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Biomaterials for *in situ* tissue regeneration: development and perspectives

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Abstract： Tissue engineering has emerged as a powerful method to treat the loss of tissues and organs in the past several decades. Many commercial products based on tissue engineering have been applied in clinical practice. In addition to classical tissue engineering strategies, *in situ* tissue regeneration (*in vivo* tissue engineering) has become a more and more important therapy for damaged tissues and organs as it avoids *in vitro* cell manipulation and takes advantage of *in vivo* microenvironment to regulate cell activities. Biomaterial is one of the key factors for *in situ* tissue regeneration and should possess unique features including physical properties, chemical composition, and biological function to modulate cell behaviors such as adhesion, proliferation, migration, differentiation and neo-tissues formation. In this review, recent development of biomaterials used for *in situ* tissue regeneration has been summarized, classified by sources and the design of biomaterials including physical design, chemical composition, and biological functionalization were highlighted. In addition, the application of biomaterials for *in situ* tissue regeneration was also reviewed. Finally, a brief conclusion and some perspectives were given in terms of the future trend of biomaterials for *in situ* tissue regeneration.

Key words: tissue engineering, biomaterials, *in situ* tissue regeneration, cell behaviors,

biomimetic design

1. Introduction

The loss of tissues and organs is one of the most serious threats to human health. Every year, millions of people suffer or die from the loss of tissues and organs caused by aging, trauma, or injury.[1] Meanwhile the restoration of damaged tissues and failed organs is still one of the most difficult problems in clinic. Organ transplantation including autograft and allograft is considered as the most effective technique and has improved the living conditions of millions of patients. Although organ transplantation remains the dominating therapy for damaged tissues, there are many limitations such as immune response, donor shortage, and donor site morbidity which compromise the application of organ transplantation.[2]

During the exploration of curing damaged tissues, scientists creatively developed the idea of "tissues engineering" which combines the principles of biology, medicine, material, and engineering to fabricate *in vitro* substitutes for subsequent *in vivo* implantation.[3] The concept of "tissue engineering" was firstly put forward in the 1980s and great achievements have been made during the past three decades.[3] For example, Dermagraft®, a full-thickness skin substitute composed of polylactic acid (PLA), polyglycolic acid (PGA), and fibroblasts have been commercialized to treat diabetic ulcers and have achieved great success.[4] However, some problems are still unsolved. The formation of new tissues needs long term *in vitro* cell isolation, expansion, and maturation. In addition, the source of autologous cells is limited and cells isolated from native tissues are heterogeneous and hard to standardize. Furthermore, tissues remolded *in vitro* cannot have integrated structures and comprehensive functions.[5] All these drawbacks of tissue engineering strategy motivated scientists to find a new approach.

Recently, with the deeper understanding of tissue regeneration and development of biomaterial science, people have tried to restore damaged tissues at the defect sites *in situ*, which were known as *in situ* tissue regeneration (*in vivo* tissue engineering). The idea of *in situ* tissue regeneration is to implant tissue-specific biomaterials alone or combining with cells and biomolecules to the sites of injury and take advantage of *in vivo* microenvironment aiming to guide the fate of cells to regenerate new tissues *in situ* without complicated *in vitro* manipulation.[6] *In situ* tissue regeneration has gained great attention since its emergence and is a promising therapeutic alternative for tissue loss due to its great advantages. For example, collagen modified chitin membranes seeded with epidermal stem cells have successfully realized the regeneration of damaged skin with all appendages.[7] Table 1 made a comparison between several common commercial products based on the concept of classical tissue engineering and *in situ* tissue regeneration.

Biomaterials play a pivotal role for the success of *in situ* tissue regeneration because biomaterials provide three-dimensional support for cell growth and extracellular matrix (ECM) formation. Biomaterial for *in situ* tissue regeneration (BITR) must possess some basic characteristics such as: (1) suitable microstructures and mechanical properties, (2) proper surface topography and chemistry, (3) biodegradability and non-cytotoxic degradation products, (4) simple and cost effective manufacturing technology.[8] Moreover, BITR in addition must provide specific microenvironment including unique physical structures, special chemical composition, surface properties, and indispensable biosignals to direct cell behaviors. The development of biomaterial design techniques, especially the application of nanotechnology in biomaterial science, enable scientists to fabricate customized biomaterials

with exquisite structures even at micro or nano-scale.[9] In this review, we highlighted the recent development of BITR focusing on the sources, physical design, chemical composition, biological functionalization as well as application. Finally, a brief conclusion and perspectives were presented.

2. Biomaterials for *in situ* tissue regeneration

Biomaterial is one of the key elements in tissue regeneration. In most cases, biomaterials serve as 3D matrix for cell activities. As mentioned above, biomaterials for tissue regeneration should be biodegradable, biocompatible, and provide physical, chemical, and biological signals to induce cell attachment, migration, proliferation, differentiation and neo-tissue formation.[10] Versatile formats of biomaterials such as porous scaffolds,[11] hydrogels,[12] membranes,[13] tubes,[14] micro and nanospheres[15] are available and can be manipulated specifically according to the requirements of different damaged tissues. Multiple preparation methods such as freeze-drying,[16] gas foaming,[17] electrospinning,[18] layer-by-layer (LbL) assembly,[19] and 3-D printing[20] make it feasible for the construction of biomaterial matrix with organized structures and well-defined functions. Typically, four main groups of biomaterials including natural biomaterials, synthetic polymers, bioceramics, and ECM-based biomaterials are employed for *in situ* tissue regeneration.

2.1 Natural biomaterials

Natural biomaterials such as collagen, gelatin, fibrin, chitosan, hyaluronic acid (HA) have been widely used for *in situ* tissue regeneration. In general, natural biomaterials have excellent biocompatibility and biodegradability. Natural biomaterials can mimic many features of ECM and the recognition sites they carried are beneficial cues for cell behaviors.

As the most abundant protein in mammals supporting connective tissues such as skin, bone, cartilage, blood vessel, and ligament, collagen has been fabricated into many formats for *in situ* tissue regeneration.[21-24] Collagen scaffolds conjugated with stem cell specific antibody could capture stem cells at the wound site and promoted cardiomyocytes regeneration in a mouse model.[25] Collagen can be easily cross-linked and modified by biomolecules to fabricate versatile derivatives with customized properties.[26]

Chitosan, a natural polysaccharide derived from chitin, is the second most abundant biosynthesized material.[27] Chitosan exhibits outstanding biodegradability, biocompatibility, and antibacterial activity.[28] Its cationic nature makes it an ideal vector for the delivery of anionic glycosaminoglycans (GAGs), growth factors, cytokines and genes.[29] Besides, advances have been made to modify chitosan to obtain various derivatives such as sulfated chitosan, trimethyl chitosan, and thiolated chitosan.[30]

HA, a glycosaminoglycan found in ECM of many tissues, is an attractive biomaterial especially for cartilage repair due to its limited immune response and positive biological significance including proliferation, morphogenesis, and wound repair.[31] HA is synthetically versatile and can be easily modified to form hydrogels.[32] HA hydrogels promoted better cartilage regeneration than PEG hydrogels.[33] Furthermore, HA-based hydrogels have been employed for delivery of growth factors, such as vascular endothelial growth factor (VEGF) and bone morphologic protein (BMP).[34, 35]

In some cases, several natural biomaterials have been used to fabricate matrix with advantages that single natural biomaterial does not have. Collagen/chitosan blend can be fabricated into nanofibers by electrospinning to facilitate endothelial cells and smooth muscle

cells growth.[36] The incorporation of HA into chitosan-gelatin scaffolds improved the adhesion of fibroblasts.[37] In addition, the crosslinking efficiency of collagen-based scaffolds could be enhanced by the addition of HA.[38] However, natural biomaterials usually possess relatively poor mechanical properties compromising their application for hard tissue regeneration. To overcome this shortcoming, people combined synthetic biomaterials or inorganic biomaterials with natural biomaterials to enhance their mechanical properties.[39]

2.2 Synthetic polymers

Synthetic polymer is another important category of BITR. Compared to natural biomaterials, synthetic polymers are easy to process under controlled conditions with mature methods which endow them with predictable and flexible physical and chemical properties including microstructure, mechanical properties, degradation rate, and functional groups.[40] In addition, synthetic polymers can be well designed to possess functional sites by conjugating biomolecules.[41] Moreover, synthetic polymers have fewer biosafety concerns including host responses and disease transfection than natural biomaterials.[42] Synthetic polyesters such as PGA, PLA, poly (lactide-co-glycolide) (PLGA) and polycaprolactone (PCL), which can be tailored with different molecular weights, mechanical properties, porous architecture, and degradation rate for specific tissue regeneration have widespread applications.[43] These polyesters can be degraded by hydrolysis into non-cytotoxic products. PLA is one of the most promising biodegradable polyester owing to its mechanical property profile, thermoplastic possibility and biological properties. It is synthesized from lactic acid, a naturally occurring organic acid that is metabolically innocuous.[44] PLGA based biomaterials are used extensively for cartilage and bone regeneration.[45]

Unfortunately, the conventional synthetic polymers generally lack of functional groups resulting in their limited capacity to combine with bioactive moieties to reinforce their cell affinity. Recently, functional synthetic polymers gain their popularity due to their easy design and modification, which enables them to provide unique bioactive signals to enhance spatiotemporal biomaterial-cell interaction. Basically, functional synthetic polymers have unsaturated bond,[46] or functional groups such as hydroxyl,[47] carboxyl,[48] amide,[49] through which functional biomaterials can be chemically modified by biomolecules to improve their bioactivity. Ring-opening polymerization and post modification is an efficient way to synthesize various functional biodegradable polymers.[50] Malemide functional biodegradable polyesters have been synthesized using ring-opening copolymerization and modified by laminin-derived peptide (IKVAV).[51] The peptide modified polyesters enhanced neurite outgrowth of PC12 cells. Those functional synthetic polymers provide versatile alternatives for tissue regeneration. Advances in synthetic chemistry are in favor of the development of novel synthetic biomaterials applicable for *in situ* tissue regeneration.

2.3 Bioceramics

Bioceramics are mainly used for bone tissue regeneration. Bioceramics have been confirmed to demonstrate biocompatibility, bioactivity, osteoconductivity, similar physical and chemical properties to native bone mineral. Moreover, they show great osteoinductivity which is vital to induce bone regeneration *in situ* even without the presence of inductive factors.[52] Macroporous hydroxyapatite scaffolds with interconnected oval shaped pores were prepared by polymer sponge replication method.[53] The porous hydroxyapatite enhanced cellular functionality and supported osteoblast differentiation in comparison to dense hydroxyapatite.

Song *et al.* demonstrated that bone-marrow-derived mesenchymal stem cells (BMSCs) can be migrated from bone marrow through blood circulation to non-osseous biphasic calcium phosphate (BCP) implants and new bone formation could be induced by BCP in a canine model without growth factors delivery.[54] Bioceramics can be fabricated into nanoscaled structures with different morphologies including nanorods, nanospheres, needle-like, flower-like, bowknot-like, and fibrous structures.[55-60] And these nanoscaled structures enhanced osteoinductivity. Zhang and coworkers used the methods of co-precipitation synthesis, microwave heating, and hydrothermal methods to create bioceramics with different nanostructure.[61-63] These nano-sized bioceramics generally have better osteoinductivity and efficacy of bone regeneration than conventional ones.

