Polymer colloids as drug delivery systems for the treatment of arthritis

by

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Abstract:

The most common types of arthritis are osteoarthritis (OA) and rheumatoid arthritis (RA) which are themain causes of disability and pain among older people. Current treatment of arthritis mainly consists of oral and intra-articular medications. Despite the efficacy of the intraarticular injections over the oral treatment, it is still limited by the rapid clearance of the injected drug. Therefore, a rational design of drug delivery systems (DDSs) able to delivery drugs in controlled manner and for required period of time to the arthritis joint is a key in developing safe and effective formulations for OA and RA. In this paper various colloidal systems like nanoparticles, liposomes, cationic carriers, hydrogels, and emulsion-based carriers were presented and discussed in light of their use and efficacy as delivery systems to transport therapeutics for arthritis treatment. Factors influencing the delivery efficacy such as size, charge, structure, drug uptake, retention and its release profile alongside with cytocompatibility and safety were addressed. Moreover, the advantages and disadvantages of the different colloidal systems were emphasised.

Keywords: osteoarthritis, rheumatoid arthritis, drug delivery system, hydrogels, nanoparticles, emulsion, liposomes, cationic carriers, PBAEs.

Contents

1. Introduction

Arthritis is a degenerative joint disease and a leading cause of disability and pain among elder people. In 2020 it was estimated that 10 million people in the UK were diagnosed with arthritis (1), and in the same year, 52.5 million Americans reported arthritis, including osteoarthritis (OA) and rheumatoid arthritis (RA), (2–4). Arthritis-related joint pain limits the functional ability and quality of people's life. There is a strong correlation between the development of functional limitations and the arthritis risk factors as age, obesity, diet, gender, genetic, occupation, physical activity or the existence of previous joint injuries. (5–8). In addition, pain commonly shows during activity, but night and rest pain can also occur. The symptoms get worse with the activities byaffecting the normal every day life style and making difficult to perform the usual tasks at work and home. In addition, the medication side effects can lead to adverse health conditions both directly and not directly related to the joint disease (8–10).

Osteoarthritis and rhumatoid arthritis are the two main type of arthritis and two of the most important inflammatory diseases. Osteoarthritis (Fig. 1) is the most prevalent joint condition that causes difficulty in joint movement, stiffness and gradual loss of articular cartilage and accompanies with moderate to severe pain (9,11,12). The incident of osteoarthritis increases with age; therefore, it has been considered as ' wear and tears' disease because of the strong association between age and osteoarthritis (13,14). rhumatoid arthritis is a chronic autoimmune inflammatory disease of the joint and the most common type of autoimmune arthritis (15,16). It causes joint pain, stiffness, swelling, and decreased joint movement, leading to structural damage, deformity, and disability (15,16). Currently, there is no treatment or cure option available, but only management of the arthritis condition that is mainly aimed to relieve the symptoms, improve physical function as well as patient quality of life and prevent arthritis complications and progression. The main available treatment option includes paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, supportive supplements , nutritional supplements, or/and surgical treatment, however, the

outcomes are limited (8,17). For example, non-steroidal anti-inflammatory drugs must be taken in caution for patients with gastrointestinal (GIT) and cardiovascular (CV) medical conditions, such as peptic ulcer, angina, heart attack, myocardial infarction or stroke as it cause gastrointestinal and cardiovascular side effects (18). Moreover, corticosteroids are more potent anti-inflammatory medications than non-steroidal anti-inflammatory drugs, especially in rheumatoid arthritis (19). They can be used widely as intra-articular injections for osteoarthritis and rheumatoid arthritis treatment, however, when considering long term usage, the benefits of the corticosteroid injections are poor and display adverse effects when were given at long durations such as hot flashes, subcutaneous atrophy, risk of infection, skin depigmentation, muscle weakness, suppression of adrenal glands and growth retardation (20–22). Consequently, the use of corticosteroids (40 mg/dose) is limited from three to four intra-articular injections annually with at least three-months gap between the injections (23,24). Therefore, they are only prescribed for a short period of time at low doses, during aggravation or flares of rheumatoid arthritis and osteoarthritis (19).

Other treatments such as surgeries, while being expensive, they also cause secondary damage to the joint tissue (25). For example, arthroplasty is the total joint replacement procedure in which joints are replaced with metals or artificial plastic parts. It is mainly done for the hip, knee, shoulder (glenohumeral joint), elbow, wrist, and ankle joint (26–28). Nowadays, around seven million Americans are living with a hip or knee joint replacement andthis number is estimated to increase over the coming years (29). In fact, it is the most effective medical procedure for the treatment of end-stage osteoarthritis and rheumatoid arthritis , especially for elderly patients who last over 20 years (26,27). The operations are cost-effective as the current cost burden to Medicare is US £19,560.39 in patients with no complications such as coagulopathy, congestive heart failure, and electrolyte imbalance while it is £38,747.35 for patients with complications (30). In the UK arthroplasty economic cost is estimated to be more than £850 million (31). These operations often lead to the

serious complications such as wound healing problems, infection, inflammation, and postoperative instability (27,32).

In addition, local delivery strategies may provide the best and effective method of treatment as they aim to reduce local joint inflammation and destruction, offer pain relief and improve patient activity and joint function (24,25). Therefore, corticosteroids and hyaluronic acid are the drugs widely used in the clinics as intra-articular (IA) injections for arthritis treatment (8,24). Intra-articular injection is a method of needle injection which is used to deliver a drug inside the joint for pain and inflammation treatment. Physicians usually tried to minimise the time between the injection and consider it to be from 2-12 weeks which may lead to ineffective treatment and patient compliance (33).

Generally speaking, the fundamental problems with the arthritis therapies are the drug retention in a pathological joint, rapid degradation and clearance of the injected drug which mainly delivered as intraarticular injections, while oral drugs can increase the risk of complications and systemic side effects thereby, do not reach the adequate therapeutic level required to treat the arthritis condition (25,33, 34). That is mainly because of the joint structure, when the drug inters to the synovial fluid, most of it is rapidly drained via the blood vessel to the systemic circulation (35). Therefore, it cleared entirely from the joint in hours or days, depending on the molecular weight of a drug. In addition, in arthritis, the treatment target for most of the modifying drugs is the articular cartilage; however, drug penetration inside the cartilage is a challenge. That is mainly because the cartilage is avascular (has no blood vessels), dense, small pore size (< 15nm), highly anionic matrix as it composes of negatively charged proteoglycans, Fig. 2 (34,36–39). Therefore, penetration of effective concentration of the drug into the cartilage is slow and mostly cleared quickly(33). For instance, the half-lives of non-steroidal anti-inflammatory drugs and soluble steroids in synovial fluid in human were approximately 1-5.2 hrs (40), and the half-life of hyaluronic acid in rabbit knee was 21.8-26.3 hrs (41). Therefore, many attempts were made to improve uptake, penetration, and retention time of drugs into the cartilage, such as combining drug

delivery systems (DDSs) and IA injections to provide effective treatment. Fig. 3 showed the difference between the release profile of an administered drug of a repeated intraarticular injection and a single injection of a drug with the drug delivery systems. Multiple injections can lead to high dose fluctuation, which ranging from toxic to subtherapeutic level while single injections plus DDSs come within required therapeutics level (dashed line) (42).

The idea of drug delivery systems was recognised for a long time since the 1960s when Folkman identified that a steady rate of drug delivery could be done by using silicone rubber tube in rabbit anaesthesia (43). Since that, many types of studies have been performed to develop an effective delivery system to deliver drugs through intraarticular route, and for this purpose many carriers were designed ranging from macroparticles to nanoparticles (43). In addition, a projected drug delivery system for intra-articular treatment of osteoarthritis had become an expanding area of concern in the late 1990s (42). Drug delivery systems are mainly aimed to enhance specificity, improve activity, reduce toxicity and maximise treatment safety (44). It is a useful method of the treatment targeting osteoarthritis joints. With that is being said, reduction of the joint clearance of a drug, and enhancement of the drug penetration into cartilage have to be considered in development of novel drug delivery systems. Using a vehicle for sustained release of the drugs inside the joints for a long time is an effective way for the treatment (42). There are different types of vehicles that facilitate drug delivery to the affected joints and organs such as matrix system and vesicles (25,42). Soft matter carriers and colloidal systems possess interesting and relevant characteristics and provide choice options for drug delivery such as emulsions, hydrogels, nanoparticles, liposome, polymeric particles and cationic carriers (8,33,44,45). Relevant treatment approaches have to be designed depending on stages of the disease progression. To illustrate some, using therapeutics such as non-steroidal anti-inflammatory drugs , corticosteroids and hyaluronic acid are mainly aimed to reduce the inflammation, to relieve pain and to improve the mobility and joint stiffness especially in the early stage of arthritis as there is no complete damage of the joint tissue (1,17). On the other hand, natural or

synthetic compounds such as growth factors can work synergistically to increase cell growth, regulate tissue development and homeostasis, and trigger cell differentiation. Based on that, these compounds can be used as adjuvant agents to promote cartilage regeneration (46,47). Considering different drug molecules and routes of their administration, various drug delivery systems were created and used for the application. This review aims to summarise the current state of the art in soft matter and colloidal systems employed for drug delivery to treat or prevent the progression of arthritis.

2. Delivery systems.

2.1. Nanoparticles.

Nanotechnology is a rapidly developing areawhere compounds in the nanoscale range are employed to deliver therapeutic agents to specifically targeted sites in a controlled manner, (48,49). They offer multiple benefits in treating diseases by site-specific and target delivery of suitable therapeutics (48). The use of large-sized materials in drug delivery is facing many challenges, such as in vivo instability, low bioavailability, and poor solubility, slow absorption in the body, issues with target-specific delivery and systemic side effects of drugs (48,50). Nanotechnology engineers nanoscale materials that are able to interact with living cells and tissues with a high degree of specificity. This property is achieved mainly due to a small size of nanoparticles comparable with cellular compartments allowing interactions with cellular components. The specificity allows advancement in designing targeted drug delivery systems with reduced toxicity and improved efficiency compared to conventional therapies (48,50). Therefore, using nanotechnology for drug delivery targeting joint tissue could be an appealing option that may solve the critical concerns associated with the use of macro-scale materials for arthritis treatment.

Table 1 summarised nanoparticulate delivery systems and their advantages and disadvantages to deliver therapeutics for arthritis treatment. In this table nanoparticle

preparation, its characterisation and efficacy to deliver drug molecules in vitro and in vivo were presented and discussed in the details in the text below.

Recently, a study in 2020 (50) used curcumin as a natural product which has potent antioxidant and anti-inflammatory activity for osteoarthritis treatment (51). In the previous study (52), curcumin was assessed to be an effective antioxidant by radical scavenging and metal chelating and possessed potent anti-inflammatory activity in musculoskeletal disease (52). As curcumin has low bioavailability, the authors prepared acid activatable curcumin polymer (ACP) in which curcumin was covalently incorporated in the backbone of the poly beta-amino ester (PBAE) polymer. The acid activatable curcumin polymer was self-assembled to form micelles rapidly releasing curcumin under the osteoarthritis joint acidic condition (Fig. 4). Histological examination of a knee osteoarthritic joint showed that acid activatable curcumin polymer micelles decreased the inflammation through suppression of the two major inflammatory cytokines; tumour necrosis factor-alpha (TNF α)and interleukin 1β (IL-1β) in a monoiodoacetic acid (MIA) induced osteoarthritis mouse model (51). In addition, this delivery system had excellent biocompatibility and allowed high drug content at the target joint as it provided 95% of curcumin released at acidic media pH 6, compared to 25% at pH 7.4 (51). Moreover, other authors, Cetin et al. (2010) (52) conducted an in-vitro study using diclofenac sodium-loaded Eudragit®L100 and Eudragit®L100/ poly (lactic-co-glycolic acid) (PLGA) nanoparticles to deliver the drug in a controlled manner and to reduce the gastrointestinalside effects of diclofenac sodium. The authors employed Eudragit® L 100 that was an anionic copolymerization product of methacrylic acid and methyl methacrylate. Cetin et al. had prepared these nanoparticles with size ranging between 241nm and 274 nm. They found that the initial burst release of diclofenac sodium ranged between 38% and 47% within four hours. The extent of the drug release from Eudragit® L100 nanoparticles was up to 92% at 12 h. A slower sustained release followed by the initial burst release at 72 h, and the cumulative drug release was 56% for Eudragit®/PLGA (20:80), 69% for Eudragit®/PLGA (30:70) and 81% for Eudragit®/PLGA (50:50) nanoparticles respectively. It was noticed that

