Expert Opinion

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Intra-articular drug delivery systems: overcoming the shortcomings of joint disease therapy

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Background: Intra-articular drug delivery is very useful for treating local disease flare-ups, synovitis and pain in joints. However, the effectiveness of drugs following intra-articular administration is limited by drug delivery issues. *Aim*: This review addresses critical drug delivery parameters that influence the biocompatibility, tolerability and efficacy of intra-articular administrations and offers an opinion on aspects of formulation design. *Methods*: The relevant literature was reviewed, focusing on factors influencing tissue targeting, safety and effectiveness of particulate formulations. *Results/conclusion*: Therapeutic applications of novel drug delivery systems for the localized treatment of joints have lagged significantly. Future innovations in the field will require the discovery of new therapeutic agents for regional delivery, combination regimens, novel biomaterials as drug carriers and targeting carriers to specific molecules.

Keywords: arthritis, biocompatibility, biomaterials, drug delivery, efficacy, intra-articular, microspheres, nanospheres, targeted delivery, tolerability

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1. Introduction

Diseases that come under the umbrella of inflammatory arthritis comprise a large number of acute and chronic inflammatory conditions of synovial joints that affect an enormous number of individuals. For example, rheumatoid arthritis (RA) is a chronic severe inflammatory and destructive joint disease, affecting 0.5 – 1% of the population in the industrialized world [1]. Osteoarthritis is a chronic joint condition in which there is a limited, low level of inflammation and it is the most common type of arthritis, primarily targeting 60 – 70% of people older than 65 years of age [2]. The inflammatory conditions termed acute gouty arthritis and pseudo gout affect between 1 – 2% and between 3 – 6% of the population, respectively [3,4]. Although these arthritic conditions are usually treated by systemic drug administration, it is recognized that intra-articular drug delivery can be very useful for treating disease flare-ups, synovitis and pain when a small number of joints are affected, or for those joints that do not respond to systemic medications [5]. Generally speaking, drugs administered intra-articularly should be retained within the joint tissues over a period of weeks to months in order to produce a remission of symptoms involving the joints. Given the discomfort and risk of infection associated with intra-articular injections [5], controlled/sustained release formulations may be considered optimal delivery systems in order to reduce the need for frequent injections.

Recently, two review papers have been published, one discussing drug delivery into osteoarthritic joints [6] and one providing a highly comprehensive and

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systematic overview of drug transport processes in joints and intra-articular controlled release delivery systems [7]. This latter publication by Larsen *et al.* [7] has compiled and summarized studies of the pharmacokinetics and outcomes following intra-articular administration of low molecular weight solutes, drugs and macromolecules and drug loaded liposomes, microspheres, nanospheres and complexes into animal model or human joints. Hence, a different approach will be taken in this review. The objectives will be to discuss how critical drug delivery considerations such as size of injected particulates, biomaterial composition, biodegradation rate, drug release and tissue targeting influence biocompatibility, tolerability and efficacy following intra-articular administration, and to offer opinions on aspects of the field that remain controversial.

2. Synovial joint structure

Synovial joints are enclosed by a fibroelastic joint capsule and within this structure lies a thin sheet of tissue called synovium (also known as synovium or synovial membrane) (**Figure 1**). Synovium generates the joint lubricating synovial fluid and, via its rich microcirculation, supplies the fluid with oxygen and nutrients for transmission to the cartilage. At the margins of the joint cavity, synovial folds or 'villi' project into the synovial space [8]. The synovium itself is very thin, typically around 15 – 20 μm thick in the rabbit knee [9,10] and 60 μm thick in the human knee [11] and possesses an extracellular matrix of collagen and glycosaminoglycans [7]. Synovium backs onto the subsynovium, containing loose connective tissue, fat cells and blood and lymphatic systems [8]. Two main types of cells are found in the synovial lining: type A synoviocytes or macrophage-like cells that have a prominent Golgi complex and many vesicles, and type B cells or fibroblast-like cells that produce a protein-rich secretion [12]. Synovial fluid is a viscoelastic liquid that fills the joint cavity and is formed via ultrafiltration of plasma, to which is added hyaluronan and lubricin from the synovial cells [13]. The bones are capped with a smooth, avascular articular cartilage which is composed of chondrocytes (occupying about 0.01 – 0.1% of the volume of the tissue) set in a matrix of collagen fibrils, proteoglycans and a number of organic and inorganic solutes [14].

