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Review Article

ELECTROSPUN NANOFIBERS IN TREATMENT OF MYOCARDIAL INFARCTION: A REVIEW

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ABSTRACT

At the present time, cardiovascular disease (also known as CVD) is one of the primary causes of death. In recent years, regenerative medicine, tissue engineering, and the development of novel materials have been the primary focuses of this field of study. Recently, the public's interest has been piqued by the use of electrospinning technology to produce nanofibrous materials for the treatment of cardiovascular diseases. The production of nanofibers may be accomplished in an easy and versatile way with the use of electrospinning. In this article, we will go through a number of different biodegradable polymers that may be used for the manufacturing of fibers. In addition, we provide the most recent information about the use of nanofibers in the management of myocardial infarction. This analysis comes to a close with a review of the limitations of the technology, its potential future applications for treating cardiovascular illness, and the technical challenges it faces.

Other selections include articles from Springer, information from Internet sources, and Online published articles from Wiley, Frontiers, etc.

Keywords: Electrospinning, Nanofibers, Myocardial infarction, Cardiac patch, Cardiomyocytes

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INTRODUCTION

Cardiovascular illnesses are among the primary causes of morbidity and death globally [1]. Cardiovascular disease continues to be the major cause of mortality in modern civilization, yearly causing upwards of 18 million deaths [2]. The American Heart Association (AHA) forecasts that by 2034, nearly half of the US populace will have CV [3], which is significant proof of the tremendous impact and burden cardiovascular illness causes on humanity. Myocardial tissue is mostly cardiomyocytes and extracellular matrix, with the excess space filled by vessels of blood, nerves, migratory, and residual cells [4]. Collagen is constituted by roughly 33 percent of the total cardiac protein, with type I comprising 80 percent of the total collagen and playing a crucial role in cell attachment, growth, and movement from embryonic through postnatal stages [5]. People with advanced heart failure are often treated with heart transplants. However, the biggest impediment to organ transplants is the rising number of patients due to the lack of available donors [6]. Interventional coronary catheterization, pharmacological therapy, and bypassing the coronary arteries are the current treatments for reviving a damaged myocardium. Patients with end-stage heart failure may need heart transplantation, which is restricted in availability, or ventricular assist devices, which are ineffective [7] because of the limited efficacy of present medical therapy. Remodeling of injured cardiac tissue using tissue-engineered methods, such as the use of cardiac patches, is an alternative treatment option. As a framework for the formation of live, functioning cardiac tissue, a cardiac patchwork might carry stem cells or differentiated heart cells [8]. Challenges in cardiac tissue engineering include the use of biomaterials for framework building as well as the delivery of coordinated patterns of extracellular matrix-like structures, which are essential in the directed production, retention, and contraction of cardiomyocytes [9]. The nanoparticles are used in many ways and can be prepared from a variety of materials, such as proteins, polysaccharides, and synthetic polymers. Recently, there has been growing interest in one-dimensional nanomaterials such as nanorods, nanowires, nanofibers, and nanotubes of various oxide materials because of their fundamental scientific interest and also because of their potential applications in a variety of functional devices [10]. Among them, the development of nanoparticles and nanofibers has greatly enhanced the scope for fabricating scaffolds that can potentially meet this challenge.

Electrospinning: principle and formation

Electrospinning is a versatile and practical technique for manufacturing ultrathin fibers. Electrospinning is an electrodynamic technique in which liquid drops are charged to create a jet that is then elongated and extended to form fibers. Electrospinning involves a relatively simple setup [11, 12]. Principal components are a source of high-voltage power, a syringe pump, a spinneret, and a conductive collector. Either DC or AC may be used as the source of power. When electrospinning occurs, liquid is expelled through the spinneret in the form of a pendant droplet due to surface tension. Upon electrification, electrostatic repulsion between the particle surfaces of the same sign distorts the droplet into a Taylor cone, from which an electron-rich jet is ejected. Due to twisting instabilities, the jet first expands in a linear fashion before rapidly whipping. As the jet is expanded into smaller dimensions, it quickly solidifies, resulting in the deposition of solid fibers on the collector's ground. Typically, the electrospinning technique consists of 4 steps-Taylor cone is formed after charging of droplet; Charged jet extended in straight line; Jet thinning caused by the presence of an electric field and the development of electrical twisting instability; Solidification and solid fibre collection of the jet on a grounded collector [11, 13, 14].

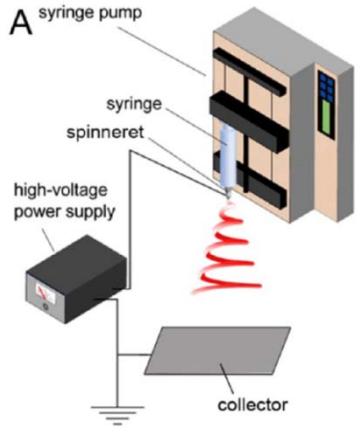

Fig. 1: Schematic illustration of a typical setup for electrospinning [15]

Materials used to produce nanofibers

Electrospinning has been used to produce nanofibers from a variety of materials. Small micromolecules include amphiphiles/

cyclodextrin derivatives. In addition [16, 17], if it assembles and forms a substantial chain attachment, it can be electrospun directly into nanofibers. Numerous composite materials, such as polyvinyl chloride (PVP) and titanium tetraisopropoxide (Ti(OiPr)4), have been electrospun directly into nanofibers [18].

Most natural polymers may be electrospun immediately if they are able to dissolve in appropriate solvents to form solutions or are melted sans degradation. A variety of organic polymers, including both natural and synthetic polymers, were then studied for solution electrospinning to produce nanofibers immediately. Synthetic polymers like polystyrene (PS) and poly(vinyl chloride) (PVC) were electrospun into nanofibers for commercial use. Several biomimetic and biodegradable synthetic polymers, including polycaprolactone (PCL), poly(lactic acid) (PLA), and poly(lactic-co-glycolic acid) (PLGA), have been electrospun instantly into nanofibers, with biomedical applications being researched. Electrospun nanofibers consist of natural biopolymers such as DNA, silk fibroin, fibrinogens, alginate, gelatin, collagen, dextran, chitin, and chitosan. Electrospinning polymers that are conductive like polyaniline (PANi) and polypyrrole (PPy) can be directly converted into nanofibers. For piezoelectric and/or pyroelectric applications, poly(vinylidene fluoride) (PVDF) and other stable polymers have been electrospun as nanofibers.

Recent works involving electrospun nanofibers for treatment of myocardial infarction

Due to its nano capabilities, high conductivity, and strong affinity for physicochemical associations with proteins and bioactive substances, carbon nanomaterials have been used in research to enhance heart function and cardiac healing. They can provide a natural, three-dimensional framework for the growth of cardiac cells. Carbon nanostructures, such as carbon nanotubes and nanofibers, are employed to strengthen collagen and various frameworks in animal models with a damaged heart due to their electrical and physical qualities. Carbon nanofibers might serve as a substrate for the development of cardiovascular cells [19, 20].

M. Tashakori et. al showed that the collagen group served as the control in this investigation of a novel bio-synthetic cardiac framework consisting of carbon nanofibers containing collagen for the restoration and rejuvenation of myocardial infarction in animal models [21].

The structural toughness of the framework improved somewhat as the density of carbon nanofibers increased.

Tissue damage and fibrosis were identified during *in vivo* testing of the framework in a rat model of myocardial infarction. As fibrosis reduced and CD31 marker indicated angiogenesis increased in the ischemic area in groups that received Collagen and Collagen-containing carbon nanofibers framework compared to the MI model, the Collagen-containing carbon nanofibers group demonstrated enhanced cardiac tissue integrity.

Staining of tissues indicated that the collagen group had much shorter intercellular gaps, which decreased cardiomyocyte deterioration and necrosis.

Actinin, a marker for the creation of new cardiac cells, increased in both collagen-and collagen-containing carbon nanofibers-treated cardiomyocytes. In addition, it was larger in the group having collagen-containing carbon nanofibers than in the group containing collagen alone.

