Intra-Articular Depot Formulation Principles: Role in the Management of Postoperative Pain and Arthritic Disorders

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ABSTRACT: The joint cavity constitutes a discrete anatomical compartment that allows for local drug action after intra-articular injection. Drug delivery systems providing local prolonged drug action are warranted in the management of postoperative pain and not least arthritic disorders such as osteoarthritis. The present review surveys various themes related to the accomplishment of the correct timing of the events leading to optimal drug action in the joint space over a desired time period. This includes a brief account on (patho)physiological conditions and novel potential drug targets (and their location within the synovial space). Particular emphasis is paid to (i) the potential feasibility of various depot formulation principles for the intra-articular route of administration including their manufacture, drug release characteristics and *in vivo* fate, and (ii) how release, mass transfer and equilibrium processes may affect the intra-articular residence time and concentration of the active species at the ultimate receptor site. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 97:4622–4654, 2008

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INTRODUCTION

Local drug action within the joint cavity is needed for the treatment of arthritic disorders and the relief of pain and inflammation. After oral or parenteral (i.v., i.m., s.c.) administration, the therapeutic agent is transported to the intra-articular site of action *via* the systemic circulation. In contrast, intra-articular (IA) injection of suitable drug delivery systems (DDSs) may enable the major part of the incorporated drug to be released in the vicinity of the target area. The most obvious advantage of this relatively simple form of targeted drug delivery is that only a minimum amount of drug is required to exert the desired pharmacological activity and thus, minimizing drug exposure to inappropriate sites. Further, direct joint instillation may constitute the only realistic route of administration for chemical entities suffering from bioavailability problems and extensive degradation *in vivo*.

Literature data indicate that dissolved small-molecule drugs are rapidly cleared from the synovial space after IA injection. Maintenance of therapeutic drug concentrations in the joint over extended periods of time can be achieved by repeated IA administrations or more ideally, by immobilization of the active agent in the form of an injectable depot formulation from which the drug is released in a controlled manner. Currently, long-lasting (weeks) corticosteroid suspen-

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sions constitute the only world wide available depot formulation type for the IA route of administration. Such depot injectables have been used for more than three decades providing symptomatic relief of pain in rheumatoid arthritis (RA) and osteoarthritis (OA).[1–3] These two major arthritic disorders strongly affect patient quality of life and present significant costs to society in terms of medical care and lost wages. The total annual burden to arthritis has been estimated to equal 1–2.5% of the gross national product of Western nations.[4] Identification of novel potential drug targets, not least in the area of OA,[5–7] gives optimism to the development of new disease-modifying therapies. These encouraging findings pose a strong incitement to the search for innovative depot principles feasible for the IA route of administration.[8]

The search for novel therapies providing local sustained drug action after IA administration is multidisciplinary of nature since it has to be founded on adequate knowledge of chemical, pharmaceutical as well as biological disciplines. This may involve the identification of new targets and subsequently, drug candidates effectively acting on such targets. Based on the knowledge of (patho)physiological conditions, it further includes the task of accomplishing (in a highly reproducible manner) the correct timing of the events leading to optimal drug action over a desired period of time.[9] The primary aims of this review are (i) to discuss advantages/limitations of various depot formulation principles in relation to the IA route of administration and (ii) to give an account on factors influencing the fate of drug molecules once released from the immobilized depot in the joint cavity. In the present review, attempts have also been made to present major knowledge gained in various research areas that is considered to constitute, at least in part, the basis for rational design of novel prolonged release DDSs feasible for IA administration. Due to the rather comprehensive literature related to some of the latter research fields, the authors have found it appropriate, in the treatise of certain topics, to give references to recent reviews dealing with these topics.

SYNOVIAL JOINTS

The bones of a synovial joint are separated by an articular cavity containing the synovial fluid (SF) (Fig. 1). The adjoining surfaces of the bones are covered with a layer of articular cartilage and a two-layer joint capsule encloses the cavity. The joint capsule consists of an outer fibrous capsule and an inner synovial membrane (synovium).[10]

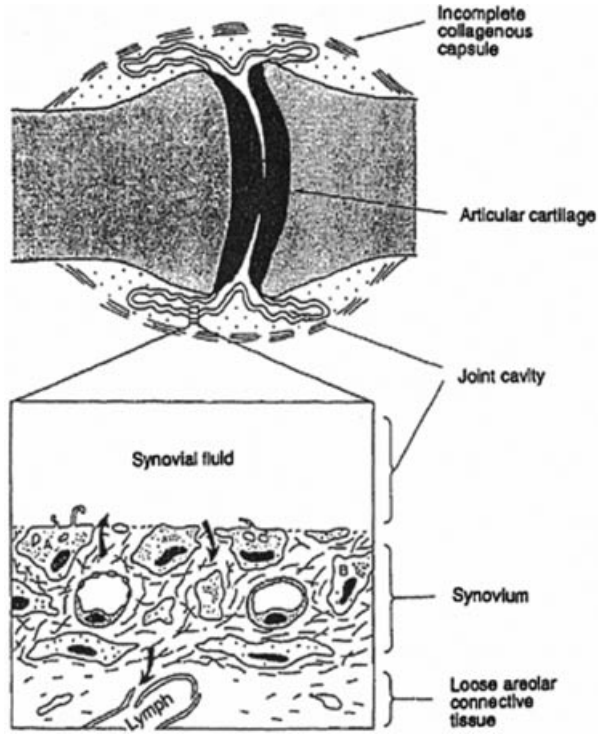

Figure 1. Schematic representation of the cross section of a synovial joint (top). Insert: An enlargement of the synovial lining. The arrows indicate ultrafiltration of fluid from the fenestrated capillaries into the joint cavity and drainage of fluid from the cavity through the synovial interstitium into subsynovial space and lymphatics. Reprinted with permission from Microcirculation.[94]

The various cell types populating the articular cartilage and the synovium as well as components of the synovial fluid are major targets for RA and OA related drug therapies. A brief account of the composition and function of these sites of drug action is given below.

The Synovium

The synovial tissue has an extensive extracellular matrix (ECM), the major components of which are collagen and glycosaminoglycan (GAG). The superficial synovial lining (synovial membrane) contains two cell types (type A/type B cells). Cellular adhesion molecules (CAMs), including β_1 integrins, probably play important roles in

synovial lining organization through cell–cell and cell–ECM interactions.[11] Immediately subjacent to the surface cells, a rich capillary network is found. Lymphatic vessels are located in the subsynovium. The synovium carries three major functions (i) it constitutes a diffusional barrier to the transport of solutes from the SF bulk solution to the fenestrated microvessels and the lymphatics (and *visa versa*), (ii) removal of foreign material or debris from the SF by phagocytic action of the type A cells of the synovial lining and, (iii) type B cell biosynthesis of hyaluronan and lubricin. The latter synovial fibroblasts are suggested to play a major role in both initiating and driving RA.[12,13] In the arthritic synovium, macrophages (type A cells) are key players in chemokine production. These molecules exert chemotactic activity towards synovium-invading leukocytes.[14] Features of the synovium related to mass transfer processes are discussed in more detail in a later section.

The Synovial Fluid

The synovial fluid (SF) fills the cavity of a synovial joint. It is a viscous, non-Newtonian fluid exhibiting thixotropic properties. The normal human adult knee joint contains on the average 2 mL SF. Under pathological conditions the volume can increase up to several hundred milliliters. The SF is considered as a plasma dialysate and the concentration of endogenous low molecular weight chemical substances resembles that of plasma. In contrast, albumin and other proteins are present in the SF at a significantly lower concentration due to the sieving action of the synovial capillary walls and matrix.[15] Inflammation associated changes of the SF may include (i) a decrease of SF viscosity due to enzyme-mediated cleavage of the hyaluronan chains, and (ii) an increase of the content of plasma derived macromolecules caused by the enhanced leakiness of the synovial capillaries.[16,17]

The Articular Cartilage

Articular cartilage furnishes each moving, bony portion of a joint with a smooth, frictionless surface. It is capable of reversible compression, distributing an applied load homogeneously, and minimizing contact stress to the underlying bone. These unique properties can be ascribed to the complex architecture of the avascular ECM of the cartilage which is composed of a highly organized water-filled network of type II collagen fibers and aggrecan-type proteoglycans. Structure and function of many ECM components have been the subject of several reviews.[18–20] The structure and biochemistry of mature articular cartilage are maintained throughout life by the resident chondrocytes.[10,21] Alteration of the normal balance between chondrocyte anabolic and catabolic activities might be effected by various stimuli including mechanical stress.[21] In addition to biomedical approaches to modulate chondrocyte activity, also the potential applicability of cartilage tissue engineering has been the subject of intense research.[22–24]

COMMON IA DRUG THERAPIES AND POTENTIAL NEW DRUG TARGETS

Postoperative Pain Management After Arthroscopic Procedures

Modern postoperative pain control focuses on early mobilization and rapid discharge of patients following surgery. Satisfactory postoperative pain relief adds to decrease patient morbidity and may reduce the risk of development of chronic pain after surgery.[25] Major sites of action of analgesics are depicted in Figure 2.[26] Drug entrance into the target area may be accomplished by (i) transport *via* the systemic circulation or (ii) direct instillation into the proximity of the site of action. Achievement of optimal localized, therapeutic activity after drug administration into discrete anatomical compartments, such as the joints, appears particularly promising. In the following, a brief overview over present IA strategies aiming

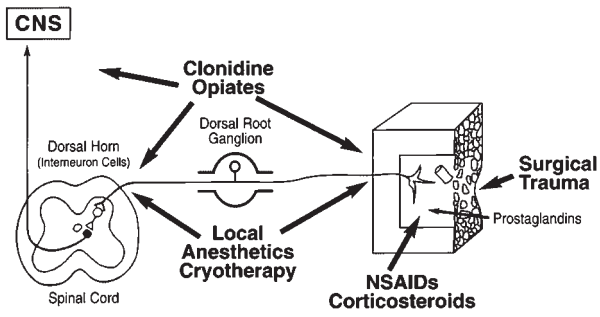

Figure 2. Schematic representation of the sites of action of analgesics along the pain pathway from the periphery to the central nervous system (CNS). Reprinted with permission from The Journal of Bone and Joint Surgery, Inc., Needham, MA, USA.[26]

at pain management after arthroscopic procedures of the knee is presented.

Although minimally invasive of nature, arthroscopic procedures do produce pain and inflammation. As a result patients may be prevented from returning to work for up to 2 weeks after surgery.[26] Findings may suggest that aggressive pain management (including local IA drug therapies) in the early postoperative period can improve convalescence after arthroscopy.[27] Over the years the efficacy of a significant number of drugs (and drug combinations) to provide pain relief after IA injection has been investigated. In recent reviews,[26,28] attempts have been made to assess the feasibility of such IA interventions. Analyses of compiled data indicate that efficacious IA monotherapeutic approaches include (i) non-steroidal anti-inflammatory drugs (NSAIDs),[29] (ii) local anesthetics[30] (primarily bupivacaine), and (iii) morphine.[31,32] A reasonable degree of consensus has, however, been reached that total postoperative pain relief is not achievable by use of a single analgesic agent or method. Therefore, multimodal analgesia (or balanced analgesia) appears to constitute a rational approach to effective pain management.[33,34] Following arthroscopic procedures promising pain alleviating effects of different IA multimodal analgesic regimens have been reported.[35–38] Most of the combinations have involved the use of 2–3 drug compounds selected from the above mentioned groups comprising local anesthetics, NSAIDs, and opiates. Further, interesting results have been reported by incorporating anti-inflammatory steroids[27,39–41] and the α_2-receptor agonist clonidine[42,43] into such multimodal IA therapies. As regard future IA multimodal therapies, particular attention has to be paid to the optimization of the duration of action of the individual therapeutic agents. Tentatively, feasible depot formulations should enable simultaneous release of analgesics (local anesthetics or opiates over a 24 h period) and anti-inflammatory agents (NSAIDs or corticosteroids over about 7 days). Potential pharmaceutical avenues to reach this ambitious goal are discussed in a later section.

Arthritic Disorders

Among the more than 100 arthritic disorders, the two major diseases are RA and OA. Despite the involvement of different (and partly unknown) aetiologies and pathogeneses,[44,45] the common thread linking RA and OA is progressive irreversible destruction of the cartilage, ligaments and bone in the affected joints.[46] In the US, OA alone leads to more than 500000 total joint replacements annually.[47] Prevalences are estimated to about 1% (RA) and 7% (OA). These diseases give rise to long-term disability and impose substantial burden to both patients and society.[48–50] No cures are available, and current pharmacological interventions addressing the pain are only moderately effective.[21] In addition to palliation of pain, current therapies primarily aim at slowing down disease progression (see below).

Rheumatoid Arthritis

Typically, RA manifests as a symmetric and erosive polyarthritis. It is suggested that antigen(s) (of unknown origin) present in the joint trigger an acute inflammation which upon an unbalanced host immune response may develop into a chronic inflammation through a complex cascade of events.[51] Briefly, in this process immune cells and macrophages home to the joint where they release inflammatory cytokines including interleukin (IL)-1 and tumor necrosis factor-alpha (TNF-α)[46] (Fig. 3). These cytokines stimulate synoviocytes to secrete matrix metalloproteinases (MMPs), the proteinases largely responsible for the irreversible destruction of cartilage in the joint.

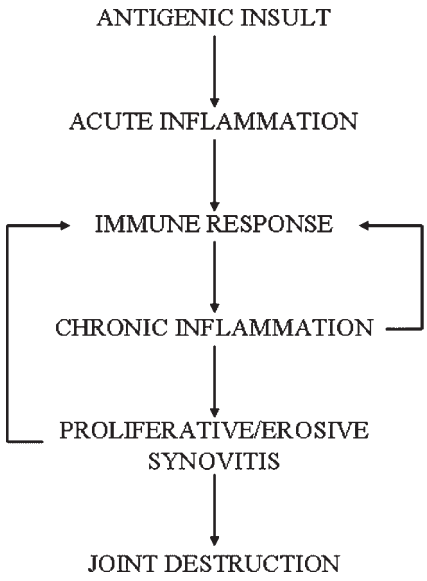

Figure 3. Pathogenesis of rheumatoid arthritis. Modified from Burrage et al.[46]

Current drug treatment options fall into four main categories[52] (i) NSAIDs, (ii) disease-modifying anti-rheumatic drugs (DMARDs), (iii) corticosteroids (occasionally), and (iv) biologics (antibodies and recombinant proteins). Aspects of the use of NSAIDs including implications of their COX-1 to COX-2 selectivity ratio have recently been reviewed.[53] Early use of DMARDs with the aim of aggressively preventing or reducing joint damage has found broad acceptance. DMARD combinations, including in particular methotrexate,[54–56] has become the cornerstone of most RA treatment regimens.[57,58] Despite management with such orally administered agents, many patients do not respond adequately. For patients with persistent disease, so-called biologics may constitute new opportunities in the treatment of RA.[59–61] These rather costly biological agents reduce various inflammatory and immunological responses by selectively blocking the effects of cytokines (TNF-α and IL-1). Six products, to be administered by injection (i.v. or s.c.), are currently approved for the treatment of RA in the US.[59]

Osteoarthritis

In contrast to RA, OA is usually located to one or a few joints. The principal morphological characteristic of OA is a slowly developing breakdown of the articular cartilage. In addition, changes occur in bone, muscle and synovium.[4,62,63] It is suggested that the major events in OA pathogenesis are located within the cartilage itself and that the chondrocytes are major contributors to the pathology of this disease.[21,46] Upon mechanical insults chondrocytes may release matrix degrading proteinases. In addition, these cells produce proinflammatory cytokines which again may stimulate neighboring chondrocytes to produce MMPs, thereby creating their own inflammatory environment[46] (Fig. 4). On disease progression, cartilage-breakdown products may give rise to episodes of (usually mild to moderate) synovitis that at the end will lead to further upregulation of MMP production.[46,62]

Current medical therapies of OA reduce the symptoms (mainly pain) but are only moderately effective. Oral treatment options embrace acetaminophen, opioid analgesics, and NSAIDs.[53] IA drug therapies to alleviate pain include long-acting glucocorticoids and the injection of various hyaluronic acid (HA) preparations.[64–67] The concept of viscosupplementation is based on the hypothesis that IA injection of HA could help restore the (lost) visco-elasticity of OA synovial fluid.[64] In contrast to RA, there are no approved disease-modifying osteoarthritis drugs (DMOADs).[47] In recent years studies have been conducted to assess the disease-modifying capabilities of nutraceuticals (such as glucosamine and chondroitin), diacerhein and doxycycline.[68,69] The efficacy of the above approaches, however, remains controversial.

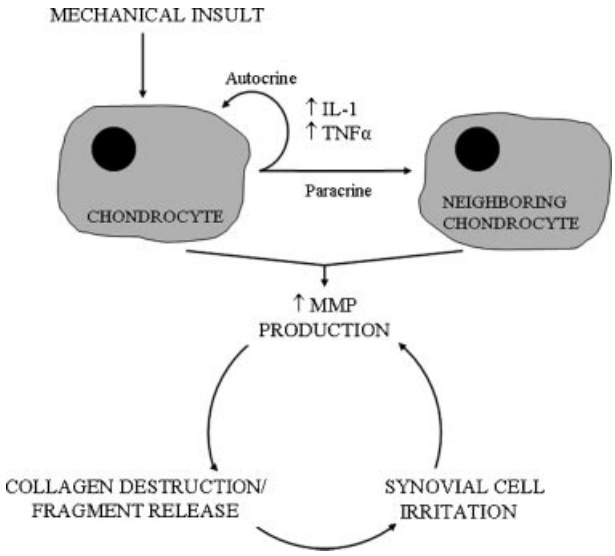

Figure 4. Pathogenesis of osteoarthritis. Modified from Burrage et al.[46]

Potential New Targets for Drug Regulation in OA and RA

In the area of arthritic disorders novel pharmacotherapeutic options may emerge from the identification of a vast number of potential targets for drug regulation. In depth treatise of this exciting area is outside the scope of the present review. Since basic knowledge about target environment and location is a prerequisite for the formulation scientist, a brief account of some major targets is presented below (see also Tab. 1 and the references given to recent reviews/original articles). The pro-inflammatory, catabolic cytokines, particularly TNF-α and IL-1, play a central role in tissue destruction in OA and RA. In RA, inhibition of the activity of these cytokines (by use of for example anti-TNF-α antibodies and IL-1 receptor antagonists) have proven effective in retarding disease progression[70] and such therapies might also be effective against OA.[71–73]

Table 1. Potential Targets for Drug Regulation in Arthritic Disorders

Targets	References
Pro-inflammatory cytokines (primarily TNF-α and IL-1)	21,51,70–72, 87,280–283
Matrix degrading enzymes	
Matrix metalloproteinases (MMPs)	46
Aggrecanases (ADAMTSs)	46,284,285
Cysteine-dependent cathepsins	77,78
Growth factors	21,82,84
Cell adhesion molecules (CAMs)	11,85

Despite significant clinical success, there is still a cohort of RA patients who do not respond to interventions involving such biological therapies.[70] This has encouraged the search for alternative anticytokine therapies including the use of inhibitors of the enzymes responsible for generation of TNF-α (TACE: TNF-α converting enzyme[70,72]) and IL-1 (ICE: IL-1 converting enzyme[7,74]).

