Review Article

NUTRACEUTICAL FORMULATIONS CONTAINING GLUCOSAMINE AND CHONDROITIN SULPHATE IN THE TREATMENT OF OSTEOARTHRITIS: EMPHASIS ON CLINICAL EFFICACY AND FORMULATION CHALLENGES

AHMED M. AGIBA

Research and Development Department, SIGMA Pharmaceutical Industries, Egypt
Email: ahmed.agiba@gmail.com

ABSTRACT

Osteoarthritis (OA) is essentially a debilitating disease symptomatized by a gradual loss of articular joint cartilage, causing painful impairment among the population of different ages, particularly patients over the age of 50 y. Nutraceuticals; namely glucosamine and chondroitin have been widely used in the treatment of OA. The chondroprotective properties of the aforementioned agents have been reported, allowing the repair and recovery of the articular surface in OA. The purpose of this review article is to report the current evidence for the use of glucosamine and chondroitin sulphate in the treatment of knee OA with emphasis on their indications for clinical use, effectiveness and safety. It also highlights the role of some advanced formulation technologies in optimizing the delivery of those drugs.

Keywords: Osteoarthritis, Glucosamine, Chondroitin, Clinical use, Formulation technologies

INTRODUCTION

Osteoarthritis (OA), also known as degenerative joint disease, degenerative arthritis or osteoarthrosis, is the most common musculoskeletal disease affecting the whole synovial joint [1]. It is believed that cartilage is not the sole organ being affected by OA, but also ligaments, synovia and bone, which undergo metabolic and structural modifications as the disease progresses.

OA is the prominent form of arthritis; its prevalence increases dramatically with age [2], and can lead to significant pain, reduced range of motion and increased disability [3]. As a result, it is considered a disabling arthritic condition that causes activity limitation and reduced quality of life among the population over the age of fifty. It has been estimated that each year, more than 75% of individuals over the age of 65 present with OA in one or more joints [4]. In addition, some studies have shown that 12.1% of American individuals over the age of 25 y show clinical symptoms of OA [5].

OA is characterised by a gradual loss of articular cartilage in synovial joints, causing articular cartilage destruction with subsequent loss of joint space. Clinical manifestations of this disease are joint pain and damage, stiffness, effusion, instability, and ultimately, deformity. Genetic and nutritional factors mainly contribute to the etiology of OA, in addition to other biological, biochemical and mechanical factors [5, 6]. It is believed that the primary OA is characterized by mechanical, repetitive overloading of the articular cartilage, which leads to a vicious circle of inflammation, degradation and loss of joint cartilage. This inflammation is mainly attributed to the secretion of interleukin-1 (1L-1) and a monocyte-derived cytotoxin (i.e. tumor necrosis factor TNFα), which increase metalloproteinases and nitric oxide synthase production, which are the main catabolic agents in joint cartilage lesions [6-8].

A demand for OA treatment is gradually increasing each year due to the rise in its prevalence caused by the dramatic increase in average life expectancy among the population, with a higher degree of degenerative joint arthritis [9]. The pathogenic complexity of OA creates a challenge for diagnosis and management of this disease, which mainly relies on symptomatic treatment and improvement of patients' functional abilities. Treatment mainly includes a combination of pharmacological and non-pharmacological methods (physiotherapy, occupational therapy, weight loss and exercise). In addition, alternative therapies, such as; homeopathy, acupuncture, phytotherapeutic medications; and surgical methods are also utilized [10]. The pharmacological therapy is directed towards the prevention of pain and improvement of function among patients with OA. The first choice medications in the pharmacological treatment of OA-related pain include analgesics (acetaminophen and paracetamol) and non-steroidal anti-inflammatory drugs (NSAIDs) [11, 12]. However, some studies have indicated that NSAIDs are only used to treat symptoms without correction of the degenerative disorder of the connective tissue. Furthermore, the long-term use of NSAIDs causes potential adverse effects on the gastrointestinal and cardiovascular systems, which are mainly popular among elderly patients [13]. Therefore it can be deduced that medical treatments available for OA are moderately effective and are mostly directed at short-term pain relief, In addition, side effects of these treatments can be quite significant.

The need for the development of new drug treatments for OA that could systemically relieve pain and potentially modify structural damage has emerged. Nutraceuticals such as glucosaminoglycans (GAGs) have recently been introduced as biological alternatives for drug treatment since there is a substantial interest in the chondroprotective effects of GAGs such as glucosamine sulphate and chondroitin sulphate. Both of these drugs have been approved as agents that modify the natural history of OA [14].

