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Biodegradable Nanoparticles for Intra-articular Therapy

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ABSTRACT

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by progressive loss of articular cartilage which leads to severe pain and restricted mobility in the patients. Age, excessive joint loading, or sports injury are the factors known to increase the risk of joint degeneration thus leading to OA. Many techniques have been employed in OA therapy but none of them has given satisfactory results in long term. Oral drugs have the disadvantage of having several side effects such as gastrointestinal problems, heart attack, and stroke associated with them. Surgical techniques lead to significant donor site morbidity. Intra-articular (IA) injections of hyaluronic acid (HA), glucocorticoids, have shown symptomatic relief but none of these treatments have been able to show disease modifying effects to an appreciable extent. Nanostructured drug delivery systems using liposomes and nanoparticles (NPs) can be administered as IA injections for sustained release and increased local concentrations of drugs in arthritis. A liposomal formulation of dexamethasone palmitate is currently available for IA drug delivery. Several other NPs of chitosan and biodegradable synthetic polymers like PLGA are in the developmental stages for delivery of steroids, NSAIDs and clodronate. This review highlights some of the promising nanostructured drug delivery systems for IA therapy, the issues involved in developing such systems and the future potential of such therapies for degenerative and inflammatory joint diseases.

Keywords: gene delivery, liposomes, NSAIDs, osteoarthritis, polymeric nanoparticles

Abbreviations: cox, cyclooxygenase; **DMOAD**, disease modifying osteoarthritis drug; **DSPC**, distearoyl phosphatidylcholine; **DSPG**, distearoylphosphoglycerol; **ECM**, extracellular matrix; **HA**, hyaluronic acid; **IA**, intra-articular; **IL-1**, interleukin 1; **IL-10**, interleukin-10; **IL-1Ra**, interleukin 1 receptor antagonist; **MLV**, multilamellar vesicles; **MMP**, matrix metalloproteinase; **MSC**, mesenchymal stem cells; **Mtx**, methotrexate; **NO**, nitric oxide; **NSAID**, non steroidal anti-inflammatory drug; **OA**, osteoarthritis; **PVA**, polyvinyl alcohol; **SF**, synovial fluid; **SLNP**, solid lipid nanoparticle; **SUV**, small unilamellar vesicles; **TNF-α**, tumor necrosis factor α; **WHO**, World Health Organization

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INTRODUCTION

Osteoarthritis (OA) is the most common degenerative joint disease characterized by damage to articular cartilage resulting in restricted joint mobility. American Journal of Rheumatology defines osteoarthritis as a heterogeneous group of conditions that leads to joint signs and symptoms which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins (Altman *et al*. 1986; Puttini *et al*. 2005). OA affects men and women of all ethnic groups at all geographic locations, more commonly in women. According to WHO estimates, 10% of world's people over the age of 60 years suffer from OA. There is a progressive loss of articular cartilage accompanied by attempted repair of articular cartilage, remodeling, sclerosis of subchondral bone, and osteophyte formation (Buckwalter *et al*. 1997) in OA joints. Currently, regularly prescribed treatment strategies include oral and topical drug delivery of various classes of drugs like NSAIDs, opioids, steroids and local anesthetics. However, current treatments do not prevent or cure OA, and symptomatic treatments often fail to provide satisfactory pain relief. The frequency and chronicity of OA and lack of effective preventive measures and cures make this disease a substantial economical and social burden for patients and healthcare systems around the world. Devising solutions to

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Invited Review

these grave problems have led to the development of newer drugs and methods of drug delivery for OA.

Intra-articular (IA) drug delivery, being one of the solutions, is eyed as a potential strategy for OA treatment. Steroids, NSAIDs and viscosupplements like hyaluronic acid are currently being administered through the IA route (Gerwin *et al*. 2006; Elron-Gross *et al.* 2008; Vanniasinghe *et al*. 2008). To achieve sustained and targeted IA drug delivery, novel approaches using biodegradable nanostructures like liposomes and NPs are being explored. Biodegradable nanostructures when administered intra-articularly offer the advantage of sustained release of the drug at the desired site of action, thus reducing the frequency of IA injections and increasing the patient compliance (Carballido *et al.* 2004; Gerwin *et al*. 2006). This paper gives a brief introduction to the current therapies in osteoarthritis and reviews the principles and applications of nanostructures for IA therapy.

ANATOMY AND PHYSIOLOGY OF A DIARTHRODIAL JOINT

A joint can be defined as a complex structure which performs the primary function of providing stability and motion to the body. On the basis of types of motion, a joint can be classified into (Gerwin *et al*. 2006)

i) Synarthroses – Limited or no mobility. e.g. skull sutures
ii) Amphiarthroes – Slight movement in all directions. e.g. vertebrae
iii) Diarthroses – Opposing bones move freely. e.g. knee joint.

Diarthrodial joints are most affected in joint degenerative diseases like OA. Fibrous capsules and ligaments surround the diarthrodial joint and hold it in place and synovial membrane lines the inner surface of the fibrous capsule. The direct contact between the articulating surfaces is prevented by the presence of hyaline cartilage and synovial fluid.

The synovial membrane (synovium) and synovial fluid

The synovial membrane is composed of two layers, one intima, and the other, subintima. Intima consists of two types of cells: Type A and B synoviocytes. Type A synoviocytes are macrophages which perform the function of removing unwanted debris within the membrane. Type B synoviocytes synthesize hyaluronic acid (a long chained glycolsaminoglycan composed of repetitive units of D-glucuronic acid and *N*-acetyl glucosamine linked through β-1, 4 and β-1, 3 glycosidic bonds). Adipose, fibrous and areolar tissue make up the subintimal layer.

The synovium serves two main functions: i) Ultrafiltration of plasma, and ii) facilitation of hydraulic resistance by the extracellular matrix of synovium. Also the ultrafiltrate along with hyaluronic acid (HA) serves to lower the coefficient of friction (Mahajan *et al*. 2005).