It appears that increased levels of ECM degrading enzymes, prostaglandins, nitric oxide and other markers in arthritic fluids and tissues are related to elevated levels of TNF-α and IL-1.[71] Whereas the formation of reactive oxygen and nitrogen species at the site of inflammation gives rise to oxidative injuries in RA,[75] a relationship between cartilage lesion severity and cartilage or blood redox state in OA has apparently not been established.[76] Major enzyme families involved in the degradation of proteins of the cartilage ECM encompass the MMPs, the ADAMTSs metalloproteinases, and cysteine-dependent proteases such as the cathepsins. Collagen degradation is mediated almost exclusively by MMPs. Members of the ADAMTSs family participate in the cleavage of cartilage proteoglycans. Traditionally, the cathepsins were believed to exert nonspecific bulk proteolysis within the acidic environment of the lysosome. However, there is growing evidence for specific extracellular functions of these enzymes.[77,78] A decrease in pH from 7.1 to 5.5 at the cartilage surface of OA patients has been observed. This low pH may favor the action of cathepsins over various metalloproteases as regards ECM degradation.[79] The ECM degrading enzymes are expressed by different cells within the joint space. Their relative contribution to disease progression may depend on disease stage and type of disease. To this end, it appears that the protease activity in RA synovial fluid, in general, exceeds that of OA synovial fluid.[80,81]

In addition to hormones and cytokines, growth factors (GFs) are endogenous molecules involved in the regulation of cellular functions.[82] The metabolism of mature articular cartilage is regulated by a number of GFs that originate from cellular production within the cartilage, as well as from the SF and surrounding tissues.[83] These signaling molecules have (among other functions) the capacity to stimulate chondrocyte anabolic activity.[21] GFs, such as insulin-like growth factor (IGF-1), the bone morphogenetic proteins (BMPs), and the transforming growth factor β (TGF-β), may therefore exert potentially reparative effects in cartilage.[21,47] In general, many GFs act over short distances and have short biological half-lives. Thus, development of DDS that are capable of maintaining sufficiently high levels of various GFs at defect sites for prolonged periods may surmount this problem.[84]

The ability of cells to adhere to other cells and extracellular matrices through CAMs plays a critical regulatory role in a variety of biological processes, including tissue remodeling and the development of inflammation. These molecules (or receptors) are classified into four families.[11] The integrin family comprises heterodimeric adhesion receptors involved in the regulation of a number of cellular processes, including cell growth, differentiation, apoptosis, as well as the regulation of cell adhesion, migration and activation.[85] Integrins are expressed in chondrocytes. Signaling through integrin adhesion molecules has been associated with cartilage damage, and with the production of proteinases and cartilage components by chondrocytes.[46] Also synovial fibroblasts and macrophages express integrins which probably are involved in synovial lining organization through cell–ECM interactions.[11] CAMs have gained acceptance as viable drug targets and anti-CAM biotherapeutics have reached the market in the treatment of autoimmune diseases like chronic plaque psoriasis, multiple sclerosis and Crohn's disease.[11,85] These antibodies have yielded effective therapies, however, in many cases patients develop antibodies against the therapeutic antibodies themselves. This has given further incitement to the search for small-molecule drugs targeting CAMs such as collagen binding integrins. Interestingly, also heparin-like molecules may inhibit the action of leukocyte integrins.[86] As discussed in the following section, drug targeting might be accomplished by exploit-

ing small-molecule ligands with affinity for CAMs as so-called functionalized transport groups in prodrug and DDS design. Examples of chemical structures of novel potential small-molecule drug candidates in the area of arthritic disorders have been reported.[7,74,78,87] Some of the above mentioned potential targets for drug intervention in OA and RA are also of major interest in the area of cancer research where several reviews have dealt with the role of proteases (including MMPs)[88–90] and integrins[91,92] in tumor invasion and metastasis. Small-molecule prodrug approaches in cancer drug targeting has recently been reviewed by de Groot.[93]

DRUG TRANSPORT AND DISTRIBUTION PROCESSES IN A SYNOVIAL ENVIRONMENT

In the joint cavity, the solute drug molecule, once released from the immobilized depot, may take part in a number of reactions and distribution (equilibrium) processes before it is eventually cleared from the synovial space. These processes, the relative importance of which is determined by the physicochemical properties of the drug substance and the barrier properties of the synovium are sketched in Figure 5. Concomitant to binding to components of the SF, (i) transport and distribution into the synovium and articular cartilage, and (ii) subsequent uptake by synoviocytes and chondrocytes, may occur. The present section will discuss the barrier properties of the synovium and the cartilage as well as mechanisms related to transport into these joint tissues. Special attention is paid to the articular cartilage containing the perhaps least accessible drug targets, that is, the chondrocytes and pharmacologically active agents released by these cells.

Transsynovial Transport

Due to its architecture, the synovium (Fig. 1) constitutes the main barrier for drug transport out of the joint cavity. The healthy synovial lining is thin (60 μm[94]) and discontinuous without intercellular junctions. Together with the ECM, the synoviocytes function as a permeable, inhomogeneous matrix.[94] The healthy joint is penetrated by capillaries close to the surface of the synovium (modal depth 35 μm in man).[94] In chronic RA, thickening of the synovium occurs. This leads to an increase in the transport path from the microvessels to the synovium—SF interface.[94] The synovial capillaries have fenestrations facing the joint cavity thus adding to efficient solute exchange.[94,95] Due to the fenestration of the capillaries, plasma protein and bound drug as well as the unbound (free) drug may enter into the synovium.[96] This tissue can be considered as two barriers placed in series, that is, the interstitial matrix and the synovial capillary endothelium.[97–99] The ECM constitutes the major diffusional barrier for entry of small molecules into the synovial cavity whereas passage of the endothelium is the critical barrier for proteins. Flanking the synovium is the subsynovium consisting of loose connective tissue and fat cells. At the border between the synovium and subsynovium are the terminal lymphatics draining fluid and macromolecules from the joint cavity (Fig. 1).

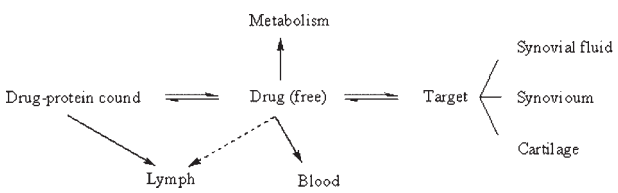

Figure 5. Schematic representation of drug transport and distribution processes of the joint.

Transsynovial transport and solute concentrations in the SF have been assessed in the evaluation of the efficiency of drug treatment regimens in relation to RA and OA where the SF concentration has been taken as a surrogate measure of drug concentrations in synovium and cartilage.[96] Upon oral drug administration, observed joint C_{max} and t_{max} values are usually lower and occur at later time points, respectively, than the corresponding parameters in plasma.[96,100,101] For many NSAIDs, plasma/SF drug concentration ratios have been found to reflect plasma/synovial protein concentration ratios. Further, free NSAID concentrations have been found to be similar in the two compartments after attainment of steady state conditions.[96,100,101] Often the SF concentrations are more sustained than plasma concentrations after oral or i.v. administration.[96,100,101] Due to continuous drug entrance from the blood compartment, IA elimination half-lives ($t_{1/2}$) estimated from pharmacokinetic profiles obtained following oral administration may tend to underestimate the "true" rate of drug disappearance from the SF after IA administration.[96] By appropriate corrections, however, reasonable estimates of rates of disappearance from joints relevant to IA

administration can be extracted from oral pharmacokinetic data. Table 2 contains a compilation of SF disappearance half-lives for some low molecular weight solutes and drugs as well as a few macromolecules. Additional data can be found in the works of Day et al.[96] (drugs) and Simkin and Nilson[102] (radio isotopes and albumin). As apparent, relatively fast disappearance from the joint is observed for small solutes with $t_{1/2}$ values in the range of about 0.1 to 6 h. In this connection disappearance kinetics from the joint cavity has often been described by simple first-order kinetics. However, several studies, for example,[103–109] have found biphasic clearance patterns after IA injection, indicating the presence of a distribution phase associated with drug distribution from the SF to other joint tissues. An initial distribution phase is probably always present upon IA instillation but may or may not be observed depending on the study design.

For small molecules diffusion through the ECM is the major barrier because such solutes readily traverse the fenestrated endothelium of the capillaries. The concentration gradient required for effective clearance of small molecules from the joint is maintained by the synovial blood flow.[110,111] Simkin and Pizzorno[112] found synovial permeability to correlate with diffusion coefficients in water for benzyl alcohol, tritiated water, urate, urea, glucose and sucrose. Among these solutes benzyl alcohol disappeared significantly faster from the joint, which was suggested to reflect the lipophilicity of the molecule, allowing diffusion to occur not only in the aqueous pores of the ECM between the synoviocytes but also across cell membranes. Collectively, these studies indicate that most small molecules cross the synovium in both directions by diffusion. In a follow up study, Simkin and Pizzorno[97] evaluated the synovial permeability of plasma protein as well as the above set of molecules in knee joints of RA patients and normal individuals. The results obtained led to the proposal of the two barrier model of the synovium. Owen et al. determined the disappearance half-lives of ^{125}I-albumin, acetaminophen, salicylate and diclofenac upon IA administration to RA patients.[103] The terminal half-lives of the drugs (listed in Tab. 2) were found to increase with the fraction of drug bound to proteins (albumin) in the SF. Lymphatic clearance of albumin was suggested to contribute to the drug efflux due to clearance of albumin bound drug amounting to 1%, 13%, and 48% of the total clearance for acetaminophen, salicylate and diclofenac, respectively. An implication of the conducted analysis was a limiting half-life of highly albumin bound drugs equal to the terminal half-life of the albumin molecule itself (13.1 h).[103] Simkin et al.[113] also investigated the effect of protein binding on articular kinetics. Based on an examination of data from oral dose studies involving highly bound NSAIDs (>99%), the authors found that synovial transport was much too fast to be accounted for by synovial clearance of albumin bound drug solely (Tab. 2). It was suggested that fast association/dissociation kinetics relative to drug transit time through the synovial capillaries would make most of the bound drug available for diffusion across the endothelium. Thus, synovial disappearance rates may not just depend on drug affinity for proteins but also on binding avidity.[98,113] Further studies are warranted to elucidate this aspect of IA drug disappearance kinetics. It is difficult to unravel the exact importance of drug characteristics (e.g., size, lipophilicity, charge, and protein binding) to synovial disappearance rates. Lipophilicity has been proposed to play a role.[100,106,112] However, a highly lipophilic drug may experience slow efflux from the joint as compared to small polar molecules. This is the case for the lipophilic gas ^{133}Xe which was found to be extensively partitioned into fat tissue of the joint.[106] Apparently, little information is available on the effect of charge on disappearance rates through the synovium. In the early study of Simkin and Pizzorno,[112] it was found that synovial permeability of magnesium and calcium was lower than for the other small molecules investigated (e.g., urea and benzyl alcohol).

Plasma proteins enter the SF (to different extents) by crossing the endothelium and diffusing through the interstitial matrix. This process is size selective, that is, albumin escapes the capillaries more readily as compared to fibrinogen and macroglobulins.[98,114–116] This size dependency is partly reflected in the SF/serum protein concentration ratios although other factors affect the concentration ratio as well.[116] Inflammation increases the endothelial permeability for proteins and consequently the SF protein concentrations as this is not fully counteracted by changes in lymphatic flow.[97,99] The permeability increase for proteins associated with inflammation is not always followed by a similar increase in synovial permeability of small solutes due to the dual barrier construction (the ECM being the critical barrier to the transport of small molecules rather

Table 2. Synovial Disappearance Half-Lives ($t_{1/2}$) and Molecular Weights (MW) of Various Solutes*

Compound	$t_{1/2}$ (h)	MW (g/mol)	Comments	References
^{131}I-cartilage proteoglycan	12.5	2.5×10^6	Rabbit knee	122
^{3}H-hyaluronan	10.2	0.09×10^6	Rabbit knee	124
^{3}H-hyaluronan	13.2	$>6 \times 10^6$	Rabbit knee	124
^{14}C-hyaluronan	20.6	2.0×10^6	Rabbit knee	286
Hyaluronan	21.8–26.3	$\sim 3 \times 10^6$	Rabbit knee	123
^{131}I-albumin	3.9	6.7×10^4	Rabbit knee	122
Albumin	1.23	6.7×10^4	Rabbit knee	123
^{125}I-ulinastatin	1.3[a]; 5.6[b]	6.7×10^4	Rabbit knee; affinity for synovial tissue	105
Sodium pertechnetate	0.82/1.78	186	Normal/arthritic rabbit knee	122
Acridine orange	0.23	370	Rabbit knee	111
Patent blue V	0.40	582	Rabbit knee	111
Evans blue	0.92	963	Rabbit knee	111
^{131}I-albumin	5.9/12.2	6.7×10^4	Canine wrist/knee	119
$^{131}I^-$	0.55/0.78	131	Canine wrist/knee	119
^{133}Xe	0.11–0.18[a]; 0.57–1.3[b]	133	Canine knee	106
Ceftiofur	5.1	524	Horse antebrachiocarpal joint	108
Procaine	0.80	236	Horse hock joint; hydrolysis occurs simultaneously	287
^{131}I-cross-linked hyaluronan	1.5; 39; 720		Triexponential kinetics, the latter $t_{1/2}$ reflecting the cross-linked HA	288
^{125}I-albumin	13.1	6.7×10^4	RA	103
D_2O	0.26	20	Normal and RA	150
THO	0.19–0.26	20	Human knee, various conditions	117
^{24}Na	0.23/0.20/0.11	24	Normal/minor/more severe RA activity	149
^{24}Na	0.39	24	Normal and RA	150
$^{131}I^-$	0.64/0.53	131	RA/OA	117
^{133}Xe	0.06[a]; 0.63[b]	133	RA	107
^{133}Xe	0.07–0.26[a];0.58–4.0[b]	133	Human knee, various conditions	106
Lidocaine	0.35	234	Arthroscopy patients	289
Methotrexate	0.59[a]; 2.90[b]	454	RA	104
Ceftazidime	0.6	547	Septic arthritis patient	290
Diclofenac	5.2	296	RA	103
Salicylic acid	2.4	138	RA	103
Paracetamol	1.1	151	RA	103
Diclofenac	1.5	296	RA/OA, oral dosing	291
Etodolac	4.1	287	RA/OA, oral dosing	291
Ibuprofen	1.9	206	RA/OA, oral dosing	291
(R)- and (S)-ibuprofen	2.6 (R) and 2.3 (S)	206	RA, OA or gouty arthritis, oral dosing	292
Indomethacin	3.3	358	RA/OA knee, oral dosing	291
Tenoxicam	2.6	337	RA/OA knee, oral dosing	291
Aclofenac	2.9		RA, oral dosing	113
Flurbiprofen	3.4	244	RA, oral dosing	113
Indomethacin	2.8	358	RA, oral dosing	113
Ketoprofen	1.9	254	RA, oral dosing	113
Naproxen (550 mg)	1.6	230	RA, oral dosing	113
Naproxen (500 mg)	1.6	230	RA, oral dosing	113
Naproxen (1000 mg)	3.1	230	RA, oral dosing	113
Tenoxicam	2.8	337	RA, oral dosing	113
Tiaprofenic acid	1.5	260	RA, oral dosing	113
Tolmetin	2.2	257	RA, oral dosing	113
^{14}C-cortisone	1.3, 1.5	360	Two RA patients	151
^{14}C-hydrocortisone	1.0, 1.46, 1.8	362	Three RA patients	151
^{14}C-hydrocortisone	0.37[a]; 2.5–4.2[b]	362	RA	109

*Values relate to intra-articular administration in human knee joint unless otherwise noted.
[a]Synovial distribution phase.
[b]Synovial elimination phase.

than the endothelium of the microvessels).[97,117] In contrast, escape of proteins from the joint occurs through the lymphatic system, a process which is not size selective for molecules with the size of plasma proteins.[102,114,116,118–120] In relation to the design of DDS it is of interest to know the size limitations to the lymphatic clearance from the joint cavity. Rodnan and MacLachlan found that the human synovium was equally permeable to albumin (67 kDa; $r_{es} = 3.6$ nm) and γ-globulin (150 kDa; $r_{es} = 5.6$ nm).[120] The r_{es} values are Einstein-Stokes diffusion radii.[99] Studies in mice revealed diminished protein clearance rates of 67–150 kDa proteins as compared to those of lower molecular weight proteins (14–47 kDa).[121] To this end it has been found that hyaluronan and cartilaginous proteoglycans are not readily cleared by lymphatic drainage (Tab. 2).[122–124] The fate of particulate and vesicular DDS upon IA administration is discussed in a following section.