diclofenac sodium release behaviour was influenced by the amount of Eudragit in the formulation. Finally, the study concluded that these nanoparticles were effective and can be used to control the release of non-steroidal anti-inflammatory drugs (53). In another study in 2013, Narayanan et al. (53) created injectable ibuprofen sodium (IbS) loaded PEGylated gelatin nanoparticles (PIG NP) of 200nm mean particle size containing 1mg/ml of ibuprofen. The in vitro experiments showed this delivery system exhibited 72% entrapment efficiency and was non-toxic as it did not trigger the cytokine release, hemocompatible as the nanoparticles did not induce hemolysis (0.01%) compared to the Triton-X100 which caused 100% lysis and non-immunogenic as it did not induce an immune response compared to Lipopolysaccharide (LPS) which stimulated the proinflammatory cytokine release. Furthermore, the in vivo intravenous administration of the formulation to rats showed a sustained release of ibuprofen sodium for seven days with its improved bioavailability, half-life (5 hr for PIG NP compared to 0.08s for ibuprofen sodium alone) and indicated its cytocompatibility compared to ibuprofen sodium alone. The authors concluded that ibuprofen sodium loaded PEGylated gelatin nanoparticles were improving ibuprofen sodium bioavailability and half-life and can be used for frequent drug administration (54). Earlier, in 2015, Zhou et al. (54) assessed the anti-inflammatory activity of berberine chloride (BBR) loaded chitosan nanoparticles (CNs) for osteoarthritis treatment. Berberine chloride had a promising protective effect against osteoarthritis, but it possessed low solubility, bioavailability and short half-life; therefore, berberine chloride loaded chitosan nanoparticles had been synthesized by the ionic cross-linking method to sustain a release of BBR. In in-vitro release and stability study performed with berberine chloride (BBR) loaded chitosan nanoparticles provided sustained released of berberine chloride for 7 days compared to fast released from the free berberine chloride within 2 days, and the delivery system was stable at 37C^{o} under 75% humidity. In in-vivo rat osteoarthritis model, the histological assessment confirmed a greater ability of berberine chloride loaded chitosan nanoparticles (0.6 mg/ml) to reverse to some degree damage of cartilage compared to free berberine chloride(60µg/ml). Furthermore, other post-in-vivo experimentation analysis such as TUNEL assay ((terminal

deoxynucleotidyl transferase dUTP nick end labelling) which used to detects the DNA breaks formed when DNA fragmentation occurs in the last phase of apoptosis), western blot (it is widely used ananalrical method in molecular biology for many reasons such as allows for the detection, localization and quantification of proteins involved in apoptotic signalling) and immunohistochemistry assays (it is a method for detecting antigens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. It is commonly used to show the presence of apoptotic cells in situ) showed that berberine chloride loaded chitosan nanoparticles possessed stronger anti-apoptotic effect, the greater decrease in caspase-3 and Bax protein expression and increased Bcl-2 expression in cartilage tissue in anterior cruciate ligament transection combined with medial menisci resection (ACLT+MMX). The authors concluded that the berberine chloride loaded chitosan nanoparticles was effective in providing a sustained release of berberine chloride and its prolong retention in synovial fluid. Therefore, IA administration of berberine chloride loaded chitosan nanoparticles could be a potent therapeutic system for osteoarthritis treatment (55).

Another study by Ding Lu et al. (2011) (55) created a novel non-viral gene delivery vector that was able to transfer a gene into the chondrocyte for arthritis treatment. They synthesized hyaluronic acid (HA)/ chitosan (CS)- plasmid nanoparticles, examined its characteristics and assessed its ability as a non-viral agent to deliver a gene into the arthritic joint tissue. The authors used different weight ratios of hyaluronic acid and chitosan ranging from 1:1 to 7:1 containing the same concentration of HA (11.25µg/ml) and varying concentrations of chitosan from 5.625 µg/ml to 78.25 µg/ml and they found that increase of chitosan concentration led to decrease of the nanoparticles size and to increase of the zeta potential charge. Specifically, the authors showed that 6:1 ratio had the smallest dimension (115.6 ±4 nm) and the highest positive charge (26.3±0.5 mV). To assess the hyaluronic acid/chitosan -plasmid nanoparticles potential toxicity, the researchers used MTT assay. MTT assay is a colorimetric assay for measuring cell metabolic activity. It is based on the ability of nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular

oxidoreductase enzymes to reduce the tetrazolium dye MTT to its insoluble formazan, which has a purple colour. This process is occurring mainly in the mitochondria of living cells, therefore, MTT assay is used widely to measure cell viability. The author found that the hyaluronic acid/chitosan plasmid nanoparticles showed more than 90% cell viability compared to Lipofectamine which showed 60% viability. In-vitro transfection efficiency of the hyaluronic acid/chitosan plasmid nanoparticles was assessed under different conditions (media pH, and plasmid dose) and the results confirmed that the highest transfection efficiency was obtained when pH was below 7, and plasmid concentration was 4µg/ml. In addition, unlike the fast expression of enhanced green fluorescence protein (EGFP) with Lipofectamine, hyaluronic acid/chitosan plasmid nanoparticles showed a gradual increase in enhanced green fluorescence protein expression between 2 – 5 days of the cell culture period. Based on the obtained results, the researchers concluded that the hyaluronic acid/chitosan plasmid nanoparticles were the safe and effective vectors for gene delivery to chondrocyte (56). In another earlier study by Ishihara et al. (2010) (56) , the authors reported the preparation of polymeric nanoparticles composed of monomethoxy polyethylene glycol (PEG)-polylactide and poly (lactic acid) block copolymer encapsulating betamethasone disodium phosphate (BP) as an anti-inflammatory agent for the treatment of arthritis. They found that these nanoparticles were gradually hydrolysed which allowed betamethasone disodium phosphate to be released steadily for 44 days in PBS at 37C^{o}. In addition, in the in-vivo rat model of adjuvant-induced arthritis, these nanoparticles gathered at the target sites and were phagocytosed by resident macrophages, letting continual release of betamethasone disodium phosphate during the period of 14 days. The authors showed that these PEG/PLA NPs enhanced the drug, betamethasone disodium phosphate, permeability and its retention, and subsequently the anti-inflammatory effect. Additionally, the researchers conducted stability studies confirming these nanoparticles stability in the freeze-dried form below 25C^{o} for 96 weeks. Hence, the authors summarised that these PEG/PLA-betamethasone disodium phosphate could be used for the purpose of clinical setting (57).

As the Folate receptor (FR) is overexpressed on the activated macrophages in both animal models and human patients with naturally occurring rheumatoid arthritis, there is a possibility of utilizing folate receptor -mediated targeting to activated macrophages of inflammation. Therefore, in the study by Chandrasekar *et al.* (2007) (57), the folate coupled poly (ethylene glycol) (PEG) conjugates of anionic dendrimer (G3.5 poly amido amine (PAMAM)) was prepared in order to get a targeted system for delivery of indomethacin into rat arthritic tissue. The researchers showed that the drug loading efficiency was increased by 10 to 20-fold, and cumulative indomethacin release reached about 95% after 24hrs. Additionally, this type of delivery systems limited indomethacin gastrointestinal side effect by decreasing its stomach uptake. Authors concluded that these folate-PEG-PAMAM conjugates werethe good options as the delivery systems able to target the inflammation and reduce the systemic side-effects of indomethacin providing higher drug efficiency (58). In another study, Han et al., 2012 (58), developed selenium chondroitin sulphate (SeCS) nanoparticles employing ultrasonic and dialysis method. The prepared selenium chondroitin sulphate nanoparticles had high selenium entrapment efficiency, less toxic to chondrocyte and more effective in reducing apoptosis in chondrocyte compared to sodium selenium and chondroitin sulphate along, respectively. The results illustrated the encouraging properties of selenium chondroitin sulphate nanoparticles and suggested that the developed system could be used in the treatment of osteoarthritis (59).

Lin et al. (2016) (59) used degradable poly(N-isopropyl acrylamide) (pNIPAM) nanoparticles as the biocompatible drug carriers, that provided protection to a drug from the enzymatic degradation and provided a drug release into target sites decreasing its systemic side effects. The authors showed the efficacy of the delivery system using KAFAK (an anti-inflammatory peptide) as a drug incorporated into the NPs, as long-term pain relief and anti-inflammatory OA therapies. Bovine cartilage knee explants stimulated with IL-6 for 8 days, were treated with KAFAK loaded PEGylated-pNIPAM nanoparticles with degradable disulfide crosslinks (named as NGPEGSS) and non-degradable NPs (PEGylated-pNIPAM),

the NPs without the drug (NGPEGSS-no KAFAK) and KAFAK alone as control groups. The results showed that NGPEGSS decreased the inflammation by suppressing the production of IL-6 ($P < 0.05$) compared to the controls on days 6 and 8 of the incubation period (60).

Overall, there are several advantages of using nanoparticle drug delivery system that include decreased dosage administration frequency, modified pharmacokinetics, increased drug solubility, prolonged-release of drug and reduction of drug unwanted side effects, even though, the regulatory mechanisms for nanoparticles along with safety and toxicity assessments still remain subjects of further development in the future (25,50,61).

2.2. Liposomes.

Liposomes are spherical vesicles composed of phospholipid bilayers and the aqueous core (25,62). They have been designed as an efficient carrier to improve and to control drug delivery as liposomes can encapsulate the hydrophilic drugs in the aqueous core and the lipophilic drugs in the lipid bilayer of their structure, Fig. 5 (25,62,63). Liposomes are biocompatible, biodegradable, flexible and right choice for local targeting of drugs to the specific site with the reduced systemic side effect (25,61,62, 63). Today, the only available liposomal formulation for arthritis treatment is Lipotalon®, Merkle in Germany. It is composed of dexamethasone-21-palmitate dissolved in soya bean oil and surrounded by a lecithin coating (64,65). This formulation is well tolerated by the patients as it is not associated with any side effects providing 4 weeks of pain relief compared to long-acting corticosteroids with a half-life of 36-72 hrs (64,65). Recently, a clinical trial (66) was conducted employing dexamethasone sodium phosphate liposomal formulation (TLC599) as a single intra-articular injection into osteoarthritis knee. This study showed that using TLC599 12mg had no treatment-related side effect regarding the safety profile and provided 30% reduction in pain over the 6 month study period compared to placebo (66). Therefore, liposome's application to deliver drugs for arthritis treatment appeared to be promising due to its safety and efficacy observed during clinical trials and in clinical practice (25,66,67).

Table 2 summarised preparation, properties, and efficacy of liposomal formulations for arthritis treatment and more details about each of this study are provided in the text below.

A study by Ulmansky R et al. (2011) (67) showed the effect of intravenous (IV) administration of Methylprednisolone hemisuccinate nano-liposome (NSSL-MPS) in rats for arthritis treatment. The arthritis score, which measures the disease progression, was significantly lower ($p<0.05$). The authors showed that in adjuvant arthritis (AA) rat model the score of the disease progression was decreased to 2 in NSSL-MPS (10 mg/kg) treated group compared to score 8 in free methylprednisolone (50 mg/kg) group ($P<0.05$). Besides, methylprednisolone hemisuccinate nano-liposome reduced the level of TNF-α by 97%, IL-6 by 75% compared to the sucrose buffer after 24hrs treatment. In addition, the subcutaneous (SC) formulation of methylprednisolone hemisuccinate nano-liposome was also used in the study where rats were treated with subcutaneous methylprednisolone hemisuccinate nano-liposome (10mg/kg), free MPS (10mg/kg) and sucrose histidine buffer of 10mM (control). The researchers found the arthritis score to be significantly reduced from 9.8 to 2.3 after 96hrs while it was 6 in free methylprednisolone and 7 in sucrose treated groups ($P<0.01$) while the level of IL-6 was decreased by 80% using subcutaneous methylprednisolone hemisuccinate nano-liposome. Based on obtained findings, the authors concluded that using liposomal formulation to deliver methylprednisolone through intravenous or subcutaneous route was shown to be effective in decreasing the arthritis progression compared to a free drug (68).

Another study conducted by Dong et al. (2012) (68) celecoxib (Clx) was loaded into liposomes and embedded in hyaluronate (HA) gel to improve osteoarthritis therapy and reduce celecoxib cardiovascular side effects. The efficacy of this formulation was demonstrated using the osteoarthritis induced rabbit model. Results showed that the intra-articular administration of liposomal celecoxib- hyaluronate formulation significantly reversed the alteration in hind rabbit paw bearing over 24 hr and 48 hr ($P<0.05$) compared to 24 hr for the celecoxib -liposome alone. Also, the celecoxib- hyaluronate liposomal formulation had

lower alleviation of cartilage degradation compared to the saline group ($p<0.05$). To conclude, the researchers verified that using the celecoxib- hyaluronate liposomal formulation was effective for pain relief and decrease cartilage degradation than using the drug alone (69).

Another research group, Elron-Gross et al. (2009) (69), prepared a liposomal formulation incorporating two drugs, diclofenac (DC) and dexamethasone (DEX), into the same bioadhesive liposomes with hyaluronan (HA-BAL) or collagen (COL-BAL) as surface anchored ligands. The study demonstrated a reduction in the percentage of joint inflammation to around 23% for COL-BAL and 12.3% for HA-BAL in vivo over 17 days compared to non-treated control ($p<0.001$, $p<0.0005$ for COL-BAL and HA-BAL), respectively. Also, the author showed that the effective treatment was obtained using the combination of DC and DEX in HA-BAL as it gave a better reduction in joint inflammation decreasing the inflammation volume to 12.3% from initial over 17 days compared to 20.4% for DC, 16% for DEX alone and 23% of COL-BAL using the same liposome. Therefore, this formulation appeared promising to be examined further in the future (70).

A study by Hofkens et al. (2011) (71), assessed the anti-inflammatory activity of liposomal formulation containing prednisolone phosphate (PLP) and compared it with a free glucocorticoid using the murine antigen-induced arthritic (AIA) model. In another study these authors performed a dose-response study and found that a single dose of liposomal prednisolone phosphate (1mg/kg) was effective in decreasing the joint inflammation by 48% equally as four repeated injections of free prednisolone phosphate (10 mg/kg) in 32 days (71). Moreover, Hofkens et al. (2011) (71) measured the corticosteroids level to evaluate the side effects of the liposomal prednisolone phosphate (1mg/kg). At day 14 of the treatment, the authors found that 1mg/kg liposomal prednisolone phosphate gave 22% suppression of glucocorticoids level more than 10 mg/kg liposomal prednisolone phosphate. Additionally, the researchers compared the activity of liposomal prednisolone phosphate with liposomal budesonide (BUP), 1mg/kg. They found that during the first day of AIA, liposomal BUP

represented a higher suppression of joint swelling compared to liposomal prednisolone phosphate (98% and 79%, respectively) and at day 21 of AIA, the reduction of glucocorticoids level was significant ($P<0.05$) for liposomal prednisolone phosphate while this suppression was not significant for liposomal budesonide. The authors concluded that the safety of glucocorticoids was improved by using liposomal formulation allowing use less effective dose compared to free drugs. Besides, this safety could be further promoted by encapsulating budesonide instead of prednisolone phosphate (72).