Glucosamine sulphate and chondroitin sulphate are natural nutraceutical compounds which are known as cartilage precursors. They are not only considered as symptomatic drugs for OA, but they also have a disease-modifying potential, hence, they have gained worldwide popularity over the last decades [15]. This review article focuses on those two compounds for the treatment of OA.

Preparations, dosages, bioavailability and pharmacokinetics of glucosamine

Glucosamine, 2-amino-2-deoxy-D-glucose ($C_6H_{14}NoO_5$), is a monosaccharide with a molecular weight (197.2 Da). It is naturally produced in the body and acts as a precursor for the biosynthesis of glycosylated lipids and proteins. Functionally, glucosamine is a prominent precursor for GAGs or mucopolysaccharides and is structurally belonging to glycoproteins and proteoglycans. Glucosamine helps in the maintenance of healthy joint function, primarily targeting people suffering from OA. Although glucosamine has not been approved yet by FDA as a drug of choice in the treatment of OA, it is prescribed as a first-line drug in OA treatment in Europe [16].

There are different forms of glucosamine in the market. Glucosamine preparations are either extracted from chitin (from crustacean shells) by acid hydrolysis (and thus patients with shellfish allergies should be cautioned to avoid the use of glucosamine) or chemically synthesised. Glucosamine is a weak organic base, which must first be stabilised as a salt. There are three forms of glucosamine commonly available on the market: glucosamine hydrochloride (from crab shells), glucosamine sulphate (from shrimp shells) and chemically synthesised glucosamine (as sulphate form), as illustrated in (fig. 1, 2).
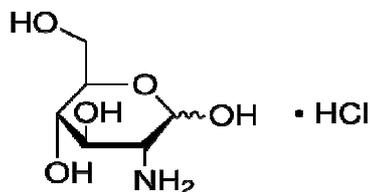
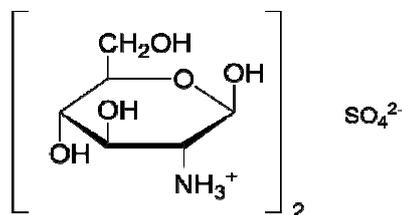**Fig. 1: Chemical structure of glucosamine HCl**

Fig. 2: Chemical structure of glucosamine So₄

The absolute daily dosing of glucosamine varies according to the preparation type, owing to the differences of the molecular size of the associated salt. The recommended daily dosing of glucosamine generally ranges from 1250 mg 1500 mg. Single oral dose of glucosamine (1500 mg) approximately produces plasma concentration of 10 µmol, while taking a dose (500 mg) three times daily produces approximately a plasma concentration of 3 µmol [17].

Several studies were conducted to determine the absorption, distribution, and metabolism of glucosamine. Clinical pharma-cokinetics of oral dose administration of glucosamine HCl at significant doses was evaluated, and glucosamine was shown to be bioavailable in the synovia and serum of beagles and horses [18, 19]. Uptake of a radiolabeled dose of glucosamine sulphate by articular cartilage following an oral dose was also detected in both rats and dogs [20, 21] and in human volunteers [22]. Absorption of radiolabeled glucosamine sulphate was estimated to be 90%, but in turn, there was a significant first-pass effect or pre-systemic metabolism resulting in a bioavailability of 26-44% [22]. Animal studies have also shown a bioavailability of approximately 19% in rats [23]. Glucosamine was estimated to have a half-life of 15 h following oral dosing [24]. Glucosamine sulphate pharmacokinetics was linear up to the standard dosing of glucosamine (1500 mg/day), while higher doses did not result in a proportionally higher increase in glucosamine maximum concentration [24].

Preparations, dosages, bioavailability and pharmacokinetics of chondroitin sulphate

Chondroitin is considered a complex macromolecule. It is a natural polymer of α-disaccharide with an alternating sequence of D-glucose and N-acetyl-galactosamine-4-sulphate or 6-sulphate residues linked together through different bonds. It is a high molecular weight GAG with a molecular weight (10000-50000 Da). There are two available forms of chondroitin sulphate in hyaline cartilage depending on the position of the sulphated monosaccharide; chondroitin-4-sulphate and

chondroitin-6-sulphate (fig. 3, 4). Interestingly, the more superficial cartilage contains higher proportions of chondroitin-6-sulphate, while the newer deeper cartilage contains more chondroitin-4-sulphate [25]. Ratios of chondroitin-6-sulphate to chondroitin-4-sulphate in cartilage with osteoarthritis were substantially lower, but the clinical significance of this finding is unclear [26].