2.4 ECM-based biomaterials

ECM is a complex, hierarchical, three-dimensional network consisting of proteoglycans, glycosaminoglycans, proteins, and other biomolecules. ECM is a dynamic system serving as a bridge for information exchange between cells and a variety of studies have suggested that ECM has great effects on cell behaviors.[64, 65] For example, ECM offers niche to induce stem cell differentiation.[66] ECM-based biomaterials have received considerable interest and are the most promising BITR because the microenvironment provided by ECM-based biomaterials is similar to native tissue, which is significantly important to mediate a wide range of cellular behaviors. Generally, ECM-based biomaterials can be derived from decellularized tissues. The advantage of this method is that the structures and components of the decellularized ECM are similar to natural tissues. However, donor shortage is still a problem. ECM-based biomaterials can also be fabricated from *in vitro* cultured cells. Xenogenic, allogenic, and

autologous ECM-based biomaterial can be obtained through this method and different types of cells can be co-cultured to provide mixed ECM-based biomaterials. Chen and coworkers did a few of work on the ECM-based biomaterials from cultured cells. They developed a novel kind of "stepwise osteogenesis/adioogenesis/chondrogenesis mimicking matrices" that mimic ECM at each stage of osteogenesis, adipogenesis, and chondrogenesis of human mesenchymal stem cells (hMSCs) respectively.[67-69] The ECM-based biomaterials from stem cells at different stages showed different effects on stem cell differentiation. They also prepared an autologous ECM (aECM) scaffold through three procedures: culture of autologous cells in a three-dimensional template, decellularization, and template removal.[70] The aECM scaffold showed excellent biocompatibility and minimal undesirable immune responses after implantation. Jia *et al.* found that the oriented cartilage ECM-based scaffolds promoted cartilage-specific ECM secretion of BMSCs compared to non-oriented ones. ECM-based biomaterials have been already successfully employed clinically for bone and cartilage regeneration.[71]

The biomaterials described above are the most common BITR. Sometimes a single kind of biomaterial cannot meet all the requirements for the repair of damaged tissues. It is increasingly popular to construct hybrid biomaterials by combining two or several types of biomaterials. A novel kind of PLGA/collagen hybrid scaffold composing of porous PLGA knitted mesh skeleton and collagen microsponges were developed (Fig. 1A).[72] The knitted mesh enhanced the mechanical properties of the hybrid scaffolds while the microsponges facilitated cell attachment, distribution and tissue formation. The hybrid scaffold exhibited induced formation of cartilage *in vivo* test.[73] Besides, growth factors could be spatially

mobilized onto the hybrid porous scaffold to reinforce osteoinductivity. Funnel-like PLLA-collagen and PLLA-gelatin hybrid scaffolds were fabricated by embossing ice particulate templates for skin tissue regeneration.[74] People usually combine bioceramics with natural and synthetic biomaterials to endow bioceramics with flexible manipulation.[75] Collagen/alginate/PCL (Fig. 1B), collagen/hydroxyapatite (Fig. 1C) hybrid scaffolds with both strength and toughness properties have been fabricated for bone repair.[76, 77] These composites demonstrate lots of advantages and provide a diverse platform for the construction of biomaterial matrix.

3. Design of biomaterials for *in situ* tissue regeneration

Biomaterial is extremely important for *in situ* tissue regeneration and must possess some unique features that can modulate cell behaviors and then induce new tissue formation. The physical properties, chemical composition, and biological functionalization of biomaterial are of great importance and were discussed below.

3.1 Physical properties

When designing BITR, the physical properties of biomaterials must be in line with the tissue of interest. The physical properties of biomaterials mainly involve mechanical property, microstructure, and surface topography.

Mechanical property means the ability of biomaterial matrix to preserve their stability and integrity. The modulus of biomaterials has great influence on cell adhesion, distribution, proliferation and differentiation. It was proved that highly elastic PLLA constructs significantly promoted chondrogenic differentiation and the deposition of chondral ECM both *in vitro* and *in vivo* compared to rigid PLA or PLGA constructs.[78] Meanwhile, many research

works have proved that increase the modulus of biomaterials can enhance osteogenic differentiation of hMSCs.[79, 80] Some chemical or physical methods are employed to enhance modulus of biomaterials such as crosslinking, addition of reinforcement phase.[81] With the development of biomaterials science and technology, people can design biomaterials with mechanical properties corresponding to either soft or hard tissues.

Microstructure is another important factor that must be taken into consideration when designing biomaterials, especially for porous scaffolds. Typically, scaffolds with porous structures have large surface area and are favorable for cell attachment, migration, infiltration, and delivery of biomolecules. Porous structures improve mechanical interlocking between the implanted biomaterials and the surrounding natural tissues, giving rise to greater mechanical stability at the interface. Porous structures can be obtained by methods of gas foaming, thermally induced phase separation, template, and electrospinning *et al* (Fig. 2A).[82-85] Pore size, porosity, and interconnectivity are the three main characteristics of porous structures. Pore size has great impact on ECM secretion and tissue ingrowth.[86] For example, collagen porous scaffolds with 150-250μm in size showed best effect on the expression of collagen type II and aggrecan as well as formation of the cartilage (Fig. 2B).[87] β-tricalcium phosphate cylinders with large pore size facilitated the growth of blood vessels while the one with pore size smaller than 400μm restricted the growth of blood vessels.[88] Even for the same tissue, different biomaterials have different optimal pore size for tissue regeneration. For instance, the optimal pore size of collagen-glycosaminoglycan scaffold used for bone regeneration was about 325μm while nano-hydroxyapatite scaffold with pore size in the range of 100-250μm has a greater ability to form new bone compared to scaffold with pore size in the range of

50-150μm or 300-500μm.[89, 90] Porosity is beneficial for the penetration of cells from the surrounding tissues. PCL nanofibers were fabricated into PLA microfibers to form a structure with 95 or 97% porosity.[91] Results indicated that higher porosity led to increased cell infiltration. Weiss *et al.* have tailored synthetic human elastin scaffolds with different porosity by controlling the electrospinning flow rate.[92] High porosity scaffolds possess high tensile strength and low Young's modulus as well as facilitate de novo collagen synthesis and early stage angiogenesis in an *in vivo* subcutaneous implantation model. Pore interconnectivity is another important trait for the optimization of BITR because interconnectivity affects biomolecules transportation and cell migration. Porous PEG hydrogel with narrow distributed pore size and varying interconnectivity was applied for the investigation of *in situ* induced vascularization. Results revealed that larger interconnectivity facilitated extensive vascularization and formation of blood vessel networks *in vivo*.[93]

Cell-material interaction is a fundamental issue in biomaterial area and significantly influenced by surface topography of biomaterials.[94, 95] Recent advances in micro and nano techniques have allowed the fabrication of biomaterials that recapitulate native topography to study the effect of topography cues on cellular function.[96, 97] Fig. 3 showed several kinds of surface topographies and their interaction with cells. Hierarchical fibrous PCL matrixes consisted of nanoscale-roughened surfaces on microscale fibers were prepared by combining the electrospinning and selective plasma-exposure treatment (SPET) techniques.[98] Compared to untreated PCL fibrous mats, the surface-roughened PCL scaffolds enhanced initial cell attachment, proliferation, and calcium deposition. Zhang *et al.* studied the impact of orientation of PLGA porous scaffolds on cartilage regeneration. The results indicated the

oriented PLGA scaffold efficiently provided clues and enhanced cartilage regeneration.[99] Micropatterned surfaces exhibited outstanding biological activities to regulate cell microenvironment and stimulate cellular responses compared to flat surfaces. For example, microgroove engineered PDMS surfaces guided neurite orientation and outgrowth.[100] And the channel width played a great role in the development and differentiation of neural stem cells. When implanted in a primary motor cortex lesion, the microgroove engineered PDMS remained structurally intact and did not induce inflammatory reaction.[101] It improved cell survival and directed axonal outgrowth for neuronal tissue regeneration.

3.2 Chemical composition

Chemical composition is another important factor for the design of biomaterial and usually dominates the mechanical properties, loading capacity and degradation rate of biomaterial and further affecting tissue regeneration process.

Component usually has crucial effect on the properties of biomaterials. A collagen-mimetic peptide GFOGER was coated onto the PCL scaffold for bone repair. The cell/growth factor free scaffold has a better effect on bone formation in non-healing bone defects of rats that pure PCL scaffold.[102] ECM-decorated polyesterurethane (PEU) scaffold can be obtained by removing the cells pre-seeded onto the PEU scaffold without damaging the deposited ECM.[103] The ECM-decorated PEU scaffold promoted the osteoblastic differentiation of newly seeded hMSCs and the formation of new bone in nude mice compared to pure PEU. Researchers can fabricate biomaterials with nanocompostion in order to mimic the nanoscale structures of native tissues.[104] Nanocomposition possesses substantially increased surface area benefiting protein adsorption, providing more sites for cell recognition and attachment, influencing cell

migration and differentiation.[105] Gogolewski *et al.* synthesized two kinds of PLA microporous membranes from poly(L/D-lactide) and poly(L/DL-lactide) while the ratio between L/D-lactide or L/DL-lactide was 95/5%.[106] The quality of the interface between the new bone and the membrane was related to chemical composition. New bone was separated from poly(L/D-lactide) membrane while poly(L/DL-lactide) membrane in some cases directly contacted with the formed new bone.

The element ratio also affects the properties of biomaterial. For example, Champion and coworkers have synthesized a single phased apatitic calcium phosphate powder with accurate control of Ca/P molar ratio ranging from 1.5 to 1.667. The ratio of Ca/P influenced the crystallinity of the nanopowder leading to variance of mechanical properties and degradation rate of calcium phosphate apatites.[107-109] Liu prepared a group of calcium phosphates with Ca/P ratios between 0.5 and 2.5 by adjusting initial amount of reactants. With the increase of Ca/P ratio, $Ca_2P_2O_7$ phase, tricalcium phosphate (TCP) phase, hydroxyapatite phase, and CaO phase were observed successively and the average pore size decreased from micro-scale to nano-scale. Moreover, calcium phosphate with Ca/P ratio below 2 promoted osteoblast alkaline phosphatase activity.[110] Wang *et al*. prepared four types of bioceramics: hydroxyapatite, β-TCP, biphasic calcium phosphate-1(BCP-1), biphasic calcium phosphate-2(BCP-2) (the ratios of hydroxyapatite to β-TCP in BCP-1 and BCP-2 were about 70/30% and 30/70%) to investigate the effect of chemical composition on protein adsorption and osteoinductive potential.[111] They found that no significant difference in BMP-2 adsorption between the bioceramics. However, after implantation for 90 days, BCP-2 enhanced the highest BMP-2 expression of cells migrated into the bioceramics and promoted the

osteoblastic differentiation of MSCs.

