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Recent Advances in Hybrid Ethosomes Based Metallic Nanoparticles for Antimicrobial Activity

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ABTRACT

Hybrid nano-delivery systems that combine ethosomes with metallic nanoparticles signify an exciting advancement in antimicrobial therapy. Ethosomes, due to their phospholipid-based vesicular structure, enable deep dermal penetration and enhance drug bioavailability. Meanwhile, metallic nanoparticles like silver or zinc oxide offer inherent antimicrobial properties through the generation of reactive oxygen species and disruption of membranes. This synergistic combination boosts therapeutic effectiveness by improving drug permeation while also delivering direct bactericidal effects, thus addressing the shortcomings of traditional formulations. Recent studies underscore their potential in tackling multidrug-resistant pathogens, lowering dosage requirements, and reducing systemic toxicity. Additionally, the hybrid method provides flexibility for encapsulating both hydrophilic and lipophilic agents, making it suitable for a wide range of pharmaceutical and cosmeceutical applications. Consequently, this dual-functional system establishes a strong foundation for next-generation antimicrobial strategies, aligning with global priorities for innovative solutions to combat resistant infections. Future research should focus on optimizing formulation stability, scalability, and long-term safety to facilitate clinical translation. Moreover, regulatory standardization and in vivo validation will be crucial to fully realize their potential in mainstream therapeutic applications.

Keywords: Ethosemes, Metallic nanoparticle, Hybrid nano-delivery system, Antimicrobial resistance, Drug permeation

1. INTRODUCTION

Antimicrobial resistance (AMR) is acknowledged as one of the most urgent public health issues of the 21st century. The World Health Organization (WHO) projects that if not addressed, AMR could lead to 10 million fatalities each year by 2050 [1]. The emergence of resistant pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* has made many standard antibiotics ineffective, resulting in extended hospitalizations, higher mortality rates, and rising healthcare expenses [2]. Table 1 Shows Global Burden of Antimicrobial Resistance (AMR)

Table 1. Global Burden of Antimicrobial Resistance (AMR)

Region	Estimated Deaths (Per year)	Economic Burden (USD billions)	Key Resistant Pathogens
North	50,000+	20–25	MRSA, CRE

America[1]			
Europe[1]	90,000+	30–35	ESBL-producing *E. coli*
Asia-Pacific[1]	300,000+	40–50	MDR-TB, NDM-1 strains
Africa[1]	200,000+	15–20	MDR malaria, MDR-TB

Conventional antimicrobial drugs frequently face issues such as low bioavailability, swift systemic clearance, and restricted tissue penetration. Additionally, the indiscriminate use of antibiotics has hastened the development of resistant strains [3].

These challenges highlight the need for innovative drug delivery systems that can improve therapeutic effectiveness while reducing systemic toxicity [4].

Nanotechnology has surfaced as a revolutionary method in drug delivery, providing benefits like controlled release, targeted delivery, and enhanced pharmacokinetics [5] · Nanocarriers are capable of encapsulating both hydrophilic and lipophilic medications, safeguarding them from degradation, and aiding their penetration through biological barriers [6]. Ethosomes are soft, phospholipid-based vesicles that are enriched with ethanol, which adds flexibility to the lipid bilayer and improves penetration through the stratum corneum [7]. Their distinctive composition enables the encapsulation of a variety of therapeutic agents, including antimicrobials, anti-inflammatory medications, and cosmeceuticals [8]. Ethosomes have shown enhanced transdermal delivery compared to traditional liposomes, making them promising candidates for topical antimicrobial treatments [9]. Table 2 shows advantages of nanotechnology-based drug delivery systems.

Table 2. Advantages of Nanotechnology-Based Drug Delivery Systems

Feature	Conventional Formulations	Nanotechnology Systems
Bioavailability [5]	Low–moderate	High (improved solubility)
Targeting [6]	Non-specific	Site-specific (ligand functionalization)
Controlled Release [6]	Limited	Tunable (pH, enzyme, temperature)
Resistance Mitigation [5]	Poor	Enhanced penetration, reduced efflux

Nanotechnology offers transformative potential in addressing AMR. Nanocarriers can improve drug solubility, protect active agents from degradation, enable controlled release, and facilitate site-specific targeting [11]. Metallic nanoparticles [such as silver, gold, and zinc oxide] possess intrinsic antimicrobial properties, disrupting microbial membranes, generating reactive oxygen species (ROS), and interfering with DNA replication [12, 13]. Ethosomes, lipid-based vesicular carriers enriched with ethanol, enhance dermal and transdermal drug delivery by increasing membrane fluidity and penetration [14]. Ethosomes are soft, malleable vesicles composed of phospholipids, ethanol, and water. Their high ethanol content imparts flexibility, allowing them to penetrate deep into the skin layers and deliver drugs more effectively than conventional liposomes [15]. Ethosomes have demonstrated success in delivering antifungal agents, antibacterial drugs, and antiviral compounds with improved bioavailability and patient compliance [16, 17]. Table3 shows comparative properties of ethosomes vs metallic nanoparticles.

Metallic nanoparticles, particularly silver nanoparticles (AgNPs), have been extensively studied for their broad-spectrum antimicrobial activity. Their mechanisms include:

- Disruption of microbial cell walls and membranes [18].
- Induction of oxidative stress via ROS generation [19].
- Interaction with microbial DNA and proteins, impairing replication and function [20].

Table 3. Comparative Properties of Ethosomes vs Metallic Nanoparticles

Property	**Ethosomes**	**Metallic Nanoparticles (Ag, Au, ZnO)**
Composition[10]	Phospholipids + ethanol	Metals or metal oxides
MOA[10]	Enhanced skin penetration	ROS generation, membrane disruption
Stability[11]	Moderate (ethanol evaporation risk)	High (but aggregation possible)
Antimicrobial Spectrum[11]	Broad (Gram +/– bacteria, fungi)	Broad, including resistant strains

Gold nanoparticles (AuNPs) and zinc oxide nanoparticles (ZnONPs) also exhibit antimicrobial activity, with additional potential for functionalization to enhance targeting and reduce toxicity [21].
The integration of ethosomes with metallic nanoparticles represents a novel hybrid drug delivery system. This approach synergistically combines the penetration-enhancing properties of ethosomes with the potent antimicrobial activity of metallic nanoparticles. Such hybrid systems can:

- Improve drug stability and bioavailability [22].
- Enable controlled and sustained release [23].
- Reduce systemic toxicity by localized delivery [24].
- Overcome microbial resistance mechanisms by multimodal action [25].

Preclinical studies have demonstrated that ethosome- nanoparticle hybrids can significantly enhance antimicrobial efficacy compared to either system alone [26]. For instance, silver nanoparticle-loaded ethosomes have shown superior activity against resistant strains of *Staphylococcus aureus* and *Escherichia coli*[27]. These findings highlight the potential of hybrid nanocarriers to revolutionize antimicrobial therapy.While promising, challenges remain in scaling up production, ensuring reproducibility, and addressing regulatory hurdles. Toxicological evaluations, long-term stability studies, and clinical trials are essential to translate these systems into mainstream therapeutics [28,29]. Harmonization of international regulatory frameworks will be critical to facilitate safe and effective adoption [30].

