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Liposome-Based Interventions in Knee Osteoarthritis

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Osteoarthritis (OA) is the most common degenerative disease of the joints, causing significant disability and socio-economic burden in the aging population. Simultaneously, however, it is a common occurrence in younger individuals, initiated by joint injuries or obesity alongside other factors. Intravenous and oral pharmaceutical OA management have both been associated with systemic adverse effects, thereby resulting in a growing interest in intra-articular (IA) treatment. IA-administered drugs circumvent the requirement for high dosage, offering immediate access to the site of interest while minimizing any unfavorable effects. Nonetheless, IA-injected drugs, administered in their free form, present low retention time in the knee joint raising the need for multiple injection dosage regimens, while their capability to target the cartilage or specific cell populations is limited. Liposomes, due to their unique characteristics and tunable nature, have proven to be excellent candidates for the management of knee OA. This review explores the last decade's research on the efficacy of various IA liposomal formulations, investigating their multifaceted properties as pharmaceutical carriers, lubricating agents, and a basis for combinatorial approaches paving the way to novel treatment solutions for OA.

1. Introduction

1.1. Knee Osteoarthritis

Osteoarthritis (OA) (from the Greek words *όστέον* (= bone), *άρθρωσις* (= joint), and the suffix-*ῖτις* which denotes inflammation) is the most prevalent joint disease, and disability cause in adults.[1] One or multiple weight-bearing joints can be involved with the knees and hips being the most affected ones. As of 2020, knee OA was estimated to have a global incidence of 203 per 10 000 persons,[2] and is generally considered the site most affected by OA, accounting for over half of all OA cases worldwide.[3] Despite predominantly being linked with the elderly, the labor force is also impacted. Reduced productivity, absenteeism, and high direct and indirect costs frame OA as a substantial socio-economic burden, alongside the personal adversity of decreased quality of life.[4] Abnormal joint mechanics[5] and systemic factors such as aging, gender, hormone imbalances, obesity,[6,7] and genetic predisposition[8] are some of the etiological factors of knee OA leading to distinct phenotypes.[9] Age appears as a crucial risk factor, due to the accumulation of incidents including reduced physical activity, aging of tissues, chondrocyte senescence,[10] and metabolic dysfunction.[11] Moreover, in the current societal landscape, the increased obesity rates lead to a higher risk of OA along with sex-based differences (≈60% of people living with OA are women) due to hormonal and genetic factors. Interestingly, studies revealed that mutations in collagen type II (COLII)[12] and matrix metalloproteinases (MMPs) genes[13] run in the same family, highlighting the less studied heritable aspect of OA.

The knee joint environment is quite complex, and OA affects its different parts in distinctive ways. The joint is surrounded by the articular capsule that holds together the articulating bones while the inner layer, called synovium (also referred as synovial membrane), is a highly vascularized layer of connective tissue. The intima layer of synovium features macrophage- (Type A) and fibroblast-like (Type B) cells performing phagocytosis and secreting synovial fluid (SF) components respectively. The subintima layer includes blood and lymphatic vessels for the exchange of nutrients and clearance of apoptotic cells, drugs, and others.[14] The articular cartilage is located at the ends of the articulating bones and is a hyaline cartilage of 1–4 mm thickness. It comprises of extracellular matrix (ECM) hosting the sole cellular type named chondrocytes in its avascular, nerve- and lymphatics-free environment. Water, collagens, phospholipids, and proteoglycans along with other proteins are its constituents.[15] It is primarily responsible for a) reducing the friction between the articulating bones with friction coefficient values ranging from $\mu \approx 0.002$–0.02 (μ = friction force/normal force),[16–18] b) protecting from wear and tear, and c) absorbing shock and distributing load applied to the area.[19] It is crucial to mention that the actual μ values may be significantly lower compared to the ones reported, given the

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challenging nature and the limitations of in vivo measurements. SF is a viscous, non-Newtonian, ultrafiltrate of blood that fills the joints, and it is mainly composed of hyaluronic acid (HA), lubricin, and albumin along with different cellular types being both a nutrient reservoir and a lubricant.[20,21]

When challenged by OA, the knee joint faces a cascade of events that advances to damage and dysfunction. The degradation of the cartilage can be attributed to wear and tear due to increased friction because of injuries, overuse, and other mechanical factors along with anabolism and catabolism imbalance in gene expressions of MMPs[22] related primarily with the mechanosensitive chondrocytes. Wear and tear begin to disrupt the smooth nature and the lubrication efficiency of the articular cartilage, with the increased friction to activate the upregulation of proteolytic enzymes (like MMPs) further degrading the proteoglycan and collagen network.[23] Moreover, synovitis (inflammation of the synovium) activates synoviocytes to produce pro-inflammatory mediators, which attract immune cells, increase angiogenesis, and induce a phenotypic shift in chondrocytes. Chondrocytes produce additional cytokines and proteolytic enzymes that eventually increase cartilage degradation, porosity, and deterioration of the collagen-proteoglycan network, inducing further synovial inflammation. Synovitis, combined with increased friction and the progressive degradation of the cartilage leads eventually to severe pain, bone loss, and other pathologic effects.[24,25] This usually follows a slow progression pattern of inconspicuous onset, however, one out of seven individuals could present rapidly evolving OA within 1 year.[26] The symptomatology of OA- joint pain, stiffness, swelling, and reduced mobility -can significantly impact an individual's well-being and ability to perform daily activities, making the development of effective treatment of outmost significance.

1.2. Intra-Articular Administration

OA management as of today mainly aims to relieve symptoms, primarily pain, as the multi-phenotype nature of the disease along with the interpatient variability hinder effective treatment. Joint surgery (e.g., knee arthroplasty) is the most effective measure to improve pain and quality of life, however, is considered the last resort especially for younger patients, as revision operations tend to be more problematic and involve a higher risk of complications.[27] Non-surgical interventions (exercise,[28] weight management,[29] and physical therapy[30]) are commonly used in the early stages of OA, but pharmacological approaches are frequently employed when severe pain is involved. Analgesics (of synthetic and natural origin), nonsteroidal anti-inflammatory drugs (NSAIDs), cyclooxygenase type 2 inhibitors, and corticosteroid injections are some of them. Gene-editing tools of CRISPR/Cas9 are under clinical trial[31] while cell therapies and others (i.e., platelet rich plasma, bone marrow aspirate concentrate, and adipose tissue) represent alternative therapeutic options to the more traditional approaches.[32]

The delivery of compounds for OA treatment are subjected to different routes of administration. The oral route is the most frequently implemented; however, particularly in the case of NSAIDs, adverse effects related to the gastrointestinal tract (hemorrhage and nausea)[33] and the renal system[34] have been reported, excluding high-risk profile patients from their use. Low bioavailability in combination with the articular cartilage's avascular nature result in the necessity for high and repeated dosing. Alternatively, topical administration for pain relief (with many market products such as Voveran Emulgel) minimizes the severe adverse effects mentioned but present limited efficacy and applicability only in mild cases of pain.

Considering the reduced efficiency and the adverse effects of topical and oral administration in addition to the site-specific manifestations of OA, intra-articular (IA) administration - direct injections into the knee joint - is considered a favorable approach. The first IA injection has been described in 1792 by the French physician Jean Gay, who administered an aqueous solution of lead subacetate (Goulard's water) in swelling knee,[35,36] while Joseph Lee Hollander[37] in the '50s re-established this administration route by IA hydrocortisone acetate in arthritic joints. Injecting medications directly into the knee joint cavity offers multiple advantages including reduced required dose; avoidance of first- pass metabolism and therefore reduced degradation of the compound; decreased adverse effects due to minimized systemic exposure,[38] increased concentration of the injected compound in the site of action,[39] faster pain relief;[40] and access to different target sites (from articular cartilage[41]to cellular sites of action[42]). Many compounds have been used in their IA injectable form,[43] with studies regarding their efficacy in different clinical trials. Corticosteroids, HA, anti-inflammatory drugs, growth factors for bone repair, cell and gene therapies are some of them. Yet, this intervention demonstrates drawbacks with the low residence time post-injection leading to multiple injections, while discomfort from needle placement, pain, bleeding, and infection risk have been also documented in clinical trials.[44]

The half-life of IA injected compounds ranges from several minutes up to few hours with the most used NSAIDs like ibuprofen having half-life of 1–2 h[39] and HA up to 48 h depending on its molecular weight and the animal model studied.[45,46] The compound's molecular weight strongly influences its residence time, as small drugs are removed from the blood vessels, while higher molecular weight molecules by the lymphatic circulation.[47] The layer of synoviocytes lining in the inner part of the capsule is discontinuous (comprising a "leaky" environment) allowing molecules to pass with size and charge dictating molecular flux.[48] Moreover, as OA progresses the pharmacokinetics alter, making the IA administration even more challenging as the increased angiogenesis transforms the area to a more permeable environment,[49] highlighting the need for systems with increased retention time.[50] Alterations in the joint lymphatics' distribution,[51] activation of inflammatory processes, decreased SF viscosity,[52] and increased macrophage presence[53] among others are additional events participating in OA's pathology.[54] Additionally, charge can dictate their electrostatic interactions with the charged components of the SF,[55] the cartilage,[56] or the cellular membranes.[57]

For all the above mentioned, numerous strategies have been followed to increase the retention time and improve the targeted delivery of compounds in the knee joints. Among them, nanosystems are ideal for this purpose as they can be tailored in terms of size, surface charge, drug loading, sustained release, viscosity, and biocompatibility. Hydrogels, liposomes, nanoparticles, and microparticles have been proposed as drug carriers, enabling

release over extended periods of time. In the present review, we focus on the advancements of liposomes and liposome-based systems, and their use in the IA management of OA over the past decade. Our analysis of the literature addresses not only their role in drug delivery but also their unique ability to act as lubricants, either as standalone strategies or when combined with other formulations to maximize the pharmacological outcomes.

1.3. Liposomes

Liposomes, since they were first introduced in the literature in 1964 by the hematologist Alec Bangham,[58] have become some of the most efficient nanosystems, especially in the field of cancer, with ≈14 systems being FDA-approved. Since 1995, the year Doxil[59] was launched, liposomes are on the market for cancer therapy, fungal diseases (Ambisome), viral vaccines (Inflexal), and photodynamic therapy (Visudyne).[60]

Liposomes are artificial, colloidal, phospholipid-based, spherical systems and can be found in multi- or unilamellar structures of lipid bilayers (lamellae). They are classified as multivesicular vesicles, multilamellar vesicles (MLVs), oligolamellar vesicles, and unilamellar vesicles while according to their size further to small-SUVs (30–100 nm), large-LUVs (>100 nm), and giant vesicles-GUVs (>1 µm). Their structural organization of lipid bilayer(s) separates the internal aqueous core from the external aqueous solution offering the systems their property of encapsulating hydrophilic, hydrophobic, and even amphiphilic compounds in their distinct areas.[61] They consist mainly of phospholipids and sphingolipids while cholesterol[62] and others are frequently incorporated for improved stability, mechanical strength, and modulation of drug release. However, they present stability issues, and administration can lead to drug leakage or aggregation, if not properly designed, compromising their efficacy. Thus, polymers such as polyethylene glycol (PEG), polyoxazolines, and other alternatives have been used for stabilization creating a steric barrier ("stealth" effect) while in many cases additional properties such as avoiding recognition by the mononuclear phagocytic system (MPS) are also applicable.[63]

Based on the compounds chosen, the systems have different properties in terms of charge, size, stability, viscosity, and stiffness. They are prepared through different processes such as thin-film hydration (Bangham method), double-emulsification method, solvent injection and others. For MLVs' downsizing in a controlled manner, membrane extrusion and microfluidics are commonly used,[64] and subsequently, their membrane can be modified by polymers,[65] peptides,[66] antibodies,[67] radiotracers,[68] fluorescent dyes[69] etc. Liposomes present numerous advantages as they are biocompatible and able to protect drugs from degradation, improving their solubility and stability. They reduce toxicity and the side effects associated with drugs, enabling controlled and sustained drug release, and improving pharmacokinetics. Their ability to carry large drug payloads and incorporate various molecules, including proteins and nucleic acids, expands their therapeutic potentials. Due to their modifiable nature, they can act as targeting drug delivery systems, improving cellular uptake[70] and reducing the immunogenicity[71] of encapsulated drugs. These features collectively make liposomes a powerful and adaptable platform for drug delivery across a wide range of therapeutic applications, including OA.

1.4. Liposomes in Osteoarthritis

The application of liposomes in OA management is a compelling strategy which facilitates the encapsulation of various compounds, mitigates issues related to joint clearance while participating in improved lubrication. Therapeutic agents such as analgesics[72] and anti-inflammatory drugs,[73] disease modifying OA drugs (DMOADs),[74] and even nucleic acids,[75] have been implemented in OA liposomal formulations to reduce pain and inflammation, while in some cases aimed for articular cartilage regeneration or reprogramming cellular phenotypes. These liposomes can be utilized for oral, topical, and IA delivery, with the latter one gaining notable interest due to its distinct advantages over oral or topical methods.

