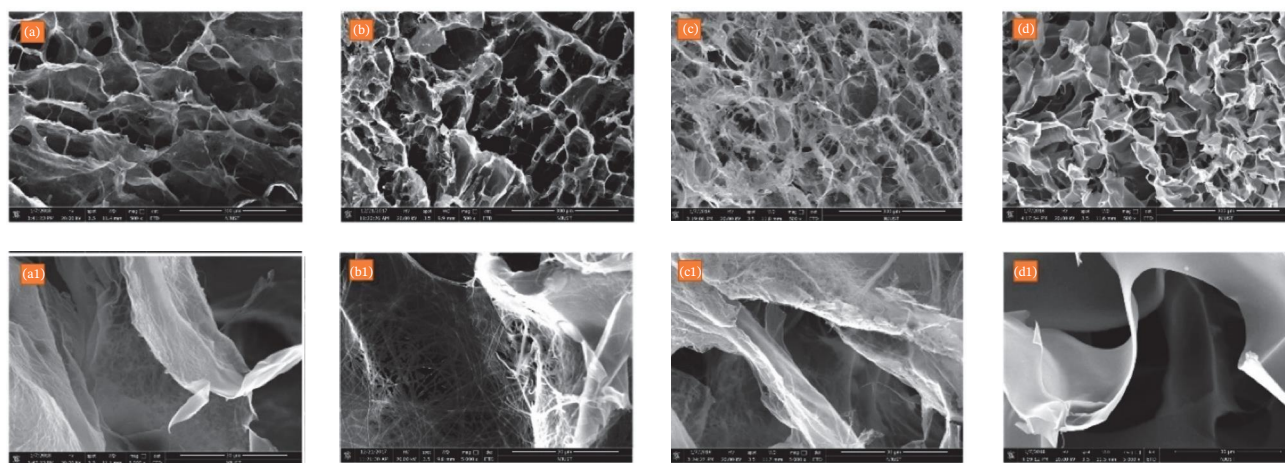


FIGURE 7 | 3D porous structure produced from different amount of fibers in ethanol solution. (a,a1) 0.5 mL-1%, (b,b1) 1 mL-1%, (c,c1) 1 mL-2%, and (d,d1) 0 mL-1% suspended in 1% or 2% CS recombinant human collagen polypeptides. Reproduced from [120]. MDPI Open Access.

The trilayered scaffolds exhibited excellent mechanical performance, with a compressive strength of ~ 1.17 MPa and an elongation-at-break of around 85.91%, indicating their strong potential for application in skin regeneration. Lou et al. [123] developed bilayer skin constructs consisting of an electrospun CS/PCL nanofibrous scaffold combined with a PLLA microporous disc fabricated via thermally induced phase separation. The design aimed to mimic the structure of human skin, which comprises a superficial stratified epidermis, primarily composed of keratinocytes, and an underlying dermis rich in fibroblasts and connective tissues. To replicate this architecture, the constructs incorporated both nanofibrous and microporous layers, offering

mechanical, chemical, and physiological properties conducive to cell expansion, biological functionality, and maintenance of the cell phenotype. CS was selected for its structural similarity to glycosaminoglycans (GAGs) found in the ECM, as well as its ability to degrade into bioactive molecules such as glucosamine, N-acetylglucosamine, and other oligomers that support ECM integration and promote wound healing. PLLA, on the other hand, was chosen for its biodegradability into nontoxic byproducts and its capacity to provide a favorable environment for cell adhesion and proliferation. The study found that the bilayer constructs created a microenvironment closely resembling the native ECM during the early stages of wound healing, effectively

supporting cell proliferation. The nanofibrous layer facilitated cell attachment and growth, while its integration with the micro-porous layer further enhanced cell migration and proliferation.

A wide range of fillers can be incorporated into electrospun mats to create multifunctional scaffolds [69]. As demonstrated by Chen et al. [69] and colleagues, posttreatment processes can subtly influence the morphology of the resulting mats. In their study, coaxial electrospinning was employed to fabricate aligned core-shell nanofibers composed of CS/PEO as the core and GEL as the shell. The fibers were collected using a rotating drum at 1000 rpm, followed by chemical crosslinking with glutaraldehyde. Subsequent freeze-drying transformed the mats into a 3D structure, which was then coated with hydroxyapatite (nHAP). The CS/PEO@GEL configuration mimicked Arg-Gly-Asp (RGD)-like motifs due to the cationic nature of CS and the GEL-rich shell, both of which promoted cell adhesion and proliferation. The surface decoration with hydroxyapatite further enhanced osteoblast proliferation, making the resulting scaffold a highly effective biomimetic platform for bone tissue engineering.

2.8 | 3D-Printed Collectors or Templates

3D-printed collectors can serve as effective templates for producing biomimetic scaffolds [124, 125]. Their geometry plays a critical role in directing the fiber orientation during the electrospinning process. By fabricating collectors in specific shapes, fibers can be deposited in a controlled manner to achieve the desired scaffold architecture. In many cases, a conductive coating is applied to the surface of the 3D-printed collector to ensure efficient fiber deposition and accurate shape formation.

Using tailored 3D-printed collectors, both highly oriented and randomly aligned fiber regions can be produced [124, 125]. The geometry of these collectors also influences key scaffold characteristics, including morphology, porosity, and mechanical performance [126]. Therefore, designing the collector with the target application in mind is essential to ensure optimal scaffold properties.

Furthermore, 3D-printed scaffolds can act as structural supports onto which nanofibers are deposited, enabling the fabrication of complex shapes for biomedical applications [127]. For example, different shapes of electrospun nanofiber scaffolds can be produced by electrospinning directly onto a 3D-printed support, as shown in Figure 8. Such structures can be engineered into highly complex geometries that mimic various biological forms, including internal anatomical features and organs, such as the tricuspid valve of the heart.

3 | Electrospun CS Hybridization

3.1 | 3D-Printing

3D printing has emerged as a powerful fabrication technique capable of transforming object-oriented prototyping from small-scale production into more advanced and adaptable manufacturing platforms [128–132], as shown in Figure 9. Its growing impact in biomedical applications is largely attributed to its versatility and ability to create intricate 3D architectures with interconnected pores, features that support the efficient

transport of nutrients, proteins, and oxygen within engineered tissues. These technologies can be classified into three, that is, inkjet, extrusion, and light-based 3D-printing [129]. Inkjet 3D printing is a powder-based deposition technique that falls into two categories: thermal and piezoelectric inkjet printing [129, 133]. In thermal inkjet 3D printing, heat is used to rapidly vaporize a small portion of the ink, forming vapor bubbles. The expansion of these bubbles generates pressure inside the reservoir, forcing ink droplets to be ejected through the nozzle. In contrast, piezoelectric inkjet printing relies on an applied voltage that causes a sudden deformation of the piezoelectric transducer. This deformation changes the volume of the ink chamber, producing a pressure wave that expels droplets through the nozzle [130]. Piezoelectric systems are capable of producing precise and uniform droplet sizes and can process a wide range of materials since no heat is involved. Thermal inkjet printing, however, offers higher printing speeds and lower equipment costs. Generally, low-viscosity inks are preferred for this technology; however, they often compromise the structural integrity and resolution of the printed constructs.

