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Nanomaterials for Cardiac Tissue Engineering Application

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Abstract: In recent years, the emerging cardiac tissue engineering provides a new therapeutic method for heart diseases. And in the tissue engineering, the scaffold material which can mimic the structure of the extracellular matrix properly is a key factor. The rapid expansion of nano-scaffolds during the past ten years has led to new perspectives and advances in biomedical research as well as in clinical practice. Here we search articles published in recent years extensively on cardiac tissue engineering scaffold materials and nanotechnology. And we review the traditional scaffold materials and the advances of the nano-scaffolds in cardiac tissue engineering. A thorough understanding of the nano-scaffolds would enable us to better exploit technologies to research the ideal scaffold material, and promote the cardiac tissue engineering using in the clinical practice as soon as possible.
Keywords: Cardiac tissue engineering; Nano-scaffolds; Nanomaterials

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Introduction

As the world's first major chronic diseases，coronary heart disease causes huge costs per year. The cost of this disease in America was alarming more than $500 billion last year [1]. With the development of medical treatment, the demands of the patients who are suffering the serious diseases are not only sustaining life, but also improving the quality of life. The tissue-based and cell-based strategies have come to the forefront as viable alternatives for the treatment of heart disease. One of these novel approaches is cardiac tissue engineering. The aim of cardiac tissue engineering is to understand the relationships of myocardial structure and function, design and construct cardiac structure and function, cultivate the new myocardial cells to replace the lost/dysfunctional cells and recover the normal function of the heart.

The basic strategy of the tissue engineering is the construction of a biocompatible scaffold to replace, regenerates or repairs damaged cells or tissues [2]. In cardiac tissue engineering, the ideal scaffold should mimic the structure of the extracellular matrix (ECM), which is very important for the proliferation and differentiation of the seeding cells. So, to seek the bionic myocardial extracellular matrix material in the myocardial tissue engineering for the cultured myocardial cells is a key factor for the translation from the tissue engineering into clinical practice. Now the common used scaffold materials include traditional scaffold material, nanometer scaffold material, and composited scaffold material. The nano-scaffolds have many unique advantages in the fild of cardiac tissue engineering. In this paper the research advances of the scaffold materials used in cardiac tissue engineering will be repoted for its wide perspective in myocardial cell regeneration.

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Traditional scaffold materials

The scaffold materials which serve as temporary 3D substrates, provide a proper microenvironment for seeding cells, and they have been shown to actively regulate cellular responses including attachment, proliferation, differentiation and matrix deposition [3-5]. The ECM-mimicking microenvironment is the place of getting nutrition, waste excretion, gas exchange and metabolism for seeding cells. In a regeneration strategy, the scaffold materials should be biodegraded gradually in order to promote new tissue formation by providing new adequate space. Traditional scaffold materials include biological scaffold materials and synthetic scaffold materials.

Biological scaffold materials

Biological scaffold materials include fibrin, collagen, hyaluronic acid and sodium alginate and so on. These natural polymers retained the normal grid structure, and they have a good biocompatibility, and they are beneficial to cell adhesion, proliferation and differentiation. The biological material is widespread, and the price is relatively cheap. Base on the advantages of good biocompatibility and cheap price, the biological material became one of the earliest applications of the scaffold materials in cardiac tissue engineering. In the laboratory, with collagen as the basis, researchers have fabricated the three-dimensional myocardial model which contains a variety of extracellular matrix proteins and growth factors successfully, and by using of the model, they cultivate regeneration myocardial cells which show good differentiation like the normal myocardial cells in vivo environment [6-8]. In Figure 1 the high degree of integrity and stability of the bioartificial myocardial tissue patch and cells distribution was observed. As a natural extracellular component, fibrin has ability to conduct signal between myocardial cells. In addition, different concentrations of fibrin can influence tissue density and mechanical strength, and using this characteristic can make the implantation tissue to get a better spatial distribution [9,10]. Recently, the cardiac tissue engineering scaffold materials made of hyaluronic acid and alginate should also be a worthwhile research direction.Since hyaluronic acid and alginate are not widely used in cardiac tissue engineering by now, but the function of promoting proliferation has been proved in laboratory [11-13].

