Review

China’s landscape in regenerative medicine

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**Abstract**

Regenerative medicine is a burgeoning interdisciplinary research field that can impact healthcare by offering new therapeutic strategies to replace or regenerate human cells, tissues, or organs with the ultimate goal of restoring or establishing normal human functions. The past decade has seen significant progress in regenerative medicine in China, the world’s most populous developing country. With government backing, the progress in regenerative medicine is driven by increasing medical demands of people, accompanied by the economic growth, population aging, and lifestyle change in China. Although regenerative medicine encompasses many components, tissue engineering and stem cell technology are generally considered the two key players. In this review article, we outline the representative achievements in the research and application of tissue engineering, stem cell technology, and other regenerative medical strategies attained by various research groups in China, and highlight the major contributions and features of several outstanding studies made by leading Chinese researchers. Where possible, we discuss the unique opportunities and challenges for advancement of regenerative medicine in China. It is our hope that this review will stimulate new research directions for regenerative medicine in general, and encourage strategic collaborations between the east and the west in particular, so that the clinical translation of regenerative medicine can be accelerated to benefit mankind.

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1. Introduction

Although the concept of tissue/organ regeneration is not new and can date back to an ancient Greek myth about Prometheus [1], it was not until recent decades that regenerative medicine emerged as a new interdisciplinary field, which integrates biological principles, engineering approaches, and translational medicine to create therapeutic strategies for replacing or regenerating the diseased and injured tissues/organs, and restoring or establishing normal function [2].

With the economic growth, population aging, and lifestyle change, there have been huge unmet medical needs for treating chronic degenerative diseases and acute traumas in China as in the whole world. Briefly, according to a 2015 national health data report in China, the injuries, accidents, and other joint traumas accounted for 5% of hospitalizations, ranking third after only respiratory diseases (12%) and digestive diseases (10%). Meanwhile, many people suffer from various chronic diseases and cognitive decline. The increased incidence of physical traumas and age-related degenerative disorders, together with tumor ablation and iatrogenic wound, leads to various tissue/organ damage and dysfunction in a growing number of cases, estimated to be approximately 100 million annually [3], a scale unimaginable in the western world. This presents a formidable challenge to deal with this large number of chronic diseases, but at the same time inspires a new solution, such as the application of regenerative medicine as a superior alternative to the classical approaches of tissue/organ repair and regeneration [4,5]. In other words, China pays great attention to the research and application of regenerative medicine [3,6] because the country must develop innovative solutions to improve healthcare of the aging population and to alleviate the associated social and economic burden.

Under strong support from government and society, China is approaching the leading countries in the world in terms of regenerative medicine research, as reflected by the gross expenditure, issued patents, and scientific publications in the field. In the National Medium to Long-term Plan for Scientific and Technological Development (2006–2020), regenerative medicine is listed as one of the five biotechnology fields (http://www.gov.cn/gzdt/2009-08/21/content_1398305.htm). In the Science & Technology on Public
Health in China: A Roadmap to 2050, issued by the Chinese Academy of Sciences (CAS), and the Study on the medium- and long-term Development Strategy of Engineering Technology in China, issued by the Chinese Academy of Engineering (CAE), regenerative medicine is defined as a cutting-edge science and technology field (http://news.sciencenet.cn/htmlnews/2012/12/273300-3.shtm). In addition, the Ministry of Science and Technology (MoST) of China, the National Natural Science Foundation of China, and the Ministry of Health (MOH) of China have jointly formulated a series of policies to stimulate the growth of regenerative medicine in China. In particular, the National Basic Research (973) Programme of China and the National High-Tech Research and Development (863) Programme of China have sponsored a myriad of projects for regenerative medicine research.

The Xiangshan Science Conference sponsored by MoST of China and CAS is considered the top science forum in China. Fortunately, many key issues of regenerative medicine, including its scope, current state and future prospect, and ethical concern, were discussed in 2005, 2010, and 2015 Xiangshan Science Conferences. The Xiangshan Science Conference has also organized several seminars and workshops on specific topics of regenerative medicine.

As another example, in 2016 the National Center for Biotechnology Development (CNCBD) of MoST of China announced a plan to provide 4.545 and 2.159 billion Chinese yuan RMB of grant funding for supporting the 2017 research and development of 197 and 116 projects, which belonged to 2 key programs entitled “stem cell biology and clinical translation” and “biomaterial development and tissue or organ repair” respectively (http://www.cnbcid.org.cn/).

Regenerative medicine represents an innovative paradigm shift in medicine, and the field is generally composed of (but not limited to) two major components: tissue engineering and stem cell-based technology, albeit some overlapping between the two components [7,8]. In this review article, we attempt to outline the current progress of regenerative medicine research and clinical translation in China by listing the main achievements and features of representative research groups. These descriptions are categorized according to the above two components of regenerative medicine. We also discuss the future work directions of regenerative medicine in China.

2. Tissue engineering for regeneration

The terms of tissue engineering and regenerative medicine are often considered as synonymous expressions, although the latter also encompasses another subfield—stem cell-based technologies. Tissue engineering emerged in 1980s in response to the difficulties associated with tissue/organ transplantation, such as donor shortage and immune rejections [9]. Traditionally, tissue engineering refers to the use of biomaterials-based scaffolds, seed cells, and bioactive molecules for engineering the biomimetic tissue-like constructs, which can be implanted into the body to repair the failing tissue/organ and restore the function. In China, tissue engineering for various tissues/organs has been extensively studied.

2.1. Cartilage tissue engineering

Surgical treatments, including microfracture, mosaicplasty, and autologous chondrocyte implantation, are used clinically to repair cartilage defects but without desired outcomes. In contrast, cartilage tissue engineering shows a great potential to effectively restore cartilage structure and function [10].

As early as the late 1990s, Yilin Cao was working with Professor Vacanti at Massachusetts General Hospital (Boston, MA), and the group made headlines by reporting on the growing of a human ear on a mouse through implantation with a polymer-chondrocyte construct [11]. In 1997, Cao returned to China and established the first tissue engineering center in Shanghai ninth people’s hospital (Shanghai). His experience in USA helped persuade Chinese government to pay attention to and invest in tissue engineering, a brand-new research field in China.

Since 1997, Cao and colleagues have constructed tissue engineered cartilage products to repair the defects in large mammals. Their tissue engineered cartilage depends on the use of scaffolds, cells, biochemical factors or the combination thereof. The group led by Cao proposed that the component and structure of cartilage scaffolds determine the mechanical property, biocompatibility, and degradability of the scaffolds and critically influence the outcome of 3-D cartilage regeneration. Cao and Guangdong Zhou and colleagues (Shanghai) used electrospun gelatin/polycaprolactone (GT/PCL) membranes to induce the regeneration of 3-D cartilage, and adopted a relatively suitable ratio of GT/PCL 70:30 for ear-shaped cartilage formation [12]. They also developed in vitro pre-cultivation of tubular constructs, which were made up of a tricopolymer of chondrocyte-polyactic acid (PLA)/polyglycolic acid (PGA), to obtain satisfactory tubular cartilage for tracheal reconstruction with the alleviation of post-implantation inflammatory reaction [13].

Xingdong Zhang and colleagues (Chengdu) investigated the effects of injectable collagen I/III composite hydrogels on chondrocyte behaviors, and their results lay a solid foundation for the application of collagen hydrogel in cartilage tissue engineering [14]. There have been other researchers who reported on a pullulan-based multicomponent injectable hydrogel used as an engineered scaffold for cartilage tissue regeneration [15].

Zhiyuan Zhang et al. (Shanghai) reported on the preparation of uniform chitosan microspheres with a nanofibrous microstructure by applying microfluidic technique to chitosan microspheres with an extracellular matrix (ECM)-mimicking nanofibrous structure, and suggested that the resulting microspheres could be used as the bottom-up cell-carrier components for cartilage tissue engineering [16].