In 1976, for one of the first IA injected liposomes, Dingle and coworkers reported a liposome-based formulation of cortisol to treat acute experimental arthritis in rabbits.[76] One of the primary benefits of IA liposome delivery is the ability to directly target the therapeutic agents to the affected joint. Liposomes enhance drug retention within the joint space,[69,77] offering sustained release[78] and prolonged therapeutic effects.[79] To increase the residence time in the joint space different parameters should be considered such as the size,[80] the surface properties,[81,82] and possible targeting strategies implemented.[82] The size of liposomes plays a crucial role in their joint residence time. Larger liposomes (>10 µm) cannot cross the "leaky" synovial membrane and escape the joint, thereby prolonging their presence within the joint space. Microparticles of ≈3 µm range can penetrate the inflamed synovial membrane but are retained within the healthy synovium (**Figure 1**).[80] The size dependence was reported from the very first studies where Bonanomi et al.[83] found that increasing the size of egg phosphatidylcholine/phosphatidic acid liposomes from 160 to 750 nm improved the joint retention of encapsulated dexamethasone palmitate (DMP) by 2.6-fold in healthy rabbit joints.

The addition of polymer moieties such as PEG or pMPC (poly[2-(methacryloyloxy)ethyl phosphorylcholine]) could lead to increased retention time by limiting cell interactions, while changes of the surface charge can benefit interaction with the SF increasing the concentration in the joint. In parallel, sustained release of drugs incorporated in liposomes prolongs exposure to target tissues, extending their effect. Targeting is another option that liposomes could benefit from for increased half-life and better results. Passive and active targeting approaches are used, with liposomal "intrinsic" properties to be the ones responsible for passive targeting. Cationic surfaces bind electrostatically to negatively changed glycosaminoglycans (GAGs) and collagen in the cartilage.[84] However, as OA progresses and GAGs are reduced, this may not be the most efficient strategy. In parallel, size can affect the targeting properties of a system due to the different porosity properties of the cartilage. In the superficial zone of the cartilage the COLII network creates a mesh[85]

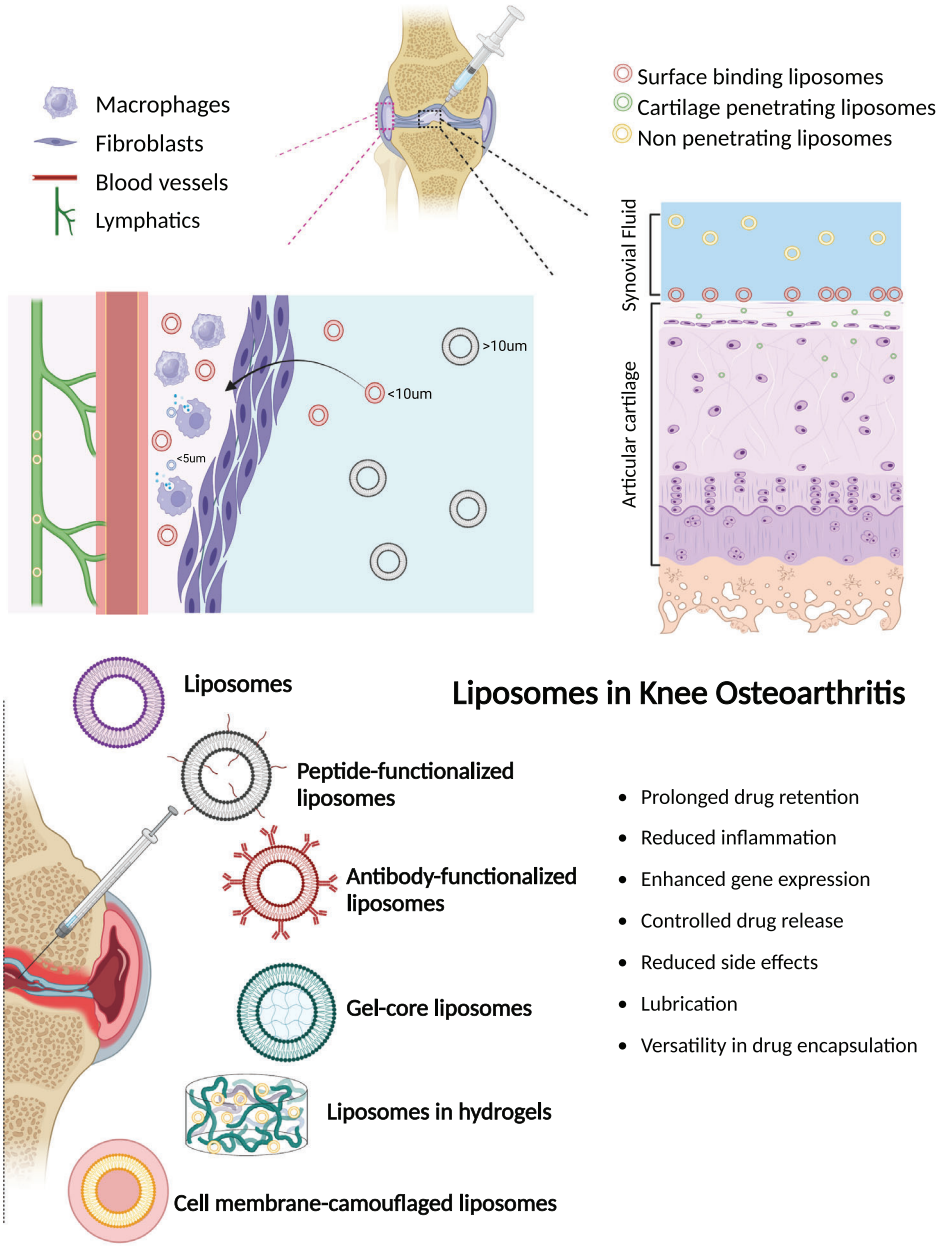

Figure 1. Upper panel: Clearance of liposome upon IA injection depends strongly on their size. Clearance via synovium includes macrophage uptake, blood vessels, and lymphatic drainage. Additionally, their distribution in the distinct areas of the joint varies based strongly on surface modifications and size. Lower panel: Addressing OA with liposomes and liposome-based systems through IA administration. Created in BioRender. Mitsou, E. (2025) https://BioRender.com/d09w772.

where systems >60 nm cannot penetrate,[86] while micelles < 20 nm are able to reach deeper depths where chondrocytes are located,[87] especially if they are of cationic nature;[88] however, such small entities still face the problem of quick clearance due to their size. To address this issue active targeting is increasingly used in liposome design, with certain peptides (such as WYRGRL[86] or TAT[87]) and antibodies against COLII showing great potential. For example, when OA progresses, and collagen is exposed, intravenously injected 200 nm liposomes modified with monoclonal anti-collagen antibodies were localized in OA lesions, proving their importance in early diagnosis and targeted treatment.[89]

Liposomes' interactions with cells, along with their physicochemical properties, play a decisive role in determining the system's biological fate and therapeutic efficacy following IA administration. Synovial cells, chondrocytes, immune cells (e.g., mast cells), endothelial cells (located in the blood vessels), and mesenchymal stem cells are the main cell populations present in the knee joint environment.[90] In OA interventions, synoviocytes have been targeted to mitigate inflammation,

endothelial cells to inhibit angiogenesis, chondrocytes to enhance ECM production, and immune cells to alleviate oxidative stress. Specific properties of the liposomes, including composition, size, membrane fluidity, charge, and surface functionalization, are the ones dictating their interaction with the cellular entities. OA joints contain macrophages of two phenotypes, namely M1 and M2. While M1 macrophages exhibit a pro-inflammatory phenotype, M2 macrophages possess anti-inflammatory and tissue repair functions.[91] The balance between these phenotypically different macrophages significantly influences the progression of OA. Targeting these cells does not consist the biggest challenge in the joint, as they are programmed to recognize and phagocytize any antigen, with an inverse relation between size and uptake. Component changes such as addition of cholesterol, surface functionalization (concentration-dependent PEG, ligands), or embedded liposomes in gels (such as HA)[92] could inhibit macrophage phagocytosis for prolonged effect of the drug, while changing composition by adding phosphatidylserines can have the opposite effect. In parallel, positively charged particles are taken up more compared to negative or neutral NPs,[93,94] while targeting macrophage receptors such as folate receptor β is also a delivery strategy.[95] Fibroblast-like and macrophage-like synovial cells are present in a healthy synovium, while in OA inflammatory cells (B- and T-cells) also infiltrate.[96] Synovial macrophage-like cells (Type A synoviocytes) behave in a similar way as the other macrophages in OA joint. In inflamed synovium, pro-inflammatory mediators are produced from fibroblast-like cells (Type B synoviocytes), followed by immune cell infiltration and vascular hyperplasia, leading to synovial inflammation.[97] Target binding to these cells has been achieved with aptamer-functionalized liposomes for eliminating senescent cells[98] or NF-kB blocking peptides, leading to more targeting-centralized approaches[99] redirecting the uptake from the predominant macrophages. Endothelial cells lining the blood vessels within the joint play also an important role in inflammation and can interact with liposomes, however mainly through targeted approaches such as cellular adhesion molecules and growth factor receptors.[100] Furthermore, liposomes' interactions with chondrocytes are extensively studied and require further rational design due to the cell's location within the articular cartilage. The ability of nanoparticles to diffuse into the cartilage matrix is critical with small liposomes (<150 nm),[101] particularly those modified with peptides,[102] to penetrate the cartilage and target chondrocytes. IA injections of negatively charged or neutral carriers are particularly affected, as electrostatic repulsion or the lack of electrostatic attraction limits drug penetration into the negatively charged cartilage. Larger liposomes larger tend to adhere to the cartilage surface, as SUVs can penetrate the cartilage, whereas MLVs and LUVs remain on the surface, providing lubrication and adhesion according to Pawar et al.[103]

In all cases, the goal is for the liposomes to approach and deliver their cargo to the intended cell population. This is achieved through liposome's degradation following their internalization via mechanisms such as (receptor-mediated) endocytosis[95] or membrane fusion.[104] During endocytosis, liposomes are transported to endosomes or lysosomes, where enzymatic activity and pH changes break them down to release their cargo. In addition, as nucleic acid delivery is emerging, endosomal escape strategies are of interest, though with limited application to date in joint diseases.[105] In contrast, fusogenic liposomes release their contents directly into the cytoplasm through membrane fusion like in liposome-facilitated mitochondrial delivery where the fusion mechanism (achieved by tailoring the lipid composition) was preferred to minimize the risk of lysosomal degradation associated with endocytosis.[106]

Beyond the points discussed above—and the ones covered in the next part of this review—it is crucial to underline an important yet neglected aspect: the structural changes imposed on the IA administered liposomes by the knee environment. Upon IA injection, liposomes encounter the highly viscous SF composed of HA, albumin, glycoproteins (e.g. lubricin), and globulin.[107] Subsequently, the rapid formation of a protein corona is the result of the biomaterial–biofluid interaction (whose composition depends on the liposome's surface functionalization). Obtaining SF, for further experimentation (such as studying changes in the properties of the injected liposomes), is challenging due to its limited availability (low fluid volume, limited donors, and ethical considerations) and the complexity of re-constituting its composition in vitro, leading to only few studies addressing its use.[108–110] Simplified models have been used instead, such as albumin solutions of physiologically relevant concentrations, to examine liposomal behavior. For example, Lin et al. have proved by dynamic light scattering (DLS) that PEG and pMPCylated liposomes retain their colloidal stability (due to their polymer hydrated steric barrier) for at least 5 days in bovine serum albumin.[69] Similarly, Resolvin D1 loaded liposomes have been shown to persist for ≈11 days in a 50% SF solution derived from OA patients, although without extensive structural characterization.[109] Additionally, osmotic pressure could also strongly influence liposome structure and subsequently drug release, as observed in calcein-loaded 1,2-Distearoyl-sn-glycero-3-phosphoglycerol/1,2-distearoyl-sn-glycero-3-phosphocholine (DSPG/DSPC) liposomes. Specifically, liposomes prepared with hyper-osmotic solutions led to increased leakage in SF derived from rheumatoid arthritis patients opposed to iso-osmotic environment.[111] To mitigate this risk, physiological buffers (mainly PBS) are commonly employed avoiding leakage phenomena and structure disruption even in the slightly elevated pH environment of OA. Enzymatic degradation in OA, is another critical factor which can alter the release of encapsulated drugs or induce structural changes in liposomes. Serine proteinases[112] and MMPs[113] degrade liposomal components or surface-functionalized molecules, impacting structural integrity and drug release kinetics. Hyaluronidases, can also affect HA-based systems such as gel/liposome hybrids by degrading their structure changing their release pattern and their morphology,[114] while phospholipase A2 can degrade lipids.[115] Moreover, as in knee OA reactive oxygen species (ROS) levels are elevated (due to mitochondrial dysfunction[116]), they can induce lipid peroxidation having an effect on liposomes' membranes fluidity and subsequently on cargo release.[117] Furthermore, surface deposition (on cartilage surface) and mechanical stress, subjects liposomes to structural changes, meaning that a robust design is necessary. Hydrogenated soy phosphatidylcholine (HSPC) liposomes when IA injected may form a stable, multi-layered liposomal boundary on the cartilage surface, reducing friction and protecting against mechanical wear via the hydration lubrication mechanism.[118,119]

Approaches such as surface force balance (SFB) have been used to prove the stability under the high mechanical loads of hydration layers acting as "ball bearings".[119] Upon additional load, further, to flattening, merging of liposomes could happen releasing their encapsulated contents.

