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Sterosome: An Advanced Non-Phospholipid Vesicular Drug Delivery System

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ABSTRACT: The growing number of problems linked to pharmaceuticals that have emanated from varied chemical and biological backgrounds has prompted scientists to seek out newer molecules and develop new methods and means of delivering them. Using unique drug delivery systems or techniques, both old and new compounds can be delivered to the site of need in a defined manner. Liposomes are regarded as an almost ideal drug-carrier system due to their morphology, similar to that of biological membranes, as well as their ability to incorporate drugs and transport both hydrophilic and lipophilic molecules, making them the most studied drug nanocarrier. However, liposomes face several limitations, including low encapsulation efficiencies, premature release of hydrophilic pharmaceuticals, and variable stability depending on the specific encapsulated drugs. These challenges have driven the need for modifications, leading to the development of a novel class of liposomes known as sterosomes. Sterosomes are composed of non-phospholipid, single-chain amphiphilic molecules with high sterol content, offering enhanced properties compared to conventional liposomes. At the nanoscale, sterosomes are produced as massive unilamellar vesicular systems with a diameter that can be modified based on the pore size of the filter employed in the extrusion system. They also exhibit the unique benefit of monomodal dispersion and low permeability of bilayers and have proven to be more stable than conventional liposomes. As a result, sterosomes hold significant potential to overcome the limitations associated with traditional liposomes. However, only a limited number of studies have explored their use as nanocarriers for enhancing the treatment of various diseases. Despite this, sterosomes represent a promising and innovative non-phospholipid liposomal platform for drug delivery, offering unique advantages that warrant further investigation. This review focuses on sterosomes as an emerging drug delivery system, highlighting their advantages, preparation methods, characterization techniques, and recent applications in disease treatment. In addition, it examines their inherent functionality and provides recommendations for future applications, as sterosomes have demonstrated considerable potential in addressing a wide range of diseases.

KEYWORDS: **Vesicular drug delivery system; Liposomes; Sterosomes; Cancer treatment; Bone regeneration; Gene delivery; Dental delivery.**

1. INTRODUCTION

Creating novel drug delivery systems (NDDS), which are unique formulation designs, addresses the flaws in conventional drug delivery systems. Active pharmaceutical ingredients (APIs) associated with conventional dosage forms are limited by poor solubility, permeability, side effects, need for frequent administration, low efficacy, and toxicity; these issues can be addressed using advanced systems. These cutting-edge drug delivery systems include nanoparticles, microspheres, micelles, vesicular systems, microemulsions, and dendrimers [1-5]. The physicochemical, pharmacodynamic, and pharmacokinetic properties of drugs are altered by their entrapment in these colloidal carriers, improving their stability, bioavailability, bio-distribution, targeted or specific-site delivery, controlled release, extended-release, anticipated therapeutic outcomes, and prevention or reduction of adverse drug events in the biological environment [6,7].

When amphiphilic components interact with water, they form highly structured units known as vesicular drug delivery systems (VDDSs). According to Lafleur and Keckeis [8], Bnyan et al. [9], and Alenzi et al. [10], they

typically consist of one or more concentric lipid or non-lipid bilayers enclosing an aqueous interior. Vesicular systems can deliver both hydrophilic and lipophilic APIs. For instance, hydrophobic drugs like paclitaxel and water-soluble drugs like doxorubicin are trapped between the outer concentric bilayer and encapsulated in the aqueous interior compartment, respectively (Figure 1) [8,9,11]. Due to their capacity to target drugs to their sites of action, these novel systems have become increasingly popular [12]. This will allow a reduction in dosage, improve pharmacological efficacy, increase bioavailability, and prevent drug toxicity.

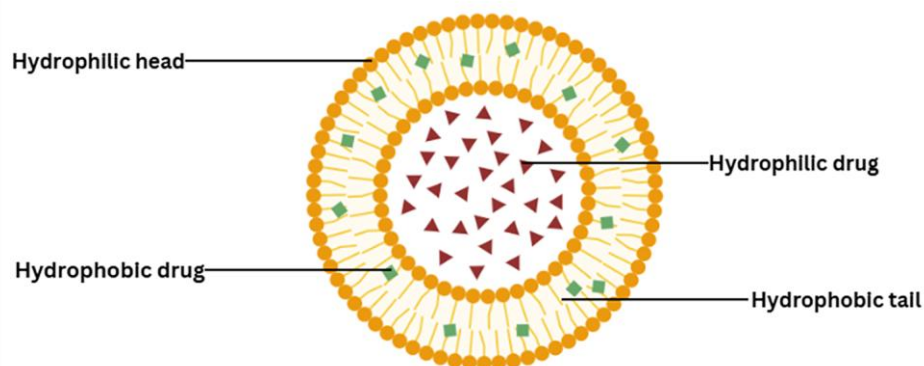

Figure 1. A diagrammatic representation of a vesicular drug delivery system loaded with hydrophilic and hydrophobic drugs.

In 2001, Paré and Lafleur explored the complex nature of cholesterol and its impact on the micromechanical properties of phospholipid membranes, particularly in the transition between the liquid-disordered and gel phases. The distinct characteristics of cholesterol inspired the development of drug-delivery liposomes that maintain fluidity while offering improved impermeability, enhanced *in vivo* stability, and adjustable molecular recognition capabilities when combined with saturated fatty acids such as palmitic acid. This vesicular drug delivery system (VDDS) represents an innovative non-phospholipid liposome derived from naturally occurring and cost-effective molecules. It demonstrates resistance to various enzymatic degradations, offers a neutral surface, and holds the potential for pH sensitivity, opening new possibilities in drug delivery systems [13].

Therefore, a sterosome is a non-phospholipid liposome (NPL) created from various biocompatible monoalkylated amphiphiles combined with a high cholesterol concentration. Multiple studies have shown that native sterosomes demonstrate enhanced characteristics and functionality and can solve the shortcomings of traditional liposomes, including low encapsulation efficiencies, premature release of hydrophilic pharmaceuticals, and variable stability contingent upon the specific drugs encapsulated [14-16]. Two key characteristics of sterosomes are their sensitivity to pH and their stability. The pH sensitivity of native sterosomes enables pH-activated release, a crucial factor for targeted drug delivery, depending on their non-phospholipid components' protonated or unprotonated state [14]. Furthermore, the improved stability of sterosomes, a significant limitation in the application of liposomes, ensures their reliability and functionality, paving the way for an optimistic future for this drug delivery system [14,17]. This review examines the key findings concerning the production, characterization, and modification of sterosomes, along with the innovative and suggested application for these novel biomaterials.

1.1. Description of Liposomes

Liposomes, the colloidal artificial vesicles made from natural or synthetic phospholipids, hold great potential for the future of drug delivery and nanotechnology. They were discovered and investigated in 1964 at the Babraham Institute, University of Cambridge, by A. Bangham and his colleague R.W. Thorne [18]. Depending on the application, phospholipids—natural or synthetic—make up most of the membranes, and cholesterol or other stabilizers help keep them stable. They are created by dispersing phospholipids in water and are observed using

an electron microscope [19-21]. The amphiphilic characteristics of phospholipids allow them to form lipid bilayers. They are made up of two hydrophobic fatty acid “tails” and a hydrophilic phosphate 'head,' which are joined by a glycerol molecule, with the hydrophilic head drawn to water and the hydrophobic tails, which are made up of two long fatty acid chains that are repelled by water [22]. The most commonly used phospholipids in the production of liposomes are phosphatidylcholine (PC), also known as lecithin, phosphatidylethanolamines (PE), phosphatidylserines (PS), and phosphatidylglycerols (PG) [23,24]. Liposomes have several advantages, including biocompatibility, biological breakdown, non-toxicity, and the ability to contain water-soluble and hydrophobic pharmaceuticals. They also acquire several morphological and biophysical features that can be modified to influence their biological function [25,26]. Liposomes' considerable therapeutic and production properties, biocompatibility with other nanoparticulate systems, ease of synthesis, chemical composition variability, and potential to encapsulate a wide range of active compounds have led to the Food and Drug Administration's approval of numerous liposome-based formulations [27]. Despite their benefits, liposomal drug delivery technology has shown some significant disadvantages.

1.2. Shortcomings of conventional liposomes

Liposome fabrication demands many methods, including low-pressure drying of a solvent system, a thin lipid film, and ultrasound treatment. These processes, notably the formation of thin films, are challenging to execute on a large scale. Consequently, transitioning from laboratory to large-scale production of liposomes is complex. Following regulatory guidelines, introducing organic solvents, including chloroform, methanol, and others, to solubilize and mix lipids is also not recommended at such high amounts [28].

Liposomes offer the benefits of increasing the stability of volatile drugs like tretinoin, but the phospholipids used to create these particles are highly susceptible to degradation and hydrolysis. As a result, lipid-based products cannot be stored for a long time. In some cases, the products are frozen and must be reconstituted before use; hence, they are chemically and structurally unstable. Moreover, the disintegration of chemicals such as proteins and enzymes encountered in in-vivo applications is a significant challenge to electrostatic liposome stabilization, which cannot guarantee adequate liposome stability [28].

As soon as liposomes are delivered into the bloodstream, they are swiftly eliminated from circulation by the reticuloendothelial system (RES), mainly composed of macrophages in the liver and spleen [29]. Liposomes are unsuitable for oral delivery due to their inability to withstand intestinal tract conditions. In addition, they are rapidly recognized as foreign particles and cleared by the mononuclear phagocytic system (MPS), also known as the reticuloendothelial system (RES) [29]. Following systemic delivery, liposomes are often endocytosed by MPS cells, such as Kupffer cells in the liver and spleen [29-33]. This rapid uptake and clearance pose a significant challenge for therapeutic applications unless modifications are made to prolong their circulation time. Liposomes can be given as a solution, an aerosol, or in a semi-solid form like lotion, a gel-like or powdered form. Furthermore, they have become multifunctional due to their ability to encapsulate hydrophilic, hydrophobic, or amphiphilic compounds and their easily modifiable physicochemical characteristics (such as surface charge, particle size, membrane permeability, membrane rigidity, and loading capacity) [30].

1.3. Classification of vesicular drug delivery systems (VDDs)

The two main groups of VDDs are lipid and non-lipid vesicular systems. Lipid vesicular systems (LVS) primarily comprise phospholipids, while non-lipid vesicular systems (NLVS) do not contain phospholipids. Phospholipids are the main constituents of LVS, while non-phospholipids, such as free fatty acids and single-chain primary amines, are found in NLVS [10]. Examples of LVS include liposomes (made from phospholipids and cholesterol), ethosomes (prepared from phospholipid, ethanol and propylene glycol), and transferosomes (created from phospholipid, edge activator surfactant and ethanol) [34-36]. Examples of NLVS include niosomes (manufactured from nonionic surfactants and cholesterol), ufasomes (produced from unsaturated fatty acid and cholesterol), and novasomes (fabricated from surfactants, cholesterol, and free (unsaturated) fatty acids [37-39].