Drug Transport into Cartilage

As revealed above, drugs are likely to distribute from the SF into various joint tissues after IA administration. Calculations on glucose transport, to feed the chondrocytes of the articular cartilage, have revealed that diffusion of this nutrient in the relatively viscous SF, *per se*, might be too slow for reaching the center of larger joints. Instead joint motion is suggested to generate additional solute transport by convection.[94,125] Upon attainment of a uniform glucose concentration in the SF, diffusion into cartilage is sufficiently fast to supply the chondrocytes with this nutrient. Similar considerations may be of relevance for drug distribution in the joint cavity. At the SF-cartilage interface two major parameters govern the efficiency of solute transport into the cartilage ECM, that is, the size and the charge of the solute. Quantitative descriptions relating rates of diffusion and the latter intrinsic diffusant (drug) properties are lacking. Physicochemical aspects of the effect of molecular size and charge on drug accumulation within the cartilage are briefly presented below.

For solutes, the effective (apparent) diffusion coefficient for transport in cartilage, $\overline{D}$, can be related to the diffusion coefficient in aqueous solution D *via* a so-called turtuosity factor λ (expresses the actual solute diffusion length per unit thickness of cartilage) and a solute-membrane friction coefficient F_{SM} (a size exclusion effect):[126]

$$\frac{\overline{D}}{D} = \frac{1}{\lambda^2}\left(\frac{1}{1 + DF_{SM}}\right) \quad (1)$$

For macromolecules like dextran 10 and 40 (about 10 and 40 kDa, respectively) $\overline{D}$ is very small compared to D due to a significantly enhanced friction between the solute molecules and the pore walls ($DF_{SM} \gg 1$). In this case (Eq. 2) may apply:[126]

$$\overline{D} = \frac{1}{\lambda^2}\left(\frac{1}{F_{SM}}\right) \quad (2)$$

Interestingly, studies may indicate that even very large molecules such as hemoglobulin (68 kDa) and dextran 40, may diffuse into cartilage albeit at very low rates.[126] This might be ascribed to a rather inhomogeneous structure of the cartilage.[127–130] In case of large molecules, transport efficiency into cartilage might to some extent be influenced by convection due to dynamic compression.[131–134]

Cartilage proteoglycans are composed of a core protein to which at least one GAG chain is covalently attached. The latter negatively charged polysaccharides are mainly chondroitin and keratin sulfate.[18] Since overall electroneutrality has to be maintained in cartilage the fixed negative charges of the proteoglycans have to be balanced by more mobile cations. Consequently an osmotic pressure gradient as well as a potential difference are created. The phenomenon corresponds to a Donnan exclusion effect. Therefore attempts have been made to model drug salt distribution in cartilage within classical ion exchange theory.[126,135–137] Within the cartilage, the total concentration of cations for an electrolyte salt can be expressed as:

$$Z_{cation}\overline{C}_{cation} = Z_{anion}\overline{C}_{anion} + \overline{C}_{immobile\ anion} \quad (3)$$

where $\overline{C}$ is the molar concentration in cartilage. Due to the fixed negative charge of the collagenous fiber network, cationic drug molecules have a higher tendency to distribute into cartilage compared to anions.[135] Based on these early observations efforts have been devoted to exploit the ion exchange properties of the ECM to enhance drug accumulation in the cartilage tissue. Various positively charged organic molecules have been observed to distribute into cartilage.[138–147] This includes (i) radiodiagnostic agents for joint imaging containing a N-quaternary functional group,[142,143] (ii) N-quaternary

analogues of anti-inflammatory oxicams,[140,144] and (iii) a derivative of the potential DMOAD doxycycline.[147]

Modeling Joint Escape Kinetics

The elimination of low molecular weight drugs in the solute state from the synovial cavity has been described by first-order kinetics (Tab. 2). This is consistent with the view that the interstitial matrix is the limiting (diffusional) barrier.[97,99] Accordingly, Ficks first law may be a suitable starting point for describing the rate of drug disappearance from the synovial cavity:[112]

$$\frac{dN}{dt} = -\frac{DA}{x}(C_s - C_p) \quad (4)$$

where N is the number of drug molecules in the synovial cavity, C_s and C_p are the drug concentrations in the SF and plasma, respectively, D is the diffusion coefficient, A the available area for diffusion and x is the length of the diffusion path. Introducing V_s as the SF volume and assuming that the systemic circulation acts essentially as a sink ($C_p \approx 0$) (Eq. 4) may be rewritten

$$\frac{dC_s}{dt} = -kC_s \quad (5)$$

where k ($=DA/xV_s$) is the synovial escape (clearance) rate constant due to simple diffusion. Difference in chemical potential is the driving force of diffusion and in simple systems, such as a dialysis cell, this can be approximated by the concentration gradient. Pursuing the analogy between the joint and a dialysis apparatus with a porous membrane impermeable to proteins and macromolecules, it is apparent that C_s in (Eqs. 4 and 5) should be the free drug concentration rather than the total analytical concentration normally measured. A number of experimental findings complicate this simplified view: the interaction of drug substances with the macromolecules of the SF and articular cartilage as well as the transport of protein bound drug out of the joint cavity.[103,113] As regard binding to tissue components and macromolecules in the SF, direct proportionality between the free and total drug concentration may be found in case (i) the drug concentration is much smaller than the concentration of binding sites[148] and (ii) the attainment of the distribution equilibria are fast. It may be difficult to evaluate whether these prerequisites are fulfilled. However, it may be the case for NSAIDs since the therapeutic drug concentrations are much smaller than that of their primary transport protein (albumin). The clearance kinetics of the SF proteins by the lymphatics has been found to obey first-order kinetics[119,122] and, consequently, this can also be expected for protein bound drugs.[103] Altogether, drug efflux from the joint cavity should follow first-order kinetics when the following (idealized) conditions prevail: the drug binding phenomena are concentration independent and the various contributing transport processes can be characterized as first-order processes. However, the rate constant k in (Eq. 5) should be replaced by a composite rate constant k', which describes the observed time dependence of the total synovial drug concentration upon IA instillation. Support to the adequacy of (Eq. 5) to describe synovial kinetics may be found in the series of studies following first-order kinetics for several half-lives for various solutes[119,122,149–151] This equation, however, does not take into account the distribution phase observed in some studies (e.g.[103,104]) upon IA administration of drug solutions.

The transport equation outlined above may be combined with knowledge on the drug release mechanism of a specific drug delivery system, to estimate the IA drug concentration versus time profile. Two limiting cases can be envisioned. For delivery systems providing a constant rate of drug release (k_{rel}; mole drug released per time and unit SF volume) an approximately constant synovial drug concentration may be achieved for a period of time:

$$\frac{dC_s}{dt} = k_{rel} - k'C_s \quad (6)$$

In contrast, for a DDS where release is governed by drug partitioning between the vehicle and the SF phase, the overall joint escape can be expected to apply to first-order kinetics. Delivery systems approximating the former case (zero order drug release) may be suspensions of poorly soluble steroids. Indications, that this is the case, are the almost constant plasma steroid concentrations found for prolonged time periods following an initial phase of rapid drug appearance in the systemic circulation.

ON FUTURE IA DRUG DELIVERY STRATEGIES

In this section a rationale for localized drug delivery (based on pharmacokinetic considerations) is presented. Several parenteral depot

formulation technologies have been investigated for the IA route of administration. A more comprehensive treatise of aspects of the applicability of major depot formulation principles is given. This includes prolonged release properties, *in vivo* fate and issues related to their manufacture. These considerations are also expected to be of relevance for other mentioned depot DDS types of potential utility for novel IA depots in the area of postoperative pain control and OA. Further, the role of *in vitro* models for quality control and the assessment of the controlled release capabilities of potential IA depot formulations is discussed. The intriguing concept of gene transfer to arthritic joints has been the subject of several reviews[152–156] and is not considered further in the present context.

Rationale for Localized Drug Delivery

For drugs that have to be transported to a discrete site of action (e.g., a knee joint) by the systemic circulation, the fraction of the administered dose that enters the target site is dependent on the extent of drug distribution to other tissues of the body.[148] After completion of the drug distribution phase, the amount of drug in the body at (steady state) equilibrium (CV_D) is given by

$$CV_D = CV_p + C\sum_{i=1}^{n} V_i K_i \quad (7)$$

where C is the plasma concentration, V_D is the apparent volume of distribution and, V_p refers to the plasma volume. V_i and K_i represent the volume and the tissue-to-plasma ratio of drug concentrations for the specific tissue (i), respectively, and n refers to the total number of tissue compartments. Thus, the fraction of a given drug dose that reaches the particular joint (f_{joint}) might roughly be estimated from

$$f_{joint} = \frac{V_{joint}K_{joint}}{V_D} \quad (8)$$

For major weight-bearing joints K values close to unity are to be expected for most small-molecule drugs. Although the magnitude of V_{joint} might vary to some extent with type and severity of disease, the key parameter influencing the size of f_{joint} is V_D that may vary widely (7–40000 L/70 kg body weight) depending on the physicochemical properties of the individual drug compound.[148] To this end it can be mentioned that after oral dosing, a steady state V_D of about 90 L has been reported for the selective COX-2 inhibitor rofecoxib.[157] This drug has now been withdrawn from the market due to severe side effects after oral administration. In addition to drug mixtures for multimodal analgesia, local administration might therefore also be considered for anti-arthritic drug candidates that are prohibited from oral administration due to severe systemic side effects or bioavailability problems. Direct IA instillation of depot formulations appears most realistic for future anti-osteoarthritic drugs based on the fact that in OA only few joints are affected. The clinical course of RA is characterized by a variable disease activity of spontaneous remissions and exacerbations (flare-ups) of the chronic inflammatory joint process. In the case of episodes of discrete joint flare-ups[158] and to treat resistant knee monoarthritis[159–161] local anti-inflammatory therapy might also be indicated.

Potential IA Depot Drug Delivery Systems

Aqueous glucocorticoid suspensions remain the only world wide, commercially available depot type for IA injection. In addition, the performance of a variety of depot principles have been investigated *in vivo* (Tab. 3). Most of these studies have been carried out in animals with the rabbit being the most frequently used animal model. The adequacy of employed injection methodologies to minimize trauma, tissue damage and extra-articular leakage[162–164] has not been optimally addressed in any of these experimental studies. Another common feature is the apparent lack of information about the robustness of the obtained *in vivo* data to minor changes in depot characteristics such as size (particles/droplets) and charge. In several studies it was reported that the IA administered formulation in question was well tolerated. Although the lack of significant local toxicity has to be documented through more comprehensive studies, feasible biocompatibility might, in general, be anticipated since pharmaceutical excipients of several of these formulations already constitute the building blocks of marketed depots intended for other parenteral routes of administration.[165]

As apparent from Table 3, far the majority of the investigated therapeutic systems have involved liposome or microsphere-based DDS. Numerous reports indicate that advanced drug delivery liposomes might be of potential utility to overcome

Table 3. Overview of Experimental Drug Delivery Systems Administered Intra-Articularly

Drug/Model Compound	Animal Model/Human	Comments	References
Liposomes			
Cortisol 21-palmitate	Rabbit (induced arthritis)	Drug incorporated in lipid bilayer.[177] Particle size not defined. Sustained anti-inflammatory effect of 3 days	171,173
Cortisol 21-palmitate	RA patients	Drug incorporated in lipid bilayer. Particle size not defined. Sustained anti-inflammatory effect of 14 days	174
Triamcinolone acetonide 21-palmitate	Rabbit (induced arthritis)	Drug incorporated in lipid bilayer. Particle size not defined. Prolonged retention in the joint cavity (38% remaining after 8 h) compared to free triamcinolone acetonide in solution	172
Dexamethasone 21-palmitate	Rabbit (healthy)	Drug incorporated in lipid bilayer. Particle size range: 0.1–30 μm. Optimal endocytosis in particle size range: 0.75–5 μm	175
Dexamethasone 21-palmitate	Rabbit (healthy and induced arthritis)	Drug incorporated in lipid bilayer. Particle size range: 160–750 nm. The amount of drug remaining in the joint increases with particle size. Prolonged retention in the joint (36% remaining after 6 h) compared to microcrystalline triamcinolone acetonide. Uptake of liposomal drug in the synovium 1–2 days after injection. Retention enhanced in healthy joints compared to inflamed joints	176
Methotrexate	Rabbit (healthy and induced arthritis)	Drug entrapped in the aqueous phase. Mean particle size: 1.07 μm. Rapid leakage of some drug from liposomes. Prolonged retention in the joint (46% remaining after 24 h) compared to an aqueous solution of the free drug. Low uptake of liposome (dose dependent) by synovium	180
Methotrexate	Rat (induced arthritis)	Methotrexate analogue (a phospholipid covalently bound to methotrexate *via* amide bond) incorporated into lipid bilayer. Mean particle size: 100 nm (small) and 1.2 μm (large). Sustained anti-inflammatory effect for 21 days (large liposomes). Prolonged retention in the joint (6% and 29% remaining after 24 h for small and large liposomes, respectively) compared to the free drug	293
Transforming growth factor-β1 (TGF-β1)	Rabbit (full-thickness articular cartilage defects)	Indications of an accelerated early-stage repair of the articular cartilage defects	294
Lactoferrin	Mouse (induced arthritis)	Drug most likely entrapped in the aqueous phase. Positively and negatively charged liposomes. Particle size not defined. Fast drug release from liposomes. Prolonged retention time in the joint of the positive charged liposome (15% remaining after 24 h) compared to an aqueous solution of the free drug	295
Lidocaine	Rabbit (healthy)	No characterization of liposomes. Fast drug transfer to the blood for free drug as well as liposome entrapped lidocaine	179

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Diclofenac	Rabbit (induced arthritis)	Drug entrapped in the aqueous phase. Liposomes and niosomes incorporated in hydrophilic polymer gels (lipogelosomes and niogelosomes). Particle size: 200–250 nm. Fast drug release from liposomes (93% after 48 h). Slower release from lipogelosomes (50% after 48 h). Longest retention in the joint with lipogelosome (50–70% remaining after 24 h)	178
Clodronate	Sheep (induced arthritis)	Drug most likely entrapped in the aqueous phase. No characterization of liposomes. Uptake of liposomes in the synovium. No therapeutic effect	296
Clodronate	Mouse (healthy and induced arthritis)	Drug most likely entrapped in the aqueous phase. Mean particle size: 1 μm. Depletion of phagocytic lining cells within 3 days	297,298
Clodronate	Rabbit (induced arthritis)	Drug most likely entrapped in the aqueous phase. Particle size: 200–300 nm.[195] Uptake of liposomes in synovium temporary anti-inflammatory and antierosive effect	299
Clodronate	RA patients	Drug most likely entrapped in the aqueous phase. Particle size: 120–160 nm. Depletion of synovial lining macrophages. Decrease expression of adhesion molecules in the synovial lining (reduced inflammation)	300
^{51}Cr (radionuclide)	Rabbit (induced arthritis)	A complex of ^{51}Cr and a lipophilic chelator was incorporated into the lipid phase. Mean particle size: 100 nm. More than 41% remaining in the joint after 21 days. Accumulation of radioactivity in the synovium	301
Benzoporphyrin derivate-monoacid ring	Rabbit (induced arthritis)	No characterization of the liposomes. High association of the drug to the synovium. Possibility of using photodynamic therapy in management of autoimmune disorders	302

Drug/Model Compound	Animal Model/Human	Comments	References
Microspheres			
None	Rabbit (healthy)	Polylactic acid, polybutylcyanoacrylate, gelatin and albumin microspheres. Particle size: 1–10 μm. Albumin microspheres best tolerated by the synovial tissue. Uptake of microspheres into the synovium. Maximum uptake for particle size <5 μm	303
Rose Bengal	Rabbit (induced arthritis)	Albumin microspheres. Mean particle size: 3.5 μm. Rapid initial release. Prolonged retention in the joint (9–25% remaining after 7 days) compared to the free model compound. Uptake of microspheres into the synovium	304

(*Continued*)

Table 3. (*Continued*)

Drug/Model Compound	Animal Model/Human	Comments	References
Prednisolone or triamcinolone	Rabbit (healthy)	Heat denaturated albumin microspheres. Mean particle size: 23 μm. Decreased plasma absorption rate from microspheres compared to that of suspensions. No uptake into the synovium. Potential adsorption of microspheres to synovium	305
Diclofenac	Rabbit (induced arthritis)	Albumin and poly(lactide-co-glycolide) microspheres. Particle size: 5–15 μm. Significant initial burst release (*in vitro*). No sustained release effect *in vivo*	306,307
Celecoxib	Rat (induced arthritis)	Chitosan microspheres. Particle size: 8–16 μm. Significant initial burst release (*in vitro*). Limited sustained release effect *in vivo*	308,309
Naproxen	Rabbit (induced arthritis)	Albumin and poly(lactide-co-glycolide) microspheres. Particle size: 5–10 μm. Some initial burst release (*in vitro*). PLGA microspheres more promising than albumin microspheres	310
Dexamethasone	Rabbit (healthy)	Poly(D,L-lactide) microspheres. Particle size: 40–110 μm. Increased molecular weight of polymer resulted in slower *in vitro* release from microspheres. No drug detected in serum within 24 h. Drug detected in synovial cavity (not in the SF) up to 7 days	311
Coumarin (fluorescent dye)	Rabbit (healthy)	D,L-lactic acid oligomer microspheres. Particle sizes: 20–100 μm; 100–200 μm. Microsphere localized in popliteal fat tissue (independent on particle size and molecular weight of oligomer)	312
Paclitaxel	Rabbit (induced arthritis)	Poly(lactide-co-glycolide), poly(L-lactide), poly(caprolactone) microspheres. Particle sizes: 1–20 μm; 35–100 μm. Short burst release (5–7%) followed by sustained release for 30 days (*in vitro*). Inflammatory response using small particle sizes whereas larger particle sizes were found to be biocompatible	313
None	Rat (healthy)	Poly(D,L-lactide-co-glycolide) micro-and nanospheres with mean particle sizes of 265 nm and 26.5 μm, respectively. Dispersion of microspheres in the joint cavity (potential adhesion to the surface of the synovium). Uptake of nanospheres in the synovium.	314
Betamethasone sodium phosphate	Rabbit (induced arthritis)	Poly(D,L-lactide-co-glycolide) nanospheres. Particle size: 300–490 nm. Decrease of initial burst release by increasing of the molecular weight of the polymer. Sustained anti-inflammatory effect for 21 days	315