Overall, most studies predominantly emphasize the biological outcomes of liposomes, while neglecting the structural changes that occur in biologically relevant environments. Nonetheless, the reported factors are taken into consideration especially in favor of the new system's design to create more resilient or stimuli-responsive formulations. For example, HA has been used in liposomes design as it adheres to phospholipid membranes and protects from lysis by phospholipase A2, contributing to a more stable environment,[120] while in other cases its structural degradation in the presence of hyaluronidase prolongs the release of drugs (e.g., liquiritin) from liposome loaded microspheres.[114]

As the understanding of the IA administered liposomes for OA progresses, market products have emerged. These include the prolonged-release injectable liposomal formulation of the local anesthetic bupivacaine (Exparel; Pacira Pharmaceuticals, Inc., Parsippany, NJ, USA) designed for IA injection at the surgical site to provide postsurgical analgesia, for a non-opioid pain management approach.[121] More recently, MLVs containing TLC-599 (dexamethasone sodium phosphate) advanced to phase III clinical trial[122] while Lipotalon (dexamethasone palmitate, 4 mg mL^{-1}) is the only liposomal product available on the German market for IA injection, further highlighting the growing potential of liposomes in OA management.

In the following part of this review, we present the last decade's advancements in IA administration of liposomes and liposome-based systems for OA management (Figure 1).

2. Liposomes in Intra-Articular Drug Delivery

2.1. Drugs

IA administered liposomes were first reported in 1976[76,123] and since then numerous drugs have been incorporated within the liposomal environment with increased efficiency and reduced side effects. For instance, celecoxib (Clx) a prostaglandin synthesis inhibitor, is a NSAID with analgesic and anti-inflammatory activity reported for adverse cardiovascular events when orally administered.[124] After its incorporation in LUVs of 4.98 um size composed of soybean phosphatidylcholine and cholesterol, a single IA injection transiently reduced pain in surgically induced OA rabbit model. The outcomes were further enhanced when Clx-liposomes were incorporated into a HA hydrogel (1:1 ratio) which synergistically alleviated cartilage degeneration, because of both sustained release and resistance to liposome phagocytosis facilitated by the HA network.[92] Pawar et al., in order to increase the sustained release and the retention time of the NSAID drug 6-methoxy-2-napthylacetic acid (6-MNA, pro-drug of nabumetone known as Relafen), created a lipid-drug conjugate [6-MNA 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE)—Na double salt] as an alternative to the straightforward encapsulation approach. Highly polydisperse positively charged liposome, upon 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) addition co-loaded with the free form and the conjugate, resulted in five-fold enhancement in retention time, compared to the 6-MNA solution alone after a single IA injection, attributed to its interaction with the negatively charged environment of the articular cartilage. Furthermore, it reduced inflammation a week earlier than the free form, thereby decreasing the cartilage tissue damage in an arthritic rat model.[103] Steroid-based medications such as glucocorticoids are also used IA for pain and inflammation management.[125] Liposomal dexamethasone has demonstrated reduced osteophyte formation, decreased synovial inflammation, and superior pain management compared to free dexamethasone. In parallel, this corticosteroid mediated the polarization of macrophages to the anti-inflammatory M2 subtype in a severe OA model induced by surgical destabilization of the medial meniscus (DMM) in double knockout mice for miR-204/-211, genes crucial for joint homeostasis.[75] Clodronate, another drug acting on macrophages and belonging to bisphosphonates, acts on Type A macrophage-like synoviocytes leading to their apoptosis, while reducing pro-inflammatory mediators attenuating cartilage damage. In an obese associated OA (OOA) model (induced by DMM surgery along with high fat diet), 0.05 mg clodronate liposomes reduced significantly synovial inflammation when IA administered 1 week before DMM surgery, and 1 and 6 weeks upon surgery (prophylactic regimen), which subsequently lead to increase in proteoglycan production and reduction in OA severity according to histological evaluation.[126]

In addition to the commonly used synthetic drugs, the focus of the preclinical research has shifted to naturally occurring substances regulating inflammation and pain, known as endogenous mediators. Resolvin D1 (RvD1), lipid mediator derived from ω-3, in its free form induced macrophage polarization and increased anti-inflammatory gene expression in OOA.[126] Dravid et al.[127] included RvD1 in liposomes of various sizes (150 to 900 nm) with those of ≤350 nm diameter being retained longer at the injection site of non-OA mice (≈14 days), a contradictory finding to the common observation where larger liposomes demonstrate higher retention times.[80] Interestingly, IA injection in DMM-induced OA mice led to a transient pain relief, which was no longer present after 8 days, a possible indicator for insufficient RvD1 concentration or/and a different retention profile between the normal and the OA model tested. Therefore, it is evident that using OA models and appropriate in vitro techniques (SF release profile) would achieve realistic and more accurate results in the context of the disease. The addition of 7% DOTAP lipid introduced a positive charge (+8.1 mV opposed to −30 mV) however it did not improve further the retention time, credited to the PEGylation shield and the resulting steric repulsion between the particles and the cartilage. In a post-traumatic OA (PTOA) model liposomal RvD1 increased the proportion of the synovial M2 cells promoting the resolution of inflammation with substantial limited cartilage damage, osteophyte formation, and pain upon a prophylactic dosage regimen.[127] In an OOA model, the same formulation but modified for higher surface charge (−1.92 mV) in a prophylactic regimen, achieved OARSI (histopathological grading system by the Osteoarthritis Research Society International) scores comparable to the sham group, opposingly to free drug where values were higher however significantly different to the DMM group. As OA is not immediately discernible, the liposomes were additionally administered following a therapeutic dosage scheme. In this scheme, injections were performed 4 weeks after DMM surgery (where OA was established), with

positive results reported for the liposomal RvD1, while drug in its free form was ineffective. The efficacy (even limited) of the free drug in the prophylactic regimen opposed to the treatment one, highlights the differences between the dosage regimens chosen for assessing drug efficacy in disease progression. Liposomal RvD1 resulted in reduced expression of the enzymes related to matrix degradation (MMP13 and ADAMTS5), synovitis, and allodynia, underscoring the importance of dose frequency and the treatment initiation time.[109] Adenosine, an ATP metabolite, regulates inflammation and cartilage homeostasis, and has been encapsulated in phosphatidylcholine/cholesterol liposomes to prevent its enzymatic degradation and extend its half-life. IA injection of 10 mg kg^{-1} liposomal adenosine in a PTOA rat model showed superior joint protection and lower OARSI scores in the treatment group compared to the prevention group, indicating the importance once again of injection timing. This unusual, enhanced effectiveness in an established OA model, was attributed to increased adenosine 2A receptor (A2AR) expression.[128] Co-administration with an A2AR antagonist reversed the effect, underscoring the receptor's role in OA therapy. Although the study lacked detailed structural information about the liposomes, it is listed among the few assessing solely the carrier as control. The same system was tested in more challenging models, including a murine model of OOA, where chondrocyte proliferation and matrix production were activated.[129]

Drugs for targeted therapies encapsulated in liposomes can modulate disease progression through certain molecular pathways. CGS21680, a selective A2AR agonist, can exert anti-inflammatory effects and promote tissue repair. It was found to reduce OA severity in a PTOA rat and in an OOA mouse model with decreased expression of matrix degradation genes in chondrocytes.[128] Drugs of immunosuppressive and even chemotherapeutic properties (i.e., Paclitaxel[130]) have been used in OA with rapamycin (RAPA) to be the most representative interacting with the mammalian target of rapamycin (mTOR) which if inhibited or genetically deleted can reduce the OA severity.[131] In a spontaneous OA model of guinea pigs (age related model), liposomal RAPA (DSPC/cholesterol/octadecylamine) minimized the toxicity of the free drug and significantly reduced OARSI scores from 7.08 ± 0.54 (control) to 3.38 ± 0.59. This formulation enhanced COLLII and GAGs production and reduced MMP13 levels, a fact the free drug solution could not achieve even with the assistance of low-intensity pulsed ultrasound (LIPUS). Blood count and biochemical examinations on guinea pigs showed no adverse effects caused by the system upon single injection, one of the few studies that does not overlook the importance of in vivo toxicity tests to reveal potential adverse effects beyond the local area.[132] Bordon et al.[133] have developed DPPC/DSPG negatively charged MLVs for RAPA delivery and irreversibly aggregating liposomes were developed, in contrast to much of the existing literature. Zinc ($ZnCl_2$) was used as the aggregation factor along with its anti-inflammatory and antioxidant properties. The 90 µm aggregates presented sustained drug release (86% in 7 days versus 90% in 3 days for non-aggregated liposomes) and assumed to have longer retention time by evading phagocytosis, without in vivo data reported. Notably, the authors pointed out that the addition of 30% cholesterol decreased the encapsulation efficiency, avoiding its use, while specific lipid-to-drug ratio resulted in desired properties. Dasatinib, a tyrosine kinase inhibitor, and quercetin were co-encapsulated in liposomes to target senescent fibroblast-like synoviocytes in the synovial intima. This approach aimed to increase intracellular drug delivery while facilitating a targeting aptamer along with a −20 mV charge, reduced cartilage interaction and increased interaction with synovial cells. These cationic HSPC/DSPE-PEG2000 liposomes (120–150 nm) increased retention in the knee joint of non-OA mice from 3 to 5 days compared to the dye solution. Twice-weekly injections over 4 weeks, starting after OA establishment, confirmed reduced cartilage degeneration. Interestingly, even without the targeting aptamer, the system decreased cartilage degradation.[98]

2.2. Natural Compounds

Natural compounds, including dietary supplements and plant derived substances (resveratrol[134] and curcumin[135]), are currently under examination in OA due to their safety, anti-inflammatory properties, cost-effectiveness and involvement in multi-signaling pathways involved.[136] The most common natural viscosupplementation is HA with presumed lubricating properties, however, an approach still debatable with results that in cases report a placebo effect.[137] Glucosamine sulfate (GS), a dietary supplement composed of glucose and an amine, can stimulate proteoglycan and collagen synthesis in the ECM but its effectiveness is hindered by limited oral bioavailability or low residence time after IA injection. PEGylated cationic liposomes of tetradecyl quaternized carboxymethyl chitosan along with 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and cholesterol were prepared via emulsification method. The systems of ≈236 nm diameter were non-cytotoxic toward macrophages while the IA injection did not affect liver function related biochemical parameters (alanine transaminase-ALT, aspartate transaminase-AST) satisfying the in vivo biosafety aspect. DMM induced OA mice model received injections of liposomal GS at 4-day intervals for 6 weeks (first injection 7 weeks after surgery). This administration scheme resulted in surface thickening and reduced presence of hypertrophic chondrocytes, along with a decrease in anti-inflammatory factors.[138] Ji et al. loaded the same drug in DSPC liposomes possessing anti-inflammatory effect, apart from their boundary lubricating properties, leading to increased mRNA expression of aggrecan, COLLII and decreased interleukin-1-beta (IL-1β), MMP1 and even the pain related gene preprotachykinin 1 gene in chondrocytes in vitro.[78] The effect of the free GS in vitro was higher compared to its encapsulated form; however further in vivo studies are needed to examine the advantages the liposomal encapsulation offers in the challenging environment of increased friction. Urolithin A, a dietary supplement known for its ability to enhance mitophagy, reduce inflammation, and decrease ROS production in chondrocytes, was encapsulated in liposomes composed of lecithin, cholesterol, and DSPE-PEG–peptide. Incorporation of these liposomes into a HA methacrylate (HAMA) matrix resulted in increased ROS scavenging activity with improved osteophyte production and subchondral bone quality following DMM-induced OA in rats.[139] Triptolide, a diterpenetriepoxide from *Tripterygium wilfordii*, encapsulated in a liposomal formulation of egg yolk lecithin and cholesterol has shown anti-inflammatory properties among

others;[140] in this study, retention time was measured in OA mice, diverging from the misrepresentative, nonetheless conventional, practice of studying retention in normal mice. Upon a single injection in the monosodium iodoacetate (MIA)-induced OA rat model, extended residence time was observed in the knee joint. Repeated injections 7 days post OA induction resulted in smooth cartilage with strong proteoglycan expression, accompanied by reduced swelling. Furthermore, in vivo biosafety was confirmed through histological and blood analysis tests.[141] Interestingly, this paper proposed the incorporation of liposomes into microneedles as an alternative to IA administration, offering an effective transdermal delivery approach, however without explaining the mechanism by which the compounds traverse from the skin to the joint. Gallic acid, a phenolic compound with strong antioxidant properties from *Olea europea* has also been studied by its encapsulation in a lecithin, cholesterol liposome stabilized by pMPC-octadecylamine (pMPC-ODA). pMPC (MW ≈47kDa) provided stability to the liposomes, while at the same time acting as a lubricant with increased retention time and resistance to phagocytosis (3.6-fold less macrophage uptake). Intracellular ROS levels in chondrocytes, along with increased COLII and aggrecan expression was a result of the liposomal gallic acid formulation. The OARSI scores were significantly reduced compared to PBS injected animals, with smooth cartilage surface, less injury and regular chondrocyte distribution, but still higher than the sham groups. Interestingly, the liposome in the absence of the bioactive and potential therapeutic compound, nonetheless benefited the cartilage in the OA model, indicating a link between effective lubrication and protection of cartilage in OA.[142] Resveratrol in a mitochondrial targeting liposome proved that its incorporation in liposomes—even in the absence of cartilage/mitochondria targeting peptide—could reduce erosion and deformation in cartilage surface by preventing oxidative damage, while fish oil and others are implemented for their antioxidant properties in OA treatment.[143,144]

The potent antioxidant and anti-inflammatory properties of natural compounds are being exploited in OA treatment. Co-administration strategies using these natural compounds could enhance the efficacy of drugs and treatments while minimizing the side effects associated with chemically synthesized drugs when incorporated in liposomes.[98] This approach not only paves the way for cost-effective and more natural treatments but also serves as a basis for synthesizing new drugs inspired by effective natural compounds.

