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Stem Cell-Based Tissue Engineering for Regenerative Medicine

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The applications of stem cells in tissue engineering will show great promise in generating tailor-made tissue/organs for clinical applications. This paper gives a review on a broad spectrum of areas in stem cell-based tissue engineering including neuron repair, cardiac patches, skin regeneration, gene therapy and cartilage tissue engineering. This paper is intended to serve as an informative tutorial for scientists and physicians from fields other than stem cells and tissue engineering. It will shed light on various strategies of target tissue/organ repair involving stem cells.

Keywords: Stem cells; tissue engineering; regenerative medicine; neuron repair; cardiac patches; skin regeneration; gene therapy; cartilage tissue engineering.

1. Introduction

Tissue engineering is a newly emerging field that combines the use of cells, scaffolds and biological factors for the purpose of tissue/organ repair/regeneration.[1,2] Earlier known as a subset of the biomaterials area, now it can be looked at as a distinct field owing to its rising importance. It has three main facets — biology, biomaterials and cell culture. Figure 1 shows the tissue engineering cycle.

As can be seen in Fig. 1, stem cells are biological cells abundant in all multicellular organisms that can divide by mitosis and branch out into all types of specialized cell types, such as nerve, heart, liver, skin and bone cells, and are capable of self-renewal to generate more stem cells. Figure 2 shows the stem cells and their types. In mammals, the two main types of stem cells are embryonic stem cells (ESCs), isolated from the inner cell mass of blastocysts, and

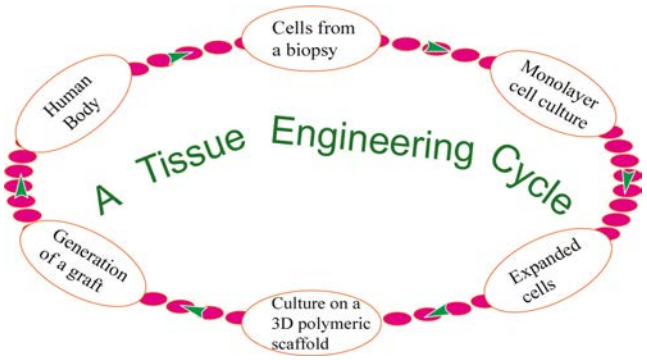


Fig. 1. A tissue engineering cycle (color online).[1]

adult stem cells, found in various tissues. The three major accessible sources of autologous adult stem cells in humans are:

(1) Bone marrow, extracted by drilling into bone,
(2) Adipose tissue, extracted by liposuction and
(3) Blood, extracted through pheresis equipment.[3]

ESCs are capable of differentiating into all types of tissue.[4] However, their availability is limited and has ethical issues associated with their use. With the advances in cell biology, now researchers are capable of producing induced pluripotent stem cells (iPSCs) from adult nonpluripotent cells. iPS cells

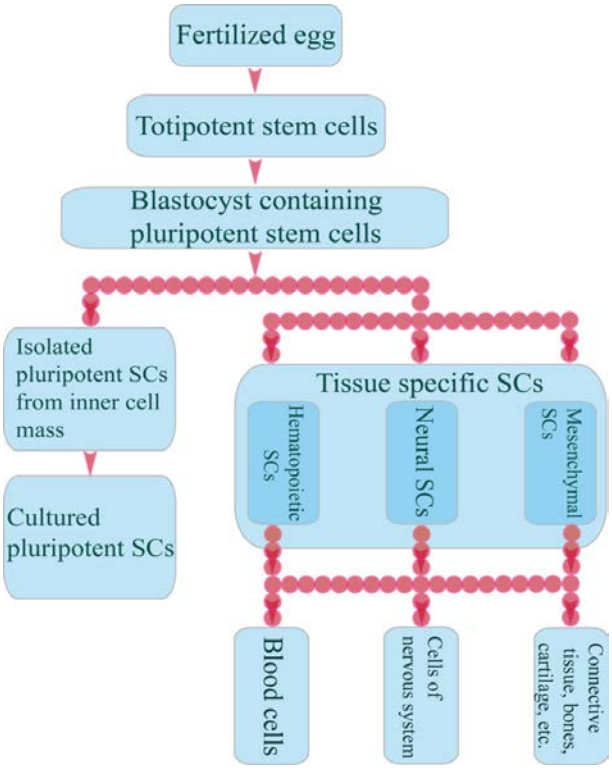


Fig. 2. Stem cells and their types (color online).[4]

appear identical to ESCs but can be created from adult cells without using an egg.[5]

Cell potency is described as the ability for cells to differentiate into various types. iPS cells can be artificially derived from any biological cell forming the body of an organism. The derivation from a nonpluripotent stem cell is typically accomplished via a "forced" expression of specific genes. iPS cells have been made from adult stomach, liver, skin cells and blood cells.[5] The use of iPS cells in tissue engineering could potentially open a new door toward tissue/organ regeneration.