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Methotrexate	Rabbit (healthy)	Poly(L-lactide) microspheres. Particle size: 60–190 μm. Rapid burst release (*in vitro*). Indication of a slightly prolonged drug release compared to the free drug in a solution. Recovery of small drug amounts in the synovial tissues after 24 h. Potential deposition of microspheres in the adipose layer	316,317
Insulin	Mouse (healthy)	Poly(D,L-lactide-co-glycolide) microspheres. Rapid burst release (*in vitro*). Insulin activity (stimulation of ECM synthesis) after release from microspheres	318
Holium-166	Rabbit (healthy)	Poly(L-lactide) microspheres. Particle size: 2–13 μm. Retention in the joint space (98% after 120 h). No uptake by the lymph nodes	319
Holium-166	Patients with RA	Chitosan microspheres. Particle size not defined. Most of the injected dose remains located in the joint cavity	320,321

DDS	Drug/Model Compound	Animal Model	Comments	References
Miscellaneous DDS				
Superparamagnetic iron oxide nanoparticles	Fluorescent dye (Cy3.5)	Sheep (healthy)	Superparamagnetic iron oxide nanoparticles coated with polyvinyl alcohol. Enforced uptake into the synovium by an extracorporally applied magnet	322
Zyn-Linkers™	Re-186 or Y-90	Rabbit (healthy)	Synthetic lipid–like molecules designed for local delivery, for example, of radionuclides for radiosynovectomy. Rapid binding of Zyn-Linkers to the synovium. Localization and retention in the knee region (>90% after 4–6 days)	323
Complex	Gadolinium tetraazacyclododecane tetraacetic acid	Dog (healthy)	Gd-complex as contrast agents in MR imaging of the joints. Mean IA half-life of the complex close to 2 h	324
Elastin-like polypeptides (ELP)	None	Rat (healthy)	Thermogelling biopolymers (ELP) that aggregate upon IA injection. Prolonged retention in the joint cavity (nonaggregating ELP, $t_{1/2} = 3.4$ h; aggregating ELP, $t_{1/2} = 3.7$ days)	201
Calcium alginate	Transforming growth factor-β	Rabbit (osteochondral defects)	Implantation of alginate beads into osteochondral defects. Slow *in vitro* release of TGF-β from alginate (30% to 40% retention after 5 days). Improved repair of the articular cartilage defects	325

(*Continued*)

Table 3. (*Continued*)

DDS	Drug/Model Compound	Animal Model	Comments	References
Gelatin hydrogel microspheres	Basic fibroblast growth factor (bFGF)	Rabbit (healthy and induced arthritis)	Mean particle size: 70 μm. Retention in the joint cavity (3% remaining after 7 days). Localization of bFGF in soft tissue (including synovium) but not in articular cartilage. Indicate induced anabolic effects on cartilage and suppression of the progression of OA	326
Gelatin/chondroitin 6-sulfate microspheres	Albumin and catalase (model proteins)	Mouse (healthy)	No phagocytosis of the microspheres. Microspheres were found to be noninflammatory *in vivo*	196
Latex particles	None	Rat (healthy)	Polystyrene latex average particle size: 240 nm. Phagocytosis of synovial cells at the synovium-cartilage junction was studied. Potential involvement of both type A (macrophage-like) and type B (fibroblast-like) synovial cells, chondrocytes and tendon cells in the phagocytosis	327

various barriers to drug delivery including biocompatibility, fast elimination and target access.[166,167] A safety concern to be considered is, however, that one of the most frequently encountered clinical problems after infusion of PEGylated liposomes into certain subjects is the initiation of non-IgE-mediated hypersensitivity reactions. These pseudoallergic reactions are believed to arise *via* complement activation.[168,169] In liposome-based product development also critical technology-oriented challenges might emerge. These might relate to the large-scale (aseptic) manufacturing process as well as to the achievement of acceptable long-term physical stability of the formulation.[170] Water-soluble drugs are mainly entrapped in the aqueous space, whereas more lipophilic compounds can be intercalated into the phospholipid bilayer. In the latter case only modest drug loads can be accomplished. Liposomal preparations containing lipophilic corticosteroid derivatives (Tab. 3) afforded sustained anti-inflammatory activity after IA injection in the rabbit and in man.[171–176] The steroids were most likely incorporated into the phosholipid bilayer. Shaw[177] observed a diminished retention of cortisol derivatives in dipalmitoyl phosphatidylcholine liposomes as a function of decreasing lipophilicity of the investigated compounds after applying such liposomes to aqueous buffer. In comparison to the steroid preparations, the more polar drugs methotrexate, lidocaine and diclofenac experienced a much less pronounced prolongation of the IA residence time from liposome formulations.[178–180] Despite the limited number of studies performed (involving different experimental conditions) it appears that the inherent sustained release capability of conventional liposome preparations is questionable. For drugs dissolved in the aqueous core, drug release is expected to proceed through diffusion across the intact bilayer whereas burst release may result from destruction of the phospholipid membranes. Analogously, drug liberation from degraded lipid membranes, in case of the steroid liposome formulations, will most likely lead to precipitation of the poorly soluble steroid derivative. Since adequate reference formulations containing the respective steroid derivative were not included in the above mentioned studies it cannot be excluded that the observed sustained anti-inflammatory effects were afforded by slow dissolution of the precipitated drugs in the joint cavity. In this context it should be mentioned that the Depo-Foam® technology, that may show some resem-

blance to multivesicular liposomes, may be useful for sustained drug delivery.[181]

Sustained release properties of biodegradable microsphere preparations (with the active agent dissolved or dispersed in a polymer-based matrix) are well-established. A number of microsphere-based products (duration of therapeutic activity ranging from about 2 weeks to 3 months) are approved for parenteral administration in areas such as cancer and schizophrenia.[182] Owing to their excellent biocompatibility, the biodegradable polyesters poly(lactide-co-glycolide) (PLGA) are the most frequently used materials for the microencapsulation of drugs. Despite the fact that PLGA-based products have been launched, the development of therapeutic PLGA microspheres might, however, be far from straightforward as discussed in more detail elsewhere.[183,184] Other synthetic polymers frequently used in the design of microparticles include polyanhydrides,[185,186] poly(ortho esters)[187] and poly-ε-caprolactone.[188] Polymeric matrices comprising polymers of natural origin, such as albumin[189] and chitosan,[190] have also been investigated. The *in vivo* studies performed (Tab. 3) do not provide a clear-cut picture of the capability of the microsphere approach to substantially prolong the IA residence time of drugs. In general, modification of drug release can be accomplished by the employment of copolymers consisting of synthetic polymers endowed with different susceptibilities to undergo hydrolytic degradation or through change of drug lipophilicity by prodrug formation.[191] Basically, after an initial burst, drug liberation from such depots may involve (i) drug diffusion out of the matrix, (ii) erosion of the matrix or (iii) a combination of these two release mechanisms. Diffusion controlled release is to be expected for small-molecule water-soluble drugs imbedded in hydrophilic polymer networks such as chitosan microspheres.[192] As to the more hydrophobic synthetic polymers, poly-ε-caprolactone[188] and poly(ortho ester)[187] microspheres degrade mainly by bulk hydrolysis and surface erosion, respectively. In contrast PLGA (and probably also polyanhydride-based) microspheres may undergo both surface and bulk erosion. The underlying mechanism for bulk erosion (and the accompanying significant decrease in core pH) is not fully elucidated.[183,193,194] Like liposomes, microspheres might be taken up by synovial macrophages after instillation into the joint space. Size-dependent phagocytotic uptake have been observed (Tab. 3) but parameters like surface charge might also influence the efficiency of endocytosis where apparently a net negative surface charge prohibits extensive phagocytosis.[195,196]

Seemingly, glucocorticoids represent the only drug class that has been injected intra-articularly in the form of depot suspensions. As mentioned in a previous section such formulations are reasonably well tolerated although "steroid flare" reactions occasionally occur.[197] *In vitro* phagocytosis of steroid crystals by leukocytes has been reported,[198] however, information about uptake of solid steroid particles by synovial cells *in vivo* is apparently lacking. Drug appearance in the SF after IA injection of aqueous suspensions is mainly governed by the dissolution rate of the poorly soluble agent. According to the Noyes-Whitney equation, the dissolution rate is directly proportional to the drug solubility and the total surface area of the solid particles. The parenteral suspension may be an advantageous dosage form from the perspective that high drug load can be achieved and only a minimum of pharmaceutical excipients is needed. However, in spite of the simplicity of this formulation type, parenteral suspensions pose certain challenges to the manufacturing process and physical stability.[199,200]

Interestingly, aqueous solutions of certain elastin-like polypeptides (biopolymers with molecular weights in the order of 50 kDa) are observed to exhibit a phase transition above a given transition temperature characterized by the formation of micron or submicron size aggregates (potential drug vehicles). *In situ* aggregate formation after IA injection of aqueous solutions of such biopolymers in rats resulted in a significantly prolonged IA residence time as compared to that of the dissolved biopolymer.[201] Also patented depot DDS principles based on *in situ* gel formation might be of potential interest for the IA route of administration.[202,203] In fact, the *in situ* forming parenteral DDS approach may, in general, constitute a means to circumvent various critical issues, such as physical stability and sterilization, related to the development of microparticulate systems.[204–206]

On Novel IA Approaches for Postoperative Pain Control

It is to be expected that future IA depots acting in a multimodal fashion should comprise at least one analgesic agent and one anti-inflammatory drug. In addition to the IA depot principles dealt with above, it can be mentioned that in the area of

local anesthetics several approaches to achieve sustained drug action after various parenteral routes of administration have been investigated. The therapeutic agents, mainly bupivacaine and lidocaine, have been incorporated into microspheres,[207–216] liposomal formulations,[217–223] lipid solutions (iophendylate),[224–226] vegetable oil,[227] liquid synthetic polymers,[228] lipid–protein–sugar particles,[229] injectable gels,[230] dry emulsions to be reconstituted prior to use,[231] and lipospheres.[232] Interestingly, inclusion of small amounts of dexamethasone (about 0.04%) in bupivacaine microspheres has been demonstrated to prolong bupivacaine analgetic activity significantly in animal studies.[211,212] Similar effects have been observed in man as regards intercostal blockade[207] and after subcutaneous infiltration of bupivacaine microspheres.[208] The mechanisms behind the blockade-prolonging effect of glucocorticoids are far from fully elucidated.[208]

Assuming that optimal pain relief after minor joint surgery requires analgetic and anti-inflammatory action locally at the site of trauma over about 1 and 7 days, respectively, the first step in the search for achievement of this therapeutic goal might be to investigate the possibility of using combinations of already marketed drug products. Although sufficiently prolonged anti-inflammatory activity can be accomplished by using suspensions of glucocorticoid ester derivatives such as methylprednisolone acetate (Depo-Medrol®), their onset of action might be relatively slow. Also suspensions of poorly soluble ester prodrugs of the more potent steroid betamethasone (e.g., Diprospan®)[233] are used for IA injection. In addition to the 17,21-dipropionylester derivative, the latter product also contains betamethasone in the form of the water-soluble 21-phosphate ester. The rationale for incorporation of the water-soluble prodrug is apparently to ensure fast onset of drug action. After entrance into the systemic circulation, 21-phosphate esters of various glucocorticoids are cleaved relatively fast to give the parent drug.[234,235] After i.v. injection of dexamethasone phosphate ester the *in vivo* conversion rate exceeded that observed after prodrug incubation in full blood by a factor of about 25, suggesting that the major sites for phosphate ester bond cleavage are the highly perfused organs, that is, the liver and kidney.[236] In this connection, determination of betamethasone pharmacokinetics after combined IA injection of the acetate and phosphate 21-esters of the corticosteroid led to the suggestion that the phosphate ester did seem to be an unnecessary part of the combination due to rapid clearance from the joint cavity.[237] Aqueous solutions of morphine and bupivacaine are marketed, and both drugs (and in combinations) have demonstrated positive analgesic effects after IA injection. However, as discussed in more detail elsewhere[26] their effectiveness in the immediate postoperative period are relatively short-lived (at best 8–12 h in case of morphine).

In summary, it appears conceivable to suggest that optimal IA multimodal analgesia is presently not achievable by combinations of existing products due to the lack of sufficiently long-acting therapies for abolishment of the acute pain following surgical injuries. Although speculative, prolongation of the effects of morphine and bupivacaine might potentially be achieved by administering these agents in the form of poorly soluble salts. It is well known that pamoic acid forms poorly soluble salts with many drugs containing an amine functional group.[238,239] Also other aromatic *ortho*-hydroxycarboxylic acids have been observed to render relatively poorly soluble amine salts[240,241] including the NSAID diflunisal, that forms salts with morphine, bupivacaine as well as lidocaine. Use of the common ion effect for prolonging the release of bupivacaine from mixed salt suspensions *in vitro* have recently been reported.[242] Compared with the use of combinations of drug products, an IA depot type capable of controlled parallel release of an analgesic and an anti-inflammatory agent may, *a priori*, exhibit therapeutic advantages. To this end lipid solutions might be of potential interest. Oil depot injectables (mainly ester prodrugs of antipsychotics and steroid hormones dissolved in vegetable oils) for intramuscular and subcutaneous administration have been in the marketplace for decades.[243–245] The fact that drug release rates from such oil vehicles, at least in part, are influenced by the oil–water distribution coefficient of the drug[246,247] opens for the possibility of design of depots with feasible delivery characteristics since manipulation of distribution coefficients might be accomplished through prodrug formation (e.g., morphine ester prodrugs[248]) or optimization of oil vehicle composition.[249]

On Novel IA Approaches in Arthritic Disorders

Whereas inhibition or modification of the activity of components of the SF might be of relevance in

the treatment of arthritic disorders, the synovium and the articular cartilage are considered additional major target tissues for drug action as regard RA and OA, respectively. In a recent review,[250] approaches for targeting the inflamed synovium *via* the systemic circulation have been dealt with in a highly competent manner. In addition to liposome approaches to provide synovial macrophage depletion following endocytosis,[251] some of the strategies to confer regional or cell-specific homing of therapeutic agents might also give inspiration to the development of novel IA depot formulations providing sustained as well as targeted drug action. Based on results obtained by using integrin antagonists,[252–255] the prospects for the use of novel small-molecule antagonists of cell-specific CAM molecules as pro-moieties in joint-targeted prodrug design appears especially attractive. Also synovial-specific transduction peptides may be used as transport vectors to facilitate drug access to activated synovial fibroblasts.[256] In case the ultimate target is located in the SF or within the articular cartilage, nonspecific phagocytotic uptake of the injected depot DDS by cells of the synovial lining is unwanted. Due to the architecture of the cartilage, electrically neutral solute molecules with molecular weights above approximately 10 kDa have been shown to get modest access to the inner space of the ECM. It is therefore less likely that even nanosize DDS should accumulate to any significant extent within the cartilage microenvironment. These considerations may suggest that prolonged delivery to cartilage sites of drug action can only be accomplished when the drug is released from the immobilized depot directly into the SF or perhaps preferably, at the interface between the SF and the cartilage. In addition to size, also the charge of the active agents is expected to influence transport into the ECM of the cartilage. The high negative charge density of this matrix may limit the entrance of anionic drug compounds. Transient masking of such negative charges by prodrug design might abolish or at least minimize this barrier to effective drug distribution into the cartilage. Since nonspecific phagocytosis, at least theoretically, can be avoided by careful control of particle size (distribution) and overall surface charge, it appears conceivable to suggest that a number of microparticulate-based depot types possesses the potential of enabling slow drug release into the SF after IA instillation. More detailed information about the *in vivo* fate of such depot types including the kinetics of drug release from the depot has, however, to be established through future studies. Interestingly, modulation of drug release might also be accomplished by using depot building blocks that are susceptible to degradation mediated by enzymes, the SF level of which is expected to vary with the state of inflammation. To this end it has been shown that model protein release rates from gelatin-chondroitin 6-sulfate microspheres varied with the concentration of matrix metalloproteases in the SF.[196] Although speculative, chitosan-based drug delivery systems might provide some degree of drug release at the SF-cartilage interface. Despite the fact that the amine groups of this polysaccharide are only partly ionized at physiological pH (pK_a 6.3[190]) some affinity to the anionic ECM of the cartilage can not be excluded. Further, this polysaccharide is of significant interest in the design of *in situ* forming depot systems, since the physical properties of polymeric chitosans change with the environmental pH. Under acidic conditions it forms a viscous solution, whereas the compound transforms into a gel at pH 7.4.[257]

In Vitro Release Models for Quality Control and Formulation Development Purposes

From a regulatory point of view the drug product specifications is a key document through which reproducible product quality, or in other words batch to batch consistency of product performance, has to be documented. This documentation may, dependent on the formulation type in question, embrace different product characteristics. On the other hand control of drug release rate from the formulation has to be demonstrated for all parenteral depots. In this field, therefore, development of suitable *in vitro* release models (for quality control as well as formulation development purposes) constitutes a significant challenge, Currently, no regulatory approved standard methods exist for testing drug release from controlled release parenteral products. As regard such quality control methods, accelerated *in vitro* release testing might be of particular utility for parenteral depots characterized by a duration of action exceeding a few weeks.[258–260]

Basically, employed *in vitro* release methods might be divided into three broad categories (i) sample and separate methods, (ii) continuous flow methods, and (iii) dialysis techniques.[261,262] Two USP (United States Pharmacopeia) apparatus,

the reciprocal cylinder (apparatus 3) and the flow through cell (apparatus 4) have been recommended for controlled release parenterals.[258–260] In addition to the use in quality control and formulation development, the *in vivo* relevance of employed *in vitro* methods should also be considered.[260] Dialysis membrane-based models are in general considered feasible only for the study of drug release from depots intended for administration sites for which drug release under nonsink conditions is prevailing.[262,263] The dialysis technique has been used to study drug release from a variety of formulations, for example, oil solutions,[247] aqueous suspensions,[241,242] microspheres,[264,265] liposomes,[266–268] nanoparticles,[269] emulsions,[270,271] and *in situ* forming DDS.[272] Different experimental setups have been used, with the relatively simple dialysis bag technique being the most widely used model.[268–272] This includes the Float A Lyzer® tube system that recently has gained considerable interest.[261,264,265] A bulk reverse dialysis bag technique[270,271] has been applied in order to circumvent problems related to the maintenance of sink conditions during the release studies. Other model modifications include the side-by-side diffusion cell,[270] a fractional dialysis method,[267] the reciprocating dialysis tubes[273] and the rotating dialysis cell.[241,242,246,247,249,274–279] Since model related factors vary from model to model, rate constants obtained by using different methods are not directly comparable.