2. STEROSOMES

Sterosomes (Figure 2) are liposomes that do not contain phospholipids [14,40]. These vesicular systems differ from liposomes in that monoalkylated amphiphiles are used in place of the phospholipid component of liposomes [40,41]. Single-chain amphiphiles and high levels of sterols (50–75%) are the two main components of sterosomes; in fact, the name of this vesicular system was derived from its high cholesterol content, hence the name "Sterosomes" [40-42]. According to several studies, the combination of monoalkylated amphiphiles, such as alkylated primary amines or saturated fatty acids, and sterols, such as cholesterol, at a ratio of 30:70 or equal molarity (Table 1) can result in the development of very stable liquid-ordered (LO) lamellar phases or large unilamellar vesicles (LUVs). This stability is a key feature of sterosomes, providing reassurance of their reliability and functionality [14,17,40,43].

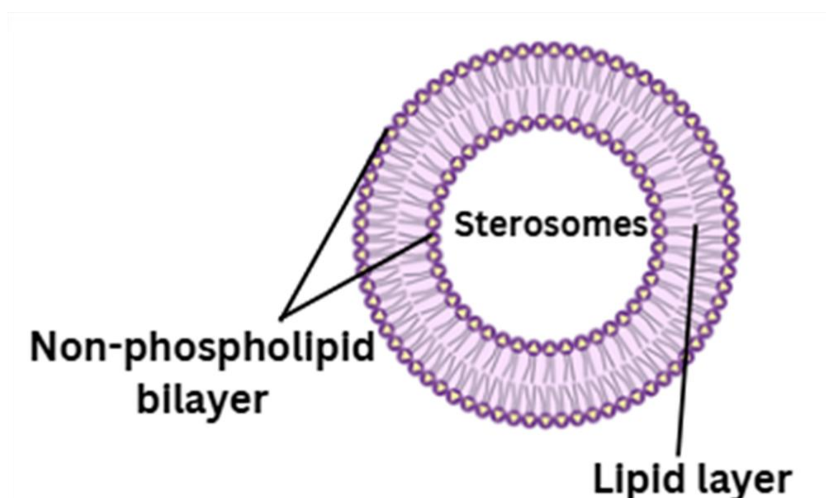

Figure 2. Basic structure of sterosomes.

Table 1. The different excipients used to prepare sterosomal vesicular systems.

Monoalkylated amphiphiles (30-50%)	Sterols (50-70%)
Palmitic acid [15,43,46-51]	Cholesterol [40-43,48-59]
Stearylamine [14,40-42,52,53,60]	Cholesterol sulphate [46,55,56,61]
Octadecyl methyl sulfoxide [54]	Dihydrocholesterol [44]
Cetylpyridinium chloride [16,55-57]	7-dehydrocholesterol [43]
Lyso-palmitoylphosphatidylcholine [59]	Stigmastanol [44]
N-acylethanolamine [59]	β-Sitosterol [15]
Lauric acid [15]	20(S)-hydroxycholesterol or oxysterol [14,16,60]
Stearic acid [15]	
Myristic acid [42]	
Azobenzene [61]	

The hydrophobic match between the long axis of the sterol and the acyl chain of monoacylated amphiphiles at the 14–18 carbon atoms, or the apolar portion of the two constituents, is responsible for the formation of the bilayered, stable lamellar fluid-ordered phases known as sterosomes [40,43,44]. This hydrophobic matching builds up strong bonding or electrostatic interactions between the two molecules, preventing the phase separation of the created vesicles and ensuring a stable bilayer structure; the ensuing fluid bilayers have some of the highest orientational orders ever seen, which significantly limits their permeability [17,40,43,45]. One of the most significant characteristics of these vesicles is their pH sensitivity, which can be harnessed for pH-activated release due to the protonated or unprotonated state of its non-phospholipid constituents [14,40,43,46]. The pKa value of monoalkylated amphiphiles can influence the pH-triggered release of these advanced vesicles, whereas the characteristics of the sterol constituent modulate the overall pH stability of the vesicles. This pH sensitivity is a key factor in the controlled release of active agents, sparking new ideas for drug delivery systems [40,47,48].

2.1. Characteristics and Advantages of Sterosomes

Table 2 summarizes the distinct characteristics of sterosomes and highlights the benefits of these VDDS compared to traditional liposomes. The characteristics and advantages outlined position sterosomes as an innovative drug delivery system capable of achieving multifaceted drug encapsulation, significant passive permeability of the payload(s), sustained release, inherent bioactivity, reduced administered dose, targeted delivery, and a substantial reduction in dosing frequency. This feature of sterosomes will ensure patient adherence and minimize the risk of missed doses. Sterosomes have the potential to be used for multiple synergistic mechanisms to enhance the therapeutic activity of drugs with low potency and to combat drug resistance in the treatment of infectious diseases.

2.2. Formulation of sterosomes

The primary method of sterosome preparation is lyophilization, a process known for its thoroughness and precision. This approach involves dissolving the non-phospholipid constituents in the selected organic solvent. The solution is frozen in liquid nitrogen and lyophilized for several hours to allow the organic solvent to sublimate thoroughly. For the formation of sterosome multilamellar vesicles (MLVs), freeze-dried lipid mixtures are hydrated with a buffer, and the suspension is usually subjected to several cycles of freezing and thawing (from liquid nitrogen temperature up to 70 °C) and vortexed between successive cycles to ensure adequate hydration of the samples. Repeated extrusion of the MLV dispersions at ambient temperature yields large unilamellar vesicles [48]. This approach may take up to two days to complete, during which up to 95% of the water can be removed. Moisture condenses in this process in a volatile state rather than a liquid. Compared to other drying processes, the quality of the dried sterosomes produced by this approach is significantly higher. Its goal is to remove the chemically bound water that has not frozen and is still present in the dried product [48]. However, the intricacy of this procedure has led to a limited number of commercially available lyophilized sterosome products since it is challenging to select excipients and process variables that can preserve the membrane integrity against freezing and dehydration stresses [48].

Another method of sterosome preparation, which showcases innovation in the field, employs a modified classical thin film hydration procedure. The non-phospholipid components are mixed in a suitable organic solvent, and the organic solvent is evaporated using rotary evaporation equipment to form a lipid film of the mixture. The resulting lipid film mixture is hydrated in TRIS buffer, and sterosomes are produced using sonication with a high-power dis-membrator [53]. Drug loading into the systems can be accomplished by dissolving lipophilic drugs in the phospholipid mixture before the thin film forms or by entrapping hydrophilic cargo in the hydration medium and then incorporating them into the sterosomes during the hydration process. Reduction of size and lamellarity is the next stage in the preparation process. Small-scale production, low entrapment efficiency, and the challenge of eliminating the organic solvent are the primary disadvantages of this method [53].

Table 2. A summary of the characteristics and advantages of sterosomes.

Characteristics	Advantages	References
Non-phospholipid composition with high sterol content	- Enhanced stability and rigidity compared to traditional liposomes. - The sterol-influenced rigidity and thickness of the vesicles allow for superior extended drug release of encapsulated solutes.	[17,40,46]
The type, molecular weight, and chain length of the fatty acid composition are adjustable or interchangeable	- The type and molecular weight of saturated fatty acids used to form sterosomes can affect active agent entrapment efficiency and sterosomes particle size, respectively. - The chain length of the fatty acids used to form sterosomes can influence the rate of solute release from their bilayer; the shorter a fatty acid's chain length, the faster the drug is released due to its lower melting point and lipophilicity. - The two ingredients used in the formation of sterosomes can easily be substituted with analogue monoalkylated amphiphiles and sterol molecules, resulting in the preparation of vesicular systems whose stability, permeability, and drug release are pH-dependent, and functionalized sterol constituents can be used in place of cholesterol; these sterosomes are biologically active in the absence of active agents	[14,15,60]
Easy surface modification	- Reduced recognition by the MPS, leading to prolonged circulation time.	[48,50]
Thermal and pH-responsive properties	- Controlled drug release in targeted pH environments (e.g., tumor sites). - Unlike traditional liposomes, which have a short shelf-life due to aggregation, flocculation, hydrolysis, and oxidation, sterosomes are highly stable over a wide pH and temperature range.	[13,40,41,43,49,50]
Highly stable sterosomes formed under specific conditions	- Compared to palmitic acid/cholesterol sterosomes or palmitic acid/cholesterol sulphate sterosomes, the palmitic acid/cholesterol/cholesterol sulphate sterosomes are stable over a more comprehensive pH range. - The high membrane permeability active agent 5-fluorouracil (5-FU), which is poorly retained within the aqueous interior compartment of conventional liposomes, can be successfully encapsulated in sterosomes due to their superior limited permeability.	[13,45,48,50,63]
Low passive leakage	- Sterosomes have been shown to significantly increase the biological half-life (t1/2) of drugs, and the solubility of hydrophobic molecules entrapped within their bilayer. - Sterosomes improve the therapeutic efficiency of drugs encapsulated within their bilayer, promoting potential dose reduction to reduce side effects or preventing toxicity of drugs used to treat diseases such as HIV/AIDS, cancer, and tuberculosis. - The vesicular system allows for high drug entrapment and loading capacity (above 70% and 90%, respectively). - Sterosomes enhance poorly soluble neutraceuticals' solubility, bioavailability, and therapeutic effect.	[15,42,48,50,52,53]
Self-assembling, spherical, fluid bilayer-structured, large unilamellar vesicles with diameters greater than 100 nm (at the nanoscale)	- The vesicular system's preparation is simple and inexpensive.	[16,17,40,49,50,52,53]
Either positively charged and protonated or negatively charged and unprotonated	- Increased cellular uptake into biological membranes for targeted drug delivery reduced or prevented drug toxicity and adverse effects in healthy cells due to surface charge.	[14,41,43,53]
Efficient multiple payload delivery	- These vesicles can be used for simultaneous gene and active agent delivery in combination therapy.	[52]

2.3. Characterization of sterosomes

The physicochemical properties, including size, surface charge, and activity of nanoparticles are all critical considerations when using functional payload as a delivery platform. Size, shape, surface charge, lamellarity, and various other features influence the biological activity and fate of sterosomes. As a result, before utilizing sterosomes, these characteristics must be thoroughly characterized to assure possible *in vitro* or *in vivo* performance [20,62].