2. Strategies

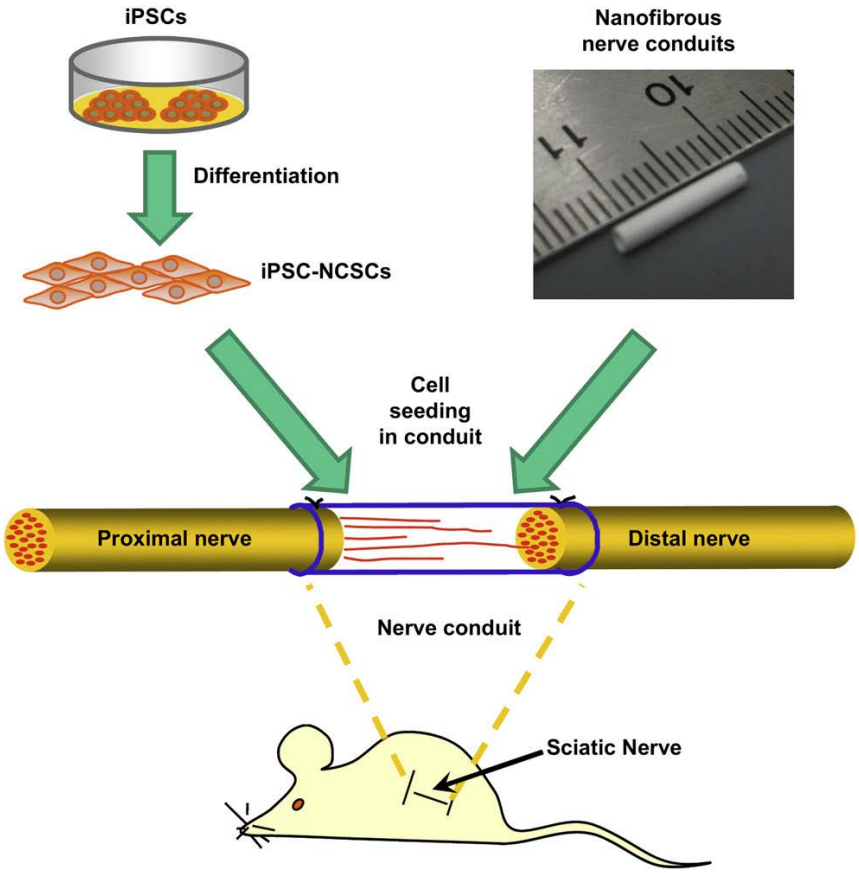
The following review provides some of the recent experimental approaches in stem cell-based tissue engineering.

2.1. *Nerve tissue engineering*

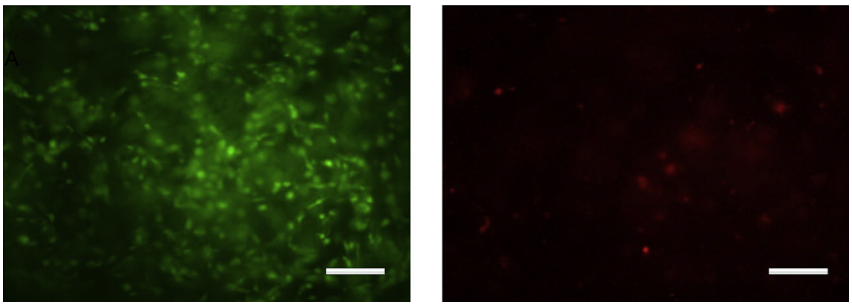
The use of iPSCs for cell therapies and tissue engineering was reported by Wang *et al.*[6] They extracted neural crest stem cells (NCSCs) from human iPSCs and ESCs, and investigated their prospects for neural tissue engineering.[7–11]

In this study, cells were allowed to migrate and grow to confluence followed by dissociation and culture as a monolayer. To evaluate the multipotency of NCSCs, cell differentiation into neural cells and mesenchymal cells was executed. Nanofibrous nerve conduits were made using the electrospinning technique.[12,13] A rotating mandrel assembly with two electrically conductive ends and a central nonconductive section was applied to produce tubular scaffolds. The tissue-engineered constructs were sterilized and stored in an incubator for a specific time interval. Electrophysiology testing was done prior to euthanasia, to assess the regeneration of the sciatic nerve across the conduits, followed by histological analysis and evaluation of myelination.[6]

As shown in Fig. 3, tissue-engineered nerve conduits are fabricated in order to investigate the prospects of iPSC-derived NCSCs for tissue engineering applications. The results show that more than 90% of the cells survived in the nerve conduits after one-day of *in vitro* culture. The electrospinning system was designed to make bilayered nanofibrous nerve conduits from biodegradable polymer poly (L-lactide-co-caprolactone). After cutting the sciatic nerve in rats, the gap was joined by a



(a)



(b) (c)

Fig. 3. Tissue engineered nanofibrous nerve conduits for peripheral nerve regeneration. (a) Schematic outline of tissue engineering approach by combining NCSCs and a nanofibrous nerve conduit. (b) and (c) The NCSCs were mixed with matrigel, injected into the nerve conduits, and cultured for one day. The viability of cells was tested by using live/dead assay. Live cells (calcein staining, in green) are shown in (b). Dead cells (ethidium homodimer-1 staining, in red) are shown in (c) (color online). [Copyright: Ref. 6]

tissue-engineered nerve conduit. A well-controlled microenvironment for analysis of NCSC differentiation was created by the nerve conduit. To study the regeneration of sciatic nerves in the animals, electrophysiological tests were carried out. Compound muscle action potentials (CMAPs) were preserved at both the operated and un-operated sides for each animal.

Detailed histological analysis was carried out to identify the operating mechanism of peripheral nerve generation. Their results indicate that the transplanted NCSCs improved the regeneration of peripheral nerves by assisting the myelination of regenerating axons.[6] To ascertain the contribution of engrafted cells, the group analyzed the outcome of the transplanted NCSCs in the regenerated nerve.

