Cartilage-targeting drug delivery: can electrostatic interactions help?

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Abstract | Current intra-articular drug delivery methods do not guarantee sufficient drug penetration into cartilage tissue to reach cell and matrix targets at the concentrations necessary to elicit the desired biological response. Here, we provide our perspective on the utilization of charge–charge (electrostatic) interactions to enhance drug penetration and transport into cartilage, and to enable sustained binding of drugs within the tissue’s highly negatively charged extracellular matrix. By coupling drugs to positively charged nanocarriers that have optimal size and charge, cartilage can be converted from a drug barrier into a drug reservoir for sustained intra-tissue delivery. Alternatively, a wide variety of drugs themselves can be made cartilage-penetrating by functionalizing them with specialized positively charged protein domains. Finally, we emphasize that appropriate animal models, with cartilage thickness similar to that of humans, must be used for the study of drug transport and retention in cartilage.

No disease-modifying osteoarthritis drugs (DMOADs) are currently available. Several drugs have potential to inhibit cartilage degeneration associated with osteoarthritis (OA) and post-traumatic osteoarthritis (PTOA), and to promote cartilage repair; however, none of these drugs have yet translated to clinical practice, owing in part to the lack of effective delivery systems that enable local, safe administration in low doses without off-target effects. Direct intra-articular administration of drugs can minimize adverse systemic side-effects. But even intra-articular injection remains inadequate, as small compounds and large macromolecules are rapidly cleared from the joint space via subsynovial capillaries and lymphatics, respectively. For example, the mean half-lives of NSAIDs in the synovial fluid are only 1–4 h. As a result, multiple injections of high-dose drugs are sometimes used in attempts to suppress pain, inflammation and cartilage destruction, an approach that can cause systemic toxicity.

Drugs need to penetrate the full depth of cartilage to reach the chondrocytes and extracellular matrix (ECM) targets involved in OA-associated cartilage pathogenesis. Drug penetration into cartilage is especially important following traumatic joint injury, which can result in damage to articular cartilage, subchondral bone and nearby soft tissues, initiating a sequence of inflammatory events that can progress to PTOA. Biopsy-obtained samples of cartilage from anterior cruciate ligament injury have revealed degradative changes to cartilage as early as 3 weeks after injury, including loss of superficial zone proteoglycans and cell viability, even in cases where there is no obvious damage to cartilage or its collagen network, as visualized by arthroscopy.

New drug-delivery systems have been proposed for sustained delivery in the synovium and synovial fluid using polymeric nanoparticles, microparticles, liposomes, drug-loaded hydrogels, phase transitioning elastin-like polypeptides, silk constructs, and electrospun fibres. These drug carriers have prolonged residence times due to their large size (micron) or viscous and/or aggregating properties that prevent them from leaving the joint space rapidly, thereby enabling rapid drug release within the synovial fluid and/or synovium. These techniques might be useful for delivering drugs for relieving pain and joint inflammation. However, these approaches do not guarantee drug penetration into cartilage (or other target tissues) or reversible binding of drugs inside cartilage. Both mechanisms are necessary to elicit the prolonged biological response needed for cartilage protection.

Drug penetration and retention inside cartilage is a challenging problem. The tissue’s ECM contains densely packed, highly negatively charged aggrecan proteoglycans enmeshed within a complex collagen network; the ECM prevents sufficient drug penetration, thereby enabling rapid clearance of the drug from the joint space.

In this Perspectives article, we first describe candidate drugs for the treatment of OA and PTOA, and then focus on mechanisms by which charge–charge interactions can increase drug penetration, transport kinetics and retention within charged, avascular tissues such as cartilage. We compare three approaches to intra-articular cartilage-targeted delivery, and end with a discussion on the appropriate animal models to use for testing these systems.

Candidate disease-modifying drugs

Current therapies for OA provide only short-term relief of pain and inflammation (for example, analgesics and hyaluronic acid lubricants), but no protection against further degeneration of cartilage and OA progression. Several therapeutics have been identified as having the potential for disease-modifying inhibition of cartilage breakdown, including anticitabolic glucocorticoids (such as dexamethasone and triamcinolone), cytokine blockers, proanabolic growth factors (including insulin-like growth factor (IGF) 1 and bone morphogenetic protein (BMP) 7) and chondrogenic biomolecules.

Given that OA affects the entire joint, DMOAD development and associated clinical trials have targeted cartilage breakdown (with protease or cytokine blockers), bone remodelling (with bisphosphonates, BMP7 or calcitonin), and synovial and inflammatory mediators.
Table 1 | Examples of potential drugs for OA treatment under experimental or clinical trial testing