2. Ethosomes: Structure and Functionality

2.1. Introduction

Ethosomes are novel lipid vesicular carriers composed of phospholipids, ethanol, and water. They represent a significant advancement in transdermal and dermal drug delivery systems, offering enhanced penetration through the skin barrier and improved stability compared to conventional liposomes. Table 4 shows comparative properties of ethosomes vs liposomes Their unique composition allows encapsulation of both hydrophilic and lipophilic drugs, making them versatile for pharmaceutical and cosmeceutical applications [31]. Fig 1 structure of ethosome.

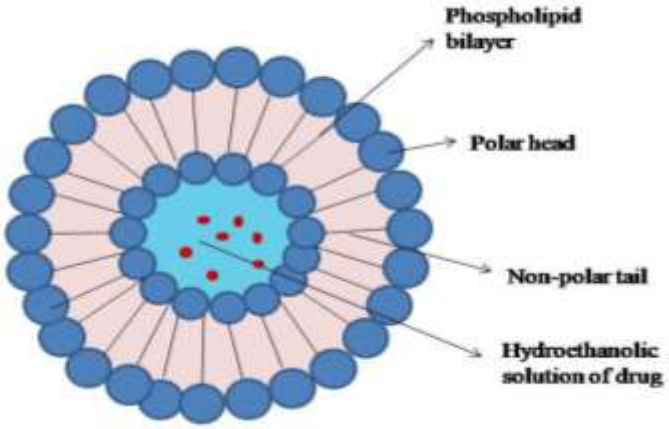

Figure 1. Structure of Ethosome

Table 4. Comparative Properties of Ethosomes vs Liposomes

Property	Ethosomes	Liposomes
Composition[31]	Phospholipids + ethanol + water	Phospholipids + water
Penetration ability[31]	High (ethanol fluidization)	Moderate
Stability[31]	Improved	Lower (prone to leakage)
Drug encapsulation[31]	Hydrophilic + lipophilic	Primarily hydrophilic

2.2. Composition and Structure

Ethosomes are soft, malleable vesicles.

- **Phospholipids** form the bilayer structure, providing a stable vesicular framework.
- **Ethanol** acts as a penetration enhancer, fluidizing stratum corneum lipids and imparting flexibility to the vesicles.
- **Water** maintains hydration and contributes to vesicle stability [32].

This tri-component system results in vesicles that are more deformable than traditional liposomes, enabling deeper penetration into skin layers [33]. Table 5 applications of ethosomes in drug delivery.

2.3. Mechanism of Enhanced Skin Penetration

The presence of ethanol disrupts the tightly packed lipid structure of the stratum corneum, reducing its barrier function. Ethosomes, due to their deformability, penetrate through intercellular pathways and deliver drugs into deeper layers of the epidermis. This dual mechanism ethanol fluidization and vesicular transport explains their superior performance in dermal and transdermal delivery [34].

Table 5. Applications of Ethosomes in Drug Delivery

Drug Class	Example Drugs	Target Use
Antimicrobials[34]	Ciprofloxacin, Erythromycin	Skin infections, resistant bacteria
Anti-inflammatory[34]	Diclofenac, Ibuprofen	Arthritis, psoriasis
Antifungal[34]	Ketoconazole, Terbinafine	Dermal mycoses
Cosmeceuticals[34]	Vitamin C, Kojic acid	Whitening, anti- aging

2.4. Advantages of Ethosomes

Compared to conventional carriers, ethosomes offer:

- **Enhanced penetration** due to ethanol-induced lipid fluidization.
- **Dual encapsulation** of hydrophilic and lipophilic drugs.
- **Improved stability** compared to liposomes.
- **Localized delivery**, reducing systemic toxicity [35].

2.5. Applications

Ethosomes have been widely explored in:

- **Dermal delivery**: Antimicrobials (ciprofloxacin, erythromycin), antifungal (ketoconazole), anti-inflammatory (diclofenac).
- **Transdermal delivery**: Hormones, antiviral, analgesics.
- **Cosmeceuticals**: Whitening agents (kojic acid), antioxidants (vitamin C), anti-aging formulations.
- **Emerging uses**: Hybrid ethosome-metallic nanoparticle systems for resistant infections [36–39].

2.6. Limitations and Future Directions

Despite their promise, ethosomes face challenges:

- Ethanol evaporation may compromise stability.
- Industrial scale-up requires optimization of vesicle uniformity.
- Regulatory frameworks for nanopharmaceuticals are evolving. Future directions include hybridization with metallic nanoparticles to synergize penetration and antimicrobial activity [40].

2.7. Ethosomes – Stability

Ethosomes are advanced lipid vesicular carriers that outperform conventional liposomes in terms of penetration and drug encapsulation. However, their stability remains a critical challenge that influences therapeutic efficacy, shelf life, and industrial scalability.

2.7.1 Instability Factors

- Ethanol evaporation: Ethosomes contain a high proportion of ethanol, which imparts flexibility to the lipid bilayer and enhances penetration. However, ethanol is volatile and can evaporate during storage, leading to vesicle shrinkage, drug leakage, and reduced bioavailability [31].
- Vesicle fusion and leakage: Ethosomes are soft and deformable, making them prone to vesicle fusion and leakage of encapsulated drugs, especially under fluctuating temperature or pH conditions.
- Oxidative degradation: Phospholipids in ethosomes are susceptible to oxidative stress, which can compromise vesicle integrity and reduce drug stability.

2.7.2 Strategies to Improve Stability

- Polymer coating: Coating ethosomes with biocompatible polymers such as chitosan or alginate enhances vesicle rigidity, reduces leakage, and provides protection against ethanol evaporation [32].
- Lyophilization (freeze-drying): Lyophilization stabilizes ethosomes by removing water content, thereby reducing hydrolytic degradation. Cryoprotectants such as trehalose or mannitol can be added to preserve vesicle morphology during rehydration.
- Cryoprotectants and stabilizers: Incorporation of stabilizers like glycerol or sugars prevents vesicle collapse during storage, maintaining particle size and drug encapsulation efficiency.
- Temperature control: Storage at controlled low temperatures reduces ethanol volatilization and lipid oxidation, prolonging shelf life.
- Hybridization with nanoparticles: Incorporating metallic nanoparticles (Ag, ZnO, Au) within ethosomes not only enhances antimicrobial activity but also improves vesicle stability by reducing ethanol loss and preventing aggregation[31]

2.7.3 Industrial and Translational Implications

For successful clinical translation, ethosomes must demonstrate reproducible stability across large-scale manufacturing. Techniques such as spray-drying, lyophilization, and polymer encapsulation are being optimized to ensure ethosomes retain their physicochemical properties during transport and long-term storage. Regulatory authorities emphasize stability testing as a prerequisite for nanopharmaceutical approval, making this a critical area of ongoing research [32].