But no evidence was found about NCSCs differentiating into neurons. It was found that NCSCs are more suited for mature neurons and Schwann cells have poor survival rates. NCSC differentiation *in vivo* is possibly controlled by microenviromental factors, since they preferentially differentiate into Schwann cells according to peripheral nerve regeneration studies conducted by Wang *et al.*

The experimental results show that adult somatic cells (e.g., skin fibroblasts) can be reprogrammed into iPSCs, which in turn are favorable for patient-specific cell therapies using ESCs as a cell source. The findings of the study also indicate the following: (1) differentiation efficiency is a key parameter for both ESCs and iPSCs, and (2) significant difference exists in the expansion potential of NCSCs extracted from iPSCs and ESCs.

In the current research of tissue engineering, iPSCs have proven to be important cell sources. The study by Wang *et al.* shows that NCSCs derived from iPSCs and ESCs have synonymous characteristics and therapeutic effects on nerve regeneration and multipotent NCSCs can be straight-forwardly utilized for tissue repair. Most importantly, the study shows that the union of stem cells and engineering scaffolds yields great therapeutic effect and this strategy has promising prospects for regenerative medicine and tissue engineering applications.[6]

2.2. *Cardiac tissue engineering*

A new approach to create scaffold-free human cardiac tissue patches using human embryonic stem cells (hESCs) was described by Stevens *et al.*[26] Crucial issues linked to human cell sourcing and tissue fabrication were addressed by scaffold-free human myocardial patches in this study.

A considerable portion of cardiac tissue engineering work rests on the basis of using exogenous scaffolds or extracellular matrix-based materials. Another important aspect of the system is the utilization of human cells, which is a significant step toward the application of engineered cardiac tissues. There are numerous benefits by hESC-derived cardiomyocytes for use in cardiac regeneration post-myocardial infarction: (1) indefinite propagation in the undifferentiated state in culture[14] and thus high potentials in human therapy[15]; (2) hESC-derived cardiomyocytes functionally coupling with host myocardium after transplantation,[16,17] and (3) recent progress in direct differentiation of hESCs toward cardiac lineage leading to abundance of hESC-derived cardiomyocytes.[18]

For uniform cellular composition, the human cardiomyocytes grew in the patches, while non-cardiomyocyte cell populations decreased with culture time. Efforts are in progress to optimize the purity of cell populations, utilized to create human cardiac tissue patches.

Variations were observed in the composition of patches with respect to time in culture and every patch itself, and with patch edges being improved for cardiomyocytes examined against patch centers. This observation is in consistence with the prior work, in which the creation of muscle seeping greater than 100–200 μm into the engineered tissue is prevented by the diffusion limits of nutrients and oxygen.[19,20]

Numerous studies prove that hESC-derived cardiomyocytes are capable of growing both in culture and post-implantation into animals.[21–24] Stevens *et al.* reported that hESC-derived cardiomyocytes are able to proliferate in scaffold-free human cardiac tissue patches. Strategies to monitor the growth of graft cells post-implantation (currently under development[25]) could permit the expansion of human cardiac tissue patches in a controlled fashion after implantation.

It was observed by Stevens *et al.* that patches were conducting calcium impulses and the excitation of cardiomyocytes activated calcium release and caused muscle contraction in normal cardiac tissue. In order to contribute to host contraction, the engineered cardiac tissue must impede and conduct impulses simultaneously. It is evident from their findings that tissue-engineered cardiac patches can be connected with and transmit impulses with the host.

Based on the previous experimental studies on cardiac tissue engineering, one may conclude that these patches could have an important effect on therapies and treatments for human heart diseases. The unique approach of making scaffold-free patches with an abundance of hESC-derived cardiomyocytes may also have great promise in drug development.[26]

2.3. *Skin tissue engineering*

Xie *et al.* examined epidermal stem cell (ESC)-based candidates for reconstructing tissue-engineered skin for a skin defect repair.[35] The group anticipated that the selection of appropriate seed cells to ascertain the function of regenerated epidermal

tissue would be the primary problem of tissue engineered skin (TES). Based on prior work, ESCs, connected to the underlying basement membrane, were thought to play a significant role at the time of skin formation. In addition, the growth prospects and pluripotency are found to be the major characteristics of an ESC.[27–29] Thus, they investigated the application of reconstructed TES using ESCs as seed cells.

In the study, dermal substrates were prepared using donor cryopreserved porcine skin specimens, which were subjected to continuous shaking to remove the epidermis and other cellular components from the dermal matrix.[30] This was followed by the isolation of keratinocytes and fibroblasts from human foreskin biopsies.[31] Using the method explained by Dunnwald *et al.*, adhesion of keratinocytes to Type IV collagen was performed[32–34] followed by their cultivation, as shown in Fig. 4.

Equivalent ESC and fibroblast cell suspensions were made and seeded onto the acellular dermal substrates simultaneously; all composites were cultured, removed and sections were extracted for histological examination. A total of 16 full-thickness defects were created on the dorsum of each nude mouse and each wound was implanted with TES. Figure 4 exhibits that in approximately one month, a differentiated epidermis is observed having a clearly defined dermis with numerous morphological features of the skin.

From the immunohistological assay that was carried out to locate the origin, it was observed that keratinocytes of all grafted TES demonstrated positive expression proposing that they originated from transplanted human ESCs and not from mice. Electron microscopy and light microscopy were used to affirm that grafted TES combined well into the host tissue.