3. Inflammatory arthritis and synovial permeability

RA is a chronic inflammatory disease resulting from complex intercellular interactions initiated by the interaction between antigen presenting cells and $CD4^+$ T cells. Macrophage activation ensues with secretion of proinflammatory cytokines such as tumor necrosis factor and interleukins. These cytokines then stimulate synovial fibroblasts and chondrocytes resulting in inflammation of the synovial tissues [15,16]. The tissue transforms into an aggressive tissue, the pannus [17]. Growth and proliferation of the pannus is accompanied by new blood vessel growth (angiogenesis or neovascularization). Neutrophils accumulate within the synovial fluid and participate in the degradative processes. Degradation of cartilage is mainly mediated by the matrix metalloproteinases secreted within the joint.

Osteoarthritis is characterized by degeneration and loss of cartilage, caused by cytokines, growth factors and matrix metalloproteinases. There is hyperplasia of synovial lining cells, infiltration by lymphocytes and mononuclear cells, bone remodeling and new bone formation at the margins of the articular cartilage [6].

Crystal-induced arthritis is an acute inflammation that results from the interaction between neutrophils that are recruited to the synovial fluid and inflammatory microcrystals. Neutrophil activation leads to a number of responses including phagocytosis of the crystals, respiratory burst activity, lysosomal enzyme release and release of inflammatory mediators [4,18].

The synovium offers the main resistance to fluid escape from joints when there is a rise in joint pressure caused by inflammatory infiltrate. There are irregular gaps (2 – 3 μm wide) in the discontinuous layer of synoviocytes, occupied by an interstitial matrix that offers the hydraulic resistance to fluid outflow [19]. The volume of synovial fluid is regulated by exchange between synovial capillaries and the cavity and by drainage from the cavity into the lymphatic vessels in the subsynovium [20]. Fluid movement is a passive process dependent on local intra- and extravascular pressures. The volume of synovial fluid in a normal, non-inflamed knee joint is small, less than 2 ml in humans [21] and about 0.5 ml in rabbits. Transsynovial flow or absorption occurs from the joint cavity through synovial intercellular spaces and into the subsynovium and increases at high intra-articular pressures.

In inflammatory diseases, the balance of intravascular and synovial interstitial pressures is disrupted, resulting in a marked increase in synovial fluid volume and edema of the adjacent synovial tissue. There is an increase in the passage of proteins from blood to synovial fluid as the degree of inflammation increases and a linear relationship has been demonstrated between synovial fluid to serum protein concentration ratios and molecular radius of the protein [21]. The protein levels in inflammatory synovial effusions range from 30 – 70% of that found in plasma and reflect a new steady state equilibrium between increased microvascular protein permeability and increased lymphatic protein removal.

The factors involved in the exchange of drugs and small solutes between plasma and synovial effusions are complex and are related to synovial pathophysiology, transsynovial absorption rates and the properties of the drug [22]. For most low molecular weight drugs, the barrier for transsynovial exchange between synovial fluid and plasma is the synovial tissue interstitium [22]. Many types of synovitis are characterized by synovial cell proliferation, cellular infiltration, villous