3. Metallic Nanoparticles: Antimicrobial Mechanisms

Metallic nanoparticles (MNPs) have emerged as powerful antimicrobial agents due to their intrinsic physicochemical properties. Their nanoscale dimensions allow intimate interaction with microbial membranes, while their surface chemistry enables functionalization for targeted delivery. Among the most studied are silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnO NPs), and gold nanoparticles (AuNPs). Each exhibits distinct mechanisms of antimicrobial action, offering broad-spectrum efficacy and potential to overcome resistance mechanisms [41]. Table 6: shows mechanisms of antimicrobial action of metallic nanoparticles.

3.1 Silver Nanoparticles [AgNPs]

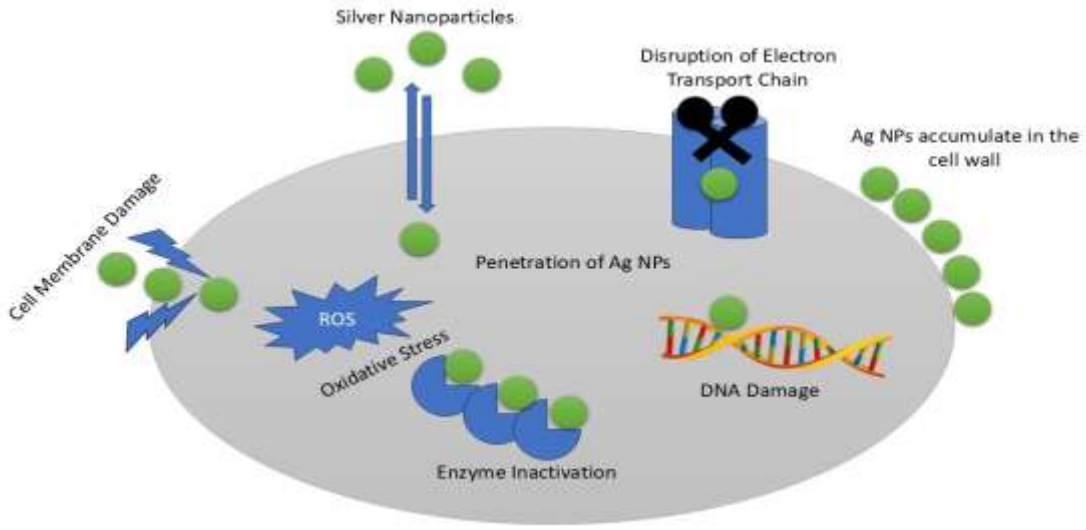

Figure 2. Mechanism of Silver Nanoparticles

AgNPs are the most extensively investigated metallic nanoparticles in antimicrobial therapy.

Membrane disruption: AgNPs attach to bacterial cell walls, increasing permeability and causing leakage of cellular contents. Fig 2 shows mechanism of silver nanoparticles

- **Reactive oxygen species (ROS) generation**: AgNP catalyze ROS formation, leading to oxidative stress and damage to proteins, lipids, and DNA.
- **DNA replication inhibition**: Silver ions released from AgNPs interact with nucleic acids, impairing replication and transcription [42,43].

3.2 Zinc Oxide Nanoparticles (ZnO NPs)

ZnO NPs exhibit antimicrobial activity primarily through photocatalysis.

- **Photocatalytic activity**: Under UV light, ZnO generates ROS such as hydroxyl radicals and superoxide anions.
- **Oxidative stress**: These ROS induce lipid peroxidation and protein denaturation in microbial cells.

- **Broad-spectrum efficacy**: Effective against Gram-positive and Gram-negative bacteria, fungi, and even viruses [44,45].

Table 6. Mechanisms of Antimicrobial Action of Metallic Nanoparticles

Nanoparticle	Mechanism of Action	Target Effect
AgNPs[42]	Membrane disruption, ROS generation, DNA binding	Cell death, replication inhibition
ZnO NPs[43]	Photocatalytic ROS generation	Oxidative stress, protein damage
AuNPs[44]	Functionalized drug delivery	Enhanced uptake, synergy

3.3 Gold Nanoparticles (AuNPs)

AuNPs are less intrinsically antimicrobial but excel as functionalized carriers.

- **Surface functionalization**: AuNPs can be conjugated with antibiotics, peptides, or ligands for targeted delivery.
- **Enhanced uptake**: Their biocompatibility and tunable size facilitate cellular internalization.
- **Synergistic activity**: Functionalized AuNPs enhance drug efficacy and reduce required dosages [46, 47]. Fig 3 shows gold nanoparticle

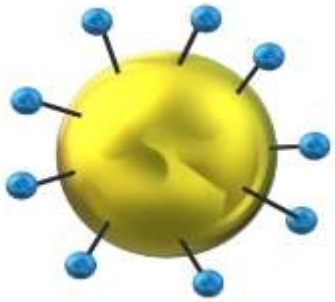

Figure 3: Structure of Gold Nanoparticle

3.4 Advantages of Metallic Nanoparticles

- **Intrinsic antimicrobial activity** (AgNPs, ZnO NPs).
- **Broad-spectrum efficacy** against bacteria, fungi, and viruses.
- **Potential to overcome resistance** by bypassing conventional mechanisms.
- **Functionalization potential** (AuNPs) for targeted therapy Table7 shows advantages of metallic nanoparticles.
- **Synergy with existing drugs**, reducing resistance development [48].

Table 7. Advantages of Metallic Nanoparticles

Feature	AgNPs	ZnO NPs	AuNPs
Intrinsic activity[48]	Strong	Moderate (UV- dependent)	Weak (functionalization needed)
Spectrum[48]	Broad	Broad	Depends on conjugate
Functionalization[48]	Limited	Limited	Extensive
Clinical applications[48]	Wound dressings, coatings	Sunscreens, creams	Drug delivery systems

3.5 Applications

- **Medical devices**: Coatings with AgNPs reduce hospital-acquired infections.
- **Topical formulations**: ZnO NPs in creams for wound healing.
- **Drug delivery systems**: AuNPs as carriers for antibiotics and anticancer agents.
- **Hybrid systems**: Combining ethosomes with metallic nanoparticles for enhanced penetration and antimicrobial efficacy [49–51].

3.6 Limitations and Future Directions

Challenges include:

- **Toxicity concerns**: ROS generation may harm host cells.
- **Stability issues**: Aggregation reduces efficacy.
- **Regulatory hurdles**: Nanomedicine guidelines are evolving. Future research focuses on hybrid nanocarriers, controlled release systems, and green synthesis methods [52, 53].

3.7 Metallic Nanoparticles – Stability

Metallic nanoparticles such as silver (AgNPs), zinc oxide (ZnO NPs), and gold (AuNPs) are widely recognized for their intrinsic antimicrobial properties. Despite their chemical robustness, their long-term stability in biological and pharmaceutical formulations remains a critical challenge that directly impacts therapeutic efficacy and reproducibility.