The other group, Craciunescu et al. in 2013 (72), prepared and characterized a liposomal formulation containing chondroitin sulphate (CS) for its use in the treatment of inflammatory and degenerative disorders in arthritis. The encapsulation efficiency of chondroitin sulphate in liposomes was 86.8%, confirming the high ionic attraction between them. The results demonstrated excellent cytocompatibility of liposomal chondroitin sulphate formulation in L929 fibroblast cell culture shown by MTT and LDH cytotoxicity assays; additionally, the liposomes appeared providing protection to the cells against oxidation. More importantly, the liposomal chondroitin sulphate formulation showed higher anti-inflammatory activity than chondroitin sulphate in H2O2 stimulated cells by reducing the level of IL-8 and TNF-a proinflammatory cytokines. The overall results suggested that using the liposomal formulation to deliver chondroitin sulphate to the affected joint is a promising system for intra-articular treatment of inflammatory and degenerative joint disorders and should encourage its further examination in a relevant animal model (73).

Another study by Harigai *et al.* (2007) (73) examined the efficacy of prednisolone phosphate (PSLP) in 3,5-dipentadecyloxybenzamidine hydrochloride (TRX-20) liposomes for rheumatoid arthritis (RA) treatment. The experiments performed using human fibroblast-like synovial (HFLS) cells showed that prednisolone phosphate -containing TRX-20 liposomes was interacted with HFLS cells approximately 40 times higher than that with prednisolone phosphate -containing liposomes without TRX-20. It was bound to HFLS cells mainly via chondroitin sulfate, then TRX-20 liposomes were taken up by the cell and localized to acidic

compartments. Therefore, the prednisolone phosphate -containing TRX-20 liposomes decreased the production of IL-6 and IL-8 more effectively than the prednisolone phosphate -containing liposomes without TRX-20 ($P<0.001$), to a level proportional with the free prednisolone phosphate. The author stated that prednisolone phosphate -containing TRX-20 liposomes showed a promise as a novel drug delivery system that could promote the clinical use of glucocorticoids for treating rheumatoid arthritis (74). In fact, further in-vivo studies must be done in the future to confirm their finding.

Xiuling Ji *et al.* (2019) (74) developed a glucosamine sulphate (GAS) distearoyl phosphocholine (DSPC) liposomes for osteoarthritis treatment. This kind of drug-loaded liposome combined the anti-inflammatory effect of the glucosamine sulphate and the lubrication ability of DSPC liposomes which expected to sustain anti-inflammation and reduced cartilage damage. Fig. 6 represented a simple diagram for DSPC–GAS liposomes preparation, characterization and release, lubrication, and anti-inflammatory activities. The best encapsulation efficiency (50.1%) was given by DSPC–GAS liposomes prepared with the molar ratio of 2:8 and the loading capacity was 29.3%. In addition, experimental release results indicated that the GAS-loaded DSPC liposomes could release GAS in a sustained manner in pure water (H_2O) and PBS for 14 days (Table 2). The glucosamine sulphate release behaviours and lubrication properties of the DSPC–GAS liposomes indicated that the salts (PBS) mainly enhanced the release properties and the lubrication through the electrostatic interaction, and the enhanced electrostatic interaction helped to stabilize the DSPC–GAS liposomes. To illustrate, in the PBS, the salt ions interacted electrostatically with the oppositely charged headgroups of the lipid molecules leading to the more tight packing of the DSPC liposomes than DSPC–GAS liposomes prepared in H_2O as summarized in Fig. 7 (75). The viability of chondrocytes using live/dead and CCK-8 assay was done and found that in the DSPC–the GAS group the cell viability was almost the same as the control (PBS) group for all incubation times (day1, 3 and 5), indicating that the DSPC–GAS liposomes had no cytotoxic effect on the chondrocytes. Moreover, the authors

demonstrated the anti-inflammatory effect of the DSPC–GAS liposomes through inhibition of the pro-inflammatory cytokine TNF-α, which caused the increase of the mRNA expression of IL-1β and IL-6 as well as the expression of a pain-related gene and catabolism protease (TAC1 and MMP1) associated with cartilage degradation. The results presented that DSPC–GAS liposomes reduced the production of IL-1β and IL-6 for TNF-α treated chondrocytes compared to a free glucosamine sulphate ($P < 0.01$). Addition of DSPC–GAS liposomes reduced the production of TAC1 ($P < 0.001$) and MMP1 ($P < 0.01$) of the TNF-α treated chondrocyte compared to free glucosamine sulphate. Lastly, the authors concluded that the DSPC–GAS liposomes were biocompatible and possessed a protective effect for inflammation-induced degeneration of chondrocytes. GAS-loaded DSPC liposomes could provide a new strategy for the treatment of OA in the future (75).

Liposomes are of great interest to researchers as they offer good biocompatibility and ability to protect the encapsulated drug, and the ability to encapsulate both lipophilic and hydrophilic agents (45,63). In addition, they are non-toxic as it is already used in the clinic for arthritis treatment (Lipotalon®), and there is already some formulation in the clinical trials to be used soon in the clinics. Overall, the liposomal formulation to targeted synovial delivery offers increased therapeutic activity and improvement in the arthritis treatment (63).

2.3. Emulsions.

Emulsion based drug formulations are mainly applied for topical or transdermal applications (76). The emulsion is a colloidal system composed of the oil phase, aqueous phase, surfactant, and co-surfactant (77–79). It keeps the drug in solubilized form as able to highly solubilize both lipophilic and hydrophilic compounds (80). In addition, using of nanoemulsion and microemulsion were well established now as drug delivery systems. It has been found that nanoemulsion provides an advantage over emulsion as in terms of the droplet size and stability, Fig. 8. The main problem with the conventional emulsions is the stability which is due to the larger size (≥ 1μm) of the emulsion droplets. These droplets provide high gravitational force and less repulsive force between the droplets. Therefore, fast

sedimentation can occur; the oil phase separated from the aqueous phase and moves toward the upper side of the bottle, Fig. 8 (81). On the other hand, nanoemulsion provides small size (10-250 nm) of the emulsion droplets and repulsion developed based on the charge on the surface of the droplets (81).

Moreover, micro and nano-emulsion have the size of a small droplet, range of 10–1000 nm, usually maintain a large amount of drug absorbed on the applied area which mainly due to the penetration enhancement effect of the carrier, mostly composed of saturated and unsaturated fatty acids as the oil phase. (78,82). The presence of oil in the formulation helps to improve drug bioavailability and to enhance its permeability into the tissue. Emulsion delivery systems could allow a controlled release profile by an increase in the pharmacological drug action at the site of application and decrease the systemic side effects (77–79). However, these formulations retain low viscosity; therefore, gelling agents such as carbomer 940 and xanthan gum are widely added to make it a suitable dosage form for topical applications (78,79). In Table 3, emulsion-based systems to deliver therapeutics for arthritis treatment were presented, and their preparation, characterisation and efficacy in relevant models were shown. Detailed information on the key studies was provided in the text below.

For transdermal administration at the joint inflammation site, Jagdale et al. (2018) (76) developed and optimized Nabumetone formulations based on microemulsion delivery system. The optimization was carried out using 32 factorial (F) design and F7 (factorial design number 7) among eight other batches was optimized which contained 0.124% w/w Carbopol 934 and 0.187% w/w HPMC K100M as a gelling agent, 0.71% w/w tween 80 as a surfactant, 0.35% w/w propylene glycol as a co-surfactant, 4.3% w/w liquid paraffin as an oil, 0.2% w/w drug and 11.68% w/w water. The factorial design suggested that drug release and gel viscosity values were strongly dependent on the concentration of the Carbopol 934 and HPMC K100M, respectively. F7 batch contained a high concentration of the gelling agent. The authors conducted diffusion study showing that the increase in the level of gelling

agent delayed the drug release from formulations which may be attributed to the increase in viscosity of formulations. In-vitro diffusion study for F7 batch presented 99.16±2.10 % drug release over egg membrane, around 95% drug release using cellophane membrane and 99.15±2.73% drug release in an ex-vivo study using rat skin. To conclude, transdermal delivery of Nabumetone can potentially alternate the oral formulation of Nabumetone to overcome its gastrointestinal side effect and to provide better patient compliance. An animal study should be done to evaluate Nabumetone microemulsion efficacy in arthritis animal model and to be a candidate for the clinical trial. (77). Another study was done by Goindi et al. (2016) (78) who prepared Tenoxicam (TNX) microemulsion topical formulation to overcome the gastrointestinal side effect associated with prolonging oral tenoxicam use. The formulations were prepared using Captex 300/oleic acid as oil, Tween 80 as a surfactant and n-butanol/ethanol as co-surfactant. The authors compared two TNX microemulsion formulations one containing 99.403 drug content (TNX03) and another one containing 99.801 drug content (TNX04) with conventional cream (TNX 01) and aqueous suspension (TNX 02) which both containing 1.5 mg of TNX. The ex vivo permeation study using mice skin showed that tenoxicam microemulsion formulation (TNX03 AND TNX04) had significantly higher ($p<0.001$) cumulative permeation value compared to TNX01 and TNX02. In addition, microemulsion formulations exhibited a significant ($p<0.001$) anti-arthritic and anti-inflammatory efficacy in mice and rat models of arthritis and inflammation, and their therapeutic effect as compared to an oral formulation. Histopathology studies using mice skin confirmed the dermal safety of both microemulsion formulations as no pathological changes were found on the mice skin microscopic structure. In conclusion, the study demonstrated that topical microemulsion formulations of tenoxicam could be used as an effective delivery system of tenoxicam and a potential alternate to the oral drug formulation (79).

Other researchers, Gokhale et al. (2019) (77), prepared and evaluated the efficacy of Quercetin (QCT) loaded nanoemulsion (NE)-based gel (QCT-NE) for the rheumatoid arthritis

treatment. The QCT-NE formulations were prepared using arachis oil as an oil phase, oleic acid as a permeation enhancer and oil phase, Tween 20 as a surfactant and PEG 400 as a co-surfactant. The cytotoxicity study and the effect on TNF-α production were evaluated in vitro using RAW264.7 cells. The results showed that QCT-NE had no toxic effect on synoviocytes and significantly inhibited LPS induced TNF-α production (P= 0.041) as compared to free QCT. The authors showed that QCT-NE gel twice improved drug permeation compared to free QCT gel. Besides, the topical application of QCT-NE gel in Wistar rats was found to be not causing any skin irritation symptom such as erythema and/or oedema within 72 h. In addition, it is significantly inhibited paw oedema in rats (51.13 mm) compared to the free CFA control group (71.21 mm) over 24hr (p=0.006). Hence this study confirmed that QCT-NE gel could be an efficient topical formulation for the arthritis treatment (78).

To conclude, using soft matter drug delivery systems such as emulsions target and improve the delivery and efficacy of arthritis drugs, and at the same time reduce toxicity and provide treatment safety (83). As examples above showed (Table 3), emulsion-based drug delivery systems provide good bioavailability of therapeutic actives, especially those with poor water solubility, low permeability, and short resident time inside the affected area (83,84).

2.4. Cationic carriers.

Cartilage consists of a highly anionic matrix as it composes of negatively charged glycosaminoglycans (GAGs) in their structure, Fig. 9 (36). This provides a high ability of binding sites for the positively charged agents, which will give an advantage by enhancing arthritis drugs uptake and prolong retention time inside the tissue (22,85,86). By binding drugs to positively charged carriers, cartilage can be act as a drug reservoir instead of a drug barrier for sustained intra-joint delivery (85).