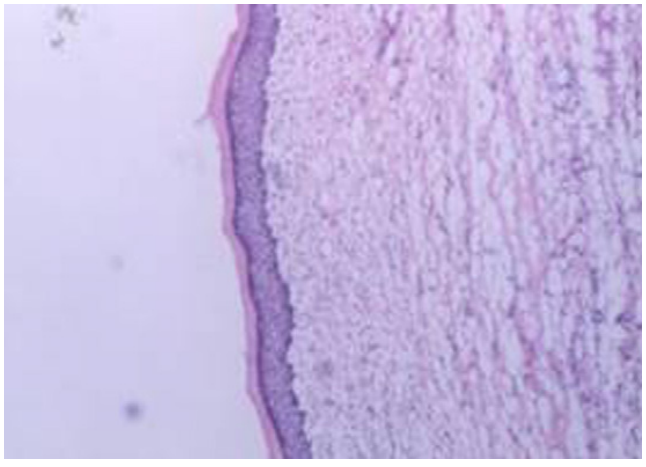


Fig. 4. The transplanted skin developed a well-stratified epidermis and clearly defined dermis (color online). [Copyright: Ref. 35]

The findings by Xie *et al.* demonstrate that a permanent coverage from epidermal stem cells *in vitro* can be created. Their study shows that the reconstructed TES has created a well-differentiated epidermis and dermis. Great textural and histological qualities of the reconstructed TES are also confirmed due to a continuous basement membrane, keratin filaments, anchoring fibril, demosomes and hemidesmosomes.[35]

2.4. *Bone tissue engineering*

The impact of 3D-culture system on gene transfection of mesenchymal stem cells (MSCs) and *in vivo* bone formation was studied by Hosseinkhani *et al.*[36] The group investigated bone regeneration by scaffold implantation and carried out histological and biochemical studies.

For successful tissue regeneration, the renewable source of cells is required such as stem cells. In particular, MSCs have been widely investigated to use either by themselves or in combination with the scaffold for their applications to regenerative medicine. An emerging research approach with MSCs is the combinational therapy of MSCs with exogenous gene expression. The cells cannot only function as the carrier vehicle to target genes to the site of action, but also positively participate in the process of tissue repair. Therefore, several studies have investigated therapy via tissue regeneration by genetically engineered MSCs. Over the last decade, gene therapy has captured the scientific and public interest with the promise to deliver genes and proteins to specific tissues or to replace deficient host cell populations. Gene modification is preferred over addition of growth factor to the cell as, typically, (1) the half-life of the selected growth factor is short; (2) a single administration is usually not sufficient for a biological effect, (3) the quantities required are prohibitively expensive, and (4) continuous protein production increases the likelihood that a desired outcome will be achieved. In planning gene therapy strategies for tissue engineering, one must consider two major avenues: direct gene delivery *in vivo* using viral or nonviral vectors or *in vitro* cell-mediated gene therapy. In both cases, the aim is to

deliver a therapeutic gene of a growth factor or cytokine, into the target tissue. This paradigm provided a proof of principle for the use of tissue engineering techniques to improve methods of gene transduction. The 3D transgene cell construct can be potentially used as therapeutic cell-based gene delivery or as an *in vitro* model system for testing of genetic manipulations to study the effects of gene expression on tissue development.

Therapeutically important genes are used for *in vitro* pharmacological, physiological and developmental researches as well as *in vivo* cell-based gene therapy applications. Gene transfection with 3D tissue constructs is a new field and few studies have been published on the *in vitro* transfection of genes into 3D tissue constructs.

The efficiency of transfection with expression vector has been shown to be related to cell cycle activity.[37] Attachment of cells to substrata is a major factor controlling their structure, function and cell cycle.[38] At first, cells attach to the surface of fibers throughout the polymer scaffold, then spread and proliferate. The 3D fibrous matrices can provide greater available surface area for cell attachment and spreading than 2D surfaces (i.e., tissue culture plate). Moreover, the 3D scaffold surface affects cell adhesion, spreading and proliferation, and controls the spatial arrangement of cells and their transmission of biochemical and mechanical signals. Cell adhesion, spreading and cytoskeletal reorganization initiate signaling cascades that govern gene expression.[39] It has been demonstrated that hematopoietic cell adhesion to the cell matrix might govern the expression of transgene.[37] The results indicated that, in 2D culture, the initial rate of cell growth was higher, but their proliferation was not maintained once the cells reached confluence.[40] However, the cell growth in 3D matrices continued for longer time periods than those of 2D matrices.[40] The higher the cell growth, the better the gene transfection for the cells.[41] It has been found that effective transfection of multicellular hepatocyte spheroids required the process of spheroid formation.[42]

Currently, the combinational strategy of tissue engineering principles with gene therapy involves the introduction of foreign genes into cell grown *in vitro*, selection for stable tranfectants and seeding the modified cells into polymer scaffolds.[43–45] This process is slow and requires considerable time to establish stable transfectants. Xie *et al.*[41] indicated that direct application of cDNA into cell-scaffold constructs is more efficient than transfection of cells grown on 2D tissue culture plate. Thus, establishing a tissue-like construct on polymer scaffold, followed by stable or transient expression plasmid transfection may offer a superior method to the conventional *in vitro* gene transfection and then application of cells directly into a host or seeding them into scaffolds.

The experimental steps by Hosseinkhani *et al.* involved preparation of plasmid DNA followed by acetylation and characterization of polyethylenimine. DNA nanoparticles were prepared at room temperature and their formation was confirmed by electrophoresis followed by particle size measurement. Preparation and culture of MSC was carried out by fabrication of poly(glycolic acid) (PGA) fiber-incorporated collagen sponge. The mechanical properties (compression modulus) were characterized by performing a compression test on sponge and MSCs were seeded into collagen sponge scaffolds. The bone mineral density was assessed for the *in vivo* experiment followed by a histological analysis. The experimental method was concluded with an *in vitro* and *in vivo* osteogenic evaluation by determining intracellular alkaline phosphatase (ALP) activity and bone osteocalcin (OCN) content.