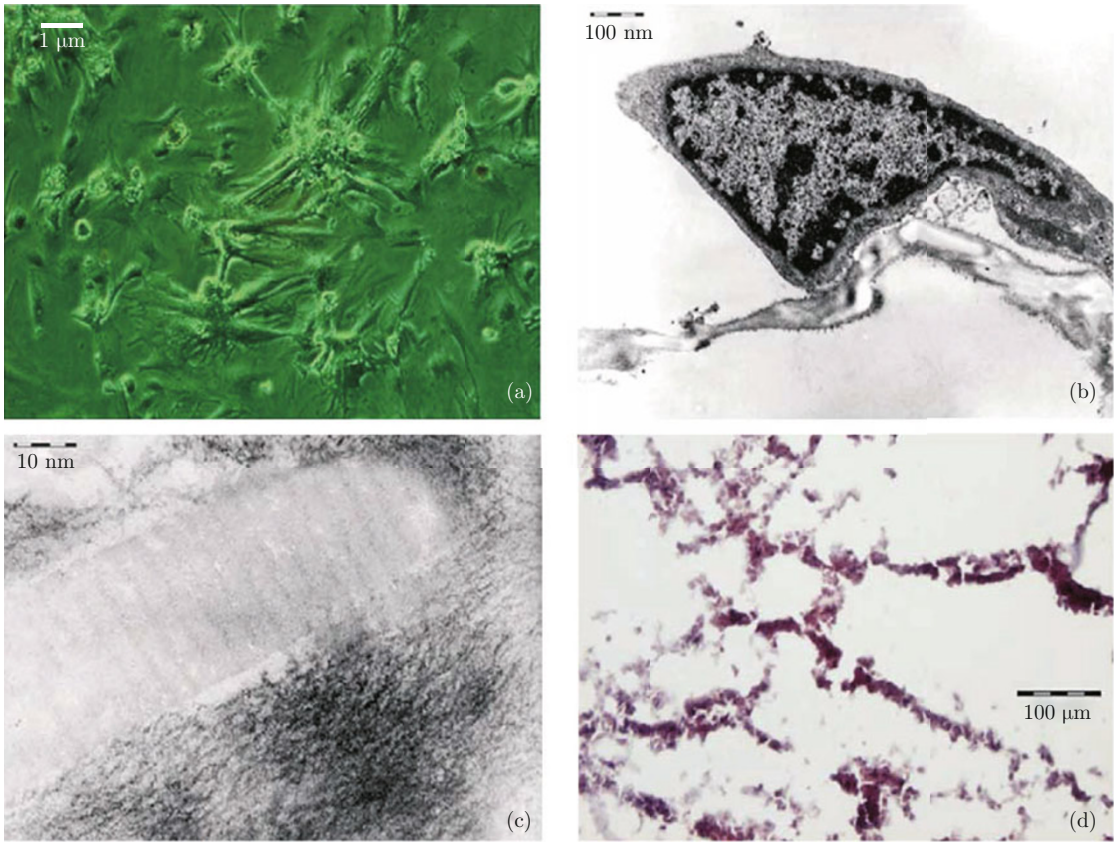

Fig. 1 Celluar seeding of the bioartificial myocardial tissue patch: (a) Arrangement of cells in 3D collagen structure; (b) Junction between cellular body and collagen fibril, demonstrated by electron microscopy (x8000); (c) Collagen fibrils are embedded in amorphous collagen matrix (x63000); (d) Cells attach along collagen fibrils and are distributed homogenously even in deeper layers of the bioartificial myocardial tissue patch (MF-20 stain) [4].

Natural tissue or cell derived ECM, is currently being tried for cardiac tissue engineering [14-16]. The origin of ECM needs to be considered, as different tissues exhibit different ECM compositions and ultrastructure, which may affect the formation of the desired tissue. So suitable ECM for cardiac regeneration may be the one that originates from heart tissue. It provides the appropriate cell guidance and differentiation signals, but this material is still far from mature [17].

Synthetic material

Synthetic materials include polyesters，elastomers and so on. The polyesters such as polylactic acid (PLA) and polyglycolic acid (PGA) are common used for myocardial tissue engineering. The reasons why they are popular are mostly depended on the good biocompatible of their degradation products, their mechanical properties and simple to manufacture. The pore size distribution of the polymer foam is analyzed with the public domain NIH Image program. Figure 2 is an example of this image analysis procedure, and it provide a better understanding of the meaning of pore size calculated by this method [18]. But at the same time, the degradation products may cause inflammatory response [19]. What's more, the bulk degradation kinetic of the polyesters may lead to a sudden loss of structural integrity, and this is bad for the target tissue. The elastomers are synthetic mimicals of natural rubber. A 3D cardiac construct made out of polyurethane shows good cell adhesion, and in-vitro or in-vivo evaluations on tissue inflammation [20-21]. But to our disappointed, the polyurethane release a toxic byproduct-diisocyanate [19], and this disadvantage limits its clinical use.