<table>
<thead>
<tr>
<th>Drug type and/or target</th>
<th>Drug action</th>
<th>Examples</th>
<th>Molecular weight</th>
<th>Mechanism of therapy</th>
<th>Target location inside joint</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSAIDs</td>
<td>Pain relief</td>
<td>• Ibuprofen&lt;br&gt;• Naproxen&lt;br&gt;• Celecoxib</td>
<td>&lt;500 Da</td>
<td>Inhibit COX enzymes</td>
<td>• Vasculature of the joint capsule and cartilage–bone interface&lt;br&gt;• Free nerve endings of sensory neurons in soft tissues (e.g. patella ligament and below the synovial layer)</td>
</tr>
<tr>
<td>Monoclonal antibodies against NGF</td>
<td>Biologic agents for pain relief</td>
<td>• Tanezumab&lt;br&gt;• Fluranumab</td>
<td>~150 kDa</td>
<td>Bind to and inhibit NGF, which is produced by OA synovial cells and chondrocytes and acts directly on sensory neurons</td>
<td></td>
</tr>
<tr>
<td>Monoclonal antibodies against inflammatory cytokines</td>
<td>Biologic agents as DMOADs</td>
<td>TNF inhibitors*&lt;br&gt;- Infliximab&lt;br&gt;- Adalimumab&lt;br&gt;- Etanercept&lt;br&gt;- IL-1β inhibitors*&lt;br&gt;- Canakinumab</td>
<td>~150 kDa (except Etanercept, ~50 kDa)</td>
<td>Directly bind target cytokines, preventing them from binding with their respective cell-surface receptors to initiate signalling</td>
<td>Cytokine targets hypothesized to be in the synovium, the synovial fluid and found throughout the full depth of the cartilage extracellular matrix</td>
</tr>
<tr>
<td>Receptor antagonists</td>
<td>Biologic agents as DMOADs</td>
<td>IL-1 receptor antagonists*&lt;br&gt;- Anakinra</td>
<td>~17 kDa</td>
<td>Competitively bind with cell-surface cytokine receptors thereby blocking cytokine activity</td>
<td>Full depth of cartilage as well as neighbouring soft tissues and synovium</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>Pain relief at high doses (and anticaatabolic effects in cartilage at low doses)</td>
<td>Salts of dexamethasone, triamcinolone and prednisone</td>
<td>&lt;1 kDa</td>
<td>Bind with intracellular glucocorticoid receptors and inhibit cytokine-induced catabolic activity</td>
<td>Full depth of cartilage, meniscus and other tissues</td>
</tr>
<tr>
<td>Growth factors</td>
<td>Biologic agents as DMOADs</td>
<td>IGF-1, FGFs, BMPs</td>
<td>10–20 kDa</td>
<td>Bind with cell surface growth factor receptors to stimulate repair</td>
<td>Full depth of cartilage, meniscus and other tissues</td>
</tr>
<tr>
<td>Protease inhibitors and pro-protein convertase blockers</td>
<td>DMOADs</td>
<td>Inhibitors of MMPs, aggrecanases (ADAMTS-4, ADAMTS-5), cathepsins, PACE4, and others</td>
<td>&lt;1 kDa</td>
<td>Bind with the catalytic zinc atom at the MMP active site (for MMP inhibitors) to inhibit cartilage ECM breakdown</td>
<td>Full depth of cartilage, synovium and joint capsule space</td>
</tr>
<tr>
<td>Viscosupplements</td>
<td>Pain relief and joint lubrication</td>
<td>Hyaluronan1, lubricin (proteoglycan 4) and others</td>
<td>2–6 MDa</td>
<td>Intended to restore joint lubrication, and hypothesized to bind with CD44 receptors to induce chondroprotection</td>
<td>Synovial fluid, joint capsule, synovial membrane and superficial zone of cartilage</td>
</tr>
</tbody>
</table>

ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; BMP, bone morphogenetic protein; COX, cyclooxygenase; DMOAD, disease-modifying osteoarthritis drug; ECM, extracellular matrix; FGF, fibroblast growth factor; IGF-1, insulin like growth factor 1; MMP, matrix metalloproteinase; NGF, β-nerve growth factor; OA, osteoarthritis. *Currently used for systemic treatment of rheumatoid arthritis. †Approved but no longer recommended for patients with symptomatic knee OA, according to American Academy of Orthopaedic Surgeons 2013 evidence-based guidelines.

For example, a multicentre, randomized, double-blind, placebo-controlled study (NCT00110916 [REF. 38]) was performed to evaluate the clinical response, safety and tolerability of a single intra-articular injection of anakinra (an IL-1 receptor antagonist (IL-1Ra), molecular weight ~17 kDa) in patients with symptomatic knee OA. Although significant improvement was observed at day 4, anakinra did not improve OA symptoms after 1 month when compared with placebo. The results suggest that the drug had cleared out from the joint space rapidly following intra-articular injection, and furthermore showed a serum half-life of only 4 h. The investigators speculated that multiple injections would be needed in any attempt to achieve cartilage protection. Another randomized, double-blind, placebo-controlled, multiple-dose study (NCT00110942) used subcutaneous injection or infusion of a monoclonal antibody (AMG 108) that binds the IL-1 receptor type 1 (IL-1R1), thereby inhibiting the activity of IL-1β. The results showed statistically insignificant but numerically greater improvement in WOMAC (Western Ontario and McMaster Universities Arthritis Index) pain score compared with the placebo group, but the clinical relevance was stated to be unclear. The authors stated that it was not possible to evaluate the penetration of AMG 108 into the deeper cartilage layers, and that the availability of the drug to chondrocytes in cartilage remains a possible limitation of this strategy for IL-1 inhibition.