The extent of acetylation was determined by H^1 nuclear magnetic resonance spectroscopy (NMR) and the morphology of PGA fiber-incorporated collagen sponge was analyzed by light microscopy and scanning electron microscopy (Fig. 5). As can be seen in Fig. 6, every collagen sponge has an interconnected porous structure and synonymous intrastructural appearance, although a few PGA fibers are exposed in the pores of PGA-incorporated sponges. This was achieved by osteogenic differentiation of MSC transfected with DNA nanoparticles by 2D- and 3D-culture methods.

It is now recognized that one of the tissue engineering strategies is proceeded by the following three principal steps: the proliferation of cells, the seeding of cells into a suitable scaffold and the maintenance of the differentiation phenotype of the engineered tissues.[46] A biocompatible porous scaffold plays an important role in seeding cells and serves as a template for tissue regeneration. The scaffolding materials should be biocompatible, biodegradable and osteoinductive to accept the attachment and migration of osteoblasts. Among many materials currently used as cell scaffolds, collagen has been widely used because of the good

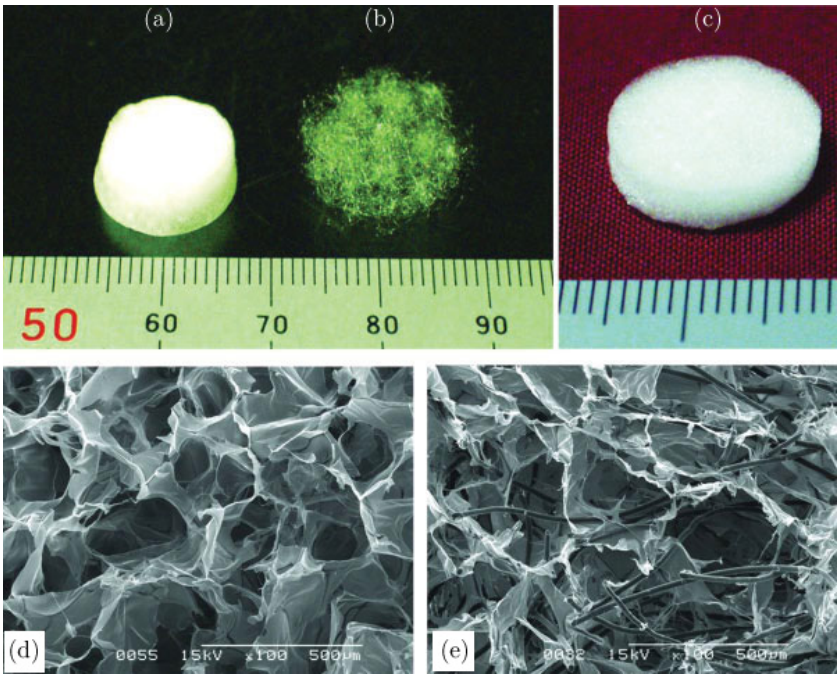


Fig. 5. Light microscopic photographs of a collagen sponge without PGA fiber incorporation (a), a frame structure of PGA fiber incorporated (b), a collagen sponge incorporating PGA fiber at a collagen/PGA weight ratios of 0.2 (c), and cross-sectional scanning electron microscopy (SEM) photographs of a collagen sponge without PGA fiber incorporation (d) and collagen sponge incorporating PGA fiber at collagen/PGA weight ratios of 0.2 (e) (color online). [Copyright: Ref. 36]

cell compatibility and its processability into various shapes.[47–49] Collagen sponges have been widely used as scaffolds to demonstrate the feasibility in inducing the regeneration of skin, connective tissue, trachea, adipose tissue and peripheral nerve.[50–55] Collagen is inherently biocompatible with cells because collagen is one of the main components of extracellular matrix. The structure of collagen sponge is highly porous with an interconnected pore structure, which is effective in cell infiltration and supplying oxygen and nutrients to cells, while the bioresorbability can be regulated by changing the formulation conditions. The drawback of collagen sponge as a scaffold for cell proliferation and differentiation is its poor mechanical strength. To overcome the inherent nature of sponge, the combination with other materials has been attempted.[53] In addition, the materials to be combined should be bioabsorbable. From the viewpoint of clinical application, it is preferable to select a material that has been clinically used. Several biodegradable synthetic polymers, such as PGA and its copolymerswith L-lactic acid, DL-lactic acid, and ε-caprolactone, have been fabricated into scaffolds of nonwoven fabric and sponge shapes for tissue engineering. The mechanical resistance of the scaffolds to compression is acceptable in a practical sense for tissue engineering applications, because of their hydrophobic nature. However, the cell attachment to the surface of synthetic polymer scaffolds is poor compared with that of collagen. PGA has been approved by US Food and Drug Administration for human clinical applications. The previous studies revealed that incorporation of PGA fiber enabled collagen sponges to increase the resistance to compression *in vitro* and *in vivo*.[56] The *in vitro* culture experiment revealed that the number of MSCs attached increased with the incorporation of PGA fiber to a significantly high extent compared with that of the original collagen sponge.[40] Because the PGA fiber incorporation also suppressed the shrinkage of collagen sponge, it is possible that the volume available for cell attachment was larger, resulting in a higher number of cells attached. It has been shown that mouse fibroblast L929 cells infiltrated into the collagen sponge incorporating PGA fiber more deeply than the collagen sponge.[56] This phenomenon can also be explained in terms of suppressed shrinkage of sponge by PGA fiber incorporation. The penetration depth of cells into a matrix is a good parameter for scaffold–cell interaction analysis in tissue