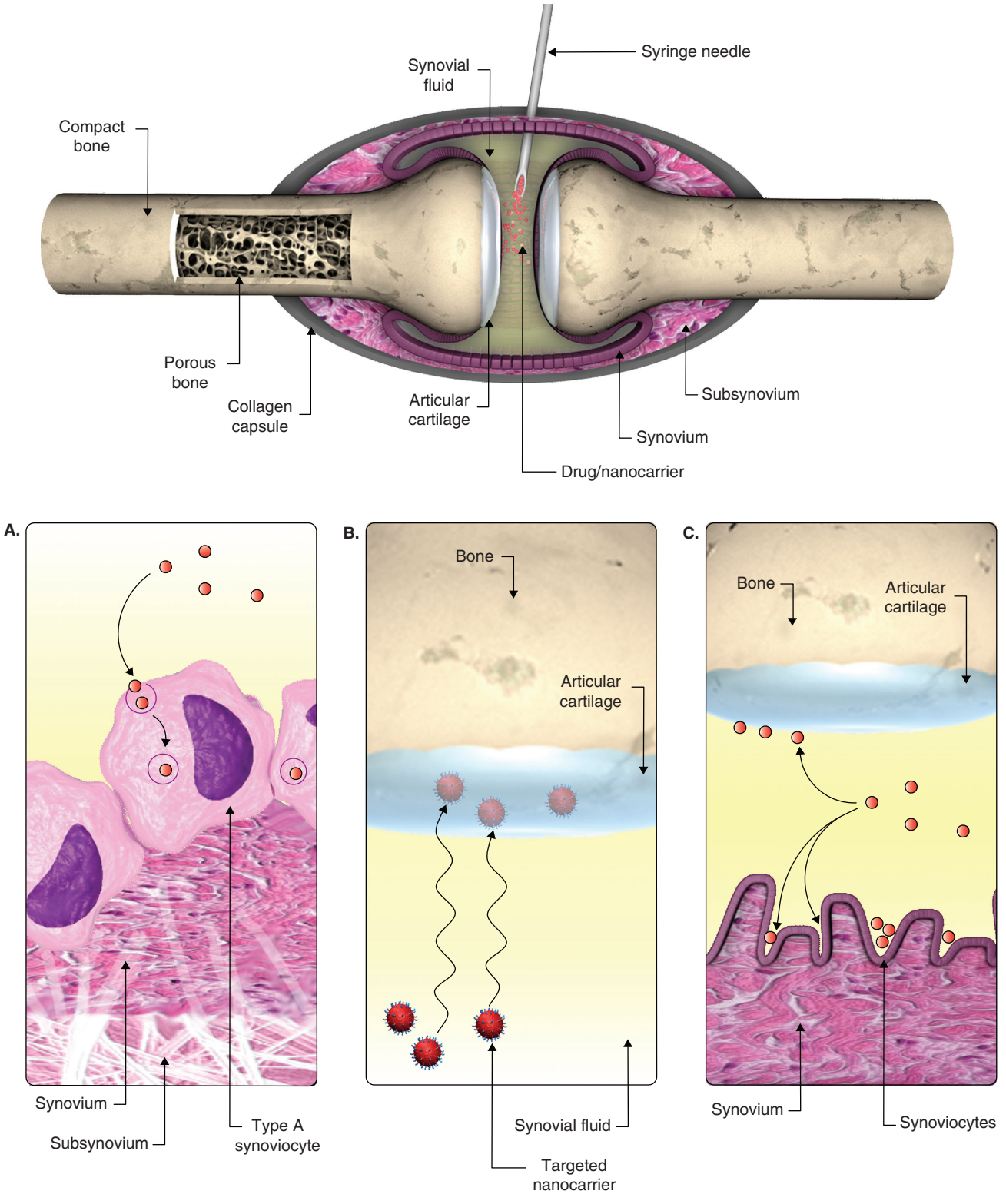

Figure 1. Illustration of the structure of a synovial joint. Suspensions of particulate drug delivery systems are injected into the synovial fluid (intra-articular injection). Particulates may be transported by the following pathways: **A.** This pathway shows phagocytosis by synovial membrane macrophages (Type A synoviocytes). Encapsulated drug may be released within these target cells. **B.** This pathway demonstrates entry of targeted nanoparticulates into the cartilage matrix by convective transport. **C.** Microparticulates remain within the synovial fluid, adhere to the cartilage and synovium or become entrapped within synovial folds.

hypertrophy and dilatation and the evidence suggests that small solutes cross the altered barrier at the same or slightly higher than normal rates. Interestingly, it has also been suggested that, in RA joints, the synovial proliferation characteristics of the disease may increase the barrier effects of the interstitium and restrict synovial permeability to smaller molecules [21,22]. There are several factors related to the nature of the solute or drug molecules that are known to influence the transport of drugs into and out of the synovial fluid, including drug dissociation constant, molecular radius, serum half-life, protein binding and drug solubility [22]. Protein bound drugs cross the synovium slowly compared to free drug, lipophilic drugs are trapped in perisynovial fat tissue and the effects of synovial fluid volume are profound. Higher intra-articular pressures caused by increased synovial fluid volumes result in greater transsynovial absorption rates of fluid carrying dissolved solutes/drugs [21,23]. The intra-articular volumes in osteoarthritis and RA patients' synovial effusions were determined using a radiolabelled albumin distribution method. Protein clearances were greater from RA knee joints than osteoarthritis joints, but effusion volumes were similar, of the order of 100 ml [24].