(with cytokine blockers)46. Table 1 is a representative list of such therapeutics that are currently being considered for OA treatment. Biologic agents such as monoclonal antibodies against IL-1β (canakinumab) and TNF (infliximab, adalimumab), and other anti-IL-1 or anti-TNF agents (anakinra, etanercept), have been used successfully for the treatment of rheumatic diseases via systemic delivery. Notably, monoclonal antibodies and similarly sized therapeutics are probably much too large to penetrate cartilage sufficiently before being rapidly cleared from the joint47. Although some of these agents are being considered for intra-articular delivery to treat OA, clinical trials have lacked evidence of either sustained benefit or effective cartilage targeting.3
A new class of RNA interference (RNAi)-based therapeutics has also emerged to target transcription factors (for example, NF-κB and HIF-2α) and their target genes, including those encoding matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) aggrecanases, but these therapeutics can become clinically relevant only if there is a way to deliver them to chondrocytes. To summarize thus far, certain drugs intended for relief of pain and general joint inflammation might do well with delivery and retention in the synovium or synovial fluid. However, to achieve cartilage protection (that is, to protect chondrocyte viability, inhibit cartilage matrix degradation and stimulate cartilage matrix biosynthesis), preclinical and clinical studies to date strongly suggest that appropriate therapeutics must be delivered to chondrocytes (especially in the middle and deep zones of cartilage) or to cartilage matrix-associated targets.

**Delivering drugs to cartilage**

**Cartilage: a barrier to drugs**

Articular cartilage is a highly complex, avascular, alymphatic and aneural tissue whose matrix is made of a dense network of collagen fibrils (50–60% dry weight of tissue), aggrecan proteoglycans that contain highly negatively charged glycosaminoglycan (GAG) chains (30–35% tissue dry weight) and dozens of additional extracellular macromolecules, which are continuously synthesized by a low density of chondrocytes (1–5% tissue dry weight). The collagen fibril network (mostly type II collagen with some type IX and XI collagen) has an approximate pore size of 60–200 nm (REF. 44). Collagen fibrils are aligned parallel to the surface in the superficial zone (about 10–20% of total cartilage thickness), but are randomly oriented in the middle zone (40–60% tissue thickness) and perpendicular to the subchondral bone in the deep zone (30–40% tissue thickness). The collagen network is filled with ~300 MDa aggregates formed mainly of aggrecan; each aggregate comprises a central hyaluronan GAG chain to which as many as one hundred 2–3 MDa aggrecan monomers are noncovalently bound via G1 binding domains, an interaction further stabilized by a link protein (FIG. 1). The sulfated GAG chains covalently linked to the aggrecan monomers are spaced only 2–4 nm apart along the monomer core protein. Thus, these bottle-brush structured aggrecan monomers are so densely packed within the collagen network that the GAG chains on adjacent aggrecan monomers are essentially as close to each other as GAG chains along the core protein. Taken together, this matrix composition presents substantial steric hindrance to the penetration of therapeutic molecules. In addition, the density of aggrecan increases with depth into cartilage, which further...
reduces the effective pore size and restricts the ability of solutes to penetrate and diffuse within the tissue. Given that the majority of chondrocytes reside in the middle and deep zones of the tissue, drug delivery to chondrocytes is a challenge, and avascular cartilage is clearly a barrier to drug and/or drug-carrier entry.

**Drug transport into cartilage**

Drug penetration and retention in cartilage depends on two competing rates of transport (FIG. 2): first, the net flux of drug carriers entering cartilage from synovial fluid, \( N_{\text{entry}} \); and second, the rate of exit from the lymphatics and subsynovial capillaries, \( N_{\text{exit}} \). \( N_{\text{entry}} \), \( N_{\text{exit}} \) should be fast enough to achieve intra-cartilage therapeutic levels before the drugs are cleared from the joint space\(^6\). Clearance is rate-limited first by elimination through the synovial membrane and then by the systemic circulation\(^6\). Detailed pharmacokinetic models of the escape kinetics of drugs from the synovial cavity can be found elsewhere in the literature\(^4,48\).

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**Figure 2** | **Distribution of drugs or drug carriers inside the joint space following intra-articular administration.** The concentration of injected drugs in the synovial fluid (\( C_{\text{sf}} \)) is assumed to be homogenous throughout the joint space shortly after injection (for example, due to joint flexing). Penetration of the drug into cartilage (\( N_{\text{entry}} \)) competes with clearance of the drug through the synovium membrane into the lymphatics and vasculature (\( N_{\text{exit}} \)) (part a). A simplified one-dimensional model depicting the transient drug or drug-carrier concentration profiles at various time points (T1 to T5) is shown during drug accumulation into cartilage and depletion from cartilage (part b). These chosen time points are also illustrated in a graph depicting the drug concentration in the cartilage or synovial fluid over time, following injection (part c). The time period during which the drug stays above the critical therapeutic level inside cartilage (denoted by *) is denoted as \( \tau_{\text{eff}} \), corresponding to the period during which the drug is effective in eliciting a biological response.
The manner in which electrically neutral drugs and/or drug carriers distribute between regions of the joint space following intra-articular injection is depicted schematically in FIG. 2. To reduce the mathematical complexity, this scheme has been simplified to a one-dimensional transport system and depicts the transient concentration profiles of the drug (or drug carrier) in these regions at various time points during its accumulation (FIG. 2b) and depletion (FIG. 2c) inside cartilage. Intra-articular injection immediately increases the synovial fluid concentration (\(C_{SF}\)) of the drug or drug-carrier. Using the simplest model in which drug or drug-carrier concentration becomes uniformly distributed in the synovial fluid volume (for example, by flexing the joint), the \(C_{SF}\) at the interface of synovial fluid and cartilage can be assumed to be approximately equal to that at the interface of synovial fluid and synovial membrane. Initially the drug concentration inside cartilage (\(C_{C}\)) will continue to increase with time even as \(C_{SF}\) begins to decrease with clearance from the synovial fluid. When the drug concentration in the synovial fluid finally becomes lower than that inside cartilage, a net outward diffusion from cartilage back into synovial fluid follows (FIG. 2c), unless there is a mechanism by which the drug or drug carrier can bind to sites inside cartilage. From standard diffusion theory, the diffusion time across cartilage is proportional to the square of the cartilage thickness (\(L\)); hence, cartilage thickness is extremely important to the success of intra-cartilage delivery (see discussion of animal models, below). The time period during which the drug stays above the critical therapeutic level inside cartilage (\(t_{wp}\), denoted by * in FIG. 2b–d) corresponds to the period during which the drug is effective in eliciting the desired biological response.