Surface functional groups play great role in biological behaviors including protein adsorption, cell attachment and so on. Self-assembled monolayers (SAMs) were employed to prepare hydroxyl (-OH), carboxyl (-COOH) and amine ($-NH_2$) functionalized surfaces. More endothelial cells were adhered onto the hydroxyl (-OH) functionalized surfaces than that of carboxyl (-COOH) and amine ($-NH_2$).[112] PLA membranes with hydroxyl and amide have better cytocompatibility than pure PLA membrane, while PLA membrane with carboxyl has worst cytocompatibility.[113] Besides, it has been proved that hydroxylated surface promotes Ca-P nucleation and growth relative to carboxyl and amine-terminated surfaces.[114] There are study showed that carboxyl (-COOH) group density influenced both the adhesion and differentiation of C17.2 cells,[115] while functional polyester carrying free hydroxyl groups supported the attachment, proliferation, differentiation as well as mineralization of hMSCs both *in vitro* and *in vivo*.[116] The differences of biological behaviors between different functional groups can be attributed to the differences of chemistry properties and surface wettability.

3.3 Biological functionalization

Biological functionalization is an indispensable issue in the design of BITR. People are trying their best to fabricate bioactive materials in a reproducible, safe, and cost-effective way. Biomolecules such as peptides, growth factors, cytokines, chemokines, and genes are widely used for their properties on regulating cell activities by providing signals to stimulate or inhibit cellular adhesion, recruitment, migration, growth, differentiation and gene expression.[117, 118] Several common biomolecules used in tissue regeneration and their

application were summarized in Table 2. These biomolecules can be introduced into biomaterials either by physical incorporation or chemical immobilization (Fig. 4).

Physical incorporation is the most common method to combine biomolecules with biomaterials because it is easy to manipulate. Hossein and his coworkers have synthesized a kind of peptide-amphiphile (PA) with negative charge at pH 7.4.[119] When mixing with positive charged growth factors suspension, growth factors will be absorbed onto the PA. The screen electrostatic repulsion among PA results in self-assembly of PA by hydrogen bond and the unfavorable contact among hydrophobic segments and water molecules. This system can form an injectable hydrogel meanwhile fulfill the encapsulation of growth factors. They used this hydrogel to incorporate BMP-2 and basic fibroblast growth factor (bFGF) to investigate their impact on *in situ* angiogenesis and bone repair. BMP-2 and bFGF showed sustained release from the hydrogel.[120, 121] In order to better control the release profile of biomolecules, some special formats for physical incorporation were designed. A common method is to load biomolecules to particles followed by injection into biomaterials.[122] *N,N,N*-trimethyl chitosan chloride (TMC) was used as a vector to be associated with plasmid DNA to form nanoparticles by electrostatic interaction.[123] The loading amount could be mediated by the feeding concentration and the release profile was more sustained. In comparison with naked plasmid DNA, TMC/DNA complexes had more positive effects *in vivo*. In addition, core-shell structures have been fabricated through electrospinning.[124] PCL and transforming growth factor-β1 (TGF-β1) were first used to prepare coaxial fibers with core-shell structures while proteins were evenly distributed in the core and PCL shells were subsequently modified with BMSCs specific affinity peptide.[125] This system promoted adhesion and the chondrogenic

differentiation of BMSCs, and synchronously improved glycosaminoglycan, aggrecan and collagen type II expression required for cartilage regeneration. Besides, a recently developed method is to combine growth factors with specific domain of ECM to form growth factor variants with promiscuous high-affinity to the ECM. These ECM high-affinity growth factor variants induced better repair of chronic wound and bone *in situ* than wild type growth factors. This unique method to deliver growth factors could be clinically useful.[126, 127] All of these unique designs realize more controllable and longer release of biomolecules which is important for *in situ* tissue regeneration in the long term.

However, some problems of physical incorporation method to deliver biomolecules still exist. First of all, loading efficiency is relatively low. In addition, the half-life of biomolecules in circulating blood is short and can be easily degraded. To overcome these defects and improve the stability and long-term release of biomolecules, chemical immobilization has emerged as a promising approach. Immobilization can prolong the release of biomolecules, increase the efficacy, allow spatial distribution, and reduce the required amount thereby reduce the cost of treatment. Although most of biomolecules are water soluble and can be damaged in organic solvent narrowing the options available for the conjugation of biomolecules, but this limitation has not impeded the application of chemical immobilization for *in situ* tissue regeneration. Immobilization of biomolecules to both 2D and 3D biomaterials can be achieved by various methods. Basically, biomolecules possess carboxyl, amino, or sulfydryl, so researchers create special biomaterial systems with functional moieties to react with these groups. The common chemical reactions between biomaterial substrates and biomolecules include sulfydryl-maleimide,[128, 129] sulfydryl-double bond,[130] carboxyl-amino[131] amino-maleic

anhydride,[132] sulfydryl-disulfide bond[133] and so on. These chemical reactions can be conducted under mild conditions and have been widespread used to realize the control release of biomolecules. For example, the release of conjugated BMP-2 from the PCL scaffolds was significantly slower than that from BMP-2 adsorbed PCL scaffolds over 15 days.[134] Furthermore, gene expression of osteogenic markers was up-regulated in the BMP-2 conjugated PCL scaffolds. Similarly, TGF-β1 was covalently conjugated to injectable, visible blue light inducible chitosan hydrogel via succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker.[135] *In vitro* test showed that the release of covalently conjugated TGF-β1 was more stable than the absorbed one. Moreover, covalently conjugated TGF-β1 achieved the most efficient cartilage regeneration of chondral defects *in vivo*. Epidermal growth factor (EGF) was chemically immobilized onto the surface of the PCL-PEG block copolymers nanofibers.[136] Compared to soluble EGF, EGF-conjugated nanofibers significantly enhanced the expression of keratinocyte-specific genes and had better effects on wound healing process *in vivo*.

As we all known that cell migration is an important behavior in tissue regeneration and gradient signals are important cues to induce cell migration.[137] Over the past several decades, many kinds of technologies have been employed to fabricate spatio-temporal gradients. Gao and his coworkers have done a number of work on the fabrication of gradient biomaterials to investigate their effects on cell migration. They constructed unidirectional gradient signals such as rigidity,[138, 139] thickness,[140, 141] molecular weight,[142] chain density,[143] peptide,[144] growth factors[145, 146] to enhance the directional migration of chondrocytes, smooth muscle cells, endothelial cells. To move forward, they fabricated complementary gradients of polymer

brushes and peptide to selectively regulate specific cells directional migration while the migration of other cells was not influenced or even suppressed (Fig 5).[147, 148] There are also some studies which showed that gradient signals affected cell differentiation.[149, 150] These results are good instruction for the fabrication of 3D biomaterial systems to modulate cell recruitment and migration.

It is widely accepted that many kinds of stem cells that contain self-renewal and differentiation capability reside in "stem cell niche" and can be activated to migrate into the defect sites in response to biological signals that originate from tissue injury. It will benefit *in situ* tissue regeneration if we can utilize the *in vivo* microenvironment and special properties of biomaterials to recruit endogenous stem cells to the injury sites to achieve damaged tissues regeneration while avoiding the introduction of donor cells. With the aim of recruiting sufficient host stem cells to the biomaterial after implantation, researchers have paid great attention to use biomolecules to enhance cell recruitment. Stromal cell derived factor-1 (SDF-1), a member of the CXC chemokine family, have been identified to be a potent factor to recruit stem cells. PLGA scaffolds incorporated with SDF-1α can recruit more stem cells, increased angiogenesis, decreased fibrotic responses, and down-regulate inflammatory cell responses compared to pure scaffolds.[151] The sustained release of SDF-1α from engineered HA hydrogels enhanced the migration of BMSCs to the remodeling myocardium.[152] Another important feature of biomaterials is that stem cells recruited from the host tissues should be adhered onto biomaterials. For instance, Arg-Gly-Asp (RGD), a cell-adhesion recognition motif, is usually used for cell adhesion. BMSCs adhered to and differentiate into neuron exclusively in RGD modified alginate gel microchannels.[153] Recently, a peptide sequence

(EPLQLKM) with high specific affinity to BMSCs was identified for BMSCs homing and adhesion.[154] When covalently conjugating this peptide onto the synthetic PCL mesh, it can specifically enhance the adhesion of BMSCs *in vivo*.

4. Application of biomaterials for *in situ* tissue regeneration

4.1 Bone

Bone is a highly vascularized connective tissue with a complex structure mainly composed of calcium phosphate and collagen. Bone possesses the intrinsic capacity for regeneration in response to injury or trauma. However, mal-union or non-union in serious fractures inhibits the self-healing of damaged bone. Autografts are the "gold standard" to treat bone defects but require multiple surgeries. Metal implants are attractive for their mechanical strength and integrity but may induce stress shielding, infections, and chronic pain.[155] *In situ* bone regeneration is an important method to treat bone defects. Lots of biomaterials have been employed for the study of bone regeneration. For example, chitosan has been applied for bone regeneration owning to its ability to accelerate proliferation and mineral deposition of osteoblasts.[156] However, for *in situ* bone regeneration, the most widely utilized biomaterials are collagen and bioceramics because they can mimic the key features of natural bone. Zhang and coworkers tried a lot of biomaterials including collagen, hydroxyapatite, calcium phosphate, titanium, to evaluate their efficacy on *in situ* bone regeneration.[61, 157-159] They found that osteoinduction and bone regeneration can be influenced by phase composition, nanostructures, and biological molecules.[160] They fabricated three-level hierarchical calcium phosphate/collagen/hydroxyapatite (CaP/Col/HAp) scaffolds for bone regeneration (Fig. 6A).[161] Porous CaP severs as substrate to mimic the porous bone structure. Then collagen

network was composited into porous CaP bioceramics to form a second-level structure. Finally, a third level hydroxyapatite layer was fabricated by biomimetic mineralization. The three-level hierarchical biomimetic scaffolds demonstrated enhanced mechanical strength, biocompatibility, and osteoinductivity. Faster and increased bone formation was observed after a six-month implantation in rabbit model, which indicates that this biomimetic scaffolds exhibits better osteoinductivity than common CaP scaffolds (Fig. 6B). To enhance the bioactivity of biomaterials for bone regeneration, many biomolecules are combined with these matrixes. Matrix extracellular phosphoglycoprotein (MEPE) peptide, which has been shown to stimulate osteoblast differentiation, was covalently immobilized on hydroxyapatite/β-tricalcium phosphate (HAp/β-TCP) particles.[162] *In vivo* test showed that MEPE peptide-immobilized HAp/β-TCP significantly accelerated bone regeneration and increased bone area by enhancing osteoblast differentiation. BMP is the most extensively investigated biomolecules for their potential to induce differentiation of osteoblasts which plays a great role in the formation of bone. BMP-2 and BMP-7 have been licensed for clinical application since 2002 and 2001 respectively.[163] BMP-2 released from collagen-hydroxyapatite (CHA) scaffolds can enhance ALP activity and calcium production of osteoblasts.[164] After implantation, the scaffold loaded with BMP-2 had the potential to achieve bone regeneration while using 30 times less BMP-2 than INFUSE®, a collagen based sponge used for bone regeneration produced by Medtronic. There are studies to investigate the synergistic effects of BMP and other biomolecules. Reinforced bone regeneration was observed by the gelatin hydrogel encapsulated with SDF-1 and BMP. BMSCs can be recruited by SDF-1 released from the hydrogel and induced osteogenic differentiation by BMP.[165] In

contrast with single release of SDF-1 or BMP, the combination release showed significantly synergistic effects on the regeneration of both the critical-sized bone defect and subcutaneous site.[165]