Compared with natural materials, synthetic material has its advantages and disadvantages [22]. Synthetic material have a wide range of properties that may be obtained and customized with respect to mechanics, chemistry, and degradation, but may be limited in functional cellular interactions and other biological characteristics. But the disadvantages of synthetic material can be improved with adhesion peptides or designed to release biological molecules [23,24].

Nano-scaffolds

Nanotechnology is defined by the size of a material (generally 1-100 nm) or manipulation on the molecular level, and the 3 D space of the nano-scaffold should be at least one dimensional in nanometer level. The electrospinning process produced polymer fibers in the nanometer range with an approximate diameter of 100 nm (Fig. 3). The rapid expansion of nano-scaffolds during the past ten years has led to new perspectives and advances in biomedical research as well as in clinical practice. Nano-scaled materials have been widely applied to the fields of regenerative medicine, including tissue engineering (TE), cell therapy, diagnosis and drug and gene delivery.

Nano-scaffold used in cardiac tissue engineering

The microscopic and submicroscopic structure of the scaffold surface has very important influence in adhesion and growth of the myocardial cells [25]. The nano-scaffold has a better specific effect of bulk and surface advantages, which is much superior than millimeter and micrometer scaffold materials. The collagen fibers with diameters in the nanometer and submicron range are the major component of the ECM, so fabricating the nanoscaled scaffold becomes the pursuits of the researchers. While some studies have found that the smallest fibers (near 100 nm) produced by electrospining are superior [26,27], others have concluded that slightly larger, submicron fibers (near 400 nm) offer the best performance [28]. Although there is a debate about which nanoscaled materials will show better, there is no doubt nano-diameter fiber scaffolds provide a significant increase in functional surface area compared with

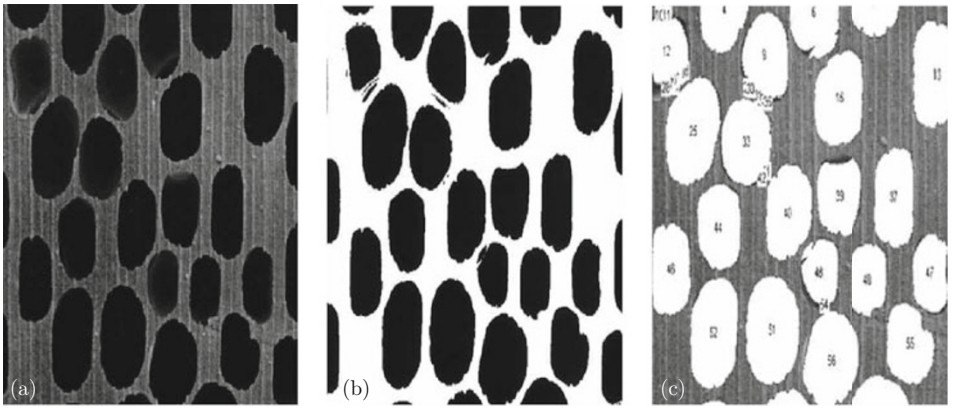

Fig. 2 Thresholding process of image analysis of LDI-glycerol-PEG-AA polymer foam: (a) Initial SEM image; (b) Thresholding SEM image; (c) Labeled pores of an SEM image of LDI-glycerol-PEG-AA polymer foam [24].