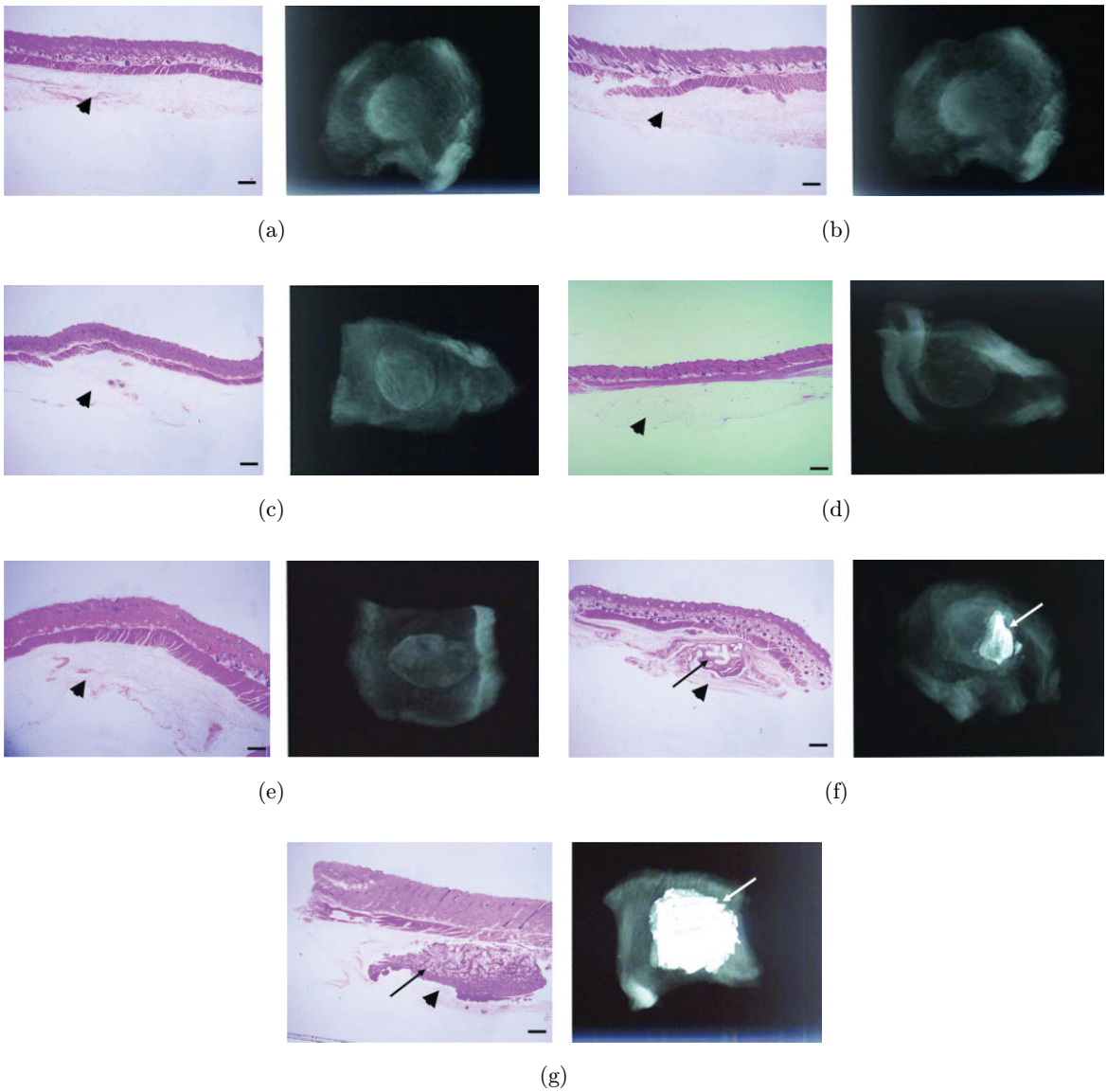


Fig. 6. Histological cross sections and soft X-ray photographs of ectopically formed bone three weeks after implantation of collagen sponges with PGA fiber incorporation at collagen/PGA fiber weight ratio of 0.2 impregnated with phosphate-buffered saline (PBS) (a), MSC (b), naked plasmid DNA (c), DNA nanoparticles formed with PEI (d), DNA nanoparticles formed with PEI-Ac_{80} (e), transfected MSC by 3D method by DNA nanoparticles formed with unmodified PEI (f), and transfected MSC by 3D method by DNA nanoparticles formed with PEI-Ac_{80} (g). The dose of plasmid DNA was 0.01 μg/μL. Each specimen was subjected to H&E staining. Arrows and arrowheads indicate the newly formed bone and the residual collagen sponge, respectively. The scale bar measures 1 mm in full cross section (color online). [Copyright: Ref. 36]

engineering.[57] It has been reported that the *in vivo* formation of adipose tissue was more significant as the penetration of cells into the scaffold increased.[58] Collagen sponge with this advantageous feature is a promising scaffold for tissue regeneration. The pore structure and interconnectivity of collagen sponge cannot be impaired by the PGA fiber incorporation. Irrespective of the fiber amount incorporated, the fiber-incorporated collagen sponges possessed a similar interconnected porous structure with an average pore size of 180 μm.[56] The incorporation of PGA fiber enabled the sponge to increase the resistance to compression. On comparing *in vivo* degradability, the collagen scaffold is generally digested faster than the PGA fabric. This greatly depends on the cross-linking extent of collagen

sponge, the PGA molecular weight and formulation. A combined cross-linking method of dehydrothermal, glutaraldehyde and UV was used to prepare collagen sponges with or without PGA fiber incorporation. Weadock *et al.*[59] evaluated physical, mechanical and biological behaviors of collagen sponge cross-linked by physical (UV irradiation and dehydrothermal) and chemical (carbodiimide and glutaraldehyde) or combination of physical (dehydrothermal) and chemical (carbodiimide). The results revealed that combination of physical (dehydrothermal) and chemical (carbodiimide) cross-linking of collagen reduced significantly the swelling ratio and increased the collagenase resistance time to low and high strain modulus compared with single cross-linking by UV, dehydrothermal and carbodiimide. Glutaraldehyde cross-linking itself showed the same physical and mechanical property as combination of physical (dehydrothermal) and chemical (carbodiimide) cross-linking. Based on these experiments, the combined cross-linking of dehydrothermal, glutaraldehyde and UV was superior to other methods.