Using CASPASE-3 and TUNEL, apoptosis was assessed in cells. CASPASE-3 expression was drastically decreased in both groups. TUNEL results using carbon nanofibers containing collagen demonstrated a decrease in the number of dead cells.

Collagen alone as a substrate displayed moderate recovery in cardiac ischemia-damaged tissue; however, collagen frameworks with collagen-containing carbon nanofibers exhibited much improved results.

Promotion of angiogenesis is a critical step that may ameliorate cardiac dysfunction after infarction of the myocardium. Enhanced vascularization and oxygen supply to the injured ischemia zone may reduce cardiomyocyte death and scarring [22, 23] and are crucial for minimizing the development of an infarction leading to heart failure. Injection of angiogenic GAG mimetic peptide nanofibers [24] improved the functioning of the heart in a rat model of myocardial infarction by preserving increased heart muscle and promoting the formation of new blood vessels. The rat model of myocardial infarction had GAG mimicking nanofibers/control nanofibers intramyocardially administered to the location of the infarct. Before and after infarct development and injection, the heart function was evaluated by echocardiography. Echocardiography revealed that LV remodelling, important for avoiding heart dysfunction, was reduced in the group treated with GAG mimic nanofibers.

The GAG mimetic nanofiber treatment group had a superior change in fractional area, end-systolic/diastolic volume than the saline treated group. Hemodynamic study revealed that the GAG mimicking nanofiber injected group and the control nanofiber group exhibited clinically substantial increases in contractile index, blood flow, and mean LV pressure relative to the group treated with saline.

Semiquantitative analysis revealed that the size and area of the infarct were smaller in the group treated with GAG mimicking nanofiber, along with a large proportion of living cardiomyocyte tissues in the peri-infarct section in both the GAG mimicking nanofiber and control nanofiber treated groups, leading to an increase in the thickness of the walls in comparison to the saline treated group. The impact of peptide nanofibers on the overexpression associated with specific cardiac marker genes [25-27] were studied to comprehend the engagement of progenitor cells to the cardiomyocyte line. Mlc-2v, a cardiac-specific marker, was expressed at a higher level in H9C2 cells grown on GAG-mimetic nanofibers, according to the findings. On both GAG-mimic nanofiber-wrapped substrates, the important cardiac-specific protein cTnT was detected playing a role in relaxation and contraction of the heart muscle.

Benjamin W. Streeter *et al.* showed that electrospun nanofiber-based frameworks and cardiac progenitor cells were coupled to generate cardiac patches that might restore heart function lost because of CHD and subsequent palliative surgery [28].

By covering each framework's surface with fibronectin, the random and aligned orientations of electrospun nanofiber frameworks formed comprising polycaprolactone or a combination of polycaprolactone and gelatin were effectively adjusted. On each framework, cardiac progenitor cells were grown, and the resulting patches were shown to emit paracrine signals that were anti-fibrotic and pro-angiogenic. Cardiac progenitor cells lose their reparative capability as early as one year of age, demonstrating an age-dependent variation in cardiac progenitor cells' behavior. Neonatal cardiac progenitor cells administered into a rat model with right ventricular heart failure raised RV fraction of ejection, reduced RV thickness of walls, and improved tricuspid annular plane systolic excursion, but child cardiac progenitor cells lacked these reparative capabilities.

In relation to the addition of adherence factors to frameworks, the data revealed age-dependent alterations in the alignment and morphology of cardiac progenitor cells. Although the addition of gelatin and Fibronectin to nanofibers facilitated alignment in newborn cardiac progenitor cells, only the addition of Fibronectin to random nanofibers enhanced orientation in paediatric cardiac progenitor cells on the patch. In addition, combining gelatin and Fibronectin did nothing to enhance the ability of newborn cardiac progenitor cells patches to stimulate angiogenesis, but the inclusion of Fibronectin to oriented nanofibers enhanced this ability in paediatric cardiac progenitor cells patches. The study discloses the nanofiber-based patches that may be optimum for a certain patient's cardiac progenitor cells based on the effects of several factors on cardiac progenitor cells from various donors.

Cardiomyocytes produced from human induced pluripotent stem cells (hiPSC) with a high degree of purity have promise for drug development and cardiac regeneration. Junjun Li *et al.* demonstrated that by growing hiPSC-cardiomyocytes on low-thickness bio-degradable poly (D, L-lactic-co-glycolic acid) nanofibers, cardiac

tissue-like constructs of superior quality were produced [30]. Aligned nanofibers displayed considerably larger amounts of b-MHC, a contractile velocity associated cardiac maturity marker, based on cardiac tissue-specific markers immunostaining [31]. cTnT-positive myofilaments and a-Actinin-positive sarcomeres were positioned along aligned nanofibers. Many genes involved in sarcomere structures (MYL2, HAND2) and ER-Ca2+function were amplified in cardiomyocytes grown on aligned nanofibers compared to those grown on (PLN and RYR2), as determined by mRNA expression analysis. Consistent with the alterations in sarcomeric architecture suggested by electron microscopy data, hierarchical clustering results indicate that the expression profile of cardiac tissue-like structures was distinct from those of random nanofibers and flat cells.

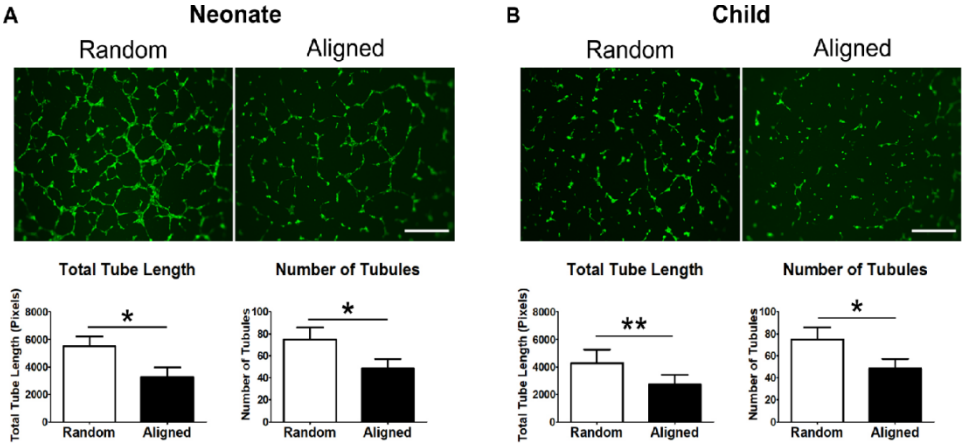

Fig. 2: HUVEC tube formation on matrigel following 6 h of incubation in conditioned media collected at day 2 from random and aligned patches with (A) neonatal or (B) child CPCs, respectively. Calcein-AM staining is shown in green. Scale bar = 200 µm. Total tube length and number of tubules are quantified for HUVECs [29]

Analyzing the electrical properties of cardiomyocytes grown on highly dense aligned nanofibers, low-density aligned nanofibers, random nanofibers, and flat, a field potential of greater amplitude was observed for cardiomyocytes coated on aligned nanofibers and random nanofibers than those cultivated on flat, indicating superior cell adhesion to nanofibers. Field potential amplitude was smaller for the high-density aligned nanofiber samples compared to the low-density aligned nanofiber samples, most likely as a result of a denser fiber layer, decreasing the amount of contact between electrodes and cardiomyocytes, hence boosting resistivity. Due to weaker cell attachment and homogeneity, the cardiomyocytes on the Flat cells detach from the electrodes, resulting in a lower proportion of T-wave channels in the microelectrode array. But cardiomyocytes grown in high-density aligned nanofibers exhibited a greater proportion of channels, which is essential for researching the long-term effects of drugs on cardiomyocytes. Following this, *in vitro* engrafting tests were conducted to evaluate the capacity of cardiac tissue-like structures to make contact with and adhere to the sheets of cardiomyocytes. Cardiac tissue-like structures have the potential to treat re-entrant cardiac arrhythmias by fusing with the cardiomyocyte sheets of the host and establishing a fast electrical connection between separated heart regions.