In addition to endeavors of improving the scaffold design, tissue engineered cartilage also benefits from the introduction of seed cells, as manifested in many Chinese researchers’ studies. For example, Wei Sun et al. (Beijing) fabricated an oriented cartilage ECM-derived scaffold with thermally-induced phase separation technique, and the resulting scaffold, in combination with differentiated bone mesenchymal stem cells (BMSCs), successfully repaired full-thickness articular cartilage defects in rabbits, and significantly improved the mechanical property of the regenerated cartilage [17]. As another example, Qiong Wu et al. (Beijing) designed a controlled double-duration inducible gene expression system to regulate the anti-apoptotic gene bcl-2 and the chondrogenic master regulator Sox9, thus improving cartilage tissue engineering [18].

Chinese researchers have noted the favorable effects of BMSCs in cartilage tissue engineering, which included the effect of inducing chondrogenic differentiation [19], ameliorating the scaffold induced inflammation [20], and improving hypertrophy and elastic modulus of engineered cartilage [21]. Moreover, Barbara Pui Chan et al. (Hong Kong) adopted a microencapsulation technique to entrap BMSCs in collagen microspheres, thus engineering a fibrous meshwork scaffold, which was used to investigate the effects of cell density and differentiation status of BMSCs on cartilage repair in rabbits [22]. They also found that other stem cells, such as dermal fibroblasts [23] and adipose-derived mesenchymal stem cells (ADMSCs) [24], were suitable as seed cells within the scaffold to promote cartilage tissue engineering.

Importantly, Hongwei Ouyang et al. (Hangzhou), in
collaboration with the researchers from University of Pittsburgh School of Medicine, (Pittsburgh, PA), reported their new findings that the stem/progenitor cells, which had been derived from adult human chondrocytes, termed chondrocyte-derived progenitor cells (CDPCs), showed similar phenotype as BMSCs but exhibited greater chondrogenic potential. They used specifically-cultured CDPCs for cartilage formation both in vitro and in vivo and, particularly, for repairing large knee cartilage defects (6–13 cm²) in 15 patients with some success [25].

To advance cartilage tissue engineering in China, growth factors and cytokines are introduced into the scaffold to enhance cartilage regeneration, which include basic fibroblast growth factor (bFGF) [26,27], transforming growth factor (TGF)-β1 [28], Chondromodulin-I (Chm-I) [29], insulin-transferrin-selenium (ITS) [30], and Stromal cell-derived factor-1 (SDF-1) [31].

2.2. Chemical tissue engineering

From a chemical point of view, the bone can be regarded as a mineralized composite of inorganic (mostly carbonated hydroxyapatite) and organic (mainly type I collagen) phases having the hierarchical nanostructure and excellent mechanical properties. Tie Wang et al. (Beijing) discussed the mechanisms involved in bone biomineralization and examined the relationships between bone hierarchical structures and bone deformation process [32].

Fuzhai Cui and colleagues (Beijing) developed nanostructured scaffolds to closely mimic the natural bone ECM niche for bone regeneration. Cui’s group prepared a nano-hydroxyapatite/collagen/poly (lactic acid) composite by using a hierarchically biomimetic technique [33,34] or constructed a porous collagen-based bone scaffold [35,36] by adding chitosan/chitin-based reinforcing fibers. To engineer a novel 3-D unwoven macroporous nanofibrous (MNF) scaffold, Xiaohui Zou and colleagues (Hangzhou) fabricated a 3-D unwoven macroporous nanofibrous scaffold with electrospun PLA/polycaprolactone (PCL) nanofibers through sequential yarns manufacture and honeycombing process [37].

Although the commonly-used biopolymers, either of natural or synthetic origin, still represent main options for fabricating scaffolds in bone tissue engineering [38–40], bioactive silicate ceramics have recently gained attention for use as a scaffold biomaterial because they have an excellent ability for apatite mineralization and their ionic products can enhance the proliferation and osteogenic differentiation of osteoprogenitor cells [41]. Keron Dai et al. (Shanghai) and Jiang Chang et al. (Shanghai) have respectively reported their exciting results in ceramic-based bone tissue engineering. They examined the feasibility of porous calcium magnesium silicate bioceramic (akermanite) scaffolds for bone tissue engineering in terms of the preparation, characterization, and in vitro bioactivities of the scaffold [42]; they evaluated the effects of akermanite on bone regeneration in vitro and in vivo [43]; they observed that akermanite, as the osteoinductive material used for bone grafts, possessed an ability to enhance angiogenesis in bone regeneration [44]; they developed mesoporous bioactive glass nanolayer-functionalized, 3D-printed scaffolds for bone regeneration [45]; they performed in vivo tests to show that akermanite bioceramics dramatically stimulated osteogenesis and angiogenesis in an ovariectioned rat critical-sized calvarial defect model [46]. In addition, other inorganic polymers have been extensively evaluated for bone tissue engineering recently in China [47–49].

Several biometals also exhibit the advantages of inorganic biomaterials for bone tissue engineering. Due to their good mechanical properties, biometals may become potent options for preparing the scaffolds with cortical bone-like mechanical properties. Among these biometals, magnesium has the density and mechanical strength similar to those of cortical bone, thus catching the eyes of researchers due to its potential application in bone tissue engineering [50].

Meanwhile, some Chinese researchers attempted to use cellular cues in bone tissue engineering. Cui et al. (Beijing) investigated the osteogenic capacity of different seed cells, including BMSCs, induced-periosteal ADMSCs, and umbilical cord-derived menenchymal stem cells (UCMSCs). And other researchers identified different cell sources for constructing tissue engineered bone products [51,52].

Zhengguo Wang and colleagues (Chongqing) have made substantial progress in using genetically modified, allogenic ADSCs with low immunogenicity (called “universal” stem cells) to construct tissue engineered bone for repairing bone defects in minipigs or pigs [53,54]. They further demonstrated that ADSCs could modulate immunity and induce immune tolerance, providing a mechanistic understanding of the use of ADSCs in bone tissue engineering [55].

In fact, either BMSCs or ADSCs have been extensively studied for cell-based bone tissue engineering by many Chinese research groups, which has been reviewed in details elsewhere [56]. Likewise, molecular cues are also introduced to scaffolds to improve bone regeneration [57–59].

2.3. Tendon tissue engineering

Tendon/ligament injury is a common and debilitating clinical problem in athletes and aged people, and tendon tissue engineering is being developed to treat these injuries. In China, similar strategies are employed for constructing different connective tissues, including cartilages, bones, and tendons/ligaments. Hongwei Ouyang and colleagues (Hangzhou) have made contributions to tendon tissue engineering. The major features of their studies were embodied in (1) the preparation of a nano-microfibrous polymer scaffold by electrospinning nanofibers onto a knitted matrix, where the polymers used were poly(lactic-co-glycolide) (PLGA) [60] and silk/collagen [61,62]; (2) the use of appropriate stem cells including MSCs [63], human embryonic stem cells (hESCs) [64], and hESC-derived MSCs (hESC-MSCs) [62], as supplements to tenocytes and dermal fibroblasts.

On the basis of long-term studies on tendon healing, Huiqi Xie and colleagues (Chengdu) claimed that tissue engineered tendons derived from in vitro co-culture of seed cells and scaffold materials could enhance tendon replacement [65]. They seeded transformed human embryonic tenocytes (THETCs) onto the ECM components-coated poly(lactic-co-glycolide) (PLGA) scaffold to construct human tendons [66,67].

Yilin Cao and Wei Liu and colleagues (Shanghai) implanted an engineered construct, which had been prepared by seeding autologous dermal fibroblasts and tenocytes onto a PGA unwoven fibrous scaffold, to repair a defect of flexor digital superficial tendon in a porcine model, enabling the injured tendons to exhibit the histology similar to that of natural tendon [68].