4. Intra-articular administration and pharmacokinetic studies

Studies of the pharmacokinetics of drugs delivered intra-articularly are limited and have been thoroughly reviewed by Larsen *et al.* [7]. Direct intra-articular administration of anti-rheumatic drugs has generally resulted in kinetics that can be interpreted as rapid intra-articular distribution followed by slower transsynovial removal [22]. Soluble drugs administered intra-articularly typically show rapid absorption into the blood [25]. In a study by Wigginton *et al.*, [26] RA patients were injected intra-articularly with 5 mg methotrexate (MTX) (a disease modifying anti-arthritic drug) solution with a second dose after 24 h. The elimination of MTX from the joint was biexponential over 24 h, with half-lifes of 0.54 and 2.90 h. However, the authors concluded that intra-articular MTX was clinically ineffective, primarily because the intra-articular half-life of methotrexate was too short relative to the probable synovial cell cycle generation time [26]. Repeated intra-articular MTX doses have produced better results [27]. Rheumatoid and psoriatic arthritis patients were administered 10 mg MTX every week for 8 weeks, resulting in a significant reduction in synovial thickness and joint effusion [27].

Sustained release formulations of insoluble drug suspensions injected into healthy or RA patient joints showed slow absorption and long mean residence times in the joint [28,29]. Concentration–time profiles were described by a triexponential expression and the terminal part of the curve represented systemic absorption rather than elimination (termed 'flip-flop case'). High molecular weight drugs (hyaluronan and a glycoprotein, ulinastatin) administered into joints also demonstrated slow elimination from the joint or 'flip-flop' kinetics [30,31].

5. Controlled release intra-articular drug delivery systems

Intra-articular suspensions of corticosteroids have been used to treat a variety of rheumatic diseases for decades and can be very helpful in reducing pain and inflammation [5]. Methylprednisolone and triamcinolone esters provide local relief from inflammation for days to weeks following intra-articular injection [5].

There is significant interest in the development of nano- and micro-particulate systems as drug carriers for the delivery of drugs into the synovial space in order to localize the drug to the site of action and minimize systemic toxicity. The ideal characteristics of these particulate systems are provided in **Table 1** and critical aspects of these properties will be discussed in greater depth in a later section. The different types of delivery technologies that have been explored to date include liposomes, solid–lipid nanoparticles, polymeric nano- and micro-spheres and complexes and are reviewed in Larsen *et al.* [7].

5.1 Lipid-based delivery systems

In some early studies, liposomes showed limited efficacy following intra-articular injection due to rapid release of drug and leakage into the circulation [32,33]. However, Williams *et al.* [34] were able to demonstrate efficacy in a rat antigen induced arthritis model by using liposomally conjugated MTX to reduce the rapid loss of MTX from the liposomes and the joint.

Turker *et al.* [35,36] prepared so-called 'lipogelosome' formulations composed of diclofenac sodium encapsulated in liposomes of dimyristoylphosphatidylcholine : cholesterol : dicetyl phosphate (7:1:2) dispersed in either a carbopol or sodium carboxymethylcellulose gel, to provide bioadhesion and prolonged drug release. In an antigen-induced arthritis rabbit model, the lipogelosome formulations showed enhanced joint retention and decreased inflammation compared to diclofenac sodium solution [35,36].

Using a radio contrast agent, iohexol, loaded into liposomes, the biodistribution and clearance following intra-articular administration into a large animal model (sheep) was investigated [37] with the goal of passively targeting the liposomes to the synovial macrophages. The liposomes were in the 3 – 5 μm size range and were located intra-articularly within synovial pouches, synovial fat bodies and sequestered within macrophages, and extra-articularly, along muscle fascial planes [37]. The terminal half-life of liposomal iohexol was 134 h compared to 3 h for free iohexol [37].

The biocompatibility and biodistribution of celecoxib loaded solid lipid nanoparticles based on glycerol behenate (mean particle size of 257 nm) were evaluated in a rat

Table 1. Ideal properties of a controlled release micro- or nano-particulate intra-articular drug delivery system.

Size range of particulate should be appropriate for target tissue within the joint
Carrier biomaterial must be biocompatible and well tolerated
Particles must be biodegradable
Degradation products must be biocompatible and non-toxic
Particles must have sufficient drug loading capacity
Sterilizable
Drug should be released and retained at the target site over a prolonged period of time

antigen-induced arthritis model [38]. The biocompatibility of the solid lipid nanoparticles was demonstrated and the celecoxib nanoparticles were retained longer in the inflamed joint compared to celecoxib solution, likely due to phagocytosis of the nanoparticles by the synovial macrophages [38].