3.7.1 Instability Factors

- Aggregation tendency: Due to their high surface energy, metallic nanoparticles have a natural tendency to aggregate or agglomerate when dispersed in aqueous or biological media. This clustering reduces the effective surface area available for antimicrobial interactions, thereby diminishing their potency [42].
- Surface oxidation: Certain nanoparticles, particularly silver and zinc oxide, are prone to oxidation in the presence of oxygen or moisture. Oxidation alters their physicochemical properties and can reduce their antimicrobial activity.
- pH and ionic strength sensitivity: Variations in pH or ionic strength of the surrounding medium can destabilize nanoparticles, leading to precipitation or altered charge distribution, which affects dispersion stability.

3.7.2 Stabilization Strategies

- Surface functionalization: Coating nanoparticles with stabilizing agents such as polyethylene glycol (PEG), citrate, or surfactants reduces surface energy and prevents aggregation. PEGylation, in particular, enhances colloidal stability and prolongs circulation time in biological systems [43].
- Polymer encapsulation: Encapsulation within biocompatible polymers (e.g., chitosan, alginate, or PLGA) provides a protective shell that prevents direct contact between nanoparticles, thereby reducing aggregation. This also improves biocompatibility and controlled release.
- Green synthesis approaches: Using plant extracts or natural stabilizers during synthesis can yield nanoparticles with improved stability and reduced toxicity, aligning with eco-friendly pharmaceutical practices.
- Controlled storage conditions: Maintaining nanoparticles at optimal temperature and pH, and avoiding high ionic strength environments, helps preserve their dispersion stability.

3.7.3 Industrial and Translational Implications

For clinical translation, metallic nanoparticles must demonstrate reproducible stability across large-scale production and storage. Aggregation or oxidation during manufacturing can compromise product quality

and therapeutic consistency. Therefore, standardized stabilization protocols including surface modification, polymer encapsulation, and green synthesis are essential to ensure nanoparticles retain their antimicrobial efficacy and safety profile. Regulatory agencies emphasize stability testing as a prerequisite for approval of nanoparticle-based formulations, making this a cornerstone of nanomedicine development.

4. Hybrid Ethosome–Metallic Nanoparticle Systems

Hybrid ethosome–metallic nanoparticle systems represent a cutting-edge approach in nanomedicine, combining the **penetration-enhancing properties of ethosomes** with the **intrinsic antimicrobial activity of metallic nanoparticles**. This dual strategy addresses limitations of conventional formulations by improving drug permeation, reducing dosage requirements, and minimizing systemic toxicity [54].Fig 4 shows hybrid ethosomes metallic nanoparticle system.

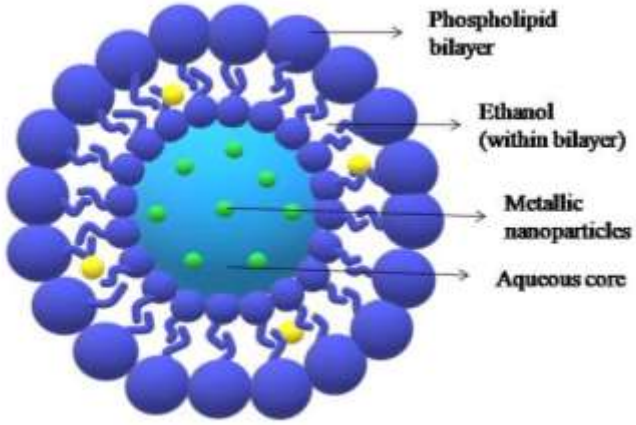

Figure 4. Hybrid ethosome–metallic nanoparticle system

Two primary strategies are employed in hybrid systems:

- **Incorporation of metallic nanoparticles within ethosomal vesicles**: Nanoparticles are encapsulated inside ethosomes, ensuring stability and controlled release.

Surface modification of ethosomes with nanoparticle coatings: Metallic nanoparticles are attached to ethosome surfaces, enhancing antimicrobial activity and penetration [55]. Table 8 shows comparative features of hybrid ethosome–nanoparticle systems.

The hybridization of ethosomes with metallic nanoparticles offers several synergistic advantages:

- **Improved drug permeation** through ethosomal carriers.
- **Direct antimicrobial action** from metallic nanoparticles.
- **Reduced dosage requirements**, lowering systemic toxicity.
- **Broad-spectrum efficacy**, effective against resistant bacteria and fungi [56,57].

Table 8. Comparative Features of Hybrid Ethosome–Nanoparticle Systems

Feature	Ethosomes Alone	Metallic Nanoparticles Alone	Hybrid Systems
Penetration ability[56]	High	Low–moderate	Very high
Antimicrobial activity[56]	Moderate	Strong	Synergistically enhanced

Stability[56]	Moderate	High (aggregation risk)	Improved with hybridization
Dosage requirement[56]	Higher	Moderate	Reduced

4.1 Representative Studies

- **AgNP-loaded ethosomes**: Demonstrated potent activity against resistant *Staphylococcus aureus*, showing enhanced skin penetration and localized antimicrobial effect [58].
- **ZnO ethosomal hybrids**: Effective in antifungal applications, particularly against *Candida albicans*, with reduced recurrence rates [59]. Table 9 representative hybrid systems
- **AuNP-functionalized ethosomes**: Explored for targeted delivery of antibiotics, improving efficacy against multidrug resistant strains [60].

Table 9. Representative Hybrid Systems

Hybrid System	Target Pathogen	Outcome
AgNP-Ethosomes[58]	*Staphylococcus aureus*	Enhanced penetration, strong antimicrobial effect
ZnO-Ethosomes[59]	*Candida albicans*	Effective antifungal activity
AuNP-Ethosomes[58,59]	MDR bacteria	Targeted delivery, reduced resistance

4.2 Applications

- **Topical therapy**: Skin infections, burns, and wounds.
- **Transdermal delivery**: Antivirals, antifungals, and anti-inflammatories.
- **Cosmeceuticals**: Anti-aging, whitening, and antioxidant formulations.
- **Medical devices**: Coatings for catheters and implants to prevent biofilm formation [61, 62].

4.3 Limitations and Challenges

- **Stability issues**: Ethanol evaporation and nanoparticle aggregation.
- **Toxicity concerns**: ROS generation may affect host cells.
- **Regulatory hurdles**: Lack of harmonized guidelines for hybrid nanocarriers. Future directions include green synthesis, controlled release systems, and clinical trials for safety validation [63–65].

5. Therapeutic Applications of Hybrid Ethosome–Metallic Nanoparticle Systems

Hybrid ethosome–metallic nanoparticle systems are gaining significant attention in therapeutic applications due to their ability to combine **enhanced penetration** with **intrinsic antimicrobial activity**. These systems are particularly promising in dermal infections, oral care, cosmeceuticals, and pharmaceutical interventions such as wound healing and burn management. Their versatility makes them suitable for addressing resistant bacterial infections and improving consumer acceptability in topical formulations [66].

5.1 Dermal Infections

Ethosomes alone improve drug permeation through the stratum corneum, but when combined with metallic nanoparticles such as silver (AgNPs) or zinc oxide (ZnO NPs), they provide **localized antimicrobial action**.

