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Recent Advances in Tissue Engineering and Regeneration Medicine for Treatment for Osteoarthritis

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Osteoarthritis is a global degenerative joint disease involving the cartilage and many of its surrounding tissues. The World Health Organization's Scientific Group on Rheumatic Diseases estimates that 10% of the world's populations aged 60 years or older have significant clinical problems that could be attributed to osteoarthritis [1]. Take the United States for example, symptomatic knee OA occurs in 10% men and 13% in women aged 60 years or older [2]. There are estimates that Osteoarthritis **(OA)** may become the fourth-highest impact condition in women and the eighth most important condition in men in the developed world [3]. The pain and loss of function can be debilitating; in developed countries the resultant socioeconomic burden is large, costing between 1.0% and 2.5% of gross domestic product [4]. However, the prevention and treatment for OA remains a worldwide medical problem. Current guidelines for OA therapy in the following defined order: first, behavioral interventions; second, simple analgesic such as acetaminophen (paracetamol); third, nonsteroidal anti-inflammatory drugs, including COX-2

inhibitors; fourth, intra-articular injection of hyaluronic acid or corticosteroid; and finally fifth, total joint replacement [5-7]. The goals of these treatment are to reduce pain, improve joint mobility, reduce disease progression and seek joint replacement surgery after failure of a series of nonsurgical therapy that are not ideal treatment options. Osteoarthritis **(OA)** is a chronic and progressive destructive joint disease, especially degeneration of cartilage, and preventing the degeneration and promoting the regeneration of cartilage is the ideal therapeutic strategy. However, the strategy remains a huge challenge due to the poor self-renewal and regeneration potentials of chondrocytes. After years of research and development, tissue engineering and regeneration medicine makes possible preventing the degeneration and promoting the regeneration of cartilage. In this article, we review recent advances in tissue engineering and regeneration medicine for the treatment for osteoarthritis. Firstly, an improved understanding of the pathogenesis combined with improved assays of disease activity is facilitating a shift in focus to the prevention and treatment of early osteoarthritis, and in the first part we introduce injectable treatments for early osteoarthritis without focal cartilage lesions, such as stem cells, Platelet-Rich Plasma **(PRP)**, injectable materials, and cartilage regenerative factors. Second, cartilage focal lesions are also common in the adult population and may lead to the progression to arthritis, hence early treatment of focal cartilage lesions is imperative to prevent progression of osteoarthritis. However, the primary treatment for focal cartilage lesions, arthroplasty surgery, is not an ideal treatment which has the limitations including the possibility of adverse outcomes and the finite lifespan of prostheses. In the second part, we introduce scaffold-based treatment in tissue engineering and regeneration medicine strategy, including cell-free scaffolds, cell-scaffolds without induction *in vitro* and engineered cartilage. We also cover future trends for early osteoarthritis and cartilage lesions in tissue engineering and regeneration medicine strategy.

INJECTABLE TREATMENTS FOR EARLY OSTEOARTHRITIS

Viscosupplementation

The term viscosupplementation was proposed in the late 1970s by Dr. Endre Balazs to refer to the concept of synovial fluid replacement with intra-articular injections of hyaluronic acid into joints for the relief of pain associated with OA [8]. In addition to its viscoelasticity, anti-inflammatory, anabolic, and antinociceptive effects for osteoarthritis, intra-articular HA turned out to have chondroprotective potential which may play an important role in the treatment for early osteoarthritis. Takahashi et al. show that HA injections enhance cartilage matrix production by down-regulation Matrix Metalloproteinase (**MMP**-3) and IL-1β which degrade articular cartilage acellular matrix molecules, including proteoglycan and type II collagen during early development of OA. [9] Listrat et al. systematically evaluated the potential structure-modifying effects of HA and found that repeated intra-articular injections of HA might delay the structural progression of the disease. [10] Frizziero et al. evaluated the structural effects of HA injections by microarthroscopy and morphological analysis of biopsy samples and found an improvement in the chondrocyte

density and vitality, as well as a statistically significant reconstitution of the superficial amorphous layer of the cartilage. The evidences indicate the potential of HA, preventing the degeneration and promoting the regeneration of cartilage [11] The multiple effects of HA made it an useful treatment option for OA. In recent years, the safety and efficacy of HA for OA has been determined [12-14] At present, HAs are United States Food and Drug Administration **(FDA)**-approved for the treatment of pain associated with OA, and remommened as a valuable medical management of osteoarthritis of the hip and knee by the American College of Rheumatology Subcommittee in the 2000 treatment guidelines for knee OA. HA has different forms, and the field of HA derivatives is evolving [15,16]. Therefore, the treatment guideline including standardization of the HA products classification as well as the classification for indications is essential.