2.4. Skin tissue engineering

Skin is the largest tissue of the body and serves as a barrier to the environment and for thermal regulation and hydration retention, thus being indispensable for the survival of the organism. Although skin is constantly undergoing renewal with a capacity of repairing wounds, tissue engineering is still applied to fabricate skin substitutes for promoting the healing of acute and chronic skin wounds, which are usually caused by burn or freezing injuries, radiation, surgical procedures, chronic skin ulcers (e.g., venous, pressure, and diabetic foot ulcers), or other dermatological
conditions. In China, the high and constantly increasing incidence of skin wounds, as reported by an epidemiologic study [69], fuels the development of tissue engineered skin substitutes. Several Chinese research groups have made significant progress in skin tissue engineering.

To engineer full-thickness skin substitutes, skin-derived cells (such as keratinocytes, dermal fibroblasts, epidermal stem cells, and melanocytes) and non-cutaneous cells (such as inducible pluripotent stem cells (iPSCs), MSCs, endothelial cells, and amniotic cells) can be used as seed cells; meanwhile, different synthetic and natural biomaterials are selected and optimized to fabricate the scaffold. Many interesting results have been reported by Chinese researchers [70]. For instance, Yan Jin and colleagues (Xi’an) evaluated the outcomes of skin regeneration after implantation of porous gelatin-based implantable particles or bilayered skin substitutes consisting of ECM and microspheres-containing gelatin hydrogel [71,72], and they prepared a tricopolymer scaffold for dermal tissue engineering by crosslinking collagen, chondroitin sulfate, and hyaluronic acid to imitate ECM structure [73]. Jin’s group also investigated the multiple functions of BMSCs as seed cells in skin tissue engineering, such as the promotion of full-layer cutaneous wound vascularization and regeneration [74] and the prevention of scar formation through inflammatory regulation [75]. Moreover, Jin et al. reported their attempts of using other seed cells, such as a combination of keratinocytes, melanocytes, and dermal fibroblasts [76], a combination of human amniotic mesenchymal cells and human amniotic epithelial cells [77], or a combination of fibroblasts and adipose tissue-derived stem cells [78], to improve skin wound repair.

Usually, artificial skin substitutes fail to completely replicate the anatomy and physiology of natural skin due to difficulties in reconstructing skin appendages (including sweat glands, sebaceous glands, and hair follicles). The group led by Xiaobing Fu (Beijing) has engineered a 3-D human skin construct by culturing sweat gland cells (SGCs) on the epidermal growth factor (EGF)-containing gelatin microspheres and then delivering the resulting complex into the collagen-based, seed cells-containing skin scaffold. Importantly, this engineered construct improved the quality of skin repair and maintained homeostasis during the process of skin regeneration and wound healing after being implanted into full-thickness cutaneous wounds in an athymic murine model [79]. Accordingly, the work of Fu and colleagues represents a pioneering example in developing the tissue engineered skin with cutaneous appendages (Fig. 1). Recently, Fu’s group designed a 3-D bioprinted ECM that provided the spatial inductive cues for enhancing specific differentiation of epidermal lineages to regenerate sweat glands, which is critical for treating deep burns or other wounds. The novelty and significance of the work lies in the overwhelming advantages of the 3-D bioprinting construct over other cell delivery technologies in maintaining high cell proliferation; another interesting finding is that adult tissue components retain a gland lineage-inductive power as embryonic tissue, which can facilitate translation. This study may also lead to the development of a new generation of engineered skin with functional sweat glands [80].

Fu’s group found that epidermal cells could be dedifferentiated into epidermal stem cells in vitro and in vivo under growth factor stimulation [81], and evaluated the possibility of using several stem cells for seed cells in skin tissue engineering [82]. Also, Fu’s group successfully induced human BMSCs to differentiate into SGC-like cells with cell phenotype and functions similar to normal SGCs by using a unique protocol (mainly using shock stimulation and temperature control) [83–85]. In addition to the studies on epidermal cell dedifferentiation and stem cells-based sweat gland regeneration, Fu’s group has made multifaceted contributions to skin tissue engineering, including the elucidation of growth factor regulation of wound healing (for the details see the following section 4), the analysis and prevention of serious complications of skin trauma and burn, and the establishment of education program for chronic wound care in China [86].

2.5. Corneal tissue engineering

Corneal damage results from a variety of clinical disorders or chemical, mechanical, and thermal injuries. Tissue engineered corneal stroma represents a promising strategy to overcome donor shortage in cornea replacement with allografts. Yan Jin and colleagues (Xi’an) seeded rabbit stromal keratocytes onto a scaffold made up of decellularized porcine cornea, thus engineering a corneal stroma to be implanted into a model of corneal ulcer [87]. They also prepared another type of engineered cornea composed of amniotic epithelial cells and acellular porcine cornea for treating corneal alkali burn [88]. Zhichong Wang and colleagues (Guangzhou) used phosphopase A(2) to allow enzymatic decellularization of native porcine cornea for preparing acellular porcine corneal stroma (APCS), which, as a tissue engineering scaffold, showed no significant difference compared to native corneas after subcutaneous implantation [89]. In a later study [90], they seeded a combination of corneal epithelial cells (CECs) and genetically modified embryonic stem cells (ESCs) between the acellular porcine corneal stroma and the amniotic membrane, and the formed engineered lamellar cornea showed the better epithelial barrier functions and wound healing abilities in a rabbit model of amellar transplantation. Yilin Cao et al. (Shanghai) demonstrated that intrastromal implantation of a PGA-based, corneal stromal cells-containing scaffold was successfully used for corneal stromal tissue reconstruction without compromising the tissue transparency [91], and the researchers also reported on an implantation with a sandwich-like combination of acellular corneal stroma sheets and keratocytes for corneal stroma regeneration in a rabbit corneal stroma defect model [92].

2.6. Tooth tissue engineering

Although tooth damage or loss is one of the most common diseases and affects the quality of life of people, current tooth replacement methods can not lead to biological restoration and satisfactory outcomes. Tooth tissue engineering involving the recapitulation of the embryonic environment has attracted interest worldwide as well as in China.

Yan Jin et al. (Xi’an) prepared a dentin matrix (TDM) scaffold loaded with dental follicle stem cells (DFCs) and implanted it into the alveolar fossa, thus successfully regenerating tooth roots [93]. The researchers noted that (1) skin epithelium-derived cells from postnatal rats were able to convert to functional ameloblasts under effective induction [94]; (2) dental-derived and some non-dental-derived mesenchymal stem cells were both capable of periodontal regeneration under certain conditions with induced differentiation, proliferation, cellular secretion, and their interactions [95]; (3) stem cells from root apical papilla (SCAPs)-based scaffold-free stem-cell sheet-derived pellets (CSDPs) with a mount of endogenous ECM were capable of forming a heterotopic dental pulp/dentine complex in empty root canals, thus providing an alternative treatment approach for pulp disease [96].

Weidong Tian et al. (Chengdu) focused on the application of dentin matrix (TDM) scaffold in tooth engineering. In their studies, the TDM scaffold was used in combination with human dental follicle cell sheets (DFCs) for tooth root regeneration [97], in combination with dental follicle cells (DFCs) for regeneration of complete dentin tissues [98], or in combination with aligned PLGA/
gelatin electrospun sheets and native dental pulp ECM for tooth root regeneration [99], respectively. They also optimized the shape of TDM scaffold, which was combined with dental stem cells for tooth root regeneration [100].