Cardiac tissue-like constructs were administered to the hearts of rats with infarction, whereas nanofiber frameworks were supplied to the control group. A few weeks after transplantation, the implanted constructs were present on the surface of the rat heart, but the acellular nanofiber frameworks were scarcely discernible. Using CD31 immunostaining, it was discovered that the cardiac tissue-like construct group had a higher density of small blood vessels than the control group. Rats treated with cardiac tissue-like constructs showed a rise in ejection percentage after a few weeks. The transplantation of cardiac tissue-like constructs improved the left ventricle's fractional shortening and end-systolic diameter. The transplantation of functioning, well-organized cardiomyocytes from cardiac tissue-like constructions led to a significant operational enhancement in the heart of a patient with myocardial infarction.

Hitscherich P *et al.* revealed the viability of a conductive graphene-bearing framework for cardiac tissue engineering since conducting nanomaterials such as graphene and carbon nanotubes have surfaced as very attractive candidate biomaterials [32, 34]. Electrospinning graphene-dispersed PCL solutions successfully created a 3D conductive nanocomposite framework. Because conductive nanoparticles prefer to agglomerate, it is difficult to adequately handle 3D electroconductive frameworks. The addition of up to 0.05 percent conductive graphene to an insulating PCL polymer matrix resulted in an equal dispersion of graphene particles throughout the 3D PCL matrix, which improved volume conductivity owing to the percolation effect [35, 36]. This is advantageous because electrical stimulation can improve cardiomyocyte function and differentiation potential [37, 38]. To investigate signal transmission across PCL+G frameworks, a device for delivering focused electrical stimulus at a single site was constructed. By causing contractions in the cells in proximity to the stimulation electrode, targeted point stimulation more closely imitates this process. Signal transmission across PCL+G frameworks containing graphene reveals the possibility of adding further electrical stimulus to these tissues. Therefore, frameworks containing 0.01 percent graphene were selected to determine whether this quantity of graphene is adequate to provide functional benefits while maximizing biocompatibility. The excellent cellular vitality and adherence of cardiomyocytes obtained from stem cells suggested that 3D PCL+G scaffolds are compatible with stem cell-derived cardiomyocytes [39-41]. Mouse embryonic stem-cardiomyocytes naturally contracted, responded well to an inotropic medication, generated cardiac-specific markers, and verified the phenotype for genuine cardiomyocytes on graphene frameworks. The greatest increase in cardiac-specific protein expression and contractility was seen in mouse embryonic stem cardiomyocytes cultured on an aligned PCL+G framework, showing a synergistic effect of fiber orientation and graphene inclusion. Specifically, the increased expression of Cx43 by mouse embryonic stem-cardiomyocytes on aligned PCL+G frameworks is suggestive of enhanced cell–cell coupling [42–44], which is likely due to signal propagation aided by graphene supplying local conductive sites. In addition, aligned PCL+G frameworks induced the greatest fractional release and spontaneous beating frequency of all conditions.

Mehrabi. A *et al.* created electrospun Carbon nanofibers/Gel frameworks for cardiac tissue engineering and combined the biological compatibility of Gel with the conductive property of Carbon nanofibers, as it was hypothesised that Gel matrix with carbon nanofibers could enhance cardiomyocyte functions by

increasing gene expression in the absence of external electrical stimulation [46]. Gel matrix incorporated with carbon nanofibers enhanced the conductivity of the manufactured frameworks. The conductivity of plain gel increased when carbon nanofibers were added. The obtained values for the electroactive framework fell within the semiconductor range, which is enough for cardiac patch applications. The addition of carbon nanofibers to the gel boosted the gel nanofibers' tensile strength, and their elastic modulus was greater than that of pure gel matrices. Following cardiomyocyte culture, qRT-PCR was used to assess the influence of carbon nanofibers/gel on cardiac-specific gene expression. Comparison of gene expression in conductive frameworks with Gel (non-conductive framework) revealed a threefold and fourfold increase in Conx43 and Actn4, respectively. However, the TrpT-2 alterations were found to be statistically insignificant. Gel/carbon nanofibers' cardiac gene expression was compared to that of the control, TCP. According to the data, the expression of all genes rose. Only the variations in Actn4 were statistically significant. The angiogenesis of the samples was evaluated by removing the subcutaneously implanted frameworks. After implantation, the number of vasculatures that grew on both frameworks increased while tissue integrity was maintained. Both frameworks exhibited clear cellularization in HandE pictures. However, cell migration was substantially stronger on Gel/Carbon nanofibers frameworks than on Gel frameworks. These findings reveal that Gel/Carbon nanofiber frameworks maintain a cell-infiltrating and migration-friendly condition. Vascular endothelial growth factor immunofluorescence labeling of tissue slices was also utilized to examine newly created blood vessels. More capillaries were discovered in the frameworks containing carbon nanofibers, correlating with the results reported for cell movement inside these frameworks. The quantitative measurements also demonstrated that the vessel density in the carbon nanofibers-containing framework was much higher than in the Gel framework, which is advantageous for tissue engineering.

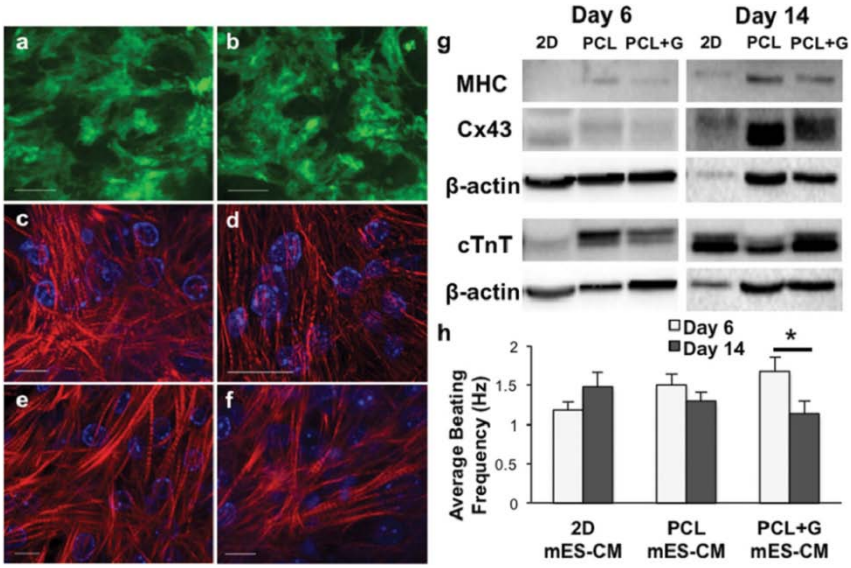

Fig. 3: MES-CM on random PCL+G scaffolds: High expression of cTnT-eGFP by mES-CM cultured on (a) PCL or (b) PCL+G was seen on day 6 of culture (green, scale bars = 100 µm). F-actin staining with rhodamin-conjugated phalloidin (red) demonstrated well-registered sarcomeres by mES-CM cultured on (c, e) PCL and (d, f) PCL+G scaffolds, respectively, on day 6 and day 14. Scale bars = 10 µm. (g) Western analysis revealed similar expression of MHC, cTnT, and Cx43 among all the groups on day 6 of culture. However, upregulation of cTnT expression in mES-CM on PCL+G scaffolds on day 14 was demonstrated. (h) Average beating frequency for mES-CM cultured on PCL+G scaffolds trended higher than those in 2D and on PCL scaffolds on day 6. However, the average beating frequency was significantly lower on day 14 in mES-CM cultured on PCL+G scaffolds when compared to day 6 [45]