Autologous-Conditioned Serum and cartilage regenerative factors

Autologous-Conditioned Serum **(ACS)** is considered as a potential candidate for treatment of OA, including a variety of cartilage regenerative factors such as Basic Fibroblast Growth Factor (**bFGF)**, transforming growth factor, Insulin-Like Growth Factor (**IGF-1)**, IL-1 receptor antagonist, IL-4, IL-10, and IL-13. Frizziero et al. found that ACS can lead to enhancement of tissue regeneration and to reduction of degenerative mechanisms, and Astolfi et al. found that an ACS injection is more efficient at producing a reduction in symptoms than HA or a saline injection in the treatment of osteoarthritis [17,18].

The homeostasis and repair of articular cartilage is regulated by a variety of cartilage regenerative factors such as growth factors, cytokines, and other biologics. Growth factors have an important role in regulating the behavior of all cells, including articular chondrocytes. By modulating the local microenvironment, the anabolic and anticatabolic effects of growth factors have demonstrated potential in the treatment for both local cartilage defects and osteoarthritis [19]. Researches on pathogenesis of osteoarthritis demonstrated that a mumber of cytokines are closely related to OA and OA progression and one of them, IL-1 beta is the main factor leading to the degenerative arthritis process. Growth factors- and cytokines-related injections may become main treatment options for early OA [20]. Lohmander et al. evaluated the efficacy and safety of intraarticular recombinant human fibroblast growth factor in the treatment of symptomatic knee osteoarthritis in a clinical trial, and found that it was associated with statistically significant reductions in loss of total and lateral femorotibial cartilage thickness and volume [21]. Hayashi et al. found that weekly intra-articular injections of BMP-7 inhibited progression of osteoarthritis in rabbits, and Hunter et al. reported phase 1 safety and tolerability of BMP-7 in symptomatic knee osteoarthritis [22]. Genemaras et al. demonstrated the efficacy of Interleukin-1 receptor antagonist protein treatment in modulating catabolic microRNA and mRNA expression in cartilage which indicated the potential as an early intervention strategy for the prevention of cartilage degeneration after impact injury [23]. Although each of these cartilage regenerative factors plays an important role in the cartilage regeneration and may be used as one treatment option, there is

no doubt that more than a single growth factor will be needed to achieve cartilage regeneration for the OA patients. Furthermore, these cartilage regenerative factors have a short half-life and can be cleared rapidly *in vivo*, which hinders their optimal therapeutic effects of the direct intra-articular injection [24-26]. Therefore, to achieve the best treatment effect of these factors, the combined application and appropriate controlled delivery strategy will be the research emphasis and future trends.

Platelet-Rich Plasma (PRP)

PRP is prepared by withdrawing peripheral blood and by centrifugation to obtain a highly concentrated sample of platelets [27]. In the past decade, Platelet-Rich Plasma **(PRP)** has emerged as one of the research hotspots in the treatment for osteoarthritis and cartilage defects. Its therapeutic effects are based principally on three functional components: 1) growth factors: a variety of growth factors are stored in and secreted from platelet α-granule, such as PDGF, TGF-beta, IGF-1, bFGF, and BMP-2, which stimulate chondrocyte proliferation, promote chondrocytes to synthesize extracellular matrix; 2) inflammatory mediators: a number of inflammatory mediators ,such as IL-1 receptor antagonist, TNF receptor, IL-4, and IL-10, may suppress inflammation in osteoarthritis and prevent progression of osteoarthritis; 3) scaffolding activity: a variety of plasma proteins, such as fibrinogen, promote fibrin gel formation *in vivo* which may contribute to the controlled delivery of growth factors and inflammatory mediators and then achieve better therapeutic effects for early OA [28]. Saito et al. investigated the therapeutic potential of PRP In a traumatic OA model in rabbits and found that Intraarticular injections of PRP in gelatin hydrogel microspheres stimulated chondrocyte GAG synthesis and significantly suppressed progression of OA morphologically and histologically [29]. Kwon et al. investigated the effects of intra-articular platelet-rich plasma injection in a collagenase-induced OA model, and found that PRP promoted cartilage regeneration. Furthermore, a number of preclinical studies and clinical trials suggest that PRP is a promising clinical treatment for OA [30]. Xie et al. summarized the reports of PRP treatment of degenerative cartilage diseases from four levels: level IV, case series; level III, retrospective comparative studies; level II, prospective comparative studies or lesser quality randomized control trials (RCTs); and level I, high-quality RCTs [27]. Laudy et al. investigated the efficacy of PRP injections for treatment of osteoarthritis of the knee in a systematic review and meta-analysis. PRP is a mixture of multiple growth factors and cytokines, some of which, such as TGF-beta and IL-1 beta, have negative effects on the OA joint [31]. Future directions of PRP application in OA therapy may concentrate on the researches controlling its negative effects.