Synovial fluid (SF) is present between the articulating surfaces to reduce the coefficient of friction. Volume of SF in a normal joint is 0.5-2.0 ml and its pH is 7.4. SF has a resemblance with plasma with respect to its composition, because it is formed from HA and ultrafiltrate of plasma. Synovial fluid also consists of lubricin, proteins, phospholipids, high molecular weight fatty acid, leukocytes and cholesterol, etc. HA forms intramolecular hydrogen bonds and thus maintains the viscoelasticity of the SF (Moreland 2002). HA is an effective lubricant at low shear rates. At high shear rates lubricin works as a lubricant which interacts with surface of phospholipids. HA also stimulates the production of Matrix Metalloproteinases (MMP) inhibitors. MMPs (1, 8 and 13) are responsible for degradation of native collagen in cartilage, of which MMP 13 preferentially degrades collagen II. SF in general performs two functions: one, provides low friction coefficient between articulating surface, and second, provides nutrition to the chondrocytes, as cartilage is avascular and alymphatic.

Articular cartilage

Cartilage is an avascular, aneural structure of 1-5 mm thickness synthesized by the chondrocyte cells present in the joints. Collagen and proteoglycans along with 80% water (contributing to the wet weight of cartilage) are the predominant constituents forming the cartilage matrix, with chondrocytes embedded within this matrix. The matrix components impart the articular cartilage with the property of high tensile strength, load distribution and compressive stiffness (Aigner *et al*. 2006; Gerwin *et al*. 2006). Based on the arrangement of cells and matrix fibrils, cartilage is divided into four different zones: superficial, radial, deep, calcified. The calcified layer separates the cartilage and the underlying bone.

Bone

The subchondral bone (bone below the cartilage) differs from the cortical bone in that the former is thinner and contains haversian systems which run in parallel to the joints. The unique structural feature of subchondral bone makes it more susceptible to deformation than cortical bone shaft (Gerwin *et al*. 2006).

Articular cartilage and synovial fluid undergo several pathological changes in joint diseases like osteoarthritis. A brief description of the normal and diseased constituents of these structures is described in the next section.

BIOCHEMICAL COMPOSITION OF CARTILAGE

Chondrocytes

Chondrocytes are the only cells present within the articular cartilage occupying less than 10 % of the total volume. They are derived from mesenchymal stem cells (MSCs) found in the bone marrow of mature individuals and are essential for maintaining the extracellular matrix (ECM), size and mechanical properties of the tissue. The MSCs start differentiating during embryogenesis and pass through various lineages before forming hypertrophic chondrocytes, producing proteins that are important for matrix calcification. Chondrocytes are metabolically active and respond to various environmental stimuli, mechanical loads, osmotic pressure changes, and injury and degenerative arthritis (Temenoff *et al*. 2000; Flik *et al*. 2007).

Extracellular matrix

The extracellular matrix (ECM) present in the synovium consists of collagen types II, III, V, VI, IX, X, XI, which forms the interfibrillar mesh, proteoglycans-hyaluronic acid, chondroitin sulfate, keratin sulfate, dermatan sulfate and heparin sulfate which facilitate interaction with water, decorin, biglycan and non-collagenous proteins such as fibronectin and tenascin (Gerwin *et al*. 2006).

Tissue fluid

Tissue fluid comprises of water, metabolites, gases and inorganic dissolved salts of sodium, calcium, chloride, and potassium. The interaction between tissue fluid and ECM provides a high compressive strength and the ability to withstand high loads to the articular cartilage. Tissue fluid provides nutrients and oxygen to the avascular cartilage through exchange with synovial fluid (Temenoff and Mikos 2000).

ARTICULAR CARTILAGE PATHOLOGY

Cartilage defects can be classified as partial thickness lesions, full thickness lesions and osteoarthritis, depending on the depth of cartilage layer affected. In partial thickness lesions, the body responds by accumulation and proliferation of chondrocytes near the defect site, however, the defect

filling process ceases before it is repaired. With full thickness defect, MSCs from the subchondral bone quickly penetrate the defect area and start differentiating into chondrocytes. The chondrocytes then begin synthesizing ECM constituents- collagen and proteoglycan. However, the new tissue formed is an intermediate between the fibrocartilage and the hyaline, and is less stiff and more permeable than the native cartilage which ultimately degrades over a period of time (Holland and Mikos 2003).

Exact reasons for the development of OA are unknown but the possible risk factors which can cause the disease include age, sex, obesity, genetics, hormonal status, metabolic and nutritional factors (e.g. hyperglycemia), joint injury, occupational factors and sports, etc. OA progression starts with an increased degeneration of matrix and cartilage and increased production of the same, so there is a state of compensated osteoarthritis. However with time, the repair process slows down, and cartilage and matrix degeneration continues, leading to OA. Structurally, OA is characterized by cartilage degeneration, bone sclerosis, osteophyte (branched outgrowth of a bone) formation and bone cysts development. There is a continuous loss of important constituents of SF and cartilage like proteoglycans, decorins, biglycans. Synthesis of HA and its molecular weight is reduced resulting into lower viscocity of SF. Increased activity of Matrix metalloproteinases (MMP) results in cartilage degradation. A few members of 'Disintegrin and a Metalloproteinase Domain with Thrombospondin Motif' (ADAMTS) family are considered responsible for degradation of aggrecans. Degradation leads to a fall in pH resulting in activation of proteolytic enzymes called Cathepsins B, L and K, which further causes cartilage degradation. Various other factors like Cytokines (Interleukin and TNF-α), production of Nitric oxide (NO), prostaglandins (PGE_2) also play an important role in cartilage degradation (Pelletier *et al*. 2001; Puttini *et al*. 2005). The synovial fluid is decreased in its volume, viscosity and has altered protein and lipid levels and is infiltrated by inflammatory cells and markers in osteoarthritis.