Extrusion-based 3D printing involves the controlled movement of a dispensing head along the X- and Y-axes, depositing ink onto a substrate as a continuous filament [129, 130, 134]. Structures are created layer by layer, either by moving the print head or adjusting the position of the stage along the Z-axis. This technique can be divided into melting-based and nonmelting-based processes. Melting-based extrusion, such as fused deposition modeling (FDM), melts a filament before deposition. It is simple and cost-effective but limited to thermoplastic materials, which restricts its use in biomedical applications [130, 131, 135]. Non-melting extrusion systems include pneumatic and mechanical (screw- and piston-based) mechanisms [135]. Pneumatic extrusion uses compressed air or gas to push the ink through the nozzle, enabling printing with a broad range of ink viscosities. However, this method can suffer from delayed response times, making precise control difficult. Piston- and screw-based extrusion rely on mechanical force, either a linearly driven piston or a rotating screw, to deliver the ink. Mechanical systems provide superior spatial control and can process high-viscosity inks, though the high shear forces may damage sensitive materials such as living cells.

Light-based 3D printing techniques are nozzle-free and use controlled light exposure to induce photopolymerization of a photo-sensitive resin, enabling solidification in a layer-by-layer manner [129, 130]. The most widely used technologies in this category include digital light processing (DLP), stereolithography (SLA), and two-photon polymerization (2PP) [130, 136]. These methods differ primarily in their light sources and imaging systems, while their motion control and layer-stepping mechanisms are generally similar. Light-based approaches offer high precision and excellent surface finish and are suitable for complex geometries, particularly in biomedical and microfabrication applications. Table 4 summarizes advantages and limitations associated with 3D printing technologies toward biomedical applications.

Beyond the limitations outlined in Table 4, current 3D-printing technologies are unable to fabricate filaments with the resolution required for scaffolds tailored to different tissue types [109, 130, 132, 137–139]. The resulting pore sizes are typically larger than individual cells, which reduces the cell-seeding efficiency and

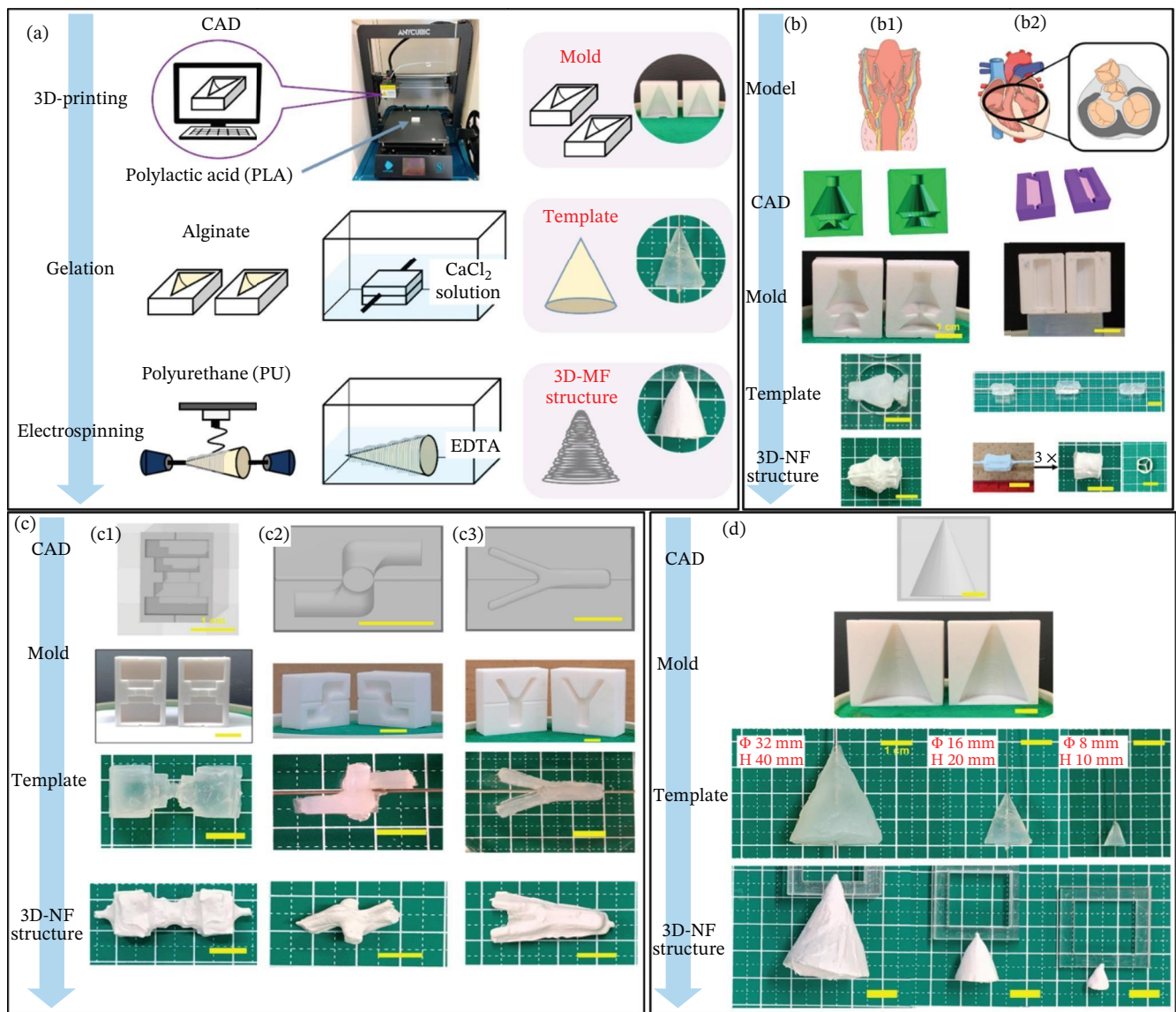


FIGURE 8 | (a) Schematic illustration showing the step-by-step fabrication of the electrospun scaffold. Fibers were deposited onto a rotating 3D-printed collector. (b) Examples of 3D-printed scaffolds inspired by anatomical forms, including (b1) a larynx (voice box) and (b2) a tricuspid heart valve, demonstrating the ability to produce complex, anatomy-like designs. (c) Fabrication of hollow 3D-printed templates featuring (c1) a textured surface for uneven geometries, (c2) intersecting macroscale structures, and (c3) a branched architecture. (d) Conical scaffolds of various dimensions, highlighting the versatility of the method in generating templates of different sizes. Reproduced from [127].