4.2 Cartilage

Cartilage, especially articular cartilage, is of significant importance for physical mobility. However, cartilage possesses limited capacity to self-repair because of the lack of blood vessels and neural connections. Surgical treatments such as microfracture, drilling, osteochondral grafting, and autologous chondrocyte implantation are frequently applied for the regeneration of injured cartilage.[166] Tissue engineering is also applied to restore injured cartilage. However, the neo-cartilage regenerated by conventional tissue engineering strategies has been shown to have shortcomings including lack of complex structure, mechanics mismatching with native tissues. *In situ* cartilage regeneration can be achieved by well-designed biomaterials. In a study, chitosan was modified by graft of glycolic acid (GA) and phloretic acid (PA) to form hydrogel by enzymatic crosslinking with horseradish peroxidase (HRP) and H_2O_2.[167] Chondrocytes cultured in the hydrogel are viable and maintain their round shape after 2 weeks. HA has been proven to maintain the phenotype of chondrocyte and support cell proliferation.[168] There are evidences that indicated collagen or alginate hydrogels encapsulated with HA increase the production of cartilaginous matrix.[169, 170] PLGA microspheres were incorporated into a hybrid matrix containing gelatin/chitosan/hyaluronan for *in situ* cartilage regeneration.[171] PLGA microspheres can provide suitable mechanical properties meanwhile maintaining the biocompatibility of the original gelatin/chitosan/hyaluronan matix with chondrocytes. A composite construct has been

developed by incorporating fibrin gel loaded with TGF-β1 and BMSCs into PLGA sponge to repair full-thickness articular cartilage defects in rabbit model. After implantation for 12 weeks, compared with TGF-β1 absent constructs, neo-cartilage was formed and integrated well with its surrounding tissues and the cartilage specific genes were significantly up-regulated.[172] Further study demonstrated that the molecular weight of PLGA used in the composite constructs affects the *in vivo* cartilage regeneration.[173] To achieve long-term release of TGF-β1, *N,N,N*-trimethyl chitosan chloride (TMC), were employed to combine plasmid DNA encoding TGF-β1 (pDNA-TGF-β1) to realize the long term expression of TGF-β1(Fig. 7A).[174] In comparison with BMSCs or TMC/pDNA-TGF-β1 constructs, both the cartilage and subchondral bone were perfectly regenerated and integrated with the host tissues in the BMSCs/TMC/pDNA-TGF-β1composite constructs (Fig. 7B). This kind of composite constructs have great potential for *in situ* cartilage regeneration.

4.3 Skin

As the biggest organ in mammals, skin serves as a protection for internal tissues from the external environment. However, skin loss has already become a severe threat to human health. Skin substitutes including wound dressings, autografts, and allografts have been employed to repair damaged skin, but they are far from satisfactory. Skin substitutes based on the principle of *in situ* tissue regeneration are a promising approach. Ma and Gao have developed a collagen/chitosan hybrid scaffold cross-linked with glutaraldehyde (GA) for skin regeneration.[175] Collagen and chitosan evenly distributed in the scaffolds with high porosity and good interconnectivity. *In vivo* test revealed that the scaffold could induce the fibroblasts infiltration from the surrounding tissues. Furthermore, silicone membranes were used to cover

the hybrid scaffolds to form a collagen/chitosan-silicone membrane bilayer dermal equivalent (BDE) (Fig. 8A).[176] After transplanted into a bama miniature pig for 4 weeks, it was found that the BDE could regenerate new dermis with similar structure of the normal skin and facilitated angiogenesis of regenerated dermis.[177] Moreover, the regenerated dermis supported the transplantation and survival of thick skin. This dermal equivalent can be functionalized by TMC/pDNA-VEGF complex (Fig. 8B). The TMC/pDNA-VEGF complex functionalized BED can significantly promote the VEGF expression resulting in encouraging regeneration efficacy of full-thickness incisional wounds and burn (Fig. 9A).[178] What's more, TMC/siRNA complexes which could suppress transforming growth factor-β1 (TGF-β1) pathway were incorporated into the BDE to interfere in TGF-β1 signal pathway and ultimately inhibit scarring (Fig. 9B).[179] All these proofs revealed that the BDE can be easily tailored and has great potential for clinical application for *in situ* skin regeneration.

Although epidermal, dermal or full-thickness skin substitutes such as Dermagraft®, Apligraf® have been applied in clinical use and Integra® (the first product for *in situ* skin regeneration) has also been commercialized, the appendages such as hair follicle, sweat glands and sebaceous glands are still very difficult to be reconstructed. The trend of biomaterials for skin regeneration is to induce *in situ* regeneration of appendages. Hepatocyte growth factor (HGF) stimulating hair follicle regeneration was usually used for hair follicle regeneration.[180, 181] Fu and his coworkers did lots of studies on *in situ* sweat gland regeneration. They used collagen-based biomaterials loaded with gelatin microspheres containing EGF and sweat gland cells (SGCs) or BMSCs to improve the quality of skin. This engineered skin construct can regenerate sweat gland-like structures both *in vitro* and *in vivo*.[182, 183]

Dextran-based hydrogels facilitate the infiltration of inflammatory cells, angiogenic cells and endothelial cells into the healing wounds.[184] Mature epithelial structure with hair follicles and sebaceous glands was regenerated after 21 days. Moreover, new hair was observed 5 weeks later after the implantation of the hydrogel. These inspiring outcomes make us believe that we can achieve *in situ* regeneration of skin with integrated structures in the future.

4.4 Nerve

Nerve regeneration remains a great challenge. Current clinical treatments involve the end-to-end anastomosis and utilization of autografts. However, these approaches are often ineffective because of gap length between the injured nerves, formation of neuromas, and shortage of donor sources. Therefore, artificial nerve grafts are becoming a more and more important alternative to repair nerve injury. Since the regeneration of damaged nerves is a complicated biological process that requires multiple signals to facilitate neurocyte survival and stimulate neurite growth, nerve grafts that simultaneously possess multiple cues including topography guidance, electrical activity, and neurotrophic activity are desirable alternative for the regeneration of injured never tissues. Topography plays a fundamental role in nerve repair.[185] Electrospinning is the most common technique to fabricate aligned topography that support adhesion and regulate the growth of neuron. Besides, electrical stimulation is closely related to nerve regeneration as well. Great attention have been paid to electrically conducting polymers such as polypyrrole (PPy) and its derivatives because of their biocompatibility and conductivity.[186] PPY/ poly(d, l-lactic acid) (PDLLA) composite nerve conduits with different conductivities were fabricated using emulsion polymerization.[187] With the increase of the content of PPY, increase of both the percentage of neurite-bearing cells and the median

neurite length of PC12 was observed after being stimulated with 100 mV for 2 h. More importantly, PPY/PDLLA conduits for rat sciatic nerve defects demonstrated functional recovery close to autologous grafts. Neurotrophic agents, such as acetylcholine,[188] laminin-derived peptides including IKVAV and YIGSR,[189, 190] nerve growth factor (NGF),[191] and GAG mimics[192] are important for neural differentiation. Biomaterials combining these cues of electrical stimulation, topographical guidance, and neurotrophic activities have great potential for *in situ* neural regeneration. Schmidt *et al.* have developed a neural conduit that displays submicrometer-scale features, electrical conductivity, and neurotrophic activity by coating polypyrrole (PPy) onto the PLGA nanofibers followed by chemical immobilization of NGF onto the surface of the fibers.[193] These NGF-immobilized PPy-coated PLGA (NGF-PPy-PLGA) fibers facilitated PC12 neurite formation and neurite growth. A NGF gradient was immobilized within poly(ε-caprolactone)-block-poly(l-lactic acid-co-ε-caprolactone) (PCLA) nanofibrous nerve conduits (nNCs) by combining the differential adsorption duration of NGF and silk fibroin coating (Fig. 10A). [194] A rat sciatic nerve defect model was used to evaluate the efficacy of nNCs containing NGF gradients *in vivo*. After 12 weeks implantation, the NGF gradient-immobilized nNCs achieved positive results with morphological and functional improvements, which was similar to autograft and better than empty and uniform NGF nNCs (Fig. 10B, C).

4.5 Other tissues

Except for the above tissues, the concept of *in situ* tissue regeneration was also applied in some other tissues. HA hydrogel containing SDF-1 and angiogenic peptides (Ac-SDKP) for stem cell recruitment and angiogenesis respectively was used for the regeneration of

chronically infracted myocardium (CMI). The biomimic hydrogel exhibited extraordinarily improved left ventricle function, enhanced vascularization, increasing wall thickness after 4 weeks post-treatment, which indicated the combination of SDF-1 and Ac-SDKP had therapeutically beneficial effects for CMI.[195] *In situ* cross-linkable gelatin-poly(ethylene glycol)-tyramine hydrogel incorporated with human adipose derived stem cells (hADSCs) and bFGF can enhance muscle regeneration with minimal fibrosis in lacerated muscle.[196, 197] Poly(propylene fumarate)-co-2-hydroxyethyl methacrylate (PPF-HEMA) networks were synthesized for tarsal plate repair in rabbit eyelids.[198] Results turned out that PPF-HEMA scaffolds are satisfactory substitutes for the repair of tarsal plate with mild tissue response and good biocompatibility to fibroblast. Tubular cell-free tissue engineering scaffolds consisting of PGA knitted fibers and poly(lactide-co-caprolactone) (PLCL) sponge with outer poly(glycolide-co-caprolactone) (PGCL) monofilament reinforcement was used for inferior vena cava and pulmonary artery regeneration. The results showed that the cell-free scaffolds can be a promising platform for the repair of pediatric cardiovascular.[199, 200]

5. Conclusion and perspectives

In situ tissue regeneration is a more convenient approach avoiding *in vitro* cell manipulation and taking advantage of *in vivo* microenvironment, making it a promising strategy for future clinical application of tissue regeneration. BITR have made remarkable progress during the past two decades and are still evolving in terms of category, design, composition, and functionalization.

A key point for *in situ* tissue regeneration is to recruit enough host stem cells to the defect sites and induce them to differentiate into targeted cells to avoid the introduction of

exogenous cells as possible as we can. Meanwhile, the reprogramming including lineage reprogramming and pluripotent reprogramming of somatic cells has gained great attention around the world.[201] Reprogramming approaches, especially pluripotent reprogramming is becoming a novel avenue for *in situ* tissue regeneration. Many researches have proved that mouse or human fibroblasts, human fetal and adult blood $CD34^+$ cells can be converted into induced pluripotent stem cells (iPSC) by elaborate biomaterial system such as using microRNAs and stem cell factors.[202-204] Moreover, these induced pluripotent stem cells can re-differentiate into targeted cell types under specific environment.[205] New biomaterial fabrication strategies are needed to guarantee the safe and efficient production of iPSC, and to induce their differentiation into the desired lineages at the defect sites.

It is becoming increasingly important to design "smart biomaterials" to provide instructive/inductive cues to cells or stimulate target cell responses in the processes of tissue regeneration.[206] "Smart biomaterials" can be achieved by modulating the properties of biomaterials either in special physical design, specific chemical composition, or by using biomolecules in a way that allows sustainable and spatio-temporal release. At the same time, the capacity of biomaterial responding to the internal or external stimuli, such as temperature, pressure, pH, and magnetism, is another important development tendency for *in situ* tissue regeneration.[207] We believe that biomaterials are of fundamental importance to *in situ* tissue regeneration and will accelerate its development.