2.3.1. Size and polydispersity

Measuring the size and polydispersity of the nanocarrier is a vital aspect, enabling the assessment of batch integrity and repeatability. A diverse range of analytical methods can be used to assess the size of delivery systems, such as sterosomes [24]. These techniques, which include dynamic light scattering (DLS), size exclusion chromatography (SEC), nuclear magnetic resonance (NMR), transmission electron microscopy (TEM), cryogenic TEM (cryo- TEM), and atomic force microscopy (AFM), each offer unique insights into the properties of the delivery systems. However, DLS stands out as the most widely used technique for measuring size and polydispersity, evaluating incident light scattering induced by the Brownian motion of dispersed particles, which creates a time-dependent fluctuation in the scattered intensity of light. This allows for calculating the sterosomes distribution diffusion coefficient, which is subsequently translated into a size distribution employing established hypotheses.

However, DLS implementation is not without its challenges. The system's inability to distinguish individual particles from aggregates can lead to misleading results, with clumps sometimes being mistaken for single particles [20,24]. Researchers need to be aware of these limitations. In contrast, HPLC-SEC, a size measurement technique that involves advancing samples via columns with either perfect or porous sealing under pressure from HPLC pumps, offers a reliable and reproducible alternative. This device can effectively segregate particles of various sizes, from nanoscale to larger sizes. On the other hand, sterosomes are malleable structures that can compress through apertures, potentially leading to erroneous results [24]. The formulation can also be nebulized spontaneously with the jet nebulizer to estimate the particle distribution. This is accomplished by simply attaching the nebulizer to the coolant Anderson Cascade impactor with the plates in place and determining the mass per unit of median aerodynamic diameter (MMAD) of aerosol droplets, acceptable particle dose (FPD), and fine particle fraction (FPF%) from the cumulative mass distribution utilizing the Inhaler Testing Data Analysis Software [42].

Electron microscopy, particularly cryo-TEM and TEM, is crucial for determining the size of sterosomes. It provides a more precise size measurement by facilitating direct observation of the particles. During TEM analysis, a negative stain using a solution of uranyl acetate or phosphotungstic acid is applied on a small copper grid. The aqueous medium is allowed to dry. Sterosomes typically appear as bright dots on a dark background or as blank spots on a white background, depending on whether a staining agent was used. Although TEM has shown considerable potential for structural characterization, its application may be limited by the production of artefacts in the resulting image due to the technique's requirement for removing sterosomes from their natural environment. These can be examined in their native environment through cryo-TEM, which utilizes liquid nitrogen to freeze the sample and prevent particle damage, compression, or deformation. Nevertheless, adopting these solutions is limited since they require a costly infrastructure [20,24]. AFM, a superior resolution technique, is also used to detect particle sizes. This method is trustworthy, quick, and does not require sample pre-treatments. The scattering of laser light is analysed to identify the presence of the nanoparticles in nanoparticle tracking analysis (NTA). This method works by introducing sterosomes into the laser beam path and photographing the illuminated cell containing the nanoparticle in the medium using a camera with a digital sensor. This approach quickly evaluates nanoparticles ranging in diameter from 10 to 2000 nm using the nano-sight instrument software [24].

2.3.2. Zeta potential (ZP)

Determining the zeta potential involves calculating and measuring the electrical charge on the surface of sterosomes in dispersion. Depending on the composition of the vesicular system and the surface functionalization, sterosomes may be negatively, positively, or neutrally charged. Highly charged sterosomes do not assemble due to repulsive forces that push them apart in the medium, but lowly charged sterosomes merge over time, coming together due to attractive forces. The charge of this lipid nanocarrier can predict and regulate stability under preservation conditions. Surface charge is routinely determined using DLS, where the variations in dispersed light induced by the applied electric field are measured [24].

2.3.3. Encapsulation efficiency (EE)

Encapsulation efficiency (EE) is typically used to denote the total amount of encapsulated drug present in the sterosome solution compared to the original amount used for encapsulation. Separation of drug-loaded sterosomes from the unloaded drug is achieved via centrifugation, dialysis, or column chromatography, followed by quantification of the encapsulated drug and replacement of the aqueous phase with an organic solvent (e.g., acetonitrile, ethanol, methanol, Triton X-100). A wide range of approaches are used for to estimate the drug content. These include ultraviolet (UV) spectrometry, fluorescence spectroscopy, gel electrophoresis, enzyme or protein-based assays, HPLC, UPLC, or LC-MS, depending on the physical and chemical properties of the drug [24,64].

2.3.4. Lamellarity

Lamellarity, the number of bilayers that form a sterosome, is a critical property that significantly influences the behaviour of nanoparticles *in vivo*. This property is often estimated using chemically labelled reagents or radiolabelled ions to determine the number of lipids on the exterior vesicle layer [24]. The complexity of studying lamellarity is evident in the various techniques used, including Cryo-TEM, 31P NMR, and X-ray scattering (SAXS) [24,64,65]. The collective efforts in mastering these techniques and understanding the significance of lamellarity in drug delivery are crucial for advancing this field.

2.3.5. In vitro drug release

The pharmaceutical release profile is determined using dialysis. Sterosome dispersion is inserted in a dialysis bag with specific molecular weight cut-offs, which is subsequently immersed in the release media (usually a buffer), then kept at an appropriate temperature (37 °C) and constantly stirred within a sealed chamber to mimic the *in vivo* environment. The release medium is removed and replaced with a new buffer component at predefined intervals. A range of reliable techniques, including UV spectrometry, fluorescence spectroscopy, HPLC, UPLC, LC-MS, are used to analyse the drug. The cumulative release% vs. time graphs depict the percentage of drug release [24].

2.3.6. Phase behaviour

Sterosomes` phase behaviour can be investigated utilizing a comprehensive suite of methods, including the use of differential scanning calorimetry (DSC), X-ray diffraction (XRD), and thermogravimetric analysis (TGA). These approaches ensure the formulation's thermally stable and crystalline properties [66]. DSC is a fundamental method for studying the direct absorption of heat energy in a sample when exposed to a controlled temperature increase or decrease. DSC is characterized by a simultaneous energy flow into the sample cell and uses a metal pan made of aluminium, zinc, or tin [67,68]. DSC differs from standard thermal analysers (DTA) in one fundamental way. DTA records and measures the variations in temperature between the reference and sample channels. At the same time, DSC detects and records the energy variation required to maintain both the sample and reference channels at the same temperatures throughout the analysis process. However, DSC is often used because most physical or chemical transitions are triggered by a change in heat [69,70]. The resulting heat flow vs. temperature map contains knowledge regarding first-order transitions like melting or crystallization and second-order transformations like the glass transition temperature in the samples [70]. The most common application of XRD is the characterization of crystalline materials. This knowledge includes the crystal structure's

phase, preferred crystal orientation (texture), and other structural variables. The XRD apparatus produces sample-specific peaks through constructive interference of homogeneous X-ray beams diffracted from every pair of lattice planes in a sample at precise angles. The arrangement of atoms inside the lattice determines the intensity of the peaks. The diffraction of X-rays from atoms produces a pattern known as diffraction that contains information about the atomic configuration. XRD is widely used in the pharmaceutical industry for polymorph characterization, stability monitoring, creation of methods, and validation for the quantification and identification of drugs. In nanotechnology, XRD is used to determine crystallinity and particle size [71,72]. TGA is a thermal technique used to characterize a substance, compound, or blend by measuring weight changes at high temperatures. TGA equipment includes of a precision balance and a furnace. The device's internal temperature is scheduled to rise linearly over time. The results of TGA experiments are represented by a weight loss curve that depicts the profile of mass changes as an indicator of temperature. This is complemented by a derivative curve (DTG), which provides a better understanding of the recorded decomposition stages in the studied material. This comprehensive research process enables the elimination of gaseous products from the vicinity of the sample to maintain a stable environment composition during the experiment, preventing the reaction between the sample and air from forming [71,73].

3. MODIFIED STEROSOMES

Studies have shown that pre- and post-modifications of native sterosomes increase circulation and residence time in the bloodstream and reduce clearance [48,50]. Modified sterosomes are summarized in Table 3.

3.1. Pre-modification of sterosomes

To improve the circulation of non-phospholipid sterol-rich liposomes in the bloodstream, Cui et al. pioneered the pre-insertion of PEG into the mixture of single-chain amphiphile molecules (palmitic acid) and cholesterol (Figure 3) to create ground-breaking prolonged-circulation sterosomes [48]. These pre-modified sterosomes were a significant leap in overcoming the critical disadvantages of conventional liposomes, particularly their uptake by the mononuclear phagocyte system (MPS) after parenteral administration, which resulted in poor bioavailability and therapeutic efficacy of drugs. The combined properties of the interfacial PEG and the highly liquid-ordered bilayer-structured core led to the creation of modified sterosomes by hydrophobically or covalently anchoring PEG to cholesterol before mixing with palmitic acid. These modified sterosomes achieved extended bloodstream circulation lifetimes, potentially enhancing the therapeutic activities of encapsulated active agents. The increased systemic lifetime of these modified nanovectors and the limited passive permeability of sterosomes, enabled by the high sterol content and the presence of PEG, facilitated sustained drug delivery after intravenous administration.

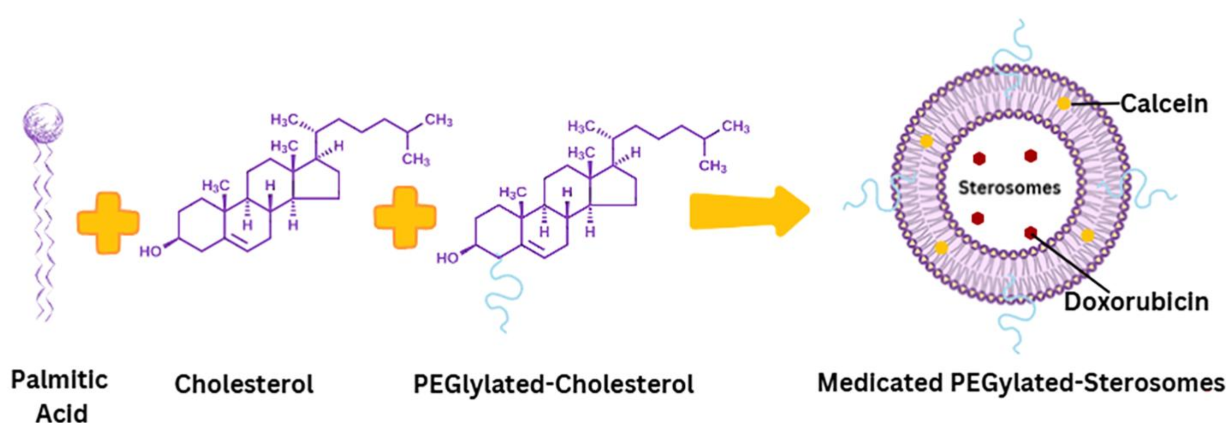

Figure 3. A diagrammatic representation of PEG pre-insertion into palmitic acid, cholesterol, and PEG-cholesterol mixture to form PEGylated sterosomes loaded with calcein and docorubicin.