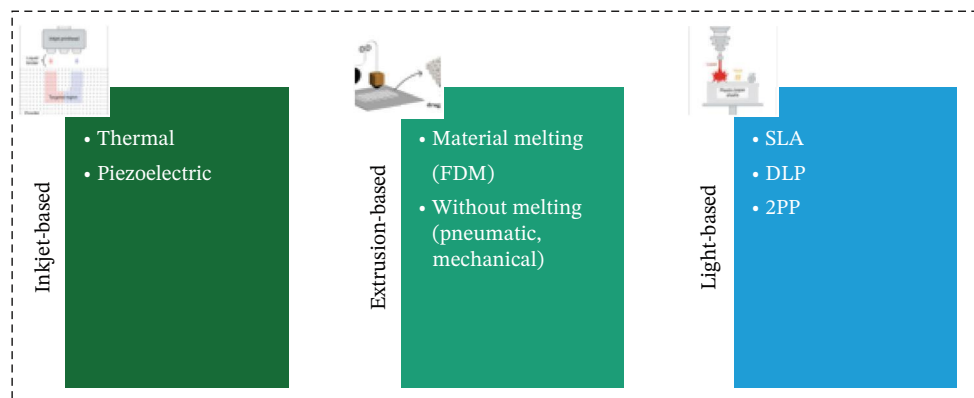


FIGURE 9 | 3D printing technologies [130].

TABLE 4 | Limitations and advantages associated with 3D-printing technologies toward tissue engineering.

Technology	Advantages	Limitations	References
Extrusion-based 3D	• It is compatible with a wide range of inks • Capable of producing large scaffolds thanks to its high deposition speed • Simple, efficient, and cost-effective • Supported by readily available open-source designs	• Optimization is complex, making it challenging to print multiple materials simultaneously • It is best suited for highly viscous inks • High mechanical stress during printing can reduce cell viability compared to gentler technologies such as inkjet-based systems • Limited resolution, typically below 100 μm • They are prone to aggregation and sedimentation of materials	[131, 132, 136].
Ink-jet	• Multiple nozzle heads can be used, enabling the printing of multifunctional materials suitable for heterogeneous tissue structures • Provides acceptable resolution, typically greater than 50 μm	• Only low-viscosity inks (typically <10 MPa·s) can be used • Supports only low cell densities, generally below 10^6 cells/mL • They are prone to material aggregation and sedimentation • Slow printing speed and often associated with significant material wastage	[131, 132, 135]
Light-based 3D printing	• Nozzle-free technique, eliminating mechanical stress on cells and preventing cell damage • Produces mechanically robust constructs without introducing artificial interfaces • Allows rapid fabrication of complex, multilayered structures, often within seconds • Delivers high resolution, reaching approximately 1 μm	• High cost of printers • Limited availability of photopolymerizable biomaterials often requiring modification before they can be used as inks for these technologies • Absence of a nozzle head leads to increased ink wastage, as the material must occupy the entire printing area, even regions not intended for polymerization • Often requires additional post-processing steps	[131, 132, 137].

impairs tissue formation. These challenges can be addressed by integrating 3D-printing methods with electrospinning. While electrospinning excels at producing fibrous, ECM-like structures, its main drawback is the difficulty in generating fully 3D constructs with adequate mechanical strength. Therefore, combining both techniques leverages their respective strengths, achieving scaffolds with suitable porosity, interconnected architecture, enhanced mechanical properties, controllable geometry, ECM-mimicking niches, and improved bioactive cues for cell growth [109, 138].

There are few methods that are used to combine both electrospinning and 3D printing [130, 139, 140]. The most commonly used simple and cheaper method involves electrospinning onto the surface of 3D-printed scaffolds [139, 140]. In this case, the printable ink, which is mostly biocompatible polymers, is printed into the desired shape, and then fibers are spun onto the printed constructs. The second approach involves 3D printing onto electrospun nanofibers [139, 140]. This approach is often employed when complex hybrid materials are produced. The third approach involves a layer-by-layer fabrication process through the alternating of 3D printing and electrospinning to afford 3D living structures with the desired internal structure [139]. The fourth strategy involves utilizing electrospun nanofibers as printable inks for 3D-printing [139]. This method provides the advantage of producing scaffolds with enhanced mechanical strength,

well-controlled geometries, tailored pore sizes, and defined microstructures. The fifth approach focuses on integrating or coating 3D-printed constructs with electrospun nanofibers [140, 141]. In one example, a reinforced composite framework with predefined internal channels was fabricated by coprinting PCL and Pluronic F-127 as a sacrificial material [141]. Short nanofibers composed of hydroxybutyl CS, along with human MSCs, were subsequently injected into the printed framework to form the final construct [141]. The resulting 3D-printed composite exhibited a rapid gelation time of under 15 s at 37°C. The inclusion of nanofibers significantly enhanced cell proliferation, gene expression, and ECM deposition, indicating robust chondrogenic differentiation. For instance, Long et al. [142] developed 3D-printed CS-pectin scaffolds loaded with lidocaine hydrochloride for use as wound dressings. The scaffolds were fabricated using an extrusion-based 3D printer, which enabled excellent printability, dimensional stability, and inherent self-adhesion to the skin. The printed structures retained their geometry and mechanical integrity after both printing and subsequent lyophilization. The lyophilized scaffolds exhibited the necessary flexibility for pain management applications, allowing patient mobility and comfort during recovery while maintaining continuous contact with the wound surface. In addition, the scaffolds demonstrated self-adhesive behavior, showing bio-adhesion strengths in the range of 87–127 g, which is comparable to commercially available wound dressings. Drug-release studies further