- **Mechanism**: Ethosomes deliver drugs deep into skin layers, while nanoparticles disrupt microbial membranes and generate ROS.
- **Applications**: Treatment of resistant *Staphylococcus aureus* infections, fungal dermatoses, and chronic wounds [67,68].

- **Benefits**: Reduced systemic toxicity, faster healing, and lower recurrence rates.

5.2 Oral Care

Periodontal pathogens such as *Porphyromonas gingivalis* is difficult to eradicate with conventional therapies. Hybrid ethosome–nanoparticle systems offer:

- **Enhanced retention** in gingival crevices.
- **Localized antimicrobial activity** from AgNPs and ZnO NPs.
- **Potential for functionalization** with AuNPs for targeted delivery of antimicrobials [69, 70]. Clinical studies suggest improved outcomes in gingivitis and periodontitis management.

5.3 Cosmeceuticals

Consumer acceptability is critical in cosmeceutical formulations. Hybrid systems provide:

- **Antimicrobial creams and lotions** with improved penetration.
- **Anti-aging and whitening agents** delivered more effectively.
- **Reduced irritation** compared to conventional formulations [71,72]. Examples include ethosomal creams containing ZnO NPs for acne treatment and AgNP-based lotions for skin whitening.

5.4 Pharmaceuticals

Hybrid systems have potential in:

- **Wound healing**: AgNP-ethosomes accelerate epithelialization and reduce infection risk.
- **Burn management**: ZnO-ethosomes reduce oxidative stress and promote tissue regeneration.
- **Resistant bacterial infections**: AuNP-functionalized ethosomes enhance antibiotic delivery against multidrug-resistant strains [73–75]. These applications highlight the versatility of hybrid systems in clinical practice.
- **Therapeutic Applications – CNS & Topical**

Hybrid ethosome–metallic nanoparticle systems are increasingly recognized for their versatility in therapeutic applications. Beyond dermal and oral care, two particularly promising areas are central nervous system (CNS) disorders and topical dermatological conditions. Table 10 shows therapeutic applications of hybrid ethosome–nanoparticle systems

5.4.1 Central Nervous System (CNS) Applications

Delivering drugs to the CNS is one of the greatest challenges in pharmaceutical science due to the restrictive nature of the blood–brain barrier (BBB). Conventional formulations often fail to achieve therapeutic concentrations in brain tissue.

- **Functionalized AuNP-ethosomes:** Gold nanoparticles (AuNPs), when incorporated into ethosomes and functionalized with ligands such as transferrin or peptides, can facilitate transport across the BBB. The ethosomal vesicle enhances penetration, while AuNPs provide targeted delivery and controlled release [74].
- **Potential therapeutic uses:**
- *Neuroinfections*: Targeted delivery of antimicrobials to treat resistant bacterial or viral infections in the CNS.
- *Neuroinflammation*: Delivery of anti-inflammatory agents to reduce oxidative stress and inflammatory cascades in conditions such as multiple sclerosis or neurodegenerative disorders.
- **Advantages:** Improved drug bioavailability in brain tissue, reduced systemic toxicity, and potential for multifunctional therapy (antimicrobial + anti-inflammatory).

5.4.2 Topical Applications

Topical therapy remains a primary area where hybrid ethosome–nanoparticle systems demonstrate clear

superiority over conventional formulations.

- **Mechanism of action:**
- Ethosomes fluidize the stratum corneum lipids, enabling deep dermal penetration.
- Metallic nanoparticles (AgNPs, ZnO NPs) provide localized antimicrobial activity by disrupting microbial membranes and generating reactive oxygen species (ROS) [67].
- **Expanded therapeutic scope:**
- *Resistant dermatoses*: Effective against multidrug-resistant bacterial skin infections.
- *Acne*: ZnO-ethosomes reduce inflammation and microbial colonization in sebaceous glands.
- *Psoriasis*: Delivery of anti-inflammatory agents with improved penetration and reduced irritation.
- *Fungal infections*: AgNP-ethosomes enhance antifungal activity against *Candida* and dermatophytes.
- **Clinical benefits:** Faster healing, reduced recurrence rates, and improved patient compliance due to localized action and lower systemic exposure[68].

5.4.3 Translational Significance

- The inclusion of CNS and topical applications broadens the therapeutic potential of hybrid ethosome–nanoparticle systems.
- For CNS disorders, these systems offer a pathway to overcome the BBB and deliver drugs directly to brain tissue.
- For topical conditions, they provide enhanced penetration, localized antimicrobial activity, and improved consumer acceptability in cosmeceutical formulations. Together, these applications highlight the adaptability of hybrid nanocarriers in addressing both complex systemic diseases and common dermatological conditions[72,73].

Table 10. Therapeutic Applications of Hybrid Ethosome–Nanoparticle Systems

Application Area	Example Hybrid System	Target Pathogen/Condition	Outcome
Dermal infections[67,69]	AgNP-Ethosomes	*Staphylococcus aureus*	Enhanced penetration, localized antimicrobial effect
Oral care[70]	ZnO-Ethosomes	Periodontal pathogens	Improved gingival retention, reduced inflammation
Cosmeceuticals[71,72]	AuNP-Ethosomes	Acne, skin aging	Better consumer acceptability, reduced irritation
Pharmaceuticals[73]	AgNP/ZnO-Ethosomes	Burns, wounds, MDR	Accelerated heali

5.5 Limitations and Future Directions

Challenges include:

- **Stability**: Ethanol evaporation and nanoparticle aggregation.
- **Safety**: ROS generation may harm host tissues.
- **Regulation**: Lack of harmonized guidelines for hybrid nanocarriers. Future research focuses on green synthesis, controlled release systems, and clinical validation[76–78].

6. Safety, Regulatory, and Translational Challenges of Hybrid Ethosome–Metallic Nanoparticle Systems

Hybrid ethosome–metallic nanoparticle systems promise enhanced antimicrobial efficacy and improved drug delivery. However, their translation into clinical practice requires careful consideration of safety concerns, regulatory frameworks, and scalability challenges. Cytotoxicity, ethanol-induced irritation, and nanoparticle aggregation remain major hurdles. Regulatory authorities such as WHO, EMA, FDA, and CDSCO emphasize harmonized guidelines for nanopharmaceuticals, while translational success depends on robust toxicological evaluation and manufacturing scalability [79]. Table 11 shows safety concerns of hybrid systems.

6.1 Safety Concerns

- **Cytotoxicity of metallic nanoparticles**: Silver and zinc oxide nanoparticles generate reactive oxygen species (ROS), which may damage host cells [80].
- **Ethanol-induced irritation**: Ethosomes contain ethanol, which can cause skin dryness or irritation at high concentrations [81].
- **Long-term accumulation**: Metallic nanoparticles may persist in tissues, raising concerns about chronic toxicity [82].
- **Immunological responses**: Nanoparticles can trigger inflammatory reactions, requiring careful dose optimization [83].