Functionally, chondroitin sulphate is a sulphated GAG, and structurally, it is found attached to proteins as part of a proteoglycan. Chondroitin sulphate is an essential component of joint cartilage which also helps to inhibit the enzymes that cause its decomposition. It is approved in Europe as a symptomatic slow-acting drug for OA [16]. It is marketed together with glucosamine in different concentrations [27].
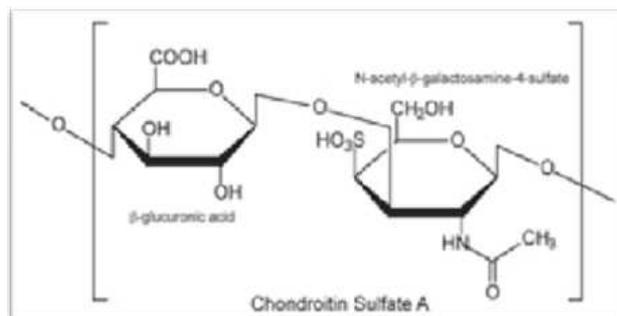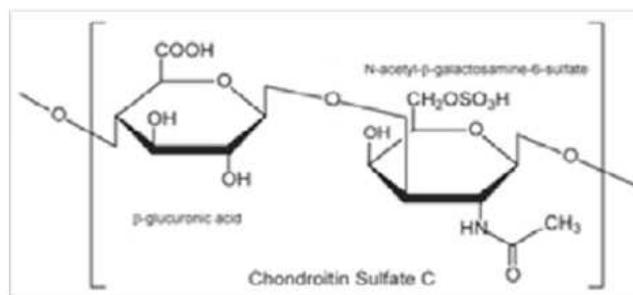
Fig. 3: Chemical structure of chondroitin-4-So₄

Fig. 4: Chemical structure of chondroitin-6-So₄

Chondroitin sulphate sodium salt is the most commonly used salt of chondroitin sulphate in oral preparations. It is sold in the United States as a dietary supplement and does not require a prescription. It either comes from bovine or porcine tracheal cartilage or occasionally from fish or avian cartilage. Although no enough dosing studies have been conducted, recommended daily dosages of chondroitin sulphate are between 800 to 1200 mg [17].

The oral bioavailability of chondroitin sulphate ranges from 5 to 15% with a half-life of 6 h and plasma peak levels from 2 to 28 h after oral administration. A published study on the human pharmacokinetics of clinically relevant doses of both glucosamine HCl and chondroitin sulphate sodium salt did not reveal a detectable difference of circulating endogenous plasma levels of chondroitin sulphate after a single oral dose of 1200 mg at all-time intervals 0.25 to 36 h [28].

Modes of action of glucosamine and chondroitin in OA

The possible modes of action of glucosamine and chondroitin in the treatment of OA are described in (table 1). Glucosamine and chondroitin promote the biosynthesis of proteoglycan and inhibit the degradative enzymes which are responsible for the premature breakdown of cartilage in OA [29, 30].

Table 1: Possible modes of action of glucosamine and chondroitin in the treatment of OA

Glucosamine	Chondroitin
Inhibits cytokines (interleukin-1 β)	Inhibits cytokines (interleukin-1β)
Inhibits proteases (matrix metalloproteinase and collagenase)	Inhibits proteases (collagenase)
Interferes with binding of nuclear factor $_kB$	Increases bone mineralisation and repair
Reduces prostaglandin E_2 production	Increases viscosity of synovial fluid
Increases proteoglycan synthesis	Increases proteoglycan synthesis by chondrocytes

Clinical studies conducted on glucosamine in the treatment of OA

Efficacy of glucosamine in the treatment of OA was reported in earlier clinical trials provided by Rottapharm [31]. In general, these studies declared a positive effect of glucosamine both symptomatically and radiographically in the treatment of OA. The first study [32] evaluated and studied 212 patients with knee OA and randomized a group to receive a daily dose of glucosamine sulphate, 1500 mg, versus placebo. Western Ontario and McMaster Universities OA Index (WOMAC) scores were used to evaluate pain and function as percent change on a visual analog scale (VAS).