Using the mixing procedure to encapsulate polypyrrole in silk fibroin electrospun fibers was anticipated to boost the electrical conductivity of the silk fibroin electrospun fibers. Additionally, the biologically functional growth of cardiomyocytes present in electrospun Polypyrrole-Silk fibroin mat was investigated *in vitro* to establish its Cardiac Tissue engineering potential [47]. Polypyrrole was successfully encased in Silk fibroin electrospun fibers with a negatively charged N-terminus and hydrophilic negatively charged amorphous regions in the heavy chain [48], allowing for electrostatic bonding with positively charged modified Polypyrrole. The hybrid conductive Polypyrrole-Silk fibroin fibers enhance cardiomyocyte alignment, similar to that of natural myocardium, and contributed to the development of achieving strong contraction. The incorporation of polypyrrole decreased the diameter of the fiber relative to the pure silk fibroin fiber, which more closely mimics the fibrils of native myocardium. It was hypothesized that the provided negative voltage would cause polypyrrole to migrate to the spinneret wall, resulting in more severe fiber stretching and a coarser fiber surface for cell attachment. The findings of the research indicated that rough polypyrrole-silk fibroin fibers increased cell attachment and that different cardiomyocytes engaged with the fibers. Based on the concentrations of polypyrrole and silk fibroin, the conductance of polypyrrole-silk fibroin fibers was equivalent to that of native myocardium in this study. The electrical conductivity of the fibre did not display a positive correlation with Polypyrrole concentration, as the 30:70 Polypyrrole-to-Silk fibroin ratio led to superior electrical conductivity than the 40:60 ratio. The optimal framework should possess mechanical properties that are suitable for cardiomyocytes. However, conductive materials are often fragile/hard, inhibiting the capacity of the cardiomyocyte to compress. Therefore, the physical qualities of composites can be enhanced by using soft natured bio-materials such as gelatin. It has been discovered that conductive electrospun mats containing decreased graphene oxide and carbon nanotubes stimulate cardiomyocytes to produce a larger cell aspect ratio, a longer sarcomere, and a larger cTni expression area [49-51]. The research showed similar results. However, we also observed that a low amount of polypyrrole promoted the functional development of cardiomyocytes more efficiently, but a higher level of polypyrrole increased conductivity.

7 percent Polypyrrole15 had the greatest impacts on cardiomyocyte adherence, morphology, alignment, cardiac-specific protein expression, and contraction compared to mats with greater Polypyrrole concentrations and mats without Polypyrrole. Highly ordered cardiomyocytes in the native heart result in synchronized action potential propagation and contraction. In terms of functional development, the NR cardiomyocyte-seeded 7 percent Polypyrrole-15 mat and the hiPSC cardiomyocyte-seeded 7 percent Polypyrrole-

30 mat exhibited the most forceful contractions and synchronous electrophysiological responses. In contrast, the cardiomyocyte-seeded pure Silk fibroin mat exhibited no contraction, which may be attributed to the variations in the framework's conductivity and mechanical characteristics. External electrical stimulation and the nanofibrous framework promoted contraction synergistically, and electrical stimulation increased CX43 expression. In addition to its synergistic benefits on contraction enhancement, the conductive ES (Polypyrrole, Silk fibroin) mat was able to protect cells from possible harm caused by external electrical impulses. Also, the majority of polypyrrole-containing composites implanted *in vivo* exhibited no significant inflammatory reactions.

Dan Kai *et al.* showed that polycaprolactone–gelatin nanofibers supported the cardiomyogenic specialization of human mesenchymal stem cells (hMSC) [52]. Nanofibrous frameworks were infused with vascular endothelial growth factor to stimulate the cardiac development of MSC implanted on the frameworks. Due to its short half-life, vascular endothelial growth factor cannot meet the long-term demands of tissue engineering applications. It was hypothesized that the synergistic effect of the topography signals of nanofibers and the biological signals of vascular endothelial growth factor could stimulate the cardiomyogenic differentiation of human mesenchymal stem cells (hMSC) for the use of polycaprolactone-gelatin–vascular endothelial growth factor as a substrate for myocardial restoration.-ACT is an essential component of the contractile apparatus in native cardiomyocytes, whereas TP-T governs the strength and speed of cardiac contraction [53]. Immunostaining results reveal that MSC on PCL-gelatin nanofibers expressed much more cardiac-specific proteins than MSC on TCP. The quantification results demonstrated that the expression of-ACT by MSC-differentiated cardiomyocytes on Polycaprolactone-gelatin was higher than its expression on TCP, whereas TP-T was expressed three times larger than on TCP, suggesting that the cardiac substrate with nanofibrous topography could provide additional chemical and mechanical cues to enhance the cardiac differentiation of MSC. As an important angiogenic growth factor, vascular endothelial growth factor was included into the nanofiber to promote cardiac differentiation, as it has been used to enhance heart function after MI in animal models and to stimulate angiogenesis with the formation of vascular structures in the infarcted area [54, 55]. The incorporation of vascular endothelial growth factor into the core of Polycaprolactone-gelatin nanofibers had no discernible effect on the nanofibers' mechanical properties. vascular endothelial growth factor-containing nanofibers boosted human mesenchymal stem cell proliferation much more than Polycaprolactone-gelatin nanofibers alone. The rate of cell growth is crucial in tissue engineering since it is connected with the rate of regeneration of new tissue, and a high proliferation rate would naturally result in greater tissue development. vascular endothelial growth factor might affect cellular differentiation in addition to promoting cell proliferation. Results suggest that cells on Polycaprolactone-gelatinV/B and Polycaprolactone-gelatinV/CS nanofibers exhibited greater amounts of-ACT than those on TCP and Polycaprolactone-gelatin nanofibers, and the enhanced protein expression of cells on vascular endothelial growth factor-containing nanofibers revealed that vascular endothelial growth factor plays a crucial role in the embryonic phases of cardiomyogenesis. Experiments performed on Polycaprolactone-gelatin nanofibers containing vascular endothelial growth factor demonstrated the beneficial effect of vascular endothelial growth factor on the cardiomyogenic development of human mesenchymal stem cells (hMSC).

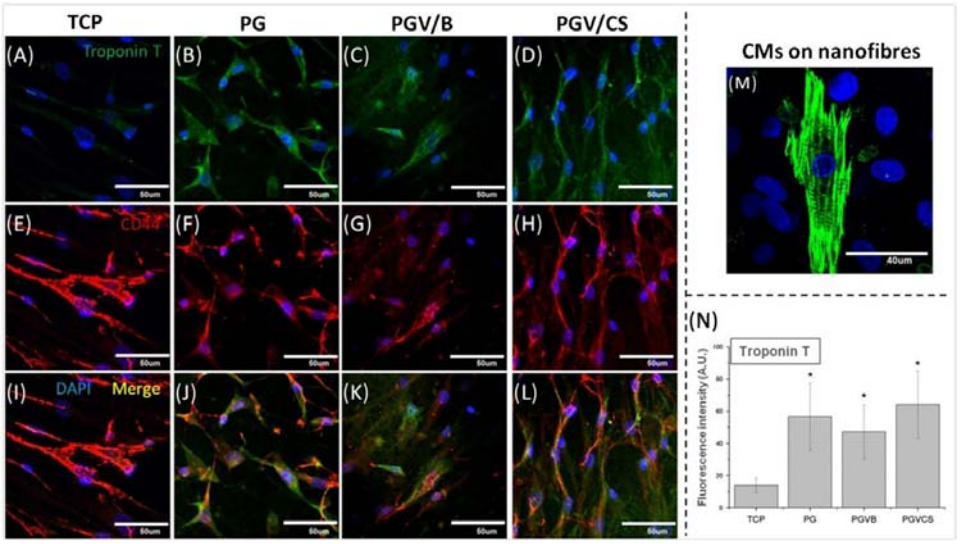

Fig. 4: Dual immunocytochemical analysis for the expression of (A–D) TP-T and (E–H) CD44. (I–L) Merged images showing the dual expression on (A, E, I) TCP, (B, F, J) PG, (C, G, K) PGV/B, and (D, H, L) PGV/CS nanofibers; scale bar = 50 µm. (M) Expression of TP-T in CMs on nanofibers as a positive control; scale bar = 40 µm. (N) Signal intensity of TP-T expressed in differentiated MSCs on TCP and PG, PGV/B, and PGV/CS nanofibers [56]

Ling Wang *et al.* performed a study in which a number of nanofibrous frameworks were fabricated that are electroactive in order to generate heart tissue [57]. Due to their biocompatibility, biodegradability, and conductivity, respectively, poly(L-lactic acid) and polyaniline (PANi) were chosen as raw materials. Using electrospinning, PANi/poly(L-lactic acid) nanofiber mats with variable polyaniline concentrations were produced.