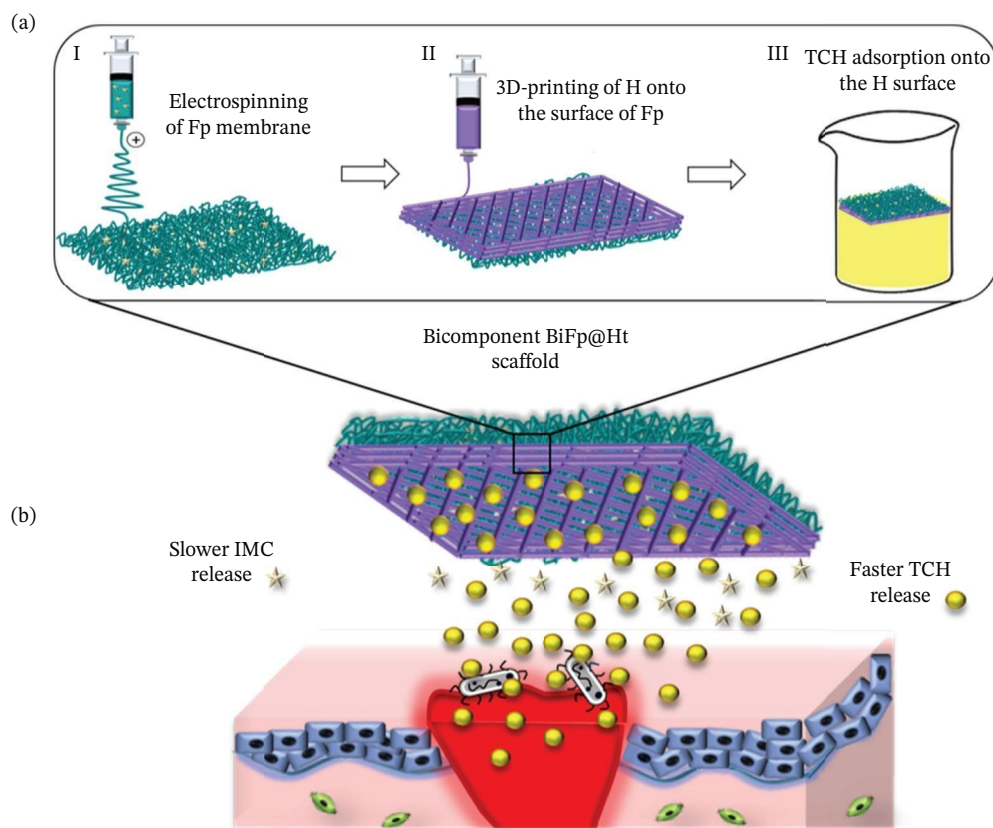


FIGURE 10 | (a) Schematic illustration of the bicomponent scaffold fabricated using electrospinning and 3D-printing techniques, and (b) its corresponding in vitro application. Reproduced with permission from [137].

revealed a rapid yet controlled release of lidocaine hydrochloride over approximately 5 h, highlighting the scaffold's potential as an effective wound-care material.

Cojocaru et al. [137] reported the development of an electrospun/3D-printed hybrid scaffold incorporated with active agents to achieve antibacterial and immunomodulatory functionality, as illustrated in Figure 10. In their approach, a hydrogel was 3D-printed directly onto an electrospun nanofibrous mat and subsequently subjected to a dual-crosslinking process. The resulting bicomponent structure was then lyophilized to form a composite scaffold comprising electrospun CS/PEO fibers loaded with a prodrug, together with a drug-loaded hydrogel layer.

As hybrid fabrication enables the development of multifunctional biomaterials, Rezaei et al. [128] investigated this approach to produce double-layer dermal scaffolds by integrating electrospinning with 3D printing. In their work, PVA/CS/sildenafil citrate (PVA/CS/SC) nanofibers were electrospun onto a 3D-printed PCL layer to create a hybrid wound dressing material. The PCL layer was first fabricated using a fused-filament 3D printing system equipped with an ink cartridge and positioned directly on the electrospinning collector. Subsequent electrospinning of CS/PVA and CS/PVA/SC solutions onto the printed PCL yielded a bilayer hybrid scaffold. The resulting structures demonstrated no cytotoxic effects, as confirmed by the MTT assays. Notably, the scaffold containing 2% (w/v) sildenafil citrate exhibited enhanced cell viability, attributed to its positive surface charge, increased hydrophilicity, and improved interfacial contact with L929 fibroblast cells. Microscopic observations revealed that fibroblasts

initially displayed a spherical morphology upon seeding but later spread extensively and adhered firmly to the electrospun nanofibers. This behavior indicates that the hybrid scaffold provided a conducive environment for cell attachment, proliferation, and growth. In in vivo wound-healing evaluations, the double-layer electrospun/3D-printed construct significantly accelerated tissue repair compared to both the drug-free scaffold and control samples. Wounds treated with the hybrid material progressed without complications or signs of necrosis. By Day 14, nearly complete wound closure was observed in the treated group, whereas lesions in the control group remained incompletely healed. The inclusion of the bioactive compound further enhanced the healing response, leading to a more developed dermis and better-organized collagen fibers after 14 days relative to the untreated controls.

3.2 | Other CS-Based Structures

Beyond electrospinning of CS-based materials to afford their application in tissue engineering, researchers have used CS as a coating layer to afford attachment, growth, and migration of cells on the electrospun nanofibers. Zhang et al. [143] produced nerve grafts with dual biomimetics from the composition and micro-physical structure perspective. In this case, aligned PLA nanofibrous structure was rolled onto the inner core of the annular conduit mold in the longitudinal direction and inserted into CS solution followed by freeze drying as schematically shown in Figure 11a. The shell was then removed to ensure that the CS hollow conduit is produced through the freeze drying process.