There was a significant improvement in pain and function, but no improvement in stiffness. The second study [33] evaluated and studied 202 patients with knee OA, using a daily dose of glucosamine sulphate, 1500 mg, concluding a significant change in WOMAC scores for pain, function and stiffness as well. Towheed *et al.* [34] evaluated the role of glucosamine in the treatment of OA, including 9 trials of glucosamine prepared using Rotta preparation method and 8 trials using non-Rotta preparation method. The results showed a clinical significance of improvement in pain relief and efficacy in function with a significant change in Lequesne index (assessing the severity of OA through evaluation of daily activity and function). In addition, there was a significant improvement in WOMAC scores reported in subgroup patients taking glucosamine prepared by Rotta preparation method. The Glucosamine/chondroitin Arthritis Intervention Trial (GAIT) [27] randomly evaluated 1583 patients suffering from knee OA and randomized them to receive a daily dose of glucosamine HCl (1500 mg), sodium chondroitin sulphate (1200 mg), both at the same doses, celecoxib (200 mg), or placebo for 24 w. Results showed a 20% decrease in WOMAC scores for pain after 24 w. Despite the finding that glucosamine had a significant effect on the subgroup of patients with moderate to severe pain; interpretation of this result was limited by the small number of patients in this subgroup. However, a GAIT study which randomly evaluated 662 patients with knee OA and treated with the same treatments, declared no clinical improvement in WOMAC scores for pain and function versus placebo [35]. Although glucosamine and celecoxib showed an improvement in pain and function, they did not reach the statistical significance. Tables 2 and 3 summarize the randomized clinical trials (RCTs) and meta-analyses of glucosamine sulphate and HCl salts respectively.

Table 2: Summary of randomized clinical trials (RCTs) and meta-analyses studies of glucosamine sulphate (GS)

Reference	No. of patients	Duration	Dosage regimen	Parameters evaluated	Results and conclusions
[36]	200	4 w	400 mg GS 3 times daily vs ibuprofen	Lequesne's severity index	GS was as effective as ibuprofen and better tolerated
[37]	252	4 w	500 mg GS 3 times daily vs placebo	Lequesne's severity index	GS was significantly more effective than placebo in revealing OA symptoms
[38]	155	6 w	400 mg GS (IM) 2 times daily for 6 w	Lequesne's severity index	Duration was too short to make significant long-term conclusions
[32]	212	3 y	1500 mg GS once daily vs placebo	WOMAC pain scores and minimal joint space	GS protected the joint space against the narrowing effects of OA
[39]	80	6 mo	1500 mg GS once daily vs placebo	Pain scores on visual analog scale, WOMAC pain scores and McGill pain index	No statistically significant improvement over placebo as a symptom modifier
[33]	202	3 y	1500 mg GS once daily	Lequesne's index, WOMAC pain scores and minimal joint space	Statistically significant effects of GS on both radiographic progression and WOMAC scores
[40]	212	3 y	1500 mg GS once daily	Joint space narrowing	Disease-modifying effects were found in postmenopausal women
[41]	137	6 mo	1500 mg GS once daily vs placebo	Lequesne's index and WOMAC pain scores	No clinical evidence of symptomatic benefits from continued GS treatment during this 6 mo study
[42]	414	3 y	1500 mg GS once daily vs placebo	Minimal joint space width and WOMAC pain scores	GS showed non-statistical effects compared to placebo
[43]	318	6 mo	1500 mg GS once daily vs placebo	Lequesne's index and WOMAC pain scores	GS found to be better than placebo and acetaminophen

Table 3: Summary of randomized clinical trials (RCTs) and meta-analyses studies of glucosamine HCl (GH)

Reference	No. of patients	Duration	Dosage regimen	Parameters evaluated	Results and conclusions
[44]	101	8 w	GH vs placebo	WOMAC pain scores	No statistically significant changes in WOMAC pain scores, i.e. GH was as effective as placebo
[45]	205	12 w	15 gm GH once daily vs placebo	WOMAC pain scores	GH was safe but not more effective than placebo in relieving symptoms of knee arthritis