The aim of the study by Hosseinkhani *et al.* was also to improve the gene expression level by combining a 3D tissue-engineered scaffold and nonviral gene carrier. Their observations show that the gene expression level was enhanced by the DNA nanoparticles and by collagen-sponge 3D-culture method. These DNA nanoparticles were made with PEI-Ac_{80} (acetic anhydride reacted with polyethylenimine to acetylate 80% of primary and 20% of secondary amines).

Figure 6 shows images of histological cross sections and soft X-ray photographs of ectopically formed bone. The bone structure is formed three weeks after implantation of collagen sponges with PGA fiber incorporation. Note that the collagen/PGA fiber is incorporated at weight ratio of 0.2 impregnated with PBS. As can be seen in Fig. 6, the implantation of collagen sponges with PGA fiber incorporation is accomplished under various conductions. In particular, DNA nanoparticles made with PEI-Ac_{80} are utilized. Upon comparison, it is found that the bone formation is considerably enhanced in the sponges seeded with transfected MSC by using DNA nanoparticles formed with PEI-Ac_{80}.

By amalgamation of DNA nanoparticles made with PEI-Ac_{80}, integrating DNA nanoparticles into the PGA fiber reinforced collagen sponge and 3D-culture method, the gene expression level was improved. The study by Hosseinkhani *et al.* demonstrates an interesting approach of tissue regeneration by combining 3D tissue engineering scaffolds, stem cells and gene therapy.

2.5. *Cartilage tissue engineering*

The application of adult stem cells in tissue engineering was assessed by Li *et al.* to target tissue/organ shortage.[74] They made a poly (ε-caprolactone) (PCL) nanofibrous scaffold (NFS) and investigated its use for *in vitro* chondrogenesis of MSCs.

Cartilage defects lead to a string of problems such as loss of mobility and joint pain. Prior work shows that the utilization of tissue engineering for articular cartilage defect repair holds promise.[60–63] Different natural and synthetic biodegradable materials have been used previously[64–69] as fiber-, sponge- and gel-based structures to culture MSCs for making biomaterial scaffolds.[68,70–74] This study analyzes chondrogenic activities of cultured bone marrow-derived MSCs, seeded in PCL NFSs, versus high density cell pellet (CP) cultures.

Human bone marrow-derived MSCs were isolated followed by formation of three-dimensional CPs (cell/pellet). Three-dimensional NFSs were studied using SEM. RNA extraction, reverse transcription polymerase chain reaction (RT-PCR) analysis, sulfated glycosaminoglycan assay and cell proliferation assay were performed prior to histological, immunohistological and statistical analysis.

The experimental findings show that while a 3D environment and TGF-β1 (growth factor) are essential to induce chondrogenesis, the PCL-based NFS considerably improve the chondrogenic differentiation of MSCs compared to CP culture. No polymer degradation within PCL nanofibrous mat was confirmed through the structural morphology of NFS (Fig. 7) and the MSC constructs. The deposition of ECM in different culture conditions was also investigated by SEM (Fig. 8).

In the cellular proliferation that was studied through a DNA assay, a similar pattern was observed by Li *et al.* in CP and NFS cultures. The DNA content was found to be no different in the presence of TGF-β1 (Fig. 9). H&E staining was carried out for histological examination of the morphology of NFS cultures (Fig. 10).

These results by Li *et al.* clearly show an improvement in the level of MSC chondrogenesis in the NFS as against the CP culture.[74] Thus, with advantages such as ease of fabrication and desirable mechanical properties, the three-dimensional PCL-based NFS is an

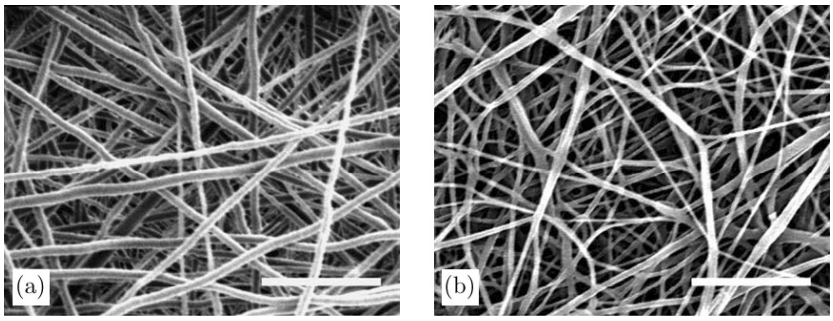


Fig. 7. Ultrastructural morphology of PCL NFS examined by SEM. (a) A PCL NFS produced by the electrospinning process showing random orientation of ultra-fine fibers with a diameter ranging from 500 nm to 900 nm, defining a matrix with interconnecting pores. (b) A NFS incubated in culture medium at 37°C for 21 days. Both fibers and pores are maintained and no significant structural degradation is evident. Bar 10 μm. [Copyright: Ref. 74]