In addition, Songling Wang et al. (Beijing) reported on the use of allogeneic hydroxyapatite tricalcium phosphate (HA/TCP)/dental pulp stem cell (DPSC)/periodontal ligament stem cell (PDLSC) sheet for building a tooth root, whose structure and function were similar to those of natural tooth root [101]. Xinquan Jiang et al. (Shanghai) demonstrated that implantation of a tissue engineered bone equivalent made up of porous beta-tricalcium phosphate (β-TCP) and osteogenically induced BMSCs could repair alveolar cleft and allowed subsequent orthodontic tooth movement [102].

2.7. Cardiovascular tissue engineering

Cardiovascular disease, such as coronary artery disease, stroke, and congestive heart failure, is a leading cause of mortality in China as well as worldwide, and cardiovascular tissue engineering aims to create functional substitutes for heart valves, arteries, and myocardium [103]. Just like other subfields of tissue engineering, cardiovascular tissue engineering uses biomaterial-based scaffolds, seed cells, soluble mediators, or a combination of these for recapitulating the normal structure and function of cardiovascular tissues.

Changyong Wang and colleagues (Beijing) mixed embryonic stem cell (ESC)-derived cardiomyocytes with type I collagen to construct engineered cardiac tissue, which was able to beat synchronously and similar to neonatal native cardiac muscle in the structure and function [104]. Wang et al. generated an engineered heart tissue from nuclear transferred embryonic stem cells (ntESCs)-derived cardiomyocytes in a home-made mould through the improved techniques, and found that after implantation, the engineered heart tissue could integrate and electrically couple to host myocardium to improve the left ventricular function of infarcted rat heart [105]. They also investigated the favorable effect of telocytes on the quality of engineered heart tissue [106]. On the other hand, Wang et al. used an injectable cardiac tissue engineering approach, and showed that chitosan and oligo(poly-ethylene glycol)fumarate (OPF) hydrogels could constitute an effective injectable scaffold to deliver stem cells (including ESCs, nt-ESCs, ADSCs, and iPSCs) or some therapeutic agents into ischemic myocardium [107–110].

Despite the effectiveness of metallic stents in preventing acute occlusion and reducing late restenosis after coronary angioplasty, bioabsorbable sirolimus-loaded poly-L-lactic acid (PLLA) stents (Xinsorb™) have been engineered by Junbo Ge and colleagues (Shanghai), and Xinsorb™ was used as an alternative to metallic stents to overcome their adverse effects. Ge’s group further evaluated the preclinical and clinical outcomes of their product [111–114].

Over past years, Chinese researchers have focused considerable attention on the development of biomimetic vascular substitutes, which had both mechanical and functional qualities for bypass surgery to treat coronary artery and peripheral vascular pathologies. Qiang Zhao and colleagues (Shanghai) applied an electrospun nano–polycaprolactone coating to a decellularized rat aorta and used exogenous heparin to modify the acellular vascular intima, thus engineering a hybrid small-diameter vascular grafts with good mechanical properties, physical stability, anticoagulation properties and biological compatibility [115]; Yilin Cao et al. (Shanghai) constructed a small diameter (less than 6 mm) elastic vessel wall by using smooth muscle cells (SMCs) differentiated from human
adipose-derived stem cells (hASCs) under pulsatile stimulation in a bioreactor [116].

2.8. Neural tissue engineering

Traumatic and degenerative injuries to the central or peripheral nervous system (CNS or PNS) constitute a common and challenging clinical problem. Following nerve injury, a series of pathophysiological changes occur around the lesion site, leading to nerve degeneration and functional loss. The adult mammalian CNS is unable to regenerate on its own, while the PNS has a certain capacity for axonal regeneration but with poor functional recovery [117]. In China, neural tissue engineering has been actively developed to advance the treatment of neural injuries [118].

To construct a neural scaffold for implantation into a lesioned area of the brain or spinal cord, Xiaoguang Li and colleagues (Beijing) screened for chitosan, a polysaccharide derived from chitin, as an optimal biomaterial, and studied the enhancing effect of neurotrophin-3 (NT-3), a neurotrophic factor on proliferation and area of the brain or spinal cord, Xiaoguang Li and colleagues (Beijing) have been actively contributing to the treatment of neural injuries [118]. In China, neural tissue engineering has been actively developed to advance the treatment of neural injuries [118].

To construct a neural scaffold for implantation into a lesioned area of the brain or spinal cord, Xiaoguang Li and colleagues (Beijing) screened for chitosan, a polysaccharide derived from chitin, as an optimal biomaterial, and studied the enhancing effect of neurotrophin-3 (NT-3), a neurotrophic factor on proliferation and differentiation of neural stem cells [119,120]. Thus, they prepared a chitosan/collagen scaffold or a chitosan-based, NT-3-containing scaffold for implantation into the injured site of the adult rat thoracic spinal cord or into the CA1 region of the adult rat hippocampus, respectively, and observed that axon regeneration and functional recovery were promoted following the repair of spinal cord injury (SCI) or brain injury [121,122]. Later, they observed that after being implanted into a 5 mm gap of transected thoracic spinal cord in rats, NT3/chitosan elicited robust activation of endogenous neural stem cells (NSCs) to interconnect the severed ascending and descending axons, thus improving the sensory and motor behavioral recovery [123]. Li et al. further performed the weighted gene coexpression network analysis, and concluded that the enhanced new neurogenesis and vascularization, as well as the reduced inflammatory responses, were responsible for the effect of NT3/chitosan on regeneration [124].

Jianwu Dai and colleagues (Beijing) have engineered two types of functionalized collagen-based nerve grafts, which were used to repair a completely transected SCI in canine and rodent model, respectively. The grafts promoted axonal regrowth and facilitated spinal cord regeneration, holding great promise in clinical treatment of SCI paralysis or other movement disorders caused by neurological diseases [125,126]. Moreover, Dai et al. engineered collagen/BMSCS constructs for repairing severe uterine injury in rats, achieving desired results [127]. Their findings have been translated into clinical trials in several successful cases.

Yuanshan Zeng and colleagues (Shanghai) demonstrated that BMSCs seeded onto a three-dimensional gelatin sponge scaffold could attenuate inflammation, promote angiogenesis, and reduce cavity formation in the transected rat spinal cord [128], and that BMSCs on a gelatin sponge scaffold could differentiate toward neuronal lineage under the induction of Schwann cells through the mediated secretion of growth factors, such as brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), bFGF, and glial cell line-derived neurotrophic factor (GDNF) [129]. Based on these findings, Zeng et al. developed a NT-3/fibroin-coated gelatin sponge scaffold as a controlled release vehicle to facilitate the regeneration of injured spinal cord in rats or dogs [130]. In their another study, BMSCs and Schwann cells were seeded and cultivated onto a gelatin sponge scaffold to allow the deposition of differentiating BMSC-secreted fibronectin on the scaffold surface, and the resulting scaffold induced neurite elongation in vitro and promoted nerve fiber regeneration in transected SCI [131].

Fuzhai Cui et al. (Beijing) focused on the development of hyaluronic acid (HA)-based neural scaffolds for central neural tissue engineering. They modified HA-based hydrogels with poly-L-lysine (PLL) and nogo-66 receptor antibody (antiNgR), and implanted the prepared construct of HA-PLL/antiNgR to rats with lateral hemisection of the spinal cord, thus enhancing axonal regrowth [132]. They also reported that the laminin- or Ile-Lys-Val-Ala-Val (IKVAV) peptides-modified HA hydrogels helped neural regeneration to a certain degree in the CNS after implantation into the lesioned cortex or cerebrum of rats, respectively [133,134].

In the past decades, Chinese researchers have devoted much efforts to tissue engineering used for peripheral nerve repair and made significant progress toward clinical translation [135]. Peripheral neural tissue engineering aims to create innovative biomimetic nerve grafts as a promising alternative to autologous nerve grafts, a golden standard for peripheral nerve repair.