**Utilizing electrostatic interactions**

The high density of negatively charged GAGs inside cartilage provides a unique opportunity to use electrostatic interactions to augment transport, uptake and binding of drugs and drug-carriers. Such interactions, however, have not yet been fully exploited for local intra-articular delivery (BOX 1). Three complementary mechanisms have been identified by which electrostatic interactions can enable cartilage-targeted drug delivery and retention (BOX 2).

**Donnan partitioning.** Within cartilage, the partition coefficient of a solute, \(k\), is defined as the equilibrium concentration of unbound, free solutes inside cartilage normalized to the solute concentration in the surrounding bath (that is, synovial fluid)\(^{48}\). \(k\) depends on solute size, charge and the composition of the cartilage ECM. A small electrically neutral solute that is not sterically hindered by cartilage ECM will have a partition coefficient of ~1: that is, when in equilibrium, solute concentration in the tissue and the surrounding synovial fluid is nearly equal. The high negative fixed-charge density of GAGs inside cartilage results in a drop in the electrical potential (\(\Delta \Phi\)) at the tissue interface, causing a strong, inwardly pointing electric field (FIG. 3a) that enhances transport of positively charged species into cartilage and excludes penetration of negatively charged solutes. This intra-tissue distribution of charged solutes within charged tissues is quantified by Donnan’s theory\(^{42,51}\), which states that, in equilibrium, all freely moving charged solutes will distribute (that is, ‘partition’) into charged tissues on the basis of the difference between the mean electrical potential inside the tissue compared with that of the surrounding bath and, additionally, that the net charge inside the tissue must be zero, including the charges of all mobile solutes as well as the fixed-charge groups of the ECM (for example, the charges on GAGs). Thus, under physiological conditions, Na\(^+\) ion concentration is higher inside cartilage while Cl\(^-\) ion concentration is lower compared with their concentrations in the synovial fluid at equilibrium (FIG. 3b).

Transport of large-molecular-weight solutes into cartilage is sterically hindered, typically resulting in partition coefficients <1. For example, Maroudas et al. showed that serum albumin (molecular weight 69 kDa, diameter ~7 nm, isoelectric point (pI) 4.7) is sterically hindered in normal human cartilage, with \(k < 0.05\) (REFS 49-51). Similarly, neutravidin (66 kDa, electrically neutral) has \(k = 0.5\) in normal cartilage\(^{33}\). By comparison, avidin (66 kDa, net charge +20, pI = 10.5), the same-sized positively charged counterpart of neutravidin, has a much higher partition coefficient (\(k = 6\))\(^{33}\). Most importantly, when a highly positively charged drug (or a potential drug carrier such as avidin) is injected intra-articularly\(^{52,53}\), there is an immediate sharp increase in its concentration just inside the cartilage due to Donnan partitioning at the synovial fluid–cartilage interface\(^{30}\) (FIG. 3a). As depicted in FIG. 3a, the resulting increased concentration (from C to KC) causes a steep intra-tissue concentration gradient (from the superficial zone inward), which greatly accelerates transport of positively charged drugs and/or drug carriers deeper into the negatively charged cartilage. This accelerated transport enables drugs and/or drug carriers to penetrate into cartilage faster than their clearance rate from the synovial fluid. For example, avidin fully penetrates through 1 mm-thick bovine cartilage in ~24 h, whereas neutravidin only partially penetrates by 4 days; avidin also shows 400-fold higher uptake into cartilage compared with neutravidin\(^{24}\).