CURRENT TREATMENT FOR OA

The primary goals of the currently available therapy for OA are to relieve pain, delay disease progression, maintain or improve joint function, and prevent or correct deformity. A large number of medications are available for the treatment of OA administered through oral, intravenous and IA routes. Surgical procedures are inevitable when drugs fail to provide relief and progression of disease cannot be averted.

Oral drug delivery

Analgesics like Paracetamol or Ibuprofen are used for relieving mild pain in OA. Paracetamol is known to induce hepatotoxicity and potential renal damage. Non Steroidal Anti-inflammatory Drugs (NSAIDs) are non-selective cyclooxygenase (COX) inhibitors which are used to suppress pain and inflammation in OA patients. NSAIDs may be given as oral drugs or topical application. Many NSAIDs, differing in activity are prescribed regularly to OA patients depending on the severity of disease e.g. Piroxicam, Diclofenac, Naproxen, ketoprofen, Indomethacin. Ulcers, bleeding, obstruction, gastrointestinal intolerance, dyspepsia, abdominal pain, nausea, nephropathy and impaired platelet aggregation are the most common side effects of systemic or oral NSAIDs. There have been safety concerns for long term treatments with NSAIDs on bone and cartilage. Reports on acceleration of joint degradation on long term usage of indomethacin and inhibition of reparative bone remodeling are some of the issues of concern for use of oral NSAIDs (Courtney and Doherty 2006; Gerwin *et al* 2006).

Coxibs: These are selective COX-2 inhibitors, do not cause gastrointestinal toxicity and platelet aggregation like the NSAIDs and are similar in efficiency to NSAIDs. Coxibs act by inhibiting the enzyme COX 2, one of the two isoforms of COX, involved in inflammatory cascade. Rofecoxib, valdecoxib and celecoxib are some widely used coxibs. However, reports of cardiac problems have led to a ban on these drugs (Courtney and Doherty 2006; Gerwin *et al*. 2006).

Opioids*:* When severity of disease is high, opioid analgesics are used, but their use is limited due to tolerance and dependence e.g. Dextropropoxyphene, Tramadol, etc.

Disease Modifying OA drugs (DMOADs): Drugs listed above alleviate the symptoms of OA but do not retard the progression of disease. This class of drugs is believed to alter the progression of disease. Various DMOADs under development are (1) MMP inhibitors e.g. CP-544439 (Pfizer), AZD-8955 (Astra Zeneca), tetracyclines and its derivatives like Doxycycline (2) Growth factors and drugs inhibiting cytokines e.g. Anakinra (IL-1R antagonist), Diacerein (inhibitor of IL-1), Adalimumab, Infliximab (humanized monoclonal antibodies to tumor necrosis factor α (TNF α) (3) Bisphosphonates (3) Gene therapy (4) Glucosamine sulfate (precursor for glycosaminoglycan and helps rebuild cartilage) (Puttini *et al*. 2005; Qvist *et al*. 2008).

IA DRUG DELIVERY

IA injections are given directly into the joints and thus have a localized and targeted effect with fewer incidences of systemic side effects and higher bioavailability and efficacy (Ayral 2001). Currently, glucocorticoids, hyaluronic acid and a few other drugs such as local anaesthetics and NSAIDs are used for IA treatment of osteoarthritis. Most of the IA therapy is restricted to knee and ankle joints. However, the technology also holds promise in temporomandibular joint diseases (Mountziaris *et al*. 2009).

IA steroids

Glucocorticoid preparations are the majority in this class and are available as suspension or solution depending upon the solubility of glucocorticoid used. Kenalog™ (triamcinolone acetonide), Depo-Medrol® (methyl prednisolone acetate), Celestone® (betamethasone sodium phosphate) are some of the currently available commercial formulations (Schumacher and Chen 2005). Most of these are in solution, suspension or emulsion form. Glucocorticoids alleviate symptoms but do not alter disease progression. Besides this, glucocorticoids cannot be used for a longer period of time, because of their serious side effects. Even when given IA, glucocorticoids have been shown to cause facial flushing, hypertension, suppression of immunity, mild synovitis, and cartilage degeneration on excessive use. There is a risk of Cushing's syndrome and anaphylactic reaction also (Carballido *et al*. 2004).

IA hyaluronic acid or Hylans

Viscoelastic, high molecular weight HA is given as an IA injection in OA, because there is a decrease in the viscosity of synovial fluid. HA is given as a viscosupplement to restore viscosity values back to normal. The currently available HA preparations are marketed as high molecular weight hylan (6-7 MDa, Synvisc®, hylan G-F20) and low molecular weight HA (0.5-2 MDa, Hyalgan®, Supartz®) (Ayral 2001, Moreland 2002). Synvisc® is a marketed viscosupplement containing Hylan G-F20, which is claimed to be effective for 6 months after single IA injection, has been approved by US FDA. The side effects of HA injection are low, except some local reactions.

Miscellaneous IA injections

Mainly local anaesthetics, NSAIDs and sometimes opioids are used for local therapy. Local anaesthetics when used provide temporary relief from pain. NSAIDs also alleviate the symptoms of pain and inflammation temporarily. A Cu-Zn superoxide dismutase, Ogotein is undergoing a number

Table 1 Advantages of NPs for IA therapy.

Localised and targeted drug delivery	Horisawa *et al*. 2002; Carballido *et al.* 2004
Less amount of drug required	Gerwin *et al.* 2006
Low systemic side effects	Horisawa *et al.* 2002
Can improve bioavailability of drugs	Monkkonen *et al.* 1995
Long duration of action due to sustained release	Puttini *et al.* 2005
Better cellular internalization due to nanosize	Panyam and Labhasetwar 2003
Can escape rapid macrophage uptake and clearance	Sanvicens *et al.* 2008

of clinical trials, for treatment of OA. However, its mechanism of action is unclear, but it is thought to inhibit phagocytic response to hydroxyapatite crystals (Gerwin *et al*. 2006). However, the currently available IA therapy has certain drawbacks such as low residence time, therefore requires repeated injections which in turn increases the risk of infections.