Clinical studies conducted on chondroitin sulphate in the treatment of OA

Efficiency of chondroitin sulphate over placebo for the treatment of OA was reported in clinical studies as well. GAIT [46] was the biggest and most rigorous study designed to evaluate the efficiency of chondroitin sulphate in symptomatic knee OA. A post-hoc analysis of GAIT demonstrated a potential benefit of chondroitin sulphate treatment on joint swelling [46]. Reichenbach *et al.* [47] published a meta-analysis study assessing the effect of chondroitin sulphate on pain in knee or hip OA. They reported substantial heterogeneity among trials, rendering the interpretation of results conflicting. A published RCT which included 662 patients evaluated the effect of chondroitin sulphate on the structural progression of knee OA using VAS and WOMAC. Pain as a secondary outcome was measured over 2

y. Results showed a significant improvement in pain relief; although statistical significance did not persist for long-term [48]. Messier *et al.* [49] evaluated 89 patients with knee OA over 12 mo by measuring the effects of a combinatorial daily dose of 1500 mg glucosamine and 1200 mg chondroitin sulphate along with exercise over the placebo. The results did not show any difference in pain and function between patients taking drugs over placebo.

Another study [50] evaluated 100 patients with knee OA receiving a combinatorial daily dose of 250 mg glucosamine sulphate and 200 mg chondroitin sulphate by using JSW and Lequesne index over 12 mo. Results of this study showed significant effects on patients taking drugs over placebo. Summary of randomized clinical trials and meta-analyses of chondroitin sulphate alone or in combination with glucosamine is illustrated in (tables 4, 5), respectively.

Table 4: Summary of randomized clinical trials (RCTs) and meta-analyses studies of chondroitin sulphate (CS)

References	No. of patients	Duration	Dosage regimen	Parameters evaluated	Results and conclusions
[51]	80	6 mo	400 mg CS twice daily vs placebo	Lequesne's index, and visual analog scale	A statistically significant improvement in patients taking CS over placebo, with no difference in side effects
[52]	127	3 mo	1200 mg CS vs 400 mg CS 3 times daily vs placebo	Lequesne's index, and visual analog scale	A reduction in Lequesne's index and spontaneous joint pain scores over placebo
[53]	104	1 y	800 mg CS vs placebo	Lequesne's index, and joint space loss	A significant improvement in patients taking CS over placebo
[54]	130	6 mo	1 gm CS once daily vs placebo for 3 mo	Lequesne's index	Lequesne's index significantly improved and remained elevated after the treatment for 1 mo
[55]	300	2 y	CS vs placebo	Joint space loss	CS reduced the radiographic progression of knee OA
[56]	120	1 y	800 mg CS once daily for 3 mo	Lequesne's index	A significant improvement in pain and function in patients taking CS over placebo
[57]	300	2 y	800 mg CS vs placebo	Joint space narrowing, and Lequesne's index	CS retarded the radiographic progression of knee OA
[58]	307	6 mo	1000 mg CS once daily	Lequesne's index	No significant improvement in patients taking CS versus placebo

Table 5: Summary of randomized clinical trials (RCTs) and meta-analyses of combination therapy of glucosamine and chondroitin

Reference	No. of patients	Duration	Dosage regimen	Parameters evaluated	Results and conclusions
[59]	90	6 mo	500 mg GH, 500 mg CS and Diclofenac Sodium twice daily for 1 mo vs Diclofenac Sodium	WOMAC pain scores	WOMAC score decreased after 3 mo of the treatment and 3 mo after the supplements had been discontinued. The combined medications offer a high evidence of safety and effectiveness in the short-term treatment of pain with long-lasting effects
[27]	>1000	6 mo	1500 mg GH vs 1200 mg CS vs GH plus CS vs 200 mg Celecoxib vs placebo	WOMAC pain scores and other indexes	Celecoxib showed a significant effect on 6 of the outcome measures, whereas GH and CS were found to have a significant effect on 14 of the outcome measures
[49]	80	1 y	1500 mg GH and 1200 mg CS	WOMAC pain scores and other parameters (6 min' walk, knee strength, and functional)	No significant difference between groups for the 6-minutes' walk, knee strength, and functional over the placebo

Safety profile of glucosamine and chondroitin

Safety studies conducted on glucosamine, chondroitin sulphate or their combination were not reported to cause systemic adverse effects compared with other analgesic or non-steroidal anti-inflammatory drugs (NSAIDs) in short or long-term treatments. However, NSAIDs including COX-2 inhibitors for pain relief in OA is accompanied by increased risk of adverse effects in long-term treatment such as gastrointestinal problems and cardiovascular risks [13]. Furthermore, no evidence of any clinically significant drug interactions for glucosamine or chondroitin was reported as well. This created an increased

demand for optimizing the delivery of glucosamine and chondroitin sulphate as safe treatments for OA.