In case of IA injection, the depot is administered into a small compartment (the joint cavity) in which the drug is released under nonsink conditions. Due to the noncontinuous nature of the synovial lining, small-molecule drugs are mainly transported out of this compartment and into the blood driven by a diffusional process.

In particular the rotating dialysis cell model, consisting of a small donor compartment (max. 10 mL) and a large acceptor compartment (max. 1000 mL) might appear attractive for investigation of some key parameters influencing IA drug residence times after local instillation of various depot DDS.[241,242,247,276] In the area of *in vitro–in vivo* correlations, however, the latter method is expected to be applicable primarily for simple drug formulations. To this end the disappearance of drugs (molecular weight range 137–2068 Da), applied to the donor cell of this model in the form of aqueous solutions, was found to obey strict first-order kinetics with half-lives in the range of 0.2–8 h.[276] Interestingly, quite similar IA elimination half-lives have been reported in the literature (Tab. 2). The applicability of the rotating dialysis cell model to simulate the drug transfer rates out of the joint cavity is presently under investigation in our lab.

CONCLUDING REMARKS

Compared to oral administration, the advantage of intra-articular drug instillation is that only a minimum amount of drug is required to exert local activity within the joint space. This relatively simple form of localized drug delivery minimizes drug exposure to inappropriate sites. Since drugs dissolved in the synovial fluid are rapidly eliminated from the joint ($t_{1/2}$ of about 0.1–6 h), maintenance of therapeutic concentrations over extended periods of time necessitates the administration of the drug in the form an injectable depot formulation. There is still an unmet need for IA therapies providing optimal pain relief after minor joint surgery. Such alleviation of postoperative pain, based on the concept of multimodal analgesia, may involve concomitant release of analgesics and anti-inflammatory agents from the IA delivery system over about 1 and 7 days, respectively. In the area of osteoarthritis, approved disease-modifying therapies are lacking. Novel treatment options may arise from the identification of a vast number of potential targets for drug action. Since OA usually affects a single or only a few joint(s), long-lasting IA technologies comprising future anti-arthritic agents hold promise. Several formulation principles are used in marketed (non-IA) parenteral depots despite the fact that, for example, their manufacture and physical stability are far from straightforward. The performance of these depot types (and many others) after IA injection has been investigated primarily in animal models. Promising results are achieved but need to be substantiated by future studies. To this end, it has to be realized that localized drug delivery not necessarily is synonymous with targeted drug delivery, especially when the drug target is located in the articular cartilage which may constitute a significant barrier to high molecular weight drugs. However, already available results suggest that intra-articular depot formulations, containing drug mixtures for multimodal analgesia or future arthritic disease-modifying agents, are likely to emerge within a reasonable time horizon.

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REFERENCES

1. Caldwell JR. 1996. Intra-articular corticosteroids. Guide to selection and indications for use. Drugs 52:507–514.
2. Hunter JA, Blyth TH. 1999. A risk-benefit assessment of intra-articular corticosteroids in rheumatic disorders. Drug Saf 21:353–365.
3. Bellamy N, Campbell J, Robinson V, Gee T, Bourne R, Wells G. 2006. Intraarticular corticosteroid for treatment of osteoarthritis of the knee. Cochrane Database Syst Rev 2:CD005328.
4. Buckwalter JA, Martin JA. 2006. Osteoarthritis. Adv Drug Deliv Rev 58:150–167.
5. Baker CL, Ferguson CM. 2005. Future treatment of osteoarthritis. Orthopedics 28:S227–S234.
6. Evans CH. 2005. Novel biological approaches to the intra-articular treatment of osteoarthritis. BioDrugs 19:355–362.
7. Wieland HA, Michaelis M, Kirschbaum BJ, Rudolphi KA. 2005. Osteoarthritis. An untreatable disease. Nat Rev Drug Discov 4:331–344.
8. Abramson S. 2006. Drug delivery in degenerative joint disease: Where we are and where to go? Adv Drug Deliv Rev 58:125–127.
9. Tomlinson E. 1987. Theory and practice of site-specific drug delivery. Adv Drug Deliv Rev 1:87–198.
10. Gardner E. 1972. The structure and function of joints. 8 edition. Philadelphia: Lea and Febiger. pp. 32–50.
11. Agarwal SK, Brenner MB. 2006. Role of adhesion molecules in synovial inflammation. Curr Opin Rheumatol 18:268–276.
12. Pap T, M-Ladner U, Gay RE, Gay S. 2007. Fibroblast biology. Role of synovial fibroblasts in the pathogenesis of rheumatoid arthritis. Arthritis Res 2:361–367.
13. Stanczyk J, Ospelt C, Gay RE, Gay S. 2006. Synovial cell activation. Curr Opin Rheumatol 18:262–267.
14. Szekanecz Z, Szucs G, Szanto S, Koch AE. 2006. Chemokines in rheumatic diseases. Curr Drug Targets 7:91–102.
15. Evans CH, Gouze E, Gouze JN, Robbins PD, Ghivizzani SC. 2006. Gene therapeutic approaches-transfer in vivo. Adv Drug Deliv Rev 58:243–258.
16. Jessar RA. 1972. The study of synovial fluid. 8 edition. Philadelphia: Lea and Febiger. pp. 67–81.
17. Tercic D, Bozic B. 2001. The basis of the synovial fluid analysis. Clin Chem Lab Med 39:1221–1226.
18. Carney SL, Muir H. 1988. The structure and function of cartilage proteoglycans. Physiol Rev 68: 858–910.
19. Aigner T, Stove J. 2003. Collagens—Major component of the physiological cartilage matrix, major target of cartilage degeneration, major tool in cartilage repair. Adv Drug Deliv Rev 55:1569–1593.
20. Hardingham TE, Fosang AJ. 1992. Proteoglycans—Many forms and many functions. FASEB J 6:861–870.
21. Goldring MB. 2006. Update on the biology of the chondrocyte and new approaches to treating cartilage diseases. Best Pract Res Clin Rheumatol 20:1003–1025.
22. Vinatier C, Guicheux J, Daculsi G, Layrolle P, Weiss P. 2006. Cartilage and bone tissue engineering using hydrogels. Biomed Mater Eng 16:S107–S113.
23. Nesic D, Whiteside R, Brittberg M, Wendt D, Martin I, Mainil-Varlet P. 2006. Cartilage tissue engineering for degenerative joint disease. Adv Drug Deliv Rev 58:300–322.
24. Lee S-H, Shin H. 2007. Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. Adv Drug Deliv Rev 59: 339–359.
25. Brown AK, Christo PJ, Wu CL. 2004. Strategies for postoperative pain management. Best Pract Res Clin Anaesthesiol 18:703–714.
26. Reuben SS, Sklar J. 2000. Current concepts review. Pain management in patients who undergo outpatient arthroscopic surgery of the knee. J Bone Joint Surg 82-A:1754–1766.
27. Rasmussen S, Larsen AS, Thomsen ST, Kehlet H. 1998. Intra-articular glucocorticoid, bupivacaine and morphine reduces pain, inflammatory response and convalescence after arthroscopic meniscectomy. Pain 78:131–134.
28. Gupta A. 2003. Update on intra-articular analgesia. Techniq Reg Anesth Pain Man 7:155–160.
29. Rømsing J, Møiniche S, Østergaard D, Dahl JB. 2000. Local infiltration with NSAIDs for postoperative analgesia: Evidence for a peripheral analgesic action. Acta Anaesthesiol Scand 44: 672–683.
30. Møiniche S, Mikkelsen S, Wetterslev J, Dahl JB. 1999. A systematic review of intra-articular local anesthesia for postoperative pain relief after arthroscopic knee surgery. Reg Anesth Pain Med 24:430–437.
31. Kalso E, Smith L, McQuay HJ, Moore RA. 2002. No pain, no gain: Clinical exellence and scientific rigour—Lessons learned from IA morphine. Pain 98:269–275.
32. Gupta A, Bodin L, Holmström B, Berggren L. 2001. A systematic review of the peripheral

analgesic effects of intraarticular morphine. Anesth Analg 93:761–770.
33. Kehlet H, Dahl JB. 1993. The value of multimodal or balanced analgesia in postoperative pain treatment. Anesth Analg 77:1048–1056.
34. Kehlet H, Werner M, Perkins F. 1999. Balanced analgesia—What is it and what are its advantages in postoperative pain? Drugs 58:793–797.
35. Elhakim M, Nafie M, Eid A, Hassin M. 1999. Combination of intra-articular tenoxican, lidocaine and pethidine for outpatient knee arthroscopy. Acta Anaesthesiol Scand 43:803–808.
36. Talu GK, Özyalcm S, Koltka K, Ertürk E, Akmer Ö, Asik M, Pembeci K. 2002. Comparison of efficacy of intraarticular application of tenoxicam, bupivacaine and tenoxicam: Bupivacaine combination in arthroscopic knee surgery. Knee Surg Sports Traumatol Arthrosc 10:355–360.
37. Uysalei A, Kecik Y, Kirdemir P, Sayin M, Binnet M. 1995. Comparison of intraarticular bupivacaine with the addition of morphine or fentanyl for analgesia after arthroscopic surgery. Arthroscopy 11:660–663.
38. Reuben SS, Connelly NR. 1995. Postoperative analgesia for outpatient arthroscopic knee surgery with intraarticular bupivacaine and ketorolac. Anesth Analg 80:1154–1157.
39. Rasmussen S, Lorentzen JS, Larsen AS, Thomsen ST, Kehlet H. 2002. Combined intra-articular glucocorticoid, bupivacaine and morphine reduces pain and convalescence after diagnostic knee arthroscopy. Acta Orthop Scand 73:175–178.
40. Rasmussen S, Kehlet H. 2000. Intraarticular glucocorticoid, morphine and bupivacaine reduces pain and convalescence after arthroscopic ankle surgery: A randomized study of 36 patients. Acta Orthop Scand 71:301–304.
41. Kizilkaya M, Yildirim OS, Dogan N, Kursad H, Okur A. 2004. Analgesic effects of intraarticular sufentanil and sufentanil plus methylprednisolone after arthroscopic knee surgery. Anesth Analg 98: 1062–1065.
42. Buerkle H, Huge V, Wolfgart M, Steinbeck J, Mertes N, Van Aken H, Prien T. 2000. Intraarticular clonidine analgesia after knee arthroscopy. Eur J Anaesthesiol 17:295–299.
43. Reuben SS, Connelly NR. 1999. Postoperative analgesia for outpatient arthroscopic knee surgery with intraarticular clonidine. Anesth Analg 88: 729–733.
44. Sandell LJ, Aigner T. 2001. Articular cartilage and changes in arthritis. An introduction: Cell biology of osteoaerthritis. Arthritis Res 3:107–113.
45. McIlwraith CW. 2005. Use of synovial fluid and serum biomarkers in equine bone and joint disease: A review. Equine Vet J 27:473–482.
46. Burrage PS, Mix KS, Brincherhoff CE. 2006. Matrix metalloproteinases: Role in arthritis. Front Biosci 11:529–543.
47. Abramson SB, Attur M, Yazici Y. 2006. Prospects for disease modification in osteoarthritis. Nat Clin Pract Rheumatol 2:304–312.
48. Cooper NJ. 2000. Economic burden of rheumatoid arthrtis: A systematic review. Rheumatology (Oxford) 39:28–33.
49. Elders MJ. 2000. The increasing impact of arthritis on public health. J Rheumatol 27:6–8.
50. Reginster JY, Khaltaev NG. 2002. Introduction and WHO perspective on the global burden of musculoskeletal conditions. Rheumatology (Oxford) 41:1–2.
51. Choy EH, Panayi GS. 2001. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med 344:907–916.
52. Cannella AC, O'Dell JR. 2006. Early rheumatoid arthritis—Pitfalls in diagnosis and review of recent clinical trials. Drugs 66:1319–1337.
53. Steinmeyer J, Konttinen YT. 2006. Oral treatment options for degenerative joint disease-presence and future. Adv Drug Deliv Rev 58:168–211.
54. Haagsma CJ, Russel FG, Vree TB, Van Riel PL, Van de Putte LB. 1996. Combination of methotrexate and sulphasalazine in patients with rheumatoid arthritis: Pharmacokinetic analysis and relationship to clinical response. Br J Clin Pharmacol 42: 195–200.
55. Proudman SM, Conaghan PG, Richardson C, Griffiths B, Green MJ, McGonagle D, Wakefield RJ, Reece RJ, Miles S, Adebajo A, Gough A, Helliwell P, Martin M, Huston G, Pease C, Veale DJ, Isaacs J, van der Heijde DM, Emery P. 2000. Treatment of poor-prognosis early rheumatoid arthritis. A randomized study of treatment with methotrexate, cyclosporin A, and intraarticular corticosteroids compared with sulfasalazine alone. Arthritis Rheum 43:1809–1819.
56. Wilke WS, Mackenzie AH. 1986. Methotrexate therapy in rheumatoid arthritis current status. Drugs 32:103–113.
57. Mullan RH, Bresnihan B. 2003. Disease-modifying anti-rheumatic drug therapy and structural damage in early rheumatoid arthritis. Clin Exp Rheumatol 21:S158–S164.
58. Smith RJ. 2005. Therapies for rheumatoid arthritis: Hope springs eternal. Drug Discov Today 10: 1598–1606.
59. Gartlehner G, Hansen RA, Jonas BL, Thieda P, Lohr KN. 2006. The comparative efficacy and safety of biologics for the treatment of rheumatoid arthritis: A systematic review and metaanalysis. J Rheumatol 33:2398–2408.
60. Fleurence R, Spackman E. 2006. Cost-effectiveness of biologic agents for the treatment of auto-

immune disorders: Structured review of the literature. J Rheumatol 33:2124–2131.
61. Christodoulou C, Choy EH. 2006. Joint inflammation and cytokine inhibition in rheumatoid arthritis. Clin Exp Med 6:13–19.
62. Aigner T, Sachse A, Gebhard PM, Roach HI. 2006. Osteoarthritis: Pathobiology-targets and ways for therapeutic intervention. Adv Drug Deliv Rev 58: 128–149.
63. Pelletier JP, Martel-Pelletier J, Abramson SB. 2001. Osteoarthritis, an inflammatory disease—Potential implication for the selection of new therapeutic targets. Arthritis Rheum 44:1237–1247.
64. Watterson JR, Esdaile JM. 2000. Viscosupplementation: Therapeutic mechanisms and clinical potential in osteoarthritis of the knee. J Am Acad Orthop Surg 8:277–284.
65. Uthman I, Raynauld JP, Haraoui B. 2003. Intraarticular therapy in osteoarthritis. Postgrad Med J 79:449–453.
66. Gerwin N, Hops C, Lucke A. 2006. Intraarticular drug delivery in osteoarthritis. Adv Drug Deliv Rev 58:226–242.
67. Gossec L, Dougados M. 2006. Do intra-articular therapies work and who will benefit most? Best Pract Res Clin Rheumatol 20:131–144.
68. Curtis CL, Harwood JL, Dent CM, Caterson B. 2004. Biological basis for the benefit of nutraceutical supplementation in arthritis. Drug Discov Today 9:165–172.
69. Hogenmiller MS, Lozada CJ. 2006. An update on osteoarthritis therapeutics. Curr Opin Rheumatol 18:256–260.
70. Tremoulet AH, Albani S. 2006. Novel therapies for rheumatoid arthritis. Expert Opin Investig Drugs 15:1427–1441.
71. Goldring MB. 2001. Anticytokine therapy for osteoarthritis. Expert Opin Biol Ther 1:817–829.
72. Abramson SB, Yazici Y. 2006. Biologics in development for rheumatoid arthritis: Relevance to osteoarthritis. Adv Drug Deliv Rev 58:212–225.
73. Chevalier X, Giraudeau B, Conrozier T, Marliere J, Kiefer P, Goupille P. 2005. Safety study of intraarticular injection of interleukin 1 receptor antagonist in patients with painful knee osteoarthritis: A multicenter study. J Rheumatol 32: 1317–1323.
74. Cornelis S, Kersse K, Festjens N, Lamkanfi M, Vandenabeele P. 2007. Inflammatory caspases: Targets for novel therapies. Curr Pharm Des 13: 365–383.
75. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84.
76. Henrotin Y, Kurz B, Aigner T. 2005. Oxygen and reactive oxygen species in cartilage degradation: Friends or foes? Osteoarthr Cartil 13:643–654.
77. Vasiljeva O, Reinheckel T, Peters C, Turk D, Turk K, Turk B. 2007. Emerging roles of cysteine cathepsins in disease and their potential as drug targets. Curr Pharm Des 13:385–401.
78. Yasuda Y, Kaleta J, Bromme D. 2005. The role of cathepsins in osteoporosis and arthritis: Rationale for the design of new therapeutics. Adv Drug Deliv Rev 57:973–993.
79. Konttinen YT, Mandelin J, Li TF, Salo J, Lassus J, Liljestrom M, Hukkanen M, Takagi M, Virtanen I, Santavirta S. 2002. Acidic cysteine endoproteinase cathepsin K in the degeneration of the superficial articular hyaline cartilage in osteoarthritis. Arthritis Rheum 46:953–960.
80. Nakano S, Ikata T, Kinoshita I, Kanematsu J, Yasuoka S. 1999. Characteristics of the protease activity in synovial fluid from patients with rheumatoid arthritis and osteoarthritis. Clin Exp Rheumatol 17:161–170.
81. Mantle D, Falkous G, Walker D. 1999. Quantification of protease activities in synovial fluid from rheumatoid and osteoarthritis cases: Comparison with antioxidant and free radical damage markers. Clin Chim Acta 284:45–58.
82. Holland TA, Mikos AG. 2003. Advances in drug delivery for articular cartilage. J Control Release 86:1–14.
83. Schmidt MB, Chen EH, Lynch SE. 2006. A review of the effects of insulin-like growth factor and platelet derived growth factor on in vivo cartilage healing and repair. Osteoarthr Cartil 14:403–412.
84. Issack PS, DiCesare PE. 2003. Recent advances toward the clinical application of bone morphogenetic proteins in bone and cartilage repair. Am J Orthop 32:429–436.
85. Vanderslice P, Woodside DG. 2006. Integrin antagonists as therapeutics for inflammatory diseases. Expert Opin Investig Drugs 15:1235–1255.
86. Ludwig RJ, Alban S, Boehncke WH. 2006. Structural requirements of heparin and related molecules to exert a multitude of anti-inflammatory activities. Mini Rev Med Chem 6:1009–1023.
87. Paul AT, Gohil VM, Bhutani KK. 2006. Modulating TNF-alpha signaling with natural products. Drug Discov Today 11:725–732.
88. Reuning U, Magdolen V, Wilhelm O, Fischer K, Lutz V, Graeff H, Schmitt M. 1998. Multifunctional potential of the plasminogen activation system in tumor invasion and metastasis (review). Int J Oncol 13:893–906.
89. Curran S, Murray GI. 2000. Matrix metalloproteinases: Molecular aspects of their roles in tumour invasion and metastasis. Eur J Cancer 36:1621–1630.
90. Overall CM, Lopez-Otin C. 2002. Strategies for MMP inhibition in cancer: Innovation for the post-trial era. Nature Rev Cancer 2:657–672.