5.2 Polymer-based delivery systems

Larsen *et al.* [7] have reviewed the relatively small number of *in vivo* studies where polymeric microspheres were used as carriers for delivering drugs to the synovial joints in animal models. In these studies, the drugs encapsulated in either naturally occurring polymers (albumin and chitosan) or synthetic, biodegradable polyesters were: prednisolone, triamcinolone, celecoxib, naproxen, dexamethasone, beta-methasone, methotrexate, insulin, holmium-166 and paclitaxel. Interestingly, Larsen *et al.* noted only one clinical study in rheumatoid arthritis patients with a holmium-166-chitosan complex [7]. They also suggested that the reported *in vivo* animal studies 'do not provide a clear-cut picture of the capability of the microsphere approach to substantially prolong the IA [intra-articular] residence time of drugs' [7]. This opinion is likely based on the varied success reported in the literature in sustaining either efficacy or drug localization in the joint cavity following intra-articular administration of these formulations.

In initial work with non-drug loaded microparticles composed of polycyanobutyl ester, gelatin, albumin or poly(lactic acid) (PLA), Ratcliffe *et al.* [39] showed that with the exception of albumin, these biomaterials elicited various levels of inflammation following their intra-articular injection. The study indicated that commonly used biodegradable polymers, such as PLA, might not be suitable drug carriers for the intra-articular treatment of arthritis. However, the study did not investigate the effect of microsphere size or dose on the degree of inflammation induced by the different polymers. In a follow-up study, Ratcliffe *et al.* [40] investigated the retention of radiolabelled albumin microspheres, with a mean diameter of 3.5 μm, in normal and arthritic rabbit knee joints and found that there was no difference between normal and inflamed joints, but that 10 days post-intra-articular injection, the majority of the microspheres were found within the synovium.

Betamethasone was loaded into poly(lactic-co-glycolic acid) (PLGA) nanospheres (300 – 490 nm) and the efficacy evaluated after intra-articular injection in an antigen-induced inflammatory arthritis rabbit model [41]. Joint swelling and cell infiltration was reduced over 21 days.

Tuncay *et al.* [42] prepared diclofenac-loaded PLGA microspheres (5 – 10 μm diameter) and monitored arthritic lesions using technetium-labelled polyclonal human IgG and gamma scintigraphy. There was no difference in efficacy between control and diclofenac-loaded microsphere treated groups.

Intra-articular injection of paclitaxel-loaded PLGA microspheres with a mean diameter of 50 μm had no adverse effect on the normal function of horse joints [43]. These authors used an equine isolated, extracorporeal metacarpophalangeal joint model that allowed for simultaneous measurement of the hemodynamic state and transsynovial fluid flows in the joint. Hemodynamic parameters were not affected by intra-articular injection of paclitaxel-loaded microspheres, but there was greater synovial fluid flow, intra-articular pressure and permeability to fluid transport compared to control joints [43].

Plasma levels of flurbiprofen following intra-articular administration of flurbiprofen-loaded gelatin microspheres in the size range 2 – 12 μm into healthy rabbit joints showed significantly lower maximum plasma concentrations compared to flurbiprofen solution, indicating greater retention in the joints for drug-loaded microspheres [44].

Our group conducted biocompatibility and pharmacokinetic studies in healthy rabbit joints using intra-articular control (MTX solution) or MTX-loaded poly(L-lactic acid) (PLLA) microspheres in the size range 62 – 83 μm [45,46]. The microspheres were well tolerated [45] and retained much greater amounts of MTX in the joint tissues compared to MTX solution over 24 h [46].

Indomethacin was solubilized in polymeric micelles of polyphosphazene substituted with poly(*N*-isopropylacrylamide) and ethyl glycinate and administered intra-articularly into an antigen induced arthritis rat model [47,48]. Based on measurements of rat paw edema, the copolymer micelles appeared to be biocompatible and indomethacin-loaded micelles achieved a significant reduction in inflammation [47,48].

5.3 Factors influencing biocompatibility/tolerability and efficacy

Based on the review of lipid-based and polymer-based particulate delivery systems provided above, it becomes apparent that there are a number of critical drug delivery considerations when designing a particulate delivery system for the joint and these will be discussed in greater depth.