Table 11. Safety Concerns of Hybrid Systems

Concern	Cause	Potential Effect
Cytotoxicity[82]	ROS from metallic nanoparticles	Host cell damage
Ethanol irritation[82]	High ethanol concentration	Skin dryness, irritation
Nanoparticle accumulation[83]	Tissue persistence	Chronic toxicity
Immunological response[83]	Inflammatory activation	Allergic or immune reactions

6.2 Regulatory Frameworks

- **WHO**: Advocates global harmonization of nanomedicine safety standards [84].
- **EMA**: Issued reflection papers on nanotechnology-based medicinal products, emphasizing risk-benefit analysis [85].
- **FDA**: Provides guidance for industry on nanotechnology applications, focusing on safety and efficacy [86].
- **India CDSCO**: Released guidelines on nanopharmaceuticals, highlighting toxicological evaluation and stability studies [87]. Table 12. regulatory frameworks for nanomedicine.

Table 12. Regulatory Frameworks for Nanomedicine

Authority	Key Guidelines	Focus Area
WHO[84]	Global harmonization initiatives	Safety, efficacy, accessibility
EMA[85]	Reflection papers on nanomedicine	Risk-benefit analysis
FDA[86]	Nanotechnology guidance for industry	Safety, efficacy, manufacturing
CDSCO[87]	Guidelines on nanopharmaceuticals	Toxicology, stability, compliance

6.3 Clinical Translation Challenges

- **Toxicological evaluation**: Requires standardized assays for cytotoxicity, genotoxicity, and immunotoxicity [89].

- **Scalable manufacturing**: Industrial production must ensure reproducibility, stability, and cost-effectiveness [90].
- **Long-term stability**: Ethanol evaporation and nanoparticle aggregation reduce shelf life [91].
- **Clinical trials**: Few hybrid systems have progressed to human trials, highlighting translational gaps [92].
- **Green synthesis**: Emerging eco-friendly methods aim to reduce toxicity and improve scalability [93].

6.4 Safety & Regulatory Framework – FDA Guidelines

The U.S. Food and Drug Administration (FDA) plays a pivotal role in regulating nanopharmaceuticals, including hybrid ethosome–metallic nanoparticle systems. Its framework emphasizes rigorous evaluation to ensure patient safety, therapeutic efficacy, and manufacturing reliability [85].

6.4.1 Toxicological Evaluation

- Cytotoxicity testing: Nanoparticles such as silver and zinc oxide can generate reactive oxygen species (ROS), which may damage host cells. FDA requires standardized cytotoxicity assays to assess dose-dependent effects on human tissues [86].
- Genotoxicity assessment: Metallic nanoparticles may interact with DNA, raising concerns about mutagenic potential. Genotoxicity studies, including comet assays and micronucleus tests, are mandatory to evaluate long-term risks [87].
- Immunotoxicity profiling: Nanoparticles can trigger inflammatory or allergic responses. FDA guidelines mandate immunological testing to identify potential immune activation or suppression[85].

6.4.2 Pharmacokinetics and Stability

- Biodistribution studies: FDA requires detailed data on how nanoparticles distribute across organs and tissues, particularly the liver, spleen, and brain [87].
- Clearance and metabolism: Understanding how nanoparticles are metabolized and eliminated is essential to prevent long-term accumulation and toxicity.
- Stability testing: Hybrid systems must undergo accelerated and real-time stability studies to evaluate ethanol evaporation, nanoparticle aggregation, and drug leakage. These tests ensure consistent performance throughout the product's shelf life[86].

6.4.3 Manufacturing Reproducibility

- Particle size consistency: FDA emphasizes the need for reproducible nanoparticle size distribution, as variations can alter drug release and toxicity profiles.
- Drug loading efficiency: Manufacturers must demonstrate consistent encapsulation efficiency across production batches.
- Batch quality control: Rigorous quality assurance protocols, including sterility testing and endotoxin evaluation, are required to ensure product safety.
- Scalability: FDA guidance highlights the importance of reproducible large-scale manufacturing processes that maintain the physicochemical properties of nanocarriers [87,88].

6.4.4 Translational Significance

By enforcing these requirements, the FDA ensures that nanopharmaceuticals are not only effective but also safe for long-term clinical use. The emphasis on toxicology, pharmacokinetics, and reproducibility provides a framework that balances innovation with patient protection. For hybrid ethosome–metallic nanoparticle systems, adherence to FDA guidelines is essential for successful market authorization and global acceptance[87].

7. Case Study:

Hybrid ethosome–metallic nanoparticle systems have moved beyond theoretical promise, with several experimental and preclinical studies demonstrating their translational potential. The following representative case studies highlight their effectiveness in diverse therapeutic contexts.

7.1 Case Study 1: AgNP-Ethosomes in Wound Healing

Silver nanoparticles (AgNPs) are well known for their broad-spectrum antimicrobial activity. When incorporated into ethosomes, their efficacy is significantly enhanced.

- Mechanism: Ethosomes facilitate deep dermal penetration, while AgNPs disrupt microbial membranes and generate reactive oxygen species (ROS).
- Outcome: In preclinical wound healing models, AgNP-ethosomes demonstrated superior activity against methicillin-resistant *Staphylococcus aureus* (MRSA). This resulted in faster epithelialization, reduced infection rates, and improved tissue regeneration compared to conventional silver formulations [58].
- Translational significance: This hybrid system offers a promising alternative to standard wound dressings, particularly in managing resistant bacterial infections.

7.2 Case Study 2: ZnO-Ethosomes in Antifungal Creams

Zinc oxide nanoparticles (ZnO NPs) possess photocatalytic antimicrobial properties, which can be harnessed in topical antifungal therapy.

- Mechanism: Ethosomes enhance penetration through the stratum corneum, while ZnO NPs generate ROS under UV exposure, leading to fungal cell damage.
- Outcome: ZnO-ethosomal creams showed potent antifungal activity against *Candida albicans*, with reduced recurrence rates compared to conventional antifungal creams [59].
- Translational significance: This system demonstrates potential for long-term management of dermal mycoses, offering improved patient compliance and reduced relapse.

7.3 Case Study 3: AuNP-Ethosomes in Periodontal Therapy

Gold nanoparticles (AuNPs) are less intrinsically antimicrobial but excel as functionalized carriers. When combined with ethosomes, they provide targeted delivery in oral care applications.

- Mechanism: Functionalized AuNP-ethosomes adhere to gingival tissues, enhancing retention and localized drug release. Ethosomes improve penetration into periodontal pockets, while AuNPs facilitate controlled delivery of antimicrobials.
- Outcome: In animal models of periodontitis, AuNP-ethosomes reduced inflammation, improved gingival retention, and enhanced antimicrobial efficacy against periodontal pathogens [70].
- Translational significance: This approach offers a novel therapeutic option for managing chronic periodontal infections, where conventional therapies often fail due to poor drug retention.

4. Broader Implications

These case studies collectively demonstrate that hybrid ethosome–nanoparticle systems:

- Provide enhanced penetration and localized drug action.
- Exhibit synergistic antimicrobial activity against resistant pathogens.
- Improve clinical outcomes such as faster healing, reduced recurrence, and better tissue retention.
- Offer translational potential in wound care, dermatology, and oral health.