91. Thorpe PE. 2004. Vascular targeting agents as cancer therapeutics. Clin Cancer Res 10:415–427.
92. Mizejewski GJ. 1999. Role of integrins in cancer: Survey of expression patterns. Proc Soc Exp Biol Med 222:124–138.
93. de Groot V. 2007. Targeting: Cancer—Small molecules. In: Stella VJ, Borchardt RT, Hageman MJ, Oliyai R, Maag H, Tilley JW, editors. Prodrugs: Challenges and rewards. New York: Springer. pp. 447–506.
94. Levick JR. 1995. Microvascular architecture and exchange in synovial joints. Microcirculation 2: 217–233.
95. Levick JR, Smaje LH. 1987. An analysis of the permeability of a fenestra. Microvasc Res 33:233–256.
96. Day RO, McLachlan AJ, Graham GG, Williams KM. 1999. Pharmacokinetics of nonsteroidal anti-inflammatory drugs in synovial fluid. Clin Pharmacokinet 36:191–210.
97. Simkin PA, Pizzorno JE. 1979. Synovial permeability in rheumatoid arthitis. Arthritis Rheum 22:689–696.
98. Simkin PA. 2005. Synovial physiology. In: Koopman WJ, Moreland LW, editors. Arthiritis and allied conditions: A textbook of rheumatology. 15 edition. Philadelphia: Lippincott Williams and Wilkins. pp. 175–187.
99. Levick JR. 1981. Permeability of rheumatoid and normal human synovium to specific plasma proteins. Arthritis Rheum 24:1550–1560.
100. Wallis WJ, Simkin PA. 1983. Antirheumatic drug concentrations in human synovial fluid and synovial tissue. Observations on extravascular pharmacokinetics. Clin Pharmacokinet 8:496–522.
101. Netter P, Bannwarth B, Royer-Morrot M-J. 1989. Recent findings on the pharmacokinetics of nonsteroidal anti-inflammatory drug in the synovial fluid. Clin Pharmacokinet 17:145–162.
102. Simkin PA, Nilson KL. 1981. Trans-synovial exchange of large and small molecules. Clin Rheum Dis 7:99–129.
103. Owen SG, Francis HW, Roberts MS. 1994. Disappearance kinetics of solutes from synovial fluid after intra-articular injection. Br J Clin Pharmacol 38:349–355.
104. Wigginton SM, Chu BC, Weisman MH, Howell SB. 1980. Methotrexate pharmacokinetics after intraarticular injection in patients with rheumatoid arthritis. Arthritis Rheum 23:119–122.
105. Ohzawa N, Takahashi Y, Ogihara T, Nakai Y, Ishiguro J. 1997. Metabolic fate of ulinastatin (2); pharmacokinetics in rabbits following intra-articular administration. Biol Pharm Bull 20:732–738.
106. Phelps P, Steele AD, McCarthy DJ. 1972. Significance of xenon-133 clearance rate from canine and human joints. Arthritis Rheum 15:360–370.
107. St Onge RA, Dick WC, Bell G, Boyle JA. 1968. Radioactive xenong (^{133}Xe) disappearance rates from the synovial cavity of the human knee joint in normal and arthritic subjects. Ann Rheum Dis 27:163–166.
108. Mills ML, Rush BR, St Jean G, Gaughan EM, Mosier D, Gibson E, Freeman L. 2000. Determination of synovial fluid and serum concentrations, and morphologic effects of intraarticular ceftiofur sodium in horses. Vet Surg 29:398–406.
109. Winter JA, Sandberg AA, Saroff J, Slaunwhite WR. 1967. Disposition and metabolism of intra-articularly injected 4-C^{14}-cortisol in rheumatoid arthritis. Arthritis Rheum 10:352–356.
110. Harris R, Millard JB. 1956. Clearance of radioactive sodium from the knee joint. Clin Sci 15:9–15.
111. Poli A, Scott D, Bertin K, Miserocchi G, Mason RM, Levick JR. 2001. Influence of actin cytoskeleton on intra-articular and interstitial fluid pressures in synovial joints. Microvasc Res 62: 293–305.
112. Simkin PA, Pizzorno JE. 1974. Transsynovial exchange of small molecules in normal human subjects. J Appl Physiol 36:581–587.
113. Simkin PA, Wu MP, Foster DM. 1993. Articular pharmacokinetics of protein-bound antirheumatic agents. Clin Pharmacokinet 25:342–350.
114. Brown DL, Cooper AG, Bluestone R. 1969. Exchange of IgM and albumin between plasma and synovial fluid in rheumatoid arthritis. Ann Rheum Dis 29:644–651.
115. Wallis WJ, Simkin PA, Nelp WB. 1987. Protein traffic in human synovial effusions. Arthritis Rheum 30:57–63.
116. Weinberger A, Simkin PA. 1989. Plasma proteins in synovial fluids of normal human joints. Semin Arthritis Rheum 19:66–76.
117. Simkin PA, Bassett JE, Koh E-M. 1995. Synovial perfusion in the human knee: A methodologic analysis. Semin Arthritis Rheum 25:56–66.
118. Bauer W, Short CL, Bennett GA. 1933. The manner of removal of proteins from normal joints. J Exp Med 57:419–433.
119. Simkin PA, Benedict RS. 1990. Iodide and albumin kinetics in normal canine wrists and knees. Arthritis Rheum 33:73–79.
120. Rodnan GP, MacLachlan MJ. 1960. The absorption of serum albumin and gamma globulin from the joint of man and rabbit. Arthritis Rheum 3:152–157.
121. van Lent PL, van den BL, Grutters GJ, van den Berg WB. 1989. Fate of antigen after intravenous and intraarticular injection into mice. Role of molecular weight and charge. J Rheumatol 16: 1295–1303.
122. Page-Thomas DP, Bard D, King B, Dingle JT. 1987. Clearance of proteoglycan from joint cavities. Ann Rheum Dis 46:934–937.

123. Coleman PJ, Scott D, Ray J, Mason RM, Levick JR. 1997. Hyaluronan secretion into the synovial cavity of rabbit knees and comparison with albumin turnover. J Physiol 503:645–656.
124. Brown TJ, Laurent UBG, Fraser JFE. 1991. Turnover of hyaluronan in synovial joints: Elimination of labelled hyaluronan from the knee joint of the rabbit. Exp Physiol 76:125–134.
125. Maroudas A, Bullough P, Swanson SAV, Freeman MAR. 1968. The permeability of articular cartilage. J Bone Joint Surg Br 50B:166–177.
126. Maroudas A. 1970. Distribution and diffusion of solutes in articular cartilage. Biophys J 10:365–379.
127. Gonsalves M, Barker AL, Macpherson JV, Unwin PR, O'Hare D, Winlove CP. 2000. Scanning electrochemical microscopy as a local probe of oxygen permeability in cartilage. Biophys J 78:1578–1588.
128. Gonsalves M, Macpherson JV, O'Hare D, Winlove CP, Unwin PR. 2000. High resolution imaging of the distribution and permeability of methyl viologen dication in bovine articular cartilage using scanning electrochemical microscopy. Biochim Biophys Acta 1524:66–74.
129. Leddy HA, Guilak F. 2003. Site-specific molecular diffusion in articular cartilage measured using fluorescence recovery after photobleaching. Ann Biomed Eng 31:753–760.
130. Stolz M, Raiteri R, Daniels AU, VanLandingham MR, Baschong W, Aebi U. 2004. Dynamic elastic modulus of porcine articular cartilage determined at two different levels of tissue organization by indentation-type atomic force microscopy. Biophys J 86:3269–3283.
131. Evans RC, Quinn TM. 2006. Dynamic compression augments interstitial transport of a glucose-like solute in articular cartilage. Biophys J 91:1541–1547.
132. Evans RC, Quinn TM. 2006. Solute convection in dynamically compressed cartilage. J Biomech 39: 1048–1055.
133. Quinn TM, Morel V, Meister JJ. 2001. Static compression of articular cartilage can reduce solute diffusivity and partitioning: Implications for the chondrocyte biological response. J Biomech 34:1463–1469.
134. Quinn TM, Kocian P, Meister JJ. 2000. Static compression is associated with decreased diffusivity of dextrans in cartilage explants. Arch Biochem Biophys 384:327–334.
135. Maroudas A. 1968. Physicochemical properties of cartilage in light of ion exchange theory. Biophys J 8:575–595.
136. Nimer E, Schneiderman R, Maroudas A. 2003. Diffusion and partition of solutes in cartilage under static load. Biophys Chem 106:125–146.
137. Maroudas A. 1980. Physical chemistry of articular cartilage and the intervertebral disc. In: Sokoloff L, editor. The joints and synovial fluid. New York: Academic Press. pp. 239–291.
138. Olsen GD, Chan EM, Riker WK. 1975. Binding of D-tubocurarine di[methyl-C-14] ether iodide and other amines to cartilage, chondroitin sulfate and human plasma-proteins. J Pharmacol Exp Ther 195:242–250.
139. Maurizis JC, Ollier M, Nicolas C, Madelmont JC, Garrigue H, Veyre A. 1992. In vitro binding of oxime acetylcholinesterase reactivators to proteoglycans synthesized by cultured chondrocytes and fibroblasts. Biochem Pharmacol 44:1927–1933.
140. Bannwarth B, Netter P, Lapicque F, Mainard D, Fener P, Gillet P, Gaucher A. 1991. Tenoxicam concentrations in synovium and joint cartilage in humans. Agents Actions 32:295–298.
141. Giraud I, Rapp M, Maurizis JC, Madelmont JC. 2002. Synthesis and in vitro evaluation of quaternary ammonium derivatives of chlorambucil and melphalan, anticancer drugs designed for the chemotherapy of chondrosarcoma. J Med Chem 45:2116–2119.
142. Giraud I, Rapp M, Maurizis JC, Madelmont JC. 2000. Application to a cartilage targeting strategy: Synthesis and in vivo biodistribution of C-14-labeled quaternary ammonium-glucosamine conjugates. Bioconjug Chem 11:212–218.
143. Maurizis JC, Rapp M, Nicolas C, Ollier M, Verny M, Madelmont JC. 2000. Disposition in rats of N-pyridinium-propyl-cyclam, N-triethylammonium-propyl-cyclam, and N-[Triethylammonium]-3-propyl-[15]ane-N5, potential cartilage imaging agents. Drug Metab Dispos 28:418–422.
144. Nicolas C, Verny M, Giraud I, Ollier M, Rapp M, Maurizis JC, Madelmont JC. 1999. New quaternary ammonium oxicam derivatives targeted toward cartilage: Synthesis, pharmacokinetic studies, and antiinflammatory potency. J Med Chem 42:5235–5240.
145. Rapp M, Giraud I, Maurizis JC, Madelmont JC. 2003. Synthesis and pharmacokinetic profile of a quaternary ammonium derivative of chlorambucil, a potential anticancer drug for the chemotherapy of chondrosarcoma. Bioorg Med Chem 11:5007–5012.
146. Rapp M, Giraud I, Maurizis JC, Galmier MJ, Madelmont JC. 2003. Synthesis and in vivo biodisposition of [C-14]-quaternary ammonium-melphalan conjugate, a potential cartilage-targeted alkylating drug. Bioconjug Chem 14:500–506.
147. Vidal A, Sabatini M, Rolland-Valognes G, Renard P, Madelmont JC, Mounetou E. 2007. Synthesis and in vitro evaluation of targeted tetracycline derivatives: Effects on inhibition of matrix metalloproteinases. Bioorg Med Chem 15:2368–2374.

148. Rowland M, Tozer TN. 1989. Clinical pharmacokinetics. Concepts and applications. 2nd edition. Philadelphia-London: Lea and Febiger.
149. Harris R, Millard JB, Banerjee SK. 1958. Radiosodium clearance from the knee joint in rheumatoid arthritis. Ann Rheum Dis 17:189–195.
150. Scholer JF, Lee PR, Polley HF. 1959. The absorption of heavy water and radioactive sodium from the knee joint of normal persons and patients with rheumatoid arthritis. Arthritis Rheum 2:426–432.
151. Peterson RE, Black RL, Bunim JJ. 1959. Disposition of intra-articularly injected cortisone and hydrocortisone. Arthritis Rheum 2:433–439.
152. Moritz F, Distler O, Ospelt C, Gay RE, Gay S. 2006. Technology insight: Gene transfer and the design of novel treatments for rheumatoid arthritis. Nat Clin Pract Rheumatol 2:153–162.
153. Evans CH, Ghivizzani SC, Robbins PD. 2006. Gene therapy for arthritis—What next? Arthritis Rheum 54:1714–1729.
154. Keller M. 2005. Lipidic carriers of RNA/DNA oligonucleotides and polynucleotides: What a difference a formulation makes. J Control Release 103:537–540.
155. Evans CH, Gouze JN, Gouze E, Robbins PD, Ghivizzani SC. 2004. Osteoarthritis gene therapy. Gene Ther 11:379–389.
156. Gelse K, Schneider H. 2006. Ex vivo gene therapy approaches to cartilage repair. Adv Drug Deliv Rev 58:259–284.
157. Matheson AJ, Figgitt DP. 2001. Rofecoxib—A review of its use in the management of osteoarthritis, acute pain and rheumatoid arthritis. Drugs 61:833–865.
158. van de Loo F, de Hooge A, Smeets RL, Bakker AC, Bennink AC, Arntz OJ, Joosten LAB, van Beuningen HM, van der Kraan PK, Varley AW, van den Berg WB. 2004. An inflammation-inducible adenoviral expression system for local treatment of the arthritic joint. Gene Ther 11:581–590.
159. Schatteman L, Gyselbrecht L, De Clercq L, Mielants H. 2006. Treatment of refractory inflammatory monoarthritis in ankylosing spondylitis by intraarticular injection of infliximab. J Rheumatol 33:82–85.
160. Conti F, Priori R, Chimenti MS, Coari G, Annovazzi A, Valesini G, Signore A. 2005. Successful treatment with intraarticular infliximab for resistant knee monarthritis in a patient with spondylarthropathy—A role for scintigraphy with Tc-99m-infliximab. Arthritis Rheum 52:1224–1226.
161. Veale DJ, Reece RJ, Parsons W, Radjenovic A, O'Connor PJ, Orgles CS, Berry E, Ridgway JP, Mason U, Boylston AW, Gibbon W, Emery P. 1999. Intra-articular primatised anti-C D4: Efficacy in resistant rheumatoid knees. A study of combined arthroscopy, magnetic resonance imaging, and histology. Ann Rheum Dis 58:342–349.
162. Holland T, Jones R, Basford A, Child T, Smith A. 2000. An intra-articular method for assessing topical application to a synovial joint. Lab Anim 34: 298–300.
163. Wind WM, Smolinski RJ. 2004. Reliability of common knee injection sites with low-volume injections. J Arthroplasty 19:858–861.
164. Ingpen ML. 1995. Joint injections: Intra-articular and periarticular techniques. Modern Med 38: 20–27.
165. Shi Y, Li LC. 2005. Current advances in sustained-release systems for parenteral drug delivery. Expert Opin Drug Deliv 2:1039–1058.
166. Torchilin VP. 2005. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov 4:145–160.
167. Metselaar JM, Storm G. 2005. Liposomes in the treatment of inflammatory disorders. Expert Opin Drug Deliv 2:465–476.
168. Moghimi SM, Hamad I, Bunger R, Andresen TL, Jorgensen K, Hunter AC, Baranji L, Rosivall L, Szebeni J. 2006. Activation of the human complement system by cholesterol-rich and pegylated liposomes—Modulation of cholesterol-rich liposome-mediated complement activation by elevated serum LDL and HDL levels. J Liposome Res 16: 167–174.
169. Moghimi SM, Hamad I, Andresen TL, Jorgensen K, Szebeni J. 2006. Methylation of the phosphate oxygen moiety of phospholipid-methoxy(polyethylene glycol) conjugate prevents PEGylated liposome-mediated complement activation and anaphylatoxin production. FASEB J 20:2591–2593.
170. Sharma A, Sharma US. 1997. Liposomes in drug delivery: Progress and limitations. Int J Pharm 154:123–140.
171. Phillips NC, Thomas DP, Knight CG, Dingle JT. 1979. Liposome-incorporated corticosteroids. II. Therapeutic activity in experimental arthritis. Ann Rheum Dis 38:553–557.
172. Lopez-Garcia F, Vazquez-Auton JM, Gil F, Latoore R, Moreno F, Villalain J, Gomez-Fernandez JC. 1993. Intra-articular therapy of experimental arthritis with a derivative of triamcinolone acetonide incorporated in liposomes. J Pharm Pharmacol 45:576–578.
173. Dingle JT, Gordon JL, Hazleman BL, Knight CG, Page Thomas DP, Phillips NC, Shaw IH, Fildes FJ, Oliver JE, Jones G, Turner EH, Lowe JS. 1978. Novel treatment for joint inflammation. Nature 271:372–373.
174. de Silva M, Hazleman BL, Thomas DP, Wraight P. 1979. Liposomes in arthritis: A new approach. Lancet 1:1320–1322.