2.3. Non-Conventional Approaches

While conventional drugs have been the cornerstone of the OA management, emerging gene delivery approaches grow in popularity for disease modifying options, personalized treatment and long-term effects. He et al.[145] co-administered lornoxicam with microRNA (miRNA) to act at post-transcriptional level. MiRNAs, single stranded noncoding RNAs, can play a significant role in OA as they are able to participate in the regulation of cellular processes by binding, and silence complementary sequences in mRNA molecules. MiR-140, has been involved in the regulation of IL-1β, playing a significant role in different aspects of OA from chondrocyte differentiation to aggrecan production. A lecithin/cholesterol cationic liposome of ≈286 nm diameter, supplemented with ODA and Tween 80, was used and electrostatically bound with the negatively charged miR-140, with relative stability against RNase. The co-administration with the NSAID lornoxicam facilitated a stepwise treatment approach where the drug reduced inflammation and subsequently miR-140 reached chondrocytes. This approach is carefully designed as the absence of lornoxicam, led to inflammation and higher MPS clearance, making the miRNA unable to reach its target. Another microRNA treatment approach has been studied by Jin et al. with liposomes of undisclosed composition incorporating miR-9-5p and subsequent IA injection into OA ankle joints of rats, where related injuries were recovered.[146] Double transfection using IA injected interleukin-1 receptor antagonist (IL-1Ra) and transforming growth factor-beta 1 (TGF-β1) genes into joints, in combination with the commercially available Lipofectamine 2000 (3:1 mixture of 2,3-dioleoyloxy-*N*-[2(sperminecarboxamido)ethyl]-*N*,*N*-dimethyl-1-propaniminium trifluoroacetate and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE)), has proved beneficial in OA rabbit model for cartilage repair after 2 injections in 1 week.[147] Except miRNA, small interfering RNA (siRNA) has also been studied in OA treatment by disrupting the mitochondrial DNA and its inflammatory response. Positively charged liposomes of DOTAP and cholesterol (size ≈100 nm), efficiently incorporated the negatively charged siRNA, and subsequently integrated in an in situ injectable chitosan hydrogel system which increased the joint space width while minimizing severe surface abrasion and disorganized chondrocytes (irregularly positioned). It must be noted that the authors attribute the reduced cartilage erosion to the lubrication properties of the systems, a claim not supported by appropriate carrier controls and the absence of literature data regarding DOTAP's lubricating properties.[148]

Peptides and hormones represent another category extensively studied in vitro and/or in preclinical studies along with liposomes regarding their possible effect in OA. These range from common factors like TFG-β1[149] to more sophisticated synthetic structures such as chimeric peptides like REG-O3[150] which has an amino acid sequence of growth hormone factor and somatostatin combined for improved joint function. Uniquely, organelles such as mitochondria, due to earlier observations that mitochondrial dysfunction in chondrocytes contributes to cartilage degeneration during OA progression, have been lately incorporated in liposomes. Systems designed to fuse with the membrane of target cells were developed by DSPE, DOTAP and a fluorescently labeled lipid (phosphoethanolamine) for the delivery of exogenous functional mitochondria to chondrocytes. This strategy avoids the digestion of macrophages from lysosomes, which occurs following endocytic cellular uptake of non-liposomal mitochondria. Organelles obtained from healthy donors were successfully encapsulated, driven by the positive charge of DOTAP as opposed to the anionic mitochondrial surface, with sizes of 610 nm and highly positive surface charge. Despite their stability issues (mitochondria stable up to day 3), in vivo studies using OA animal models (MIA-induced) show that "mitofusosomes" localized and were taken up by chondrocytes in the articular cartilage, significantly outperforming mitochondria alone and restoration of the damaged cartilage. Given that

the system's size exceeds the collagen network pores in cartilage, their use is possible in environments where the cartilage surface is already degraded with increased pore size, to reach chondrocytes. Micro-CT scans indicated that the highest cartilage recovery occurred with mitochondrial liposomes, restoring mitochondrial bioenergetics and biosynthetic capacity, ultimately slowing, or reversing OA progression.[106] As mitochondria are in the range of 0.5–10 um, their involvement in early stages of OA may not be applicable due to inability to reach chondrocytes in the different depths of the articular cartilage.

2.4. Liposomes with Targeting Moieties in Intra-Articular Drug Delivery

Upon IA injection in the synovial cavity a nanoparticle has a specific target: the cartilage, the chondrocytes, the synovial cells or its retention and interaction with the SF. Targeting the cartilage may entail focusing on the surface to provide boundary lubrication (see Section 2.5) or embedding in the cartilage and subsequently penetrating to reach chondrocytes. Peptides and antibodies have been used as active targeting molecules to guide liposomes in the site of the desired action. Peptides, such as the HAP-1 (SFHQFARATLAS) for targeting fibroblast-like synovial cells at sites of inflammation[82] or CKPFDRALC named ART-2[151] and others,[99] have been developed, where the majority was administered intravenously. Recently, IA administered liposomes have been modified with peptides for targeting chondrocytes, and specifically the subcellular mitochondria which play a significant role in oxidative stress and energy supply for the cell. WYRGRL and SS-31 peptides, with cartilage and mitochondria targeting activities respectively, were attached through lipid-PEG spacer-chemistry in the membrane of lecithin/cholesterol liposome with different in length PEG-spacers. It is assumed that the longer PEG spacer is related to the targeting of the WYRGRL peptide, which must attach first on the cartilage for subsequent action. WYRGRL is a peptide well known for its COLII binding properties (binds to the α1 chain of COLII), discovered by phage display method,[86] which increases targeting and/or retention time of nanoparticles from polymeric ones[152] to exosomes and liposomes[139] in OA joints. Additionally, SS-31 peptide, was used for increased mitochondrial internalization, probably due to its binding to key components of the ADP/ATP transport and production pathways[153] in parallel with its cardiolipin affinity, a phospholipid mainly found in the inner mitochondrial membrane. This 130 nm "mito"-battery, protected OA chondrocytes, even in the absence of its bioactive compound-resveratrol, by restoring mitochondrial electron transport chain. Upon two injections in OA rats in a 6-week dosage scheme, oxidative stress was reduced leading to restoration of the cartilage.[143] CAP (chondrocyte affinity peptide) has also been used to decorate particles for IA administration. In a hybrid exosome–liposome system, peptide modification was facilitated for the delivery of CRISPR/Cas9 MMP13 plasmids to chondrocytes for knocking out the corresponding MMPs.[154] The liposome offered extended stability to the exosomes while retaining the exosomal membrane proteins for chondrocyte targeting, providing fluorescent signals for a minimum of 7 days upon IA injection in DMM induced OA rat model (**Figure 2**). This study is among the few evaluating the retention time of the systems in the exact OA model that the efficiency assessment was afterward tested upon IA injection, in contrast to the commonly used non-OA mice.

Actively targeting distinct cell population at well-defined phenotypic states, could also prove beneficial in OA. Chen et al.,[98] to address senescence in synovial cells and its participation in joint degradation, formulated systems that exclusively target senescent synovial cells leaving non-senescent ones unaffected. Inability to divide and resist apoptosis leads to an inflammatory environment in the surrounding cells and tissues. The drugs of choice were incorporated in an aptamer functionalized liposome. Aptamers, like antibodies, have a unique 3D recognition with their targets. In this case CX3 aptamer selected to bind to the fibroblast-like synoviocytes (FLSs). FLSs senescence has an earlier onset in OA and is more critical than chondrocyte senescence. HSPC and DSPE-PEG$_{2000}$-maleimide composed liposomes were used for the attachment of the aptamer with a negative charge. Dir (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide, lipophilic far-red dye) incorporating liposomes upon IA injection had higher retention time in the joints when functionalized with aptamer and lower signals reported in the internal organs compared to plain liposome. DMM induced OA mice upon multiple injections lead to significantly lower OARSI score attenuating cartilage degradation.

Targeting through cell membranes is an innovative targeting approach, still in its infancy, currently finding applications in OA management. Nanoparticles are being coated with cell membranes, for targeting and immune evasion. Deng et al.[155] addressed the challenges of rapid clearance and, most importantly, the tissue and cell-targeting limitations of commonly used glucocorticoids. To address this issue, liposomes were coated with membranes derived from apoptotic neutrophils, which are the first immune cells recruited to inflamed joints in OA, facilitating recognition and phagocytosis by both activated synovial fibroblasts and pro-inflammatory M1 macrophages. The nanoparticles, loaded with the anti-inflammatory drug triamcinolone acetonide (TA) had a more negative surface charge than the uncoated systems, avoiding cartilage attachment while increased the interaction with the SF components. In vitro studies demonstrated that TA-loaded, camouflaged liposomes (TA-NM@Lips) effectively repolarized M1 macrophages to their anti-inflammatory M2 phenotype and deactivated pathological synovial fibroblasts. Retention studies, in OA joints, showed 28 days retention upon a single injection. Multiple injection effectively attenuated synovitis, and simultaneously provided long-lasting pain relief in an OA-related pain model induced by MIA, while proving biologically safe. It is worth mentioning that antibodies have even been used to surface functionalize liposomes, also in terms of OA, mainly for monitoring disease's progression.[156] As COLII is exposed in the early stages of cartilage degradation, antibodies were used for its detection upon IV administration in PTOA[157] and DMM OA[158] in spontaneous OA in guinea pigs.[89,159] These 200 nm DOPC/DSPE-PEG2000-antibody liposomes were efficiently bound only in the load-affected knee, making them able to detect early stages of OA. Exploiting the unique properties of this system with the monoclonal anti-COLII antibody (MAbCII) also via IA administration could be of great advantage not only for

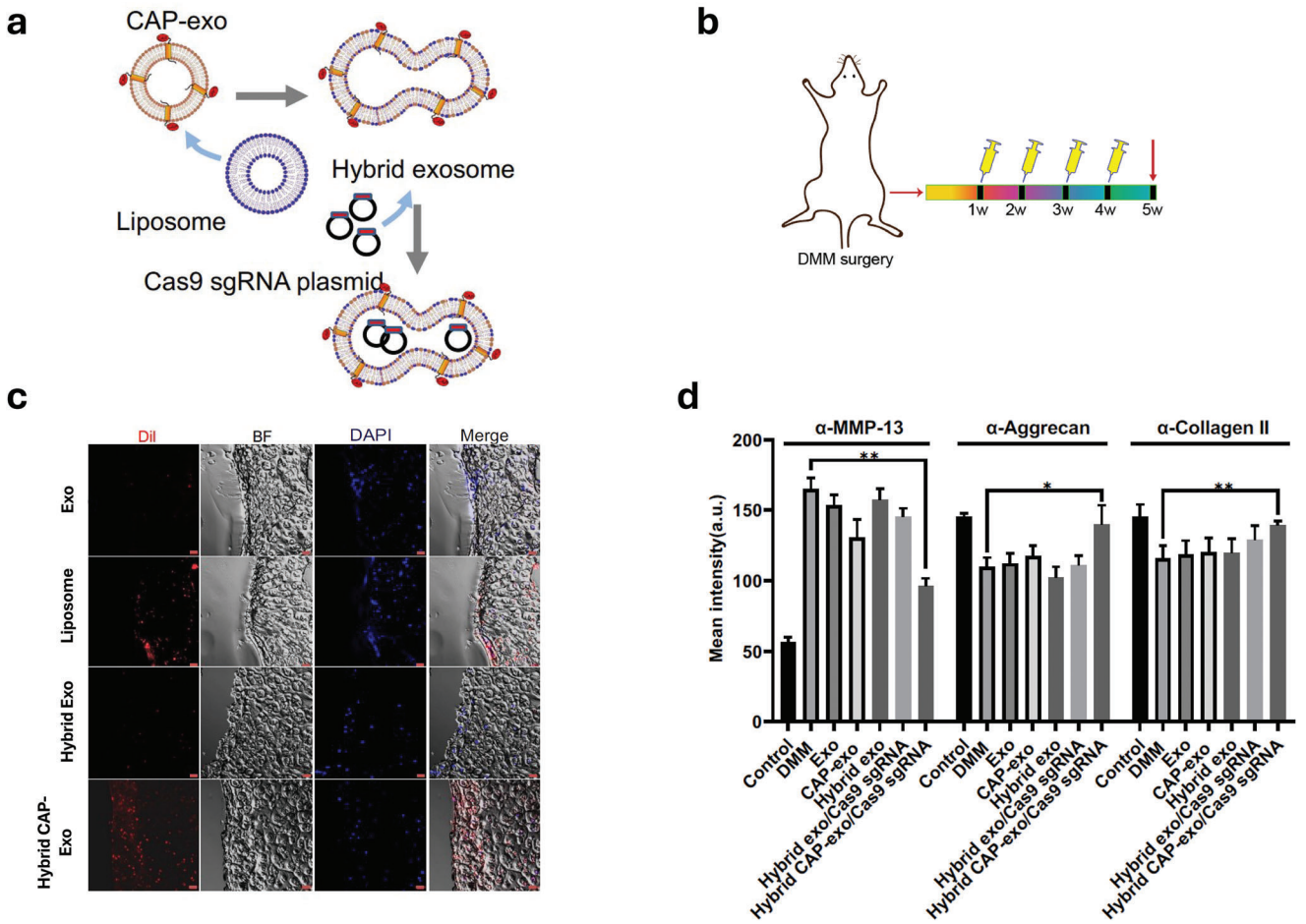

Figure 2. a) Schematic illustration of chondrocyte-specific genome editing by hybrid exosomes (Exo). b) Schematic illustration showing the dosage regimen. c) Representative confocal imaging shows the DiI signal of hybrid CAP-Exo and Hybrid Exo in the rat cartilage. Scale bar, 20 μm. d) Measurement of MMP13, aggrecan, and COLII levels in the knee joints. Quantification of the fluorescent signals in different groups. Adapted from ref.[154]; Reproduced with permission from the authors.

research reasons but also as a non-invasive diagnostic tool and possible targeting drug delivery.