5.3.1 Joint tissue targeting

Larsen *et al.* [7] have discussed the uptake and distribution of free drug (once released from a depot system) into

synovial fluid, synovium and cartilage. However, it is clear that intra-articular micro- and nano-particulate drug delivery systems possess the ability to target different joint tissues, primarily by virtue of their size. As illustrated in **Figure 1**, particulates may be transported by the following pathways: i) Phagocytosis by synovial membrane macrophages where the encapsulated drug may be released within the targeted synovial macrophages [37,38,40,41,49]. There is also evidence that phagocytosed nanoparticles may be transferred through the cell-junction and penetrate into the subsynovium [49]; ii) Entry of nanoparticulates into the cartilage matrix by convective transport during cartilage compression and penetration of the nanoparticulates between the collagen fibres of the extracellular matrix [50]. There is evidence for internalization of nanoparticles within chondrocytes by unknown mechanisms. Surface modification of the nanoparticles with a peptide with specific binding to collagen II resulted in increased retention in the cartilage extracellular matrix [50]; iii) Microparticulates remain within the synovial fluid, adhere to the cartilage and synovium, or become entrapped within synovial folds. Drug is released from the microspheres into the synovial fluid and free drug is transported via passive diffusion into joint tissues, lymphatics and capillaries and into the systemic circulation [43,45,46,51,52]; and iv) Extra-articular migration of particulates along muscle fascial planes [37].

The bulk of the literature reviewed would suggest that in the treatment of inflammatory arthritis conditions, the primary target tissue is the synovium. However, in diseases such as osteoarthritis, the primary site of the disease process is the cartilage matrix and targeting to this tissue with agents to inhibit cartilage degradation may be an important goal of drug delivery into the joint.

5.3.2 *Size of particulates*

Although the transport and ultimate location of particulates within joint tissues following intra-articular injection is determined by their size range, the importance of considering the effects of the size range of drug delivery particulates on biocompatibility and tolerability in the joint cannot be emphasized enough. Horisawa *et al.* [41,49] demonstrated that both PLGA microspheres (3 – 60 μm) and nanospheres (110 – 670 nm) were safe and well tolerated in rat joints. PLGA nanospheres were phagocytosed by macrophages in the synovium and produced slight hyperplasia of the synovial membrane and widespread infiltration of cells throughout the synovial lining, whereas the microspheres were adhered to the surface of the cartilage and synovium. An upper limit for macrophage phagocytosis of approximately 5 μm diameter particles was suggested.

The interaction of particles of standard sized latex beads (0.4, 15, 45, 90 μm) with synovial fibroblast cell cultures has been investigated by measuring the collagenase synthesis induced by the particles [53]. The ability of latex beads to induce collagenase was strongly size dependent, such that particles that were 0.4 μm were readily phagocytosed by the cells and induced collagenase production, while 15 μm latex beads were not readily phagocytosed, but did induce collagenase synthesis if the beads were internalized. Latex particles of 45 and 90 μm were too large for uptake by synovial fibroblasts and did not induce collagenase production.

Nishide *et al.* [52] found that intra-articular poly(D,L-lactic acid) microspheres of different sizes (< 20 – 200 μm) in rabbit joints produced no inflammatory responses to the microspheres by the synovial tissue within 3 days after injection.

Our group has conducted studies of the effects of size and dose of microspheres on biocompatibility or tolerability in rabbit joints [54]. We have shown that PLGA microspheres in a smaller size range of 1 – 20 μm produced a greater inflammatory response in rabbit joints than larger sized microspheres (35 – 105 μm). The inflammatory response at higher doses of the smaller microspheres was accompanied by cartilage proteoglycan loss [54]. We suggested that phagocytosis of a large fraction of small microspheres by synovial macrophages was accompanied by cell activation and some local inflammation. Certainly, phagocytic cells such as neutrophils or mononuclear cells may recognize polymeric surfaces as foreign material and cell activation may lead to an attempt to phagocytose the polymer with resulting local inflammation [55]. Studies in which human neutrophils were incubated with polymeric microspheres showed low levels of neutrophil activation (measured by respiratory burst activity) [56]. However, adsorption of opsonizing proteins from plasma onto the microsphere surface resulted in significant enhancement of neutrophil activation. It is interesting to speculate that the first event following injection of particulates into the synovial space may be opsonization by synovial fluid proteins onto particulate surfaces, triggering phagocytic cell activation and uptake. To what extent protein adsorption onto particulate surfaces occurs in synovial joints, if at all, and whether the initial processes of activation of synovial macrophages to phagocytose particulates, results in release of inflammatory mediators, remains an area where more research is needed. Tabata and Ikada [57] published an excellent review of the phagocytosis of polymer microspheres by macrophages. They studied both the degradation and phagocytosis of numerous polymeric microspheres, including PLGA and PLLA microspheres with diameters less than 2 μm by mouse peritoneal macrophages *in vitro*. They also evaluated the influence of protein precoating of the microspheres with albumin, gelatin, fibronectin and IgG on phagocytosis and showed that the extent of microsphere phagocytosis by macrophages was decreased with albumin coating but enhanced by IgG coating, consistent with opsonization.