A study by Bajpayee et al. (2014, 2016) (36,86) prepared and characterized avidin as a cationic carrier for treating post-traumatic osteoarthritis. In addition, they conjugated Avidin with dexamethasone (DEX) and tested the activity of avidin- DEX in inhibiting the catabolic

effects in cytokine-challenged cartilage relevant to post-traumatic osteoarthritis. Avidin has been shown to has around 10 nm size with a net charge of +20 mV which was able to penetrate 1000 μm thick ex-vivo bovine cartilage within 24 hr. Therefore, it was able to release dexamethasone (0.5 mg) inside the cartilage tissue, which significantly inhibited IL-1 production, thus decreasing GAGs loss over three weeks compared to free dexamethasone ($p < 0.05$). To conclude, avidin containing delivery carrier exhibited ideal characteristics for targeted intra-cartilage drug delivery as small size, and optimal positive charge allowing rapid penetration inside full-thickness cartilage improving drug uptake and retention time inside the cartilage tissue (36,87). A year later, another research group, Perni and Prokopovich (2017) (22) developed a targeted system to deliver drugs into cartilage tissue. The authors used poly-beta amino esters (PBAEs) as nano-vehicles to covalently bind a model steroidal drug, dexamethasone (DEX). The authors hypothesized that positively charged poly-beta amino esters nano-vehicles will stay in the cartilage tissue for longer due to electrostatic interactions with the negatively charged tissue components, glycosaminoglycans (GAGs). The efficacy of the developed system was confirmed in an ex-vivo bovine cartilage model where it was shown that uptake of DEX covalently bond to PBAEs by healthy cartilage was significantly higher ($p<0.05$) compared to a control, free Dex-phosphate (Dex-P) and the uptake was doubled by glycosaminoglycans depleted cartilage (simulated early stage of osteoarthritis) compared to free Dex-phosphate ($p<0.05$). The beneficial results of DEX conjugated to poly-beta amino esters were also observed on the retention of DEX by the cartilage matrix compared to the control (free Dex-P). The authors concluded that using poly-beta amino esters as a delivery system was biocompatible, biodegradable, effective and non-expensive to deliver drugs into cartilage tissue (22). In addition, in 2020, Perni and Prokopovich (85) published another study where they conjugated DEX to poly-beta amino esters, which was amine end-capped ethylene-diamine (e1) and with diethylene-triamine (e2). The uptake studies were performed using the ex-vivo bovine cartilage explant model, and the results showed that end-capping with e2 resulted in higher uptake than e1 and all formulations had a significantly higher uptake (p

<0.05) compared to DEX-P. Moreover, among all the PBAEs formulations, A5–e2 (A5 is the PBAE prepared from 1,4-butanedioldiacrylate and 3-(di-methylamino)propylamine which followed by e2 end-capped with diethylene-triamine) returned a DEX uptake almost 8 times higher than DEX-P after 10 mins of contact between cartilage explants and fluid containing the poly-beta amino ester –DEX conjugate. Additionally, the authors tested the efficacy of A5-e2 to prevent cartilage degradation by IL-1α (interleukine1α) and found that the addition of A5–e2 to the medium containing IL-1α led to the reduced osteolytic activity of IL-1α and after 8 days the cartilage sample observed the same amount of glycosaminoglycan as at time 0. When DEX was administered continuously no degradation of glycosaminoglycan was observed, and the tissues had the same amount of glycosaminoglycan as the controls ($p < 0.05$), no difference was observed between DEX-P or the same amount of steroidal drug conjugated to A5–e2 (86).

Another study was performed by Vedadghyami *et al.* (2019) (87) who designed cartilage penetrating and binding cationic peptide carriers (CPCs) able electrostatically bind to the high negative fixed charge density (FCD) of cartilage. The idea of making drugs positively charged was assumed to be used to convert cartilage from a barrier to a drug entry into a depot. These researchers prepared cationic peptide carriers with different charges of +8, +14, +16 and +20mV and measured their uptake and retention using the ex-vivo cartilage bovine model. The uptake results showed that cationic peptide carrier uptake increased with an increasing net charge up to +14mV, but it was decreasing as the charge increased further. This could be mainly due to stronger binding interactions between the cationic peptide carrier and glycosaminoglycan in the cartilage that prevented cationic peptide carrier penetration and uptake; therefore, that weak-reversible binding was important to enabling their penetration through full tissue thickness. In addition, when GAG amount was depleted in cartilage explants by 90%, the uptake of CPC (+14mV) was not hindered but it was reduced by 50%. Therefore, the charge-based binding occurred even in arthritis cartilage. This work suggested that the rational design of using cationic carriers based on

electrostatic interaction with fixed charge density can be extended to a drug delivery for other avascular, negatively charged joint tissues. In addition, an appropriate charge has to be determined for a drug carrier to enable a drug diffusion into an oppositely charged tissue. As in this work, the increasing net charge did not necessarily provide an increased uptake; therefore, an optimal charge range for a carrier of a given size can effectively target a tissue. Weak and reversible binding interactions were ideal for carriers to penetrate through the tissue zones, so they can reach joint cell and matrix. As illustrated in Fig. 10, arginine-rich cationic peptide carriers bind more strongly with the intra-cartilage negatively charged aggrecan- glycosaminoglycans compared to the lysine-rich cationic peptide carriers because of short-range H-bond and hydrophobic interactions that stabilized electrostatic binding (88). More information about various delivery systems using cationic carriers were provided in Table 4 in details.

To conclude, cationic carriers that form an electrostatic interaction with the anionic glycoprotein in the cartilage can augment drug penetration and retention within the cartilage (22,33). They are important to enable rapid drug penetration inside the cartilage and sustained delivery to the chondrocyte cell and matrix target within the joint tissue (33,87). In addition, electrostatic interactions can be used to significantly advance the era of targeted drug delivery for avascular, negatively charged cartilage tissue. They are biocompatible and can be used to convert cartilage from a barrier to a drug entry into a drug depot that can prolong drug uptake and penetration providing sustained drug doses over several weeks.

2.5. Hydrogels.

Hydrogels are a 3D dimensional polymer network act as a drug reservoir which extended the residence time of therapeutic agents (33,61,65). They are mainly used as an intra-articular injection to enhance hyaluronic acid formulations delivery in the joint tissue, which provides joint lubrication and arthritis pain relief. Hydrogels are composed of water-swollen natural or synthetic polymeric compounds that hold drug, therapeutics agents, proteins or even cells Fig. 11 (33,65,89). Hyaluronic acid (HA) is a complex glycosaminoglycan compound present

widely in the body tissues with the highest concentration in the synovial fluid. It is one of the major components of articular cartilage matrix (90). The primary function of hyaluronic acid is to provide the viscoelasticity and lubricating properties to synovial fluid, allowing normal fluid flow, joint motion and reducing articular cartilage damage (91,92). The normal concentration of hyaluronic acid in synovial fluid is 297 mg of hyaluronic acid per 100 ml, which is decreasing in osteoarthritis joint to 141 mg/100 ml (93). Therefore, the main objective of treatment osteoarthritis with hyaluronic acid is to restore the viscoelastic properties of the synovial fluid. Hydrogel loaded HA or hyaluronans (HAs) have been used for the treatment of painful osteoarthritis, and it is known as viscosupplementation treatment for osteoarthritis (91,92). hyaluronic acid injection (single dose 20mg/2ml) usually have a long duration of action around six months. On the other hand, it may cause transient pain, redness, tenderness at the site of injection, swelling, stiffness and difficulty of moving (94). Generally, hydrogels as active drug delivery systems for osteoarthritis reduce oral medication side effect, provide lubrication, anti-inflammatory and chondroprotective effects to the affected joint (25,61,65,95).

Summary information regarding drug delivery through hydrogels for arthritis was shown in Table 5 and detailed in the text below. A study by Ghosh et al. (2018) (94) developed and optimized aspasomes of methotrexate with ascorbyl palmitate as an antioxidant for rheumatoid arthritis treatment. The authors took the best working formulation and loaded it into a hydrogel for further assessment in vitro and in vivo employing adjuvant-induced arthritis (AIA) rat model. The results indicated that the formulation did not induce any irritation to the rat skin. Using AIA model, transdermal treatment with methotrexate aspasome loaded hydrogel led to reverse in paw diameter values (0.63) up to day 21 compared to methotrexate -free drug hydrogel (0.70), Aspasome hydrogel without methotrexate (0.72) and Arthritis control (after induction of arthritis, no treatment was given to this group of animals) (0.80). The histological evaluation of ankle joints of arthritic rats showed the degree of inflammation in periosteum region was mild in case of aspasome

hydrogel without methotrexate treated and moderate in methotrexate -free drug hydrogel-treated groups while methotrexate aspasome treatment showed near to normal control architecture of the synovial area. To conclude, the mixed effect of ascorbyl palmitate as an antioxidant with methotrexate anti-inflammatory activity led to the improved effect of methotrexate against rheumatoid arthritis. The study showed that methotrexate aspasome loaded hydrogel was a therapeutically active system providing an effective controlled drug release through the transdermal route with a drug loading of 21.46%, and possessing better disease modifications against rheumatoid arthritis than the free drug, thereby providing a more efficient therapeutic design for rheumatoid disease treatment (96).

In another study conducted by Chejara et al. (2017) (95), a novel microporous hydrogel was developed based on sodium alginate and 4-aminosalicylic acid (4-ASA) as potential viscosupplementation for arthritis treatment. The authors conducted cytotoxicity analysis using human dermal fibroblast-adult (HDF) cells, and the results indicated non-toxic characteristics of Alg-4-ASA hydrogel (95% cell viability). Drug release profiles presented 49.6% drug release in the first 8 h and 97.5% within 72 h, similar to the alginate gel which displayed 42.8% drug release in first 8 h and 90.1% within 72 h. Moreover, after applying external stimuli, the Alg-4-ASA hydrogel displayed significant structure recovery behaviour and gelling properties confirmed using rheological study (viscosity 8095.3 mPas and thixotropic area of 26.23%). The modified hydrogel, thus, provided a good possibility for improved synovial lubrication for joint-related injuries and arthritis-induced conditions. In addition, it was non-toxic, and have high drug release profiles permitting potential viscosupplementation for clinical application (97).

Recently, Yin et al. (2020) (96) developed a hydrogel-based delivery system for rheumatoid arthritis treatment composed of polyethyleneimine as a carrier for loading indomethacin and methotrexate, and then, this system was loaded into a temperature-sensitive hydrogel (D-NGel). The obtained hydrogel system effectively improved arthritis disease progression while indomethacin reduced pain and joint swelling in arthritis. Drug release profiles using D-NGel

were compared to the drug release from the carrier only, their results were 83.20% indomethacin and 70.69% methotrexate release within the first 2 h from the carrier, while 10.31% of the indomethacin and 33.10% of the methotrexate from the D-NGel. The prepared in situ hydrogel system was administrated intra-articular, and its anti-inflammatory activity was measured in a collagen-induced arthritis rat model, as detailed in Table 5. In addition, the authors assessed the formulation toxicity, liver and kidney function of treated rats and found that the drug-loaded hydrogel did not appear to have side effects on liver or kidney function. Overall, this work demonstrated the synergistic effect of indomethacin and methotrexate loaded in D-NGel, effectively improving the rheumatoid arthritis conditions by releasing these drugs in a controlled manner into the joint tissue (98).

Other researchers, Lu et al. (2013) (97), evaluated the effect of the hydrogel containing hyaluronic acid and doxycycline (HA-DOX hydrogel) through intra-articular injections using osteoarthritis rabbit model. The authors hypothesised that combining HA and doxycycline in the hydrogel may provide a synergistic effect through the anti-inflammatory and analgesic effect of doxycycline, hyaluronic acid and the lubricant effect of the hydrogel. The results showed that the HA-DOX hydrogel exhibited low cytotoxicity in vitro on the human chondrosarcoma cells SW1353 using a tetrazolium-based cell viability assay. The relative percentage of surviving cells was quantified and compared to the control (non-treated) group, which represented 100% survival. After the intra-articular HA-DOX hydrogel was injected in a surgically osteoarthritis induced rabbit, the percentage of weight distribution was significantly ($p<0.05$) reduced compared to the non-treated group indicating the analgesic effect of HA-DOX hydrogel. In addition, the macroscopic examination of articular surfaces of the femoral condyles and the tibia plateau found that the HA-DOX hydrogel significantly inhibited ($p<0.05$) the progression of osteoarthritis as measured by a loss of superficial layer, features, osteophyte, fibrillation and cartilage erosion compared to the non-treated group. Histological examinations of haematoxylin and eosin-stained sections of the cartilage in the femoral condyles confirmed the effectiveness of the HA-DOX hydrogel in

reducing osteoarthritis pathology ($p<0.05$). Overall, the HA-DOX hydrogel may act as an effective drug delivery system for osteoarthritis treatment as it can prevent osteoarthritis cartilage disease progression and reduce pain and inflammation (99).

In another study, Hui et al. (2007) (98) evaluated the efficacy of intra-articular injection of chondroitin sulfate (CS) loaded into a hydrogel for the treatment of chondral defects in the adult rabbit model. The authors studied five types of hydrogel carriers (α-CD-EG 4400, α-CD-EG 8400, α-CD-EG 13300, α-CD-PEG 20000, and α-CD-PEG 35000) and their results showed no sign of redness or swelling around the rabbit knee observed on days 3, 7, and 21 after injection of the CS-hydrogel or normal saline. The release study demonstrated 80% chondroitin sulfate released in one week, and the remaining 20% was retained for one month from the hydrogel. According to the results obtained, the authors concluded that α-CD-EG4400 was the best hydrogel carrier of chondroitin sulfate for the treatment of joint defect in rabbits (100). Additionally, intra-articular injection of chondroitin sulfate (100 mg/mL) carried by a-CD-EG 4400 hydrogel was effective in improving both the biomechanical and histological properties of the knee joints. On day 50 after the treatment, microscopic observation of the knee joint showed that in the saline-treated group, the surface of the lesion appeared uneven and showed no signs of healing while in the chondroitin sulfate -hydrogel group, the area of the lesion was almost completely covered with cartilage-like tissue and showed fewer signs of deterioration changes. In addition, there were no signs of tissue reaction or inflammation observed. Overall, this delivery system was effective in retaining the drug inside the knee joint for a long time and could be effective in the treatment of cartilage defect. However, further study in the large animal models has to be conducted before moving it to the clinical trials (100).

To conclude, hydrogels have been of great interest to researchers either to intraarticular use for hyaluronic acid preparation or as a carrier to deliver osteoarthritis drugs to the joints such as chondroitin sulfate. Their three-dimensional structure prevents the diffusion of the encapsulated drugs from it, thus increasing the retention of drugs in the joint tissue (11,45).

