Natural Polymeric Nanoparticles for Drug Delivery in Osteoarthritis

Subjects: Orthopedics

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Natural polymers include several polysaccharides of plant-based origin that are either positively or negatively charged. They may have linear or branched configurations with amine groups that can be protonated under acidic conditions. Their main advantages as drug delivery vehicles are the biodegradable and biocompatible properties, unique chemical variety and presence of adjustable active sites that confer improved physicochemical properties to different biological applications. As opposed to synthetic polymers, natural polymers can have bioactive effects (e.g., anti-inflammatory) in tissues like cartilage, thus holding an intrinsic therapeutical activity apart from the one provided by the encapsulated drugs. In addition, the inherent antioxidant and anticoagulation effects of polysaccharides ensure a low immunogenicity for in vivo applications. The main limitation in the use of natural polymers is the batch-to-batch variation, because they are derived from natural sources that have a less controlled composition.

Keywords: nanoparticles ; polymers ; osteoarthritis ; cartilage

1. Chitosan

Chitosan is a linear polymer that can be naturally found in the shells of crustaceans and some other organisms. It contains a carbohydrate backbone similar to cellulose that consists of β-1,4-linked D-glucosamine, thus carrying a positive charge from amine groups. In recent years, chitosan has been widely used as a biodegradable and pH-responsive non-viral vector for drug delivery, exhibiting important antioxidant and anti-inflammatory properties [1][2]. Chitosan nanoparticles (CNPs) have been the most common type of natural polymer explored for OA treatment, either alone or combined with other polymers or inorganic compounds, with some examples of preclinical studies so far.

In a post-traumatic rabbit model of Osteoarthritis (OA), local delivery of CNPs loaded with plasmid DNA encoding IL-1Ra (IL-1 receptor antagonist) was able to significantly reduce severity of histological cartilage lesions compared to plasmids alone, and remarkably sustained the expression of this anti-inflammatory factor in the knee joint synovial fluid for at least 14 days [3]. In primary rabbit chondrocytes, chitosan–pDNA nanoparticles encoding an shRNA targeting MMP-3 and MMP-13 showed greater suppression in mRNA and protein levels, compared to empty NPs and plasmids alone [4]. Glycol chitosan/fucoidan nanogels loaded with the anti-inflammatory peptide KAFAK slowed down cartilage degeneration in a rat OA model, reducing the Osteoarthritis Research Society International (OARSI) score and structural changes in subchondral bone in the KAFAK-loaded NPs to the levels observed in normal rats [5]. The KAFAK-loaded nanoparticles also inhibited expression of inflammatory factors IL-6 and TNF-α in the rat models more than empty NPs and KAFAK alone [5].

Chitosan nanoparticles complexed with hyaluronic acid (Ch-HA NPs) were shown to promote efficient targeting by HA binding to the chondrocyte CD44 receptor. For example, Ch-HA NPs loaded with a plasmid encoding for anti-apoptotic CrmA (Cytokine response modifier) significantly decreased cartilage damage and synovial inflammation in rat models when compared to empty NPs, as demonstrated by a downregulation of IL-1β, MMP-3, and MMP-13 expression, and attenuated the loss of collagen type II [6]. Loading of Ch-HA NPs with curcuminoid suppressed inflammation and chondrocyte apoptosis in rat OA knee via repression of the NF-κB pathway [7]. Catabolic activity was also reduced as indicated by lower expression of MMP-1 and MMP-13, whereas collagen type II expression was increased in comparison with direct curcuminoid treatment [7]. Similarly to HA, chondroitin sulfate (CS), a negatively charged glycosaminoglycan present in the natural cartilage tissue, also can be recognized by the CD44 surface marker [8]. Chondroitin sulfate can be functionalized with chitosan nanoparticles by substitution of tripolyphosphate (TPP) for chondrocyte targeting [9]. Indeed, loading of these chitosan-CS NPs with GFP plasmid DNA showed significantly higher transfection efficiency than chitosan-TPP NPs in human arthritic chondrocytes, as well as downregulation of MMP13 expression when chitosan-CS NPs were loaded with MMP13 siRNA [9].

Chitosan nanoparticles have been tested with a variety of functional groups. Grafting of chitosan NPs with hydrophilic SO_3^- groups improved hydration capacity of NPs and provided efficient lubrication under a wide range of loads [10]. Given the wide buffering capacity provided by the amine groups of polyethyleneimine (PEI), chemical functionalization of chitosan NPs with PEI can further improve endosomal escape and protein expression levels of delivered plasmids. Indeed, pEGFP-loaded nanoparticles showed comparable transfection efficiencies with Lipofectamine 2000 in primary rabbit chondrocytes and synoviocytes [11]. In addition, functionalization of a chitosan nanogel with type A endothelin receptor antagonist triggered a decrease in inflammatory and catabolic markers in an OA equine organoid model, after seven days of culture [12]. This therapeutic effect was further enhanced by the combination of a hyaluronic acid nanogel functionalized with a type B1 bradykinin receptor antagonist [12].