Xiaosong Gu (Nantong) proposes the essential principles about the construction of tissue engineered nerve grafts [136], and his group has long contributed to the search and development of desirable biomaterials for fabricating neural scaffolds for peripheral nerve regeneration [137], thus becoming one of the earliest research groups to characterize and utilize chitosan and silk fibroin protein in peripheral neural tissue engineering [138–141]. Gu et al. engineered two types of nerve grafts by seeding BMSCs onto silk fibroin- and chitosan-based neural scaffolds to bridge 10 mm sciatic nerve gaps in rats [142], and 50 or 60 mm long sciatic nerve gaps in dogs [143,144], respectively. They further developed chitosan-based, BMSCs-containing tissue engineered nerve grafts to bridge 50 mm long medium nerve gap in rhesus monkeys (Fig. 2), inducing successful nerve repair and functional restoration and suggesting a safety of BMSC-based cell therapy [145]. They also carried out a preliminary study for an in vivo application of molecular cues in peripheral neural tissue engineering [146]. In addition, Gu's group isolated an effective plant ingredient (called Achyranthes bidentata) from a traditional Chinese medicine, and included it into tissue engineered nerve grafts to serve as a growth factor-like biomolecule [147,148].

With the backing of abundant experimental data, Gu et al. have launched a prospective randomized controlled multicenter study in four Chinese public hospitals after receiving the approval from the Chinese State Food and Drug Administration (CFDA), and thus they became the first pathfinders in China to translate peripheral neural tissue engineering research into the clinic [149]. They reported two human cases, where 30 and 35 mm long medium nerve gaps were repaired by a chitosan-based neural scaffold, and the three-year follow up confirmed overall functional recovery of injured nerves in both patients [150,151].

Recently, Gu's group has been studying the optimization of a regenerative microenvironment within a nerve graft by modifying a neural scaffold with the seed cell-derived ECM structure [152,153].

In fact, acellular nerve grafts prepared by decellularization of allogeneic and xenogeneic nerve (or non-nerve) tissues are also regarded as ECM modified nerve grafts, but the ECM herein is derived from tissues rather than cells. Xiaolin Liu and colleagues (Guangzhou) engineered acellular nerve allografts loaded with platelet-rich plasma or etifoxine to bridge 15 mm long gap in rat sciatic nerve, and these tissue engineered grafts enhanced nerve regeneration and functional recovery and produced the outcomes nearly as well as autografts [154–157]. Furthermore, Liu et al. performed a prospective multicentre controlled clinical trial, which involved 72 patients enrolled and had a 1–6 month follow-up, to evaluate the safety and efficacy of human acellular nerve grafts in the repair of 1–5 cm long human digital nerve injury [158]. More recently again, they provided another case report where a total of 64 patients from 13 hospitals were subjected to a 3 year follow-up after the nerve injury repair in the hand and upper extremity with human acellular nerve allografts [159].
Shibi Lu and Jiang Peng and colleagues (Beijing) investigated the effect of adenoviral transfection with hepatocyte growth factor (HGF) on the functional outcome of repairing rat transected sciatic nerves using acellular nerve grafts [160]. They introduced BMSCs [161], Schwann cell-like cells derived from BMSCs or ADSCs [162], and BMSC-containing fibrin glue [163] to acellular nerve allografts respectively, which were implanted into rodents to bridge a sciatic nerve gap. The results suggested that addition of cellular cues to acellular nerve allografts was helpful at improving nerve regeneration and maintaining nerve structure.

2.9. Organ engineering

Regenerative medicine focuses on the engineering of artificial tissues or whole organs for clinical repair applications. As is well known, end-stage organ failure caused by diseases or traumas is one of the most devastating problems in health care. Accordingly organ transplantation has been becoming a viable therapeutic strategy for an increasing number of patients suffering from irreversible organ failure, but this therapeutic strategy is limited by a severe shortage of donor organs. To overcome this challenge, organ engineering is developed through decellularization of donor organs to provide an acellular, naturally-derived biologic scaffold and then recellularization of the scaffold with stem cell populations [164,165]. In this respect, Chinese researchers have made several interesting attempts.

Shusen Zheng and Ming H Zeng and colleagues (Hangzhou and Hong Kong) used a by-pass circulation through the portal vein and infra-hepatic vena cava with a perfusion chamber system to decellularize the single liver lobe and recellularize it with allogenic primary hepatocytes in rats. The decellularization process in vivo preserved the vascular structural network and functional characteristics of the native liver lobe. After efficient recellularization with allogenic primary hepatocytes, blood circulation was reestablished, the function of liver lobe was regained, and the allogenic hepatocytes could secret albumin [166].

Mei Jin et al. (Wenzhou) reported a simple method by which rat kidneys were perfused with detergents through the abdominal aorta to obtain decellularized kidney scaffolds with vascular structure [167]. Shengtian Zhao et al. (Jinan) also performed decellularization with sodium dodecyl sulfate solution and Triton X-100 to produce renal ECM scaffolds of porcine kidneys, which preserved intact microarchitecture, including the renal vasculature and essential ECM components [168].

Hong Bu and colleagues (Chengdu) prepared whole porcine liver and kidney by decellularization with detergent perfusion via the portal vein and renal artery of the liver and kidney, respectively. They found that cellular components and xenoantigens were...
cleared and ECM composition was retained in the resulting liver and kidney scaffolds [169].

3. Stem cells for regeneration

Regenerative medicine attempts to repair the diseased or damaged tissues/organisms and restore their normal functions by providing specific healthy cell population, alone or included within a tissue engineered scaffold, to stimulate an intrinsic healing capacity of the body. Accordingly, tissue engineering and cell therapy are the two wings of regenerative medicine. Since living cells specific to various tissues do not fully meet the requirements of in vivo implantation, stem cells, used as seed cells having a capacity for self-renewal and a potential to differentiate into various cell lineages specific to various mature tissues, have provoked great interest for regenerative medicine use. Stem cells are often divided into two broad types: embryonic stem cells (ESCs) found in the germ lineages of mature animals and induced pluripotent stem cells (iPSCs) generated from somatic cells. iPSCs have been shown to acquire the pluripotent level of ESCs and iPSCs [198].

3.1. Stem cell transplantation

Besides hematopoietic stem cell transplantation for leukemia and other hematological disorders, more types of stem cell transplantation have been used to recover the normal cellularity, although they are still at the experimental or preclinical stage. In China, some research groups have attempted to directly inject stem cells to the injured or diseased tissue/organ for treating coronary heart diseases [170,171], diabetes [172], liver failure [173,174], skin trauma [175–177], intracerebral hemorrhage and ischemic stroke [178–180], neurological disorders [181–185], and other diseases. In this way, the researchers wanted to build a solid foundation for clinical application and product commercialization.

Here, two examples of stem cell transplantation for skin repair deserve to be highlighted. For sweat gland regeneration after deep burn injury in patients, Fu’s group (Beijing) enabled BMSCs to acquire the phenotype of sweat gland cells (SGCs) by induced differentiation in vitro, and then transplanted these SGC-like cells into fresh skin wounds harvested from the excised anhidrotic scars after healing the deep burn injury in five patients. Histological and biochemical observations confirmed the recovery of perspiration in the transplanted areas. This is the first report of successful transplantation of MSCs for regenerating functional sweat glands in the world and ten-year follow-up in some typical cases confirmed that these regenerated sweat glands remain to play the role of sweating [176].

Similarly, to reconstruct normal skin tissues, Jun Wu and colleagues (Chongqing) systematically investigated the growth potential and differentiation capacity of porcine embryonic skin precursors, and they observed that transplantation of porcine embryonic skin precursors (PEPSs) of different gestational ages to nude mice could generate the integral skin, including epidermis, dermis, and skin appendages [177].