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Table 1 Several commercial products based on classical tissue engineering and *in situ* tissue regeneration.

	Name	Biomaterials	Application	Cells	*In vitro* construction	Manufacturer
Products based on classical tissue engineering	Dermagraft®	PLA,PGA	Dermal substitute	Fibroblasts	2-3 weeks	Advanced Tissue Sciences, USA
	Apligraf®	Collagen	Full-thickness skin substitute	Fibroblasts, epidermal cells	3 weeks	Organogenesis, USA
	BIOSEED®-C	ECM	Cartilage repair	Chondrocytes	4-5 weeks	Biotissue, Germany
	MyoCell®	ECM	Heart repair	Muscle stem cells	1-2 weeks	Bioheart, USA
Products based on *in situ* tissue regeneration	Norian SRS®	Calcium phosphate	Bone regeneration	/	/	Synthes Inc. USA
	Actifit®	PCL, polyurethane	Meniscus repair	/	/	Orteq Bioengineering, UK
	Hyalograft®C	Hyaluronan	Cartilage repair	Chondrocytes	/	Fidia Advanced Bio-polymers, Italy
	Integra®	Collagen, 6-chondroitin sulfate silicon	Dermal substitute	/	/	Integra Life Sciences Corporation, USA
	NeuraGen®	Collagen	Nerve repair	/	/	Integra Neuro-Sciences, USA
	Avance®	ECM	Never repair	/	/	AxoGen, Inc. , USA

Table 2 Common biomolecules for *in situ* tissue regeneration.

Biomolecules	Name	Application	References
Growth factors	BMP	Bone regeneration	35, 120, 134
	EGF	Angiogenesis/vascularization, cartilage repair	136, 182, 183
	TGF-β1	Cartilage regeneration	124, 135, 172
	VEGF	Angiogenisis/vascularization	34, 178
	HGF	Skin regeneration	180,181
	NGF	Nerve regeneration	191, 193, 194
Peptide	RGD	Cell adhesion	153
	IKVAV	Never regeneration	189
	YIGSR	Neurite growth	190
Chemokine	SDF-1	BMSCs recruitment	152, 165, 195

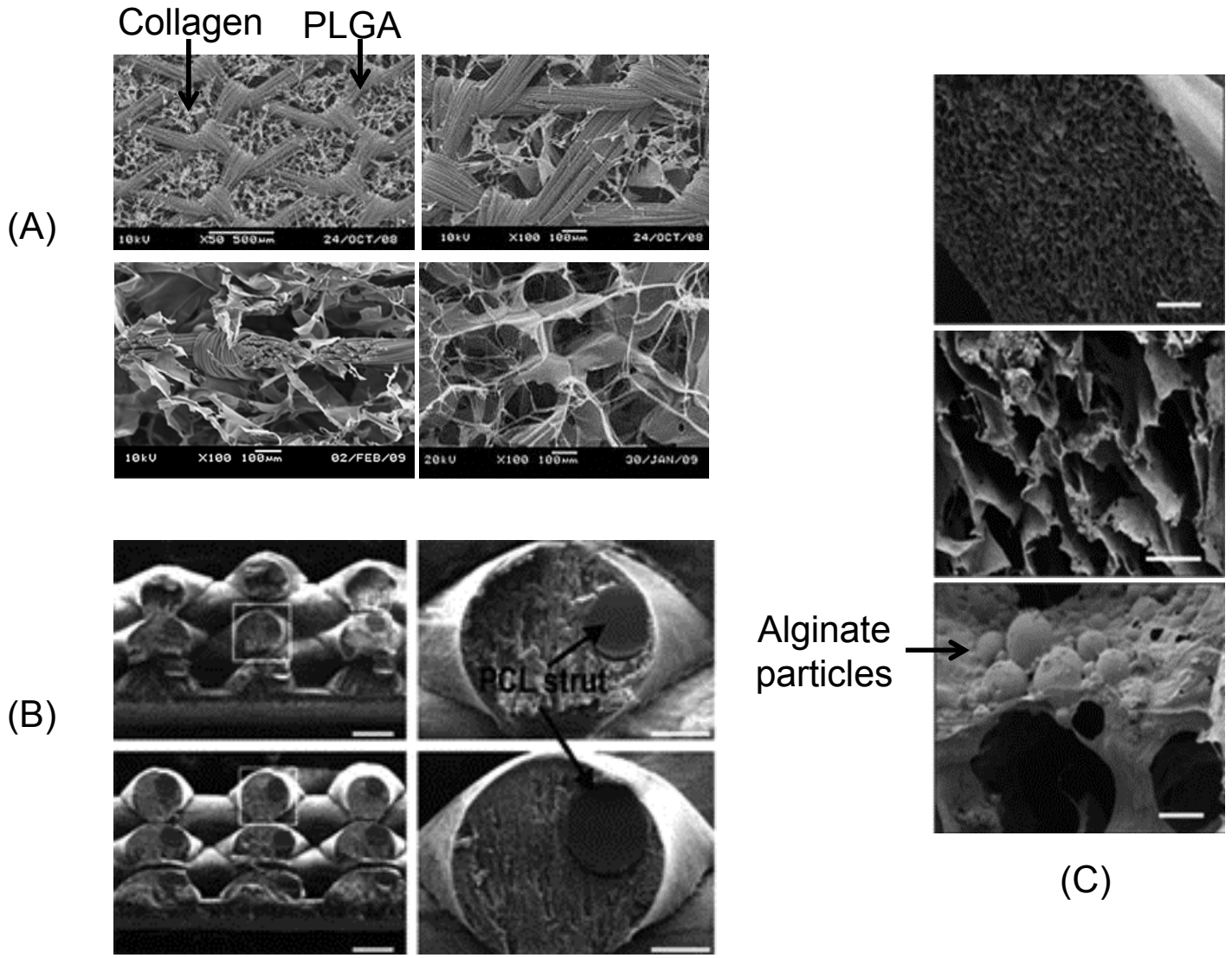


Fig. 1 SEM images of (A) PLGA/collagen hybrid scaffolds,[67] copyright 2010 Elsevier; (B) collagen/alginate scaffolds comprising PCL core,[71] copyright 2013 Royal Society of Chemistry; (C) collagen-hydroxyapatite scaffolds incorporated with alginate particles,[72] copyright 2013 American Institute of Physics.

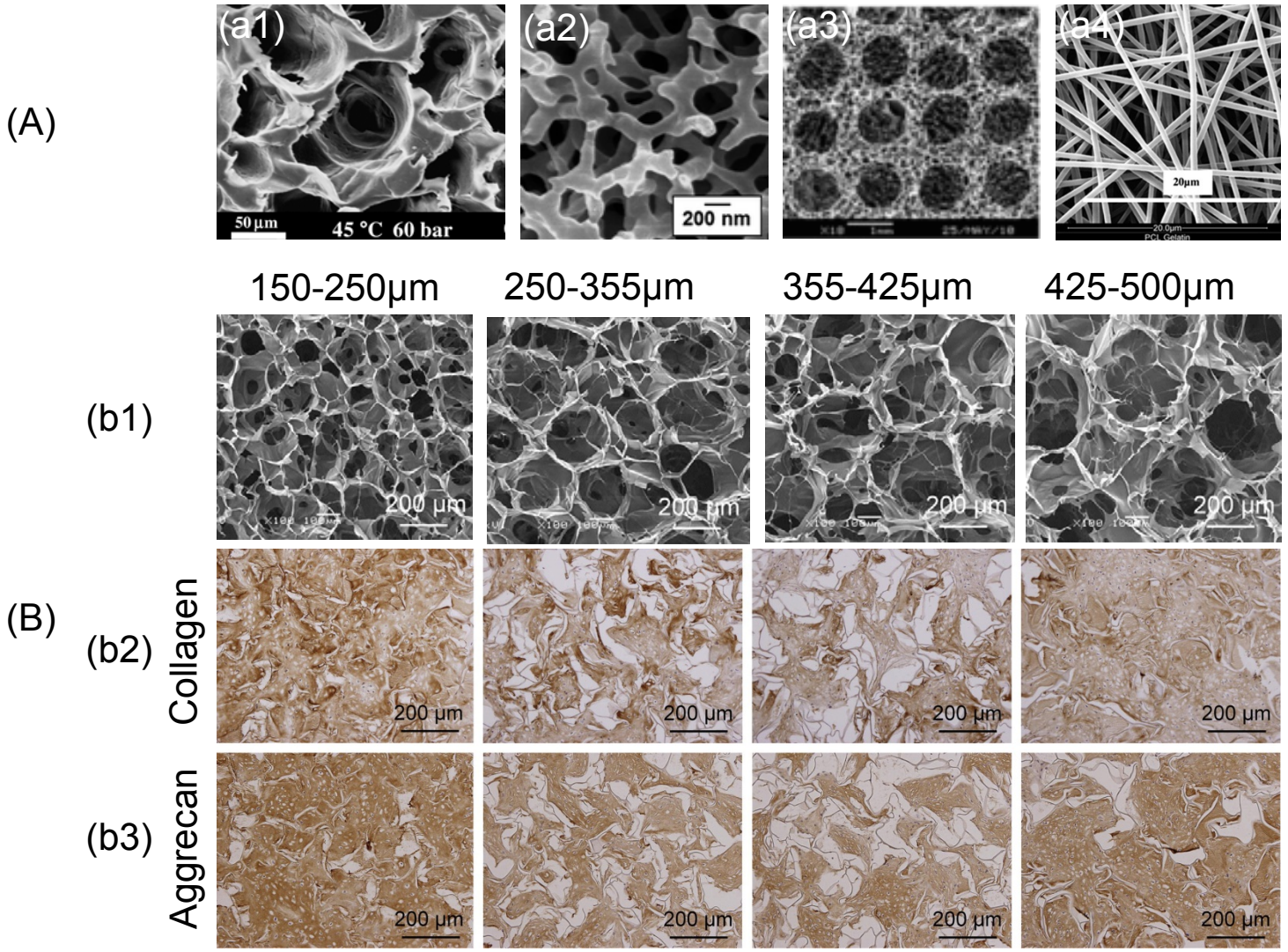


Fig. 2 (A) Several porous structures fabricated by (a1) gas foaming,[76] copyright 2012 Elsevier; (a2) ice thermally induced phase separation,[77] Royal Society of Chemistry; (a3) ice template,[78] copyright 2012 Wiley; and (a4) electrospinning,[79] copyright 2012 Future Medicine. (B) Collagen scaffolds with different pore size and the effect of pore size on cartilage regeneration.[81] (b1) SEM images of collagen scaffold, immunohistochemical staining of type II collagen (b2), aggrecan (b3) of cells in them, copyright 2014 Elsevier.