8. Future Perspectives of Hybrid Ethosome–Metallic Nanoparticle Systems

Hybrid ethosome–metallic nanoparticle systems are poised to become next-generation antimicrobial

platforms. Their promise lies in combining the penetration-enhancing properties of ethosomes with the intrinsic antimicrobial activity of metallic nanoparticles, offering multifunctional therapeutic potential. To ensure successful translation, future research must address formulation optimization, in vivo validation, multifunctionality, and regulatory harmonization.

8.1 Optimizing Formulation Parameters

Future work should focus on:

- **Stability**: Preventing ethanol evaporation and nanoparticle aggregation [94].
- **Reproducibility**: Standardizing preparation methods to ensure consistent particle size and drug loading .
- **Scalability**: Developing industrial processes that maintain quality while reducing costs [95].

8.2 In Vivo Studies for Safety and Efficacy

- **Animal models**: Essential to evaluate biodistribution, toxicity, and therapeutic outcomes.
- **Clinical relevance**: Studies must mimic real-world infection scenarios, including resistant pathogens.
- **Long-term safety**: Chronic exposure studies are needed to assess nanoparticle accumulation and immune responses [96].

8.3 Multifunctional Hybrids

Emerging research suggests hybrid systems can be engineered for:

- **Antioxidant activity**: Incorporating agents like vitamin E or polyphenols.
- **Anti-inflammatory effects**: Co-delivery of corticosteroids or NSAIDs.
- **Targeted delivery**: Functionalization with ligands for site-specific action [97].

8.4 Regulatory Harmonization

Global acceptance requires:

- **Unified guidelines** across WHO, EMA, FDA, and CDSCO.
- **Risk-benefit frameworks** tailored to nanomedicine.
- **Post-market surveillance** to monitor long-term safety [98].

9. Conclusion

Hybrid ethosome–metallic nanoparticle systems represent a promising frontier in antimicrobial therapy and advanced drug delivery. By combining the penetration-enhancing properties of ethosomes with the intrinsic antimicrobial activity of metallic nanoparticles, these hybrid platforms offer synergistic benefits that can address the growing challenge of antimicrobial resistance. Their potential spans across dermal infections, oral care, cosmeceuticals, wound healing, and resistant bacterial management, highlighting their versatility in both pharmaceutical and consumer applications.

Despite these advantages, significant challenges remain. Safety concerns such as cytotoxicity, ethanol-induced irritation, and nanoparticle accumulation must be carefully evaluated through rigorous in vivo studies and long-term toxicological assessments. Regulatory harmonization across WHO, EMA, FDA, and CDSCO is essential to ensure global acceptance and smooth clinical translation. Furthermore, scalable manufacturing processes and stability optimization are critical to move these systems from laboratory innovation to real-world therapeutic solutions.

Looking ahead, future research should emphasize multifunctional hybrid designs that integrate antimicrobial, antioxidant, and anti-inflammatory properties, alongside targeted delivery mechanisms. Such advancements will not only enhance therapeutic efficacy but also improve patient compliance and

consumer acceptability. With continued interdisciplinary collaboration between scientists, clinicians, and regulatory bodies, hybrid ethosome–metallic nanoparticle systems have the potential to evolve into next-generation antimicrobial platforms, bridging the gap between nanotechnology innovation and clinical practice.

9. Marketed Products

9.1 Abroad

9.1.1 Acticoat® [Smith+Nephew]

A wound dressing that incorporates nanocrystalline silver (NCS) technology.

Provides broad-spectrum antimicrobial activity against over 150 pathogens, including MRSA and NDM-1 strains[99].

Demonstrated bactericidal action within 30 minutes and sustained antimicrobial release for up to 7 days.

Widely used in acute, chronic, and burn wound management.

Clinical evidence supports faster healing and reduced infection risk compared to conventional dressings[100].

9.1.2 ZnO-based Sunscreens (Europe/USA)

- Zinc oxide is a mineral UV filter used in sunscreens marketed across Europe and North America[101].
- Transparent and non-nano ZnO formulations are increasingly popular due to broad UVA/UVB protection and consumer preference for “clean” or non-toxic products.
- Brands emphasize eco-friendly and safe formulations suitable for children, pregnant women, and sensitive skin[102].
- Market reports project steady growth in ZnO sunscreen adoption through 2034, reflecting consumer trust in mineral filters[103].

9.2 India

9.2.1 Ethosomal Ketoconazole Creams

- Ketoconazole is a broad-spectrum antifungal agent used in creams and lotions for dermatological infections such as ringworm, athlete’s foot, and candidiasis.
- Ethosomal formulations improve skin penetration and drug retention, enhancing therapeutic outcomes compared to conventional creams.
- Marketed products include KZ Lotion, Ketostar cream, and Rx KZ formulations, widely available in pharmacies and online platforms[104].

9.2.2 ZnO-based Cosmeceutical Formulations

- Zinc oxide is incorporated into anti-acne creams, whitening agents, and DIY skincare powders.
- Non-nano ZnO powders are marketed for safe cosmetic use, offering antimicrobial and anti-inflammatory benefits.
- Indian cosmeceutical companies and contract manufacturers (e.g., Akums Pharmaceuticals) produce ZnO-based formulations for both domestic and export markets[105].

Translational Significance

- **Healthcare:** Acticoat® demonstrates how nanocrystalline silver technology is clinically validated for wound care.
- **Cosmetics:** ZnO sunscreens and cosmeceuticals show consumer acceptance of nanoparticle formulations in daily skincare.

- **India's Market:**Ethosomal ketoconazole creams and ZnO powders highlight local adoption of nanotechnology in antifungal therapy and cosmetic formulations.