Using cyclic voltammetry, the electrochemical characteristics of poly(L-lactic acid)/PANi samples were examined (CV). The conductance of these nanofiber mats improved as the polyaniline concentration grew, as the higher polyaniline concentration aided in the creation of a conducting network inside the blending system.

H9c2 cell lines were cultured upon poly(L-lactic acid)/PANi nanofiber mats to see whether they might be used for cardiac tissue regeneration. H9c2 cells were chosen because they have been used extensively in several studies investigating the effect of biomaterials on cardiac cell growth.

The majority of cells on poly(L-lactic acid)/PANi 1.5 and poly(L-lactic acid)/PANi 3 nanofiber mats displayed green fluorescence, suggesting that the proliferation of live H9c2 cells was explored in this work. At each time point, the rate of cell development did not vary significantly across nanofibers. Similar to poly(L-lactic acid) nanofiber mats, PANi/poly(L-lactic acid) nanofiber mats exhibited high cell survival and proliferation, according to this research.

Similar to poly(L-lactic acid) nanofiber mats, PANi/poly(L-lactic acid) nanofiber mats exhibited high cell survival as well as proliferation, according to this research.

On these nanofiber mats, the myogenic differentiation of H9c2 cells was examined further. After induction, multinucleated merging myotubes with such a finely organized structure developed on PANi/poly(L-lactic acid) 1.5 and PANi/poly(L-lactic acid) 3 nanofiber mats, which were detected by immunofluorescent labeling with MYH2.

PANi/poly(L-lactic acid) 1.5 and PANi/poly(L-lactic acid) 3 myotube lengths were significantly longer than PANi/poly(L-lactic acid). H9c2 cells grew more myotubes on PANi/poly(L-lactic acid) 1.5 and PANi/poly(L-lactic acid)3 nanofiber mats than on poly(L-lactic acid) nanofiber mats. The maturity index was used as an additional distinguishing criterion to evaluate the growth of myotubes. According to the results, PANi/poly(L-lactic acid) 1.5 and PANi/poly(L-lactic acid)3 created more mature myotubes. All of these results suggest that these conductive PANi/poly(L-lactic acid) nanofiber mats have a positive effect on the differentiation of H9c2 cells in terms of myotube quantity, length, fusion and maturation index, indicating their enormous potential for use in cardiac tissue engineering.

The cell behavior of primary cardiac myocytes (cardiomyocytes) on these nanofiber mats was explored since cardiomyocytes are the predominant cardiac muscle cells and have been extensively studied for cardiac regeneration [58–61]. According to the findings of live/dead staining, the majority of cardiomyocytes on PANi/poly(L-lactic acid) nanofiber mats were lively and vivid. Comparing the cell viability of cardiomyocytes on PANi/poly(L-lactic acid)1.5 and PANi/poly(L-lactic acid)3 nanofiber mats to that on poly(L-lactic acid) nanofiber mats, the quantitative study demonstrated that cell survivability of cardiomyocytes PANi/poly(L-lactic acid)1.5 and PANi/poly(L-lactic acid)3 nanofiber mats is greater than that on poly(L-lactic acid) nanofiber mats.

To analyse the effect of PANi/poly(L-lactic acid) nanofiber mats on cell architecture, confocal fluorescence images of F-actin and DAPI staining were taken to assess the cytoskeletal structure of cultured cardiomyocytes. On poly(L-lactic acid) nanofiber mats, cardiomyocytes displayed restricted F-actin fibre expression and a rectangular shape. In contrast, cardiomyocytes on PANi/poly(L-lactic acid)1.5 and PANi/poly(L-lactic acid)3 nanofiber mats had greater F-actin fibers in all aspects. Furthermore, on PANi/poly(L-lactic acid)1.5 and PANi/poly(L-lactic acid)3 nanofiber mats, elongated cardiomyocytes with well-defined stress fibers were identified, but they were illusive on poly(L-lactic acid) nanofiber mats. In addition, rapid Fourier transform (FFT) analysis of F-actin fibre demonstrated that cardiomyocytes on PANi/poly(L-lactic acid) 3 were clearly connected and spatially oriented, suggesting enhanced cell-cell interactions. Upon PANi/poly(L-lactic acid) nanofiber mats, immunofluorescence labelling for cardiac-specific proteins sarcomeric-actinin and connexin 43 revealed the formation of cardiomyocytes (CX43). CX43 is a necessary gap junction protein for cell-cell communication and synchronized heartbeat rhythm in cardiomyocytes. On PANi/poly(L-lactic acid)1.5 and PANi/poly(L-lactic acid) 3 nanofiber mats, cardiomyocytes exhibited widespread connected sarcomeric configurations with insufficient uniaxial alignment. The sarcomeric structure of cells on poly(L-lactic acid) nanofiber mats was, however, scattered and poorly organized. This indicated that cardiomyocytes on PANi/poly(L-lactic acid) nanofiber mats expressed sarcomeric-actinin with a more homogeneous structure than cardiomyocytes on poly(L-lactic acid) nanofiber mats. The quantitative analysis demonstrated that the area coverage of sarcomeric-actinin on PANi/poly(L-lactic acid) 1.5 and PANi/poly(L-lactic acid) 3 nanofiber mats was much larger than on poly(L-lactic acid) nanofiber mats.

CX43 was likewise substantially expressed and demonstrated a considerably more uniform distribution on PANi/poly(L-lactic acid)1.5 and PANi/poly(L-lactic acid)3 nanofiber mats. On PANi/poly(L-lactic acid)1.5 and PANi/poly(L-lactic acid)3, CX43 was largely diffused between neighbouring cardiomyocytes, as seen in high-magnification photographs. In contrast, the distribution of CX43 in cardiomyocytes on poly(L-lactic acid) nanofiber mats was shown to be sparse. The area coverage of CX43 was larger on PANi/poly(L-lactic acid)1.5 and PANi/poly(L-lactic acid)3 nanofiber mats than on poly(L-lactic acid) nanofiber mats. Increased expression of CX43 in PANi/poly(L-lactic acid) nanofiber mats improved cell-cell contact. Immunofluorescence labelling revealed well-developed sarcomeres and gap junction networks, and also the ability to induce synchronised beating of cardiomyocytes on PANi/poly(L-lactic acid) nanofiber mats, which provide good conductance and an extracellular matrix-like nanostructure.

To analyse the biological activity of cardiomyocytes on PANi/poly(L-lactic acid) nanofiber mats, further research was conducted on the spontaneous beating behaviour of cardiomyocytes. cardiomyocytes produced on PANi/poly(L-lactic acid) 1.5 and PANi/poly(L-lactic acid) 3 nanofiber mats beat more synchronously and at a substantially greater rate than those grown on poly(L-lactic acid) nanofibrous. This was mostly due to the excellent cell-cell interaction.