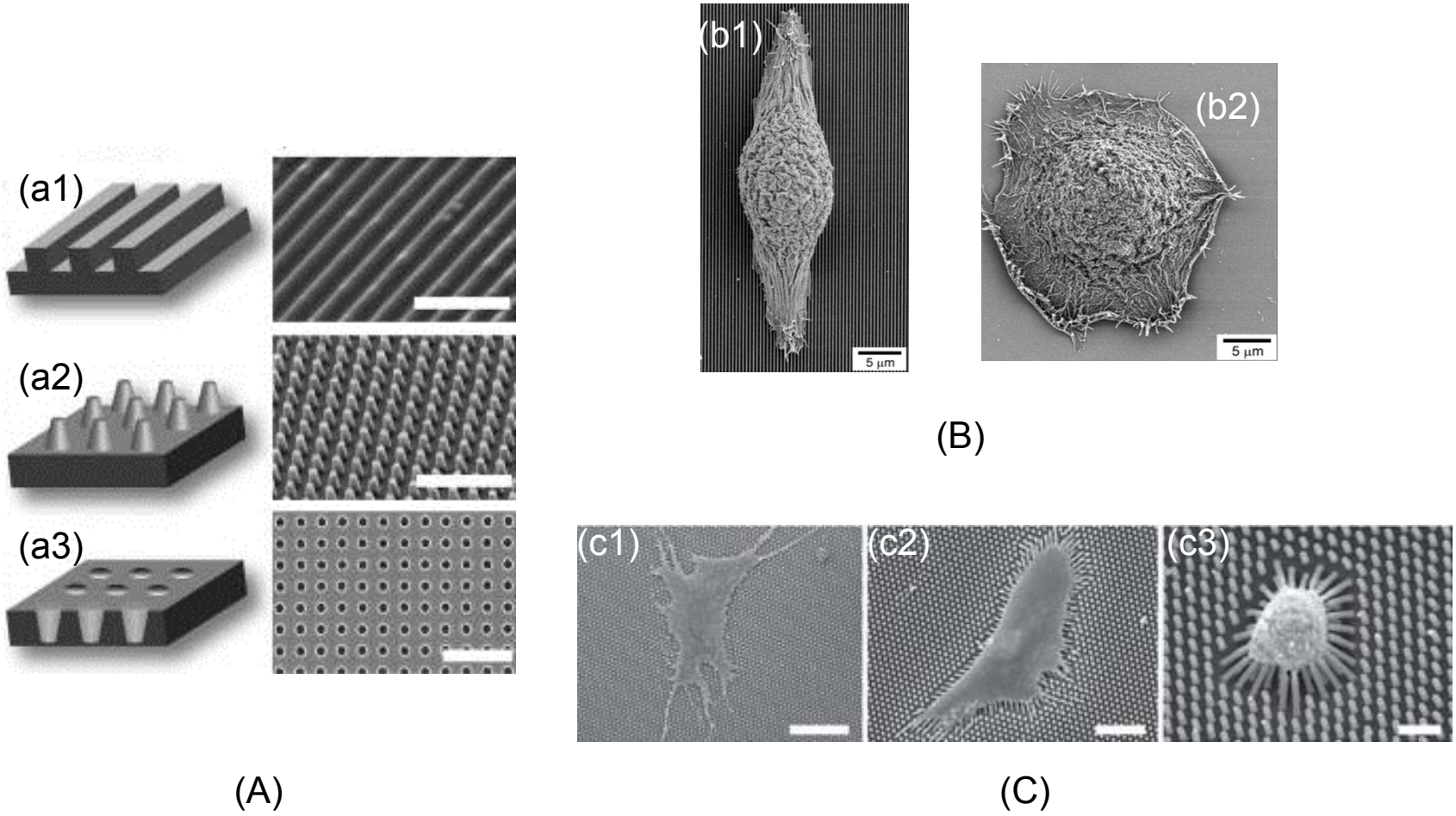


Fig. 3 (A) Schematic presentation of grating (a1), post (a2), and pit (a3) surface topography,[88] copyright 2009 Wiley. (B) Epithelial cells morphology on grating (b1) and flat surfaces (b2),[90] copyright 2003 The Company of Biologists Ltd. (C) hMSCs cultured on different micropost arrays,[91] (c1) 0.97μm, (c2) 6.1μm, (c3) 12.9 μm, copyright 2010 Nature Publishing Group.

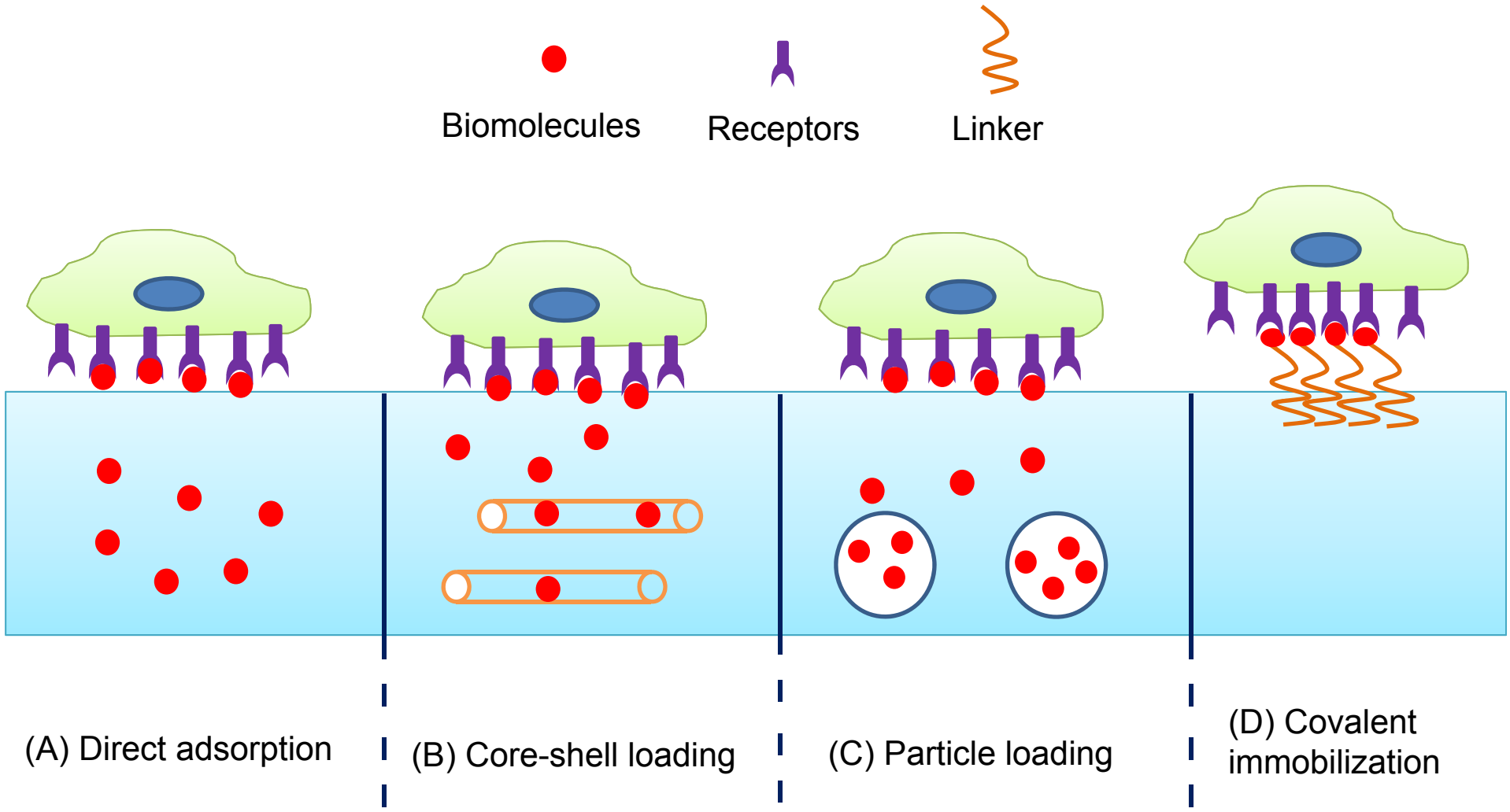


Fig. 4 Schematic presentation of several methods for the control release of biomolecules and their interaction with cells. (A) Direct adsorption, (B) core-shell loading, (C) particle loading, and (D) covalent immobilization.