It has been reported that some technical measures are adopted to enhance the efficacy of direct injection of stem cells. Xuetao Pei et al. (Beijing) developed a microencapsulation-mediated intra-peritoneal injection to overcome the immune incompatibility between donor and recipient in transplantation of a cell mixture of human fetal liver stromal cells (hFLSCs) and rat hepatocytes into mice with acute liver failure, thereby improving liver function and survival rate [186]. They used a novel small molecule, Me6TREN (Me6), to immobilize endothelial progenitor cells (EPCs) into the blood circulation, and found that local intramuscular transplantation of Me6-immobilized EPCs significantly attenuated ischemic disease in a mouse model of hind limb ischemia [187]. Gang Li and Jinfeng Zhang et al. (Shanghai) developed a novel co-culture system of BMSCs and tendon-derived stem cells (TDSCs) and showed that the cell sheets formed by this co-cultured cell system promoted tendon healing compared to those by a single cell source in a rat patellar tendon window injury model, suggesting the feasibility of transplantation of a two-stem cell co-culture for tendon tissue engineering [188]. Besides, Gang Li and Yan Wang et al. (Hong Kong) reported on a proof-of-concept study, in which an engineered scaffold-free tendon tissue was produced by treating TDSCs with connective tissue growth factor and ascorbic acid.

Interestingly, the researchers observed that implantation of their engineered tissue significantly promoted tendon healing in a rat model of patellar tendon window injury, suggesting that application of stem cells (or plus active molecules) but without scaffold may also be a potentially new approach for tendon repair and regeneration [189].

In addition, Hongyun Huang and colleagues (Beijing) applied cell transplantation of olfactory ensheathing cells (OECs) for clinical trials to treat cerebral palsy and complete chronic spinal cord injury, and they have reported the follow up results of their randomized controlled clinical trials [190,191].

3.2. Stem cell engineering

In China, stem cell-based therapy, either via direct transplantation or combined use with tissue engineered constructs, has been greatly advanced by modification of stem cells and selection of stem cell source, which may be loosely categorized into stem cell engineering.

Qi Zhou and colleagues (Beijing) have concentrated their research efforts on the mechanistic understanding of cellular programming, cellular reprogramming, and the customized development of pluripotent stem cells (PSCs) for regenerative medicine applications.

Qi Zhou et al. adopted the sequential culture method [192] and introduced a small molecule additive [193] to successfully improve the reprogramming efficiency of somatic cell nuclear transfer (SCNT) technology, thus enabling the generation of human nuclear transfer-embryonic stem cells (NT-ESCs) from patient autologous cells. They applied electrofused blastomeres to create a practical method as an alternative to SCNT-based therapeutic cloning for the generation of patient-specific human ESC lines [194]. Zhou’s group made a series of improvements on the technical process to extend therapeutic cloning from mouse to human [195,196]. Since the assessment of the pluripotency level of induced pluripotent stem cells (iPSCs) at early stages would help the generation and application of iPSCs, Zhou’s group identified the Dlk1-Dio3 region as a marker to identify fully pluripotent iPSCs [197]. Currently, Zhou and colleagues further reported a non-invasive method to determine the pluripotent level of ESCs and iPSCs [198].

Very strikingly, Zhou’s group generated the first viable mouse...
through tetraploid complementation with induced pluripotent stem cells (iPSCs), thus demonstrating that somatic cell-derived iPSCs had a pluripotency similar to that of ESCs [199], and also obtained the first live transgenic mouse derived from androgenetic haploid ESCs, thus showing the developmental pluripotency of androgenetic haploids [200]. Obviously, these two excellent studies, both published in *Nature*, may be rated as the outstanding examples of stem cell engineering (Fig. 3). Similarly, based on haploid ESC technology, Zhou's group further led to the simultaneous generation of multiple gene mutations in rats via the clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated (Cas) system, thus establishing rat androgenetic haploid embryonic stem cells (RahESCs) as a useful tool for genetic modification and screening [201].

Peng Xiang and colleagues (Guangzhou) have studied the application of interspecies chimeras in regenerative medicine. They showed that apodemus ESCs could fully integrate with the inner cell mass of mouse blastocysts and produced viable chimeras despite the considerable evolutionary distance between two species, and that ESCs multipotent ability could integrate into the host tissues. They concluded that interspecies chimera system raised a feasibility of differentiating pluripotent stem cells into a wide range of cell types and even tissues [202]. Xiang et al. noted that connexin43 expression significantly enhanced the reprogramming efficiency [203], and proposed a safety strategy for PSC transplantation use by pre-modifying the cells with a suicide gene [204]. Based on the understanding of stem cell regulation [205–208], they derived neural crest stem cells from cynomolgus monkey ESCs and observed the in vivo outcome of the obtained neural crest stem cells after transplantation to developing chick embryos or fetal brains of cynomolgus macaques, providing a useful tool for treating neural crest-associated human diseases [209].

Hsiao-chang Chan and Ting-yu Li et al. (Hong Kong and Chongqing) found that dedifferentiation might be a prerequisite for differentiated stem cells to undergo redifferentiation into different cell lineages [210,211], and that dedifferentiated BMSCs had an enhanced cell survival and an increased redifferentiation efficacy compared with undifferentiated BMSCs both in vitro and in vivo [212]. The researchers further proposed that epigenetic regulation in reprogramming might be responsible for an improved therapeutic potential of dedifferentiated BMSCs [212]. Similarly, Xiaobing Fu and colleagues (Beijing) also showed that the cells derived from differentiation of differentiated epidermal cells were similar to epidermal stem cells in the phenotypic and functional characteristics, thus offering a new approach to yield epidermal stem cells for wound repair and regeneration [213].

Fanyi Zeng and colleagues (Shanghai) established the chimeric animal models through in utero stem cell transplantation (IUSCT) of hematopoietic stem cells, mouse ESCs, and mouse iPSCs into fetal goats to investigate biological functions of these transplanted stem cells, and evaluate their clinical potential for tissue repair and genetic disease management [214–217].

Biao Cheng et al. (Guangzhou) used a 3-D membrane from freeze-dried BMSCs-conditioned medium to accelerate wound healing and enhance the neovascularization as well as epithelialization through the action of trophic factors in the wound bed [218].

Recently, Zhongchao Han et al. (Tianjin) investigated the angiogenic efficacy of MSCs derived from different tissues sources, noted that their heterogeneous proangiogenic properties might be dependent on the in vivo isolated environment, and concluded that MSCs derived from bone marrow and placental chorionic villi (CV) might be preferred in clinical application for therapeutic angiogenesis [219]. They used vascular cell adhesion molecule 1 (VCAM-1) to characterize MSCs and proposed that VCAM-1+CV-MSCs possessed a favorable angiogenic paracrine activity and displayed therapeutic efficacy on hindlimb ischemia. Their results suggested that VCAM-1+CV-MSCs may represent an important subpopulation of MSCs for efficient therapeutic angiogenesis [220]. In addition, although cultured human umbilical cord (hUC)-MSCs have been observed to have encouraging therapeutic outcomes in clinical trials, Han's group wondered whether in vitro expansion influenced the genomic stability of hUC-MSCs. The group reported that hUC-MSCs with genomic alterations did not undergo malignant transformation, and suggested that periodic genomic monitoring and donor management focusing on genomic stability might be needed before using hUC-MSCs in clinical setting [221].