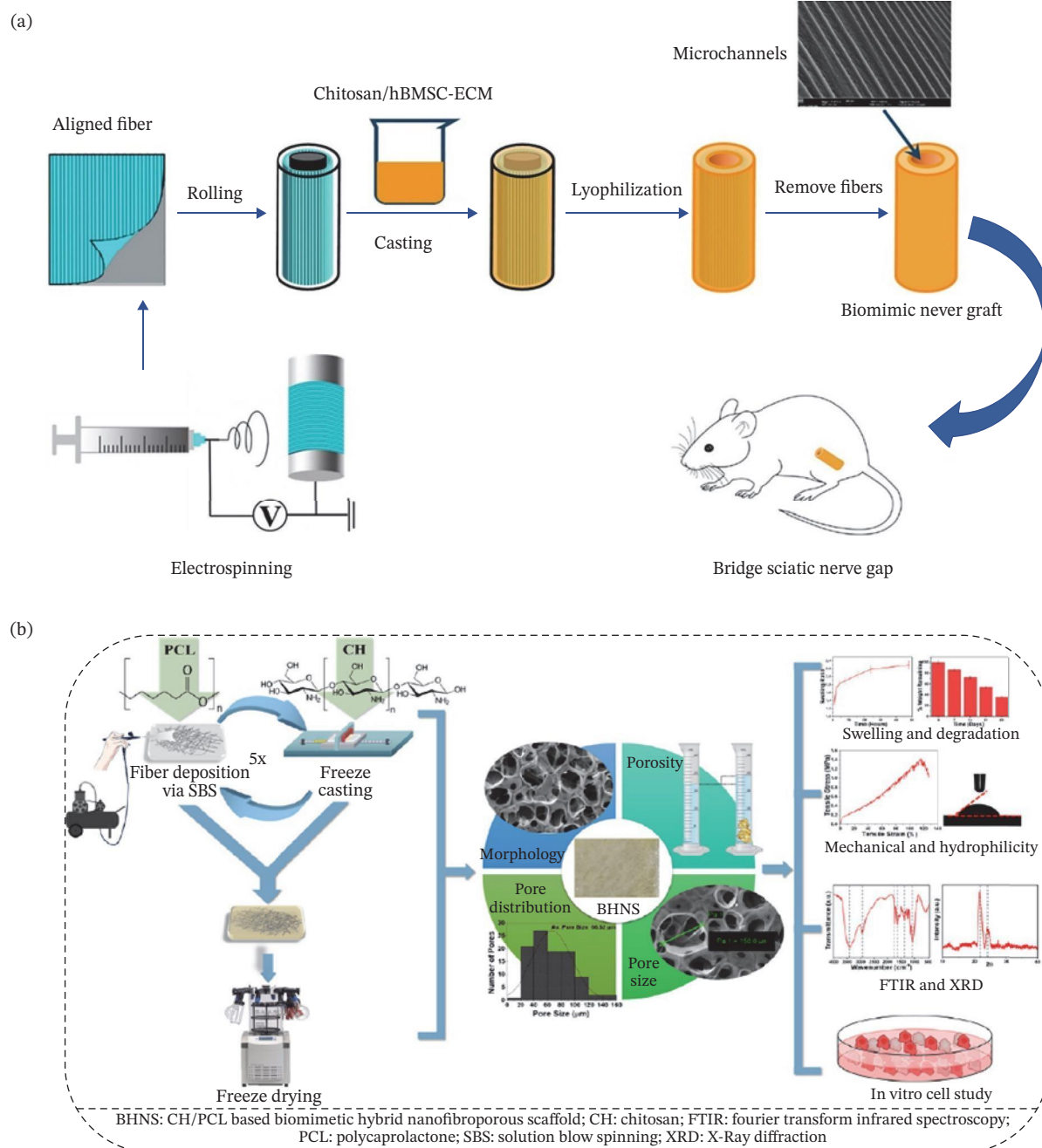


FIGURE 11 | (a) Schematic illustration of the fabrication process for biomimetic nerve graft. Reprinted with permission from [143]. (b) Schematic illustration for the fabrication process of PCL/CS fibroporous material (BHNS) highlighting its physicochemical, mechanical, and biological properties. Reprinted with permission from [144]. BHNS, CH/PCL based biomimetic hybrid nanofibroporous scaffold; CH, chitosan; FTIR, fourier transform infrared spectroscopy; PCL, polycaprolactone; SBS, solution blow spinning; XRD, X-ray diffraction.

The resultant grafts had smooth and uniform morphology, large pores, and microchannel structure with no impurities essential for nerve regeneration. The grafts exhibited good hydrophilicity, porosity, and mechanical performance with good blood compatibility without significantly affecting cell viability or migration. From histocompatibility and immunohistochemical studies, the as-prepared grafts displayed excellent biocompatibility and faster axonal regeneration and myelium recovery. The grafts reached the level of the autologous nerve in terms of sensory and motor function recovery and thus could be used in nerve regeneration.

Singh et al. [144] developed a scaffold for skin tissue regeneration using a layer-by-layer fabrication approach. The process began with solution blow spinning of PCL to form a fibrous base, followed by the application of CS onto the fibers, as shown in Figure 11b. The composite was then freeze-dried and chemically crosslinked using 3 wt% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). This resulted in a biomimetic, crosslinked hybrid nanofibrous scaffold that closely resembles the ECM, thereby promoting cell attachment, proliferation, and matrix deposition. The

combination of blow spinning and freeze-drying produced a randomly oriented, fibroporous structure with an average pore size of $\sim 67\ \mu\text{m}$, ranging from 20 to $160\ \mu\text{m}$. The scaffold exhibited a porosity of around 74.6% and a swelling ratio of ~ 2.29 . In degradation studies using PBS containing lysozyme, the scaffold showed about 36% degradation after 28 days. Mechanically, the scaffold demonstrated a tensile modulus of $\sim 11\ \text{MPa}$, a tensile strength of $\sim 2.4\ \text{MPa}$, and an elongation-at-break of $\sim 103\%$, indicating excellent flexibility and strength suitable for skin tissue applications. Biological evaluation using NIH-3T3 fibroblasts revealed enhanced cell proliferation, increasing from $\sim 18\%$ on Day 2 to $\sim 37\%$ on Day 4 and reaching $\sim 79\%$ by Day 7, compared to control samples cultured on standard polystyrene plates. Nuclear staining confirmed that cells began attaching and spreading within 24 h, with significant proliferation and dense cell coverage observed by Day 7. Overall, the hybrid nanofibrous scaffold provided essential cues for cell attachment, distribution, and organization within a 7-day incubation period, making it a promising candidate for tissue engineering applications. These favorable outcomes were attributed to the scaffold's structural, physical, and chemical properties that closely mimic the natural ECM. Shokrollahi et al. [145] investigated the development of a multilayered scaffold loaded with chamomile extract for use as a wound dressing material. The scaffold consisted of three layers: a base layer of PCL, a middle layer composed of PCL and chamomile-loaded carboxyethyl CS (CECS)/PVA, and a top layer of chamomile-loaded CECS/PVA. The resulting structure exhibited excellent mechanical strength and demonstrated strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, supporting its potential application as an effective wound dressing material. In addition, the scaffold showed notable antioxidant

capacity and good biocompatibility, as evidenced by improved cell viability over extended culture periods.

The strategic use of physical crosslinking, combined with appropriate material selection and processing techniques, enables the development of mechanically robust, biologically compatible, and architecturally viable materials [146, 147]. For example, Wang et al. [146] fabricated a hydrogel by integrating electrospinning and lyophilization methods, resulting in a mechanically strong, 3D CS-based hydrogel suitable for tissue engineering applications. Electrospun nanofibers were homogenized and dispersed in a viscous CS solution, followed by freeze-drying and lyophilization, which produced a 3D honeycomb-like structure, as illustrated in Figure 12. The incorporation of 30% cellulose acetate nanofibers preserved the cellular architecture in aqueous environments without the need for chemical crosslinking, maintaining a freestanding hydrogel structure with a low solid content of just 1%. Furthermore, the addition of SiO_2 as a reinforcing agent significantly enhanced the hydrogel's elasticity, enabling full recovery from 80% compression, a rapid recovery rate exceeding 500 mm/min, and excellent fatigue resistance in water. These architectural enhancements were found to support human MSC adhesion and elongation, highlighting the hydrogel's potential in regenerative medicine. Wright et al. [148] electrospun blends of synthetic polymers, which were subsequently introduced into a CS solution to fabricate 3D scaffolds for articular cartilage regeneration. The electrospun nanofibers contained salt, which was leached out prior to the incorporation of CS into the system. The resulting PDLA/PLLA/CS hydrogel demonstrated mechanical properties comparable to those of native articular cartilage; however, it lacked sufficient recovery during dynamic