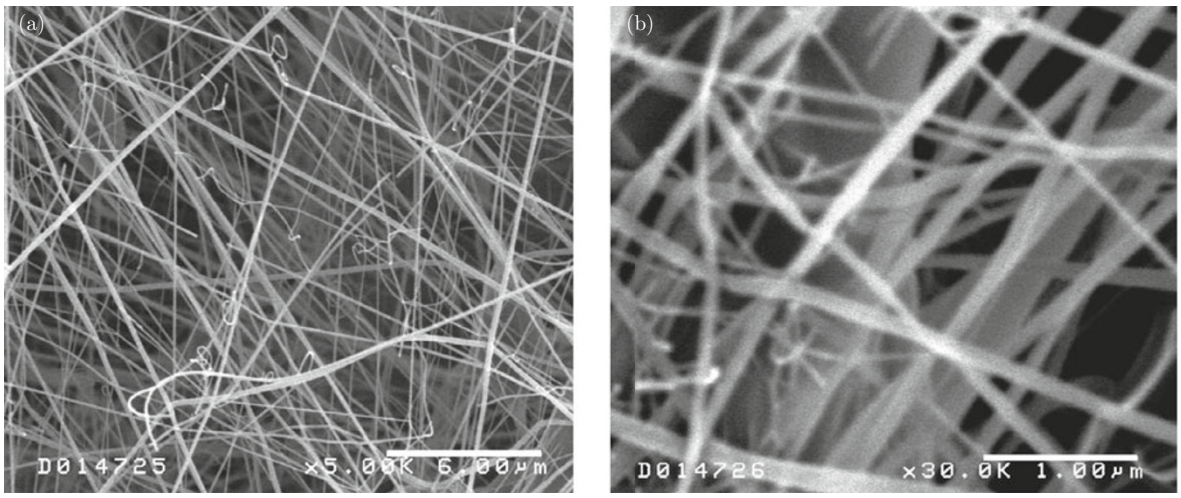

Fig. 3 Scanning electron microscope images of poly(DL-lactide-co-glycolide) nanofibers at 5000x magnification (a) and 30 000x magnification (b). The polymer fiber diameter was slightly variable with a mean diameter of approximately 100 nm [29].

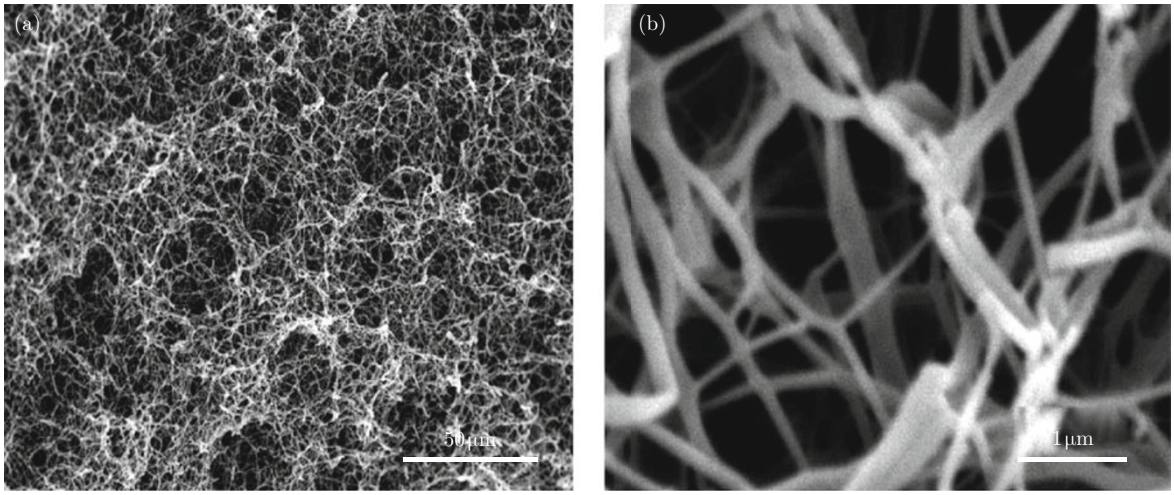

Fig. 4 Scanning electron micrographs of a PLLA nanofibrous scaffold prepared from 2.5% PLLA/THF solution at a phase separation temperature of 8℃: (a) 500% (b) 20 000% [31].

conventional materials with no roughness at the nanoscale. So the nano-scaffold is much more better for cell adhesion because of greater surface.

In contrast with traditional scaffold materials, the 3-D nanofibrous scaffolds provide a superior microenvironment for promoting cell functions. Since nanofibrous scaffolds have nanometer pore sizes, cells are unable to penetrate by themselves, so the seeding cells must be incorporated into the scaffold during fabrication to ensure proper cell distribution [29]. We can use this characteristic to guide cell distribution in order to make the regenerated tissues be more closer to normal cardiac tissue structure [30].