2.5. Liposomes in Lubrication of Joints

A healthy knee joint can sustain excessive local stresses up to even 25 MPa,[159] while simultaneously enabling low friction articulation ascribed to the superior lubrication of the articular cartilage.[160] High friction—as a result of injuries, body weight, activity, and aging—could lead to the wear of the cartilage directly, but also through the indirect upregulation of matrix degrading enzymes produced by the mechanosensitive chondrocytes under increased shear stress (mechanoflammation).[161] The term refers to acute mechanical stresses which result in inflammatory signaling and induce inflammatory genes in chondrocytes which contribute to the breakdown of cartilage in OA. As of today's understanding, the boundary lubrication via the hydration lubrication[162–165] mechanism dictates the efficient articulation ($\mu \approx 0.001$–0.02) through a synergistic action of molecules in the cartilage. HA, lubricin,[166] and aggrecan,[167] have been considered as lubricants. However, when operating independently they do not achieve the desired low friction values.[168] This redirected the interest to surface-active PC lipids, the most abundant lipids in the articular cartilage. Examination of the outer surface of cartilage in rats and dogs[169,170] found spherical lipid formations, supporting further the idea that these molecules—separately or synergistically—could lead to the protection from wear-and-tear in cartilage. Several studies revealed that indeed friction coefficients in the range of $\mu \approx 10^{-4}$, comparable with that of healthy cartilage, could be achieved with different PCs such as HSPC, DSPC, and 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC).[171–174] Extremely low friction and robustness in both artificial membranes (i.e., mica substrates) or joint replacement-related materials through different techniques such as SFB and atomic force microscopy (AFM) paved the way for the use of PC liposomes as IA lubricants, apart from their drug delivery properties.[18,175] Various liposomal systems have been studied for their lubricating properties examining parameters such as size,[108] polymer functionalization,[176] and mechanical stability (e.g., cholesterol addition[177]) on lubrication. However, in vivo studies employing these systems for OA treatment

have neglected the lubrication aspect, predominantly focusing on drug delivery. After HSPC liposomes proved to be efficient lubricants,[119] PEGylated systems were developed and IA administered in normal mice knee joints, with a half retention time of 17.2 h, remarkably increased compared to the IA injected HA (<1 h) used in the clinic.[69] Although the colloidal stability of the HSPC system was improved upon PEG addition, reduced lubrication was reported. Substitution with pMPC polymer led to particle stabilization and efficient lubrication. pMPCylated 170 nm HSPC systems significantly increased the half retention time after single injection in non-OA CD1 mice to 84.7 h. This outstanding performance can be explained by the charge–dipole interaction between the PC groups of pMPC and HA.[69] These lipid-based lubricants ($\mu \approx 0.01$–0.02 in HA coated surfaces, pressure 4 MPa measured with surface force balance apparatus) demonstrated a ≈50 h half-time retention in a different non-OA murine model, proving the effect of the animal model in the experimental results. In a DMM-induced OA model, the lubricant, efficiently decreased the MMP13, and Il-1β expression compared to PBS injection by reducing the stress the embedded chondrocytes feel, while left unaffected the non-shear responsive Timp1 (tissue inhibitor metalloproteinase).[77] The dosage scheme included a first injection 2 days before joint destabilization and a second one post-surgery. Most importantly, similar pMPCylated systems due to their increased retention time and their lubrication properties, have been preclinically tested in vivo in rats for their safety even at repeated high concentrations (twice or thrice ≈30 mM), with no toxicity observed upon 1 week (short-term) or 13 week (long-term) follow up. No blood or histology abnormalities were reported apart from minimal to mild macrophage accumulation (with no inflammatory cells, and no significant damage to the articular structure across all test groups), findings fully reversible upon testing on week 13th.[178] In parallel, in the larger and more representative animal model of sheep, no signs of toxicity documented (locally or systemically) after single IA injection, paving the way for their use in the clinic upon 6-week follow up period.[179]

Transitioning from using liposomes solely as drug delivery systems and the emerging lubrication involvement in OA treatment, recent research has directed attention on combined approaches that exploit the synergistic benefits of these functionalities. Designing systems capable of simultaneously addressing drug delivery (sustained and/or targeted) while providing lubrication represents a promising approach to alleviate OA symptoms and address the underlying disease pathology. Such dual-functionality liposomes can offer: a) reduced joint friction, alleviating pain, and decelerating mechanical wear on cartilage, while b) deliver encapsulated drugs to directly target inflammation, repair tissue damage and reduce pain. Specifically, increased friction generates cartilage debris that enters the SF, activating immune cells and triggering an inflammatory response that participates in OA progression.[180] By introducing lubricating liposomes cartilage debris is reduced, while the presence of anti-inflammatory drugs could reduce the pre-existing inflammation and prevent further progression. Furthermore, reducing friction down-regulates MMPs[77] and possibly halts ROS production,[181] thereby enhancing the therapeutic strategy. Consequently, this dual-approach design, incorporated into a single injection, allows for improved joint function and long-term disease management potentially reducing the frequency of interventions and enhancing overall therapeutic outcomes minimizing discomfort and injection-related risk.

However, as lubrication through liposomes in OA is a relatively recent approach, specific limitations, which can affect efficiency, must be acknowledged. Mainly these limitations arise from the influence of the biological environment on liposomes, possibly compromising their overall stability and structure. As previously mentioned, liposomes are prone to degradation by enzymes present in the SF, leading to premature drug release and reduced therapeutic efficacy (off target effects and reduced IA retention time), making precise control of the rate and duration of drug release crucial for optimal therapeutic effects. Additionally, different lipids lead to liposomes of different mechanical characteristics which must be taken into consideration when developing lubricants, with the lower compressibility to present higher friction coefficient, while MLVs of µm size reported as better lubricants.[108] In terms of liposome mechanical changes (ranging from deformation to fusion and aggregation), it would be useful to identify the timeframe within which the drug must be released before any shear forces applied and disrupt the liposomal structure, which has not been addressed in the literature so far.

An additional challenge arises from the different sites of action for lubrication and drug delivery in some cases. Lubrication occurs on the cartilage surface, but certain drugs are intended to target macrophages or synoviocytes, located in distinct compartments of the joint limiting the dual functionality of the system. In the case though, of drugs intended for articular cartilage targeting this could be a significant advantage, underscoring the necessity to develop systems with appropriate properties (e.g., increased size could be beneficial for lubrication and joint retention time; however, it could halt the penetration of liposomes and subsequently of the encapsulated drug into the cartilage).[108,152] Furthermore, active targeting—often employed by functionalizing liposomes with peptides—is another commonly employed strategy which can alter the membrane structure and potentially compromise the initial lubricating properties (charge interactions and steric hindrance), while the protein corona developed around the liposome can substantially change the lubricating properties identified in vitro.

Beyond these complexities, the literature has begun to study liposomes under the prism of dual functionality, as discussed below. However, we highlight the need to intensify research endeavors to optimize and refine dual-function liposomal systems for clinical application in OA.

Among these studies, Yang et al. has used the highly lubricious zwitterionic polymer pMPC attached to ODA to functionalize their gallic-encapsulated liposomes composed of lecithin and cholesterol creating a multi-purpose system for lubrication, macrophage escape, and extended retention time. The pMPCylated (47 kDa) system led to μ values of 0.03–0.04 in a mechanically destroyed pig articular cartilage (sliding velocity of 0.1–1.0 mm s^{-1}), significantly better than PBS and bare liposomes. Interestingly, in vitro, the pMPCylated systems—in the absence of gallic acid—did not improve the secretion of important proteins in chondrocytes while IA injection in a MIA-induced OA rat model resulted in a different behavior. The 162 nm negatively charged pMPCylated system—in the absence of gallic acid—showed

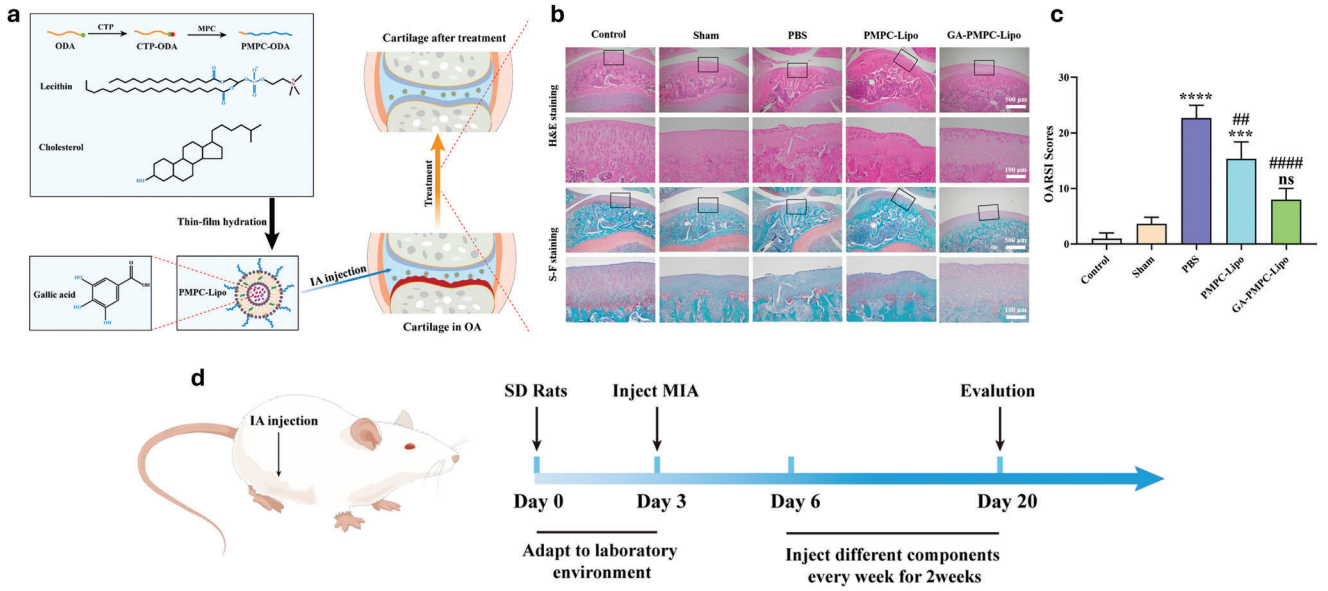

Figure 3. a) Schematic illustration of the preparation and principle of gallic acid (GA) Safranin O-fast green-PMPC–Liposomes. b) Representative images of hematoxylin and eosin (H&E) staining and safranin O-fast green (S–F) staining. c) The OARSI scores of articular cartilage of each group. ($n = 3$, the values are shown as mean ± standard deviation (SD), $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, compared with the control group; $\#\#P < 0.01$, $\#\#\#P < 0.001$, $\#\#\#\#p < 0.0001$, compared with the PBS group). d) Illustration of the in vivo dosage scheme. Reproduced with permission.[142] Copyright 2020, Elsevier Ltd.

improvement in the cartilage histology with relative integrity compared to the PBS group, while the addition of the antioxidant led to a synergistic effect with pronounced improvement (**Figure 3**). This disparity between in vivo and in vitro behavior, where normal and shear forces are not involved, supports the idea that lubrication is a key factor in the observed improvement. However, since normal pressure values in a knee joint are much higher compared to the ones applied in the present study,[142] it is important to adjust to a more realistic configuration.

One step further, liposomes have been combined with hydrogels for sustained release[182] and increased stability[183] while the hydrogel matrix can act as reservoir for lubricating materials.[184] In earlier approaches, DSPC liposomes were used to cover the outer layer of silk microspheres,[185] however the drawback of lipid removal upon shear had not been addressed in this case. Mimicking the structure of the gel-like cartilage, with additional exposed PC boundary layers, this "fortified" hydrogel can enable continuous lubrication as its wear leads to newly exposed layers of lipids. This boundary layer addition in the form of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) or HSPC MLVs at the surface of a poly(hydroxyethylmethacrylate) hydrogel reduced the friction from μ of ≈0.5 in lipid free gels to 0.01 in the case of incorporated PC-MLVs gels at the load of 1.53 MPa.[186] Lei et al.,[187] following the same rationale, combined a hydrogel HAMA matrix with the highly lubricious HSPC in a microsphere construction for a synergistic effect from both ball bearing and hydration lubrication mechanism. In this study, RAPA–loaded cationic MLVs of 102.3 ± 35.2 nm, were developed and along with microfluidics and photopolymerization resulted in a liposome/HAMA complex of 208.36 ± 7.37 µm. This system facilitated a two-step lubrication process, where initially the outer liposomes are quickly removed from the complex but form self-renewable hydration layers. This provides a stable lubrication effect, in addition to an extended retention time due to its resistance to hyaluronidase enzyme (63 days to degrade). Upon IA injection, the systems could remain at the site of action for up to 2 months while in a rat OA model, the RAPA-loaded systems prevented the decrease in joint width, yielding results identical to the sham group. Notably, in the absence of RAPA histological examination revealed that the systems contributed to the maintenance of relative integrity and regular cartilage structure compared to PBS group, indicating that the carrier, probably attributed to is lubricant properties, could protect cartilage from defects. However, Mankin score (histopathological grading system), cellular abnormalities and articular space width values did not point out any differences between the liposome–gel hybrid system in the absence of drug compared to PBS group. The same liposome was further encapsulated in an aldehyde-modified HA and proved decreased MMP13 expression compared to the PBS group in vivo even in the absence of CLX drug supporting, this time, the benefits of the liposome–gel system as an effective lubricant.[188] A study of Bordon et al.[133] combined drug release with lubrication properties using an "unorthodox" strategy, through liposome aggregation. The liposomes formed micrometer size aggregates to increase retention time in the knee joints while the tribological assessment both on silicon surfaces and ex vivo porcine cartilage demonstrated that the aggregated liposomes reduced the friction coefficient further. However, this approach relies on controlled aggregation which has not been tested in vivo and could potentially lead to application limitations. Interestingly, liposomes combining lubrication with anti-inflammatory properties were also found to benefit other OA affected joints such as temporomandibular joint.[189] The 224 nm liposomes, made of HSPC,

DSPE-PEG$_{2000}$, and cholesterol with meloxicam, inhibited chondrocyte apoptosis and prevented cartilage matrix degeneration under severe inflammation.