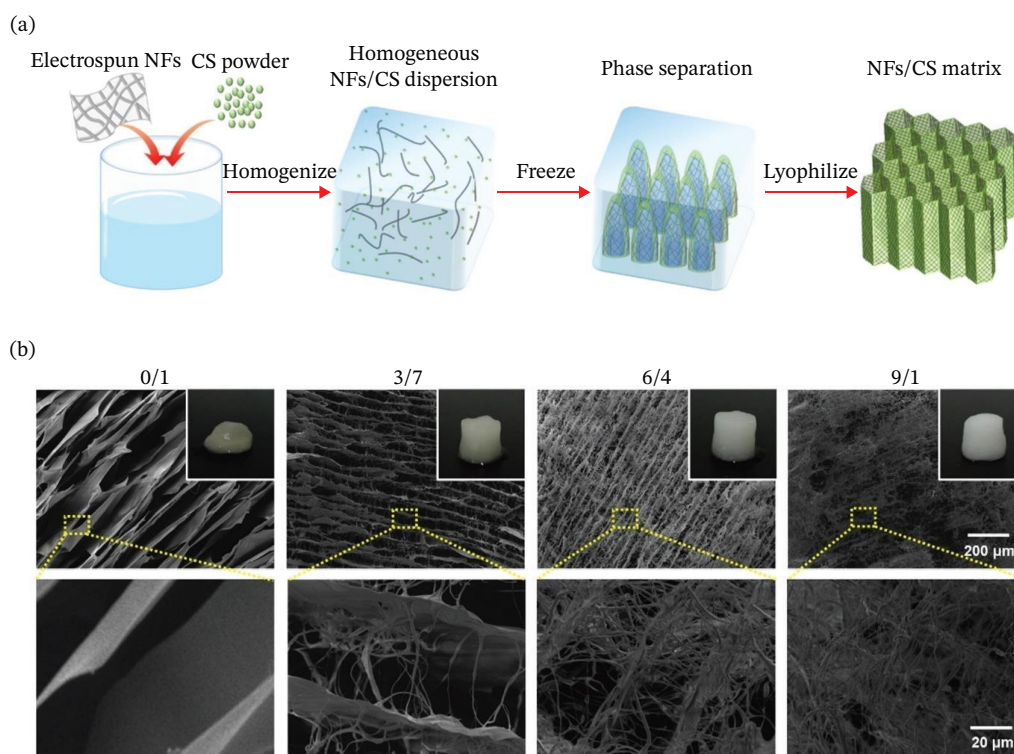


FIGURE 12 | (a) Schematic illustration of the process used to fabricate honeycomb-like 3D CS-based hydrogels. (b) Photographic images and SEM micrographs of cellulose acetate nanofiber/CS (CA NF/CS) matrices at varying ratios: 0/10, 3/7, 6/4, and 9/1 (CA NF/CS). Reproduced with permission from [146].

mechanical testing. In contrast, PLA/PCL/CS scaffolds exhibited lower mechanical strength relative to articular cartilage but showed improved recovery under dynamic conditions. Furthermore, canine chondrocytes cultured on PDLA/PCL scaffolds successfully produced the cartilage ECM, suggesting that mechanical limitations could be addressed to make these materials viable for cartilage regeneration applications.

Shen et al. [149] developed a CS-based scaffold by incorporating short fibers derived from electrospun aligned poly(lactic-co-glycolic acid) (PLGA) fibers. The inclusion of these short fibers as a reinforcing phase significantly enhanced the mechanical properties of the resulting scaffold, with a 349% increase in compressive strength and a 153% improvement in tensile modulus compared to those of the neat hydrogel. The reinforced scaffold also exhibited large pores, suitable porosity, and rapid water-absorption capacity.

Moreover, it supported chondrocyte adhesion and proliferation while promoting the formation of mature cartilage tissue characterized by a cartilage-like structure and the deposition of ECM components specific to cartilage, including a 42% increase in GAG content and a 295% increase in type II collagen content relative to the neat hydrogel. These findings highlight the potential of combining electrospinning with other fabrication techniques to produce mechanically and structurally viable scaffolds that biomimic the native tissue environments and support tissue regeneration.

Balagangadharan et al. [72] utilized the electrospinnability of PCL to fabricate a tissue scaffold for bone tissue regeneration. Sinapic acid was encapsulated within CS nanoparticles and subsequently incorporated into the PCL spinning solution, resulting in a nanofibrous scaffold with randomly oriented fibers measuring 317–372 nm in diameter. The average fiber diameter increased as the content of sinapic acid-loaded CS nanoparticles rose. The resulting scaffold exhibited no toxicity to cells, while the release of sinapic acid stimulated mMSCs differentiation into osteoblasts and enhanced bone regeneration in vivo.

3.3 | Antimicrobial and Antifungal Properties

CS-based electrospun matrices combine intrinsic contact-active antimicrobial/antifungal activity with excellent capability as controlled-release platforms [150, 151], and this dual functionality has been exploited to produce infection-resistant wound dressings and implant coatings [150]. The polycationic backbone and protonated amine groups of CS disrupt microbial membranes and bind anionic cell components, giving broad-spectrum activity, while the nanofibrous morphology increases the surface area and facilitates payload loading (e.g., antibiotics, metal nanoparticles, and antifungal agents) [150–154], producing synergistic effects. Several recent studies exemplify these strategies: (i) CS nanofibers loaded with teicoplanin provided sustained local antibiotic release for up to ~12 days, enhanced bactericidal activity against *Staphylococcus aureus* (1.5–2x compared with free drug in vitro), were cytocompatible with fibroblasts, and accelerated wound closure in a rat full-thickness model [155]; CuS nanoparticle-loaded CS electrospun membranes combined adsorption and photo-Fenton/photothermal mechanisms to degrade antibiotic pollutants and also showed antibacterial activity, with cross-linking (genipin) improving nanoparticle stability and pH-range

usability [154]; and (iii) surface-immobilized silver nanoparticles produced by plasma or in situ reduction substantially increased bactericidal activity of CS nanofibers, including activity versus *Pseudomonas aeruginosa* and MRSA [153]. Together, these approaches illustrate (a) contact-killing by CS, (b) ion/ROS-mediated activity from metal nanoparticles (Ag^+ , Cu^{2+} and photocatalytically generated ROS), and (c) potentiation of conventional antibiotics via sustained local delivery from electrospun matrices, a combination that improves efficacy while reducing systemic exposure [150, 152–154, 156]. These findings support the placement of electrospun CS systems as promising platforms for topical infection control and wound healing, and they highlight the importance of reporting both in vitro antibacterial/antifungal data and any available in vivo efficacy to strengthen translational claims.