**Weak reversible binding.** Current research in intra-articular delivery has sometimes focused on using strong binding mechanisms (for example, covalent bonds\(^{37,59}\)) to increase the residence time of drug carriers inside cartilage. However, such strong-binding mechanisms would markedly slow the penetration of these carriers into human (and large animal) cartilage as the carriers would be trapped in the surface layers well before reaching middle zone and deep zone targets (BOX 3). More precisely, diffusion-reaction transport times are inversely proportional to the effective diffusivity of drug carriers, which would be decreased by orders of

**Box 1 | Why intra-cartilage delivery?**

- Potential disease-modifying osteoarthritis drugs (DMOADs) are in preclinical development, but cartilage-targeted delivery methods for intra-articular delivery in humans are lacking
- Drugs injected directly into the joint have short residence times as they are rapidly cleared via lymphatics or sub-synovial capillaries
- The dense matrix of cartilage prevents the drug penetration necessary to elicit the desired biological response
- In the absence of cartilage-targeted intra-articular delivery, multiple injections of high-dose drugs might cause off-target effects and even systemic toxicity
- To reach chondrocyte and matrix targets throughout the full thickness of cartilage, drug carriers are needed that can penetrate to the deep zone and bind to cartilage matrix, and thereby provide sustained intra-tissue delivery of therapeutics
Box 2 | How can electrostatic interactions help?

Cationic carriers rapidly penetrate negatively charged cartilage, resulting in high uptake, binding to extracellular matrix components and sustained drug delivery to chondrocytes

**Transport rate and uptake into cartilage**

- Electrostatic interactions cause a sharp increase in concentration of cationic carriers at the synovial fluid–cartilage interface (called Donnan partitioning) following intra-articular injection
- This Donnan partitioning causes steep intra-cartilage concentration gradients that accelerate transport and enable high uptake of cationic carriers into cartilage before they exit the synovial fluid

**Depth of penetration and binding**

- Electrostatic binding is weak and reversible (that is, carriers rapidly unbind after initial binding with negatively charged groups); thus, cationic carriers continue to diffuse throughout the full thickness of cartilage
- Despite weak binding, the high negative fixed charge density of aggrecan glycosaminoglycans inside cartilage greatly increases the residence time of cationic carriers
- In the early stages of OA, despite the loss of some GAGs, the remaining negative charges inside cartilage still provide sufficient binding sites for cationic carriers

Magnitude due to tight binding. By contrast, nonspecific electrostatic interactions between positively charged drugs and/or drug carriers and negatively charged cartilage ECM lead to weak and reversible binding, which provides the distinct advantage of enabling drug carriers to rapidly penetrate through the full thickness of cartilage. Such cationic particles will be attracted to and could weakly bind negatively charged GAG constituents inside cartilage. This weak and reversible ionic binding has a correspondingly high dissociation constant, \( K_d \), so the carriers also rapidly unbind from their intra-tissue binding sites. If the particles are small enough such that they are not subject to steric hindrance by the cartilage ECM, they will continue to diffuse through the cartilage and penetrate deeper into the tissue, given the inward concentration gradient induced by Donnan partitioning at the superficial zone, as described above.

**High intra-tissue binding site density.**

The high density of GAGs in cartilage provides a high density of binding sites for certain positively charged solutes, which greatly increases their intra-tissue residence time, despite their weak binding. For example, avidin remains bound within cartilage for several weeks owing to a very high intra-cartilage binding site density (\( N_y = 2,900 \mu M \)) even though its binding affinity is very weak (\( K_y = 150 \mu M \))

However, although electrostatic interactions result in upward partitioning of cationic peptides into cartilage, they do not guarantee binding to the matrix. Binding depends on the precise chemical structure of the binding site (for example, the GAGs within cartilage) and that of the drug and/or drug carrier molecule. For example, the heparin-binding domain of heparin-binding (HB)-IGF-1 binds with a higher affinity to heparan sulfate GAGs (\( K_y = 21 \text{nM} \)) than to chondroitin sulfate GAGs (\( K_y = 160 \text{nM} \)). Similarly, avidin binds weakly to chondroitin sulfate GAGs. However, this weaker binding is compensated by the much higher density of chondroitin sulfate GAGs than heparan sulfate GAGs in cartilage (500–1,000 fold). By contrast, although the small cationic peptide therapeutic Pf-pep (Arg-Tyr-Lys-Arg-Thr, 760 Da, net charge +3, pI ~11) was found to partition upward (\( k \sim 3.5 \)) into cartilage, this peptide did not bind inside cartilage and rapidly diffused out of the tissue, thereby preventing its intra-cartilage retention at levels needed for sustained therapeutic effect. Thus partitioning and binding are two independent mechanisms that affect solute uptake and retention inside cartilage in very different ways. Separate experiments must be performed to test whether electrostatic interactions can simultaneously enable both upward partitioning and binding.

**Effects of dynamic loading**

Dynamic loading of cartilage, as would occur during walking, running or jumping, might also affect drug uptake. These effects are independent of the electrostatic interactions discussed above. Joints are mechanically loaded across a wide spectrum of frequencies (loading rates) depending on the type of physical activity, which compresses the cartilage. This dynamic loading of joints results in fluid flow within cartilage that could potentially enhance drug transport, completely independent of electrostatic effects. However, several studies have reported that the effects of dynamic loading increase transport by only about twofold for large macromolecules compared with that in the non-loaded condition. This approximately twofold increase was reported when cartilage plugs were subjected to continuous cyclic loading for several hours, a procedure not practical in clinical situations. Hence, dynamic loading has a smaller effect on transport of small molecules compared with passive diffusion. By contrast, the electrostatic effects described here can enable increases in intra-cartilage concentration of 10–100-fold, as has been reported for various cationic solutes in cartilage.