Stem Cells

MSCs are multipotent progenitor cells that can differentiate into selected lineages including chondrocytes, with capability of self-renewal, high plasticity, and then MSCs play an essential role in supplying recovery cells for injuries. Caplan and colleagues recently underlined that these cells derived from perivascular cells, have a key role in the response to tissue injuries not just

by differentiating themselves, but also by inducing regeneration processes at the injury site through the secretion of several bioactive molecules [32-34]. Murphy et al. compared MSCs from patients with end-stage OA and normal donors, and found that MSCs from patients had reduced *in vitro* proliferation and differentiation potentials along with decreased chondrogenic and adipogenic activity [35]. Chua et al. compared MSCs from old and young patients and found that MSCs from older osteoarthritic patients have lesser differentiation capacity and expression of stemness genes [36]. Meanwhile, Centeno et al. observed that synovial fluid from donors with osteoarthritis or rheumatoid arthritis inhibits the chondrogenic differentiation of MSCs from healthy donors [37]. Furthermore, the immunosuppressive and anti-inflammatory properties of some MSCs contribute to the treatment for immune-mediated OA. These results suggest that the development and progression of OA might be related to lack of healing mechanism for cartilage injuries based on MSCs. Therefore, MSCs represent an excellent candidate for OA.

There are a lot of studies about MSCs treatment for focal articular cartilage defects, but the researches about stem cell injections are relatively less and they are mainly BM-MSCs and ADSCs.. Murphy et al. observed in a caprine model that BM-MSCs injection stimulated regeneration and retards the progressive destruction of cartilage. 35Centeno et al. first reported in a clinical trial that the patient had statistically significant cartilage and meniscus growth on MRI after intra-articular injection of autologous cultured BMSCs [38]. Huurne et al. investigated intra articular injection of ADSCs for OA in a mouse model and found that ADSCs inhibits synovial thickening and cartilage destruction in the progress of OA [39]. Jo et al. investigated ADSCs injections for the treatment of generalized cartilage loss in osteoarthritis, and found that ADSCs improved function and pain of the knee joint and reduced cartilage defects by regeneration of hyaline-like articular cartilage [40].

There is consensus that the treatment of early OA should be considered before consideration of more aggressive surgical approaches. The above injectable treatments prevent the degeneration and promote the regeneration of cartilage, and then may be served as promising treatment options for early OA. Based on the complex pathogenesis of OA, the combined application, such as HA and growth factors, HA and PRP, growth factors and stem cells, rather than the single injection, can achieve a better therapeutic effect.

SCAFFOLD-BASED TREATMENT FOR ARTICULAR CARTILAGE FOCAL LESIONS

Articular cartilage focal lesions repair is far beyond the regenerative capacity of cartilage. Self-repair mechanism for articular cartilage defects, fibrous tissue repair, can't realize the function of cartilage at all. In contrast to the poor therapeutic effects of traditional treatment modalities, tissue engineering and regeneration medicine provided a preferable treatment strategy and developed rapidly in the last few decades. Injectable treatments, such as stem cells and cartilage regenerative factors, can hardly remain and then perform their functions of promoting cartilage

regeneration in the 3D-shaped lesions. The application of scaffolds is imperative in tissue engineering and regeneration medicine strategy for OA treatment [41].

Cell-free Scaffolds for Cartilage Defects

Jackson et al. found in a goat model that osteochondral defects possess a certain reparative capacity, and microfracture is an effective treatment for full-thickness osteochondral defects [42]. These are based on spontaneous reparative effect of BMSCs from the bone marrow beneath the defects. The scaffolds in the defects may provide an 3D environment for BMSCs and thus have the therapeutic potential in the treatment of articular cartilage defects [43-48].