NP FOR IA THERAPY

Studies are being carried out by various groups on different novel carriers, ranging from microparticles to NPs to improvise on the conventional IA drug delivery systems. Efforts are being made to design formulations which have a sustained release, targeted drug delivery with minimum amount of adverse effects and maximum therapeutic efficiency. Potential advantages of nanocarriers in IA therapy are summarized in **Table 1**.

NPs are particles below the submicron range. Research is constantly being conducted in the field of medicine and healthcare with an aim to improve the accuracy of diagnosis of a disease and treat a particular ailment with least or no side effects.

TYPES OF NANOSTRUCTURES

Nanostructures used for drug delivery may be classified based on structure of the carrier, composition, cellular fate etc. **Fig. 1** describes the classification of various nanostructures. For therapeutic purposes, biodegradable NPs are preferred as they can be broken down in the body and their breakdown products are also biocompatible.

Superparamagnetic NPs

Superparamagnetic NPs are synthesized using magnetic materials such as iron, nickel, cobalt and their alloys. They exhibit the phenomenon of superparamagnetism, consisting of small ferromagnetic clusters that can orient themselves in the presence of external magnetic fields and can cause a rise in temperature when exposed to an alternating magnetic field. These particles have found application catalysis, as contrast agents in magnetic resonance imaging (MRI) and can also be made biocompatible for suiting biomedical application by coating them with silica or PEG. Superparamagnetic iron oxide nanoparticles (SPION) have been used in drug delivery and gene transfection (Schulze *et al*. 2005; Azzazy and Mansour 2009; Faraji and Wipf 2009). Schulze *et al* studied uptake of PVA coated SPIONS by synovial cells and biocompatibility given as intraarticular and periarticular injections in sheep models.

Though not biodegradable, superparamagnetic NPs can be included along with suitable surface modifications for IA drug delivery. The advantage of using superparamagnetic NPs is the feasibility of increasing local concentrations and achieving prolonged joint retention by using external magnets.

Superparamagnetic iron oxide NPs and dexamethasone acetate (DXM) were co-encapsulated into PLGA microparticles for IA therapy of arthritis by Butoescu *et al*. (2009a, 2009b). The particles were found to be compatible with synoviocytes and were internalized through a phagocytic process. The retention of the particles in the joints was enhanced by external magnets.

Quantum dots

Quantum dots, also known as nanocrystals, are nanosized semiconductors typically of the size range of 2-10 nm. They

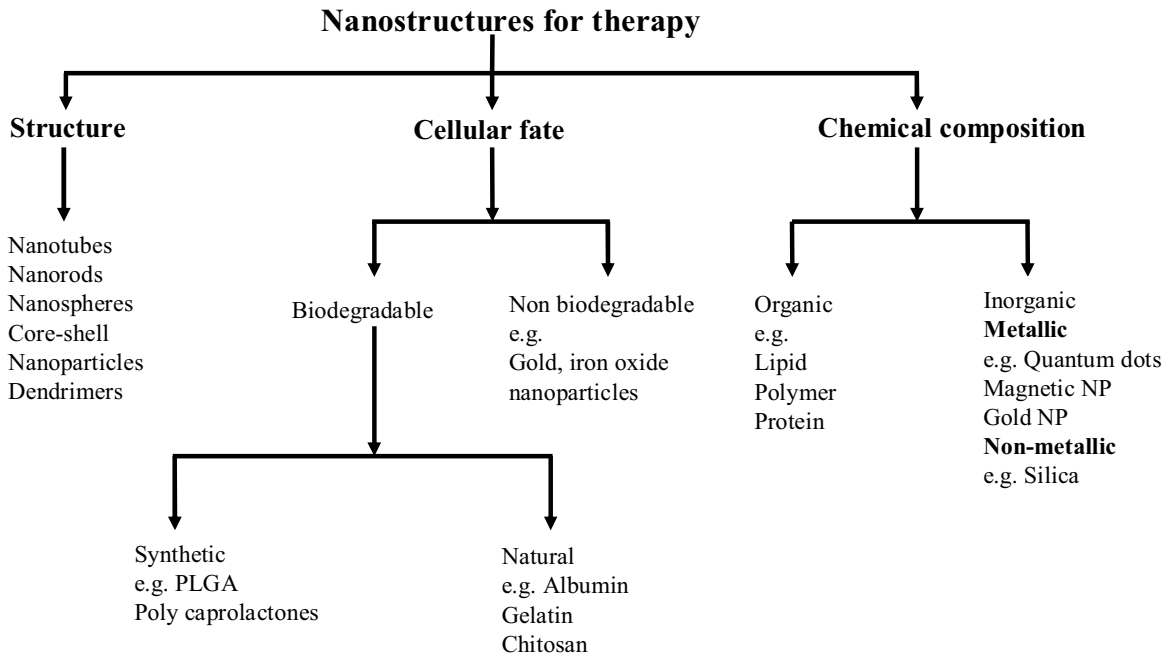

Fig. 1 Classification of nanostructures for drug delivery.

are inorganic fluorophores consisting of a core, shell and coating. Quantum dots take their name from the quantum confinement effect which is also responsible for their tunable emission spectra. They have a broad range excitation, narrow emission spectra and good photostability (Penn *et al*. 2003). Quantum dots have been used in cell imaging and immunoassays and in cancer drug delivery (Juzenas *et al*. 2008; Azzazy and Mansour 2009; Faraji and Wipf 2009).