**Drug delivery approaches**

**Cartilage-targeting drug carriers**

The current clinical standard for intra-articular delivery is direct injection into the joint. New delivery approaches being investigated in clinical trials utilize micron-sized, impenetrable, non-binding particles that remain suspended in the synovial fluid for sustained drug release into the joint space. However, a considerable fraction of the released drug might be cleared from the joint before entering cartilage. Hence the available drug concentration inside cartilage, \( C \), will be lower than the concentration of drug injected into the joint, \( C \). The time required to reach intra-cartilage therapeutic levels (\( t_c \)) can be very long (if ever attained). Most currently explored sustained-release drug delivery systems fall into this category; examples include triamcinolone-loaded PLGA (poly lactic-co-glycolic acid) microspheres, carriers or drugs crosslinked to either exogenous hyaluronan particles or endogenous hyaluronan within the joint, and elastin-like polypeptides for delivery of IL-1Ra. These drug delivery systems are most relevant if target sites are mainly in the synovial fluid or synovium, such as when mediating pain and inflammation, but are not very effective for targeting chondrocytes unless extremely high drug doses are used.

A second approach utilizes drug carriers of varying sizes functionalized to bind to cartilage surfaces. Such surface binding prevents these carriers from penetrating deeper into the tissue, although drugs released from them could penetrate effectively depending on their properties. As a result, drug concentrations could reach intra-cartilage therapeutic levels in a shorter time, \( t_c \), compared with drug-release from

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impenetrable non-binding carriers (τc < τv, FIG. 4b). This method might be best suited for delivery of novel proteoglycan 4 (PRG4)-like proteins for surface lubrication41.

In contrast to impenetrable particles, positively charged nanosized carriers <10 nm diameter (cationic drug nanocarriers) can penetrate past the superficial zone of the cartilage44 (FIG. 4c). Carriers with similar diameter but longer length (for example, rod or chain structures) might also penetrate cartilage tissue owing to matrix tortuosity65. The positive charge of nanocarriers drastically increases their partitioning and results in steep intra-tissue concentration gradients and accelerated transport. Mediated by weak reversible ionic binding, this approach can yield full depth penetration and retention of drug carriers, resulting in therapeutic levels of intra-cartilage drug concentrations in the shortest time (FIG. 4c). For example, avidin has been shown to have optimal size and charge properties for intra-cartilage drug delivery24,26. When conjugated with dexamethasone, it rapidly penetrated into full-thickness cartilage explants, releasing the drug inside, which significantly suppressed IL-1-induced GAG loss over 3 weeks compared with free drug66. In another study, cationic moieties were incorporated into DOTAM (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraaetatic acid amide)-based nanocarriers functionalized with the cathepsin D inhibitor pepstatin A, and demonstrated retention in mouse knee joints66. Thus, this approach could enable intra-cartilage delivery of potential DMOADs, which has remained a challenge. Such an approach might also offer a unique opportunity to re-examine OA drugs that have failed clinical trials65 owing to a lack of tissue-targeted delivery and resulting systemic adverse effects.

**Cartilage-targeting drugs**

Other approaches have been explored for targeting chondrocytes inside cartilage, including gene delivery66 (which often involves use of cationic viruses) and the use of ultrasonography to increase drug transport rates67. Attention has also focused on the addition of cationic domains to known protein therapeutics (for example, growth factors) to enable electrostatic partitioning, binding and retention inside cartilage in a manner similar to that pictured in FIG. 4c. Clues for this approach were garnered from the FGF family, one of the first ligands discovered to have a cationic heparin-binding domain68. This domain binds heparan sulfate GAG chains on the surface of cells in cartilage, increasing the affinity between FGF family ligands and their cell-surface receptors, crucial for stabilizing this formation and initiating cell signalling69.

In addition to facilitating growth factor signalling, heparin and heparan sulfate are of particular interest in the realm of growth factor delivery owing to the cationic nature of heparin-binding domains. The existence of this domain on FGF-18 was partly the motivation for utilizing this growth factor for ongoing OA clinical trials63.

Although some proteins (such as the FGF family and vascular endothelial growth factor (VEGF)) have naturally occurring cationic domains that bind to heparin and heparan sulfate, such heparin-binding domains can also be attached to other molecules, resulting in fusion proteins such as HB-IGF-1 that are now known as heparin-binding drugs. Studies in vitro, as well as in animal models10,31, revealed that intra-articular injection of HB-IGF-1 resulted in longer retention and bioactivity in cartilage, as well as enhanced local delivery to chondrocytes, compared with IGF-1. Furthermore, HB-IGF-1 also binds to, and is primarily retained by, chondroitin sulfate GAG chains than does avidin, although penetration of HB-IGF-1 into cartilage is still dramatically high63.