Nonetheless, while the use of PC liposomes in vivo is widespread, there is limited data available regarding the beneficial effect of sole lubrication. Indeed, with one exception, all studies have failed to show that lubrication alone can suppress upregulation of catabolic mechanosensitive enzymes with Zhu et al.[77] to be the first to show that sole liposomes are able to downregulate MMP3 with unaffected non-mechanosensitive genes. For that reason, the addition of appropriate controls, in the absence of loaded compounds could enrich our knowledge regarding lubrication and its participation in OA development and lead to reduced drug-related side effects while potentially minimize the need for repeated interventions.

2.6. Hybrid Formulation Approaches

The use of liposomes in OA treatment has been proved beneficial, however combined formulation approaches that integrate other delivery platforms with liposomes are developed seeking to improve the outcomes in OA management. As HA is a component of ECM with multiple functions from regulating inflammation to lubrication it has been used in several approaches for OA, including its hydrogel state. Alleviation of cartilage degradation through activation of mitophagy in chondrocyte targeting was made possible with the use of lecithin, cholesterol, DSPE-PEG$_{2000}$-WYRGRL, and Urolithin A (in a ratio of 4:1:0.1:0.2 w/w) incorporated in HAMA microsphere through microfluidics and photopolymerization. Liposomes assisted microspheres to resist degradation from 6 to 9 weeks creating a hydration layer acting as a physical barrier to hyaluronidase. The positively charged liposomes (+5.4 mV) "loaded" on the porous microspheres of 212.9 um were able to deliver the natural metabolite in chondrocytes. This multi-step formulation approach, even though it led to lower encapsulation, effectively reduced the ROS production. DMM induced OA rats were IA injected (2-, 4-, and 6-weeks post-surgery) and the total Mankin score along with better COLII expression were improved in the case of liposome-incorporated hydrogels, compared to the plain hydrogel.[139] HAMA was used by Chen et al.[190] also for the incorporation of a HSPC/cholesterol/DSPE-mPEG$_{2000}$ with HC-030031 inhibitor forming microspheres of 248 um with reduced encapsulation (64.29%) but with sustained release for almost 3 weeks. In an OA model the system managed to reduce MMP13 positive cells and reduce OARSI score of the cartilage. Further modification of the HAMA-liposome microgels surface with dopamine for ROS-triggered release of gallic acid, decreased the OARSI score by 50% compared to PBS in MIA induced OA model.[81]

Kartogenin is a promising compound of chondroprotective properties when IA administered and Yang et al.[191] developed a liposome-in-hydrogel approach but this time with the use of gelatin methacryloyl (GelMA). 100 um microgels of a porous external (millimeter) and internal (nanometer) structure led to the same pattern of lower encapsulation/slower release (up to 3 weeks) and resistance against collagenase for 5 weeks. DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine) IA-administered lipo-GelMA fluorescence signal remained for 3 weeks stable in non-OA mice, compared to the 2-week retention time of liposomes, leading to the highest retention time reported for related systems. In DMM OA rat model, the drug-loaded microgel system showed the most favorable histological image, closely resembling the sham group, with minimal abrasion and increased tissue cellularity. Additionally, the levels of COLII and aggrecan were positively influenced by both liposomes and hybrid system, with the second one having a sham phenotype. Chondroitin sulfate methacrylate (ChsMA) hydrogel microspheres[114] were loaded by electro spraying method with liposomes carrying the antioxidant drug liquiritin (HSPC: cholesterol: mPEG200-DSPE: liquiritin 63:30:5:2 molar ratio) presenting half retention time in non-OA rat joints of ≈16 days. Repeated IA administration (four injections in 10 weeks' time) in DMM induced rat model resulted in significant reduction in cartilage degeneration, proteoglycan loss, and OARSI scores compared to the PBS treated groups while the blank microsphere controls and the liposome had also a positive effect. Interestingly, this is another case where the MMP13 expression is reduced upon liposomal injection, possibly related to the HSPC lubricating properties. A recent study,[192] inspired and along the same lines as the previous one, presents a multifaceted approach combining three different structures into a single therapeutic system for OA. The innovative design consists of ChsMA microspheres, which form the core structure, covered with a stimuli-responsive GelMA shell, and further loaded with HSPC LUVs liposomes containing Clx. The ChsMA microspheres had a size of 195.5 µm and a pore size of 22.8 nm. The system demonstrated stronger adherence to chondrocytes while in the presence of collagenase II, the core of the microsphere remained intact releasing chondroitin sulfate for cartilage repair until day 35. In a surgery-induced OA rat model, the subjects received an injection of the microspheres 4 weeks post-surgery (well-established OA) and after an additional 4 weeks, leading to an increase in chondrocyte number. Gelatin has been also used by Li et al.,[193] who utilized gallic acid-grafted gelatin to create an injectable hydrogel by enzymatic crosslinking for delivering the FDA-approved peptide teriparatide, which exhibited significant cartilage degeneration, reduced subchondral bone erosion, and osteophyte formation compared to all control in OA.

As OA is a dynamical disease and its progression differs in each individual, stimuli-responsive hydrogels have the potential to manage disease progression on-demand. Stimuli-responsive microgels (240 um) incorporating liposomes of 92 nm (DOTAP/DOPE/Cholesterol) were developed with the use of a HAMA/MMP13 substrate peptide via UV-crosslinking. The incorporated amino acid sequence was cleaved by the presence of MMP13, releasing the liposomes when needed adapting to changes in inflammatory state. Upon OA induction in rats, two injections (1- and 4-weeks post OA induction) lead to substantial increased medial joint space while the cartilage histology and osteophyte production was close to the sham group 8 weeks post-surgery. The superiority of the stimuli-responsive system over the conventional one could be adapted as a strategy for the early-stage treatment of OA in a more effective way. The systems will be able to address precisely the problem when needed, thereby preserving the encapsulated compound from unnecessary usage.[194]

In a reverse case scenario, gels can also be integrated in the inner part of the liposomes with examples such as PEG-DMA,

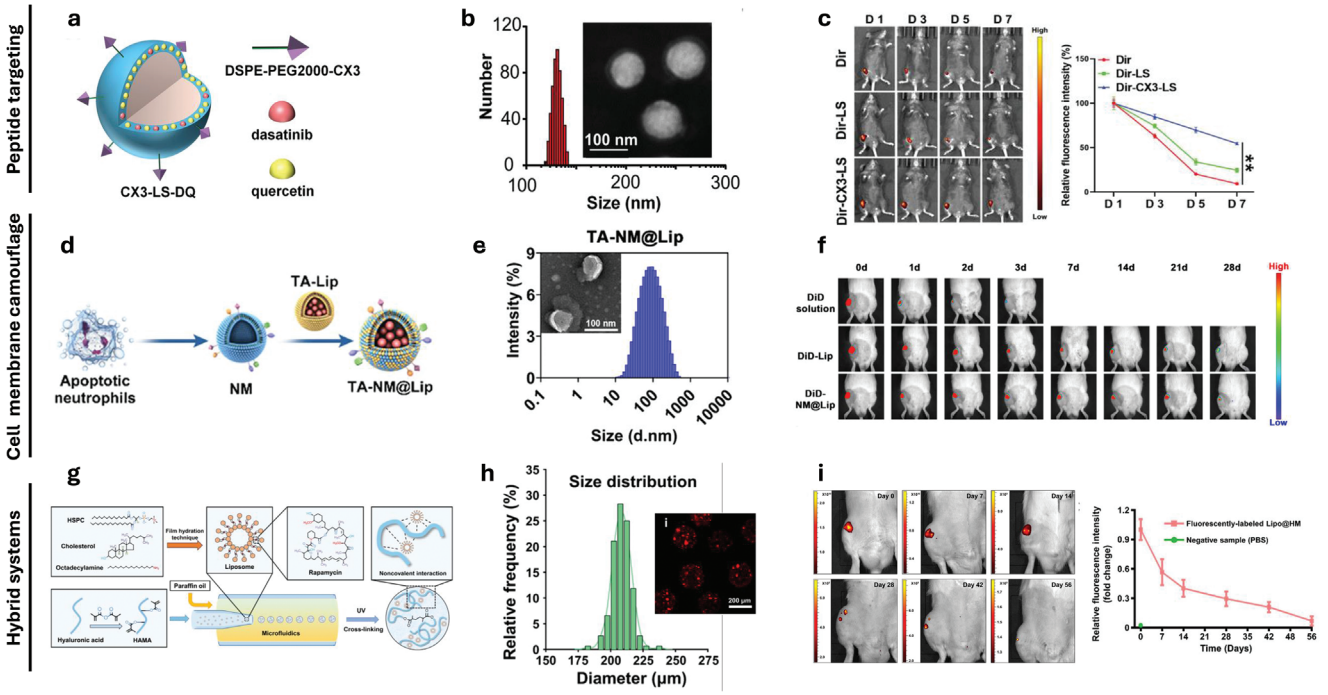

Figure 4. a) Schematic representation for CX3 modified liposome encapsulating dasatinib and quercetin (CX3-LS-DQ). b) The particle size distribution of CX3-LS-DQ by DLS. The insert image indicated the representative transmission electron microscopy (TEM) images of CX3-LS-DQ. c) Fluorescence imaging and intensity analysis with in vivo imaging system (IVIS) spectrum imaging system of knee joints from non-OA mice after IA injection with Dir, Dir-LS, or Dir-CX3-LS for 1, 3, 5, and 7 days. The fluorescence intensity was normalized to the fluorescence intensity of D1 in each group. ($n = 6$ per group, $^{**}p < 0.01$) Adapted from;[98] Copyright 2022, John Wiley & Sons, Inc. d) Schematic representation of TA-NM@Lip. e) Representative size distribution and TEM image of TA-NM@Lip. f) IVIS images of rat knee joints over 28 days after IA injections of free 1,1′-dioctadecyl-3,3,3′,3′- tetramethylindodicarbocyanine (DiD), DiD-Lip, or DiD-NM@Lip ($n = 5$) Adapted from,[155] Copyright 2023, American Chemical Society. g) The fabrication of RAPA@Lipo, photocrosslinkable HAMA matrix, and microfluidic RAPA@Lipo@Hydrogel microspheres (HMs). h) Size distribution of Lipo@HMs and laser scanning confocal microscopy (LSCM) images of Lipo@HMs. i) IVIS images of fluorescently labeled Lipo@HMs at different time points. Adapted from;[187] Copyright 2022, The American Association for the Advancement of Science.

gelatin, HA, and others, for the delivery of hydrophilic drugs, proteins, and even organelles for applications ranging from cancer therapy to OA. The properties of these liposomes can be finely tuned by adjusting hydrogel composition, crosslinking density, and molecular weight. Eladawy et al.[195] consistent with the use of HA as OA viscosupplementation, developed novel IA injectable HA gel–core vesicles (known as hyalusomes) loaded with diacerein, a DMOAD (hindering IL-1β). They achieved in OA rat model, reduced inflammation. showing the beneficial properties of the liposomes, however upon extremely high-volume injection (0.5 mL), which may not have clinical relevance.

Other than gels, liposomes have been combined with structures from nanodelivery systems to cells. A novel nanoformulation for IA delivery of curcumin was developed where the drug-cyclodextrin inclusion complex is encapsulated within the aqueous core or lipid bilayer of the liposome, depending on the complex's hydrophilicity.[196] The presence of cyclodextrins within the liposomal core modulated the release kinetics and membrane permeability, offering opportunities for tailored drug delivery. Maestrelli et al.[196] formed an inclusion complex between curcumin and hydroxypropyl-β-cyclodextrin which was then encapsulated within 3–4.3 um size LUVs composed of phosphatidylcholine and cholesterol. A positive zeta potential (31.7 mV), and a high encapsulation efficiency of over 90% for curcumin led to significant therapeutic effects, after a single dose 7 days post-OA induction with cartilage degeneration and high pain tolerance. A unique hybrid exosome–liposome system for targeted genomic editing in chondrocytes was developed with encapsulating the CRISPR/Cas9 system and RNAs, combining exosomes' targeting ability and liposomes' capacity for nucleic acid incorporation.[154] Gold nanoparticles[144] have been also encapsulated in DPPC liposomes for enhanced joint retention time with significant suppression of catabolic markers like NF-κB, and MPPs in the synovial fluid 15 days post-treatment.

More unique approaches have emerged in the last years involving cells[155] or different configurations such as microneedles[141] and others exploiting further the involvement of liposomes in OA for increased efficiency and patient compliance (**Figure 4**).

3. Clinical Studies

The extensive number of studies on liposomal interventions for OA management is not reflected in the number of clinical trials. Many reasons contribute to this including limited long-term safety and efficacy data in the literature along with regulatory hurdles.