In the study performed by Jiangwei Chen *et al.* [62], using the layer-by-layer approach, silk fibroin and chitosan were combined to electrospun cellulose nanofibers to create a new cardiac patch in the study performed by Jiangwei Chen *et al.* [62]. It was shown that the patch might be an excellent carrier for the preservation and survivability of mesenchymal stem cells produced from adipose tissue, as well as providing structural support to prevent unfavorable remodeling of the left ventricle. Nanofibers produced by electrospinning retain a 3-dimensional structure that is porous with nanometers to micrometers in diameter, and this porosity renders them suited for tissue engineering by offering stem cells a 3-dimensional growth area [63]. Nonetheless, the vast majority of natural biopolymers cannot be formed by electrospinning to generate nanofibers, while natural polysaccharides with a high electrospinning capacity, like cellulose, are incompatible with living organisms. As a result, coating methods use many layers. Silk fibroin and chitosan greatly increased biocompatibility by assembly upon the cellulose nanofibers using the layer-by-layer approach. Due to its excellent flexibility, biodegradability, and continuous release [64], silk fibroin has been extensively researched in tissue engineering. Silk fibroin is perfectly suited for tissue engineering of the functioning heart due to its unique qualities. Chitosan, the second-most-common natural biopolymer beside cellulose, has distinctive physio-chemical and biological properties, such as an excellent hydrophilic nature, increased cohesive strength, extraordinarily little toxicity, and innate antibacterial power [65, 66]. It was proven that 10.5 layers of Chitosan/Silk fibroin nanofiber mats had the highest biocompatibility; hence, 10.5-layer Chitosan/Silk fibroin nanofiber mats were selected for cardiac patches. No substantial evidence of *in vivo* nanofiber deterioration was discovered during a study of degradation. Nanofibrous cellulose was tolerated well by the host animals due to its biocompatibility, with limited immunological responses to the implants. It is simple to extract adipose tissue-derived mesenchymal stem cells [67] from adipose tissue. Adipose tissue-derived mesenchymal stem cells are paracrine, multipotent, and immunosuppressive [68]. The findings shows that a desirable extracellular matrix and biomimetic extracellular matrix enhance the retention and survivability of the engrafted adipose tissue-derived mesenchymal stem cells, and that the mechanical action of the cell nano-patches for the expanding ventricular post-MI prevents HF progression by inhibiting ventricular remodelling. Staining with Masson's trichrome and studies of morphological and physiological properties indicate that the cell nano-patch may be able to reverse infarcted myocardium remodelling. In support of this discovery, analysis of Western blot demonstrated that the cell nanopatch decreased the synthesis of b-MHC and BNP while increasing the expression of a-MHC [69, 70]. Using an acellular framework to inhibit post-infarction ventricular remodelling was supported by these findings, which were consistent with earlier research.

Shokraei *et al.* demonstrated that carbon nanotubes/polyurethane nanofibrous frameworks were fabricated using a simultaneous electrospray/spinning method with improved mechanical and electrical properties with possible applications in cardiac tissue engineering, where a composite membrane was fabricated using

electrospinning of polyurethane and electrospray of MW carbon nanotubes at various carbon nanotubes/polyurethane weight ratios [71]. Carbon nanotubes are allotropes of carbon with a tube-shaped structure. The diameter of the tubes are in the nanometer scale (can be as thin as a few nm yet having a length up to 100 microns). They have thermal conductivity, mechanical and electrical properties. Nowadays, CNTs have found use in the medical field as they can be used for gene delivery to cells and organs as well as tissue regeneration [72]. CNTs can be classified as single-walled nanotubes and multiwalled nanotubes. As the name suggests, single-walled CNTs consist of one layer forming a cylinder while the multi-walled nanotubes comprise several cylindrical layers each having a diameter greater than the other. The chemical bonding in the tubes is best described by orbital hybridization (sp2-hybrid carbon atoms) which accounts for the unique strength of CNTs. CNTS are widely researched in the field of drug delivery and biosensing methods for disease treatment and health monitoring. The average pore size and diameter of nanofibers were measured using SEM images, which demonstrated that the addition of carbon nanotubes to chitosan nanofibers decreased the nanofibers' average diameter and pore size. This is because the conductivity of carbon nanotubes might impede the electrospinning process. Using Field Emission-Scanning electron microscopy, the existence and distribution of carbon nanotubes inside polyurethane nanofibers were examined. Carbon nanotubes were uniformly coated on the surface of polyurethane nanofibers, according to the results. Field emission-scanning electron microscopy images revealed the presence of carbon nanotubes enhanced the formation of web-like structures. Conductivity characteristics of nanocomposites were tested using a four-point probe approach. Pure polyurethane nanofibers exhibited no discernible conductivity. In contrast to polyurethane nanofibers, carbon nanotubes/polyurethane nanofibers exhibited a significant degree of electrical conductivity. The electrical conductivity of carbon nanotubes/polyurethane frameworks was quite similar to that of native myocardium. According to the results, higher concentration of carbon nanotubes inside the structure of nanocomposites strengthened the resultant nanofibers and reduced the elastic nature of the nanofibrous frameworks. This is a result of the interactions between polymer chains and carbon nanotubes in nanofibers. The MTT test was utilized to quantify the cell viability upon attachment and growth on nanocomposite mats. Carbon nano tubes/polyurethane nanofibers were more cytocompatible than polyurethane nanofibers at all examined time periods. During the initial days of cell culture, it is obvious that the presence of carbon nanotubes boosted cell proliferation. Carbon nanotubes have been demonstrated to boost the survival and cardiomyocyte growth, as well as their electrophysiological abilities [73], while also dramatically enhancing the cell response and encouraging cell proliferation. Fluorescent pictures of PI-labelled cells further validated the MTT experiment results for polyurethane and carbon nanotubes/polyurethane composites. Using SEM, the morphology and adhesion of H9c2 to nanofibers were determined. The results revealed that following cell seeding, appropriate interactions between H9c2 cells and frameworks emerged. On pure polyurethane, there were fewer attachments to cells than on carbon nanotubes/polyurethane 2:10 frameworks, which was precise with the MTT assay results, which indicated that the electroconductive properties of carbon nanotubes/polyurethane enhanced cell response, along with cell development, proliferation, and interaction between cells. The MTT test was used for qualitative evaluation of cell viability of the nanofibers after they were seeded with HUVEC. The results proved that the vitality of cells on carbon nanotubes/polyurethane composites was greater than that of polyurethane and that HUVEC cultivated on carbon nanotubes/polyurethane 2:10 and 3:10 were able to multiply significantly than other groups, demonstrating the harmless nature of the materials utilised. It was also observed that electrospun nanofibrous composites of carbon nanotubes/polyurethane can imitate the morphology and function of extracellular by encouraging cell proliferation, and extracellular collagen secretion, thereby mimicking the structure and function of extracellular [74]. Using Scanning electron microscopy, the cell structure, and interactions of HUVEC on frameworks were investigated. On the polyurethane frameworks, the cells grew in the form of spindles, but on the carbon nanotubes/polyurethane frameworks, a continuous cell sheet was seen. This suggests that the presence of carbon nanotubes may significantly enhance cellular responsiveness and boost cell development. For polyurethane and carbon nanotubes/polyurethane, fluorescent pictures of cells stained with PI were produced. Compared to polyurethane samples, a greater number of cells adhered to carbon nanotubes/polyurethane samples after cell seeding.

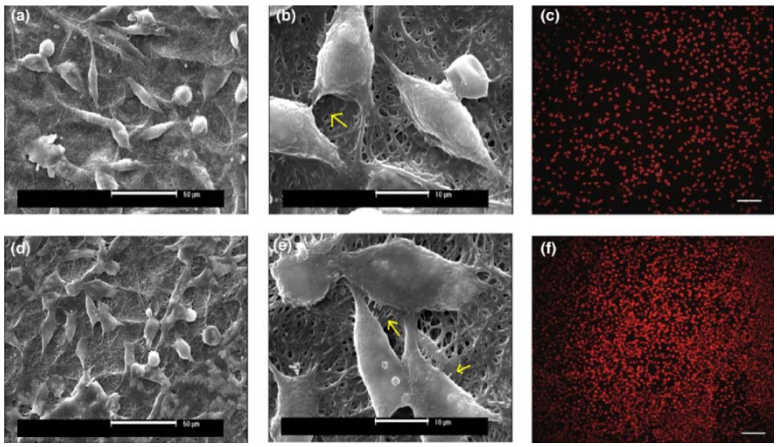

Fig. 5: SEM images of cultured HUVECs on PU nanofibers (a, b) and CNT/PU 2:10 wt% (d, e) after 2 d (scale bar: 50 µmin a and d and 10 µm for b and e). PI fluorescent images of the cells cultured on PU (c) and CNT/PU 2:10 nanocomposite (f) after 2 d. Filopodias of the cells were highlighted [75]