Table 3. Summary of modified sterosomes.

Sterosomes Archetype	Lipid Composition	Modification Strategy	Active agent	Advantages	Limitation	Ref
PEGylated sterosomes	Palmitic acid (PA) and Cholesterol (Chol)	PEGylated cholesterol (PEG-Chol) pre-insertion	Calcein and doxorubicin	PA/Chol/PEG-Chol liposomes showed a minimal permeability to calcein and doxorubicin	Blood circulation was considerably shorter than that observed for controlling PEGylated phospholipid liposomes	[48]
Stealth sterosomes	Palmitic acid (30%) and Cholesterol (70%)	PEG-modified (DSPE-PEG) post-insertion	NM	Long-term circulation in the bloodstream	Reduced *in vitro* complement activation as well as *in vitro* macrophage uptake	[50]
Ternary sterosomes	Palmitic acid (30%), cholesterol (28%), and cholesterol sulfate (42%)	Self-assembling	Ascorbic acid	Efficiently protected ascorbic acid from an oxidizing environment of iron(III)	They are only stable between pH 5 and 9.	[45]
Functional sterosomes	Stearylamine, 20(S)-hydroxycholesterol	Oxidized derivatives of cholesterol	Purmorphamine/ 20(S)-hydroxycholesterol	Promote osteogenesis and bone healing by activating the hedgehog signalling	NM	[60]
Functional sterosomes	Stearylamine (SA) and 20(S)-hydroxycholesterol (Oxy)	Oxidized derivatives of cholesterol	20(S)-hydroxycholesterol	Stimulate osteogenesis and bone formation. No cytotoxicity on cell proliferation observed up to 500 µg/mL of SA/Oxy sterosomes	NM	[14]
Functional sterosomes	Cetylpyridinium chloride, 20(S)-hydroxycholesterol	Oxidized derivatives of cholesterol	Cetylpyridinium chloride and 20(S)-hydroxycholesterol	Intrinsic antibacterial, anti-biofilm and osteogenic abilities	NM	[16]

PEG: Polyethylene glycol; DSPE-PEG: PEG-modified distearoylphosphoethanolamine; Ref: Reference; NM: Not Mentioned.

Furthermore, the interfacial PEG plays a crucial role in stabilizing the palmitic acid and preventing its protonation at lower pH, thereby reducing the pH sensitivity of modified sterosomes compared to native sterosomes. As a result, PEG pre-insertion sterosomes are more stable than conventional sterosomes, and up to pH 5, no evidence of triggered release or destabilization of the liquid-ordered lamellar system is observed. The pegylated sterosomes further improve the limited passive permeability of their encapsulated payloads and inhibit pH sensitivity, ensuring their stability. Compared to pegylated liposomes, pegylated sterosomes exhibited

a remarkable ability to maintain stability and retain encapsulated drugs (calcein and doxorubicin) over an extended period. Pegylated sterosomes demonstrate significantly enhanced blood circulation compared to conventional sterosomes, lasting six times longer, which is a remarkable accomplishment. The modified sterosomes maintain a strong affinity for blood proteins due to their charged interface, resulting in rapid and efficient clearance from the bloodstream compared to pegylated liposomes [48].

3.2. Post-modification of sterosomes

Cielak et al. set out to develop stealth sterosomes to create long-circulating nanocarriers in the bloodstream and evade capture by the monocytic phagocyte system. Their innovative approach involved the attachment of polyethene glycol (PEG) to the surface of a pre-existing native sterosome (Figure 4) composed of a palmitic acid and cholesterol mixture [50]. Despite the initial lack of clear evidence, the idea that PEGylated amphiphiles could integrate in substantial quantities into preformed sterosomes, given the highly ordered apolar core of these bilayers resulting from their elevated sterol content [13], was a promising one. The investigation conducted by Cielak et al. revealed that after parenteral administration, these post-modified sterosomes exhibited enhanced and prolonged systemic circulation compared to native sterosomes, offering hope for improved drug delivery. The storage stability of these PEG-modified sterosomes was comparable to that of their unmodified counterparts. In contrast to unmodified sterosomes, which become destabilized at lower pH (below pH-6), these post-modified sterosomes were not destabilized at different pH ranges (up to pH-3). The disadvantages of this modified nanocarrier include the loss of pH sensitivity, reduced complement activation, and decreased macrophage uptake *in vitro* and *in vivo*. The highly negative zeta potential (ZP) value exhibited by pure sterosomes was lost in the modified form; the ZP value in PEG-sterosomes was decreased and close to neutrality, which could have affected their pH sensitivity due to the interfacial PEG's ability to conceal the negatively charged carboxylic group of palmitic acid from environmental pH or the reduction of the pKa value of this saturated fatty acid [50]. As a result, PEG-modified sterosomes cannot be used as nanocarriers for pH-stimulated targeted or site-specific drug release. Furthermore, when administered via intravenous or non-invasive routes (such as inhalation and transdermal administration), the poor complement activation and macrophage uptake properties of these modified sterosomes may hinder their targeted drug delivery to biological membranes or epithelial cells, such as the lymphatic system.

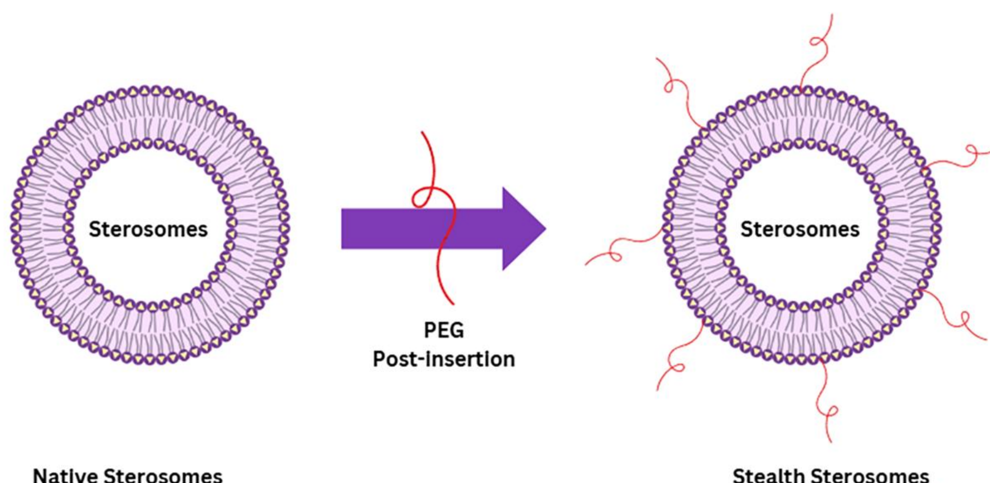

Figure 4. A diagrammatic depiction of PEG post-insertion into native sterosomes to form stealth sterosomes.

3.3. Ternary mixture sterosomes

Based on the type of electrostatic interfacial interactions between palmitic acid and cholesterol or palmitic acid and cholesterol sulphate, which are significantly regulated by the protonation state of palmitic acid in the formed sterosomes, blends of palmitic acid and cholesterol or cholesterol sulphate can form highly stable large

unilamellar vesicles with different pH-induced release profiles. When palmitic acid sterosomes are formed with cholesterol, they are deprotonated and form stable vesicles at high pH; as pH decreases, they become unstable, and their encapsulated content is released because palmitic acid becomes protonated. In contrast, palmitic acid vesicles made with cholesterol sulphate were stable and protonated at low pH and became unstable as pH increased due to the deprotonation of palmitic acid and the triggered release of the vesicles' contents [46,47].

Carbajal et al. [45] investigated the possibility of creating sterosomes from a ternary combination of palmitic acid, cholesterol, and cholesterol sulphate due to the unique behaviour of palmitic acid with the two different sterols. At molar concentrations of 30:28:42 for palmitic acid, cholesterol, and cholesterol sulphate, respectively, stable, self-assembling bilayer fluid-ordered lamellar phase vesicles were generated. These modified sterosomes efficiently protected ascorbic acid from redox-active ions and oxidative environments.

Interestingly, the stability and phase behaviour of the ternary mixture sterosomes are unaffected by the protonation or deprotonation state of palmitic acid. When the fatty acid is protonated, 42% cholesterol sulphate prevents phase separation or reduces the repulsive electrostatic force between palmitic acid (30%) and cholesterol (28%). However, at higher pH, 28% cholesterol is sufficient to properly mix protonated palmitic acid (30%) and cholesterol sulphate (42%), preventing the destabilization of the ternary sterosomes. Therefore, sterosomes composed of palmitic acid, cholesterol, and cholesterol sulphate (30/28/42) are stable over a wide pH range [45].

3.4. Functional sterosomes

The potential of using functional cholesterol derivatives in nanocarriers is highlighted because they can replace the sterol constituent in sterosomes. This replacement results in therapeutically active vesicular systems that enhance the outcomes of encapsulated active agents. The key to this enhancement is the synergistic effect between the drug and functional excipients.

Most vesicular systems have limited drug loading capacity, necessitating the utilization of high concentrations of nanocarriers, which can lead to unwanted adverse effects and increased production costs. However, the availability of a chemically active nanocarrier that reduces the use of large carrier materials while maintaining high solute loading capacity, as demonstrated by Lee et al. [60], offers a promising novel delivery platform that could potentially reduce production costs.

Studies have shown that by effectively selecting the sterol constituent of sterosomes, cholesterol derivatives with intrinsic or therapeutic potential can be used instead of native cholesterol to develop active nanocarriers in the absence of drugs. Notably, oxidized derivatives of cholesterol, known as oxysterols (e.g., 20(S)-hydroxycholesterol), were used to create sterosomes (Figure 5) that can promote osteogenesis and bone growth, as demonstrated by Cui et al. [14], Zhang et al. [16], and Lee et al. [60].