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**Figure 3** | **Electrostatic (charge–charge) interactions cause Donnan partitioning but not necessarily drug binding to cartilage matrix.** a) The high negative fixed-charge density of glycosaminoglycans (GAGs) inside cartilage results in a decrease in the electrical potential (∆Φ) from the synovial fluid into cartilage, owing to a strong, inwardly pointing electric field (E) that enhances transport of positively charged species into cartilage and diminishes penetration of negatively charged solutes such that the total net charge inside the cartilage is zero. b) The concentration of positively charged Na+ partitions upward (from CNa to kNa CNa), while negatively charged Cl− partitions downward (from CC to kCl CCl). Similarly, positively charged drug carriers (for example, avidin) partition upwards into negatively charged cartilage. k is the partition coefficient and C is the concentration of the solute particle within the synovial fluid. c) The small cationic peptide Pf-pep does not bind inside cartilage and hence rapidly diffuses out64. Avidin binds weakly and reversibly with the negatively charged GAGs with a dissociation constant (Kd) of 150 μM owing to charge–charge interactions (note that a high dissociation constant implies weak binding).64. The cationic heparin-binding (HB) domain of heparin-binding insulin-like growth factor 1 (HB-IGF-1) binds comparatively more strongly with chondroitin sulfate GAG chains than does avidin, although penetration of HB-IGF-1 into cartilage is still dramatically high63.
the C-terminal peptide of chondroadherin selectively binds to heparan sulfate chains; similarly, the GAG-binding domain of prolargin (also termed PRELP) can be fused with drugs to enable binding within cartilage ECM.

**Delivery to damaged cartilage**

In the early stages of cartilage degradation, a window of opportunity exists for drug delivery when there might be some but not yet complete loss of GAGs from cartilage.

Experiments utilizing avidin showed that its concentration inside partially degraded cartilage (40% depletion of GAG chains) was 25 times higher than that in the bathing medium. Thus, utilizing charge–charge interactions for targeting and retaining positively charged drugs and/or drug carriers is feasible even with partial GAG loss. At this stage of disease, before overt collagen fibrillation, pharmaceutical intervention could delay, prevent or even reverse progression of OA or PTOA.

**Glossary**

**Therapeutic levels**
The drug doses necessary to elicit the desired biological response. For a particular drug this level can be estimated using a combination of in vitro assays and in vivo pharmacokinetic and pharmacodynamics studies.

**Diffusion time**
Time for diffusion (t) of a drug into cartilage of thickness L’ is ~ L’/D, where D is the diffusivity of the drug inside cartilage tissue.

**Electrostatic interaction**
Non-covalent repulsive or attractive interaction between charged molecules (for example, proteins, glycosaminoglycans) in a physiologic medium (for example, saline, synovial fluid) or inside highly charged tissues such as cartilage.

**Partition coefficient**
The equilibrium concentration of unbound, free drug inside cartilage, normalized to drug concentration in the synovial fluid (denoted as Kp).

**Electrical potential**
The potential energy of a charged particle at any location divided by the particle’s charge. Sharp jumps in electrical potential result in high localized electric fields at that region.

**Steric hindrance**
When the pore size of the tissue matrix is small enough, diffusion and transport of a drug or drug carrier will be hindered simply because of its size.

**Donnan partitioning**
The change in concentration of a charged drug across the synovial fluid–cartilage interface due to the drug’s charge. The high negative fixed-charge density of glycosaminoglycans inside cartilage results in a drop in the electrical potential at the tissue interface, causing a strong inward pointing electric field that enhances transport of positively charged species into cartilage and excludes penetration of negatively charged species such that the net charge inside the cartilage is zero. Thus, the concentration of positively charged drug can increase dramatically (i.e. partition upwards) across the interface as the drug enters the negatively charged cartilage.

**Dissociation constant, Kd**
Here, the concentration of the drug at which (in equilibrium) half of the binding sites are occupied by the drug. Generally, the lower the value of Kd, the tighter the binding.

**Binding site density, Ns**
Here, the local density of sites inside a tissue that can bind drug molecules.

**Binding affinity**
Here, the strength of the binding interaction between a drug and its binding-site partner that bind together reversibly. High affinity means very tight binding.

**Dynamic loading**
The mechanical loading of joints, which can occur across a wide range of frequencies (loading rates) depending on the type of physical activity. For example, joint loading frequencies can range from ~1 Hz in slow activities such as walking to 1,000 Hz for high rate activities such as jumping and high impact sports.

**Cationic drug nanocarriers**
Biological or synthetic nanoparticles (with diameters approximately <10 nm) that can be conjugated to small or large molecule drugs to enhance delivery.

**Electrostatic binding**
Binding due to electrostatic interactions; generally nonspecific and much weaker than strong (for example, covalent) binding.

Late-stage OA is characterized by extensive damage to the collagen network of cartilage and other soft tissues, as well as substantial loss of GAGs from cartilage. These changes are accompanied by episodic synovitis, osteophyte formation and subchondral sclerosis. Although this more extreme degradation of cartilage makes the tissue much more permeable to larger sized drugs (perhaps even ~150 kDa antibodies), this advanced stage of disease might be irreversible, and treatments are probably limited to symptomatic relief of pain and inflammation. However, GAG chains are present in the menisci, ligaments and in lower concentrations in tendons. Additionally, lubricin glycoproteins are found in the superficial zone of cartilage and in the surface layers of the synovial membrane, fat pads and other gliding joint tissues, providing additional natural reservoirs for positively charged drug carriers. Hence, attaching a cationic domain to pain and inflammation relievers might still enhance their residence time in the joint through binding to negatively charged molecules in the synovial fluid, fat pads and synovium.