Aditi Jain *et al.* showed that electrospun polycaprolactone and polycaprolactone mixed with gelatin (PCLG) nanofibers were coated with cerium oxide nanoparticles [76]. Utilizing newborn primary cardiomyocytes, it is shown that cerium oxide nanoparticles/PCLG nanofibers may reduce reactive oxygen species production along with agonist-induced cardiac hypertrophy. Using a mixture of PCL and gelatin to create nanofibers ensures that the fiber mat is non-cardiotoxic. The cerium-decorated nanofiber mat created has significant promise as a cardiac patch, including for cell transport. Moreover, the ceria coating's enhanced reactive oxygen species scavenging characteristics are supported by its Ce4+-dominated oxidation state [77]. On nanofibers with nanoscale roughness that is conducive to cellular attachment, a simple cerium coating has been produced. Studies show roughness at the nanoscale significantly enhances cellular adhesion [78]. After coating PCLG nanofibers with cerium oxide nanoparticles, it was revealed that coating time and concentration of cerium affected cell adherence and vitality. Even at lower concentrations, longer incubation produced aggregates of

ceria deposits, inhibiting cell attachment. Lower cell attachment of primary cardiomyocytes or H9c2 on polycaprolactone mixed with gelatin-1200, polycaprolactone mixed with gelatin-0.1Ce1200, and polycaprolactone mixed with gelatin-0.5Ce1200 was attributed to cerium oxide-induced surface roughness and improved framework hydrophobicity. No detectable cytotoxicity was seen in primary fibroblasts and myotubes, or H9c2 rat cardiomyoblasts cultured on TCPS, polycaprolactone mixed with gelatin, polycaprolactone mixed with gelatin-Ce10, or polycaprolactone mixed with gelatin-Ce60, demonstrating the viability of the cardiac patch. In deteriorating hearts, elevated amounts of reactive oxygen species are known to trigger hypertrophic signalling kinases, promote fibroblast proliferation, and lead to extracellular remodelling of the heart. Moreover, after reperfusion, ROS levels rise in ischemic heart tissue [79, 80]. Using hydrogen peroxide, the circumstances of exposing cardiomyocytes to extrinsic ROS was reproduced. gPCLG-Ce60 protected against stress due to oxidation better compared to gPCLG-Ce10. It became probable that gPCLG-Ce60 supplied more cerium ions for ROS elimination. Therefore, gPCLGCe60 was used in the following testing. Sundaresan *et al.* demonstrated that PE induced an instantaneous rise in the levels of reactive oxygen species (ROS) in cardiomyocytes. PE is an adrenergic receptor agonist reported to induce *in vitro* myocardial hypertrophy. Hypertrophy is associated with the activation of the foetal gene program, including the rise of atrial natriuretic peptide [81]. Atrial natriuretic peptide is a quantitative indicator of the quantity of hypertrophic cardiomyocytes [82-84]. Due to the longer scavenging of intrinsic reactive oxygen species, the quantity of PE-induced hypertrophy in heart cells cultured on gPCLG-Ce60-A was much less than that on gTCPS, confirming the anti-hypertrophy efficacy of gPCLG-Ce60-A nanofibers. Therefore, gPCLG-Ce60-A nanofibers can be used as a patch to minimize cardiac hypertrophy after MI, ischemia, and other cardiac diseases. Coating cerium oxide nanoparticles on polycaprolactone mixed with gelatin nanofibers enables the targeted and localized delivery of Ce ions to the heart at a lower nanoparticle concentration. Additionally, gPCLG-Ce60-A may be used as a cardiac patch in combination with other drugs or factors targeting pathways like Wnt and TGF-, which promote heart repair and govern the transition of fibroblasts to myofibroblasts.

Nazari H *et al.* demonstrated that the silk fibroin framework was electrospun with and without SPION casein and employed for cardiac ECC differentiation [85]. Scanning electron microscopy scans revealed the effective production of monodispersed spherical SPION casein NPs. The tissue engineering micrographs validate the integration of SPIONs into the silk fibroin nanofibers during the electrospinning procedure. The XRD findings confirm the coating of SPIONs with casein protein in the core shells. SPIONs whose surfaces were modified with casein exhibited increased hydrophobicity, suggesting stronger framework interaction. The nanofibrous shape of frameworks indicated that ECC on silk fibroin/SPION casein elongates well, forms colonies, and connects to nanofibers and neighboring cells through filopodia development. Following the integration of SPIONs, the tensile strength and strain of the silk fibroin rose, but the young's modulus decreased, as determined by mechanical property testing. So, although the brittleness resistance of silk fibroin/SPIONs composites increased, their elasticity decreased marginally. Biological evaluations of the ECC planted in the framework demonstrate that inclusion of SPION casein into silk fibroin enhances the ECC's biological activities. In the presence of SPION's in the framework, the ECC has a more elongated shape, as seen by Scanning electron microscopy micrographs.

The MTT assay indicated that silk fibroin and silk fibroin/SPION casein frameworks had no detrimental effect on the viability and proliferation of ECC but modestly improved ECC proliferation. In the presence of silk fibroin/SPION casein frameworks, the expression of mature cardiac gene markers, including cTnT and MHC, was enhanced. Thus, the SP/SPION-casein framework ensured differentiation of ECC into adult cardiac muscle and maintained the proliferative and stemness characteristics of ECC. Using immunofluorescent labeling, ECC's protein expression was observed. SPION casein modified frameworks greatly increased the expression of cardiac maturity markers-MHC and c-TnT. In addition, the quantified ICC outcomes verified the increase of-MHC and c-TnT. The expression of functional genes, including cTnT and MHC, was greater in silk fibroin/SPION casein treated cells than in silk fibroin cells, as determined by real time PCR. Consequently, the increased production of these functional proteins in silk fibroin/SPION casein nanofibers validated the enhanced cardiogenic differentiation of ECC on magnetic NPs-loaded frameworks. In conclusion, the inclusion of coreshells into silk fibroin promoted cardiac differentiation without impairing the proliferation and self-renewal potential of ECC. The composite frameworks with a SPION casein core shell are thus appropriate for future *in vivo* research.

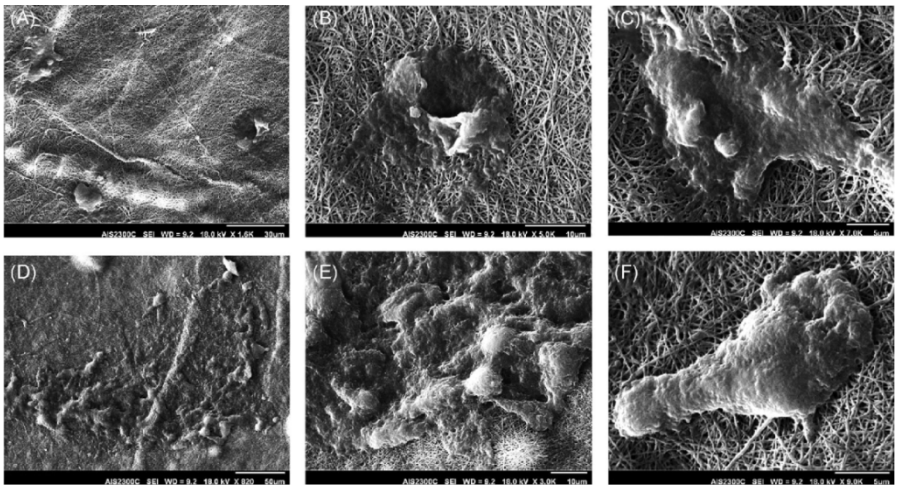

Fig. 6: The morphology of ECCs after 7 d of culture on SF (A-C) and SF/SPION-casein (D-F) scaffolds. Scale bars: a = 30 µm; b = 10 µm; c = 5 µm; d = 50 µm; e = 10 µm; and f = 5 µm [86]