3.4 | Preclinical and In Vivo Studies

Although most CS-based electrospun biomaterials remain at the laboratory stage, a growing body of preclinical work demonstrate promising in vivo biocompatibility and therapeutic performance. Subcutaneous implantation studies in rats have shown that electrospun CS microfiber scaffolds support stem-cell attachment and survival while eliciting minimal host inflammatory responses, confirming their suitability as biocompatible tissue-engineering matrices [157]. In wound and hemostasis models, electrospun CS-PEO membranes exhibited strong biocompatibility and effective bleeding control in rat liver injury models, with faster biodegradation and improved tissue repair compared with conventional CS sponges [158]. Bone regeneration studies further demonstrate translational potential: modified electrospun CS membranes stabilized via postspinning treatment supported new bone formation in rat calvarial defects at levels comparable to commercial collagen membranes while preventing soft-tissue infiltration [159]. Similarly, in full-thickness skin wound models, CS nanofiber scaffolds enhanced reepithelialization, reduced wound contraction, and promoted neodermis formation relative to comparator polymer scaffolds [85].

Collectively, these studies indicate that CS-based electrospun systems have progressed beyond in vitro validation toward robust small-animal preclinical evidence, particularly in wound healing, hemostasis, and guided tissue regeneration. However, clinical translation remains limited, with few human studies being reported to date. Future work should prioritize standardized in vivo models, long-term degradation and immune response assessments, and scalable manufacturing strategies to bridge the gap between promising preclinical results and clinical adoption.

3.5 | Commercial and Regulatory Progress of CS-Based Scaffolds

A number of CS-based wound dressings and hemostatic devices have received regulatory approval and are commercially available, demonstrating the translational potential of this class of biomaterials. However, it is important to note that most approved products are not electrospun nanofiber scaffolds but rather CS films, sponges, or impregnated gauze. Fully electrospun CS scaffolds remain largely at the preclinical or early translational stages.

Several FDA-cleared or CE-marked CS devices are already in clinical use. Examples include HemCon bandages [160], Chito-Gauze [161], Celox [162], and ChitoFlex [162], which utilize CS's intrinsic hemostatic and antimicrobial properties to rapidly control bleeding in trauma and surgical settings. These dressings act by forming a mucoadhesive barrier that promotes clotting through electrostatic interactions with erythrocytes and platelets and are widely used in military and emergency medicine. Similarly, FDA-cleared CS wound dressings such as the KA01 CS dressing are indicated for chronic and acute wounds, including ulcers, burns, and surgical injuries, highlighting regulatory acceptance of CS as a safe wound contact material [163].

Although these approved devices validate the clinical relevance of CS biomaterials, true electrospun CS nanofiber scaffolds have not yet achieved widespread regulatory approval. Most electrospun systems remain in preclinical development due to challenges related to scalable manufacturing, mechanical stability, sterilization, and regulatory standardization. Nonetheless, the success of existing CS dressings provides a strong translational foundation, suggesting that electrospun variants, offering higher surface area, an ECM-like structure, and tunable drug release, are likely candidates for future clinical translation once these manufacturing and regulatory hurdles are addressed.

Overall, while commercially approved CS wound care products already demonstrate clinical utility, the next stage of translation will involve bringing electrospun CS scaffolds from promising preclinical platforms to regulated therapeutic devices.

3.6 | Challenges

CS is one of the most extensively studied natural polymers, with applications spanning biotechnology, medicine, water remediation, cosmetics, the food industry, pharmaceuticals, and agriculture owing to its unique properties. However, its use in the biomedical field is often limited by poor mechanical strength and low solubility in common solvents. These challenges are further intensified when CS is processed via electrospinning. A promising approach to address its solubility issues is the use of CS derivatives, which enhance the processability. The mechanical limitations can also be improved by blending CS with other polymers and fillers, which not only reinforces mechanical properties but can also impart additional functionalities such as increased conductivity and antibacterial effects. Nevertheless, the electrospinnability of CS typically depends on harsh chemicals and sacrificial polymers that are unsuitable for large-scale production and commercialization. The biomimetic structures produced often require modifications to the electrospinning technique, which may restrict commercial viability due to the limited quantity of fibers that can be fabricated. Integrating electrospinning with other advanced fabrication methods offers a crucial strategy to control the morphology and architectural features of the resulting materials. This integration opens up new avenues for developing novel materials tailored for tissue engineering applications. It is important, however, to select these techniques carefully to preserve the delicate nanofibrous architecture resembling the natural ECM, which is essential for optimal cell attachment, proliferation, and growth. One of the most widely adopted strategies for combining with electrospinning is the integration of 3D-printing technology [137]. Although 3D printing offers

numerous advantages, it still presents several limitations. A major challenge lies in its difficulty in producing high-resolution filaments required for scaffolds tailored to different tissue types. Furthermore, the pore sizes generated in conventional 3D-printed structures are typically much larger than the dimensions of most cells, which can impede efficient cell seeding and subsequent tissue development.

To overcome these limitations, the hybridization of 3D printing with electrospinning has emerged as an effective solution. This combined approach merges the precise macroscale architecture provided by 3D printing with the nano to microscale fibrous networks produced through electrospinning. As a result, it enables the fabrication of scaffolds with customizable geometries, interconnected porosity, robust mechanical integrity, nanoscale topographical cues, and an extracellular-matrix-like architecture. These synergistic features enhance bioactivity and support improved cellular adhesion, proliferation, and differentiation. Constructs produced through this integrated method are often termed hybrid scaffolds or hybrid materials, reflecting their incorporation of multiple structural features into a single multifunctional system.

4 | Future Perspective

CS-based electrospun biomaterials continue to gain momentum as multifunctional platforms for wound healing, drug delivery, and antimicrobial applications. Despite substantial progress, several scientific and translational challenges remain that will shape future research directions [164–167].

One emerging trend is the development of multifunctional and stimuli-responsive CS systems capable of responding to environmental triggers such as pH, temperature, enzymatic activity, or light [168]. These systems enable on-demand drug release and improved infection control, particularly in chronic wound environments where pH and microbial burden fluctuate [168]. Integration of photothermal or photocatalytic nanoparticles (e.g., CuS, Ag, and ZnO) into electrospun CS matrices represents a promising strategy for combining structural support with externally triggered antimicrobial activity [154, 169]. Future work should focus on balancing therapeutic efficacy with long-term cytocompatibility and nanoparticle stability within biodegradable CS scaffolds.