2.6. Comparative summary

As shown in Tables 1-5 various drug delivery systems possessed different characteristics and activity affecting their ability to provide controlled and prolong drug release (Fig. 12), drug uptake and retention with optimum behaviour and efficacy in in-vivo, ex-vivo and in-vivo. These data showed that carrier characteristics (size, charge, composition and preparation methods) affected the delivery efficacy, particularly release profile, therefore the researchers made efforts to optimise such characteristics in their studies.

Specifically (Table 1), ACP micelles (loaded with curcumin) showed more than 95% of curcumin was released in 7 days (Fig. 12) and prolonged retention time of curcumin for (28 days) in osteoarthritis joint and no induced cytotoxicity in-vitro. In addition, acid activatable curcumin polymer micelles were pH dependent for curcumin release. At acidic pH 6, more than 95% of curcumin was released in 7 days while 30% was released at neutral pH of 7.4. It was mainly because of acid catalysed hydrolysis of ester linkage of the acid activatable curcumin polymer micelles in acidic media which sustained and controlled release of curcumin at acidic media. Other nanoparticular formulation (Table 1), PIG nanoparticles aided sustained release of ibuprofen sodium for 7 days which mainly occured via diffusion of the IbS from the hydrophilic gelatin matrix of the nanoparticles. In other study (Table 1), Berberine chloride (BBR) encapsulated in chitosan nanoparticles were exhibited a prolong berberine chloride retention for 4 days and continuous BBR release behaviour of 70% cumulative release achieved in 7 days, Fig. 12. These NP formulations had appropriate particle sizes (170nm for the ACP micelles 50-400 nm for the chitosans and 200nm for the PIG nanoparticles) for delivery drugs to the joint. On the other hand, another technology, folate PEG- G3.5 PAMAM dendrimer (Table 1, Fig. 12) had the shorter release profile comparing to the other NPs delivery system, it gave 95% of indomethacin release in 24

hours which was 7 times shorter time than for acid activatable curcumin polymer micelles, chitosan nanoparticle and PIG nanoparticles

In another example (Table 2), DSPC–GAS liposome showed different efficiency mainly depending on the media of preparation. The authors found that the glucosamine sulphate release rate in PBS was lower than that in H_2O during 14 days release time. Specifically, 33.5 % of glucosamine sulphate was released from the DSPC liposomes in PBS while 44.9% released in H_2O after 72 h, Fig. 12. These results were due to the electrostatic binding between the salt ions in PBS, and the oppositely charged headgroups of the lipid molecules resulted in a tighter DSPC–GAS liposomes which led to the decrease in permeability and glucosamine sulphate release. In summary, encapsulation of DSPC– GAS liposomes in PBS provided greater encapsulation efficiency, lubrication properties and lower glucosamine sulphate release, Table 2. In other study (Table 3), a microemulsion was employed for encapsulation of tenoxicam (TNX), the obtained results showed higher skin retention of tenoxicam (11.429%), which was 4.6 times higher compared to the conventional suspension formulation (2.469%) and 11.5 times higher compared to the conventional cream formulation (0.988%). In addition, tenoxicam microemulsion possessed higher permeation in mice skin for 24hr (64.647%) compared to the conventional forms, which led to the improved anti-inflammatory activity of tenoxicam, Table 3. Another examples, microemulsion of nabumetone and nanoemulsion of quercetin provided improved drug release and efficacy compared to a free drug (Fig. 12, Table 3). Overall, emulsion-based drug delivery systems displayed shorter release behaviour compared to the other drug delivery systems formulations (Fig. 12). The shortest release profile for the emulsion formulations may be attributed to the low stability of the emulsion system and its need to be combined with another delivery technique to provide better sustained drug release profile.

Other technologies (Table 4) based on use of a positively charged carriers such as avidin, poly beta amino ester polymars and the cationic peptide carriers, interacting with negatively

charged glycosaminoglycans in cartilage matrix and thus improving drug uptake and retention in the cartilage tissue were proposed.

Specifically, avidin conjugated to dexamethasone (Dex) was significantly improving the efficacy of dexamethasone by inhibiting IL-1 production and decreasing glucosaminoglycans loss over three weeks period compared to free dexamethasone ($p < 0.05$) (Table 4). In other study, poly beta amino ester polymers prolonged the uptake, retention (2.5 hr compared to 30 min for Dex-p) and release of dexamethasone for 2 days (Table 4, Fig.12). In addition, use of cationic peptide carrier showed that optimization of the carrier charge was important to prolong the retention inside of the cartilage tissue. Weak and reversible binding interactions were ideal for carriers to penetrate through the tissue zones, so they can reach joint cell and matrix. The uptake results (Table 4) showed that the drug uptake increased with increasing net charge up to +14mV, and started decreasing as the charge increased further to +20mV.

Other delivery systems, hydrogels were also provided sustained drug release, prolong drug uptake and retention inside the cartilage tissue. As illustrated in Table 5, the hydrogel loaded chondroitin sulfate was provided 80% released of chondroitin sulfate in180 hr, and 20% of the drug was retained for 30 days, Fig. 12. Other hydrogel formulations such as Alg-4-ASA hydrogel and in situ the hydrogel controlled the release of indomethacin and methotrexate for 3 days compared to 24 hr for a free drug. The uptake of these drugs increased for 6h, with increase of the anti-inflammatory activity, as a consequence of the disease progression Table 5.

Overall, nanoparticles demonstrated the longest release followed by hydrogels, liposomes, and emulsion (Fig. 12). The better way to prolong release for arthritis drugs is by encapsulating them in a carrier that prevent the immediate drug release after administration, providing biocompatibility, biodegradability, appropriate size range to allow retention in the cartilage tissue. For example, chitosan nanoparticles provided 7 days release of berberine chloride (70%) compared to 2 days release of free berberine chloride (100%). Chitosan

possessed an appropriate size range (50-400 nm), positive charge (+21.87) that allowed its interaction with negatively charged proteoglycans in the cartilage matrix in the same time providing a good biocompatibility and biodegradability. Therefore, such system can provide a sustained drug release behaviour in cartilage for arthritis treatment and prevention.

3. Conclusions and future perspectives.

3.1. Conclusions

The ability to deliver the treatment to the site of a disease is challenging for effectively treating all the arthritis conditions. Using drug delivery systems (DDSs) such as nanoparticles, liposomes, emulsions, cationic carriers, and hydrogels can be a good strategy for targeting drugs to the affected joint tissue. Nanoparticles, hydrogels, and liposomes have been studied widely, and a lot of research were done using these nanocarriers. Emulsion and cationic carriers are the developing systems, and a few ongoing studies were focusing on these types of vehicles. These delivery systems are expected to be effective for many reasons, such as capacities to improve drug bioavailability to the affected joints, to increase drug uptake and retention, and to release drugs inside the cartilage tissue in controlled and prolong manner. In addition, many factors impacted on the efficacy of the drug delivery systems for providing sustained release and resident time, including carrier charge, size, composition, preparation methods and biodegradability. Successful drug delivery systems require attention to control specific parameters such as the possibility for causing toxicity, cost, degradation and clearance of the drug from the site to avoid possible complications and systemic side effects (25,35).

Table 6, summarizes the advantages and disadvantages of the discussed drug delivery systems. The current drug delivery systems are created with attention to many of these factors, and any future investigation will need to take these factors in considerations to create an effective drug delivery system.

3.2. Future perspectives.

The problem with the OA drugs is that they lack localization and adequate uptake and retention of the drug to the area of interest which mainly due to the vasculature joint tissue nature that causes rapid clearance of drugs from the tissue (33). In addition, most of the drugs are hydrophobic, and an appropriate delivery system is required to be able to deliver them inside the affected joint, therefore delivery of these therapeutics is a challenge. Accordingly, many vehicles, materials and methods have been proposed and studied to design effective drug delivery systems with prolonged release profiles over days or month. In addition, as concurrent research in arthritis treatment and use of advanced delivery design, it seems to be that discovering and synthesizing new biocompatible and biodegradable carriers like nanoparticles, liposomes, emulsions, cationic carriers, and hydrogels are promising, as well as using combination systems composed of different types of the carriers to overcome the single drug delivery systems limitations. Nanoparticles (NPs) are the ideal candidates for targeting joint tissue especially when creating them in the appropriate size and charge or combining them with other carriers such as hydrogel or emulsion (101). It is important to design carriers that are biodegradable and non-toxic, which can naturally be eliminated from the body by natural metabolic pathways. Some of the examples of such nanoparticular systems that are FDA approved, are poly(glycolic acid) (PGA), poly(lactic-co-glycolic acid) (PLGA), and poly(lactic acid) (PLA) nanoparticles which are already used for other medical applications such as cancer, viral infection, osteoporosis and neurodegenerative disorders (102).

Rapid advances in biomedical and biotechnological sectors can improve the field of drug discovery and lead to appearance of new and efficient drug delivery systems that can effectively target drug candidates. Potent therapeutics in conjunction with effective and safe drug delivery systems are able to relieve pain, reduce the inflammation targeting the cartilage and bone conditions. Current and future research on arthritis diseases together with novel therapeutics and drug delivery systems tested in pre-clinical and clinical trials will lead to new treatment strategies that can fulfil the joint disorder needs, improve patient quality of

life and compliance. At present, many kinds of research of different drugs are reported for the management of arthritis, but a limited number of formulations entered clinical trials. The field still requires further in vivo study to be able to use technologies in the clinic successfully.

4. Acknowledgements

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5. Figures captions

Figure 1. Differences between normal and arthritis joint.

Figure 2. Articular cartilage structure.Reproduced from Ref. 34 with permission from Elsevier.

Figure 3. The therapeutic window of the administered drug. Reproduced from Ref. 42 with permission from Elsevier.

Figure 4. Schematic showing ACP micelles as a therapeutic system for osteoarthritis. Reproduced from Ref. 51 with permission from Elsevier.

Figure 5. The schematic view illustrates the structure of the liposomes. Liposomes formed by phospholipid bilayer (hydrophobic region) and the aqueous central core. Reproduced from Ref. 63 with permission from Elsevier.

Figure 6. Simplified model of the preparation and characterization of DSPC–GAS liposomes integrating sustained drug release and improved lubrication. (a) The chemical structures of DSPC and GAS. (b) Schematic diagram of the preparation, drug release and lubrication of DSPC–GAS liposomes, as well as their anti-inflammatory and chondroprotective potential in primary mouse chondrocytes treated with TNF-α. Reproduced from Ref. 75 with permission from The Royal Society of Chemistry.

Figure 7. Mechanism of the sustained drug release and the improved lubrication of the DSPC–GAS liposomes in different media (H_2O and PBS): (a) In the presence of salts, the salt ions in PBS and the oppositely charged headgroups of the lipid molecules interacted electrostatically, leading to a more compact packing of the DSPC liposomes; (b) DSPC–GAS liposomes were prepared in H_2O. Reproduced from Ref. 75 with permission from The Royal Society of Chemistry.

Figure 8. Comparison of nanoemulsion technology and conventional emulsion in terms of droplet size and stability. Reproduced from Ref. 81 with permission from Elsevier.

Figure 9. Cationic carrier and their proposed electrostatic interactions with GAGs in articular cartilage.

Figure 10. Charge based intra-cartilage delivery of CPCs at multiple length scales. A. Intra-articular (IA) injection of CPCs: Electrostatic interactions enable rapid and full depth penetration of CPCs into negatively charged cartilage. B. Tissue level transport: High upward Donnan partitioning at the synovial fluid-cartilage interface results in steep intra-cartilage concentration gradients for CPCs, thereby reducing the time (s) required to reach intra-cartilage therapeutic index (*). Weak-reversible binding of CPCs with negatively charged intra-cartilage sites enables their full depth penetration. C. Molecular-level transport: Inside cartilage, CPCs bind with aggrecans via long-range charge interactions. The electric potential of negatively charged aggrecans u(_) drops exponentially as a function of distance _ and defines the debye length or spacing between aggrecan chains. The resulting electrical fields determine intra-cartilage electro-diffusive transport and binding of CPCs. This binding is further stabilized by short-range H bond and hydrophobic interactions. Reproduced from Ref. 88 with permission from Elsevier.

Figure 11. In articular joint, hydrogels are retained within the synovial fluid and slowly release drugs. Reproduced from Ref. 89 with permission from Elsevier.

Figure 12. Release profiles of various drug delivery systems.