Divya Sridharan *et al.* [87] showcased that a coaxial polycaprolactone gelatin nanofiber framework with a gelatin shell and a polycaprolactone core was constructed and reported [88, 89], and a method for growing and transforming hiPSC into viable cardiomyocytes in a 3-dimensional environment using the scaffold was created. In addition, the differentiation and maturation efficiency of hiPSC-cardiomyocytes *in vitro* were compared between 3D and 2D cultures. 3D cultures of undifferentiated hiPSC showed higher production of cardiac progenitor-associated genes, whereas two dimensional cultures demonstrated elevated production of cardiomyocyte-associated genes. A gradual movement into the framework and a uniform dispersion of developed hiPSC

cardiomyocytes were observed in 3D cultures. Even though 2-dimensional growth of human induced pluripotent stem cells is successful, several studies [90-92] demonstrate that current culture approaches do not mimic *in vivo* differentiation. Recent studies comparing the differentiation of stem cells into cardiomyocytes [93, 94] and other cell types [90, 95] in 2D and 3D cultures revealed that 3D culture methods mimicked the *in vivo* process more closely. In addition, growing cardiomyocytes in a 3D culture boosted their contractile activity, increased the number of mitochondria, and enhanced their maturation and functioning. Polycaprolactone was blended with natural polymers such as gelatin/collagen, and the resulting structures were found to possess enhanced biocompatibility because PCL fibers have a detrimental effect on cell survivability and function owing to their hydrophobic nature and gradual degradation, which produces by-products like caproic acid, which are toxic.

Following the maturation of hiPSC grown on 3-dimensional frameworks, spontaneous contraction of heart patches in culture has been described. When iPSC matured into functional heart cells in 2-dimensional cultures as compared to 3-dimensional cultures, contractions occurred much faster. Four weeks after differentiation induction, there were no significant differences between the two culture techniques in the heartbeat frequency of cardiomyocytes. The decrease in cardiomyocyte-associated gene production in subsequent days of this research was attributed to the differentiation of hiPSC into cardiac cell types other than cardiomyocytes, such as endothelial cells. Because of in situ differentiation, the cells were able to migrate inside the scaffold throughout the EMT process, which occurs during the maturation of hiPSC into viable cardiomyocytes. The study indicated that coaxial polycaprolactone-gelatin nanofiber frameworks can serve as a 3-dimensional platform for the culturing and maturation of human induced pluripotent stem cells into functional cardiomyocytes. Also found on 3-dimensional scaffolds were the efficient movement and uniform dispersion of matured cells.

Carbon nanotubes (bl and sp). Raman spectra of polyurethane/Chitosan and polyurethane/Chitosan/Carbon nanotubes were obtained, revealing the presence of Carbon nanotubes in Polyurethane/Chitosan/Carbon nanotubes frameworks. The presence of Carbon nanotubes in the polyurethane/Chitosan/Carbon nanotubes (sp) framework was confirmed by XRD patterns obtained on Carbon nanotubes powder, polyurethane/Chitosan, and polyurethane/Chitosan/Carbon nanotubes (sp) frameworks.

Several frameworks were subjected to conductivity testing, and the results revealed that the resistivity of polyurethane/chitosan frameworks fell considerably when the nanotubes were electrosprayed onto nanofibers. The construction of a percolation network by inserting carbon nanotubes inside and on the surface of nanofibers [97] may have contributed to the enhancement of the framework's conductivity. Measuring the resistivity of polyurethane, polyurethane/Chitosan, polyurethane/Chitosan/Carbon nanotubes (bl), and polyurethane/Chitosan/Carbon nanotubes (sp) indicated that by electrospraying Carbon nanotubes onto the structures, the electrical conductivity was enhanced. Moreover, the fabrication of high-density ultrafine nanofibers in composite frameworks increased carbon nanotube-mediated conduction routes and electrical characteristics [98]. Consequently, the increase in electrical conductivity validated the uniform distribution of carbon nanotubes in polyurethane/Chitosan/Carbon nanotubes (sp) samples. As myocardial tissue is subjected to mechanical stress, the frameworks' mechanical characteristics must be able to tolerate the physiological circumstances present. The mechanical characteristics were greatly enhanced by the incorporation of Carbon nanotubes. The results indicated that the addition of Carbon nanotubes might enhance the mechanical strength of electrospun nanofibers.

For assessing the rate of degradation *in vitro*, frameworks were cultured in a PBS solution. After degradation, scanning electron microscopy images were exhibited, and the findings revealed that the generated nanofiber designs were able to successfully prevent the rapid release of the carbon nanotube content of the frameworks.

Cell adhesion to frameworks was investigated since frameworks should mirror the normal cardiac extracellular matrix (extracellular matrix). In this work, the adherence and cytotoxic nature of nanofibers were evaluated *in vitro* during three different periods using the Alamar blue test. The quantity of HUVEC cells on polyurethane/Chitosan/Carbon nanotubes was shown by the Alamar blue experiment. sp frameworks climbed much higher than polyurethane and control frameworks. Because polyurethane frameworks are hydrophobic and lack adequate cell contact sites on their surface, hydrophilic Chitosan and carboxylated Carbon nanotubes improve framework surface properties. The improved surface features of polyurethane/Chitosan/Carbon nanotube frameworks as compared to polyurethane frameworks increased HUVEC cell proliferation [99, 100]. Furthermore, Chitosan treatment may promote cell growth due to its high biological activity and hydrophilicity, which is consistent with previous research [99] indicating that a higher percentage of cells thrive on hydrophilic frameworks.

H9C2 cells grown on nanofibers validated the findings obtained with HUVEC cells. On frameworks containing electrosprayed carbon nanotubes, cell growth was much greater than on polyurethane frameworks and in the control group, which lacked frameworks. The results were consistent with findings from prior research [101] indicating integration of functionalized carbon nanotubes into nanofibrous frameworks might increase cell growth. Increased surface roughness resulting from the presence of carbon nanotubes on frameworks may boost cell attachment and growth [102-104]. Experiments using Alamar blue revealed that the population of H9C2 cells on frameworks made of polyurethane/Chitosan/Carbon nanotubes (sp) increased much more than on other frameworks and in the control group.

Future challenges and development

The fundamental goal of electrospinning technology in cardiovascular tissue repair is to develop biomimetic frameworks *in vitro* to repair myocardial or vascular tissue and reinstate their functionality, and then to investigate their biological compatibility and particular function *in vivo* [105, 106].

Many limitations must be overcome to produce highly functional and treatment-related engineered cardiac tissues, like limited porosity preventing deep seed cell invasion, Loss of synchronised contractions when cardiomyocytes are cultivated on a stiff substrate simulating post-infarct scar, Difficulties in expanding current technology.

Regarding *in vivo* applications, several obstacles must be studied. Individual physiological and physical variances need to be optimised for fabrication of nanofibers with tiny diameter, as well as considering the patient's susceptibility to these treatments for tailored diagnostics and therapies. For maximal efficacy, myocardial regeneration requires a mix of new and existing treatments to generate both endogenous and external effects.

It is possible to combine electrospinning with nanofiber hydrogels to enhance the physical properties. Additionally, new 3D-printed frameworks may be created as blueprints for cardiac cell invasion.

CONCLUSION

Tissue engineering could pave the path for the future of restorative biomedicine and encourage the invention of biological alternatives to restore damaged tissue/organ function. Electrospun nanofibers produced by electrospinning could offer the framework necessary for cardiovascular tissue engineering. Cardiac tissue can be replaced, or angiogenesis can be induced by imitating the 3-dimensional ECM structure using nanofiber production processes and technologies. Moreover, in tissue engineering, biodegradable polymeric fibre constructs, proteins, and stem cells enhance biocompatibility at the disease site. Future advancements will require the designing and manufacturing of biomaterials capable of maintaining local cardiac microenvironments to facilitate the acquisition of resident progenitor cells.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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