Clinical trials in OA focus on the alleviation of pain and function, disease progression, and replacement of affected joints

Table 1. Overview of drug-loaded liposomal formulations used preclinically for IA OA management: composition, physicochemical properties, OA animal model, dose regimen, and effects.

Formulation	Size PDI Surface charge	Encapsulated compound(s)	OA model	Dose regimen	Effects
HSPC, DOTAP, cholesterol, 6-MNA-DSPE-Na double salt	695.0 ± 10.9 nm 0.3–0.4 +33.4 ± 5.3 mV	6-MNA (4.27 mg kg^{-1})	Sprague–Dawley male rats (5–7 weeks old) Arthritis model 6 mg mL^{-1} *Mycobacterium butyricum* suspended in heavy paraffin oil	Single IA injection on the day of inflammation induction	Five times higher retention time (24 h)/higher anti-inflammatory activity (day 21) than plain 6-MNA/liposomal formulation reduce cartilage damage[103]
Soybean phosphatidylcholine (≥94%), cholesterol (1:1 with HA)	4.98 µm 0.091	Celecoxib (0.5 mg mL^{-1}) HA (10 mg mL^{-1})	New Zealand rabbits OA induction by Hulth modeling (surgical procedures of medial collateral ligament, medial meniscus, and both cruciate ligaments)	Single injection 1-week post-surgery Volume: 300 µL	Transient analgesic effect of Clx-liposome 24 h post-injection/Clx-liposome in HA gel increased analgesic effect 24 and 48 h post-injection/Alleviation of cartilage degeneration[92]
Clodrosome L-alpha-phosphatidylcholine, cholesterol	1.5–2.0 µm	Clodronate disodium salt (0.05 mg)	C57BL/6 male mice (6 weeks old) Normal or low-fat diet following DMM surgery	Injection 1 week prior to DMM and additional at weeks 1 and 6 post-DMM Volume: 10 µL	Apoptosis of macrophages in OOA/reduced inflammation/reduction in OA severity (Mankin score)/increased proteoglycans[126]
DPPC, DSPE-PEG, cholesterol	350 nm −30 ± 0.05 mV	Resolvin D1 (35.7 ng mg^{-1} of lipid, 1 mg of liposomes per joint)	C57BL/6 male mice (6–8 weeks old) DMM surgery	Injections for 3 months (1-, 4-, and 8-weeks post-surgery) Volume: 10 µL	14-days retention of the liposome/six-fold reduction of OARSI score/free drug ineffective in therapeutic regime/decreased M1/M2 macrophage ratio/MMP13 and ADAMTS5 suppression/transient reduced pain (von Frey filaments)[127]
Lecithin, DSPE-$mPEG_{2000}$	149.9 ± 7.4 nm −8.26 ± 0.25 mV	Dexamethasone (1 mg kg^{-1})	C57BL/6 male mice (2 months old) Double knock out miR-204/-211 DMM surgery	Once a week from weeks 2 to 12 Volume: 10 µL	Liposomal dexamethasone less osteophyte formation/regeneration of cartilage/protective effect on subchondral bone/reduced pain/reduced levels of IL-1b, TNF-a, IL-6/macrophage polarization to M2[75]
Phosphatidylcholine from egg yolk, cholesterol, soybean oil, glycerin	Not mentioned	Adenosine (10 mg kg^{-1})	Sprague–Dawley male rats PTOA ACL model	Preventive regimen: Injection at the time of the injury for 6 weeks (10-days interval) Treatment regimen: Injection 1 week after injury for 5 weeks (10-days interval) Volume: 100 µL	Provided more rapid and sustained pain relief up to 3 months compared to hyaluronic acid injections/good safety profile/prevented joint swelling/significantly reduced OA cartilage damage/A2AR mediates liposomal adenosine[128]

(*Continued*)

Table 1. (Continued)

Formulation	Size PDI Surface charge	Encapsulated compound(s)	OA model	Dose regimen	Effects
Phosphatidylcholine from egg yolk, cholesterol, soybean oil, glycerin	Not mentioned	Adenosine or CGS21680 (1 mg kg^{-1})	C57/Bl6 mice (12 weeks old) high fat diet spontaneously obesity induced OA Sprague–Dawley male rats PTOA model noninvasive ACL	Mice: 6 injections, 10-days interval Volume: 10 µL Rats: Injection started 4 weeks after established of OA for 8 weeks, 10-days interval Volume: 100 µL	Reduced cartilage damage, improved swelling, preserved cartilage in affected knees/cartilage regeneration/upregulation of cartilage anabolic genes (aggrecan, TGF-β2) and downregulation of genes associated with chondrocyte hypertrophy and cartilage catabolism (ADAMTS and MPPs)[129]
DPPC, DSPE-PEG, cholesterol,	312 nm −1.92 ± 0.05 mV	Resolvin D1 active loading (25 ng)	C57BL/6 male mice (6–8 weeks old) High fat diet for 8 months followed by DMM	Prophylactic regimen: Injections for 3 months (weeks 1 and 4 post surgery)	Lipo-RvD1 promoted M2 macrophage polarization/after OA induction only lipo-RvD1 reduces OARSI score/suppresses osteophyte formation/analgesic effect[109]
DOPC, DSPE-PEG_{2000}-COOH, cholesterol, HQCMC	236.3 ± 4.8 nm 0.132 +27.4 ± 4.9 mV	Glucosamine sulfate (50 ug mL^{-1})	Sprague–Dawley male rats (4 weeks old) DMM	Injections starting 7 weeks after surgery/4-day interval for 6 weeks Volume: 20 µL	Controlled release of the drug/alleviation of joint surface damage/inhibition of inflammatory factors (IL-6, TNF-a compared to DMM and free drug[138]
Lecithin, cholesterol, DSPE-PEG–WYRGRL (incorporated in HAMA microgel)	138.2 ± 27.0 nm +5.4 ± 2.2 mV	Urolithin	Sprague–Dawley male rats (8 weeks old) DMM	Injections starting 2 weeks post-surgery/2-weeks interval until 6-week post-surgery Volume: 30 µL	Mitochondria targeting/mitophagy promoted in vivo (PINK1, Parkin)/COLII levels as in the sham group[139]
Egg yolk lecithin, cholesterol	115.8 ± 2.3 nm 0.20 ± 0.02	Triptolide (200 ug mL^{-1})	Sprague–Dawley male rats MIA	Injections starting 1 week post OA induction, 2-days interval for 2 weeks Volume: 50 µL	Decreased swelling/IL-1b, TNF-a, IL-6 decreased/smooth and intact surface upon H&E staining, strong proteoglycan expression/microneedle administration better protective effect compared to IA liposomes[141]
Lecithin, cholesterol PMPC-ODA	162 ± 1.5 nm 0.105 −8.56 ± 0.58 mV	Gallic acid (0.1 mg mL^{-1})	Sprague–Dawley male rats (8 weeks old) MIA	Injection starting 3 days after MIA injection, 1-week interval for 2 weeks Volume: 100 µL	Dual: lubrication, immune escape/sustained controlled release/alleviated cartilage damage/inhibition of inflammatory factors/reduced OARSI score/histological behavior better for pMPC-lipo and pMPC-lipo–gallic[142]
Lecithin/cholesterol/DSPE-PEG_{2000}-SS31/DSPE-PEG_{5000}-WYRGRL	130 nm 0.023 +2.48 mV	Resveratrol	Sprague–Dawley male rats (12 weeks old) (ACLT + MMX).	Injection starting 2 weeks after OA establishment, injections 2nd and 5th week post-surgery Volume: 100 µL	Mitochondria targeting/AMA incorporated liposomes no significant difference with the sham group/liposomes ameliorate erosion and deformation with lower OARSI score compared to sham and PBS[143]

(*Continued*)

Table 1. (Continued)

Formulation	Size PDI Surface charge	Encapsulated compound(s)	OA model	Dose regimen	Effects
HSPC, cholesterol, DSPE-PEG_{2000} with and without DSPE-PEG_{2000}-CLX3 aptamer	120–150 nm −20 mV	Quercetin (5 mg kg^{-1}) Dasatinib (0.5 mg kg^{-1})	C57BL/6 male rats (10 weeks old) DMM	Single injection 4 weeks post-DMM Volume: 10 µL	Decreased synovitis, TNF-a, IL-1b/No histopathological abnormalities or lesions compared with saline group/eliminating senescent fibroblast-like synoviocytes (FLS)/targeting FLS over ATDC5/increased retention time in non-OA mice[98]
Egg yolk lecithin, cholesterol, stearylamine, Tween-80 N/P 7:1	286.6 ± 7.3 nm 0.262 ± 0.029 +26.5 ± 0.5	Lornoxicam (4 mg mL^{-1}) miR-140 (90 µM)	Wistar female rats OA model by repeated IA papaine (1%) and cysteine injections (0.03 M)	Injection 1-week interval for 6 weeks Volume: 60 µL	Regular arrangement and repair of chondrocytes/no infiltration of inflammatory cells in synovium/Co-administration better therapeutic efficiency/cartilage and synovium almost identical to sham group/lornoxicam improves synovial inflammation in all cases[145]
DSPC, cholesterol, stearylamine	135.2 ± 28.3	Rapamycin (5–50 µM)	Dunkin–Hartley male guinea pigs (6 months old) Spontaneous OA	Twice a week for 8 weeks Volume: 40 µL	Liposomal RAPA combined with LIPUS provides lower effective dose/nontoxic serum biochemistry and blood count/increased COLII, GAGs, and CEC/decreased OARSI score[132]
DOTAP: DSPE	610 nm +53 mV	Mitochondria	Balb/c nude mice (8 weeks old) MIA injection	Injection twice every 5 days starting 1-week post-MIA injection	Increased expression of COLII/decreased MMP13/cartilage recovery/lower Mankin score[106]
S100, HS15	76.2 ± 0.5 −14.2 ± 0.2	TA (60 ug)	Male rats (8 weeks old) MIA injection Male rats (12 weeks old) ACLT+pMMx surgical OA model	MIA model: Single injection 1-week post MIA ACLT+pMMx model: Injection 1- and 5-weeks post-surgery Volume: 60 µL	High cellular uptake in M1 macrophages and activated synovial fibroblasts/repolarized M1 macrophages to the anti-inflammatory M2 phenotype/attenuated synovitis/long-lasting pain relief/cartilage protection[155]

Abbreviations: 6-MNA: 6-methoxy-2-naphthylacetic acid; DMM: destabilization of medial meniscus; MIA: monosodium iodoacetate; ACLT: anterior cruciate ligament; MMX: medial meniscectomy; MMTL: medial meniscotibial ligament; ACL: anterior cruciate ligament; PTOA: post-traumatic osteoarthritis; DOTAP: 1,2-dioleoyl-3-trimethylammonium propane; DSPE: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine; DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine; HSPC: hydrogenated soy phosphatidylcholine; HAMA: hyaluronic acid-methacrylate.

Table 2. Summary of preclinical and clinical evaluation objectives and methods for IA-injected liposomes in OA, highlighting key tests for efficacy and safety.

Evaluation aspect	Objective	Techniques involved
Preclinical evaluation in vitro		
Efficacy	Colloidal stability in simulated biological fluids (i.e., 50% FBS[98] albumin solution)	DLS[69,98]
	Release of profile of encapsulated drug	Dialysis,[75,98,133,145,189,195] Franz diffusion cell,[141] Diffusion studies,[103] Centrifugation[132]
	Cellular interactions (i.e., chondrocyte uptake, macrophage uptake/escape[142])	Flow cytometry,[95,145] LSCM[95,106,143]
	Reduction of ROS	DPPH,[95] ABTS,[95] DCF-DA probes[142]
	Expression of biomarkers (e.g., Aggrecan, Proteoglycan, Collagen, MMP13, IL-6)	Real-time PCR,[132,192,194] Reverse RNA transcription[133]
	Lubrication properties	AFM[133] Tribometer,[133,142,186] SFB[77,110,119,168,171,173,176,177,206,207]
	Interaction with enzymatic environments	Enzymatic solution.[143] Hyaluronidase,[139] Collagenase[191]
Safety	Cytotoxicity evaluation	Calcein-AM,[139,194]WST-1,[106]XTT,[77] MTT,[103,145] CCK-8,[95,98,138] Trypan Blue[133]
	Cellular response to treatment (cell cycle)	Annexin V/PI Staining
Preclinical evaluation in vivo		
Animal Models	Rodents (mice,[126] rats,[129,138,142] and guinea pigs[132]), Rabbits,[92] Sheep[179]	
OA Models	Surgically induced (DMM,[75,77,146] Obese DMM[109]), Mechanically induced (PTOA,[128] Obese PTOA[129]), Chemically induced (MIA,[106,155] bacterial collagenase,[144,145] papain[145]), Naturally occurring (Spontaneous OA[132])	
Treatment Regimens and Study Conditions	Single or multiple injections (1[98,155] to 16 injections[132]), therapeutic[127,150] or preventive[127,128] approaches, dose frequency (once[142] to thrice[141] per week), treatment duration (up to 14 weeks post-OA induction[109]), efficacy assessed 4 days–4 weeks[127] post-treatment (1 day after the last injection[154,208] has also been reported). Mainly males, few studies address both sexes[178,179]	
Efficacy	Pain relief	Dynamic weight-bearing tests,[150] Gait Scoring,[178,193] von Frey Filaments,[75,109,127,155] Prostaglandin levels,[75] Incapacitance meter tester (IITC)[155]
	Inflammation Reduction	Joint swelling,[145] TNF-a, IL-1b,[128] IL-6 (serum, synovial)[75,126] levels, Paw edema inhibition,[103] Macrophage phenotype assessment[126,178]
	Cartilage examination	Proteoglycan content (Safranin-O,[109,127,129] Toluidine blue[154]), Collagen content, Cellular density,[109]
	Bone remodeling (i.e., bone mineral density, trabecular spacing, osteophyte reduction)	Micro-CT,[75,127,129,138,155,188] X-Ray[143,188]
	Movement-related improvement	Open-field test,[128] Rotarod test[128]
	Joint retention time	In vivo imaging[69,77,98,127,141,154,155]
	Cartilage targeting and drug localization	CLSM[77,98]
	Catabolic enzyme reduction, apoptosis	Immunohistochemistry,[109,127–129,132] PCR[129]
Safety	Pharmacokinetics	IVIS imaging,[154] γ-Imaging studies[103]
	Systemic effects	Biochemical analysis,[109,126,132,138,155,178] Blood count,[132,138] Organ histology (H&E)[139,141,155,178]
	Cartilage toxicity	Cartilage histology[103,126,194]
Clinical evaluation		
Inclusion criteria	Include both male and female participants[122,209] Age in the groups of ≥40–99[200,202] Confirmed OA diagnosis for at least 6 months[197] (moderate-severe OA), Pain VAS score ≥4[197,209] No previous treatment, overall good health	BMI requirements may be included (BMI ≤40 kg m^{-2}[197,210] while others ≤ 35[202]),
Duration	14–52 weeks including follow up period[197]	
Participants	40[209]–500[197]	