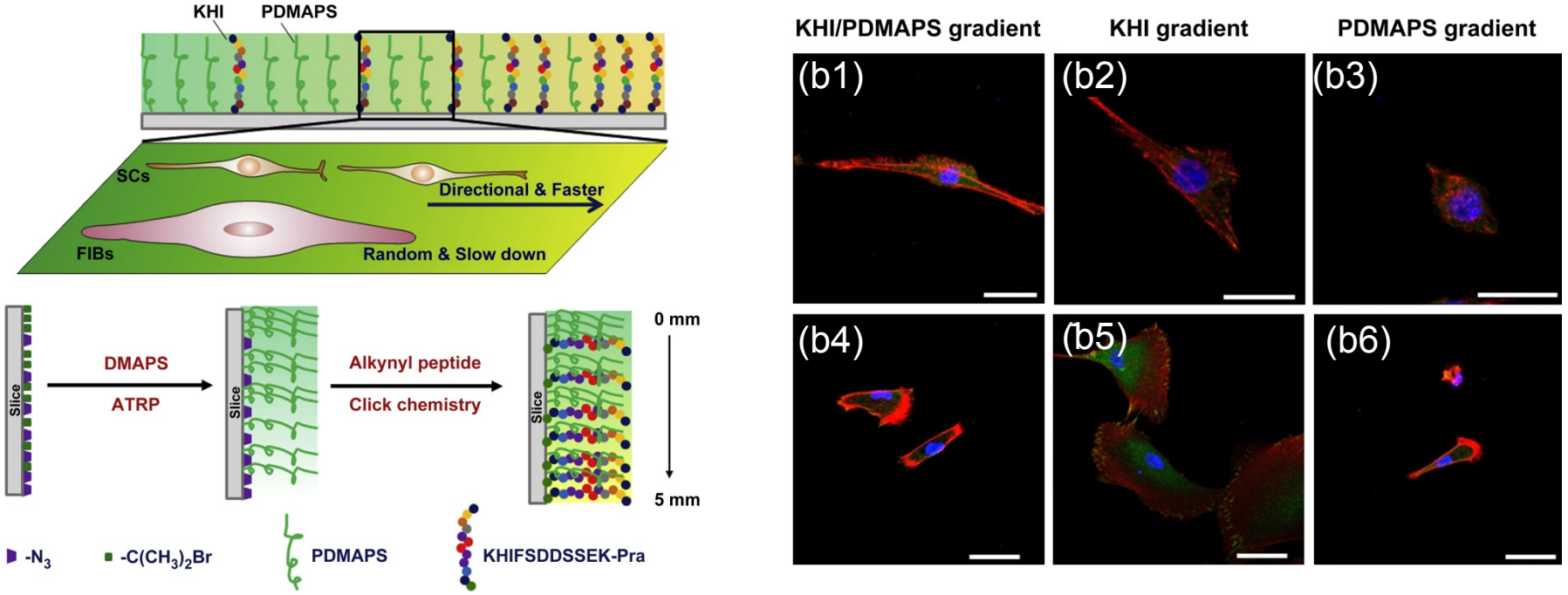
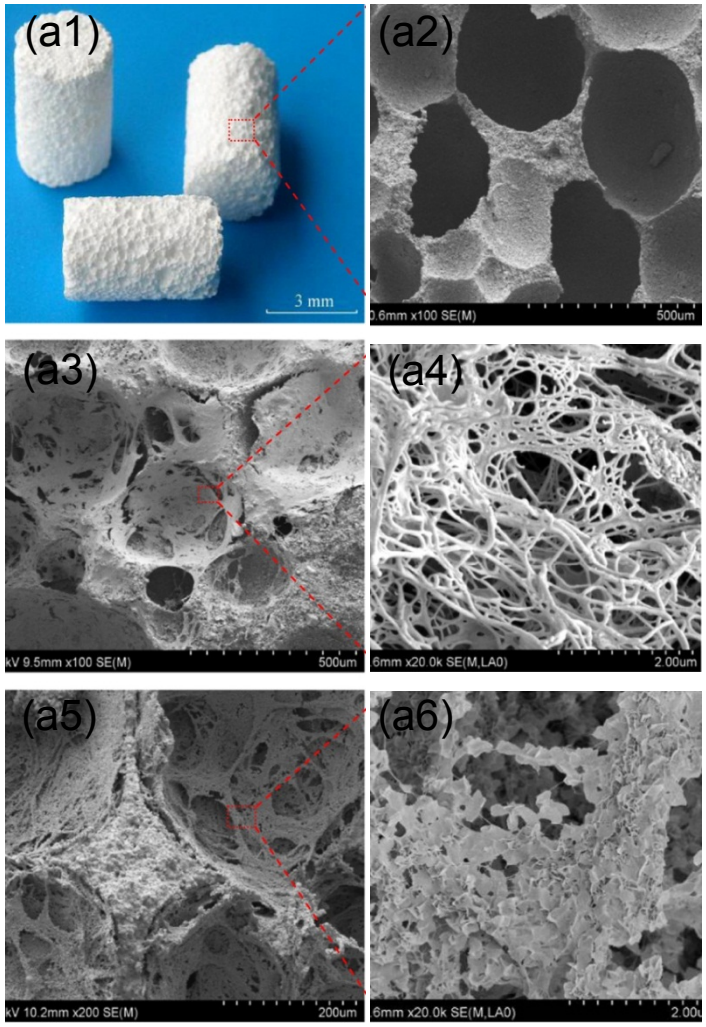
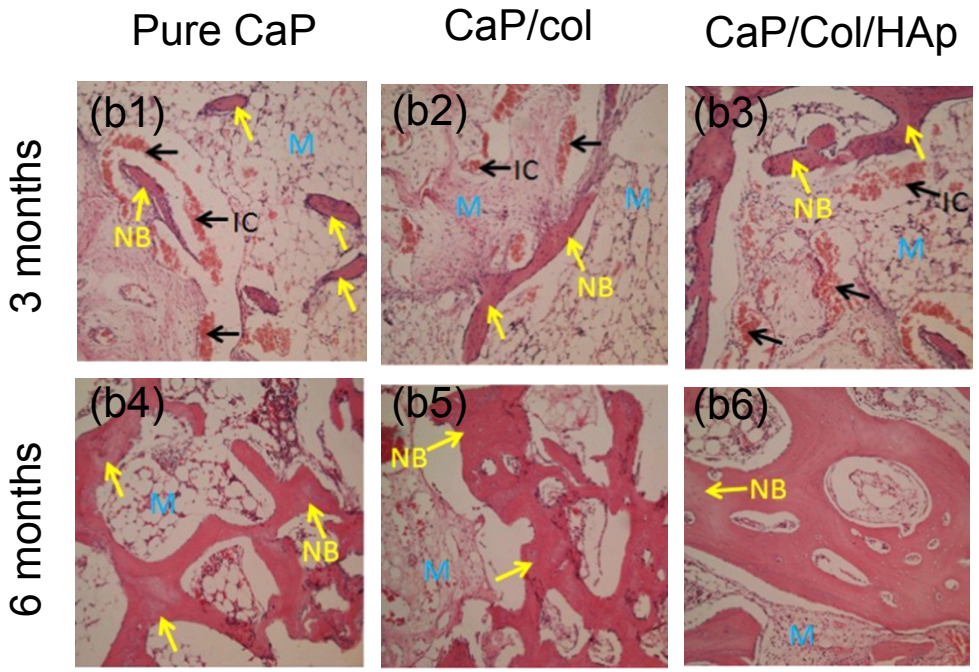


Fig. 5 (A) Schematic illustration to show the structure of a complementary density gradient of PDMAPS and KHIFSDDSSE (KHI in short). (B) CLSM images showing vinculin (green), F-actin (red), and nucleus (blue) of schwann cells (b1-b3) and fibroblasts (b4-b6) on PDMAPS/KHI complementary gradient, KHI gradient, and PDMAPS gradient.[142] Copyright 2015 Elsevier.



(A)



(B)

Fig. 6 (A) Morphology and microstructure of the three-level biomimetic scaffolds. (a1, a2) First-level structures of CaP, (a3, a4) second-level hierarchical structure of the collagen layer, (a5, a6) third-level hierarchical structure of HAp. (B) HE staining of three different scaffolds after 3 and 6 months implantation respectively. (b1, b4) Pure CaP scaffolds, (b2, b5) two level CaP/col scaffolds, (b3, b6) three level CaP/Col/HAp scaffolds. Magnification: 400 ×.[155] Copyright 2014 IOP Publishing.

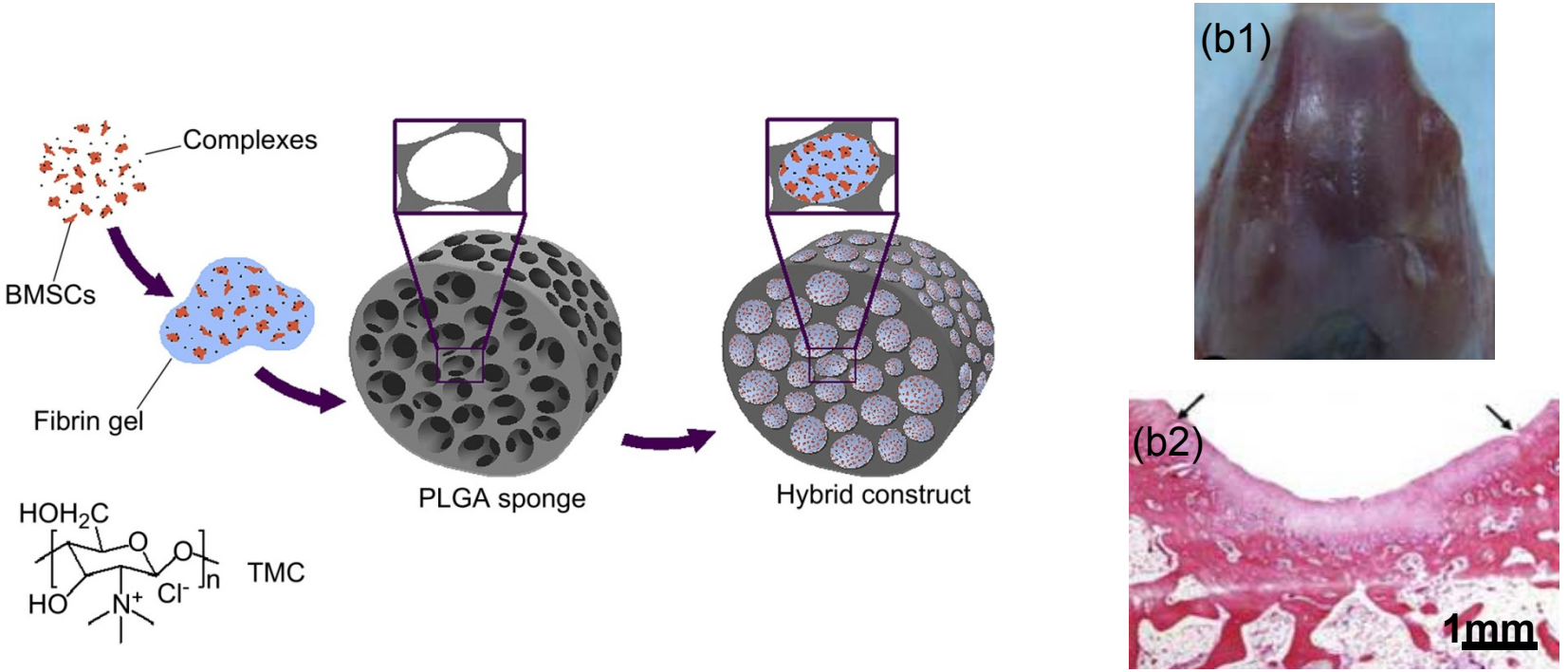


Fig. 7 (A) Schematic illustration of the fabrication procedures of PLGA/fibrin gel/BMSCs/(TMC/pDNA-TGF-β1) construct. (B) Gross view (b1) and histological images (b2) of the neo-cartilage after transplantation for 12 weeks in rabbit knee.[168] Copyright 2010 Elsevier.

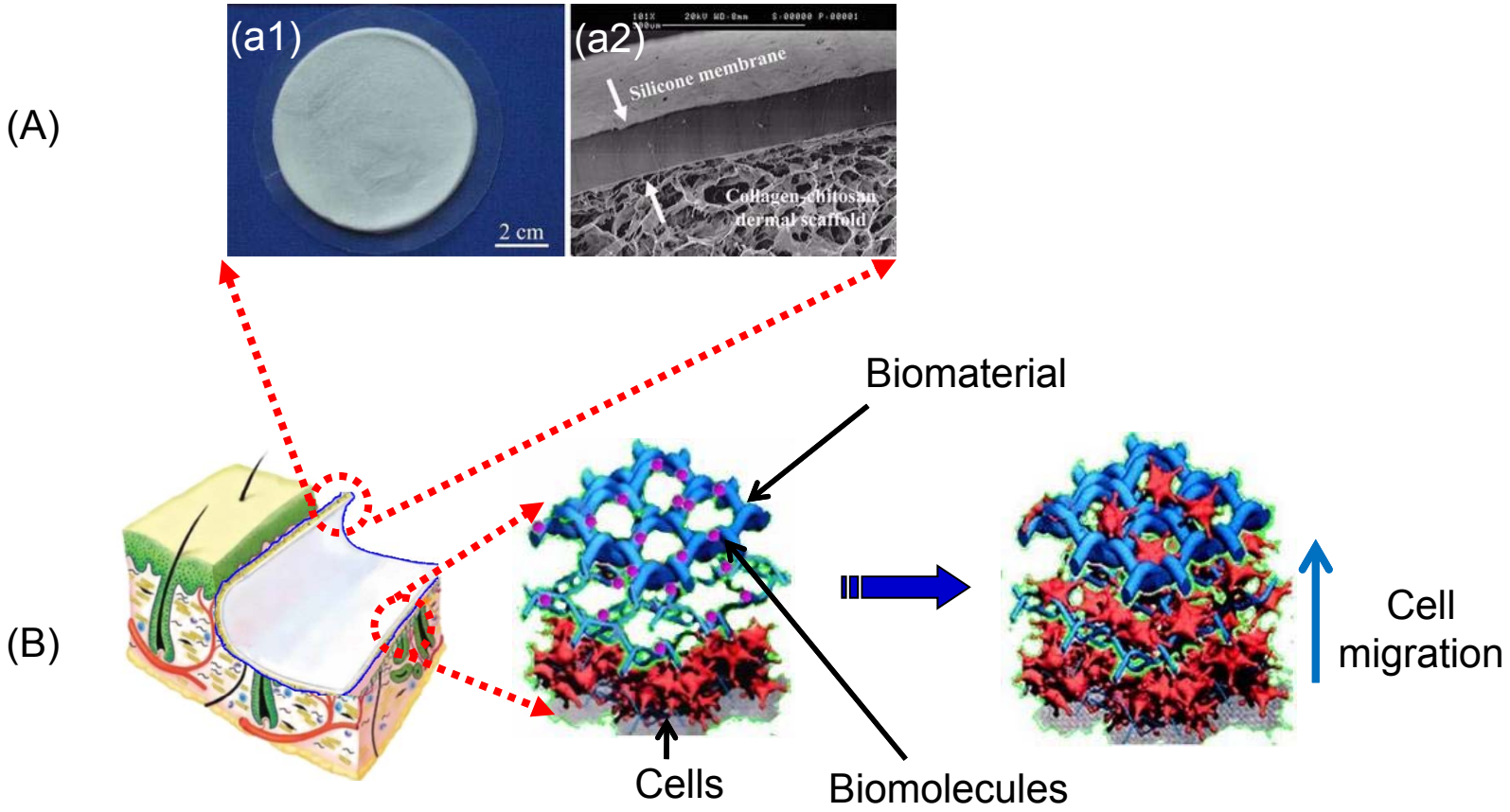


Fig. 8 (A) Macroscopic appearance (a1) and microstructure (a2) of the BDE,[170] copyright 2007 Springer. (B) Schematic presentation of functionalized BDE.