The scaffolds provide a 3D environment for cell attachment, migration, proliferation, differentiation and extracellular matrix formation, and their properties determine reparative effect of BMSCs. Therefore, there are many basic requirements for the scaffolds in the treatment for cartilage defects as follows:

1) favorable biocompatibility, non-immunogenic, and non-toxic;
2) adequate mechanical strength: the scaffolds can fulifill the defects and maintain its shape;
3) optimal porous structure for the attachment, migration, and biological activity of BMSCs;
4) appropriate degradation rate range: the scaffolds can neither degrade too rapidly which caused porous structural damages, nor degrade too slow which interfere with extracellular matrix formation [49].

Two categories of biomaterials, natural and synthetic, are applied to the fabrication of the scaffolds. Natural biomaterials, such as collagen, hyaluronan, Chitosan and fibrin, have favorable biocompatibility. The disadvantages include relatively fast degradation rate, poor mechanical properties as well as immunogenicity and a risk of potentially transmitting animal pathogens. Synthetic biomaterials, such as, poly(lacticco- glycolic acid) (PLGA), Poly(-Caprolactone) (PCL), poly(ethylene glycol) (PEG), have processable mechanical properties and degradation rate. The main disadvantage is that synthetic biomaterials and their degradation product may cause inflammatory responses and interfere with cartilage regeneration. The combination of synthetic and natural polymers on the basis of complementary advantages contributes to effective scaffolds for cartilage regeneration. Take polylactide–alginate scaffold for example, it has good compatibility due to alginate, as well as mechanical stability conferred by polylactide.

Excellent chemical and physical properties are insufficient for ideal scaffolds. Cartilage repair is a complex healing process which consists of a variety of biological events controlled by numerous signals such as growth factors and cytokines. Therefore, biomimetic composite scaffolds including regenerative signals are imperative for cartilage repair. Furthermore, since osteochondral defects are composed of cartilage and bone phase, a biphasic biomimetic scaffold is requisite. Both phases neeed to be fabricated separately, and then receive chondrogenic and

osteogenic inductions respectively. The regenerative property of BMSCs and the biomimetic scaffolds including regenerative signals make cell-free scaffolds a promising treatment for cartilage defects [50-52]. Despite the lower number of articles in comparison with cell-based scaffolds, cell-free scaffolds are gaining popularity, with a recent increase in published studies showing promising results. There are even some studies reporting no significant improvement when using cell-seeded scaffolds [53]. In fact, these results are based on the premise that BMSCs beneath the defects have relatively normal biological activities.

Cell-scaffolds without Induction *in vitro*

Elizaveta et al. reported in a systemic review that compared to cell-free scaffolds, cell–based approaches were the most investigated option for cartilage defects in the preclinical setting, showing generally superior results. In cell-free scaffolds strategy for cartilage defects, intrinsic BMSCs beneath the defects are cell source for repair. However, there are various investigations demonstrating the atypical or defective activity of MSCs during OA progression. 53 Murphy et al. founded that bone marrow MSCs isolated from end-stage OA patients exhibit deficient proliferation and differentiation potentials compared with BM-MSCs from healthy, age-matched controls. When lack of intrinsic normal BMSCs beneath the defects, cell sources from the donor may promote the repair. In the cell-scaffolds strategy the cells are isolated, expended *in vitro*, and then seeded on an biomimic scaffold for several days prior to implantation to the defects. Based on the introduction of biomimic scaffolds in the above part, we focus on the cell sources in this part, such as autologous chondrocyte, BMSCs, Synovium-derived MSCs (SM-MSCs), adipose-derived stem cells **(ADSCs)**, umbilical cord blood derived mesenchymal stem cells (hUCB-MSCs), and induced Pluripotent Stem Cells **(iPSCs)**.