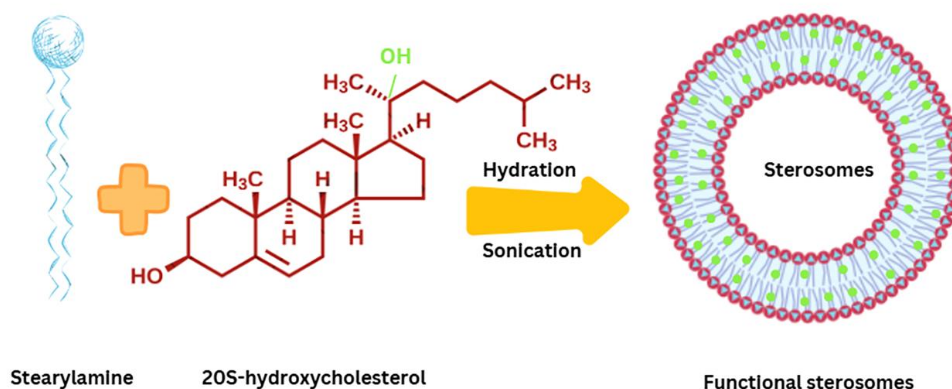

Figure 5. A schematic illustration of functional sterosomes prepared from the mixture of stearylamine and functional 20(S)-hydroxycholesterol.

The potential of using sitosterol as a functional excipient in sterosomes is highlighted by the work of AbouSamra et al. [15]. Their study found that unmedicated β-sitosterol-sterosomes had a significant anti-carcinogenic effect and that drug-loaded β-sitosterol-sterosomes significantly enhanced therapeutic efficacy. The plant Senecio fulgens was used to create β-sitosterol, which has a range of intrinsic properties, including anti-neoplastic, antimicrobial, analgesic, and anti-diabetic properties, without adverse side effects.

Furthermore, the use of intrinsically active monoalkylated amphiphiles such as cetylpyridinium chloride, a universal antibacterial, imparted antimicrobial properties to the sterosomes formed with it, enhancing the therapeutic efficacy of drugs loaded in the vesicular systems [16,55-57].

4. APPLICATIONS OF STEROSOMES

Poor physiological stability, quick clearance, nonspecific targeting, burst release, and restricted cell membrane permeability are drawbacks of using bioactive compounds without adequate delivery strategies. This is because bioactive substances frequently need supraphysiological doses to compensate for poor absorption, increasing the risk of side effects. Innovative drug delivery systems can overcome these challenges by encapsulating bioactive substances, boosting cell entry, and, most importantly, enabling controlled drug release in a spatially targeted manner. This controlled release is a key factor in ensuring the safe delivery of bioactive compound. Due to their demonstrated potential, sterosomes have been widely used in the delivery and treatment of several diseases (Figure 6). These include the treatment of cancer, dental problems, the delivery of genes, and bone regeneration, and their applications have been thoroughly reviewed below. Table 4 summarizes the applications of sterosomes as nanocarriers in treating metabolic and infectious diseases.

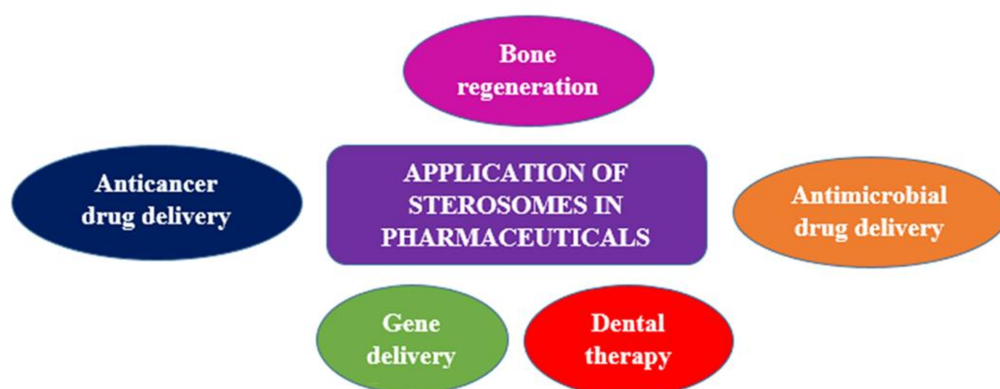

Figure 6. Schematic illustration of sterosomes applications in treating different diseases.

4.1. Bone regeneration

Sterosomes have shown considerable promise as a delivery system for pharmacological and genetic substances and exhibit inherent osteoinductive potential, even without drug loading. Cui and colleagues examined the utilization of sterosomes in the therapy of bone degeneration in 2017 by developing a sterosome formulation with osteoinductive capabilities through the successful selection of sterol, one of the sterosome components (oxysterols, an oxidized cholesterol derivative), which have been shown to improve osteogenesis and bone growth. Thus, one of the most successful oxysterols for bone regeneration, 20(S)-hydroxycholesterol, was studied as a potential candidate molecule for generating fluid lamellar phases with stearylamine, a single-chain amphiphile. Stearylamine (SA)/oxysterol (Oxy) sterosomes induced osteogenic differentiation and improved calvarial repair *in vivo* (mouse) and *in vitro* without additional therapeutic agents, demonstrating their inherent bone-forming capacity. The optimized sterosomal formulation, incorporated into a hydrogel, exhibited enhanced osteogenic differentiation of bone marrow stromal cells *in vitro*.

Table 4. Summary of the application of sterosomes to treat metabolic and infectious diseases.

Sterosome Archetype	Lipid Composition	Active Agent/ Inherent Ability	Application	Advantages	Ref
Cationic-functional sterosomes	Stearylamine (50%), 20(S)-hydroxycholesterol (50%)	20(S)-hydroxycholesterol	Bone regeneration	- Intrinsic osteoinductive therapeutic effects. - Superior gene knockdown efficiency. A stable vesicular system. No cytotoxic effects on cell proliferation up to 500 µg/mL of SA/Oxy sterosomes.	[14]
Smoothened agonist sterosomes	Stearylamine, 20(S)-hydroxycholesterol	Purmorphamine/ 20(S)-hydroxycholesterol	Bone regeneration	- Promote osteogenesis and bone healing by activating the hedgehog signalling	[60]
Multi-functional sterosomes	Cetylpyridinium chloride (50%), 20(S)-hydroxycholesterol (50%)	Antibacterial Cetylpyridinium chloride and osteoinductive 20(S)-hydroxycholesterol	Antibacterial and bone regeneration	- Intrinsic antibacterial (gram-positive and Gram-negative bacteria), anti-biofilm and osteogenic abilities	[16]
Cationic Sterosomes	Stearylamine and cholesterol	Phenamil (hydrophobic small molecules) and noggin-directed siRNA	Gene delivery for bone repair	- Osteogenic differentiation of mesenchymal stem cells and calvarial bone repair - demonstrated high phenamil encapsulation efficiency, supported sustained release of encapsulated drugs, and significantly reduced drug dose requirements to induce osteogenic development.	[52]
Cationic Sterosomes	Stearylamine and cholesterol	Small interfering RNA (siRNA)	Gene delivery to enhance osteogenic differentiation of mesenchymal stem cells	- Improved cellular uptake and gene knockdown efficiency in adipose-derived mesenchymal stem cells while causing low cytotoxicity	[41]
Antibacterial cationic Sterosomes	Cetylpyridinium chloride (CPC) and cholesterol (Chol)	Cetylpyridinium chloride	Dental treatment	- CPC/Chol sterosomes pass through the dentinal tubules and diffuse into the pulp, where dental pulp cells quickly ingest them. They also showed intrinsic solid antibacterial activity against these Gram-positive and Gram-negative endodontic pathogens and biofilms.	[57]
Phyto-Sterosomes	Lauric (25%) acid and β-Sitosterol (75%)	Rutin, β-Sitosterol	Cancer	- Unmedicated and medicated Phyto-Sterosomes exhibited anti-proliferative and tumour-suppressive effects. Increase in the bioavailability of Rutin loaded into the sterosomes compared to Rutin suspension.	[15]
Sterosomes	Stearlyamine and cholesterol (1:2)	Metformin	Lung cancer	- The biological half-life (t1/2), AUC, cellular uptake, distribution volume, and sustained release of metformin encapsulated in the sterosome reportedly increased compared to the naked drug solution.	[42]
Sterosomes	Stearylamine and cholesterol (1:2)	Vancomycin	Antibacterial	- Improved, superior, and faster bacterial killing ability against methicillin-resistant *staphylococcus aureus* (MRSA) and *Staphylococcus aureus* (*S. aureus*)	[53]
Non-phospholipid liposomes	Stearylamine (50%) and cholesterol (50%)	Cymoxanil	Antifungal	- Extended drug release was achieved over 3 days, enhancing the Cymoxanil's short duration of action.	[74]

Cationic liposomes often exhibit cytotoxic properties, producing harmful reactive oxygen intermediates and interfering with the functionality of cellular and subcellular membranes. The protonation state of SA imparts a significant positive surface charge to the sterosomes, resulting in a stable colloidal system that may pose toxicity risks to living cells. The cytotoxic effects of SA/Oxy sterosomes were assessed using mesenchymal stem cells (MSCs) *in vitro*. No cytotoxic effects on cell proliferation were detected at concentrations of up to 500 µg/mL of SA/Oxy sterosomes. This study suggests that a non-phospholipid liposomal platform with osteoinductive characteristics could deliver small-molecule drugs and other therapeutic genes for enhanced bone formation [14].

This inherent osteoinductive potential was further demonstrated by Lee et al., who created a Smoothened agonist sterosome-immobilized 3D hybrid scaffold (Figure 7). This scaffold, by triggering a Hedgehog signalling pathway with small-molecule activators [20(S)-hydroxycholesterol]/OHC and purmorphamine (PUR) of the Smoothened protein in the Hedgehog pathway serving as carrier and cargo, can stimulate osteogenesis and improve the healing of bones in an *in vivo* calvarial defect. The sterosome-immobilized hybrid scaffold not only promotes cell adhesion and proliferation but also delivers bioactive molecules in a sustained and spatially targeted manner, increasing osteogenic differentiation of bone marrow stem cells through [20(S)-hydroxycholesterol] and purmorphamine-mediated synergistic Hedgehog pathway activation. This exciting potential of sterosomes to enhance osteogenesis offers a promising future for bone regeneration. Furthermore, the OHC-sterosome combined with PUR exhibited a 1.4- and 2.1-fold increase in the expression of the PTCH1 and GLI1 transcription factors, respectively, compared to the native sterosome synthesized from stearylamine and cholesterol [60].