It is worth mentioning that the South China Institute for Stem Cell Biology and Regenerative Medicine, the Guangzhou Institutes of Biomedicine and Health, the Chinese Academy of Sciences (CAS) represents a vital research and development institute featured by a

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**Fig. 3. Generation of ICAI offspring and transgenic mice.** 

- **a.** Live E13.5 ICAI embryos produced by 2 androgenetic haploid ahES-cell lines AH129-5 (left panel, eGFP negative) and AHGFP-2 (right panel, eGFP positive). Scale bar, 50 μm. **b, c.** Comparison of the body weights (b) and placenta weights (c) of the alive (1.56 ± 0.36 g; n = 12 (mean ± s.e.m.)) and dead (0.78 ± 0.07 g; n = 17) ICAI pups with the control group (wide-type mice, 1.63 ± 0.05 g; n = 12). **d.** P < 0.001 (student t-test). **e.** Coat-colour separation among the F2 generation of an ICAI-produced adult mouse (129/Sv × CD-1 background) after mating with a CD-1 male mouse. **f.** FACS analysis of the DNA content of 2 established genetically modified ahES-cell lines. AHGFP-1 (red) maintained high proportions of haploid cells, whereas AHGFP-7 (light blue) had no haploid cells. **f.** Live normal newborn mice produced from the transgenic ahES-cell line AHGFP-1 through ICAI with the expression of the eGFP transgene detected throughout the pup body and the placenta. **g.** A PGK-neo transgenic ICAI mouse that survived to adulthood. **h.** PCR analysis of the integration of the transgene neo in 5 transgenic cell lines, and the offspring of the AHGFP-1 and AHGFP-2 cell lines. Non-transgenic cell line AHGFP-4 is used as a negative control. This figure is reproduced from Ref. [200] with permission of Nature Publishing Group.
strong international scientific collaboration. The team led by Duanqing Pei (Guangzhou) has conducted many research projects in diverse areas, including chemical biology for stem cells, somatic cell reprogramming, stem cell therapeutics, stem cell differentiation, molecular diagnosis, and animal clone and gene transfer. In 2015, Pei and colleagues proposed a complex signaling network involving the inhibition of mechanistic target of rapamycin complex 1 (mTORC1) and autophagy induction in the early phase of reprogramming, and suggested that a reprogramming efficiency was dependent on the balance of the above network [222]. They also reported that c-Jun served as a guardian of somatic cell fate and its suppression opened the gate to pluripotency [223].

4. Bioactive factors for regeneration

Bioactive factors represent the important molecular cues in support of regenerative processes. They modulate one or more regenerative and repair processes, such as cellular survival, adhesion, proliferation, migration, and differentiation. Bioactive factors include growth factors, cytokines, and other active biomolecules, while growth factors are the most widely and deeply investigated in the regenerative medicine research.

Growth factors secreted from cells are sequestered by the surrounding ECM and directly interact with the ECM for presentation to cell surface receptors. These factors are soluble signaling polypeptides capable of instructing specific cellular responses in a biological microenvironment, and upon binding to their receptors, they stimulate cellular signal transduction pathways that trigger biological events, such as cell responses involved in organism development, angiogenesis, and tissue repair, etc [224]. In the last decades, many engineered growth factors, including recombinant human epidermal growth factor, bFGF, and platelet-derived growth factor, have been produced and used in the clinic [225].

Although basic research of growth factors was recently emerged in China, their clinical application, including their genetic engineering for pharmaceutical development, has been at the advanced level in the world, and especially, outstanding achievements have been made in the repair of burn wounds and other wounds. As early as in 1998, recombinant bovine basic fibroblast growth factor (rBBFGF) was applied in the clinical trials in China [226]. Subsequently, recombinant human basic fibroblasts factor (rhBFGF), recombinant human epidermal growth factor (rhEGF), and recombinant human acid fibroblasts factor (rhAFGF) have been gradually approved and used for treating acute skin wounds and chronic ulcers. In addition, several kinds of genetic engineering drugs, namely recombinant human connective tissue growth factor, keratinocyte growth factor, have been developed for clinical and preclinical trials [227].

4.1. Growth factors

Xiaobing Fu and colleagues (Beijing) demonstrated the effectiveness of growth factors at different dosages in treating acute burns and chronic cutaneous wounds, such as the diabetic foot or venous ulcers [228]. They treated eight patients with leg ulcers by using recombinant human epidermal growth factor (rhEGF) [228]. They confirmed that bFGF could induce the terminally differentiating epidermal keratinocytes to convert into their precursor cells, thus developing a new approach for generating residual healthy stem cells for wound repair and tissue regeneration [229]. Xiaobing Fu and Xiaokun Li et al. (Beijing) developed a heparin-based coagulum, in which poly (ethylene arginylaspartate diglyceride) (PEAD) served as a storage matrix, heparin as a bridge, and fibroblast growth factor-2 (FGF2) as a cargo for wound healing. The results confirmed that a suitable delivery system could protect and release FGF2 in a sustained manner, which provides a promising therapeutic potential for topical treatment of skin soft tissues [230].

Jianwu Dai et al. (Beijing) had platelet-derived growth factor-BB (PDGF-BB) incorporated into a collagen scaffold to construct a novel system, and then observed that the collagen-binding domain (CBD)-fused PDGF and native PDGF possessed similar activity to stimulate the human fibroblast proliferation [231]. Dai’s group also indicated that bFGF could promote bladder regeneration through enhancing vascularization and smooth muscle cell ingrowth [232]. They reported that many growth factors were used, alone or in combination with tissue engineered scaffolds, for many subfields of regenerative medicine, such as bone formation [233], angiogenesis [234], nerve regeneration [235,236].

4.2. Other bioactive factors

Platelets play a pivotal role not only in the coagulation phase but also in the tissue regeneration process by releasing growth factors and cytokines [237], thus promoting tissue repair, vascular remodeling, and organ regeneration. Platelet concentrates, such as platelet-rich plasma (PRP), platelet rich fibrin (PRF), concentrate growth factors (CGF), platelet rich clot releasate (PRCR) are a volume of autologous plasma with a high concentration of platelets [238] for application in the enrich platelet therapy (EPT), which has found clinical applications in the treatment of tissue injuries by stimulating neovascularization and increasing the blood supply and nutrients needed for cell regeneration in the damaged tissue [239].

Xiaobing Fu (Beijing) and Biao Cheng (Guangzhou) et al. confirmed that PRCR could alleviate the apoptosis of BM-MSCs under stress conditions induced by hydrogen peroxide (H2O2) and serum deprivation by enhancing the expression of vascular endothelial growth factor and platelet-derived growth factor (PDGF) through activation of the platelet-derived growth factor receptor (PDGFR)/PI3K/AKT/ NF-κB signaling pathways. The researchers showed that PRCR-preconditioned BMSCs significantly inhibited apoptosis and also accelerated epithelization and blood vessel regeneration of the skin via regulation of the wound microenvironment, suggesting that PRCR might reprogram BMSCs to tolerate hostile microenvironments and enhance regenerative function [240].

Changqing Zhang and colleagues (Shanghai) have compared the chondrogenic differentiation ability of BMSCs and ADMSCs seeded within the PRP scaffold. They found that the combination of pure PRP and β-tricalcium phosphate provided a safe, simple, and effective alternative option for autogenous bone grafts in the treatment of bone defects [241]. Zhang’s group also found that PRP could be made into a candidate bioactive scaffold capable of releasing endogenous growth factors, and BMSCs and ADMSCs seeded in the PRP scaffold could differentiate into chondrocytes suitable for cell-based cartilage repair [242].

Similarly, Liming Bian and colleagues (Hong Kong) functionalized hyaluronic acid hydrogels with the N-cadherin mimetic peptide to mimic the pro-osteogenic niche, which was found to enhance osteogenic differentiation of human MSCs seeded onto the hydrogel. This study suggested that bioactive factors could also play promoting roles in tissue regeneration via their beneficial effects on seed cells within the engineered scaffold [243].

Certainly, bioactive factor-mediated tissue regeneration involves the complicated repair microenvironment, and many challenges, including stability, dosage control, sustained release, and spatial distribution, remain to be solved.