Autologous Chondrocyte Implantation **(ACI)** is one of the most widely used cell based repair strategies for articular cartilage which used autologous chondrocyte as cell soure. Brittberg et al. performed autologous chondrocyte transplantation in 23 people with deep cartilage defects in 1994 and the results revealed that cultured autologous chondrocytes can be used to repair deep cartilage defects [54] There are two major limitations in the process: 1) chondrocytes need to be covered by the periosteal flap, which resulted in many clinical complications such as flap detachment, delamination and late periosteal hypertrophy; 2) chondrocytes were in a 2D microenvironment, which is not an ideal environment for cartilage regeneration. The improved generation focused on the development of scaffold-based approaches in which scaffolds provide a 3D microenvironment for chondrocytes proliferation and cartilage regeneration. Matrix-induced Autologous Chondrocyte Implantation **(MACI)** technique used a three-dimensional collagen type I–III membrane or hyaluronic-acid as scaffolds. There are many case series of MACI demonstrating promising clinical and histological results. Zheng et al. conducted biological and histological assessment for MACI, and the results suggested that MACI may offer an improved alternative for cartilage injury by regenerating hyaline-like cartilage [55]. Marlovits et al. reported the 5-year

follow-up of clinical and radiological outcomes for MACI foe chondral defects, in which patients treated with a MACI implant demonstrated significant clinical improvement and good quality repair tissue 5 years after surgery [56].

There are two major problems with cartilage repair by autologous chondrocytes transplantation: 1) This requires a large quantity of chondrocytes from normal articular cartilage, whereas donor sites have often a limited capacity to provide the chondrocytes; 2) during the expansion phase *in vitro*, the chondrocytes tend to undergo a rapid cell de-differentiation with a loss of their chondrogenic potential and a phenotypic derangement into a fibroblast-like phenotype. MSCs become an advanced research hotspot as the alternative promising cell sources. MSCs are easily obtainable and expandable *in vitro* without losing their differentiation potential, which avoid the damage of limited cartilage. MSCs possess chondrogenic differentiation potential for cartilage while MSCs can differentiate into the subchondral bone, which promote a better integration between the subchondral bone and articular cartilage, and thus a better repair for full-thickness defects. Mesenchymal stem cells may be harvested from various tissues including bone marrow, synovium, adipose, Wharton's jelly, umbilical cord blood, and so on. Most of them are reported for treatment for articular cartilage defects. Thus far, the most frequently used is BMSCs. Wakitani et al. repaired full-thickness defects of articular cartilage in a rabbit model in 1997 [57]. Nejadnik et al. compared the clinical outcomes of patients treated with first-generation autologous chondrocyte implantation to patients treated with autologous bone marrow-derived mesenchymal stem cells, and found that BMSCs was as effective as chondrocytes for articular cartilage repair. Synovium-derived MSCs **(SM-MSCs)** have been shown to have superior chondrogenic potential [58] Koga et al. compared MSCs isolated from bone marrow, synovium, adipose tissue, and muscle in a rabbit model, and found that synovium- and bone-marrow-MSCs had greater *in vivo* chondrogenic potential than adipose- and muscle-MSCs, while synovium-MSCs had a greater proliferation potential than BMSCs. SM-MSCs are a significant promising cell source for articular cartilage repair. Although easily obtainable and many clinical trials of injection for cartilage degeneration, Adipose-Derived MSCs **(ADSCs)** demonstrated inferior chondrogenic capabilities in many studies [59]. Further investigation for ideal culture and induction system is needed before ADSCs can be applied as an ideal cell source for articular cartilage defects. Several researches have investigated the chondrogenic potential of human umbilical cord blood derived mesenchymal stem cells (hUCB-MSCs). Ha et al. repaired the articular cartilage defects in a minipig model using composites of human umbilical cord blood-derived mesenchymal stem cells and hyaluronic acid hydrogel [60]. Low immunogenicity of Wharton's jelly- and umbilical cord blood-derived stem cells makes possible allogeneic transplantation for the repair of articular cartilage defects.

Tissue-engineered Cartilage

In this strategy, chondrocytes or other cell sources were seeded in a biomimic scaffold, cultured or/and inducted *in vitro* for longer periods till the formation of the neotissue similar

to native articular cartilage. The four important parameters of tissue engineering are scaffolds, cells, soluble growth factors, and the physical environment. Compared to the microenvironment in the cartilage defects, an ideally controllable culture system may provide a better environment for neocartilage formation and cartilage repair. The cell sources, biomimic scaffolds, and cartilage regenerative factors including growth factors are introduced above. We focused on the chondrogenesis of stem cells, biophysical stimuli such as mechanical stimuli and oxygen tension, and bioreactors in this part.