175. Bonanomi MH, Velvart M, Weder HG. 1987. Fate of different kinds of liposomes containing dexamethasone palmitate after intra-articular injection into rabbit joints. J Microencapsul 4:189–200.
176. Bonanomi MH, Velvart M, Stimpel M, Roos KM, Fehr K, Weder HG. 1987. Studies of pharmacokinetics and therapeutic effects of glucocorticoids entrapped in liposomes after intraarticular application in healthy rabbits and in rabbits with antigen-induced arthritis. Rheumatol Int 7:203–212.
177. Shaw IH, Knight CG, Dingle JT. 1976. Liposomal retention of a modified anti-inflammatory steroid. Biochem J 158:473–476.
178. Turker S, Erdogan S, Ozer AY, Ergun EL, Tuncel M, Bilgili H, Deveci S. 2005. Scintigraphic imaging of radiolabelled drug delivery systems in rabbits with arthritis. Int J Pharm 296:34–43.
179. Hou SM, Yu HY. 1997. Comparison of systemic absorption of aqueous and liposomal lidocaine following intra-articular injection in rabbits. J Formos Med Assoc 96:141–143.
180. Foong WC, Green KL. 1988. Retention and distribution of liposome-entrapped [3H]methotrexate injected into normal or arthritic rabbit joints. J Pharm Pharmacol 40:464–468.
181. Mantripragada S. 2002. A lipid based depot (DepoFoam((R)) technology) for sustained release drug delivery. Prog Lipid Res 41:392–406.
182. Sinha VR, Trehan A. 2005. Biodegradable microspheres for parenteral delivery. CRC Crit Rev Ther Drug Carrier Syst 22:535–602.
183. Jiang WL, Gupta RK, Deshpande MC, Schwendeman SP. 2005. Biodegradable poly(lactic-co-glycolic acid) microparticles for injectable delivery of vaccine antigens. Adv Drug Deliv Rev 57:391–410.
184. Freitas S, Merkle HP, Gander B. 2005. Microencapsulation by solvent extraction/evaporation: Reviewing the state of the art of microsphere preparation process technology. J Control Release 102:313–332.
185. Jain JP, Modi S, Domb AJ, Kumar N. 2005. Role of polyanhydrides as localized drug carriers. J Control Release 103:541–563.
186. Kumar N, Langer RS, Domb AJ. 2002. Polyanhydrides: An overview. Adv Drug Deliv Rev 54:889–910.
187. Heller J, Barr J, Ng SY, Abdellauoi KS, Gurny R. 2002. Poly(ortho esters): Synthesis, characterization, properties and uses. Adv Drug Deliv Rev 54:1015–1039.
188. Sinha VR, Bansal K, Kaushik R, Kumria R, Trehan A. 2004. Poly-epsilon-caprolactone microspheres and nanospheres: An overview. Int J Pharm 278:1–23.
189. Patil GV. 2003. Biopolymer albumin for diagnosis and in drug delivery. Drug Dev Res 58:219–247.
190. Kumar MNVR, Muzzarelli RAA, Muzzarelli C, Sashiwa H, Domb AJ. 2004. Chitosan chemistry and pharmaceutical perspectives. Chem Rev 104: 6017–6084.
191. Sung KC, Han RY, Hu OYP, Hsu LR. 1998. Controlled release of nalbuphine prodrugs from biodegradable polymeric matrices: Influence of prodrug hydrophilicity and polymer composition. Int J Pharm 172:17–25.
192. Liu C, Desai KGH, Tang X, Chen X. 2006. Drug release kinetics of spray-dried chitosan microspheres. Drying Technol 24:769–776.
193. von Burkersroda F, Schedl L, Gopferich A. 2002. Why degradable polymers undergo surface erosion or bulk erosion. Biomaterials 23:4221–4231.
194. Schliecker G, Schmidt C, Fuchs S, Wombacher R, Kissel T. 2003. Hydrolytic degradation of poly (lactide-co-glycolide) films: Effect of oligomers on degradation rate and crystallinity. Int J Pharm 266:39–49.
195. Monkkonen J, Liukkonen J, Taskinen M, Heath TD, Urtti A. 1995. Studies on liposome formulations for intraarticular delivery of clodronate. J Control Release 35:145–154.
196. Brown KE, Leong K, Huang CH, Dalal R, Green GD, Haimes HB, Jimenez PA, Bathon J. 1998. Gelatin/chondroitin 6-sulfate microspheres for the delivery of therapeutic proteins to the joint. Arthritis Rheum 41:2185–2195.
197. Berger RG, Yount WJ. 1990. Immediate "steroid flare" from intraarticular triamcinolone hexacetonide injection: Case report and review of the literature. Arthritis Rheum 33:1284–1286.
198. Kahn CB, Hollander JL, Schumacher HR. 1970. Corticosteroid crystals in synovial fluid. JAMA 211:807–809.
199. Akers MJ, Fites AL, Robinson RL. 1987. Formulation design and development of parenteral suspensions. J Parenter Sci Technol 41:88–96.
200. Hiestand EN. 1964. Theory of coarse suspension formulation. J Pharm Sci 53:1–18.
201. Betre H, Liu W, Zalutsky MR, Chilkoti A, Kraus VB, Setton LA. 2006. A thermally responsive biopolymer for intra-articular drug delivery. J Control Release 115:175–182.
202. Zentner GM, Rathi R, Shih C, Mcrea JC, Seo MH, Oh H, Rhee BG, Mestecky J, Moldoveanu Z, Morgan M, Weitman S. 2001. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. J Control Release 72: 203–215.
203. Sartor O. 2003. Eligard: Leuprolide acetate in a novel sustained-release delivery system. Urology 61:25–31.
204. Hatefi A, Amsden B. 2002. Biodegradable injectable in situ forming drug delivery systems. J Control Release 80:9–28.

205. Matschke C, Isele U, van Hoogevest P, Fahr A. 2002. Sustained-release injectables formed in situ and their potential use for veterinary products. J Control Release 85:1–15.
206. Packhaeuser CB, Schnieders J, Oster CG, Kissel T. 2004. In situ forming parenteral drug delivery systems: An overview. Eur J Pharm Biopharm 58:445–455.
207. Kopacz DJ, Lacouture PG, Wu D, Nandy P, Swanton R, Landau C. 2003. The dose response and effects of dexamethasone on bupivacaine microcapsules for intercostal blockade (T9 to T11) in healthy volunteers. Anesth Analg 96: 576–582.
208. Holte K, Werner MU, Lacouture PG, Kehlet H. 2002. Dexamethasone prolongs local analgesia after subcutaneous infiltration of bupivacaine microcapsules in human volunteers. Anesthesiology 96:1331–1335.
209. Heller J, Barr J, Ng S, Shen H-R, Gurny R, Schwach-Abdelaoui K, Rothen-Weinhold A, van de Weert M. 2002. Development of poly(ortho esters) and their application for bovine serum albumin and bupivacaine delivery. J Control Release 78:133–141.
210. Garry MG, Jackson DL, Geier HE, Southam M, Hargreaves KM. 1999. Evaluation of the efficacy of a bioerodible bupivacaine polymer system on antinociception and infammatory mediator release. Pain 82:49–55.
211. Dräger C, Benziger D, Gao F, Berde CB. 1998. Prolonged intercostal nerve blockade in sheep using controlled-release of bupivacaine and dexamethasone from polymer microspheres. Anesthesiology 89:969–979.
212. Castillo J, Curley J, Hotz J, Uezono M, Tigner J, Chasin M, Wilder R, Langer R, Berde C. 1996. Glucocorticoids prolong rat sciatic nerve blockade in vivo from bupivacaine microspheres. Anesthesiology 85:1157–1166.
213. Curley J, Castillo J, Hotz J, Uezono M, Hernandez S, Lim JO, Tigner J, Chasin M, Langer R, Berde C. 1996. Prolonged regional nerve blockade—Injectable biodegradable bupivacaine/polyester microspheres. Anesthesiology 84:1401–1410.
214. Le Corre P, Estèbe JP, Chevanne F, Mallédant Y, Le Verge R. 1995. Spinal controlled delivery of bupivacaine from DL-lactic acid oligomer microspheres. J Pharm Sci 84:75–78.
215. Le Corre P, Le Guevello P, Chevanne F, Le Verge R. 1994. Preparation and characterization of bupivacaine-loaded polylactide and polylactide-co-glycolide microspheres. Int J Pharm 107:41–49.
216. Masters DB, Berde CB, Dutta S, Turek T, Langer R. 1993. Sustained local anesthetic release from bioerodible polymer matrices: A potential method for prolonged regional anesthesia. Pharm Res 10:1527–1532.
217. Yu HY, Sun P, Hou WY. 1998. Prolonged local anesthetic effect of bupivacaine liposomes in rats. Int J Pharm 176:133–136.
218. Grant GJ, Lax J, Susser L, Zakowski M, Weissman TE, Turndorf H. 1997. Wound infiltration with liposomal bupivacaine prolongs analgesia in rats. Acta Anaesthesiol Scand 41:204–207.
219. Mowat JJ, Mok MJ, McLeod BA, Madden TD. 1996. Liposomal bupivacaine—Extended duration nerve blockade using large unilamellar vesicles that exhibit a proton gradient. Anesthesiology 85:635–643.
220. Grant GJ, Vermeulen K, Langerman L, Zarkowski M, Turndorf H. 1994. Prolonged analgesia with liposomal bupivacaine in a mouse model. Reg Anesth 19:264–269.
221. Mashimo T, Uchida I, Pak M, Shibata A, Nishimura S, Inagaki Y, Yoshiya I. 1992. Prolongation of canine epidural anesthesia by liposome encapsulation of lidocaine. Anesth Analg 74:827–834.
222. Grant GJ, Barenholz Y, Piskoun B, Bansinath M, Turndorf H, Bolotin EM. 2001. DRV liposomal bupivacaine: Preparation, characterization, and in vivo evaluation in mice. Pharm Res 18:336–343.
223. Grant GJ, Barenholz Y, Bolotin EM, Bansinath M, Turndoft H, Piskoun B, Davidson EM. 2004. A novel liposomal bupivacaine formulation to produce ultralong-acting analgesia. Anesthesiology 101:133–137.
224. Langerman L, Grant GJ, Zakowski M, Golomb E, Ramanathan S, Turndorf H. 1992. Prolongation of epidural anesthesia using a lipid drug carrier with procaine, lidocaine, and tetracaine. Anesth Analg 75:900–905.
225. Langerman L, Grant GJ, Zakowski M, Ramanathan S, Turndorf H. 1992. Prolongation of spinal anesthesia—Differential action of a lipid drug carrier on tetracaine, lidocaine, and procaine. Anesthesiology 77:475–481.
226. Langerman L, Golomb E, Benita S. 1991. Spinal anesthesia: Significant prolongation of the pharmacologic effect of tetracaine with lipid solution of the agent. Anesthesiology 74:105–107.
227. Soderberg L, Dyhre H, Roth B, Bjorkman S. 2006. Ultralong peripheral nerve block by lidocaine: Prilocaine 1:1 mixture in a lipid depot formulation—Comparison of in vitro, in vivo, and effect kinetics. Anesthesiology 104:110–121.
228. Shikanov A, Domb AJ, Weiniger CF. 2007. Long acting local anesthetic-polymer formulation to prolong the effect of analgesia. J Control Release 117:97–103.
229. Kohane DS, Lipp M, Kinney RC, Lotan N, Langer R. 2000. Sciatic nerve blockade with lipid-protein-sugar particles containing bupivacaine. Pharm Res 17:1243–1249.
230. Paavola A, Yliruusi J, Kajimoto Y, Kalso E, Wahlström T, Rosenberg P. 1995. Controlled release of

lidocaine from injectable gels and efficacy in rat sciatic nerve block. Pharm Res 12:1997–2002.
231. Dollo G, Le Corre P, Chevanne F, Le Verge R. 2004. Bupivacaine containing dry emulsion can prolong epidural anesthetic effects in rabbits. Eur J Pharm Sci 22:63–70.
232. Masters DB, Domb AJ. 1998. Liposphere local anesthetic timed-release for perineural site application. Pharm Res 15:1038–1045.
233. Hetland ML, Stengaard-Pedersen K, Junker P, Lottenburger T, Ellingsen T, Andersen LS, Hansen I, Skjodt H, Pedersen JK, Lauridsen UB, Svendsen A, Tarp U, Podenphant J, Hansen G, Lindegaard H, de Carvalho A, Østergaard M, Horslev-Petersen K. 2006. Combination treatment with methotrexate, cyclosporine, and intraarticular betamethasone compared with methotrexate and intraarticular betamethasone in early active rheumatoid arthritis—An investigator-initiated, multicenter, randomized, double-blind, parallel-group, placebo-controlled study. Arthritis Rheum 54:1401–1409.
234. Melby JC, St. Cyr M. 1961. Comparative studies on absorption and metabolic disposal of water-soluble corticosteroid esters. Metab Clin Exp 10: 75–82.
235. Mollmann H, Rohdewald P, Schmidt EW, Salomon V, Derendorf H. 1985. Pharmacokinetics of triamcinolone acetonide and its phosphate ester. Eur J Clin Pharmacol 29:85–89.
236. Hare LE, Yeh KC, Ditzler CA, Mcmahon FG, Duggan DE. 1975. Bioavailability of dexamethasone. 2. Dexamethasone phosphate. Clin Pharmacol Ther 18:330–337.
237. Derendorf H, Mollmann H, Gruner A, Haack D, Gyselby G. 1986. Pharmacokinetics and pharmacodynamics of glucocorticoid suspensions after intra-articular administration. Clin Pharmacol Ther 39:313–317.
238. Randell TT, Ostman PLG, Flanagan DR, Perng CY, Hrdy J. 1994. Prolonged analgesia after epidural injection of a poorly soluble salt of fentanyl. Anesth Analg 79:905–910.
239. Gido C, Langguth P, Mutschler E. 1994. Predictions of in-vivo plasma-concentrations from in-vitro release kinetics—Application to doxepin parenteral (Im) suspensions in lipophilic vehicles in dogs. Pharm Res 11:800–808.
240. Nakano NI, Kawahara N, Amiya T, Gotoh Y, Furukawa K. 1978. 3-Hydroxy-2-naphthoates of lidocaine, mepivacaine, and bupivacaine and their dissolution characteristics. Chem Pharm Bull 26: 936–941.
241. Østergaard J, Larsen SW, Parshad H, Larsen C. 2005. Bupivacaine salts of diflunisal and other aromatic hydroxycarboxylic acids: Aqueous solubility and release characteristics from solutions and suspensions using a rotating dialysis cell model. Eur J Pharm Sci 26:280–287.
242. Larsen SW, Østergaard J, Poulsen SV, Schulz B, Larsen C. 2007. Diflunisal salts of bupivacaine, lidocaine and morphine Use of the common ion effect for prolonging the release of bupivacaine from mixed salt suspensions in an in vitro dialysis model. Eur J Pharm Sci 31:172–179.
243. Zuidema J, Kadir F, Titulaer HAC, Oussoren C. 1994. Release and absorption rates of intramuscularly and subcutaneously injected pharmaceuticals (Ii). Int J Pharm 105:189–207.
244. Chien YM. 1981. Long-acting parenteral drug formulations. J Parenter Sci Technol 35:106–139.
245. Benson HAE, Prankerd RJ. 1998. Optimisation of drug delivery 6. Modified-release parenterals. Aust J Hosp Pharm 28:99–104.
246. Larsen DB, Parshad H, Fredholt K, Larsen C. 2002. Characteristics of drug substances in oily solutions. Drug release rate, partitioning and solubility. Int J Pharm 232:107–117.
247. Larsen SW, Østergaard J, Friberg-Johansen H, Jessen MN, Larsen C. 2006. In vitro assessment of drug release rates from oil depot formulations intended for intra-articular administration. Eur J Pharm Sci 29:348–354.
248. Jorgensen A, Christrup LL, Fullerton A, Christensen CB, Bundgaard H. 1994. Prolonged release from the injection site of morphine from morphine esters in an oil vehicle given by intramuscular injection to pigs. Pharmacol Toxicol 75:319–320.
249. Larsen DB, Fredholt K, Larsen C. 2001. Addition of hydrogen bond donating excipients to oil solution: Effect on in vitro drug release rate and viscosity. Eur J Pharm Sci 13:403–410.
250. Garrood T, Pitzalis C. 2006. Targeting the inflamed synovium: The quest for specificity. Arthritis Rheum 54:1055–1060.
251. Richards PJ, Williams AS, Goodfellow RM, Williams BD. 1999. Liposomal clodronate eliminates synovial macrophages, reduces inflammation and ameliorates joint destruction in antigen-induced arthritis. Rheumatology (Oxford) 38:818–825.
252. Badger AM, Blake S, Kapadia R, Sarkar S, Levin J, Swift BA, Hoffman SJ, Stroup GB, Miller WH, Gowen M, Lark MW. 2001. Disease-modifying activity of SB 273005,an orally active, nonpeptide alpha(v)beta(3) (vitronectin receptor) antagonist, in rat adjuvant-induced arthritis. Arthritis Rheum 44:128–137.
253. Gerlag DM, Borges E, Tak PP, Ellerby HM, Bredesen DE, Pasqualini R, Ruoslahti E, Firestein GS. 2001. Suppression of murine collagen-induced arthritis by targeted apoptosis of synovial neovasculature. Arthritis Res 3:357–361.
254. Koning GA, Schiffelers RM, Wauben MHM, Kok RJ, Mastrobattista E, Molema G, ten Hagen TLM, Storm G. 2006. Targeting of angiogenic

