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Review

Cell-based articular cartilage repair: the link between development and regeneration

K.L. Caldwell, J. Wang*

The Harrington Laboratory for Molecular Orthopedics, Department of Orthopedic Surgery, University of Kansas Medical Center, Kansas City, KS, USA

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SUMMARY

Clinical efforts to repair damaged articular cartilage (AC) currently face major obstacles due to limited intrinsic repair capacity of the tissue and unsuccessful biological interventions. This highlights a need for better therapeutic strategies. This review summarizes the recent advances in the field of cell-based AC repair. In both animals and humans, AC defects that penetrate into the subchondral bone marrow are mainly filled with fibrocartilaginous tissue through the differentiation of bone marrow mesenchymal stem cells (MSCs), followed by degeneration of repaired cartilage and osteoarthritis (OA). Cell therapy and tissue engineering techniques using culture-expanded chondrocytes, bone marrow MSCs, or pluripotent stem cells with chondroinductive growth factors may generate cartilaginous tissue in AC defects but do not form hyaline cartilage-based articular surface because repair cells often lose chondrogenic activity or result in chondrocyte hypertrophy. The new evidence that AC and synovium develop from the same pool of precursors with similar gene profiles and that synovium-derived chondrocytes have stable chondrogenic activity has promoted use of synovium as a new cell source for AC repair. The recent finding that NFAT1 and NFAT2 transcription factors (TFs) inhibit chondrocyte hypertrophy and maintain metabolic balance in AC is a significant advance in the field of AC repair. The use of synovial MSCs and discovery of upstream transcriptional regulators that help maintain the AC phenotype have opened new avenues to improve the outcome of AC regeneration.

Introduction

An acute cartilage or osteochondral defect may be caused by a comminuted or displaced intra-articular fracture, while a chronic articular cartilage (AC) defect is often a result of AC degradation during the progression of osteoarthritis (OA). Another cause of osteochondral defects that is relatively rare is osteochondritis dissecans (OCD), a joint disease with osteonecrosis of the subchondral bone usually linked to antecedent trauma, which occurs most often in the knee of young men and athletes[1–3]. The link between AC damage and OA is undeniable, making the pursuit of clinical advancement in the area of cartilage regeneration of paramount importance. Unlike spontaneous OA, which mostly affects middle-aged and older populations, cartilage injury-induced post-traumatic OA (PTOA) often affects younger adults for whom desirable treatment is to preserve the function of the original joint by regenerating damaged AC instead of joint replacement or arthrodesis. This highlights a great need for earlier, less invasive treatment modalities for both acute and chronic AC lesions.

Many new lines of treatment for AC defects have become available over the past five decades with even more animal models on the verge of clinical trial, yet our understanding of how AC heals remains insufficient to support any given line of therapy over another. Most cartilage repair techniques have been based on a postulate that a substance, such as a graft, scaffold, or mesenchymal-cell-rich blood clot, must be interposed in order for an AC defect to be repaired. This is based on many years of success gained from the general art of using grafts to fill defects in the skin and bone. Unfortunately, grafting techniques for AC regeneration have not been as successful as for skin or bone regeneration.

The major breakthroughs in AC repair began in 1959 when Pridie published his drilling method for AC resurfacing in osteoarthritic knee joints noting that accessing the underlying bone marrow led to a clot formation which had the potential to form

* Address correspondence and reprint requests to: J. Wang, University of Kansas Medical Center, Department of Orthopedic Surgery, 3901 Rainbow Blvd., Mail Stop 3017, Kansas City, KS 66160, USA. Tel: 1-913-588-0870; Fax: 1-913-945-7773.
E-mail addresses: kenneth.caldwell1213@gmail.com (K.L. Caldwell), jwang@kumc.edu (J. Wang).

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cartilage[4]. This procedure was refined in the 1980's by Steadman *et al.* who coined the term microfracture as a method of accessing the bone marrow with a bone pick without the potentially harmful effects of drilling. A clinical follow-up revealed that 80% of the patients had significant improvement in joint function and pain[5]. However, it has become clear that the fibrocartilage-like repair tissue with hypertrophic chondrocytes generated by the bone marrow stimulation procedure was less than optimal for long term outcomes[6,7].

Osteochondral allografting was also being used during this time period and remains in use today for the treatment of large cartilage defects in young, high-demand patients in whom total joint arthroplasty was a poor option. Transplantation of mature hyaline cartilage into the affected area is an advantage of the procedure. However, disease transmission, immunological response, and the long-term viability of transplanted allografts are concerns with any allografting procedure. Graft nonunion and fragmentation may occur from months to years after the procedure[8,9]. Osteochondral autografting (mosaicplasty) affords the same advantages without the risk of disease transmission or immunologic response, but it is limited by donor site availability and morbidity. Short- (<5 years) and medium-term (5–9 years) clinical outcomes showed that patients with osteochondral defects treated with mosaicplasty maintain a superior level of athletic activity compared with those treated with microfracture. However, long-term (>10 years) clinical outcome after mosaicplasty varies greatly depending on the age, gender, and size of the lesions[10,11].