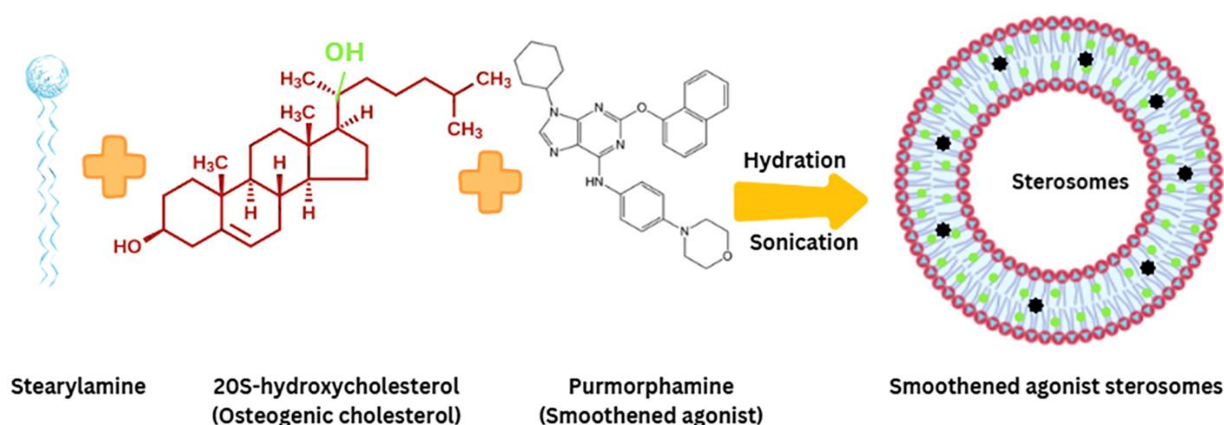

Figure 7. Schematic illustration of smoothened agonist sterosomes synthesized from stearylamine, 20(S)-hyroxycholesterol, and purmorphamine.

Broken bones and internal anchorage implants are frequently associated with bacterial infections, which can lead to osteomyelitis, a condition characterized by persistent bone infection and sequestrum formation that may result in lifelong disability or fatal sepsis. High-dose antibiotics and autologous bone transplantation are common treatments; nevertheless, they are associated with the risk of recurrence and donor site damage. The effects of antibacterial and osteogenic cetylpyridinium chloride (CPC) and osteoinductive sterol (Oxy) sterosomes on a rat model of critical-sized calvarial defects with infection were established *in vivo* by Zhang et al. Their findings showed that cetylpyridinium chloride and osteoinductive sterol sterosomes not only successfully eliminated bacterial infections but also enhanced calvarial healing without the use of antibiotics, bone formation promoters, or exogenous cells. This suggests that sterosomes could facilitate the healing of infected bone defects and be utilized alongside therapeutic genes and small-molecule drugs. No significant cytotoxic effects on bone marrow stromal (BMS) cells were detected at CPC/Oxy sterosome concentrations of 5 µg/mL. CPC/Oxy sterosomes are classified as non-antibiotic antimicrobials, offering the potential to

circumvent antibiotic resistance. CPC/Oxy sterosomes may address the challenge of balancing antibacterial activity and osteogenesis [16].

4.2. Gene delivery

Cui et al. showcased the innovative use of sterosomes, a unique drug carrier system, in bone regeneration. They combined the single-chain amphiphile stearylamine (SA) and high-concentration cholesterol (Chol) to co-deliver phenamil and noggin-directed small interfering RNA (siRNA), enhancing the osteogenic differentiation of mesenchymal stem cells. This novel approach uses a single vesicular system to activate bone morphogenetic protein-2 signalling (phenamil) and inhibit the bone morphogenetic protein-2 antagonist noggin (siRNA). Using phenamil, a small molecule bone morphogenetic protein activator, and inhibiting natural bone morphogenetic protein-2 antagonists such as noggin is a promising therapeutic strategy for promoting bone regeneration. The unique sterosomes, derived from stearylamine and high cholesterol concentration, offer a fresh perspective on fostering osteogenesis and bone regeneration by co-delivering the small hydrophobic molecule phenamil and noggin-targeted siRNA. These sterosomes demonstrated high phenamil encapsulation efficiency, prolonged drug release, and reduced dose requirements for the osteogenic differentiation of mesenchymal stem cells (MSCs). Approximately 5% drug leakage from these sterosomes was observed after 24 hours, with phenamil being released gradually in a sustained manner for up to 2 weeks. The sustained release observed can be linked to the elevated Chol content in the vesicular system, which contributes to the rigidity and thickness of their bilayers, thereby leading to reduced permeability. No apparent burst release was detected, suggesting that phenamil-embedded sterosomes maintain stability in their suspended state. This feature holds significant importance and offers convenience for immediate use and storage, ensuring no premature drug release until further clinical application. The encapsulation of phenamil using these cationic sterosomes effectively decreased the dosage by at least 10-fold to attain comparable bioactivity to free-form phenamil solubilized in DMSO. The delivery of noggin siRNA via the SA/Chol vesicular system reduced noggin mRNA levels by over 40% compared to control groups without siRNA delivery. However, the co-encapsulation of phenamil did not significantly alter the level of noggin knockdown. This suggests that embedding phenamil in Sterosomes did not affect their gene knockdown efficiency. *In vitro*, the bioactivity of SA/Chol sterosomes in a MeGC hydrogel with MSCs exhibited minimal cell death at concentrations up to 100 μg mL^{-1}. Therefore, the encapsulation of phenamil and noggin siRNA in the same nanocarrier synergistically improved mesenchymal stem cell osteogenesis *in vitro* and calvarial bone repair *in vivo*, suggesting a potential non-phospholipid liposomal approach for delivering small molecules and gene therapy for combined treatment [52].

In another study, Cui and colleagues investigated sterosomes constructed with the single-chain amphiphile stearylamine (SA) and high cholesterol (Chol) content to deliver noggin siRNA and improve osteogenic differentiation of adipose-derived mesenchymal stem cells. They examined the ability of stearylamine and cholesterol sterosomes loaded with noggin-targeting siRNA to induce osteogenic differentiation of adipose-derived mesenchymal stem cells in both two-dimensional (2D) monolayer cell culture and three-dimensional (3D) hydrogel settings *in vitro*. They also evaluated the ability of noggin siRNA-loaded sterosomes to trigger osteogenic differentiation in a mouse calvarial defect model, comparing their performance to commercially available Lipofectamine 2000. SA/Chol sterosomes and Lipofectamine 2000 demonstrated significant cell viability at concentrations of 5 μg/mL. Increasing these nanocarrier concentrations above 5 μg/mL diminished cell viability. No substantial difference in cell viability was seen between SA/Chol sterosomes and Lipofectamine 2000. Furthermore, no notable cytotoxicity was detected for either SA/Chol sterosomes and Lipofectamine 2000 when complexed with siRNA at concentrations of up to 10 μg/mL nanocarriers and 200 nM siRNA. The negative charges of siRNA may partially neutralise the positive charges of nanocarriers such as SA/Chol sterosomes, resulting in less toxic complexes than those formed with empty nanocarriers. The complexation efficiencies of Lipofectamine 2000 and the SA/Chol vesicular systems with 100 nM siRNA were 69 ± 2% and 84 ± 3%, respectively. Uptake efficiencies of 93% and 96% were recorded for Lipofectamine 2000 and SA/Chol nanocarriers, respectively. SA/Chol sterosomes-siRNA complexes showed an uptake efficiency of over 60% after 14 days of incubation at 37 °C, whereas lipoplexes showed minimal uptake. Therefore, SA/Chol sterosomes and siRNA complexes significantly improved cellular uptake and gene knockdown efficiency in adipose-derived

mesenchymal stem cells while causing low cytotoxicity. The osteogenic efficacy of these sterosomes loaded with noggin siRNA was demonstrated in both two- and three-dimensional *in vitro* conditions, as well as in a mouse calvarial defect model. This implies that siRNA transport via novel stearylamine/cholesterol sterosomes is a non-viral, practical approach for gene knockdown, potentially opening new avenues for gene therapy [41].

4.3. Dental applications

Experts investigated the trans-dentinal potential of nanoscale cetylpyridinium chloride/cholesterol cationic non-phospholipid sterosome liposomes, as well as their intrinsic antibacterial efficacy against strains linked with caries, pulpal, and periodontal illness. This might offer new insights into developing more effective and conservative pulpal disease prevention methods, reassuring patients about the safety of this innovative treatment strategy. The safety of this innovative treatment strategy is a key aspect that has been extensively researched and confirmed. Dentinal tubules are considered a primary pathway for pathogenesis in pulpal inflammation and infection, as they act as conduits for bacteria and their by-products. Cetylpyridinium chloride/cholesterol sterosomes pass through the dentinal tubules and diffuse into the pulp, where dental pulp cells rapidly internalise them, as demonstrated using human dentine blocks *in vitro* and Wistar rat molar teeth *in vivo*. They also demonstrated strong intrinsic antibacterial activity against both Gram-positive and Gram-negative endodontic pathogens and biofilms. This implies that sterosomes possess potential for multi-drug delivery, suggesting they could serve as a promising therapeutic strategy targeting specific pathways associated with pulpal diseases. This could help determine and characterise the most suitable prophylactic and therapeutic targets for early intervention in future dental applications [57].

