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Review Article

A sustained release gene delivery system based on polymerosome-entrapped injectable hydrogel for articular cartilage tissue engineering: a hypothetical approach

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Abstract:

Nowadays, Osteoarthritis (OA) is the main cause of disability, which is incurable, costly and responds poorly to treatment. Besides the common treatments, gene-activated scaffolds serve as new approach in the cartilage regenerative medicine. This hypothesis discusses a strategy for treating OA by combination of gene sustained release and tissue engineering. Possible intra-articular sites of gene transfer include the synovium and the cartilage. Most of experimental progresses have been made with gene transfer to the synovium, using viral and non-viral vectors. The studies mainly have been focused on in-vivo and ex-vivo transfer of therapeutic genes, which overexpress synthesis of the cartilaginous matrix, or inhibit its breakdown. Insulin like growth factor-1 (IGF-1) is an important growth factor for cartilage homeostasis, which leads to increase synthesis of the matrix macromolecules, decrease the catabolism of these molecules, serves as a chondrocyte survival factor and promote expression of the cartilaginous phenotype. Interleukin-1 (IL-1) known as a key mediator of cartilage loss in OA, and therefore, IL-1 receptor antagonist (IL-1Ra) gene transfer showed the therapeutic effects in experimental models of OA. Here, we argue the potential of intra-articular IGF-1 and IL-1Ra gene transfer in OA. As a scaffold delivering therapeutic genes, we hypothesized an alginate sulfate injectable hydrogel for sustained release of polymerosome, as gene carrier, for localized gene delivery in the articular cartilage defects.

Key words: Osteoarthritis; Gene therapy; Injectable hydrogel; Sustained release, Polymerosome

Introduction

Osteoarthritis (OA) is one of the most common musculoskeletal disorders. AO affected patients suffer from a huge loss in quality of life. The underlying mechanism of AO is based on the cartilage destruction accompanied by an inflammatory response, which leads to chondrocyte apoptosis and matrix degradation. However, an effective causal therapy, which provides long-term remedy, has not been found yet. Gene therapy seems as a promising approach, in which, therapeutic genes have been delivered to the targeted cells and modify the cell functions[1]. A variety of plasmid DNA (pDNAs) have been used to stimulate the biological processes, which could improve cartilage healing by (1) inducing mitosis and the synthesis and deposition

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of cartilage extracellular matrix components by chondrocytes, (2) induction of chondrogenesis by mesenchymal progenitor cells, or (3) inhibiting cellular responses to the inflammatory stimuli. The challenge is to adapt this technology into a useful clinical treatment modality [2].

Among the list of pDNAs for cartilage repair, anabolic growth factors of the transforming growth factor (TGF)-b superfamily, including TGF-bs 1–3, several of the bone morphogenetic proteins (BMPs), insulin-like growth factor (IGF)-1, fibroblast growth factors (FGFs) and epidermal growth factor (EGF) are potentially useful for AO treatment. In addition, prevention of the cartilage loss due to OA may also require inhibition of the actions of certain inflammatory cytokines, such as IL-1 and TNFa. These proteins, synthesized by synovial cells and chondrocytes, are found at elevated levels in arthritic joints and are important mediators of the cartilage degradation. Administration of proteins, which are anti-inflammatory or anti erosive, such as interleukin-1 receptor antagonist (IL-1Ra), 31 soluble TNF receptors, or inhibitors of matrix metalloproteinases may be effective in reducing the cartilage loss.

Toward the treatment and repair of damaged articular cartilage, the three primary candidate cell types, which could be targeted for the genetic modification are synovial lining cells, chondrocytes and mesenchymal stem cells (MSCs) [2].

Insulin-like growth factor-I (IGF-I) has been shown to be an important anabolic cytokine in the cartilage repair. Numerous studies in various animal models have defined the beneficial effects of IGF-I, which include increased cell survival and improved matrix quality through anabolic effects on the proteoglycan synthesis and increased type II collagen production. Of the cytokines known to control the cartilage development and homeostasis, IGF-I has emerged as one of the most critical growth factors for cartilage repair [3].

Gene therapy presents a novel and effective way to extend delivery of important anabolic peptides like IGF-I to the cells such as chondrocytes and synoviocytes. The goal is to transfer genes encoding therapeutic molecules to intra-articular tissues, so that they may then become endogenous sites for the therapeutic protein synthesis. Delivery of IGF-I through a gene-mediated protocol focuses on the stimulating an anabolic response in the synovial tissues that may then impact on the cartilage repair and synovial tissue synthetic functions. This is in contrast to the delivery of the interleukin-1 receptor antagonist (IL-1Ra) gene that has been effective in controlling the cartilage catabolic response to the injury.[4]

A most appropriate approach is to locally implant the cells associated with a supporting scaffold via surgery or injection at the injury site. In this case, the cells can either be transfected ex-vivo before association with the carrier material or first seeded in the scaffold and then transfected in the 3D environment. However, the ex-vivo transfection strategies would not provide a long-term transfection after implantation. Direct, local and sustained delivery of the nucleic acids from a scaffold can be achieved by including gene vectors into the scaffolds, thus forming a gene activated matrix, to ensure the efficient and sustainable in-vivo cell transfection.