Technologies for generating nanofibrous biomaterials

At present there is several major technologies for fabricating nanofibrous biomaterials, including phase separation, self-assembly, electrospinning and so on. Phase separation techniques have been used to prepare porous polymer membranes for purification and separation purposes. In laboratory, researchers have generated nanofibrous structures by manipulating the phase separation process (Fig. 4) [31-33]. But the outcomes of the phase-separation technique is not perfect, the generated nanofibrous materials are lack of interconnected macropores, which are critical for cell seeding and recruiting, mass transfer, vascularization and tissue organization [34]. So the phase-separation technique needs to be improved to overcome this defect. Self-assembly is a kind of technology to organize individual molecules into a well-defined and stable hierarchical structure with preprogrammed non-covalent interactions [35-40]. Self-assembly have its unique advantages. For example, the molecules concerned interact at

an atomic level driven by physical or chemical affinity self-assembly can increase the sensitivity and specificity. And because the atomic level interactions between components are retained throughout the expansion process, so we can retain nanoscale properties even in bulk materials (Fig. 5) [41]. But just as the phase separation, self-assembly also has some limitations, such as self-assembled nanofibrous scaffolds are limited to biological molecules in the form of hydrogels and the degradation of the scaffolds have not been systematically addressed. In comparison with phase separation and self-assembly, the technology of electrospinning is simple, economical and it can generate porous scaffolds with submicron diameter [42-43]. What's more, the electrospinning can be used in both synthetic and natural biology material scaffolds. Electrospinning is a well-established process that has been used to produce ultrafine fibers and it has been popular in the field of tissue engineering. There is studies demonstrated a vivid similarity between electrospun poly(caprolactone) (PCL) nanofiber matrix and native ECM in rat cornea (Fig. 6). With the advances of the technology, it is hopeful to generate nanoscaled scaffolds by using electrospinning in the future [44,45].

Nanocomposites in cardiac tissue engineering

Recently, the function of nanocomposites in cardiac tissue engineering causes a hot discussion among the researchers. The key limitation of porous matrix used for cardiac tissue engineering is that their pore walls limit the interaction of cells, and delay electrical signal propagation [46]. The 3D nanocomposites of gold nanowires within macroporous alginate scaffolds have been developed to bridge the non-conducting pore walls, and this can increase electrical signal propagation throughout the cell-seeded scaffold, and enhance the organization of functioning tissue [47]. The functional mechanism of the nanowires is unclearly now. Nanowires may create

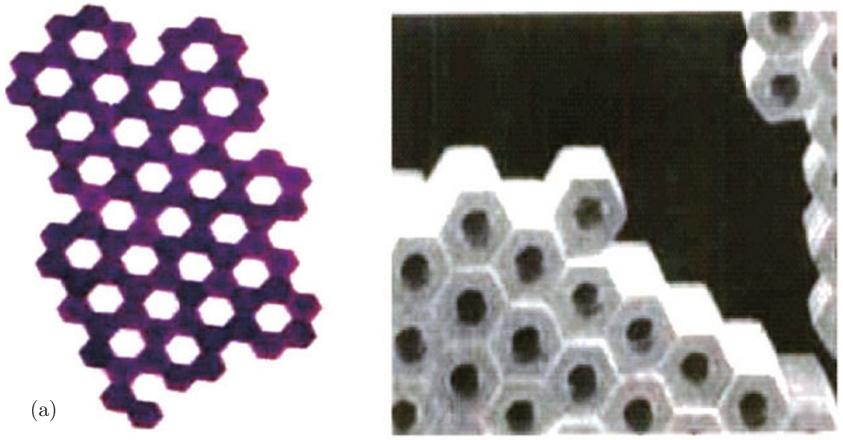

Fig. 5 Examples of static self-assembly: (a) An array of millimeter-sized polymeric plates assembled at a water/perfluorodecalin interface by capillary interactions [39]. (b) A 3D aggregate of micrometer plates assemble by capillary forces [40].