endothelial cells at sites of inflammation by dexamethasone phosphate-containing RGD peptide liposomes inhibits experimental arthritis. Arthritis Rheum 54:1198–1208.
255. Pasqualini R, Koivunen E, Kain R, Lahdenranta J, Sakamoto M, Stryhn A, Ashmun RA, Shapiro LH, Arap W, Ruoslahti E. 2000. Aminopeptidase N is a receptor for tumor-homing peptides and a target for inhibiting angiogenesis. Cancer Res 60:722–727.
256. Mi ZB, Lu XL, Mai JC, Bobby GN, Wang GQ, Lechman ER, Watkins SC, Rabinowich H, Robbins PD. 2003. Identification of a synovial fibroblast-specific protein transduction domain for delivery of apoptotic agents to hyperplastic synovium. Mol Ther 8:295–305.
257. Suzuki YS, Momose Y, Higashi N, Shigematsu A, Park KB, Kim YM, Kim JR, Ryu JR. 1998. Biodistribution and kinetics of holmium-166-chitosan complex (DW-166HC) in rats and mice. J Nucl Med 39:2161–2166.
258. Burgess DJ, Hussain AS, Ingallinera TS, Chen ML. 2002. Assuring quality and performance of sustained and controlled release parenterals: Workshop report. AAPS Pharmsci 4:E7.
259. Burgess DJ, Crommelin DJ, Hussain AS, Chen ML. 2004. Assuring quality and performance of sustained and controlled release parenterals: EUFEPS workshop report. AAPS Pharmsci 6: E11.
260. Burgess DJ, Crommelin DJ, Hussain AS, Chen ML. 2004. Assuring quality and performance of sustained and controlled released parenterals. Eur J Pharm Sci 21:679–690.
261. D'Souza SS, Deluca PP. 2006. Methods to assess in vitro drug release from injectable polymeric particulate systems. Pharm Res 23:460–474.
262. Washington C. 1990. Drug release from microdisperse systems—A critical-review. Int J Pharm 58:1–12.
263. Washington C. 1989. Evaluation of non-sink dialysis methods for the measurement of drug release from colloids—Effects of drug partition. Int J Pharm 56:71–74.
264. D'Souza SS, Deluca PP. 2005. Development of a dialysis in vitro release method for biodegradabl microspheres. AAPS Pharmscitech 6:E323–E328.
265. D'Souza SS, Faraj JA, Deluca PP. 2005. A model-dependent approach to correlate accelerated with real-time release from biodegradable microspheres. AAPS Pharmscitech 6:E553–E564.
266. Gabriels M, Plaizier-Vercammen J. 2003. Physical and chemical evaluation of liposomes, containing artesunate. J Pharm Biomed Anal 31:655–667.
267. Henriksen I, Sande SA, Smistad G, Agren T, Karlsen J. 1995. In-vitro evaluation of drug-release kinetics from liposomes by fractional dialysis. Int J Pharm 119:231–238.
268. Saarinen-Savolainen P, Jarvinen T, Taipale H, Urtti A. 1997. Method for evaluating drug release from liposomes in sink conditions. Int J Pharm 159:27–33.
269. Leo E, Cameroni R, Forni F. 1999. Dynamic dialysis for the drug release evaluation from doxorubicin-gelatin nanoparticle conjugates. Int J Pharm 180:23–30.
270. Chidambaram N, Burgess DJ. 1999. A novel in vitro release method for submicron sized dispersed systems. AAPS Pharmsci 1:E11.
271. Levy MY, Benita S. 1990. Drug release from submicronized o/w emulsion—A new in vitro kinetic evaluation model. Int J Pharm 66:29–37.
272. Kranz H, Bodmeier R. 2007. A novel in situ forming drug delivery system for controlled parenteral drug delivery. Int J Pharm 332:107–114.
273. Itoh S, Teraoka N, Matsuda T, Okamoto K, Takagi T, Oo C, Danny KH. 2006. Reciprocating dialysis tube method: Periodic tapping improved in vitro release/dissolution testing of suppositories. Eur J Pharm Biopharm 64:393–398.
274. Lootvoet G, Beyssac E, Shiu GK, Aiache JM, Ritschel WA. 1992. Study on the release of indomethacin from suppositories—In vitro-in vivo correlation. Int J Pharm 85:113–120.
275. Oribe T, Yamada M, Takeuchi K, Tsunemi S, Imasaka K, Shirakura O, Ishimaru S, Takayama K, Nagai T. 1995. Formulation and in-vivo in-vitro correlation of the dissolution property of lemildipine solid dispersions-incorporated suppositories. Int J Pharm 124:27–35.
276. Pedersen BT, Østergaard J, Larsen SW, Larsen C. 2005. Characterization of the rotating dialysis cell as an in vitro model potentially useful for simulation of the pharmacokinetic fate of intra-articularly administered drugs. Eur J Pharm Sci 25:73–79.
277. Fredholt K, Larsen DH, Larsen C. 2000. Modification of in vitro drug release rate from oily parenteral depots using a formulation approach. Eur J Pharm Sci 11:231–237.
278. Larsen DH, Fredholt K, Larsen C. 2000. Assessment of rate of drug release from oil vehicle using a rotating dialysis cell. Eur J Pharm Sci 11:223–229.
279. Schultz K, Mollgaard B, Frokjaer S, Larsen C. 1997. Rotating dialysis cell as in vitro release method for oily parenteral depot solutions. Int J Pharm 157:163–169.
280. Simmons DL. 2006. What makes a good anti-inflammatory drug target? Drug Discov Today 11:210–219.
281. Malemud CJ. 2004. Cytokines as therapeutic targets for osteoarthritis. Biodrugs 18:23–35.
282. Connell L, McInnes IB. 2006. New cytokine targetsin inflammatory rheumatic diseases. Best Pract Res Clin Rheumatol 20:865–878.

283. Fernandes JC, Martel-Pelletier J, Pelletier JP. 2002. The role of cytokines in osteoarthritis pathophysiology. Biorheology 39:237–246.
284. Glasson SS, Askew R, Sheppard B, Carito B, Blanchet T, Ma HL, Flannery CR, Peluso D, Kanki K, Yang ZY, Majumdar MK, Morris EA. 2005. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. Nature 434:644–648.
285. Stanton H, Rogerson FM, East CJ, Golub SB, Lawlor KE, Meeker CT, Little CB, Last K, Farmer PJ, Campbell IK, Fourie AM, Fosang AJ. 2005. ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. Nature 434:648–652.
286. Komatsu S, Iwata H, Nabeshima T. 1999. Studies on the kinetics, metabolism and re-utilisation after intra-articular administration of hyaluronan to rabbits. Arzneimittelforschung 49:427–433.
287. Wintzer HJ, Fitzek A, Frey HH. 1981. Pharmacokinetics of procaine injected into the hock joint of the horse. Equine Vet J 13:68–69.
288. Lindqvist U, Tolmachev V, Kairemo K, Astrom G, Jonsson E, Lundqvist H. 2002. Elimination of stabilised hyaluronan from the knee joint in healthy men. Clin Pharmacokinet 41:603–613.
289. Biswas NR, Mandal NG, PGL LK. 1995. Pharmacokinetic profile of intra-articular lignocaine during knee joint arthroscopy. Indian J Pharmacol 27:193–194.
290. Kay EA, Klimiuk PS, Ronson G, Bailie GR. 1988. Failure of intraarticular ceftazidime in a patient with Pseudomonas aeruginosa septic arthritis. Drug Intell Clin Pharm 22:315–316.
291. Elmquist WF, Chan KKH, Sawchuk RJ. 1994. Transsynovial drug distribution: Synovial mean transit time of diclofenac and other nonsteroidal antiinflammatory drugs. Pharm Res 11:1689–1697.
292. Day RO, Williams KM, Graham GG, Lee EJ, Knihinicki RD, Champion GD. 1988. Stereoselective disposition of ibuprofen enantiomers in synovial fluid. Clin Pharmacol Ther 43:480–487.
293. Williams AS, Camilleri JP, Goodfellow RM, Williams BD. 1996. A single intra-articular injection of liposomally conjugated methotrexate suppresses joint inflammation in rat antigen-induced arthritis. Br J Rheumatol 35:719–724.
294. Abe T, Yamada H, Nakajima H, Kikuchi T, Takaishi H, Tadakuma T, Fujikawa K, Toyama Y. 2003. Repair of full-thickness cartilage defects using liposomal transforming growth factor-beta1. J Orthop Sci 8:92–101.
295. Trif M, Guillen C, Vaughan DM, Telfer JM, Brewer JM, Roseanu A, Brock JH. 2001. Liposomes as possible carriers for lactoferrin in the local treatment of inflammatory diseases. Exp Biol Med (Maywood) 226:559–564.
296. Highton J, Guevremont D, Thomson J, Carlisle B, Tucker I. 1999. A trial of clodronate-liposomes as anti-macrophage treatment in a sheep model of arthritis. Clin Exp Rheumatol 17:43–48.
297. van Lent PL, van den BL, van den Hoek AE, van de EM, Dijkstra CD, Van RN, Van de Putte LB, van den Berg WB. 1993. Reversible depletion of synovial lining cells after intra-articular treatment with liposome-encapsulated dichloromethylene diphosphonate. Rheumatol Int 13:21–30.
298. van Lent PL, Holthuysen AE, Van RN, Van de Putte LB, van den Berg WB. 1998. Local removal of phagocytic synovial lining cells by clodronate-liposomes decreases cartilage destruction during collagen type II arthritis. Ann Rheum Dis 57:408–413.
299. Ceponis A, Waris E, Monkkonen J, Laasonen L, Hyttinen M, Solovieva SA, Hanemaaijer R, Bitsch A, Konttinen YT. 2001. Effects of low-dose, non-cytotoxic, intraarticular liposomal clodronate on development of erosions and proteoglycan loss in established antigen-induced arthritis in rabbits. Arthritis Rheum 44:1908–1916.
300. Barrera P, Blom A, van Lent PL, van BL, Beijnen JH, Van RN, de Waal Malefijt MC, Van de Putte LB, Storm G, van den Berg WB. 2000. Synovial macrophage depletion with clodronate-containing liposomes in rheumatoid arthritis. Arthritis Rheum 43:1951–1959.
301. Bard DR, Knight CG, Page Thomas DP. 1983. The retention and distribution in the rabbit knee of a radionuclide complexed with a lipophilic chelator in liposomes. Clin Exp Rheumatol 1: 113–117.
302. Chowdhary RK, Ratkay LG, Canaan AJ, Waterfield JD, Richter AM, Levy JG. 1998. Uptake of verteporfin by articular tissues following systemic and intra-articular administration. Biopharm Drug Dispos 19:395–400.
303. Ratcliffe JH, Hunneyball IM, Smith A, Wilson CG, Davis SS. 1984. Preparation and evaluation of biodegradable polymeric systems for the intra-articular delivery of drugs. J Pharm Pharmacol 36:431–436.
304. Ratcliffe JH, Hunneyball IM, Wilson CG, Smith A, Davis SS. 1987. Albumin microspheres for intra-articular drug delivery: Investigation of their retention in normal and arthritic knee joints of rabbits. J Pharm Pharmacol 39:290–295.
305. Burgess DJ, Davis SS. 1988. Potential use of albumin microspheres as a drug delivery system. 2. In vivo deposition and release of steroids. Int J Pharm 46:69–76.
306. Tuncay M, Calis S, Kas HS, Ercan MT, Peksoy I, Hincal AA. 2000. In vitro and in vivo evaluation of diclofenac sodium loaded albumin microspheres. J Microencapsul 17:145–155.
307. Tuncay M, Calis S, Kas HS, Ercan MT, Peksoy I, Hincal AA. 2000. Diclofenac sodium incorporated PLGA (50:50) microspheres: Formulation consid-

erations and in vitro/in vivo evaluation. Int J Pharm 195:179–188.
308. Thakkar H, Sharma RK, Mishra AK, Chuttani K, Murthy RS. 2004. Efficacy of chitosan microspheres for controlled intra-articular delivery of celecoxib in inflamed joints. J Pharm Pharmacol 56:1091–1099.
309. Thakkar H, Sharma RK, Mishra AK, Chuttani K, Murthy RS. 2004. Celecoxib incorporated chitosan microspheres: In vitro and in vivo evaluation. J Drug Target 12:549–557.
310. Bozdag S, Calis S, Kas HS, Ercan MT, Peksoy I, Hincal AA. 2001. In vitro evaluation and intra-articular administration of biodegradable microspheres containing naproxen sodium. J Microencapsul 18:443–456.
311. Ramesh DV, Tabata Y, Ikada Y. 1999. Bioabsorbable microspheres for local drug release in the articulus. J Bioact Compat Polym 14:137–149.
312. Nishide M, Kamei S, Takakura Y, Tamai S, Tabata Y, Ikada Y. 1999. Fate of biodegradable DL-lactic acid oligomer microspheres in the articulus. J Bioact Compat Polym 14:385–398.
313. Liggins RT, Cruz T, Min W, Liang L, Hunter WL, Burt HM. 2004. Intra-articular treatment of arthritis with microsphere formulations of paclitaxel: Biocompatibility and efficacy determinations in rabbits. Inflamm Res 53:363–372.
314. Horisawa E, Kubota K, Tuboi I, Sato K, Yamamoto H, Takeuchi H, Kawashima Y. 2002. Size-dependency of DL-lactide/glycolide copolymer particulates for intra-articular delivery system on phagocytosis in rat synovium. Pharm Res 19:132–139.
315. Horisawa E, Hirota T, Kawazoe S, Yamada J, Yamamoto H, Takeuchi H, Kawashima Y. 2002. Prolonged anti-inflammatory action of DL-lactide/glycolide copolymer nanospheres containing betamethasone sodium phosphate for an intra-articular delivery system in antigen-induced arthritic rabbit. Pharm Res 19:403–410.
316. Liang LS, Jackson J, Min W, Risovic V, Wasan KM, Burt HM. 2004. Methotrexate loaded poly-(L-lactic acid) microspheres for intra-articular delivery of methotrexate to the joint. J Pharm Sci 93:943–956.
317. Liang LS, Wong W, Burt HM. 2005. Pharmacokinetic study of methotrexate following intra-articular injection of methotrexate loaded poly-(L-lactic acid) microspheres in rabbits. J Pharm Sci 94:1204–1215.
318. Cai L, Okumu FW, Cleland JL, Beresini M, Hogue D, Lin Z, Filvaroff EH. 2002. A slow release formulation of insulin as a treatment for osteoarthritis. Osteoarthr Cartil 10:692–706.
319. Mumper RJ, Mills BJ, Ryo UY, Jay M. 1992. Polymeric microspheres for radionuclide synovectomy containing neutron-activated holmium-166. J Nucl Med 33:398–402.
320. Song J, Suh CH, Park YB, Lee SH, Yoo NC, Lee JD, Kim KH, Lee SK. 2001. A phase I/IIa study on intra-articular injection of holmium-166-chitosan complex for the treatment of knee synovitis of rheumatoid arthritis. Eur J Nucl Med 28:489–497.
321. Lee SH, Suh JS, Kim HS, Lee JD, Song J, Lee SK. 2003. MR evaluation of radiation synovectomy of the knee by means of intra-articular injection of holmium-166-chitosan complex in patients with rheumatoid arthritis: Results at 4-month follow-up. Korean J Radiol 4:170–178.
322. Schulze K, Koch A, Schopf B, Petri A, Steitz B, Chastellain M, Hofmann M, Hofmann H, Von Rechenberg B. 2005. Intraarticular application of superparamagnetic nanoparticles and their uptake by synovial membrane—An experimental study in sheep. J Magnetism Magn Mater 293: 419–432.
323. Ohlsson-Wilhelm BM, McDevitt MR, Gray BD, Lorinc R, McIlvain HB, Sheth K, Weeks SA, Muirhead KA. 1994. Zyn-Linker delivery of antirheumatic agents. Immunol Res 13:82–95.
324. Rahmouni A, Mathieu D, Chambon C, Dao TH, Hernigou P, Vasile N. 1995. Intraarticular tolerability and kinetics of gadolinium tetra-azacyclododecane tetraacetic acid. Acad Radiol 2:413–417.
325. Mierisch CM, Cohen SB, Jordan LC, Robertson PG, Balian G, Diduch DR. 2002. Transforming growth factor-beta in calcium alginate beads for the treatment of articular cartilage defects in the rabbit. Arthroscopy 18:892–900.
326. Inoue A, Takahashi KA, Arai Y, Tonomura H, Sakao K, Saito M, Fujioka M, Fujiwara H, Tabata Y, Kubo T. 2006. The therapeutic effects of basic fibroblast growth factor contained in gelatin hydrogel microspheres on experimental osteoarthritis in the rabbit knee. Arthritis Rheum 54:264–270.
327. Senda H, Sakuma E, Wada I, Wang HJ, Maruyama H, Matsui N. 1999. Ultrastructural study of cells at the synovium-cartilage junction: Response of synovial cells of the rat knee joint to intra-articularly injected latex particles. Kaibogaku Zasshi 74:525–535.