Two main strategies were described to incorporate the gene vectors to the scaffolds: 1) Vector-DNA complexes could be immobilized onto the scaffolds, with no release required. Here, the transfection

efficiency will depend on the spreading propensity of the attached cells. 2) The gene vector could be associated by weak or covalent (but biodegradable) interactions to/onto the scaffold to obtain a prolonged and even controlled release for efficient transfection [5].

The combination of scaffolds with the genetically-engineered cells has been proved to greatly enhance both the transfection duration and transfection efficiency of the non-viral vectors. This leads to convincing gene-activated matrixes (GAM) suitable to promote bone, cartilage, or even osteochondral regeneration. Several key steps are crucial for the design of an efficient GAM concerning a specific application, including: the identification of efficient gene targets, the appropriate time sequence for the delivery, the choice of the vector regarding transfection efficiency and interaction with the scaffold, and also the nature of the scaffold. These interconnected parameters indeed influence the final efficiency of the system to induce tissue repair [6].

Chondroitin sulfate has been shown to have therapeutic benefits for hip and knee osteoarthritis. Heparan sulfate has a high affinity to a plethora of growth factors crucial for cartilage homeostasis. Sulfation of molecules has purported effects on the biological activity of biopolymers. For instance, Alginate is one of the biopolymers, which is widely used for biomolecules encapsulation [7, 8]. Freeman et al. studied the binding affinity of 10 different heparin-binding factors (including FGF, IGF, and VEGF) to the alginate sulfate [9]. They found that except FGF most of the factors bind equally well or better to sulfated alginate compared with heparin. Re'em et al. showed that scaffolds containing a mixture of alginate and sulfated alginate caused attenuated transforming growth factor beta-1 (TGFb-1) release ,and consequently improved chondrogenesis of entrapped mesenchymal stem cells compared with scaffolds lacking alginate sulfate [10]. Alginate sulfate is a potential hydrogel for autologous chondrocyte implantation that allows cells to synthesize their own matrix, proliferate, and maintain their cartilage phenotype. In addition, it has a proven high affinity to important growth factors and can thus be loaded with relevant cartilage growth factors for improved performance [8, 11]. Therefore, alginate sulfate could be an ideal biomaterial for injectable cartilage scaffold.

Designing a suitable carrier for efficient gene delivery to the targeted cell serves as crucial step in gene therapy approach. Among the various non-viral carriers, lipid based carriers are have been shown remarkable transfection efficacy [12]. Although liposome is the most popular lipid-based gene carrier, many limitations have emerged since the first liposomal formulation appeared. In particular, liposomes have shown to exhibit low encapsulation efficiency, poor physical / chemical stability (e.g. high critical aggregation concentration) and low chemical versatility. For these reasons, block copolymers assemblies showed growing interest for gene delivery applications. While no superior encapsulation efficiency was discovered, they do offer an enhanced chemical versatility, and show improved chemical and mechanical stability [13].

Polymersomes, as a self-assembled polymeric vesicle, are able to encapsulate hydrophilic, hydrophobic and amphiphilic molecules. In addition, their thick and tough membrane provides them with superior in-vitro and in-vivo stability. The presence of a dense polyethylene glycol (PEG) brush with relatively long PEG chain on the surface of polymersomes may increase their biological stability

and prolong the circulation times in blood. Polymersomes are versatile systems and their overall properties and drug release profiles can be easily tuned by applying various block copolymers, which could be biodegradable and/or stimuli-responsive. All these advantages make the polymersomes one of the most interesting supramolecular structures for potential applications in delivery of drugs, genes and proteins [14, 15].

Cell-penetrating peptides (CPP) are a class of small peptides 5–30 amino acids in length that have the potential to transport numerous types of therapeutic agents across the cellular membrane. CPPs have been widely used as a delivery vector due to their high transduction efficiency and capacity for delivering large molecules into the cells. CPPs as vectors delivering therapeutic agents have proved effective in certain disease models. The application of CPPs for delivering a variety of agents into cells has promising clinical potential [16]. The CCP incorporation into the gene carrier structure could facilitate their cell entrance ability [17].