5.3.3 *Composition of biomaterial, drug release and biodegradability*

A wide range of biomaterial types and compositions have been evaluated in terms of their biocompatibility and tolerability

in joints, including naturally occurring materials such as gelatin, albumin and chondroitin sulfate and synthetic biodegradable materials such as the polyesters. Drug release profiles and biodegradation/bioerosion rates of particulate drug carriers will influence the lifetime of the particulates in the joint and the time course of drug availability at the site of action. Release and biodegradation rates are dependent on a large number of factors, such as composition, molecular weight and crystallinity of the biomaterial, drug loading, nature of the drug, size and many other factors which have been reviewed elsewhere [58-60].

Biocompatibility studies using microspheres of three different biomaterials, PLGA, PLLA and poly(caprolactone) and microparticles of chitosan injected into healthy rabbit joints were conducted by our group [54]. All three microsphere biomaterials were well tolerated and no difference in responses were observed. However, rabbits receiving chitosan microparticles showed severe joint swelling and cellular infiltration that persisted for 3 days, suggested to be related to effects of chitosan on inducing macrophage activities including chemotaxis [61] and so-called bioactivation effects of chitosan [62]. In contrast to these findings, Thakkar *et al.* [63] prepared chitosan microspheres using an emulsion chemical crosslinking method. The size of the microspheres was reported to be about 8 μm, 8 – 10 mg were injected in 0.2 ml saline into rat knee joints and histopathology after 3 days showed no inflammatory infiltrates or inflammatory changes in the synovium. The authors concluded that chitosan microspheres are biocompatible and suitable for intra-articular administration [63]. Whether the significant inflammatory response found in our work with chitosan in joints [54] and the biocompatibility found in this latter study [63] is due to species difference, fabrication method, microspheres versus microparticles, size, dosing, method of evaluation, etc, is not known.

Nishide *et al.* [52] examined the biodegradation of low molecular weight poly(D,L-lactic acid) microspheres in the size range 20 – 100 μm following injection into rabbit joints. All microspheres tended to aggregate and localize in the fat tissue of the popliteal region of the knee cavity and no inflammatory responses were noted. The *in vivo* degradation periods of microspheres prepared from 3000, 5600 and 7000 molecular weight polymers were approximately 5, 10 and 17 days, respectively [52].

Anderson and Shive [64] have reviewed biocompatibility and tissue/material interactions of PLA and PLGA microspheres following implantation intramuscularly, subcutaneously and injection into the eye and central nervous system. The sequence of local events, from acute inflammation to chronic inflammation, foreign body reaction and fibrosis were described. They noted that factors such as size, shape and chemical and physical properties of the biomaterial may produce variations in both intensity and time duration of the inflammatory response [64]. For biodegradable microspheres larger than 10 μm in diameter, the events described above may occur until the microspheres have degraded to less than 10 μm, at which point phagocytosis by macrophages may occur. Significant variations in tissue responses to biodegradable microspheres are also caused by different routes of administration and implant sites [64].

Although the weight of the somewhat limited evidence would suggest that microspheres composed of poly(lactic acid) homopolymers and copolymers of PLGA are biocompatible with synovial tissues following intra-articular injection [41-43,45,49,51,52,54], there are nevertheless tissue/material inflammatory 'flare' responses that take place in the joint. For example, our studies in rabbit joints have shown early inflammatory responses to PLGA, PLA and PCL microspheres, regardless of size, with mild joint swelling and some cell infiltration that began to resolve on the third day [54]. Certainly, it seems clear that there are a large number of factors influencing duration and extent of response to injected microspheres.