Nanotubes (Fullerenes, Bucky balls)

Nanotubes are self assembling sheets of atom forming single or multi-walled tubular structures. The material used for nanotubes could be either organic or inorganic for example: carbon, ceramics, metals and organic polymers. The most important group of nanotubes is carbon nanotubes which consists of single or multiple cylindrical graphite sheets of diameters ranging from 0.4 nm to tens of nanometers (Mamalis *et al*. 2004). These can be functionalized easily; however, conflicting toxicity reports limit their use in drug delivery (Yang *et al*. 2007).

Dendrimers

Dendrimers are polymer based macromolecules gaining wide use in nanomedicine due to their ease of modification and size control. They are synthesized by layer-by-layer method around a core unit and the particles formed are highly symmetrical with precise architecture and low polydispersity (Svenson 2009). They possess a highly branched structure (with or without a central core) which enables them to act as hooks for attaching various molecules such as fluorescent dyes, enzymes (Faraji and Wipf 2009). Dendrimers have been used to encapsulate drugs such as methotrexate, paclitaxel, NSAIDs such as ibuprofen, diclofenac, mefenamic acid, indomethacin, ketoprofen, and naproxen however, their use in IA drug delivery is yet to be explored (Svenson 2009).

IA half-lives of dendrimer-linked nitroxides were found to be 160–208 min which was sufficient for in vivo MR imaging. Such modified dendrimers are targeted to glycosaminoglycans and may improve cartilage imaging (Winalski *et al*. 2008)

Biodegradable polymeric NPs

Polymeric NPs are the most preferred class of nanostructures in biomedical drug delivery applications. The materials used for biodegradable NPs include chitosan, alginate, Polylactic-co-glycolic acid (PLGA), gelatin, polycaprolactones. **Fig. 2** summarises the chemical structures of some of the commonly used materials. They offer the advantage of being biocompatible and biodegradable, easy surface modification, and have a wide range of therapeutic molecules that can be entrapped and delivered using these NPs (Faraji and Wipf 2009).

Liposomes

Liposomes are biodegradable lipid-based concentric bilayered NPs of sizes ranging from 30 nm to several micrometers (Vanniasinghe *et al*. 2008). They are amphiphlic in nature and can be easily modified for increasing the half life of the drugs that they encapsulate. Liposomes have the advantage of being able to encapsulate both hydrophobic and hydrophilic drugs in the same carrier. Liposomes are

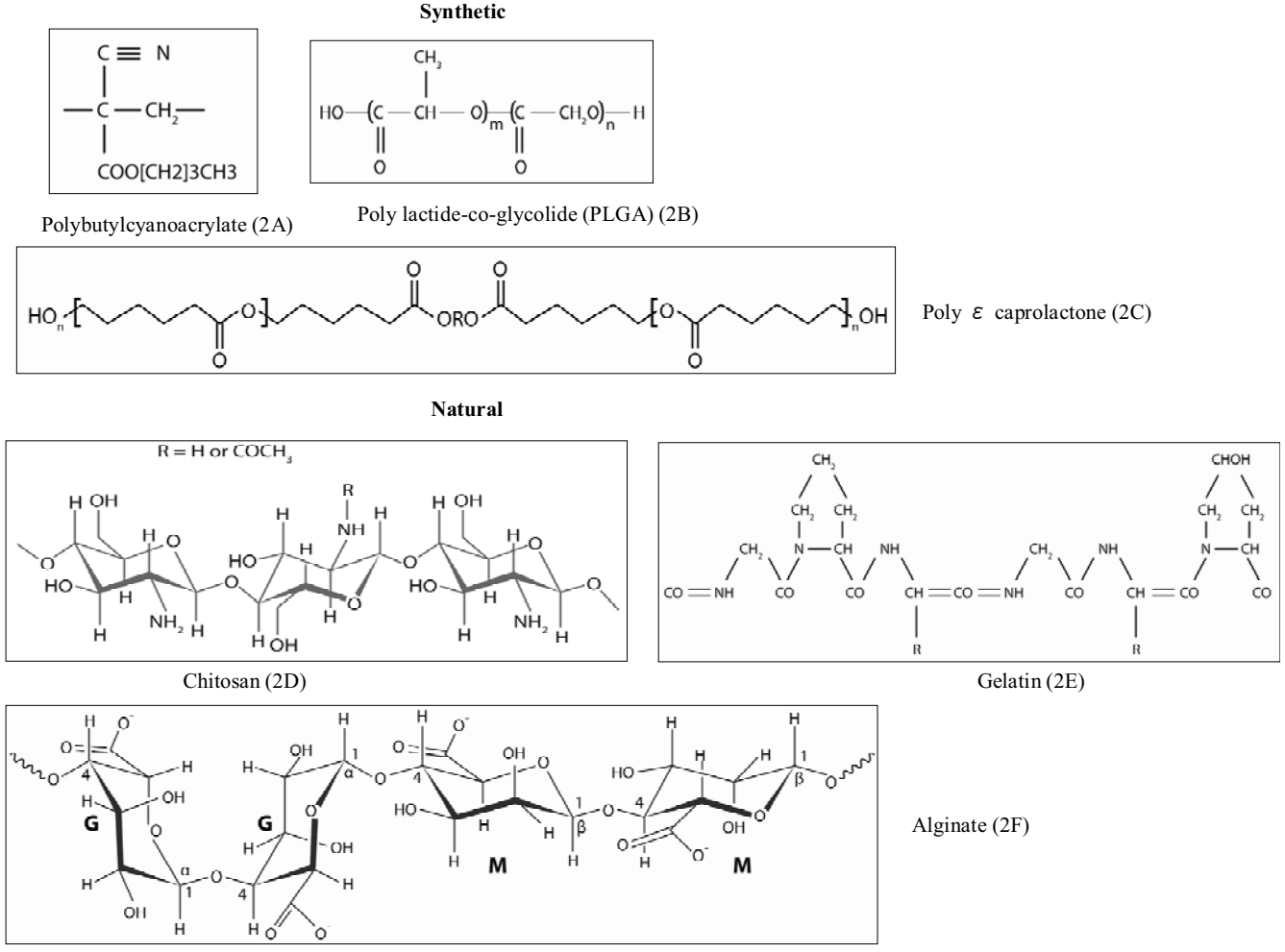

Fig. 2 Materials used for biodegradable NP preparation. Structure of polyethylcyanoacrylate (**A**), polylactidecoglycolide (**B**) and polycaprolactone (**C**), chitosan **(D)**, gelation (**E**), alginate (**F**). A-C are structures of synthetic polymers used for development of NPs. D-F are structures of natural polymers used for the development of NPs.