Water-Soluble Conjugated Polymers (WSCP) are considered as exceptional candidates to serve as carriers to deliver drugs and monitor subsequent release process. Because of the negatively charged structure of nucleic acids, cationic WSCPs hold great promise in the field of gene transfection. Considering the self-tracking properties of these polymeric structures, they are very attractive to be involved in gene carrier designing [18].

Hypothesis

We suggest a multifunctional strategy for treatment of OA in the articular cartilage. A combination of IGF-1 and IL-1Ra pDNA simultaneously delivery to the targeted cells could inhibit matrix breakdown and promote matrix regeneration, respectively. We suggest a multifunctional gene carrier based on the CPP and WSCP as a polymerosome structure, while modified by PEG and chondrocyte specific surface receptor's antibody [19]. Furthermore, the autologous chondrocyte could be harvested to culture, expand and genetically modification them. For sustained release of vectors, we offer the alginate sulfate hydrogel, which could physically entrap the designed multifunctional carrier as well as the cells. The route of administration of the hydrogel is dual syringe injection directly in the cartilage defect (Figure 1).

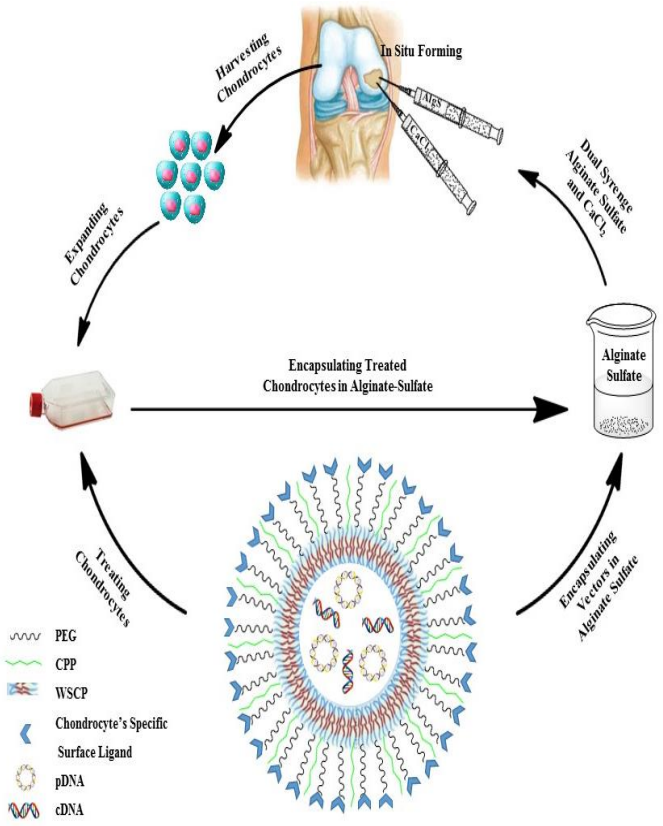

Figure 1: Schematic representation of an injectable hydrogel embedding polymerosome for sustained releasing of therapeutic genes in cartilage defect site.

Evaluation of the hypothesis

Delivery of IGF-I through a gene-mediated protocol focuses on stimulating an anabolic response in the synovial tissues that may then impact on the cartilage repair and synovial tissue synthetic functions. This is in contrast to the delivery of the interleukin-1 receptor antagonist (IL-1Ra) gene that has been effective in controlling the cartilage catabolic response to injury [4]. The delivered genes could be also tagged by a fluorescent agent to track them in the targeted tissue.

Conjugation of the chondrocyte antibodies on the PEG chains helps to target the chondrocyte or synoviocyte in the environment of defect. Using CPPs as the polymerosome compartment would result to enhance the penetration of vector to the cell. Also, the main reason for choosing WSCPs as the key polymerosome compartment is their solubility in the cytoplasm and facilitated release of the genetically cargo.

Instead of harvesting chondrocyte from non-bearing part of AC, we can use mesenchymal stem cell and differentiate them to chondrocyte in-vitro; then use them in the procedure. Based on the previous studies, cells encapsulated in the alginate sulfate exhibited remarkable spreading after 7 days of culture accompanied by a strong increase in proliferation when compared with non-modified alginate (five-fold, $p = 0.038$) [11]. Therefore, it is anticipated that the suggested hydrogel could support the MSC cells survival and proliferation.

The hydrogel including the polymerosome and cells would be directly administrated by dual syringe injection in the site of defect, where $CaCl_2$ solution would be injected simultaneously for crosslinking the hydrogel. Finally, the entrapped carries/genes in the hydrogel are expected to be release in sustained manner and the cells are locally transfected by the therapeutic genes. The hypothesized combinatorial approach seems to be effective for enhanced regeneration of the AO defeated cartilage tissue.

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