(*Continued*)

Table 2. (Continued)

Evaluation aspect	Objective	Techniques involved
Dosage scheme	Mostly single dose[197,209,210]	
	One or up to three test concentrations (high/low)[200,209]	
	Control groups: Same concentration of encapsulated drug in a solution[197]	
	Placebo group: Normal saline[197]	
Exclusion criteria	Cardiovascular disease, Diabetes, Previous joint surgeries, Recent IA (last 3 months prior) or systemic (last 30 days prior) corticosteroids injections, Recent NSAIDs (last 7 days) Autoimmune disorders, Pregnancy, Breastfeeding, Immunodeficiencies, diagnosis of infection in the index knee,[200] Pain in any other major joint[202]	
Safety	Adverse local events monitoring	Magnetic resonance imaging (MRI)[197]
	Adverse systemic effects	Physical examination, Blood count, Biochemistry levels (e.g., cortisol[197]) urinalysis, electrocardiogram (ECG)[197]
Efficacy	Pain relief	WOMAC score (physical function, pain, ad stiffness)[197]
	Change in functionality	Physical exam range of motion[202]
	Pharmacokinetics	T_{max}, maximum concentration, area under the curve[210]
	Life quality assessment	EQ-5D questionnaires,[197] Change in analgesic consumption[202]

Abbreviations: AFM: atomic force microscopy, ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), CCK-8: cell counting kit-8, LSCM: laser scanning confocal microscopy, DCF-DA: 2′,7′-dichlorodihydrofluorescein diacetate, DLS: dynamic light scattering, DMM: destabilization of medial meniscus, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ECG: electrocardiogram, EQ-5D: EuroQol 5D, FBS: fetal bovine serum, H&E: hematoxylin and eosin, IL: Interleukin, IVIS: in vivo imaging system, MIA: monoiodoacetate, MRI: magnetic resonance imaging, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, XTT: 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, WST-1: water-soluble tetrazolium salt-1, OA: osteoarthritis, PCR: polymerase chain reaction, PTOA: post-traumatic osteoarthritis, ROS: reactive oxygen species, SFB: surface force balance, TNF-α: tumor necrosis factor-α, VAS: visual analog scale, WOMAC: Western Ontario and McMaster Universities osteoarthritis index.

involving drugs, devices, and even behavioral interventions. Currently there are a few ongoing clinical trials investigating the use of IA administered liposomes for knee OA. Liposomes loaded with the local anesthetic bupivacaine have been tested to determine quantitative and qualitative differences in post-operative pain relief for patients, however in cases of total knee arthroplasty upon IA injection. TLC599 (Taiwan Liposome Co.), is an injectable liposomal formulation of the FDA-approved non-opioid corticosteroid dexamethasone sodium phosphate which has been evaluated in a Phase III study. The liposome is composed of 67.5% DOPC, 7.5% DOPG, and 25% cholesterol. The study was a randomized, double-blinded, 3-armed, placebo-, and active-controlled with 506 participants with moderate to severe knee joint pain (≥4 in 0–10 scale) continuation of a positive Phase II study.[122] The study concluded that liposomes offer benefits alleviating pain 24 weeks post-injection while a second injection provides further benefit up to week 52 (NCT04123561).[197] In the context of long-term dexamethasone release, the side effects on bone and cartilage are crucial factors to be studied while high dosages and frequent injections of dexamethasone can lead to osteoporosis and cartilage damage.[198,199] Interestingly, the adverse effects were reported at the same rate in both the study and placebo groups.

Moreover, clinical trials that evaluate the clinical outcomes of the biomechanical properties of liposomes for the benefit of knee OA patients have also been conducted. The MM-II, an IA non-opioid drug formulation developed by Moebius Medical Ltd is composed of MLVs. Recent Phase IIb drug trials (NCT04506463) of MM-II with 397 participants (including enrollees from Europe, Asia, and USA) claim reduction in average daily knee pain scores with 26 weeks efficacy paving the way toward initiation of a Phase III clinical program execution.[200] The liposomal knee joint boundary lubricant named CCoat developed by Liposphere Ltd. is currently under clinical evaluation to protect cartilage from further wear by creating a liposomal coating of unique stability on the cartilage surface. The protection of the tissue during joint articulation aims to improve functionality of the knee joint and reduce wear-induced pain for the OA patient. In parallel, the safety profile of the first-in-human (FiH) clinical trial composition (NCT05412836)[201] following a single injection of the CCoat protoype has recently been reported demonstrating a high level of safety. Following preclinical evaluation, which demonstrated a non-toxic profile, the company conducted a FiH study involving 13 participants. Notably, long-term observation over 26 weeks revealed no treatment-related adverse effects.[202] Apart from safety as the primary outcome during this FiH trial, different parameters related to efficacy of the compound such as the changes in range motion, life quality, and pain level over a 26-week period upon a single injection were assessed. Based on the successful completion of the initial trial, CCoat is currently investigated comparing two different concentrations of the formulation in a randomized control trial versus placebo injections for pain and functional outcomes in moderate OA (NCT05771948)[203] involving 120 patients in total. Both liposomal approaches appear to reach patients in the near future.

Clinical trials on IA administered liposomes remain limited, thus in-human studies regarding their safety profile are also few. Liposomal systems have demonstrated a safe profile in preclinical studies in models ranging from rodents (up to 6 weeks testing for single injections[179] while for repeated up to 13 weeks[178]) to sheep (up to 6 weeks testing),[179] with no reported toxicity neither in organs nor in joints. Although human studies are particularly important, they are far more complicated and time consuming. Reported potential side effects in the clinical trials are numerous ranging from local pain, inflammatory responses,[204] procedural complications (meniscus injury, extradural hematoma

etc.) to others such as increased blood pressure, asthma, and even endocrine disorders making important to identify which are treatment-related (in most of the cases the placebo groups present them in the same frequency). Another important point is that although allergic reactions have not been reported— to our knowledge—they remain a theoretical possibility arising from specific liposomes' components such as polymers (e.g., PEG) which has been associated with complement activation pseudo allergy (CARPA).[205] As the clinical trials are focusing on the clinical outcomes it is uncommon to assess the injected joint from histopathological perspective (biopsies for chondrocyte death, collagen matrix changes, and others) due to the highly invasive nature of the procedure. In general, detailed records should be kept during the studies, to identify early any patterns that relate composition, dose and any other factor with side effects. Unfortunately, data on multiple injections (even though it is the more relevant approach to OA management) and long-term safety are scarce. The longest reported follow-up period so far is 26 weeks,[202] while testing a single concentration upon single dose, making it difficult to draw conclusions. Therefore, careful consideration of liposome composition and dosing strategies is essential to mitigate potential risks. However, it must be pointed out that lower concentrations (of both liposomes and encapsulated drugs) are needed through IA injections compared to other interventions, automatically reducing the risk of adverse events. Even though the findings to date suggest that IA liposomal injections are generally safe, especially when administered by trained healthcare professionals, ongoing research and meticulous formulation, and experimental design are critical to ensure the long-term safety and efficacy of these drug delivery systems (Table 2).

4. Conclusions and Future Perspectives

Reflecting on the past decade's literature, the role of liposomes has been critical in opening new perspectives for the IA management of knee OA. Liposomes offer a protective environment for a range of therapeutic agents, from conventional drugs (e.g., NSAIDs, glucocorticoids) to natural compounds and - most importantly - for more advanced strategies such as gene and organelle delivery. Their biocompatibility and modifiable nature make them appropriate candidates for OA management. Components such as lecithin and DPPC are the most used in the development of the systems while cholesterol is present in ≈80% of the systems, making its incorporation of clinical relevance (see **Table 1**). It is known that one of the main disadvantages of liposomes is their reduced colloidal stability, a fact that has been overcome by the addition of polymers such as PEG or pMPC to increase steric repulsion and resist aggregation. Surprisingly, less than 40% of the systems mentioned in the literature incorporate in their formulations such stabilizing polymers. Even though this reduces their overall complexity, in a possible clinical application, the hurdle of colloidal instability will lead to increased cost and complicated handling with further implications to drug and carrier's stability. For that reason, their incorporation should be considered when new therapeutics are developed.

As multi-parameter systems (size, surface charge, membrane fluidity, polydispersity, viscosity etc.) intrinsic liposomal properties along with external modifications affect OA outcomes with results varying from subtle to significant changes, showing no differences when compared with the non-OA controls. Additionally, the variety of OA models used in the literature along with the different dosing schemes contribute to this spectrum of results (**Table 2**). Mechanical, surgical, chemical and genetic OA models have been used, each exhibiting unique onset and severity characteristics, making it difficult to draw conclusions due to the different initiation causes and phenotypes. The standardization of OA models which best represent different subtypes of human OA along with combinational models (pain and structural) and standardized dosing could benefit more accurate prediction of liposome efficacy and improved success rate. The variability within these models complicates cross-study comparisons. Also, the use of larger animal models is limited but necessary, along with advanced tissue engineering models or ex vivo human osteochondral explants which could complement in vivo studies by providing more physiologically relevant platforms for initial screening and optimization of liposomal formulations. Moreover, the timing and dosage of injections are two crucial factors that need standardization. Most studies focus on early OA stages (1–2 weeks post-induction), which may not be as beneficial as treating later stages (characterized by significant chondrocyte loss and cartilage matrix degradation). This is associated with the OA's nature and the difficulty of being spotted in its initiation. In parallel, for retention time studies, a first indicator of liposomal efficiency in drug delivery and lubrication, most of the studies have been conducted in non-OA mice, a fact that does not reflect the disease environment with possibly misleading results. In Table 2 we compiled the preclinical and clinical evaluations that have been reported in the literature for IA injected liposomes in OA, reflecting the diversity of studies conducted highlighting the need for a structured testing framework. Key preclinical tests should include—apart from the structural characterization of the liposomes—system's stability in simulated biological fluids and their resistance/responsiveness to enzymatic environments, drug release, cellular uptake and safety tests. In vivo animal studies should evaluate pain relief, cartilage protection, and inflammation reduction while motor tests are necessary. Both prophylactic and therapeutic regimens should be implemented in the studies with long-term follow-up. Finally, evaluating efficacy along with implementing careful safety monitoring protocols over extended periods, including more non-invasive imaging and biomarker monitoring, would be crucial for obtaining more robust and clinically relevant data. This comprehensive approach will ensure a thorough evaluation and would facilitate the translation of liposomal-based biomaterials from bench to patient.

While liposomes have shown promise in improving drug delivery for OA, the current literature suggests that they primarily provide symptom alleviation rather than modifying the disease. Many studies focus on the immediate benefits of pain relief and inflammation reduction, but there is limited evidence demonstrating that liposomal formulations can alter the underlying disease progression of OA, as many of the studies are dealing with OA in its initiation and not after it is well-established. This highlights a critical gap in research, as effective treatment options should ideally address both symptom management and possible disease modification. Studies, including groups long after termination of treatment could also be useful for drawing constructive conclusions. Overall, while liposomes have proven effective for drug transport, their modification and optimization remain

essential for developing better treatment options that can provide long-term benefits for OA patients.

Future perspectives on IA injections of liposomes should focus more on additional aspects beyond drug delivery. Their extremely useful lubrication properties and their effect on the mechanotranslational aspects of OA need to be explored in more depth, while their application for theranostic purposes (delivering contrast agents or imaging probes) to visualize early molecular changes associated with OA initiation, while simultaneously treating it, could be of increasing importance. In summary, while IA liposomal therapies hold significant promise, extensive clinical investigation is crucial to translate promising preclinical findings into concrete therapeutic benefits for OA patients. The multifaceted nature of OA, combined with the unique properties of liposomes, presents a powerful case for further research and development in this area.

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Conflict of Interest

The Weizmann Institute has patents on low-friction hydrogels (US20160175488A1) and on liposomic drug delivery vehicles (US20190374466A1). All other authors declare no conflict of interest.

Keywords

drug delivery, intra-articular administration, knee osteoarthritis, liposomes, lubrication

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