synthesized from naturally occurring and synthetic, non-toxic and biodegradable phospholipids such as phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PG), phosphatidyl serine (PS) and their analogs which may be used in different combinations or in combination with sterols such as cholesterol (Vanniasinghe *et al*. 2008). Liposomes are being studied extensively for their use in IA drug delivery as described later.

Solid lipid NPs

Designed as an alternative to liposomes and emulsions, solid lipid nanoparticles (SLNPs) are lipid-based colloidal carriers to be developed as drug delivery carriers in the early 1990s. These are stable, biodegradable particles consisting of a rigid core of hydrophobic lipids surrounded by a phospholipids monolayer. The core could be a homogenous matrix, a drug-enriched shell, or a drug-enriched core. Two common methods of production of SLNs are microemulsion method and high pressure homogenization. SLNPs have the advantage of an increased stability as compared to liposomes which may be particularly suitable in IA therapy as the joint is always subjected to loading and unloading conditions (Muller *et al*. 2000; Thakkar *et al*. 2007).

EFFECT OF NANOPARTICLE CHARACTERISTICS ON DRUG DELIVERY

Particle size

Particle sizes along with size distribution are important factors determining the in vivo distribution, toxicity and biological fate of NPs within the body. Particles in nanosizes have the advantage of higher intracellular uptake by cells as compared to micron sized particles. Particle sizes also affect drug release profiles and biodegradation (Mohanraj and Chen 2006).

Surface chemistry

Surface properties are crucial in the opsonization and clearance rate of the NPs. Coating NPs with polymers/surfactants or using copolymers such as Poly ethylene glycol (PEG), polysorbate 80 (Tween 80) during NP synthesis are ways of altering protein adsorption onto NPs and modulating macrophage uptake of the NPs (Mohanraj and Chen 2006). Surface coatings with biopolymers like chitosan can increase mucoadhesion which is beneficial for increasing the residence time over mucosal surfaces. Hydrophilic biopolymer coatings also play an important role in IA carriers as they influence the interactions with the extracellular matrix and cartilage.

Surface charge

The charge on NPs as determined by zeta potential measurements also influence the stability of particles in suspension. A zeta potential above +/- 30 mV indicates stable suspensions. Charged NPs also have specific interactions with cellular components and biomolecules.

Drug loading

Drug loading can be done either by incorporation method or adsorption/absorption method. The amount of drug entrapped within a NP depends on the NP composition, molecular weight, hydrophobic or hydrophilic nature of drug and matrix and interactions between drug and NPs matrix materials. Solubility of drug, desoprtion of surface-bound drug, diffusion through the particle matrix, biodegradation of the NP matrix, are factors governing rate of drug release (Mohanraj and Chen 2006).

CURRENT STATUS OF POLYMERIC NPs FOR IA DRUG DELIVERY

Microparticles of albumin were found to have an increased residence time in the rabbit joints and particles below 6 µm were found to be easily phagocytosed (Ratcliffe *et al*. 1987). The study shows the potential of using protein based NP/microparticles for IA therapy. Size and chemical nature appear to play a role in the phagocytosis process intra-articularly as is seen by the conflicting reports regarding size ranges that can escape phagocytosis by the joint macrophages.

Rothenfluh *et al.* (2008) emphasized on the specific targeting of NPs to cartilage cells by specifically binding a peptide ligand to the surface of NPs. Also, the effect of size of NPs on the ability to enter into cartilage matrix was studied. It was suggested that if NPs are able to enter the cartilage and can be attached to cartilage matrix by using a specific ligand, then it can act as a reservoir resisting rapid clearance of NPs resulting in a sustained delivery of drugs and genes. The polymer used for NP preparation was polypropylene sulphide (PPS), whereas pluronics, which are block copolymer of poly (ethyleneglycol) and poly (propyleneglycol), was used as an emulsifier. Rationale for using PPS is that it renders itself to surface functionalization and size control can be achieved easily. Also, PPS is oxidation sensitive which means in the presence of oxidative species, it is converted from sulfide (hydrophobic) to sulfones (hydrophilic) which can be used as a trigger to release drugs, as there are many oxidative species present in conditions like inflammation and tumors. Interestingly it was found that polymer NPs with a size of 96 nm were unable to enter cartilage, while 38 nm sized NPs entered cartilage efficiently.

Horisawa *et al*. (2002) studied the effect of molecular weight and ratio of lactic acid and glycolic acid of PLGA polymer on release profile of drug (Betamethasone sodium phosphate, BSP) *in vitro* and *in vivo*. It was observed from the results that the most suitable drug release profile was obtained from PLGA-7520 (LA/GA=75/25 and mol. wt. = 19,900). Low mol. wt. PLGA nanospheres were found to be able to provide prolonged release of drug *in vitro* and *in vivo*.