6. Tables captions

7. List of Figures

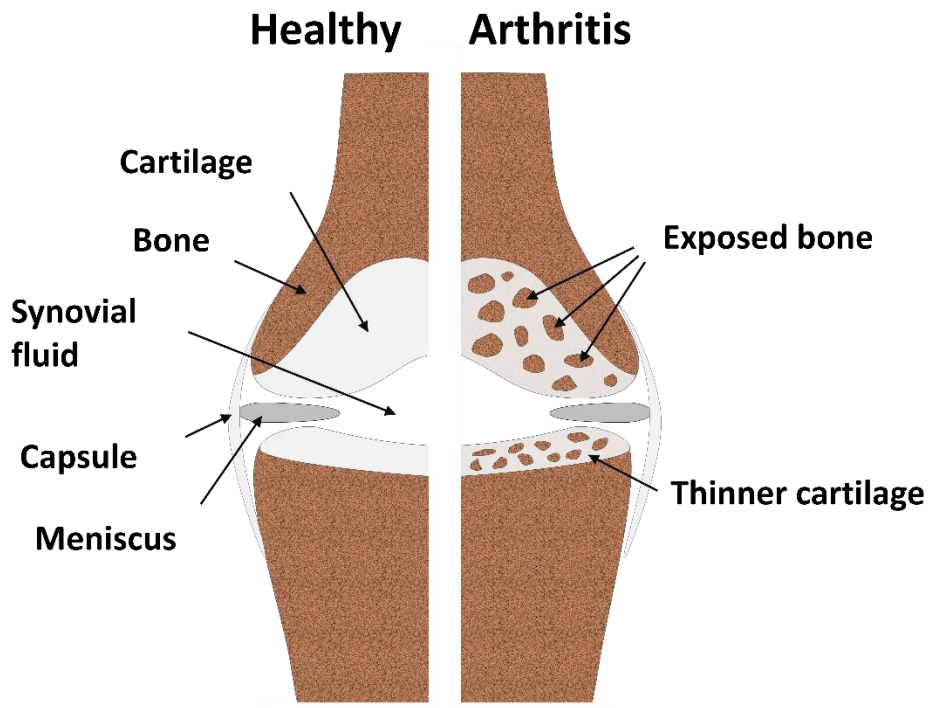
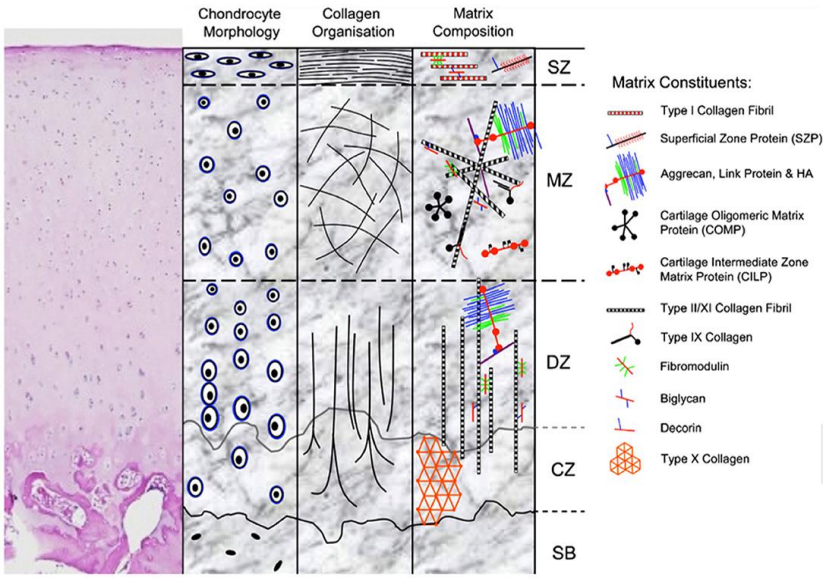


Figure 1.



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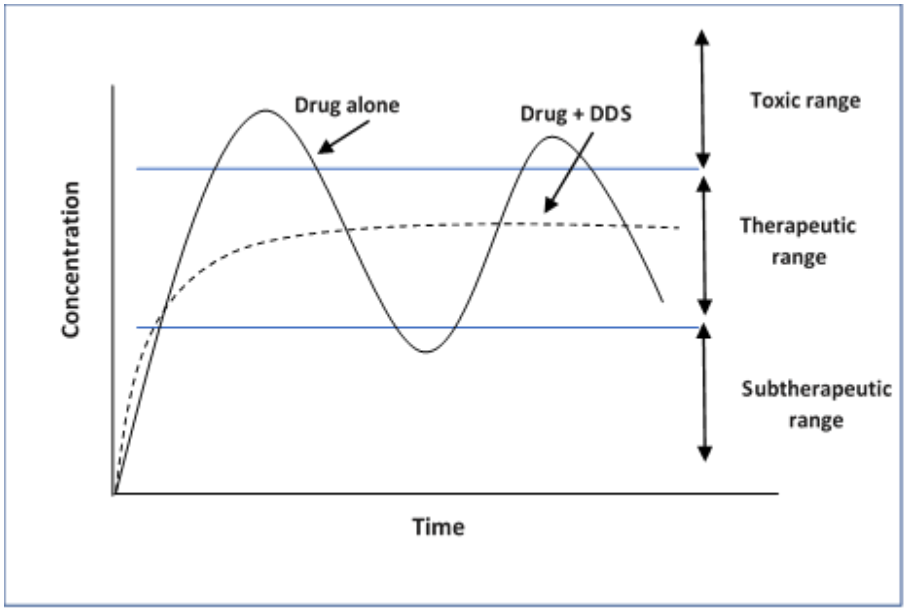

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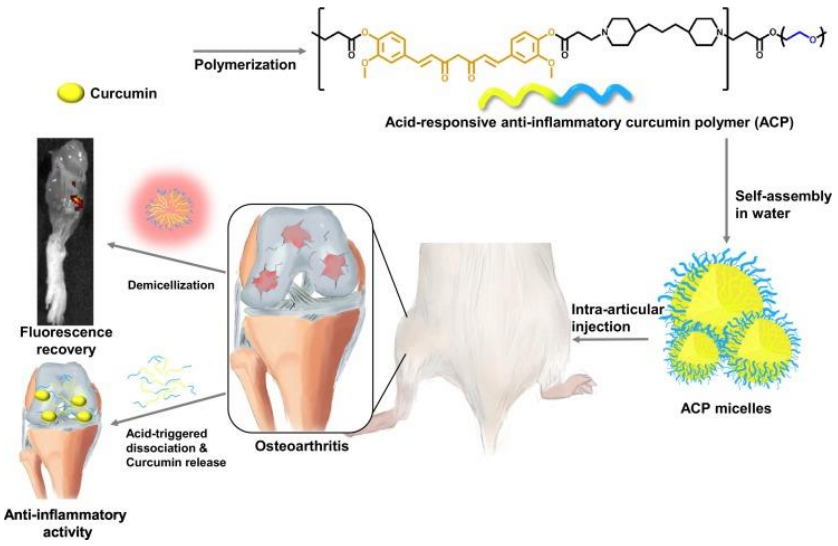

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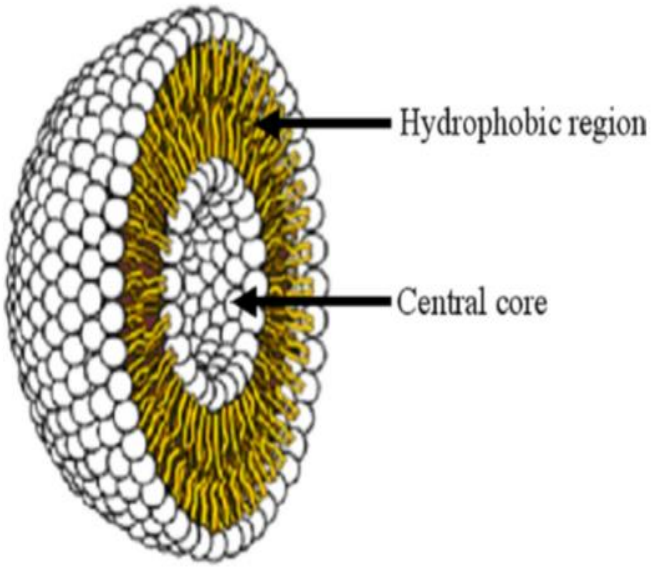

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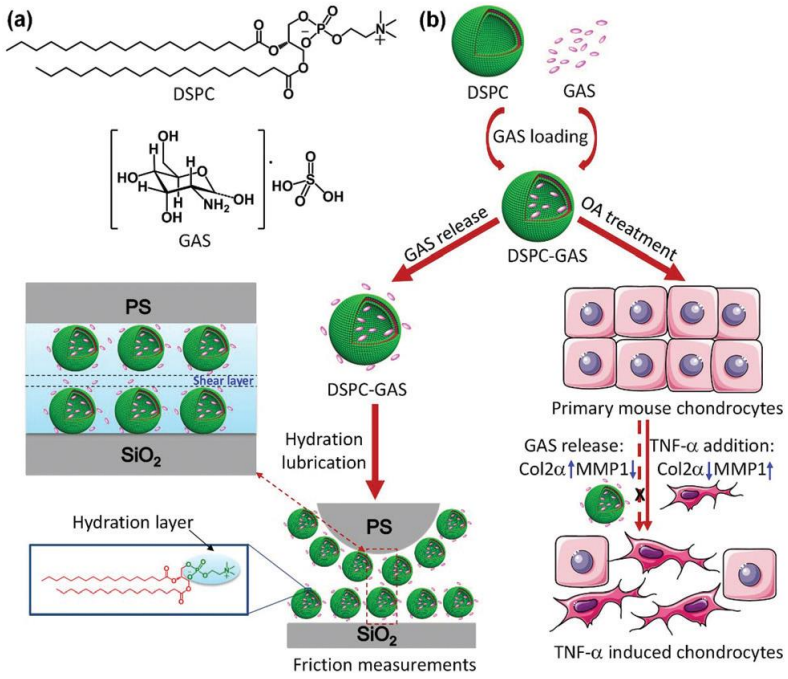

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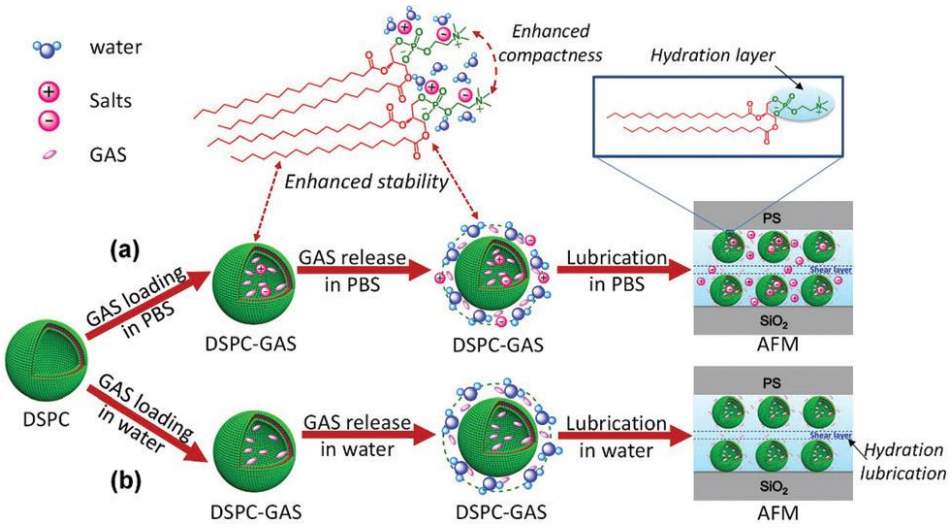

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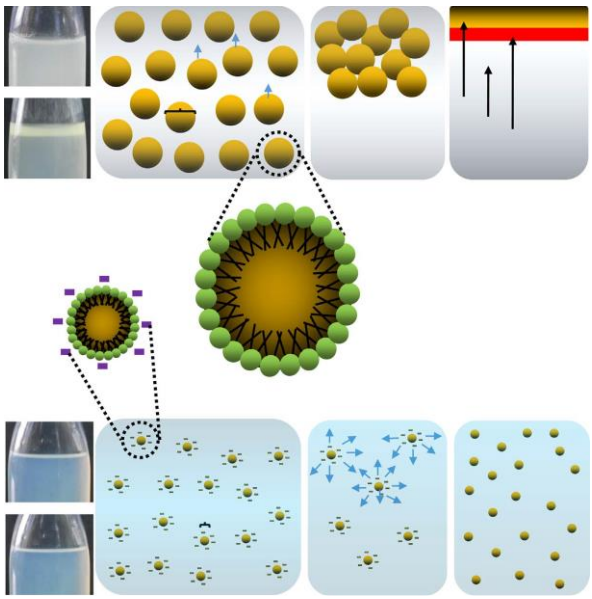

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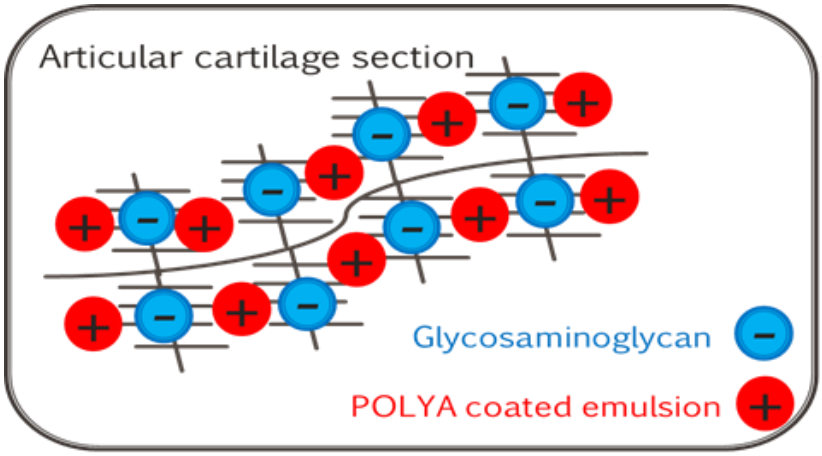