5. Miscellaneous strategies for regeneration

In recent years, there have been developed other strategies for
regenerative medicine that are not appropriately categorized into either one of two fields: tissue engineering or stem cell technologies. Several representative examples are listed below. Weizhi Ji and colleagues (Kunming) are specialized in both nonhuman pri-

mate (NHP) disease model development and contract research operation. They have made major progress in NHP research. Despite the genetic and physiological similarities between NHPs and humans, some human diseases or injuries do not occur naturally in NHPs. Accordingly, it is necessary to establish different transgenic NHPs for better understanding human diseases and their treatments [244]. To fulfill this goal, Ji’s group has produced transgenic rhesus monkeys with an improved methodology, which was featured by the use of a simian immunodeficiency virus (SIV)-based vector and a protocol for infection of early-cleavage-stage embryos [245]. Moreover, the group performed precise gene targeting in cynomolgus monkeys with the CRISPR/Cas9 system [246], and accomplished TALEN-mediated mutagenesis of two key genes in both rhesus and cynomolgus monkeys [247]. All their studies provide various efficient approaches to generating targeted gene-modified NHPs to accurately model human development and dis-

eases, which would encourage the research and development of regenerative medicine.

In an attempt to seek for an alternative approach to stem cell transplantation for treating CNS diseases and injuries, Ming Fan et al. (Beijing) focused on how to enhance endogenous neuro-
genesis by external stimuli. They investigated the roles of oxygen in CNS physiology and pathology, and indicated that intermittent hypoxia could enhance neurogenesis in the adult brain at multiple stages through affecting the proliferation of neural stem cells, the survival and migration of newborn neurons, and the spine morphogenesis of mature neurons [248–250]. Accordingly, the researchers suggested that intermittent hypoxia stimulation may be developed into an attractive candidate with therapeutic potential on brain trauma and neurodegenerative disorders.

Another series of equally interesting studies involving physical stimuli was performed by Jianxin Jiang and colleagues (Chongqing), who investigated the regulation of migration and differentiation of reparative cells by direct current electric fields. Their results indi-
cated that endogenous electric fields at the wound site played an important role in the initiation of epidermal stem cell migration from their niches to the wound site for participating in wound healing, thus suggesting a new regenerative medical approach [251–253].

6. Concluding remarks

The above overview of China’s landscape in regenerative med-

icine is surely far from exhaustive, partially due to the space limi-
tation. It is certain, however, that China has been being able to catapult herself into the international forefront of the field [254]. To date, tremendous endeavors have been made by Chinese re-

searchers, as evidenced not only by their prolific publications in international peer-reviewed journals, but also by their active efforts to push laboratory innovations into a healthcare setting. Here it should be emphasized that strong support and strict surveillance from Chinese government are key elements in ensuring the sus-
tainable development of regenerative medicine in China (for details refer to [3]).

Biomaterial-based scaffolds, seed cells, and bioactive factors constitute the essentials of tissue engineering. In the future, Chi-

nese researchers will continue to strive for developing more numerous and more functional biomaterials, either organic poly-

mer or inorganic matrix in chemical identity, either synthetic or natural in origin, either single or blended in composition, to engi-

neer tissue-specific scaffolds. Equally importantly, attention will be

concentrated on the scaffold architecture (including the dimension and shape) through the tailored design and deliberate processing, and on the vascularization and reinnervation around the repair site, because it is the most crucial that a scaffold fits the anatomy of the recipient site and replicates the intracellular niche of target cells. For the scaffold fabrication, various innovative techniques, such as 3-D printing and in vivo bioreactor [255] as well as nanotechnol-

gies, are to be further attempted, thus improving physicochemical and biological properties of scaffolds. Moreover, it is also suggested that optical imaging will be developed to track stem cell distribution and fate and that optical spectroscopy and/or imaging will be used to monitor the structural remodeling of the tissue and the resulting functional changes [256]. In order to fulfill these tasks, Chinese engineering specialists (mainly chemical and biomedical engineers) will continue to collaborate with medical experts to make valuable contributions.

Given the pluripotent differentiation potential of stem cells into various target cells, it is conceived that the use of seed cells for regenerative medicine may be updated in the scenario of stem cell technologies. Stem cells, especially PSCs, represent a particularly attractive cell source due to their scalability and versatility. One of major challenges that stem cell technologies are now facing is how to harness the broad differentiation potential of stem cells to give rise to specific cell lineages suitable for the clinic. Unfortunately, current methods for induced differentiation of human PSCs merely enable the differentiated target cells to match embryonic or fetal stages of development. The future research direction is to investi-
gate how to induce stem cell differentiation into target cells with adult-like functional properties [257].

The application of stem cells still faces more complex challenges at the scientific (e.g. directed differentiation, teratogenic or tumorigenic potential, immunosuppressive activity, etc.), techno-

logical (e.g. cell dosage, delivery route, genomic stability, etc.), and even ethical (e.g. legal, moral, and religious issues) dimensions. To overcome these hurdles, a wide range of domains in stem cell biology and stem cell engineering are to be addressed by Chinese researchers in the context of regenerative medicine, thus gaining an in depth insight into mechanistic regulation of stem cell destiny by a complicated signaling system [258–260].

In order to pursue regenerative medicine, it is also important to put better use of bioactive factors, including growth factors, cyto-

kines, chemokines, naturally-derived biomolecules, and regeneration-associated genes, to orchestrate the process of tissue/organ regeneration and exert favorable effects [261]. In addition, it is suggested to further investigate molecular mechanisms underlying specific tissue/organ regeneration [262] and to better un-
derstand and replicate the regenerative microenvironment [263] in the future perspective of regenerative medicine.

Translation of research findings into the clinic is the ultimate goal of regenerative medicine. For any new medical strategy, the commercialization and clinical translation are always impeded by a broad spectrum of challenges and complexities, and regenerative medicine is no exception. Although some tissue engineering products have now been moved to the clinic in China, they are limited to those composed of biomaterial-based scaffolds alone without addition of cellular or molecular cues, while clinical application of stem cells are mostly involved in the treatment of hematological diseases. In the light of this current status, we have got a long way to go for bringing regenerative medicine into the clinic.

Before medical products derived from regenerative medicine are commercially available and ready to replace the traditional therapy, they ought to undergo the assessment of strict regulatory systems and acquire the support of a reimbursement mechanism [264,265]. Recently, China has been establishing and perfecting the
risk-based regulatory frameworks, and the official departments have reconsidered and adjusted the access conditions and safety standards for new medical products and techniques [266]. For instance, in 2015 National Health and Family Planning Committee of China (NHFPC) and CFDA jointly issued a “Provisions for stem cell research” (trial), and in 2016 formulated a list of 30 institutes of stem cell research with the government approval, which are located in the cities of Beijing, Shanghai, and Tianjin, and the provinces of Jiangsu, Hebei, Liaoning, Henan, Hubei, Hunan, Guangdong, Sichuan, and Guizhou. It is gratifying to see that the attendants of the 6th annual meeting of Chinese Society for Stem Cell Research (2016) have assigned a self-discipline declaration for attendants of the 6th annual meeting of Chinese Society for Stem Cell Research, such as stem cell research and application, and they promise to strictly follow the rules and regulations of stem cell research, such as “Administration of Stem Cell-based Clinical Research” and “Quality Control of Stem Cell-based Medical Products and Preclinical Stem Cell Research Guidelines”. We believe that the transition from bench to bedside for regenerative medicine in China will be speeded up in a healthy and productive manner.

In closing, we would say that this review article was written with the initial intent to promote information exchange and interactive learning among all researchers in the field of regenerative medicine. At present, we hope to strengthen our bonds with foreign institutions and scholars through mutual communication and collaboration, which should contribute to the development of regenerative medicine worldwide.

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