CURRENT STATUS OF LIPOSOMES FOR IA THERAPY

Liposomes are spherical vesicles consisting of either a single bilayer (unilamellar) or more than one bilayers of phospholipids (multilamellar) ranging from 10 nm to 3000 nm in size. Liposomes can provide sustained delivery of drugs in the joints and are suitable for delivering both hydrophilic and hydrophobic drugs. Currently, Lipotalon® (manufactured by Merckle, Germany) containing dexamethasone-21-palmitate is the only available liposomal formulation for IA drug delivery. Adding palmitate to dexamethasone decreases the solubility and enables it to be incorporated into liposomes. Lipotalon has soybean oil as lipid phase and glycerol and water as aqueous phase. Rationale for using Soybean oil may be that it contains soy isoflavones which serve as anti-inflammatory agents and increase bone mineralization. Liposomes of triamcinolone acetonide-palmitate were also found to show increased IA residence time in rabbits as compared to the free steroid (Lopez-Garcia *et al*. 1993)

The drug release profile and stability of liposomes depend on factors such as type of lipids used, ratio of lipids (if used in combination), charge on the liposomal surface, type of drug used etc. Negatively charged oligolamellar vesicles (egg phosphatidylcholine) have been shown to be more effective in treatment of antigen-induced arthritis in rabbit joints than neutral multilamellar (dipalmitoyl phosphatidylcholine) vesicles. The optimal size for liposomal retention within synovial fluid was found to be 750 nm and a particle size of less than 5 µm was required to minimize macrophage endocytosis (Bonanomi *et al*. 1987).

In another study, Monkkonen *et al.* (1995) conducted studies on liposomal formulation for IA drug delivery of Clodronate. Clodronate is a bisphosphonate which inhibits the production of cytokines from macrophages and has beneficial effects when given as liposomes intra-articularly. The study concentrated on the effect of surface charge density of liposomes and internal osmotic pressure of the liquid encapsulated in liposomes on drug release. Monkkonen and co-workers tried to optimize the surface charge density using a combination of distearoylphosphoglycerol (DSPG, negatively charged) and distearoyl phosphatidylcholine (DSPC, neutral), which are biodegradable phospholipids. The liposomes formed were of the size range 200-300 nm. It was observed that formulation with 25: 75 DSPG/DSPC had least leakage of drug from liposomes. Negative surface charge density induces leakage of encapsulated material in biological fluid, and lipid vesicles are more susceptible to fusion due to Ca^{+2} causing release of material. However, negative charge is also essential for uptake of liposomes by cells (Monkkonen *et al*. 1995). The study suggests that charge on the liposomal surface needs to be optimized in order to obtain better drug release. However, another *in vivo* study suggested that liposomal clodronate had temporary anti-inflammatory and anti erosive effects on antigen induced arthritis rabbits, but there was a reduction in the loss of proteoglycan content over long term (Ceponis *et al*. 2001).

Williams *et al*. (1996) investigated the effect of single IA injection of liposomal conjugated Methotrexate (Mtx) on the joint inflammation in antigen induced arthritis in rats using multilamellar vesicles (MLV) and small unilamellar vesicles (SUV). In order to increase the incorporation of the drug into the lipid bilayer and hence increase the lipophilicity of Mtx, researchers have synthesized Mtx analogue in which dimyristoyl phosphatidylethanolamine (DMPE) is covalently bonded with Mtx. This study found that MLVs (1.2 μm) are more efficient in suppressing joint inflamemation than the SUVs (100 nm) (Williams *et al*. 1996). In another study, it was found that radioactively labeled methotrexate liposomes preferentially accumulate in the synovial membrane and allow sustained release locally (Foong *et al*. 1988)

In a similar study on MLVs, diclofenac-loaded MLVs were synthesized using soybean phosphatidylcholine (SPC) and dipalmitoyl phosphatidylethanolamine (DPPE) and covalently coated with either hyaluronan or collagen. The encapsulation efficiency of diclofenac in all coated and non-coated liposomal formulations was found to be > 50%. The study demonstrated binding of collagen and hyaluronan coated MLVs to hyaluronan specific CD44 receptors and COX inhibition in CT-26 (mouse colon carcinoma) cell lines (Elron-Gross *et al*. 2008). Metselaar *et al*. have devised a novel and promising means to increase the therapeutic activity of glucocorticoids by using long circulating polyethylene glycol (PEG) liposomes which have shown the ability of preferential accumulation in inflamed joints after intravenous administration (Metselaar *et al*. 2003).

Kaur *et al*. (2007) developed a liposomal gel (niosomal) for sustained) topical delivery of celecoxib. The celecoxib loaded liposome gel showed a significant reduction in rat paw edema when compared with conventional nonliposomal gel (celecoxib), suggesting the potential of liposomal formulations for local delivery in arthritis by penetrating through the skin.

RECENT ADVANCES IN NPs FOR IA DELIVERY OF ENZYMES AND PEPTIDES

Superoxide dismutase and its lipophilic derivatives have been used in liposomal formulations for treatment of inflammatory arthritis. The liposome-associated enzyme showed promising anti-inflammatory effects and was therapeutically superior to the free enzyme. Such liposomes are referred to as enzymosomes (Cruz *et al*. 2005; Gaspar *et al*. 2007).

Lactoferrin is an iron-binding glycoprotein from the transferrin family. It is expected to binds potentially toxic-free iron components and to modulate the inflammatory response in arthritis. Trif *et al*. (2001) encapsulated lactoferrin in negatively charged liposomes and found that they had a prolonged residence time and modulated the Th1/Th2 cytokine balance for a period of 2 weeks as opposed to the 3-4 day efficacy of the free protein.

RECENT ADVANCES IN NPs FOR IA DELIVERY OF GENES, siRNA AND REGENERATIVE THERAPIES

A study by Zhang and coworkers on chitosan NPs showed that chondrocytes can be transfected with gene of interest using NPs. Two different genes IL-1Ra (Interleukin 1 receptor antagonist) and IL-10 (Interleukin-10) were used to transfect the chondrocytes. Higher uptake of IL-1 Ra than IL-10 suggested that transfection efficiency of NPs was a function of gene product. Chitosan is a linear biodegradable polysaccharide composed of randomly distributed β-(1,4) linked-D-glucosamine (deacetylated) and *N*-acetyl glucosemine (acetylated). Chitosan NPs are better than viral constructs in terms of gene delivery, because of latter's chances of wild type reversion and immunogenicity (Zhang *et al*. 2006).