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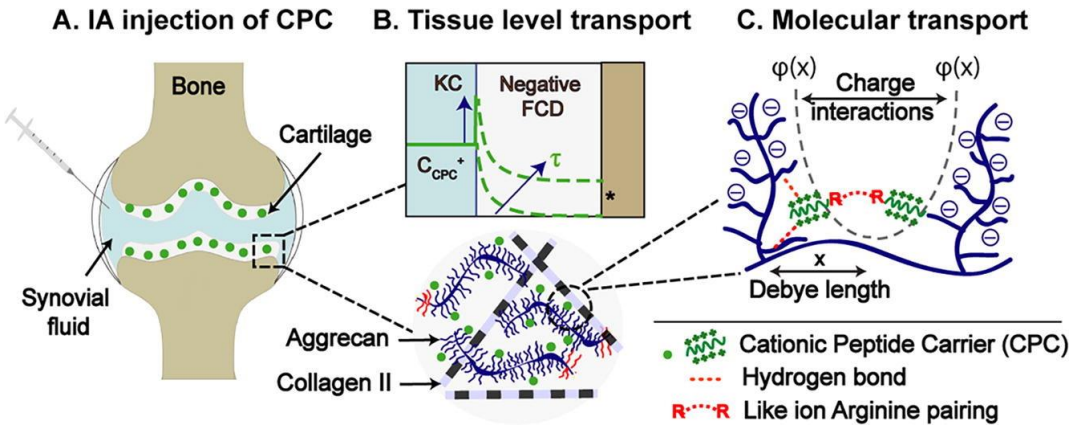

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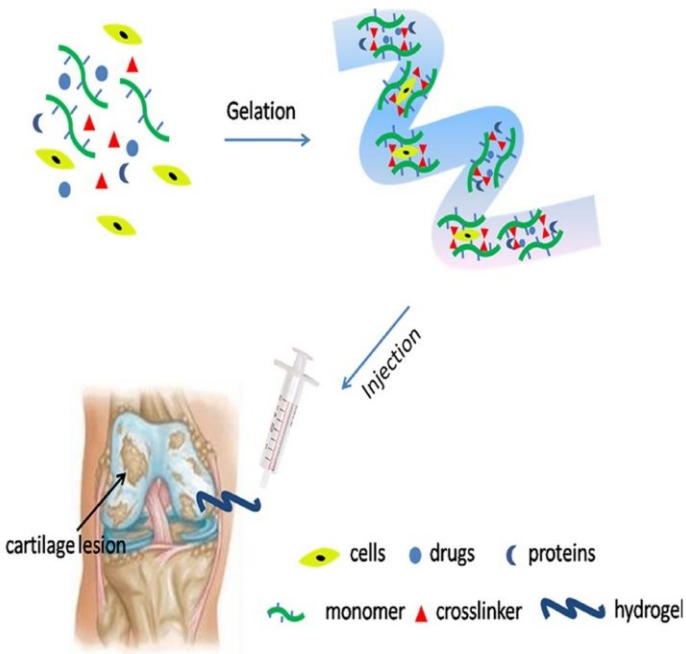
Gelation
Injection
cartilage lesion
cells
drugs
proteins
monomer
crosslinker
hydrogel

Figure 11.

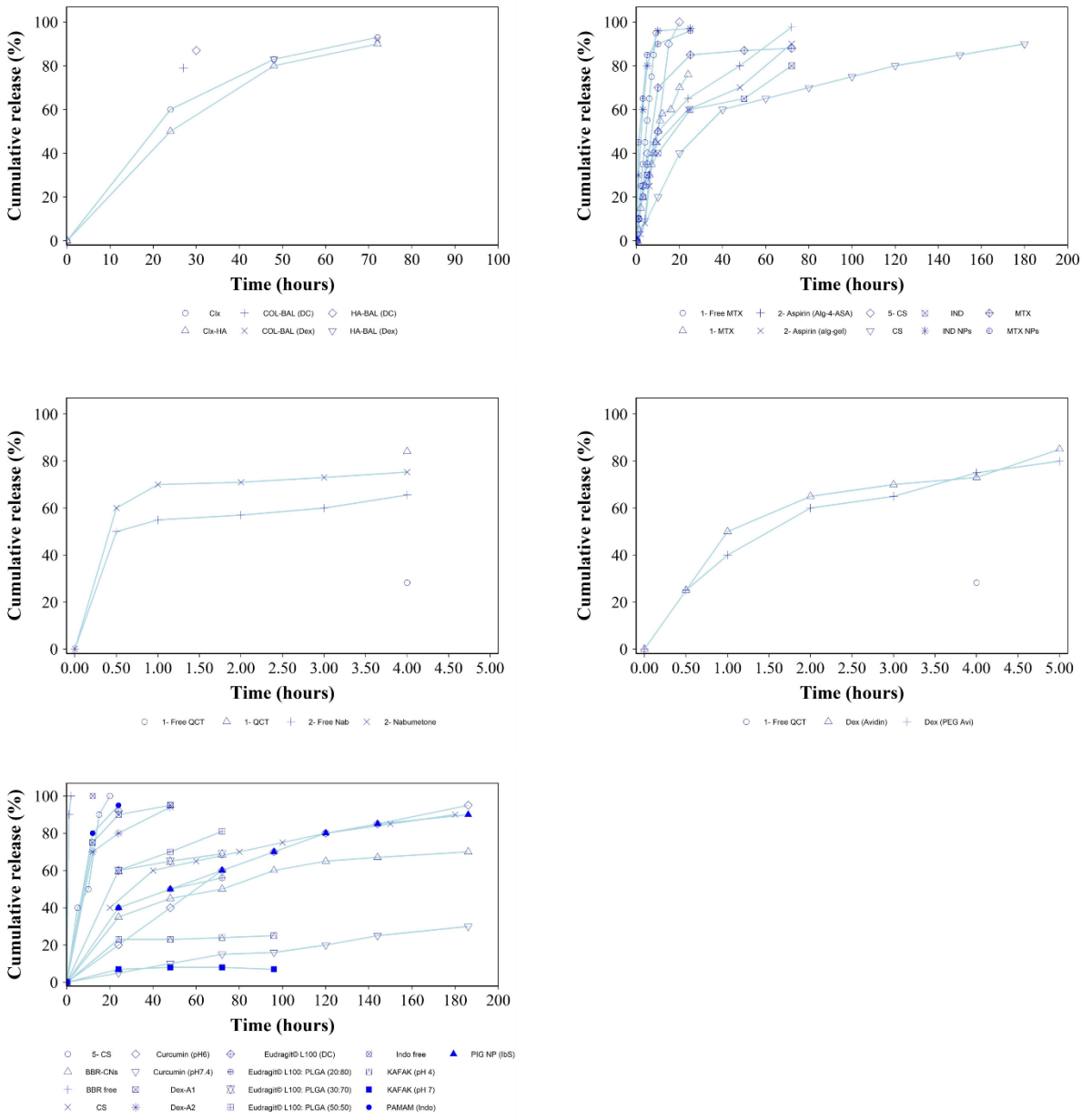

Figure 12.

8. List of Tables

Table 1.

Nanocarrier system	Size (nm)	Charge (mV)	Drug	Uptake	Retention	Activity	Cytocompatibility	In vitro	In vivo	Ex vivo	Control	Ref.
Acid activatable curcumin polymeric (ACP) micelles (10& 20mg/kg)	~170	Positive	Curcumin	In pH 6, >95% of curcumin released within 7 days.	Histological showed prolonged retention time (28 days) and released curcumin in OA joint compared to control.	Potent antioxidant and anti-inflammatory activity	It induced no cytotoxicity against RAW264.7 and chondrocyte cells at concentration < 100µg/ml	Using RAW264.7 cells and chondrocytes doing retention and toxicity says.	ACP micelles (5mg/kg) suppressed the expression of TNF-α (P<0.1) and IL-1β (P<0.01), suppressed ECM damage and maintains cartilage	NA	Curcumin (5.42 mmol). Mono-iodoacetic acid (MIA) which dissolved in PBS at 10 mg/ml.	(51)

									integrity for 28 days compared to control			
1- Eudragit ® L100	1- 274	1- −1.53	Diclofenac sodium (DC)	1- DC loading 14.26%, EE 62%	1- 92% of DC released in 12 h	Potent anti-inflammatory, analgesic, and antipyretic activity	PLGA is biocompatible and biodegradable	In-vitro drug release has been performed to pH 6.8 phosphate buffer solution at 37C^{o} using the sample and separate methods.	NA	NA	Eudragit® L100 NP	(53)
2- Eudragit ® L100: PLGA (50:50)	2- 263	2- −1.27			2- 56% of DC released in 72 h							
3- Eudragit ® L100:	3- 247.4	3- −0.46		2- DC Loading	3- 69% of DC							

PLGA (30:70)				12.21%, EE 53.1%	released in 72 h.							
4- Eudragit ® L100: PLGA (20:80)	4- 241	4- 3.47		3- DC Loading 10.51%, EE 45.3%	4- 81% of DC released in 72 h							
				4- DC Loading 5.96%, EE 25.82%								
PEGylated gelatin NP (PIG NP) 1, 2.5 &	200	-23.15	Ibuprofen sodium (IbS)	EE 72%	90% of IbS released from the NP in PBS at 37C^{o} in 5 days.	Potent anti-inflammatory, analgesic, and	Macrophage toxicity studies on RAW264.7 for 24 hr observed no	In-vitro inflammatory response of PBMCs over interaction with NP was	In the rat model, INF-γ, TNF-α, IL-8 and IL-4 level was measured	NA	Free IbS (400 mg) and non-	(54)

100 mg/kg						antipyretic activity	toxicity (90% viability) compared to media only.	observed no significant immune response	and found the activation level was not significant (P<0.05) compared to the control (media only).		PEGylated gelatin NP (IG NP)	
Chitosan -NPs (CNs) 0.6mg/ml	50-400	+21.87	Berberine chloride (BBR) 60µg/ml	NA	55.7 % of BBR released from the CNs in PBS at 37C^{o} in the first 3 days and~	Anti-inflammatory promote cell survival and matrix production.	Tunnel assay confirmed that BBR-CNs had significantly lower apoptotic effect then	In-vitro released of BBR was studied	In OA rats, BBR level decreased from the BBR solution in 2 days while it remains in the synovial fluid from	NA	BBR solution (60µg/ml in 50µl PBS)	(55)

					70% in the next 4 days		BBR (P<0.05)		BBR-CNs after 4 days. It is significantly downregulated mRNA expression of caspase-3 and Bax while upregulated Bcl-2 compared to OA-induction group (P<0.05).			

Hyaluronic acid (HA)/ Chitosan NPs (5-40µg/ml)	115.6	+26.3	plasmid	NA	NA	Gene delivery to chondrocyte	MTT assay confirmed that it is safe and showed > 90% cell viability	Transfection efficiency optimal condition was pH < 7, N/P ratio of 5 and plasmid concentration 4µg/ml. In chondrocyte cell, EGFP expression observed after 48 hr – 5 days of the cell culture period	NA	NA	Lipofectamine (5µg/ml) and CS plasmid NPs (5-40µg/ml)	(56)

PLGA/ PLA copolymer (40µg as BP/500µl of saline)	120	NA	Betamethasone disodium phosphate (BP)	NA	In-vitro BP released in PBS at 37C^{o} for 44 days. In-vivo BP released detected even after 14 days.	Anti-inflammatory	Biodegradable, biocompatible and has low toxicity	In-vitro study drug release.	Using an adjuvant-induced arthritis rat model to study BP release and retention	NA	Free BP (50µg/ml)	(57)
Folate PEG-G3.5 PAMAM dendrimer	NA	anionic dendrimer	Indomethacin (3.3 mg/kg)	Compared to PI, it shows 8.5–6.2 times less uptake in the stomach	Controlled release of indomethacin up to 24 h compared to 6 hr for the PI.	Anti-inflammatory	RBC hemolysis and MTT cell viability study showed no cytotoxicity (103)	Loading efficiency and release studies (in PBS for 37C^{o})	In arthritis rats, the half-life of conjugate indomethacin was 8.47 times higher	NA	Native dendrimer, free PAMAM–indome	(58)

				and 11 - 8 times less compared to free indomethacin					than the control.		thacin complex (PI) (3.3 mg/kg)	
Chondroitin sulphate NPs CS-S (200ng/ml)	30-200	NA	Selenium	Selenium EE~10.1%	NA	Maintain tissue structure integrity as a major component of cartilage matrix	MTT showed 98-60 % cell viability using SeCS 1.7-340 ng/ml. 8.02% apoptosis compared to 29.4% induced by T-2 toxin and	MTT and apoptosis assay on chondrocyte cells.	NA	NA	Sodium selenite, Chondroitin sulphate (200ng/ml)	(59)

							18.82% by Chondroitin sulphate					
PEGylated pNIPAM NPs with degradable disulphide crosslinks (NGPEGSS)	223µm	-3.81	KAFAK (anti-inflammatory peptide) 40 µM	Confocal microscopy confirmed that the uptake in the endosomal compartment is significantly higher in NGPEGSS compared to	In PBS, 7% of the drug was released at 24 hr and 24% released at pH 4.	KAFAK (anti-inflammatory peptide).	CellTiter assay showed no cytotoxicity after incubation NGPEGSS with chondrocyte cell for 48 hrs.	Chondrocyte NPs uptake and cytotoxicity. KAFAK suppressed pro-inflammatory TNF-α and IL-6 production compared to lipopolysaccharide (p<0.05)	NA	Bovine (knee explants.	Non-degradable NGPEGMBA NPs	(60)

				NGPEGMBA ($p < 0.01$)								

NP: nanoparticle, PBMCs: peripheral blood mononuclear cells, EE: Entrapment efficiency

Table 2.