Stem cell populations all have multilineage potential, however the chondrogenic potential needed to be highly dependent on the combination of growth factors which induce chondrogenesis, such as Transforming Growth Factor-b **(TGF-b)**, Bone Morphogenetic Proteins **(BMPs)**, Fibroblast Growth Factors **(FGFs)**, Insulin Growth Factors **(IGFs)** and so on. BMSCs have been extensively used for cartilage tissue engineering. The chondrogenesis of BMSCs is generally stimulated by TGF-b, and Indrawattana et al. also investigated that BMP-6 and IGF-1 may promote the chondrogenesis [61]. ADSCs are also capable of chondrogenesis in the presence of growth factors. While BMSCs responds more favorably to TGF-b for chondrogenesis, ADSCs shows enhanced response to Bone Morphogenetic Protein-6 **(BMP-6)**. However in the current induction system, ADSCs demonstrated lower chondrogenic potential than BMSCs. There are also other sources for engineered cartilage *in vitro*. Sakaguchi et al. compared MSCs isolated from five different tissue sources, and found that SDSCs possess greatest chondrogenic potential in the same environment [62]. Periosteum-derived stem cells (PDSCs) have been inducted for chondrogenic differentiation along with the addition of IGF-1 and TGF-b1. In general, the chondrogenic potential of stem cells are not sufficiently brought out, and an optimal system need to be investigated.

Because cartilage is exposed to many mechanical forces during joint loading such as hydrostatic pressure, compression, and shear forces, mechanical stimuli are essential for the metabolic activity of chondrocytes and chondrogenic differentiation of MSCs. Exogenous mechanical stimuli are used to enhance neocartilage formation in tissue engineering strategy [63]. Miyanishi et al. examined the effects of Hydrostatic Pressure **(HP)**, and found that HP can facilitate articular cartilage tissue engineering [64]. Furthermore, cartilage is an avascular tissue and thus in an environment of low oxygen tension. Therefore, mimicking low oxygen conditions may improve cartilage regeneration. There are investigations demonstrating that the hypoxia environment promoted proliferation, differentiation, chondrogenic phenotype, and chondrogenic matrix production of MSCs [65,66]. Chen et al. found that he hypoxia environment inhibited the expression of major marker of chondrocyte hypertrophy, and prevent calcification of engineered cartilage [67]. These researches indicated that low oxygen tension is a key regulatory microenvironment for cartilage tissue engineering. Bioreactors are devices in which biological or biochemical processes develop under a closely monitored and tightly controlled environment. Bioreactors may dynamically mimic the native mechanical loading conditions, which enhances the nutrient transport, oxygen transfer, gas exchange, promotes ECM synthesis and deposition, and then improves neocartilage

formation. There are increasing researches based on new bioreactors for cartilage tissue engineering [68-70].

CONCLUSION AND FUTURE PROSPECTS

Osteoarthritis is a destructive joint disease, but the traditional treatment has been unable to effectively solve the problem of the chronic and progressive degeneration of articular cartilage. Tissue engineering and regeneration medicine provided a promising treatment strategy for cartilage degeneration and defects. Injectable treatment prevented the degeneration and promoted the regeneration of articular cartilage by providing cartilage regenerative factors and improving the microenvironment of the joint, and thus were used in treatments for early osteoarthritis. Scaffolds combined with appropriate cell sources, growth factors, and the biophysical environment, can achieve the neocartilage formation and then be used to repair the cartilage defects, which is a far better treatment option than arthroplasty.

Although positive treatment effect in a lot of preclinical and clinical trials, many more researches are still needed for the widespread clinical application. Firstly, while each injectable and scaffold-based treatment works in cartilage regeneration, the therapeutic effects leave something to be desired. Appropriate controlled delivery strategy for cartilage regenerative factors and the combined application for injectable treatment, as well as the design of biomimic scaffolds and the optimization of culture and induction system for scaffold-based treatment, should be future focus in the next decades. Secondly, compared to the complex pathogenesis and clinical symptoms of patients in clinical, the design of animal models of OA is too simple, which provides a limited guiding significance for the clinical application. Therefore, the optimal design of animal models of OA is pivotal in the preclinical trials. Moreover, current researches were not very explicit and position-relevant for the etiologic classification, clinical grading according to Magnetic Resonance Imaging **(MRI)** and clinical symptoms of OA, but it is more reasonable that the researches of certain treatment for OA are refined to its etiologic classification and clinical grading. The goal of preclinical and clinical trials should be individualized treatment.

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