4.4. Anti-cancer drug delivery

Rutin-loaded “Phyto-Sterosomes” were successfully developed using saturated fatty acids (lauric, stearic, and palmitic acids) and β-sitosterol (a "cardioprotective" substitute for cholesterol) in 1:2 and 1:3 ratios. These newly created Phyto-Sterosomes significantly increased the solubility and cellular uptake by epithelial cells, prolonged circulation and residence time in the bloodstream, and delayed the elimination of the hydrophobic rutin (a naturally occurring anti-carcinogenic polyphenolic compound). The formulation of Phyto-Sterosomes derived from lauric acid (LA) and β-sitosterol (Sito) at a 1:3 ratio exhibited optimal physicochemical properties. They were spherical, demonstrated the highest drug encapsulation efficiency (95.7%), had the smallest particle size (250.6 nm), displayed a homogeneous distribution (PDI = 0.2), and possessed a high zeta potential (−51.5 mV). Based on a diffusion-controlled mechanism and the Higuchi model, these rutin-loaded Phyto-Sterosomes (66.79%) showed significantly improved drug release compared to rutin suspension (26.85%). After a one-month stability study conducted at various temperatures, the drug-loaded sterosomes remained physically and chemically stable. There were no signs of creaming, phase separation, or particle agglomeration. The notable stability profile of LA/Sito sterosomes can be attributed to the surface activity of β-sitosterol, which reduces interfacial free energy, thereby facilitating the formation of a stable vesicular system. In a concentration-dependent manner, drug-loaded sterosomes, pure sterosomes (unmedicated or blank), and crude rutin significantly reduced the viability of human liver cancer cell lines, with IC50 values of 73.7, 145, and 1000 μg/ml, respectively. However, the drug-loaded sterosomes were more effective due to synergistic interactions between the phytochemical and sitosterol, facilitated by the encapsulation of rutin within the LA/Sito sterosomes, which enhances drug solubility and cellular uptake, resulting in increased damage and cytotoxicity. It was demonstrated that sitosterol is valuable, as both unmedicated and medicated Phyto-Sterosomes exhibited anti-proliferative and tumour-suppressive effects. The pharmacokinetic study demonstrated a significant enhancement in the bioavailability of rutin following a single oral administration of LA/Sito sterosomes, with a peak concentration of 200 ng/mL. In contrast, the free rutin suspension showed a threefold reduction, reaching only approximately 65 ng/mL. Furthermore, Phyto-Sterosomes have the potential to significantly enhance rutin's anti-cancer effects. In conclusion, the nano-encapsulation of rutin in the novel "cardioprotective" vesicular system presents a viable therapeutic platform [15].

Osama et al. developed nebulised metformin sterosomes to treat lung cancer via inhalation. This metformin-loaded sterosome formulation was successfully prepared using a mixture of stearylamine and cholesterol in a

1:2 ratio. Human lung cancer cell lines exposed to metformin sterosomes exhibited a rapid and dose-dependent reduction in cellular growth, with viability reduced to approximately 50%. In contrast, excellent viability of these cancer cells was observed when exposed to pure sterosomes, with 94% cell survival. The biological half-life ($t_{1/2}$), AUC, cellular uptake, distribution volume, and sustained release of metformin encapsulated in these sterosomes reportedly increased compared to the free drug solution. Metformin-loaded sterosomes demonstrated a substantial enhancement in biological half-life ($t_{1/2}$), with a mean value of 7.31±1.04 hours, compared to 3.99±0.17 hours for the solution form. However, the peak plasma concentration achieved for metformin-loaded sterosome aerosols was lower than that of the free drug aerosols. This study also revealed that metformin nebulisation resulted in low T_{max} levels of 1.42 ± 0.24 hours and 1.38 ± 0.032 hours for the metformin-loaded sterosome and free drug solution, respectively. This indicates rapid metformin absorption following pulmonary administration, in contrast to the slower oral absorption documented in other pharmacokinetic studies, where T_{max} ranged from 2.0 to 3.3 hours and extended up to 6 hours for sustained-release oral tablets. Furthermore, the free metformin solution exhibited a significantly ($p < 0.05$) shorter terminal half-life compared to the sterosome formulation, indicating delayed release of metformin from the formulation, thereby maintaining a sustained therapeutic concentration of the drug. The $AUC_{(0-12)}$, $AUC_{(0-\infty)}$, and mean residence time (MRT) of the sterosomal-incorporated metformin were dramatically elevated compared to the solution form. Simultaneously, the estimated clearance (Cl) was markedly reduced relative to metformin aerosol in solution form, suggesting practical implications for drug formulation and pharmacokinetics. The suitability of sterosomes for aerosol distribution via nebulisation presents an innovative approach for administering metformin through inhalation as a potentially efficacious treatment for lung cancer. Additional studies and evaluations are required to comprehensively analyse the efficacy of this approach and its potential for large-scale industrial application [42].

4.5. Antimicrobial drug delivery

Vancomycin (VCM) was delivered using sterosomes composed of stearylamine and cholesterol in a 1:2 ratio to eradicate methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus* (*S. aureus*). After being tested for 90 days at various storage temperatures, these VCM-loaded sterosomes showed superior physical stability and no signs of haemolysis. The *in vitro* cytotoxicity of VCM-loaded sterosomes was assessed using MCF-7 and HEK-293 cell lines, with findings indicating cell viability exceeding 70% post-exposure, suggesting a high level of biosafety. VCM-loaded sterosomes also demonstrated a twofold increase in minimum inhibitory concentration (MIC) against *S. aureus* and MRSA compared to free VCM, with MIC values of 1.95 µg/mL and 0.98 µg/mL for *S. aureus* and 7.81 µg/mL and 3.91 µg/mL for MRSA, respectively. Unlike the free vancomycin solution, the drug-loaded sterosomes demonstrated improved, superior, and faster bacterial killing efficacy against the tested, highly resistant microorganisms. This study also revealed that these drug-loaded vesicular systems could significantly reduce mature MRSA biofilms; VCM-loaded sterosomes demonstrated a 27.22% reduction in MRSA biofilm, compared to free VCM, which exhibited a 2.53% reduction. About 80% of the vancomycin was released from the sterosomes over three days in a sustained manner. Vancomycin-sterosomes administered intradermally to infected mice's skin significantly eliminated MRSA compared to conventional drug forms; drug-loaded sterosomes exhibited 27-fold (7500 CFU/mL) MRSA eradication compared to 3-fold (43800 CFU/mL) for free VCM [53].

To overcome the drawbacks of traditional cymoxanil (CYM) pesticides, such as their instability, quick degradation, and brief duration of action, Zhang et al. [75] successfully created sterosomes loaded with cymoxanil. The antifungal-loaded sterosomes were prepared using a 1:1 mixture of stearylamine and cholesterol. The CYM-loaded sterosomes showed extended, sustained, and incomplete release of cymoxanil over 72 hours (20% of CYM was retained in the nanocarrier). In contrast, 80% of CYM was released from the CYM/chlorothalonil DC in 24 hours, with the loaded drug being fully released within 72 hours. The reduced drug release rate in CYM-loaded sterosomes can be linked to the surface interactions between CYM and the sterosomes. This new vesicular system demonstrated improved drug stability and superior sustained release behavior compared to commercially available products. According to an *in vitro* antifungal study, a free CYM solution was only effective for two days, while cymoxanil-loaded sterosomes inhibited the viability of yeast cells

(*Saccharomyces cerevisiae*) for five days. The unmedicated sterosomes also demonstrated a minimal inhibitory effect on the proliferation of yeast cells [74].

As previously discussed in Section 4.3, cationic sterosomes composed of cholesterol and cetylpyridinium chloride have intrinsic broad-spectrum antibacterial activity and effectively treat pulpal inflammation and infection without active agents [57].

Zhang et al. successfully developed a multifunctional sterosomal platform (Figure 8) capable of combining therapeutic genes, small molecules, and medications to treat infected bone defects. An equimolar ratio of cetylpyridinium chloride (CPC) and 20(S)-hydroxycholesterol (Oxy) was selected to prepare sterosomes. Gram-positive and Gram-negative bacteria were susceptible to the dose-dependent antibacterial effects of the CPC/Oxy sterosomes, which were comparable to the antimicrobial potential of pure cetylpyridinium chloride. Furthermore, concentrations as low as 1 μg/mL of CPC/Oxy sterosomes substantially inhibited both *S. aureus* (95%) and *E. coli* (70%). The comparative analysis in this study used stearylamine/20(S)-hydroxycholesterol sterosomes, with little evidence of antimicrobial potential observed. These unmedicated nanocarriers also effectively eliminated bacterial biofilms. At a concentration of 3 μg/mL, CPC/Oxy sterosomes successfully eliminated 85% of established bacterial biofilms within 72 hours. Hence, CPC/Oxy sterosomes, functioning as a non-antibiotic antimicrobial, can circumvent antibiotic resistance and serve as a drug delivery system for clinically utilized bone-promoting medications [16].

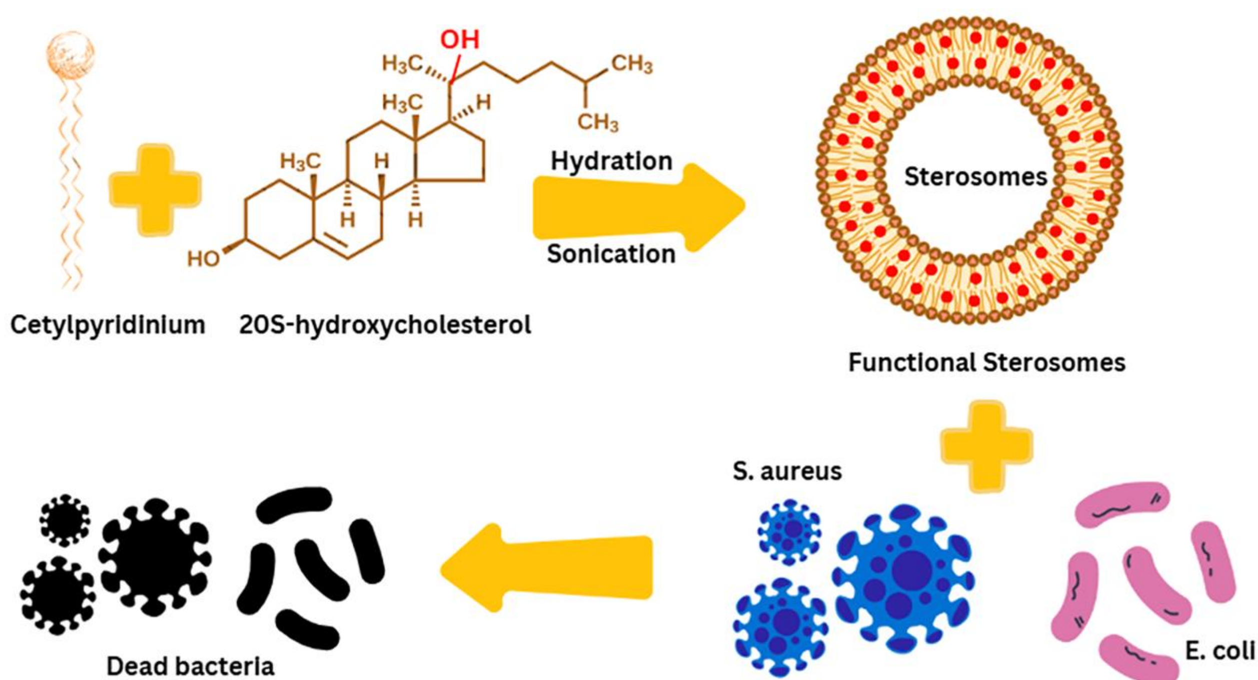

Figure 8. A diagrammatic representation of the antibacterial activity of cetylpyridinium chloride/ 20(S)-hydroxycholesterol sterosomes on Gram-positive and Gram-negative bacteria.