Liposomal system	Size (nm)	Charge (mV)	Drug	Uptake	Retention	Activity	Cytocompatibility	In vitro	In vivo	Ex vivo	Control	Ref.
IV NSSL-MPS (10mg/kg)	80	NA	Methylprednisolone hemisuccinate	NA	NA	Suppress the secretion of IL-6 (75%), TNF-α	Nontoxic	NA	Using Lewis rat, After 72 hr arthritis progression score was 2 compared to	NA	Free MPS (50mg/kg)	(68)

						(97%) and INF-γ-50%			8 for free MPS.			
SC NSSL-MPS (10mg/kg)	80	NA	Methylprednisolone hemisuccinate	NA	NA	Suppress the secretion of	Non-toxic	NA	Using Lewis rat, arthritis progression score was 2.3 compared to 9.8 for free MPS within 96h.	NA	Free MPS (10mg/kg)	(68)
Liposome loaded Clx-HA Clx (0.5mg/ml) HA	4.98µm	NA	Celecoxib (Clx)	NA	Clx release was slower compared to the control for 72hr	Analgesic and anti-inflammatory activity	Non-toxic	Drug release conducted to PBS containing 1% Tween-80 using dialysis	Using OA rabbits, % weight distribution was 57% over 48 hr	NA	Clx liposome (0.5 mg/ml), saline and 1%	(69)

(10mg/ml)								membrane for 72hr			HA (10mg/ml)	
Bioadhesive liposome with HA (HA-BAL)	NA	NA	Dexamethasone 100 nM (DEX) and diclofenac 100 nM (DC)	NA	For HA_BAL, release half-life was 1.5 for DC and 2.2 for DEX release	Analgesic and anti-inflammatory activity	Non-toxic as MRI examination showed animal weight was increased normally	Using CT-26 cell line, HA-BAL allowed 10% of COX enzyme and protein expression activity	Using OA rat, joint inflammation reduced to 12.3% for HA-BALon day17	NA	Free drugs and single encapsulated drugs (100 nM)	(70)
Bioadhesive liposome with collagen					For COL-BAL, DC release half-life was 1.3, and			Using CT-26 cell line, COL-BAL allowed 30% of COX enzyme and	Using OA rat, joint inflammation reduced to 23% for COL-			

(COL-BAL)					DEX release was 2.9			protein expression activity	BAL on day17.			
Liposomal PLP 1&10 mg/kg) and liposomal BUP (1mg/kg)	90-110	NA	Prednisolone phosphate and budesonide	NA	NA	Anti-inflammatory	Improved safety use less effective dose,1 mg/kg of Liposomal PLP compared to 10 mg/kg of PLP	NA	Using mice - AIA. Lip.PLP suppress joint swelling by 79% at day 1, and Glucocorticoids level by 24% for 21 days.	NA	Free PLP (10mg/kg)	(72)

									Lip.BUP suppress joint swelling by 98% at day 1 and Glucocorticoids level by 34% for 21 days.			
chondroitin sulphate (10mg/ml) entrapping	250.2	-9.44	Chondroitin sulphate	NA	NA	Anti-inflammatory and tissue regeneration	Cytocompatibile MTT assay: L-CS OD (0.9nm) H2O2 OD (0.1nm). LDH assay:	Using L929 fibroblast cells. L-CS increased 90% of cell viability compared to	NA	NA	Liposomes (L) and free CS (10mg/ml)	(73)

liposomes (L-CS)							L-CS OD (0.22 nm) H2O2 OD (1.8 nm)	H2O2-treated group. L-CS inhibited 4.3-fold TNF-a production compared to H2O2- group				
TRX-20 liposomes	NA	NA	prednisolone phosphate (PSLP) (1000 nM)	The uptake was increased for 48h measured in HFLS cells for 48h using fluorescenc	The interaction of HFLS cells with TRX-20 liposomes was 40 times higher than the control	Anti-inflammatory	Non-toxic	Using HFLS cells, TRX-20 liposomes significantly inhibiting the production of IL-6, IL-8, and GM-CSF compared to	NA	NA	PSLP-containing liposomes without TRX-20, PSLP and	(74)

				e microscopy				free PSLP (P<0.001).			non-PSLP.	
DSPC–GAS liposomes	In H2O (120). In PBS (109)	In H2O (2.8). In PBS (1.2)	Glucosamine sulphate (GAS) (5.0 mM)	NA	GAS released GAS in H2O (80%), and PBS (70%) was prolonged to 14 days compared to free GAS (97.2% in 2hr.)	Anti-inflammatory and the lubrication activity	The Live/Dead assay and the CCK-8 test confirmed no cytotoxicity, good viability, and proliferation of chondrocytes	Using primary mouse chondrocyte, TNF-α-induced expression of IL-1β (33.8%; $p < 0.05$) and IL-6 (25.3%; $p < 0.01$) was inhibited by DSPC–GAS liposomes compared to	NA	NA	free GAS	(75)

								the TNF-α-treated blank group				

NA: not available, EE: Entrapment efficiency

Table 3.

Emulsion system	Size (nm)	Charge (mV)	Drug	Uptake	Retention	Activity	Cytocompatibility	In vitro	In vivo	Ex vivo	Control	Ref.

Micro emulsion	157.9	-9.50	Nabumetone (10mg)	NA	67.60% release from the emulsion in 15 min compared to 47.83% for the control. 75.32 % release in 4hr compared to 65.55 % for the control.	Anti-inflammatory and analgesic effects	NA	Diffusion study for F7 batch show 95% release using cellophane and 99.15 % release using egg membrane, over 8 hr.	NA	Using rat skin, F7 batch showed 99.15 % release over 8 hr	Plain drug	(77)
Micro emulsion	TNX03-106	Near zero	Tenoxicam (TNX) 1.5 mg	NA	Skin retention of	Anti-inflammatory and	Histology study observed	NA	Using rat, the anti-inflammatory	Using mice skin, TNX permeation	The aqueous	(79)

	TNX04-122				TNX was TNX03 (11.429%), TNX01 (2.469%), TNX02 (0.988%), TNX04 (13.551).	analgesic effects	no pathological changes on mice skin confirmed the formulation safety		efficacy of TNX 03 and TNX 04 were higher (p<0.001) compared to TNX 01 and TNX 02	for 24hr was 64.647% (TNX 03), 70.829% (TNX 04), 7.31% (TNX02), 27.972% (TNX01). Xylene-induced mice ear edema was inhibited using TNX 03 (65%) and TNX 04 (70%)	suspension (TNX 02) and conventional cream (TNX 01)	

										compared to TNX 01(15%) and TNX 02(10%)		
Nano emulsion (NE)	136.8	-25.4	Quercetin (QCT) (10mg)	EE: 94.65%	84.12% of drug release from QCT-NE compared to 28.23% from free QCT	Anti-inflammatory	Non-toxic as it does not inhibit synoviocytes growth, relative growth rate at 48h in free QCT 104 % and 130 % in QCT-NE	Using RAW 264.7 cells, QCT-NE significantly reduce secretion of TNF-α compared to free QCT (p=0.041)	In rat model, paw circumference was 71.21mm in CFA treated group and 51.13% in QCT-NE gel group. Arthritic index in CFA treated group 3.7 and 1.6 in QCT-NE gel.	Using rat abdominal skin, QCT permeation for 24hr was 62.51% for QCT-NE gel and 35.87% for free QCT gel	free QCT (10mg), Complete Freund's adjuvant (CFA) model	(78)

									Stiffness score of CFA- group 2.1 and 0.7 3 for QCT-NE gel group			

NA: not available, EE: Entrapment efficiency

Table 4.

Cationic carrier	Size (nm)	Charge (mV)	Drug	Uptake	Retention	Activity	Cytocompatibility	In vitro	In vivo	Ex vivo	Control	Ref.
Avidin	10	+ 20	Dexamethasone (Dex) 100μM	400 times greater uptake of avidin compar	96% of avidin remained inside the cartilage by 15 days, 50% of neutravidin	Anti-inflammatory	Using the live-dead fluorescence assay, minimal cell death observed	Dex release in PBS at 37°C, 70% released in 3h	Using rabbit Avidin-Dex suppressed injury-induced joint swelling and catabolic gene	Using bovine cartilage explants, Dex inhibited sGAG loss (20%) in 10 days	free Dex (0.5 mg)	(36,87, 104)

				ed to neutravidin	diffused out by day 1		similar to untreated controls		expression to a greater extent than free Dex ($p < 0.05$).	compared to IL-1α (50%) alone (P<0.0001)		
PBAEs nano-vehicles	A1: 286 A2: 153	A1: +11.60 A2: +8.94	Dexamethasone	Compared to DEX-P uptake of DEX from A1-2 was increased gradually with	In A1-DEX detected in the cartilage after 2.5 hr. In A2, no drug after 90 min. In control group, no drug after 30 min.	Anti-inflammatory	Using LDH and MTT assays, the viability of chondrocyte was not affected after exposure to PBAEs for 3 days.	NA	NA	Bovine cartilage to study uptake and retention	Free dexamethasone phosphate (DEX-P)	(22)

				the incubation time (P<0.05)								
CPC	Within 10	Between +7 and +20	NA	+14 has higher uptake	In 10X PBS, 25% retention measured for CPC +8 and 83% retention for CPC +20 after 24 h desorption	NA	Using live/dead assay, chondrocyte viability was not affected over the 8-day culture period	NA	NA	Bovine cartilage to study uptake and retention	NA	(88)

Table 5.

Hydrogel	Size (nm)	Charge (mV)	Agent	Uptake	Retention	Activity	Cytocompatibility	In vitro	In vivo	Ex vivo	Control	Ref.
Hydrogel loaded aspasome MTX	386.8	−30.81	Methotrexate (MTX) aspasome (100 mg)	NA	76% release of MTX in 24 hr	Anti-inflammatory	NA	In-vitro drug release has been performed to PBS pH 7.4 using Franz diffusion cell array	Transdermal application for 12 days reduced rat paw diameter (21.25%), TNFα (33.99%), IL β (34.79%), cartilage damage (84.41%), inflammation (82.37%), pannus formation (84.38%), and bone resorption (80.52%) as compared to	NA	Free aspasome formulation. Free methotrexate-treated group	(96)

									arthritic control rats.			
Microporous Sodium alginate (alg) and 4-aminosalicylic acid(4-ASA) hydrogel	NA	NA	Alg and 4-ASA	NA	97.65% of aspirin was released Alg-4-ASA compare to 90.1% from algenate gel in 72 hr	Viscosupplementation therapy	95% of cell viability after 24hr.	In-vitro aspirin release has been performed to PBS pH 7.4 at 37C^{O}. plication. Human dermal fibroblast-adult (HDFa) cells used	NA	NA	Algenate gel	(97)

								for toxicity study				
In situ hydrogel	82.71	57.30	IND (10 mg) & MTX (5 mg)	The uptake was time depended; fluorescence signals increase with the time within 6h	80% IND and 88% MTX was released within 72 h.	Analgesic, anti-inflammatory and reduce disease progression	Nontoxic using MTT assay and has no side effects on liver or kidney function	Raw264.7 cell line for MTT assay. In-vitro drugs release has been performed to PBS pH 7.4 at 37C^{O}.	paw swelling and redness reduced, and arthritis score was 0 as normal untreated paw. D-NGel significantly inhibit ($P<0.01$) production of TNF-αand IL-1β compared to control	NA	IND & MTX in NPs, free IND & MTX.	(98)
HA-DOX hydrogel	NA	NA	HA (10mg/ml) &	NA	NA	Analgesic, anti-inflammator	100% cell survival as the non-	In vitro SW1353 cell	Using OA rabbits' model, HA-DOX hydrogel	NA	Free DOX and HA	(99)

			DOX (87.5 µg/ml)			y and viscoelasticity	treated group	cytotoxicity analysis	significantly reduced pain, OA progression (p<0.05) compared to non-treated group.			
Hydrogel loaded CS	NA	NA	Chondroitin sulfate (CS) 100 mg/ml	NA	80% of α-CD-EG4400 released in 180 hr and 20% was retained for 30 days	chondroprotective activity	Cell proliferation number increase with increase CS concentrations (0-10000ng/ml)	In vitro chondrocyte culture used to study the release.	IA injection of rabbit knee joint showed no sign of redness or swelling	NA	Different formulation of hydrogel	

Table 6.

Type of delivery system	Advantages	Disadvantages
Nanoparticle	Biocompatible Biodegradable Controlled drug release Non-toxic High drug loading capacity Decrease dosage administration frequency Modified drug pharmacokinetics Increased drug solubility Provide a high penetration rate Particle size can be manipulated to achieve drug targeting	Burst release can occur which cause local toxicity and slow drug release
Liposome	Non-toxic Ability to encapsulated both lipophilic and hydrophilic agents Provide long retention time and sustained drug release Efficient in local treatment of joint disease	Costly Low solubility Short half-life

	Can Incapsulate two drug at the same time	
Emulsion	Good tissue permeability Applied for topical, intra-articular or transdermal application Highly solubilize both lipophilic and hydrophilic compounds Maintains a large amount of drug absorption in the applied area Improve drug bioavailability Allow control release profile	Low viscosity Low stability Size may be too large
Cationic carrier	Biocompatible Provides an electrostatic interaction with cartilage proteoglycan Increase drug uptake and retention Rapid drug penetration inside the cartilage Sustained drug delivery to the chondrocyte cell	Costly
Hydrogel	Biocompatible Biodegradable	Costly Multiple injection need which leads to patient non-

	Can be injected as a liquid that gels at body temperature Provides joint lubrication Relief pain Intra-articular method of administration which reduces the oral side effect Their three-dimensional structure prevents the diffusion of the encapsulated drugs from it Increasing the retention of drugs in the joint tissue	compliance and ineffective treatment.

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