Furthermore, coating NPs with drugs has shown promise in extending drug effects. The immobilization of anti-IL-6 and anti-TNF-α antibodies on the surface of chitosan nanoparticles significantly ameliorated inflammation in rat models compared to soluble antibodies alone, thus showing a higher reduction in inflammatory cytokine production, fibrosis in the synovial membrane and osteoarticular pain [13]. Intra-articularly injected antioxidant superoxide dismutase (SOD) conjugated to functionalized chitosan (O-HTCC) significantly attenuated mechanical allodynia and suppressed gross histological lesions in OA-induced rats when compared with free SOD treatment [14]. It also enhanced the anti-inflammatory effect and the in vivo antioxidant capacity, as expressed by lower levels of synovial malondialdehyde (MDA) and increased glutathione (GSH) content in the synovial fluid [14]. Another antioxidant, berberine chloride, was loaded in chitosan NPs to evaluate its effect in OA treatment. Results showed significantly higher anti-apoptotic activity than free berberine, and a prolonged retention time in the synovial cavity of at least 96 h [15].

2. Hyaluronic Acid

Hyaluronic acid (HA) is an anionic glycosaminoglycan and a major component of the cartilage extracellular matrix. Due to its lubricating properties, HA plays an important role in maintaining the viscosity and the integrity of the joints. In fact, intra-articular injection of high-molecular-weight HA (HMW, MW > 1000 kDa) is one of the currently clinically available options for OA treatment and was suggested to promote pain relief in knee joints with mild OA [16]. While systemic adverse effects are not predominant, local inflammatory reactions are more common due to degradation of HMW into fragmented low-molecular-weight (LMW, MW < 500 kDa) HA molecules by hyaluronidases [17]. As a drug carrier, hyaluronic acid displays good biocompatibility, biodegradability, high viscoelasticity, and is suitable for cartilage targeting by specific binding to the CD44 receptor [18] highly expressed in articular chondrocytes.

Self-assembled empty HA nanoparticles (HA-NPs) without any cargo showed a chondroprotective effect by blocking the CD44-NF-κB-catabolic gene axis [19]. While CD44 expression increased in the damaged articular cartilage of patients and mice with OA, intra-articular injection of these self-assembled HA-NPs in OA mice suppressed CD44 expression more effectively than free HMW HA and protected against cartilage destruction [19]. Even in chondrocytes transfected with adenovirus carrying the mouse Cd44 receptor (Ad-Cd44) gene, both CD44 expression and the activation of NF-κB were effectively inhibited by HA-NPs. The downstream expression of catabolic genes was also inhibited, leading to attenuated collagenase activity and lower PGE_2 production [19]. Hyaluronan nanocapsules loaded with celecoxib also showed superior efficacy over celecoxib alone in attenuating certain osteoarthritis parameters in rat OA models, such as knee swelling, inflammation score and NF-κB pathway activation [20].

3. Dextran Sulfate

Dextran sulfate is a polymer of sulfated anhydroglucose that is highly water-soluble and has negatively charged branches. Recently, nanoparticles based on dextran sulfate-triamcinolone acetonide (DS-TA) conjugates were tested for their efficacy to treat OA in mice by specifically targeting scavenger receptor class A (SR-A) on activated macrophages [21]. Intra-articular injection of these conjugates not only alleviated the structural damage to cartilage, but also significantly reduced the production of proinflammatory cytokines including IL-1β, IL-6, and TNF-α in the cartilage tissue, compared to untreated OA mice [21]. However, this reduction was not significantly lower than in the groups treated with free TA or empty NPs.

4. Elastin

Elastin, like collagen, is a fibrous protein that is present in the extracellular matrix of many connective tissues, such as the cartilage [22]. Elastin-like polypeptides (ELPs) were derived from tropoelastin and comprise multiple copies of the consensus repeat of the native protein, the pentapeptide Val-Pro-Gly-Xaa-Gly (VPGXG) [2]. The repeat units enabled the ELPs to be thermo-responsive, thus having a tunable transition temperature [2]. Thermo-responsive nanoscale vesicles

from an elastin-b-collagen like peptide (ELP-CLP) showed burst release behavior by dissociating the vesicles above the unfolding temperature of the CLP domain (>50 °C), indicating the potential of combining hyperthermia treatment for release of encapsulated drugs from appropriately engineered ELP-CLPs [23]. These elastin-like peptides bound collagen and displayed excellent cytocompatibility with chondrocytes and fibroblasts, without starting an inflammatory response as shown by a lack of TNF-α production by activated macrophages [23].

5. Polyphenols

Polyphenols, such as tannic acid, are organic compounds formed by multiple phenol units which naturally occur in plants as secondary metabolites. Polyphenol nanoparticles have intrinsic antioxidant properties, thus showing great potential as a drug delivery system in the treatment of inflammatory diseases. A boronate-stabilized polyphenol–poloxamer NP (PPNPs) loaded with dexamethasone (DMX) showed ROS scavenging abilities and significantly higher IL-10 secretion in an OA mouse model, compared to free DMX [24]. The DEX-PPNPs also enabled efficient repolarization of M2 macrophages (increased expression of the M2 macrophage marker Arg-1) when compared to saline-injected mice. Angiogenesis, cartilage degradation, and bone erosion were remarkably reduced in DEX-PPNP-treated mice compared to empty nanoparticles and DMX alone [24].

6. Silk Fibroin

Silk fibroin is a natural fibrous protein derived from the silkworm *Bombyx mori*, as well as by some species of spiders and other arthropods. In addition to their unique mechanical properties and slow biodegradability for retention at the target site, silk fibroin fibers exhibit comparable biocompatibility with other commonly used biomaterials such as polylactic acid and collagen [25]. Silk fibroin nanoparticles loaded with celecoxib or curcumin showed high cell viability of IL-1β-stimulated human articular chondrocytes (hACs) and a stronger decrease in anti-inflammatory activity when compared to free drugs, as demonstrated by significant reduction in IL-6 and RANTES expression [26].

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