In 1987, it was reported that chondrocytes could be cultured and implanted into chondral defects that had not disrupted the subchondral bone[12]. Soon thereafter Brittburg and Peterson *et al.* published their first case series describing a new method of treatment termed autologous cartilage transplantation, later referred to as autologous chondrocyte implantation (ACI)[13]. Subsequent follow up studies, however, have failed to demonstrate a significant difference in structural repair at 24 months in randomized controlled clinical trials comparing ACI to microfracture[14–17].

Tissue engineering techniques for cartilage or osteochondral repair have gained a significant amount of interest over the past two decades. This technology involves three main components: biomaterial-based scaffolding, a cell source, and growth or differentiation factors. Scaffolds for repair of osteochondral defects may be fabricated with natural (e.g., collagen) or synthetic materials[18–21]. Cell sources include isolated autologous chondrocytes, minced autologous cartilage, multipotent stem cells (e.g., bone marrow-, muscle-, synovium-, or adipose-derived mesenchymal cells), pluripotent stem cells, and induced pluripotent stem cells (iPSC)[16,18,19,22–26]. Chondroinductive growth factors mainly consist of members of the transforming growth factor-β (TGF-β) superfamily, insulin-like growth factor-1 (IGF-1), and specific members of fibroblast growth factor (FGF) family. These growth factors have been used for stimulating chondrogenic differentiation of stem cells in cell culture or through controlled release, gene transduction/delivery, or nanoparticle delivery[16,25,27–30]. Bioreactors are utilized to enhance nutrient delivery and provide mechanical stimulation to tissue-engineered cartilage constructs *ex vivo* prior to *in vivo* implantation.

While cell-based therapies (e.g., microfracture, ACI) are already in clinical use for promotion of AC repair, none of these options have been proven successful in restoring the original AC structure with hyaline cartilage in humans[16,17]. Clinicians and scientists are striving for a better understanding of cartilage healing process in order to develop more reliable methods of AC repair. Here, we review the recent advances in cell-based therapies for AC repair, with a focus on the latest development in synovial mesenchymal stem cells (MSCs) as a cell source and novel TFs that may serve as potential upstream regulators for maintaining the permanent hyaline cartilage phenotype of healing AC and preventing PTOA.

Current challenges

Cartilage remains one of the most difficult tissues to heal. Several approaches including tissue engineering have been developed in the past decades to regenerate damaged AC; however, none of these approaches have been proven to effectively produce a repair tissue with the same or similar mechanical and functional characteristics of the native AC. At a cellular level the challenges we currently face in AC regeneration fall into at least two major categories:

1. *Chondrocyte differentiation problems including insufficient chondrogenic differentiation, chondrocyte dedifferentiation, and chondrocyte hypertrophy*: Although chondroinductive growth factors may induce the differentiation of various stem cells into chondrocytes, the induction process may not be sufficient to produce functional chondrocytes. Autologous chondrocytes have shown the most promise in this regard but may undergo dedifferentiation to fibroblast-like cells during the *ex vivo* expansion or *in vivo* repair process. As a result, an AC defect site may be filled with fibrous tissue or fibrocartilage-like repair tissue instead of the desirable AC containing hyaline cartilage that is uniquely organized into a complex, layered structure and physiologically tightly regulated. One of the key limitations to engineered cartilage tissues is that it is amorphous and lacks the three-dimensional organization and structural properties of native AC, thereby rendering it susceptible to physical and physiological stresses. On the other hand, it has been observed that bone marrow MSCs have an intrinsic differentiation program reminiscent of endochondral bone formation[31]. Some repair chondrocytes may undergo hypertrophic differentiation, followed by matrix calcification, vascular invasion, and endochondral ossification leading to new bone formation in an AC defect site. Because of these drawbacks researchers are searching for better repair techniques which can induce differentiation of stem cells into functional, matrix producing articular chondrocytes with less potential for dedifferentiation or hypertrophic differentiation.
2. *Cartilage homeostasis problems characterized by imbalanced anabolic and catabolic cellular activity of repair cells*: In the acute post-traumatic phase, joint trauma may lead to suppression of collagen and proteoglycan synthesis in AC. Remaining viable cells in joint tissues may respond to the injury with enhanced synthetic activity and overexpression of matrix-degrading enzymes and inflammatory mediators. During the healing of AC defects, cytokines and enzymes released by synoviocytes and chondrocytes in and around the repair tissue are required in order to initiate the repair process and eventually integrate the repair tissue within the defect. However, overexpression of catabolic factors may cause an imbalance between anabolic and catabolic activities at the defect site, leading to cartilage degradation, failed repair, and subsequent PTOA[2,32]. Therefore, the chondrocyte homeostasis in the defect is critical for the quality of healing cartilage and the integration of repair cartilage with the existing AC and subchondral bone. In addition, articular chondrocytes respond physiologically to both chemical[33–35] and mechanical[36–39] stimuli. This responsiveness could explain in part the late degradation of repair tissue which is initially hyaline-like but degenerates over time.

In order to overcome these challenges, researchers have been searching for new cell sources for AC repair by studying the link between the development and regeneration of AC and exploring