**Appropriate animal models**
Any perspective on developing new drug-delivery systems must include a discussion of animal models used to investigate in vivo biological responses and transport kinetics. Multiple studies of intra-articular delivery approaches have utilized mouse and rat models. Although rodent models continue to have an essential role in our understanding of the biological mechanisms underlying OA and PTOA, and therefore in initial drug screening and drug discovery, these models might not be informative regarding drug delivery. Transport kinetics must be investigated using larger animal models with thicker cartilage more like human, models that might also be more clinically relevant (and generally preferred by the FDA). Drug delivery and transport kinetics depend on drug carrier size and surface-functional properties, and on the biophysical properties of the animal joint and its constituent tissues. The size of joint space and, in particular, the thickness of cartilage increase with animal size. For example, average cartilage thickness for different mature animal species have been reported as follows: mouse ~50 µm, rat ~100–150 µm, rabbit ~350–700 µm, goat ~900 µm, pig ~1.5 mm, horse ~1.5–2.0 mm and human ~1.5–2.0 mm (REFS 77–80). Therefore, drug uptake, diffusion-reaction transport...
kinetics, and retention will vary markedly with animal species. Whereas drug carriers might penetrate rapidly into 50µm-thick mouse cartilage, in larger animals and humans they could easily be cleared from the joints before much penetration, as the diffusion time is proportional to the square of cartilage thickness. Conversely, once a drug reaches therapeutic levels inside cartilage and are unable to penetrate deeper into the tissue are relevant for the delivery of drugs to target sites or near the surface. c | Carriers with optimal size and positive charge can penetrate through the full thickness of cartilage and be retained owing to weak, reversible binding interactions. The sharp increase in cationic drug carrier concentration (from C to kC) at the synovial fluid-cartilage interface is caused by Donnan partitioning, which accelerates drug transport into cartilage faster than drug clearance from the synovial fluid. Thus, the time to reach intra-cartilage therapeutic levels is shortest in this case compared with large carriers or surface-binding carriers. A graph for each drug delivery system depicts the transient carrier and drug concentration profiles. τ₁ and τ₂ denote an earlier and later time point respectively. C is the concentration of drug (encapsulated in carriers) in the synovial fluid. C’ is the effective drug concentration in synovial fluid after clearance from the joint. Blue and orange curves show the concentration gradient of drug carriers. Red dotted curves show concentration gradient of the drug released from these carriers inside the cartilage. * denotes the drug therapeutic threshold.

Conclusions
Electrostatic (charge–charge) interactions provide a unique opportunity for targeted drug delivery into negatively charged tissues such as cartilage by either functionalizing drugs with cationic peptide domains or utilizing cationic nanocarriers. By designing drug-carrier conjugates of optimal size and charge, it is possible to enable their penetration and long-term retention through the full thickness of cartilage, which is necessary for drug delivery to chondrocytes and other ECM targets. This approach could enable treatment of early stage OA and PTOA when the disease is still ‘reversible’. In the case of late-stage OA, this approach might enhance the residence times of symptomatic medication by enabling binding within the synovial joint.

Figure 4 | Approaches to intra-articular drug delivery. a | Large, non-penetrating, non-binding drug carriers remain suspended in synovial fluid. These carriers are most relevant when the target sites of the drug are mainly in the synovial fluid or synovial membrane, such as with drugs used for relieving pain and inflammation. b | Large or small carriers (depicted in blue and orange respectively) that bind strongly to the cartilage surface and are unable to penetrate deeper into the tissue are relevant for the delivery of drugs to target sites at or near the tissue surface. c | Carriers with optimal size and positive charge can penetrate through the full thickness of cartilage and be retained owing to weak, reversible binding interactions. The sharp increase in cationic drug carrier concentration (from C to kC) at the synovial fluid-cartilage interface is caused by Donnan partitioning, which accelerates drug transport into cartilage faster than drug clearance from the synovial fluid. Thus, the time to reach intra-cartilage therapeutic levels is shortest in this case compared with large carriers or surface-binding carriers. A graph for each drug delivery system depicts the transient carrier and drug concentration profiles. τ₁ and τ₂ denote an earlier and later time point respectively. C is the concentration of drug (encapsulated in carriers) in the synovial fluid. C’ is the effective drug concentration in synovial fluid after clearance from the joint. Blue and orange curves show the concentration gradient of drug carriers. Red dotted curves show concentration gradient of the drug released from these carriers inside the cartilage. * denotes the drug therapeutic threshold.

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Published online 9 Feb 2017


Acknowledgements

This work was supported by NIH National Institute of Biomedical Imaging and Bioengineering grant EB017755, National Science Foundation Materials Research Science and Engineering Centers (MRSEC) grant DMR-1419807, NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) grant AR060351 and Department of Defense (DoD) Congressionally Directed Medical Research Programs (CDMRP) grant W81XWH-14-1-0544.

Author contributions

Both authors researched data for the article, provided substantial contributions to discussion of its content, wrote the article and undertook review and/or editing of the manuscript before submission.

Competing interests statement

A.G.B and A.J.G. declare that they are inventors on the US patent 9289506 B2 ‘Surface binding of nanoparticles-based drug delivery to tissue’.