5. CHALLENGES OF NATIVE AND MODIFIED STEROSOMES

Nanomedicine is still in its early stages, with most laboratory research demonstrating limited success in clinical trials or medical applications [75]. While sterosomes hold considerable promise, numerous substantial challenges require attention.

5.1. Stability Issues

The stability of sterosomes is significantly influenced by pH levels. At a pH below 5.5, the stable, fluid, and uniform liquid-ordered system of sterosomes, which is stable at neutral pH, transforms dramatically. It separates into solids and lipids in palmitic acid and cholesterol-sterosomes, primarily due to the protonation of palmitic acid at lower pH. Conversely, palmitic acid and cholesterol sulphate-sterosomes exhibit stability at acidic pH and become leaky at basic pH, a behaviour attributed to deprotonation at higher pH levels [17,43,46,49]. The stearylamine and cholesterol-sterosomes transition from their stable fluid-ordered phase to a solid state when the pH of the surrounding environment rises from 5.5 to 12 or exceeds pH 9.5. In addition, only charged stearylamine can form stable sterosomes [17,40]. The molecular characteristics of the sterol used to form sterosomes can affect the stability of the bilayer liquid lamellar phase and the permeability of the sterosomes formed [15]. Addressing these stability challenges of sterosomes through focused research and development could improve drug bioavailability, enable targeted drug release, prevent dose dumping, mitigate drug toxicity, and overcome technical, safety, stability, regulatory, and logistical barriers to implementing this nanocarrier.

5.2. Loss of Functionality

PEGylated pre- and post-modification of sterosomes can result in pH sensitivity loss [48,50]. Post-PEGylation of native sterosomes can decrease complement activation and macrophage uptake both *in vitro* and *in vivo*, reducing the targeted drug delivery potential of these modified sterosomes [50]. Compared to PEGylated liposomes, PEGylated pre-insertion-modified sterosomes exhibit a shorter biological half-life ($t_{1/2}$) due to their higher affinity for binding plasma proteins [48]. Native and PEGylated pre-insertion-modified sterosomes are easily removed from the bloodstream after systemic administration because of their charged interface. This contrasts with PEGylated post-insertion-modified sterosomes, which have a neutral interface. The charged interface of sterosomes influences their interaction with blood components, affecting their circulation time and potential for drug delivery [48,50]. Preclinical studies provide insights into the pharmacokinetics of nanocarriers, but they do not comprehensively reflect interactions within human biology. This is particularly evident in the frequent divergence of results from animal models and cell lines from human responses. It underscores the need for more accurate testing methods, making clinical trials crucial for evaluating the safety and efficacy of sterosomes and their successful clinical translation [76].

6. RECOMMENDATIONS AND FUTURE PROSPECTS

The battle against the effective and cost-effective treatment of diseases of public health, such as cancer, hypertension, diabetes, HIV, and tuberculosis, especially in developing countries, requires innovative approaches to overcome existing challenges. Sterosomes are an innovative drug delivery system that can reduce the treatment challenges of conventional drug dosage forms.

The potential of indocyanine green (ICG)-chromophore sterosomes for precision therapy and targeted drug release is a promising area for future research [51]. These ICG-loaded sterosomes can be used as a cutting-edge dual stimuli-responsive platform for precision therapy and targeted drug release (photo-temperature activated near-infrared (NIR) laser irradiation and pH-sensitive drug delivery systems made from either palmitic acid/cholesterol or palmitic acid/cholesterol sulphate or palmitic acid/cholesterol/cholesterol sulphate loaded with chromophore) for chemotherapeutic agents such as paclitaxel for breast and colon cancer [77], doxorubicin for breast and skin cancer [78,79], and doxorubicin for tumour [80,81].

The photosensitive or light-responsive sterosomes created by Cui et al. [61], allow precise control, site-specific, and switchable-manner release of the entrapped agents, due to the presence of the azobenzene moiety [61]. This adaptability, demonstrated in prior research using photosensitive groups like spiropyran, spirooxazine, and azobenezene, suggests that the azobenezene-photo-controlled sterosomes could be effective in delivering anti-cancer drugs to a variety of tumour sites due to their supramolecular and dimer-like properties [81-86].

Furthermore, targeted delivery of chemotherapeutics can be achieved using cationic sterosomes. The stearylamine/cholesterol sterosomes may be created as tumour-targeted therapeutic carriers without further modification because stearylamine can directly target phosphatidylserine existing on the outer leaflet of some

tumour cells through passive targeting. These stearylamine/cholesterol sterosomes will demonstrate improved permeability and retention (EPR) effects to allow exclusive delivery of their payloads to solid tumours due to their appropriate size (greater than 100 nm size); monomodal and monodispersed particle size distribution (which will prevent aggregation and recognition by the reticuloendothelial system); ideal surface chemistry (superior cellular uptake or accumulation in tumour tissue); positive surface charge (to help avoid opsonin aggregation); pH sensitivity and stability (to avert nonspecific release); limited passive permeability will ensure controlled and prolonged payload release (due to high cholesterol content); deformability; and biocompatibility [16,17,40,42,87,88].

Stealth sterosomes (pre- or post-insertion PEG-sterosome) successfully developed by Cui et al. [48] and Cieślak et al. [50] demonstrated prolonged bloodstream circulation time, which will enable passive accumulation in cancer tissues leading to improved permeability and retention (EPR) effects, making the entrapped payload very effective [88,89].

For successful gene delivery (siRNA) with or without drug molecules for bone regeneration, cationic sterosomes made of an equal concentration of monoalkylated primary amine (stearylamine) and cholesterol were used [41,52]. These cationic sterosomes are analogous to lentiviral particles, stable at room temperature, with an elevated positively charged surface, high complexation proficiency, and high cellular uptake efficacy [41]. These characteristics make them promising as gene-delivery nanocarriers. Comparing this study to earlier research by Sharma et al. [90], further research should be done into the use of these cationic sterosomes in the delivery of anti-miR-191 (anti-miRNA) for the treatment of resistant breast cancer with or without co-therapy with doxorubicin or cisplatin. It is worth noting that anti-miR-191 was successfully administered to breast cancer cells *in vitro* by Sharma et al. using stearylamine-cationic liposomes as the practical and intrinsic nanocarriers. Additionally, the chemo-sensitivity of breast cancer cells to pure drug solutions of doxorubicin or cisplatin was enhanced by the anti-miR-191-loaded stearylamine-cationic liposomes [91].

Conventional nanocarriers used to treat cancer, tuberculosis, and HIV, such as metallic nanoparticles, dendrimers, sterosomes, and liposomes, are linked to drug degradation, accumulation in unintended areas, and resistance development. Furthermore, detecting diseases like cancer, tuberculosis, and HIV at an advanced stage in people might lead to inefficient therapy and untimely mortality. To overcome these issues, nano-drugs, which use functionalized nanocarriers, present a promising path for improving the efficacy of medical treatments, notably in the context of individualised medicines [90]. Functionalized or biomimetic nanomedicines show promise as effective treatments for cancers, tuberculosis, and HIV at cellular reservoirs because they have good biocompatibility and can accumulate in large volumes while reducing side effects. These bioengineered innovative drug delivery systems can combine the physical and chemical properties of various functional materials with the advantages of biological materials to achieve specific goals, such as extending their circulation time in the body, specifically targeting tumour cells, macrophages, CD4+ T cells, and modulating the immune system [92,93]. The precision of cell-specific ligands, which can be altered on the surface of sterosomes to attach to corresponding proteins on cell membranes of diseased sites, offers a reassuringly accurate method for precisely targeting nanoparticles [94].

Future research should also focus on developing functionalized sterosomes for combined treatment by delivering drugs and genetic materials simultaneously. This approach offers potential advantages over single therapy and has the potential to impact public health significantly. The combined effects of multiple bioactive agents co-loaded in functionalized sterosomes, utilising the modified properties of nanocarriers, can effectively suppress drug resistance, optimise dosages of various agents, enhance immune response, promote autophagy, significantly improve the active permeability of payloads, sustain drug release, reduce side effects, and decrease the frequency of drug administration for targeted treatment of metabolic (neurodegenerative diseases, diabetes, and cardiovascular disorders) and eradication of infectious (HIV, malaria, tuberculosis, and COVID-19) diseases.

The rational design and development of high-throughput functionalities in nanocarriers, such as sterosomes for specific applications, is a crucial area of research that remains uncommon in standard practice. Their development remains nascent, primarily due to the constraints in scientists' abilities to efficiently acquire, analyse, manage, and interpret complex and expanding experimental data sets, which are essential for creating

drug delivery systems with specific functionalities. Data-driven approaches, such as high-throughput experimentation techniques, process automation, artificial intelligence (AI), and machine learning (ML), known as The Fourth Paradigm of Scientific Research, can enhance the design optimisation, stability, and functionality of both native and modified sterosomes. Integrating these approaches with nanomedicine and nanotechnology can improve the rational design and high-throughput development of efficient personalised drug-loaded sterosomes and smart sterosomes with predefined functionalities [95]. Personalised drug-loaded sterosomes entail customising medicated vesicular systems according to individual patient profiles, utilising genetic, proteomic, and metabolic data to improve treatment efficacy and minimise adverse effects. Smart sterosomes can be engineered to respond dynamically to biological environments, including variations in pH or temperature, thereby enhancing targeted delivery and controlled release [96].

7. CONCLUSIONS

Sterosomes are nanocarriers composed of non-phospholipid single-chain amphiphilic molecules with a high sterol content that have emerged as advanced drug carriers. They are recognised for producing large unilamellar vesicular systems at the nanoscale, with a diameter that can be regulated depending on the pore size of the filter employed in the extrusion system and the unique advantage of low bilayer permeability as well as monomodal dispersion. Sterosomes have demonstrated increased stability and passive permeability, thereby overcoming the limitations of traditional liposomes. The distinctive characteristics of native and modified sterosomes, including their small size, amphiphilic nature, surface charge, hydration, stimuli sensitivity, high surface modification, complexation efficiency, elevated cellular uptake, stealthiness, electrostatic interactions, limited passive permeability, enhanced penetration ability, extended biological half-life, elasticity, specificity, lentiviral properties, and intrinsic potential, set them apart as a novel nanocarrier for pharmaceuticals. Their distinctiveness can be further highlighted by their suitability for application as nanocarriers in alternative routes of administration, including intraperitoneal, nose-to-brain, transdermal, intranasal, buccal, and pulmonary delivery.

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