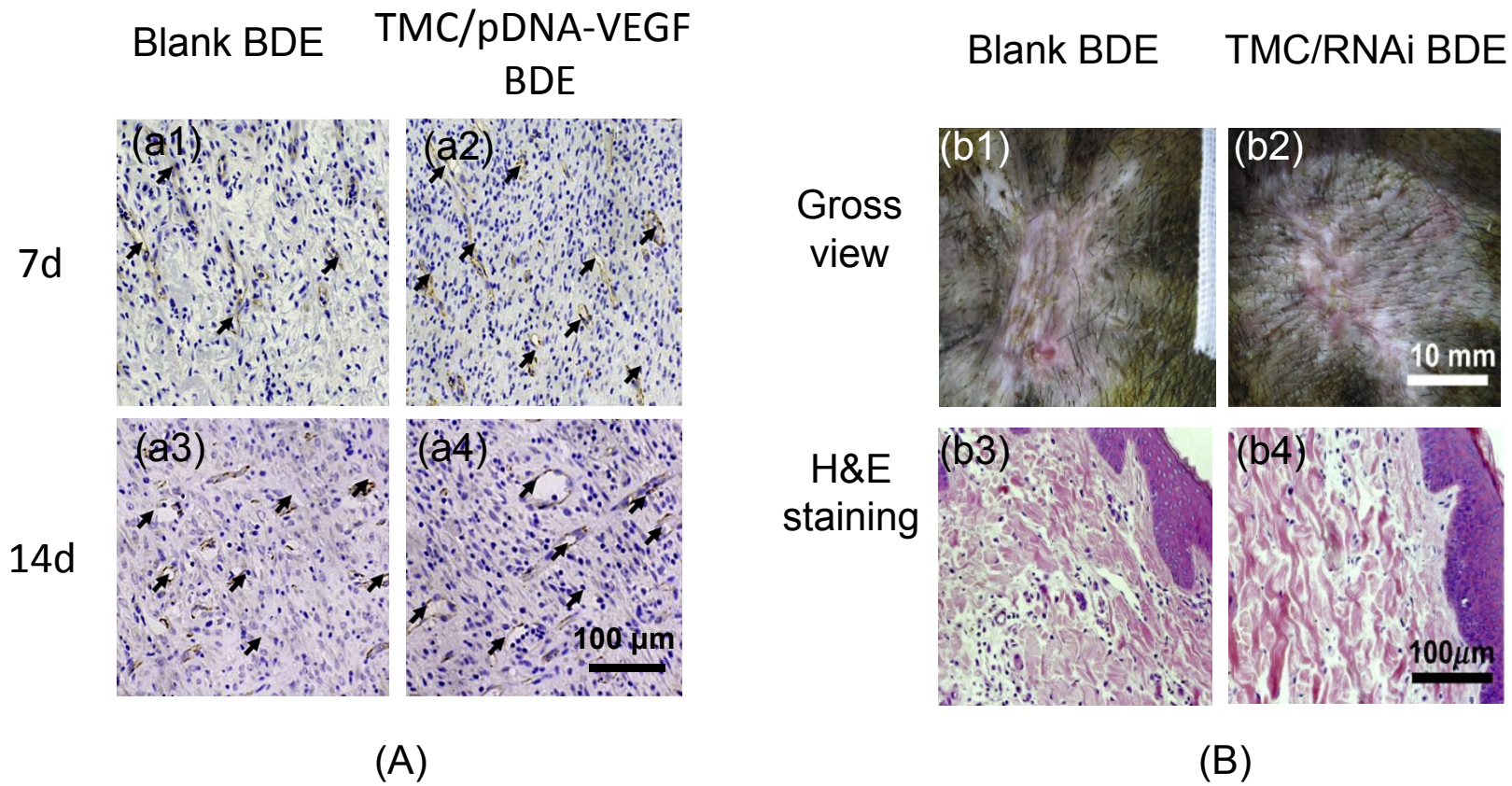


Fig. 9 (A) CD31 immunohistochemical staining of sections of burn wounds treated with blank BDE (a1, a3) and TMC/pDNA-VEGF BDE (a2, a4) at 7d (a1, a2) and 14d (a3, a4), arrows indicate blood vessels,[172] copyright 2010 Elsevier. (B) Gross views (b1,b2) and H&E staining (b3, b4) of wounds treated by blank BDEs (b1, b3) and TMC/RNAi BDEs (b2,b4) for 73 d,[173] copyright 2013 Elsevier.

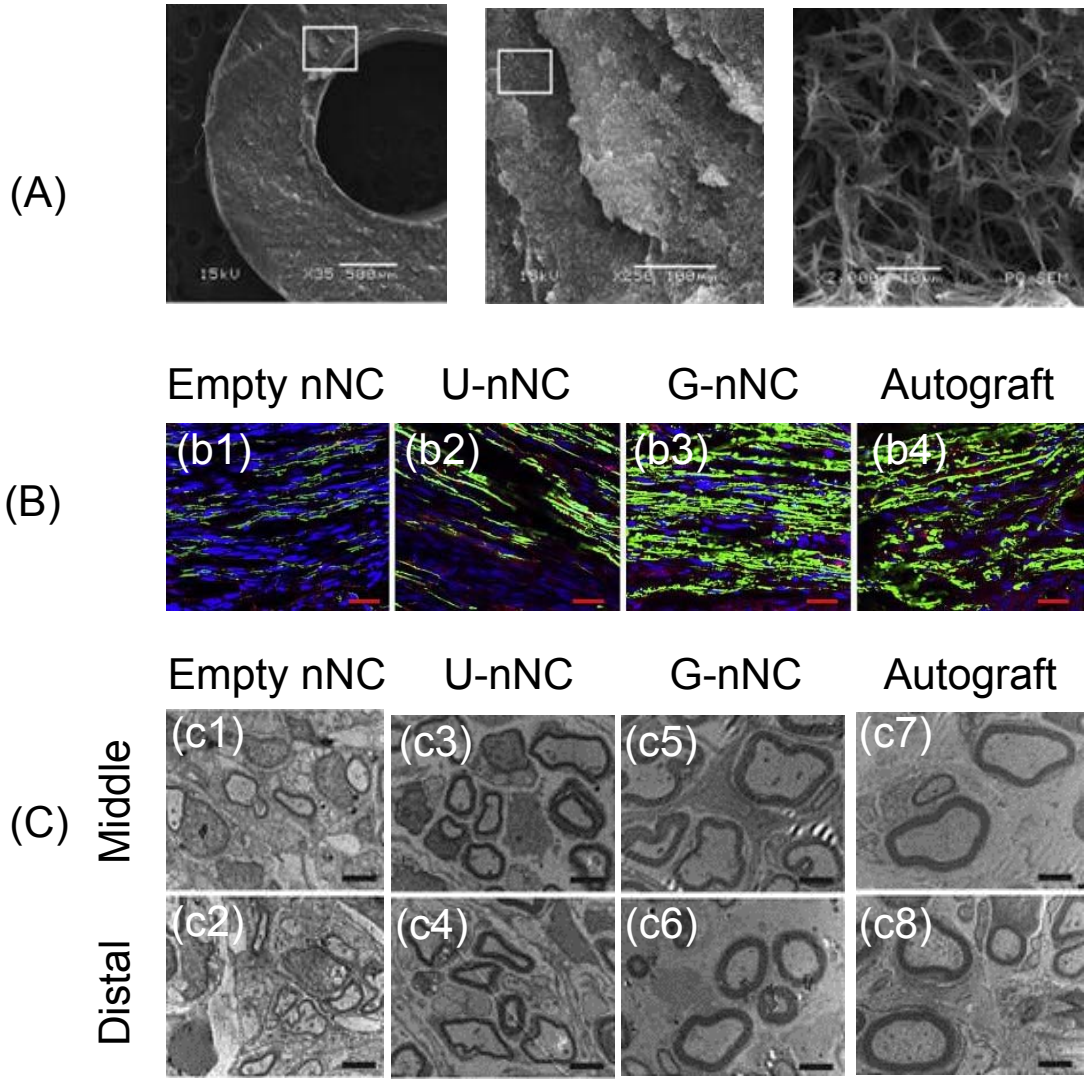
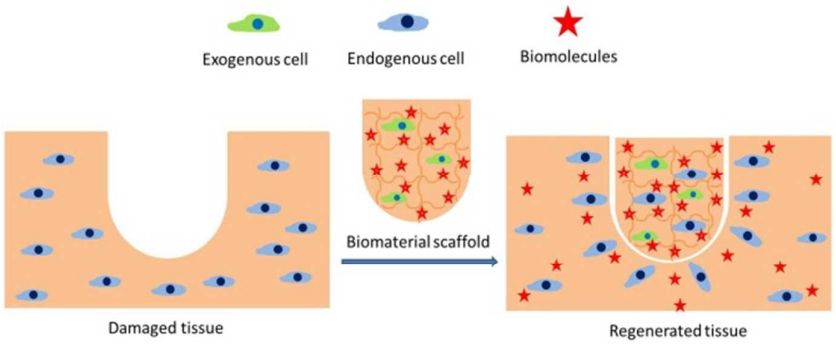


Fig. 10 (A) SEM images of PCLA nanofibrous nNCs immobilized with NGF gradients. (B) Axons regeneration 12 weeks after implantation. (b1) Empty nNC, (b2) uniform nNC, (b3) gradient nNC, and (b4) autograft. (C) TEM images of ultrathin sections showing myelinated axons at the middle and distal portion 12 weeks after implantation. (c1, c2) Empty nNC, (c3, c4) uniform nNC, (c5, c6) gradient nNC, and (c7, c8) autograft.[187] Copyrignt 2013 Elsevier.



Biomaterial is of fundamental importance to *in situ* tissue regeneration, which has emerged as a powerful method to treat tissue defects. The development and perspectives of biomaterials for *in situ* tissue regeneration were summarized.