Another key direction involves green and scalable fabrication strategies. While electrospinning is widely used at the laboratory scale, challenges remain in achieving industrial-scale production with a consistent fiber morphology, drug loading efficiency, and batch-to-batch reproducibility [164, 165, 167]. The use of benign solvents, water-based systems, and supercritical-fluid-assisted processes offers opportunities to improve sustainability and reduce residual solvent toxicity. Further exploration of scalable crosslinking strategies and solvent-free processing will be essential for clinical translation.

The standardization of antimicrobial and antifungal testing also represents an important future priority. Many studies report zone-of-inhibition or in vitro assays under varying conditions, making cross-comparison difficult [152, 156, 166]. Establishing standardized testing protocols, including biofilm models and clinically relevant microbial strains, would improve

comparability and facilitate regulatory evaluation. However, there has been a meaningful study that demonstrates the ability of these electrospun CS-based scaffolds in biofilm prevention. For example, plasma-treated CS derivatives have been investigated as anti-biofilm coatings for electrospun fibers. In this study, CS was functionalized with thiophene, furan, and 3,4-ethylenedioxythiophene using RF plasma and then electrospun into fibrous mats. Spectroscopic analyses confirmed successful surface enrichment of the functional groups, while SEM showed that the fiber morphology was preserved. Using a quartz crystal microbalance, the authors monitored *Pseudomonas aeruginosa* PAO1 adhesion and biofilm formation on coated surfaces. All plasma-modified fibers reduced bacterial attachment compared with untreated CS. The CS-furan (Ch-PFu) derivative showed the most pronounced inhibition of biofilm development. These findings demonstrate that plasma-induced surface modification of CS fibers can effectively suppress early biofilm formation without the need for added antimicrobial agents [170].

Advances in hybrid and composite systems are expected to drive the next generation of CS-based electrospun biomaterials. Combining CS with other biopolymers (e.g., collagen, GEL, alginate, and silk fibroin) or synthetic polymers (e.g., PCL and PLA) can improve mechanical strength, processability, and biological performance. The incorporation of bioactive molecules such as growth factors, peptides, and plant-derived compounds may further enhance regenerative outcomes. Future work should explore structure-function relationships between fiber morphology, surface chemistry, and biological activity to enable rational design of tailored scaffolds. From a translational perspective, regulatory approval and clinical adoption remain significant hurdles. Issues such as reproducibility, sterilization, shelf stability, and cost-effectiveness must be addressed.

The integration of electrospinning with other advanced fabrication technologies presents a promising pathway for advancing CS-based materials toward clinical translation. Such combined approaches not only enable the production of highly complex and biologically relevant structures but also allow precise control over the resulting scaffold properties. By influencing key morphological and architectural features, such as fiber alignment, pore distribution, and hierarchical patterning, these hybrid techniques offer the ability to fine-tune the mechanical performance and other functional characteristics. This level of control is essential for designing scaffolds that closely mimic native tissue environments and meet the stringent requirements of clinical applications.

Closer collaboration between materials scientists, clinicians, and industry partners will be necessary to move promising laboratory-scale systems toward commercialization. The integration of advanced characterization techniques, predictive modeling, and techno-economic analyses may help bridge this gap. Overall, CS-based electrospun biomaterials are well positioned to contribute to next-generation antimicrobial dressings and biomedical devices. Continued efforts toward multifunctionality, scalability, and clinical validation will be critical for translating these systems from experimental platforms into widely adopted healthcare solutions.

5 | Conclusions

This review highlights recent advancements in the development of biomimetic products for tissue engineering based on

electrospinning and CS-derived materials. CS is a naturally abundant polymer characterized by a unique chemical structure; it is a linear polycation with a high charge density, containing reactive hydroxyl and amino groups. Additionally, CS is known for its low toxicity, biodegradability, biocompatibility, affordability, hemostatic properties, and mucoadhesiveness, making it a highly suitable biomaterial for various biomedical applications, particularly tissue regeneration.

Recent studies indicate that electrospinning CS imparts additional advantageous properties, such as a high specific surface area and controllable architectural structures, which are critical for effective tissue regeneration. This technique provides a versatile platform for fabricating biomimetic products that promote cell attachment, growth, and regeneration.

Nonetheless, poor solubility and limited mechanical strength have been major barriers to the commercial realization of CS-based materials in tissue engineering and related biomedical fields. These challenges can be addressed by blending CS with other polymers and incorporating various fillers, which not only enhance the mechanical performance but also enable the production of multifunctional materials suitable for biomedical use. Furthermore, chemical modification of CS to produce diverse derivatives is essential to improve its solubility and electrospinnability, thereby expanding its applicability for tissue engineering. Recent advancements in modified electrospinning techniques, coupled with increased throughput capabilities, have made the industrial-scale electrospinnability of CS feasible. This progress significantly broadens its potential applications, particularly in the field of tissue regeneration. Moreover, the variability in fiber diameter observed in newly developed electrospinning methods adds an additional dimension to their suitability for biomedical applications. Such variability can be advantageous in mimicking the heterogeneous structure of native extracellular matrices, thereby enhancing the material's effectiveness in tissue engineering. The hybridization of CS-based electrospun nanofibers with complementary fabrication techniques, such as 3D printing, has significantly advanced the development of complex anatomical structures for biomedical applications. By integrating electrospinning with 3D-printing or other processing methods, it becomes possible to combine nanoscale fibrous architectures with precisely defined macroscale geometries. This synergy enables the fabrication of intricate abdominal or organ-mimetic constructs that cannot be achieved using a single technique alone. Such hybrid systems provide enhanced structural fidelity, tunable porosity, and improved biomimicry, allowing for the creation of scaffolds that better replicate the mechanical and biological characteristics of native tissues. As a result, these multifunctional constructs hold great promise for applications in tissue engineering, regenerative medicine, surgical training models, and drug-delivery platforms.

Author Contributions

Teboho Clement Mokheba: conceptualization, investigation, methodology, formal analysis, original draft preparation, review and editing of the manuscript, validation of the findings. **Asanda Mtibe:** investigation, writing – review of the manuscript. **Lerato Hlekelele, Sivuyisiwe Mapukata, and Katekani Shingange:** writing – editing and review of

the manuscript. **Amos Nhlapo**: editing, review, investigation. **Antonio Benjamim Mapossa and Mokgaotsa Jonas Mochane**: investigation, original draft preparation, review and editing of the manuscript, validation of the findings. **Eric Mathete**: writing – editing of the manuscript. **Ndivhuwo Shumbula**: drafting of the original manuscript, writing, review. **Odireleng Martin Ntwaeaborwa**: formal analysis, review and editing of the manuscript, validation of the findings.

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The authors declare no conflicts of interest.

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