Pharmaceutical strategies to enhance the delivery of glucosamine and chondroitin sulphate

As obvious from the clinical trial results reported in the previous sections of the review article, a discrepancy in the opinions of authors was reported on whether glucosamine and chondroitin are exclusively effective in the treatment of OA upon administration in the conventional ways. The oral delivery of a nutraceutical formulation containing a high dose of chondroitin sulphate and

glucosamine sulphate remains a challenge owing to the high molecular weight of chondroitin (10000-50000 Da), as well as the extensive hepatic first-pass metabolism of glucosamine sulphate. This leads to low bioavailability ranging from 5-15% for chondroitin sulphate and 26-44% for glucosamine sulphate [22, 28].

Although different classes of bioenhancer compounds such as surfactants, co-surfactants, bile salts, fatty acids, cyclodextrins, chelators, cellulose derivatives and positively charged polymers [60] have been utilized to enhance the absorption of low permeable hydrophilic drugs by acting on the mucous layer and membrane components or tight junctions of the intestinal epithelium; research studies have shown that the effective intra-articular concentration of glucosamine and chondroitin sulphate could not be achieved by oral route using conventional methods of drug delivery [18,61]. The addition of cellulose derivatives such as carboxymethylcellulose sodium (CMC-Na) and hydroxypropyl methyl cellulose (HPMC) resulted in lower absorption levels of glucosamine in rats [62]. Anionic surfactants like sodium lauryl sulphate (SLS) and non-ionic surfactants like polysorbate 80 (Tween 80) have some limitations concerning their systemic use, for example, SLS is highly toxic to the intestinal mucosa, and Tween 80 has an odoriferous smell and very bitter taste [62].

Surprisingly, the addition of positively charged mucoadhesive polymer (chitosan) 0.5% (w/v) exhibited a significant effect on glucosamine oral bioavailability [62]; therefore many studies have further been performed to investigate the possible mechanism and role of chitosan in absorption enhancement. Caco-2 permeability studies have shown that chitosan improved absorption of glucosamine by 1.9 to 4.0 fold via the reversible opening of tight cell junctions by binding tightly to the epithelium, inducing a redistribution of F-actin (an important protein that regulates paraceullular flow across epithelia) and occludin proteins [63]. Oral administration of solutions containing glucosamine coated with chitosan (Glu-CH) in rats showed that 0.5% (w/v) chitosan exhibited the highest improvement in C_{max} (2.8-fold) and AUC_0 (2.5-fold) of glucosamine. Furthermore, pharmacokinetic studies in beagle dogs showed much higher bioavailabilities of 313% for Glu-CH solution and 186% Glu-CH tablets comparing with their innovator tablets (Wellesse™ solution and Voltaflex™ tablet) [62]. Therefore, chitosan is concluded to be a promising bioenhancer for glucosamine. Chitosan was also used in the formation of hydrogels based on the formation of complexes between chitosan/chondroitin sulphates (CT/CS) then applying as devices for chondroitin release [64]. Chitosan-hyaluroan composite cross-linked with chondroitin sulphate nanoparticles (CH-HYA/nCS) was developed based on simple ionic cross-linking using N,N-(3-dimethylaminopropyl)-N-ethyl Carbodiimide (EDC) [65], followed by lyophilisation to obtain the composite sponge [66-68]. CH-HYA/nCS was structurally and physicochemically evaluated showing high porosity (60-70%). It also showed high swelling and water uptake properties, owing to the high porosity of the nanocomposite sponges, with high biodegradation profile and cell viability, indicating that CH-HYA/nCS sponge was totally biocompatible. This sponge technique was recommended as a scaffold for chondroitin sulphate release [65].

Another strategy for enhancing the delivery of glucosamine was the entrapment within fatty acid vesicles (FAV) for site-specific delivery of glucosamine sulphate [69]. Oleic acid vesicles were formulated by thin film hydration method followed by ultra-sonication for size reduction. Particle size and zeta potential were evaluated. A Stable FAV formulation was shown to have a particle size range of 100-150 nm and a zeta potential range of–20 to–23 mV. Pre-formulation studies of different FAV formulations were carried out in correlation with *in vivo* studies in rats. Results have shown that more than 60% of the glucosamine was entrapped in FAV. *In vitro* studies have shown a sustained release and a long-term stability of FAV. In conclusion, FAV exhibits better skin permeation potential and depot characteristics, as well as a reduction in joint inflammation [69].