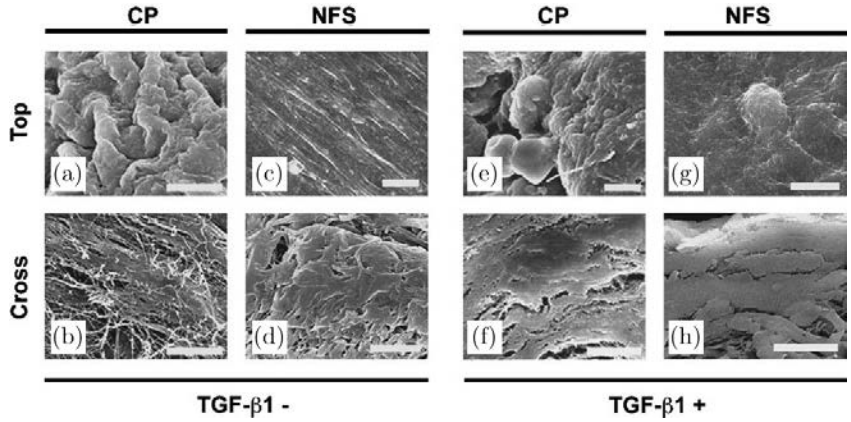


Fig. 8. Morphology of day 21 CP and NFS cultures examined by SEM. (a)–(d) cultures maintained without TGF-β1; (E)–(H) cultures treated with TGF-β1. (a) Top view of CP, revealing a roughened surface; (b) cross-sectional view of CP, showing the presence of native collagen-like fibers; (c) top view of NFS, showing fibroblast-like cells covering the surface; (d) cross-sectional view of NFS, showing cells covered with ECM and integrated with PCL nanofibers; (e) top view of CP, with round chondrocyte-like cells on the surface; (f) cross-sectional view of CP, showing thick, ECM; (g) top view of NFS, showing the presence of round, ECM-embedded chondrocyte-like cells; and (h) cross-sectional view of NFS, showing a thick and dense ECM rich layer. Bar 10 μm. [Copyright: Ref. 74]

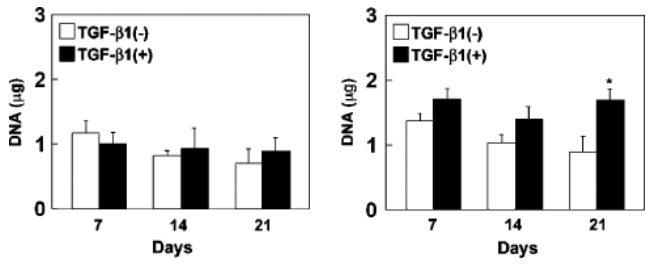


Fig. 9. Kinetics of cell proliferation in CP and NFS cultures estimated based on DNA content. (a) CP cultures; (b) NFS cultures. Cultures were treated with or without TGF-β1 as indicated. For CP cultures, cell number (based on DNA content) showed a slow decrease as a function of time in the absence of TGF-β1 treatment, but remained stable with TGF-β1 treatment. A similar trend is also seen in the NFS cultures, with TGF-β1 treated cultures on day 21 showing significantly higher cell number. Values are mean $\pm$ S.D. *, $p < 0.05$, $n = 4$. [Copyright: Ref. 74]

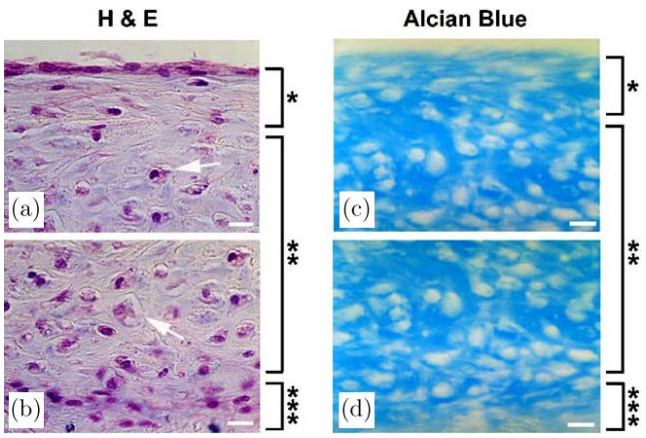


Fig. 10. Histological analysis of NFS MSC cultures maintained in a chondrogenic medium supplemented with TGF-β1 for 21 days. Sections from the upper and lower portions of the three-dimensional constructs were stained with H&E (a), (b) and Alcian blue (c), (d). H&E staining showed flat fibroblast-like cells on the top zone (bracket, *), round chondrocyte-like cells embedded in lacunae (arrows) in the middle zone (bracket, **) and small, flat cells at the bottom zone (bracket, ***). Alcian blue staining showed the presence of sulfated proteoglycan-rich ECM in the construct. Bar 10 μm (color online). [Copyright: Ref. 74]

appropriate carrier for MSC transplantation in tissue-engineering based cartilage repair.

3. Conclusion

The findings summarized in this review have provided a novel set of tissue replacement parts and implementation strategies. The critical challenges have mainly been on the multiple biomechanical properties of the laboratory-grown tissues designed for transplantation. However, for the future development of true human replacement parts, critical issues will have to be addressed on both fundamental research and clinical applications in all aspects of tissue engineering including scaffold development, tissue/organ repair/regeneration, as well as in developmental biology and materials science. Nonetheless, these previous experimental results have already shown great promise in various tissue repair strategies. The continued success of tissue engineering has paved ways for future clinical practices.

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