In view of the technical difficulties in accessing and measuring drug levels in the synovial tissues as a function of time, very few studies have attempted to measure synovial tissue or fluid levels of drug released from nano- or micro-particulates. Horisawa *et al.* [41] determined *in vivo* betamethasone release from PLGA nanospheres in the rat air-pouch model, which they considered to be a surrogate for synovial fluid levels. Betamethasone was released into the pouch and drug concentrations in the pouch gradually increased. The molecular weight of the nanospheres decreased more rapidly in the air-pouch compared to *in vitro* degradation, indicating a contribution from enzymatic degradation *in vivo*.

An intra-articular polymeric microspheres formulation of gelatin and chondroitin sulfate loaded with catalase was prepared and the release kinetics of catalase evaluated in the presence of inflammatory and non-inflammatory human synovial fluids [65]. Rate of degradation of the microspheres and the corresponding release of catalase increased with higher levels of metalloproteinase enzyme activity (gelatinase) in the joint fluids.

6. Conclusion and expert opinion

Therapeutic applications of novel targeted and/or controlled release drug delivery systems such as cancer, orthopedics, heart and vascular diseases, ocular and dental technologies have seen tremendous levels of research activity over several decades, whereas applications to the localized treatment of joints have lagged significantly. Part of the reason may be that reasonably efficacious, intra-articular, controlled release corticosteroids have been available for many years, leading to lack of a driving force for innovation. Furthermore, it may also be related to the fact that RA is considered a systemic-based disease, treated with disease modifying anti-rheumatic drugs (DMARDs) and a compelling rationale for regional delivery to the joint was not in evidence. Either way, the good news is that in the last few years, greater interest

appears to be developing. Certainly, this effort will be markedly enhanced by the discovery of new drug candidates that are peptides, proteins and antibodies [6,7], which cannot be administered safely or effectively via the systemic route and will require localized controlled release methods of delivery.

In terms of formulation design issues for intra-articular drug delivery, it is clear that size does matter and that, in our opinion, the jury is still out on the effects of size of particulates on the critically important issues of joint biocompatibility and tolerability. Several authors have concluded that nano-sized particulates, targeting the synovium via phagocytosis by synovial macrophages, are well tolerated in joints and indeed, that the synovium is the preferred target in treating inflammatory arthritis. However, there is sufficient evidence in the literature to the contrary; that particulates in the nano- or low micro-sized ranges can induce inflammation with joint swelling, cellular infiltration and even minor levels of proteoglycan loss. We suggest that retaining the particulates in the synovial fluid, by using larger micro-sized particles that provide equivalent or even better joint tolerability and a controlled release of drug into the synovial space, may also be a viable option for treating inflammatory arthritis. Well-designed, comprehensive studies incorporating a complete assessment of tolerability and biocompatibility following intra-articular injection of particulates must be an integral part of future work. The particulates must be fully characterized, such that size range, surface morphology, composition and molecular weight are well understood and documented. Biocompatibility assessments should include evaluation of swelling, cellular/inflammatory infiltrates and full histopathological analysis of joint tissues using well-defined scoring systems by skilled and blinded investigators. Since few, if any, studies have evaluated the potential effects of dose of particulates (total weight of particulates injected) on joint biocompatibility, this should also be taken into consideration.

Investigations that examine the localization and distribution of the particulate carriers, along with determinations of biodegradation, drug release profiles and *in vitro*/*in vivo* correlations are also important components of development of knowledge in the field.

It is also worthwhile commenting on the use of animal models of inflammation to evaluate the efficacy of intra-articular drug delivery systems. There are numerous models utilizing rats, mice, primarily rabbits, and occasionally, larger animals such as sheep and horses. Methods of inducing inflammation also vary. Our experience with the antigen-induced arthritis model in rabbits has been that within a day following injection of the antigen into the knee joints, development of arthritis was quite variable between animals. Histological analysis of the cartilage showed that 14 days following the induction of arthritis, there were significant levels of cell death and loss of proteoglycans, features that are considered rather severe in terms of cartilage destruction and would only be observed in patients with a late stage of RA. Thus, optimization and standardization of the arthritis animal model is important for efficacy determinations.

Finally, in addition to a clear need for a greater understanding of the formulation factors influencing biocompatibility and efficacy of these systems, future innovations in the field will likely include new therapeutic agents for regional delivery, synergistic combinations of drugs in intra-articular formulations, the use of novel biomaterials and drug carriers and the potential for targeting the drug carriers to specific target molecules, such as cartilage extracellular matrix components.

Declaration of interest

The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

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