A smart drug delivery system with controllable properties and high potential regenerative action was designed for targeting the injured site with high selectivity and specificity. Chondroitin sulphate encapsulated in a liposome (L-CS) embedded in a natural polymeric matrix of collagen (COL-L-CS) for the treatment of inflammatory disorders; especially OA was developed [70]. COL-L-CS was fabricated with pore size range between 50 and 200 μm, provided a favourable cell adhesion and proliferation; furthermore, induced osteogenic behaviour of different cell types [71]. COL-L-CS was formulated by freeze-drying technique, using thin film hydration method for the preparation of L-CS [72] followed by mixing with purified COL in a ratio of 1:1 (w/w) [70], and then frozen under controlled condition [73]. This controlled process resulted in pore formation in a three-dimensional COL sponge matrix. COL-L-CS was structurally, physicochemically and biologically evaluated. Morphologically, it was similar to COL scaffold matrix, showing a network of interconnected COL fibrils unevenly covered with white to the off-white homogenous suspension of L-CS. Structurally, as a network of nanosized COL fibrils decorated with deposits of L-CS at both sides of the sponge matrix. Physically, porosity and density were similar to COL scaffold matrix, but its biodegradability was significantly lower values (P<0.05). *In vitro* cytotoxicity testing has shown that the matrix system induced high cell viability and stimulated cell metabolism in L929 cell culture. It highlighted COL-L-CS advantage over CS alone (significantly higher (P<0.05) cytocompatibility in L929 cell culture. In addition, *in vitro* diffusion testing have shown that the released quantity of CS was significantly lower (P<0.05) after embedding of L-CS inside COL matrix. In conclusion, COL-L-CS exhibited a potential regenerative action in the treatment of OA, and could be used as a carrier for the delivery of therapeutic agents.

In a recently-published article by Umerska *et al.* [74], they successfully developed a new type of chondroitin sulfate-based nanoparticles; namely chondroitin sulfate/chitosan (positively and negatively charged), chondroitin sulfate/chitosan/calcitonin, and chondroitin sulfate/calcitonin. Chondroitin sulfate-based nano-particles offer the advantages of very high drug loading with high efficiency close to 100%. da Silva *et al.* [75] also developed a novel eco-friendly biocomposite of chondroitin sulfate/poly(vinyl alcohol)/bovine bone powder through cyclic freeze-thawing under mild conditions as a promising biomaterial for potential skin tissue engineering.

DISCUSSION

OA is the most common form of arthritis and is considered one of the most frequent causes of morbidity among the population over the age of years. Load-bearing joints (i.e. knee and hip) are considered the most affected organs, and their involvement absolutely gives rise to a high degree of functional limitations of the lower limbs. With increasing life expectancy, treatment of OA should be taken into consideration. In the United States, the scale of medications and supplements for OA treatment has risen from $86 billion in 2004 to $760 billion in 2013 [76]. The clinical treatment of OA is a matter of debate for many years. Even after many years of study and research, doubts still exists around the efficiency of glucosamine, chondroitin sulphate or both in the treatment of OA. Most studies lately published lack delineation good enough to prove secure conclusions. Recently, several studies confirmed the efficiency of glucosamine and chondroitin in the treatment of OA pain. In these circumstances, European League against Rheumatism (EULAR) confirmed glucosamine and chondroitin as symptomatic slow-acting drugs for the management of OA by modifying the structure of cartilage [77]. American College of Rheumatology (ARC) also recommended glucosamine sulphate and chondroitin sulphate under certain conditions [78]. In contrast, UK's National Institute for Health and Care Excellence (NICE) has recommended that these products mainly should not be used due to economical reasons [78].

CONCLUSION

OA is a slowly progressive disease and evidence of disease modification with an intervention may not be apparent for many years. Glucosamine and chondroitin sulphate have individually shown inconsistent efficiency in decreasing OA pain and improving joint function. A combination of oral chondroprotective glucosamine and chondroitin sulphate has proven efficiency in modulating OA and an excellent safety profile in long-term use. These components are essential substances of the cartilage metabolism and can

stimulate cartilage regeneration processes in OA. Appropriate methodology and enough clinical studies are needed in order to approve the efficiency of this combination in short-term treatment.

CONFLICT OF INTERESTS

Declare none

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