Another promising emerging technology for IA therapy is the use of small interfering ribonucleic acids (siRNAs) that silence specific genes by binding to the messenger RNA and hence preventing the translation of the associated proteins. NPs can help to internalize siRNA by overcoming various intracellular barriers and preventing the degradation of the molecule. In rodent models of knee arthritis, IA delivery of siRNA targeting tumor necrosis factor-a (TNF-a), a proinflammatory cytokine, significantly reduced inflammation and exerted a chondroprotective effect for several weeks (Inoue *et al*. 2005; Schiffelers *et al*. 2005). However, improved carriers for delivering siRNA over prolonged periods need to be developed in order to achieve promising clinical effects.

Oligodeoxy nucleotides also have a potential for local therapy in arthritis (Stevenson 2004). Liposomes of phosphatidylserine, phosphatidylcholine cholesterol encapsulated with the inactivated Sendai virus (Z strain) virion are used to deliver transcription factor decoys. Amelioration of arthritis in rats was achieved with IA delivery of nuclear factor-kappa B decoy oligonucloetides using such liposomes which are referred to as hemaglutinating virus of Japan-liposomes (HVJ-liposomes) (Tomita *et al*. 1999).

Various strategies are emerging to aid the regeneration of the diseased tissues like articular cartilage by the use of scaffolds and growth factors. A study by Inoue *et al*. (2006) found that IA delivery of basic fibroblast growth factor using gelatin microparticles in rabbits, improved knee joint swelling, proteoglycan expression, and histology of the cartilage in arthritis. There is a potential for delivering several growth factors directly into the joints and controlling their release over several weeks or months in order to achieve cartilage regeneration in arthritis.

CONCLUSIONS

OA is debilitating disease of the joint, which is not a single disease but can be called as a collection of a group of diseases, with different pathologies, ultimately leading to joint destruction. The current treatment regime involves use of oral drugs (low bioavailability), topical formulations, repeated IA injection of HA, steroids, etc. and painful surgical procedures, none of which has any disease modifying effect, thus leading to further degeneration of cartilage with each joint movement/motion.

The currently available formulations are limited in efficiency due to their low residence times, formulation problems and physicochemical properties of drugs. With limited choices of active ingredients, the therapy for OA mostly includes suspensions and solutions as the only type of for-

Table 2 Summary of some main studies of NPs for IA therapy in osteoarthritis.

Nanostructure	Material used	Particle size	Drug	References
Polymer NPs	Chitosan	150-300 nm	Gene delivery	Zhang *et al.* 2006
	Poly propylene sulphide	38-96 nm	Biomolecular ligand	Rothenfluh *et al*. 2008
	PLGA	300-490 nm	Betamethasone sodium phosphate	Horisawa *et al*. 2002
Liposomes	Combination of DSPG and DSPC	200-300 nm	Clodronate	Monkkonen *et al*. 1995
	PEG-DSPE, DPPC	90-500 nm	Prednisolone phosphate	Metselaar *et al*. 2003
	DMPE	100-1200 nm	Methotrexate	Williams *et al*. 1996
	Phosphatidic acid, Egg-PC,DPPC	750 nm	Dexamethasone palmitate	Bonanomi *et al*. 1987
	DSPG, DSPC	< 1 μm	Clodronate	Ceponis *et al*. 2001
Lipid NPs	SLNPs	< 1μm	Celecoxib	Thakkar *et al.* 2007
Iron Oxide NPs	Superparamagnetic iron oxide NPs with PLGA microparticles	10 nm for SPIONS, 10μm for PLGA	Dexamethasone acetate	Butoescu *et al.* 2009

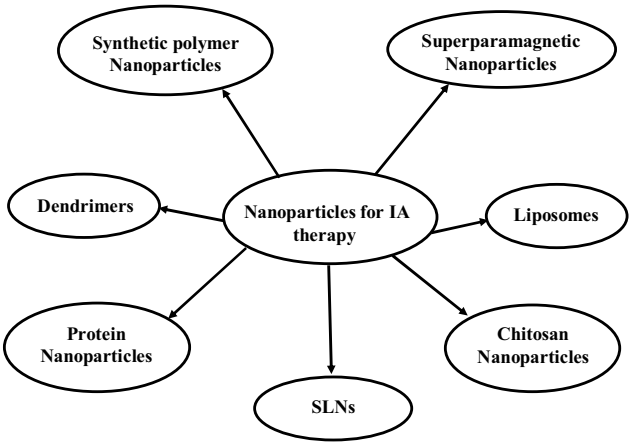

Fig. 3 NPs for IA therapy.

mulation available. These formulations, in general, have failed to provide sustained delivery of drugs hence requiring frequent injections thereby causing discomfort to the patient. IA injections of steroids and HA suffer from disadvantages of being painful procedure, low patient compliant, risk of infection and low residence time in joints.

There is a need for the development of newer techniques to deliver drugs at the target site, as well as newer active ingredients which are more effective in alleviating and curing the disease. IA therapy using nanostructures such as liposomes and NPs have demonstrated sustained drug release profiles, higher efficacies, high residence time and low systemic side effects and therefore hold a promising future for the treatment of joint degenerative diseases like OA (**Table 2; Fig. 3**). For successful development of IA NPs, the incorporation of drugs, DNA, or genes, surface functionalization, size, interactions with synovial fluid, residence time in joints and drug release in presence of loading and unloading need to be optimised. Several NPs are promising in their ability to increase joint residence time and obtain sustained release of drugs within the joints. The future may see the development of NP-based IA therapies for drugs, genes and regenerative growth factors which may help alleviate the morbidity associated currently with joint diseases.

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