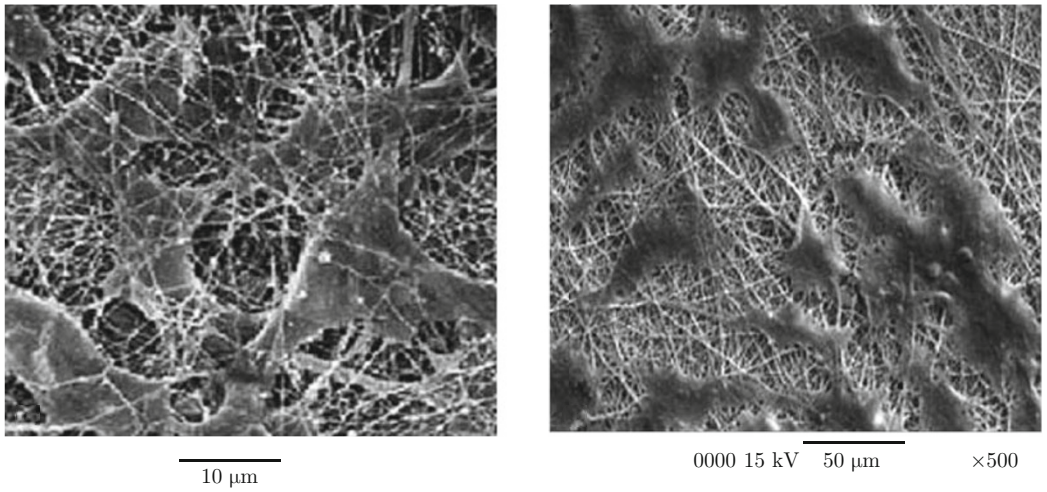

Fig. 6 Similarity between native ECM protein structure and electrospun polymeric nanofiber matrix: (a) Fibroblasts cultured on collagen fibrils of rat cornea [42]; (b) Endothelial cells cultured on electrospun PCL nanofiber matrix [43].

conductive bridges across the scaffold materials, connecting adjacent pores and/or cell bundles. Alternatively, the nanowires may enhance the expression of the electrical coupling protein connexin43. Cx-43 has been shown to regulate cell-cell communication, influence electrical coupling, and promote contractile behavior [48,49]. And Cx-43 can be upregulated under stimulated conditions, so the nanowires can be a promising candidate for cardiac tissue engineering.

Although poly(lactic-co-glycolic acid) (PLGA), one of the polymer, has desirable biodegradability and biocompatibility properties [50,51], also fails to overcome the disadvantage of the limited interaction between cells. So fabricating novel conductive, biodegradable composites became the purpose of our study. And many articles have reported that the nanocomposites can be a promising application in cardiac tissue engineering. Carbon nanofiber composites (CNM), which are conductive, have the ability to transform non-conductive polymers to conductive and they can mimic natural proteins like collagen [52,53]. And CNF possess nanoscale geometries which imitate the extracellular matrix of heart tissue, can improve cytocompatibility of pure PLGA [54].

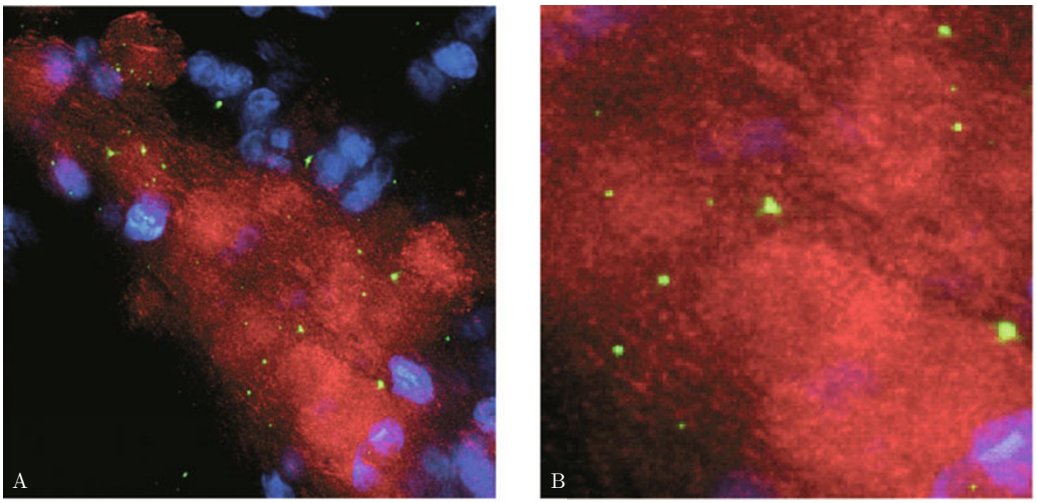

Fig. 7 Connexin 43 gap junction protein was found between cardiomyocytes in the nanowire-containing scaffolds. Nuclei are coloured in blue, B is amplification of A [47].

Conclusion

Although in the experimental stage, the cardiac tissue engineering has been made great progress, the real clinical application still has a long time to go. The ideal scaffolds should have good biocompatibility, certain tensile strength, absorbability, plasticity, chemical properties of surface and microstructure that are good for cell adhesion and can gradually degraded as the growth and differentiation of tissue, and so on.

Now there are still some theories and technology problems about the nano-scaffold need to be smoothed out, but it has shown very good application potentialities from the start. With the developing of nanomaterial technology and the advance of manufacture process, the updating nanomaterial scaffold can be produced by the application of nanotechnology to classical biomaterial-based scaffold. The future of the cardiac tissue engineering must be promising in clinical medicine.

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