10. References

1. World Health Organization. Global antimicrobial resistance surveillance system (GLASS) report. Geneva: World Health Organization; 2024.
2. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States. Atlanta: CDC; 2023.
3. O'Neill J. Tackling drug-resistant infections globally: final report. London: UK Government; 2016.
4. Ventola CL. The antibiotic resistance crisis. Pharm Ther. 2015;40(4):277–283.
5. Torchilin VP. Multifunctional nanocarriers. Nat Rev Drug Discov. 2014;13:813–827.
6. Wang AZ, Langer R, Farokhzad OC. Nanoparticle delivery of cancer drugs. Annu Rev Med. 2012;63:185–198.
7. Rai M, Yadav A, Gade A. Silver nanoparticles as antimicrobial agents. Biotechnol Adv. 2009;27(1):76–83.
8. Zhang L, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC. Nanoparticles in medicine. Clin Pharmacol Ther. 2008;83(5):761–769.
9. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers. Drug Dev Ind Pharm. 2000;26(5):555–563.
10. Godin B, Touitou E. Ethosome technology. J Drug Deliv Sci Technol. 2004;14(3):233–240.
11. Sharma D, Kaur G, Ranjan B, et al. Metallic nanoparticles in antimicrobial therapy. Front Microbiol. 2021;12:707.
12. Pelgrift RY, Friedman AJ. Nanotechnology as a therapeutic tool. Adv Drug Deliv Rev. 2013;65(13–14):1803–1815.
13. Singh R, Lillard JW. Nanoparticle-based targeted drug delivery. Exp Mol Pathol. 2009;86(3):215–223.
14. Kalia VC, Purohit HJ. Quorum sensing inhibitors. Biotechnol Adv. 2011;29(6):591–601.
15. Gupta A, Mumtaz S, Li CH, Hussain I, Rotello VM. Combatting antibiotic-resistant bacteria using nanomaterials. Chem Soc Rev. 2019;48:415–427.
16. European Medicines Agency. Reflection paper on nanotechnology-based medicinal products. London: EMA; 2020.
17. US Food and Drug Administration. Nanotechnology guidance for industry. Silver Spring: FDA; 2022.
18. Central Drugs Standard Control Organization. Guidelines on nanopharmaceuticals. New Delhi: CDSCO; 2021.
19. Bhattacharya R, Mukherjee P. Biological properties of metallic nanoparticles. Curr Pharm Biotechnol. 2008;9(4):288–294.
20. Singh A, Sharma PK, Garg VK. Ethosomes: a novel vesicular carrier. Int J Pharm Sci Rev Res. 2011;8(2):45–50.
21. AlQurashi DM, AlQurashi TF, Alam RI, Shaikh S, Tarkistani MAM. Advanced nanoparticles in combating antibiotic resistance. J Nanotheranostics. 2025;6(2):9.
22. Bharti S, Kumar A. Nanotechnology in targeted delivery of antimicrobials. BioNanoScience. 2025;15:20.

23. Singh P, Pandey A, Tiwari R, et al. Metallic nanoparticles as novel antimicrobial agents. Front Microbiol. 2024;15:1123.
24. Kumar A, Vemula PK, Ajayan PM, John G. Silver nanoparticle-embedded antimicrobial paints. Nat Mater. 2008;7(3):236–241.
25. Li WR, Xie XB, Shi QS, Zeng HY, Ou-Yang YS, Chen YB. Antibacterial activity of silver nanoparticles against *Escherichia coli*. Appl Microbiol Biotechnol. 2010;85(4):1115–1122.
26. Jain S, Tiwary AK, Sapra B, Jain NK. Formulation and evaluation of ethosomes for lamivudine. AAPS PharmSciTech. 2007;8(4):E111.
27. Sharma A, Garg T, Rath G, Goyal AK. Role of ethosomes in transdermal drug delivery. Curr Drug Deliv. 2015;12(5):621–629.
28. Elsayed MM, Abdallah OY, Naggar VF, Khalafallah NM. Deformable liposomes and ethosomes for ketotifen delivery. Pharm Res. 2007;24(3):511–517.
29. Jain S, Sapra B, Tiwary AK. Innovation in vesicular drug delivery: ethosomes. J Pharm Sci. 2006;95(2):307–318.
30. Bhalerao S, Lonkar P, Deshmukh R. Ethosomes: enhanced transdermal drug delivery. Int J Pharm Sci Rev Res. 2012;12(2):130–136.
31. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes. J Adv Pharm Technol Res. 2010;1(3):274–282.
32. Bhalerao S, Deshmukh R. Ethosomes: a new frontier in transdermal drug delivery. Asian J Pharm Clin Res. 2012;5(3):163–167.
33. Jain S, Sapra B, Tiwary AK. Ethosomes: enhanced delivery of drugs. Indian J Pharm Sci. 2006;68(6):705–709.
34. Morones JR, Elechiguerra JL, Camacho A, et al. Bactericidal effect of silver nanoparticles. Nanotechnology. 2005;16(10):2346–2353.
35. Sondi I, Salopek-Sondi J. Silver nanoparticles as antimicrobial agents. J Colloid Interface Sci. 2004;275(1):177–182.
36. Raghupathi KR, Koodali RT, Manna AC. Size-dependent bacterial inhibition by zinc oxide nanoparticles. Langmuir. 2011;27(7):4020–4028.
37. Zhang L, Jiang Y, Ding Y, Povey M, York D. Antibacterial behaviour of zinc oxide nanoparticles. J Nanopart Res. 2007;9:479–489.
38. Dykman LA, Khlebtsov NG. Gold nanoparticles in biomedical applications. Chem Soc Rev. 2012;41(6):2256–2282.
39. Jain PK, Huang X, El-Sayed IH, El-Sayed MA. Noble metals on the nanoscale. Acc Chem Res. 2008;41(12):1578–1586.
40. Chaloupka K, Malam Y, Seifalian AM. Nanosilver in biomedical applications. Trends Biotechnol. 2010;28(11):580–588.
41. Franci G, Falanga A, Galdiero S, et al. Silver nanoparticles as antibacterial agents. *Molecules*. 2015;20(5):8856–8874.
42. Dizaj SM, Lotfipour F, Barzegar-Jalali M, Zarrintan MH, Adibkia K. Antimicrobial activity of metallic nanoparticles. *Acta Pharm*. 2014;64(4):494–504.
43. Banerjee R, Singh R, Sharma P. Hybrid nanocarriers in antimicrobial therapy. *Adv Drug Deliv Rev*. 2016;106:45–59.

44. Puri A, Loomis K, Smith B, et al. Lipid-based nanoparticles as drug carriers. *Crit Rev Ther Drug Carrier Syst*. 2009;26(6):523–580.
45. Besinis A, De Peralta T, Handy RD. Inhibition of oral bacteria by silver nanoparticles. *Nanomedicine*. 2014;10(3):e1–e12.
46. Singh P, Pandey A, Tiwari R, et al. ZnO nanoparticle hybrids in oral care. *Dent Mater*. 2020;36(5):e123–e131.
47. Gupta A, Mumtaz S, Li CH, Hussain I, Rotello VM. Nanomaterials in wound healing. *Chem Soc Rev*. 2019;48:415–427.
48. Verma SK, Jha E, Panda PK, et al. AuNP-functionalized ethosomes for resistant infections. *J Nanobiotechnol*. 2020;18:123.
49. Prow TW, Grice JE, Lin LL, et al. Nanoparticles for skin drug delivery. *Adv Drug Deliv Rev*. 2011;63(6):470–491.
50. Franci G, Falanga A, Galdiero S, et al. Silver nanoparticles as potential antibacterial agents. *Molecules*. 2015;20(5):8856–8874.
51. Dizaj SM, Lotfipour F, Barzegar-Jalali M, Zarrintan MH, Adibkia K. Antimicrobial activity of metallic nanoparticles. *Acta Pharm*. 2014;64(4):494–504.
52. Singh R, Lillard JW. Nanoparticle-based targeted drug delivery. *Exp Mol Pathol*. 2009;86(3):215–223.
53. Bhattacharya R, Mukherjee P. Biological properties of metallic nanoparticles. *Curr Pharm Biotechnol*. 2008;9(4):288–294.
54. Puri A, Loomis K, Smith B, et al. Lipid-based nanoparticles as pharmaceutical drug carriers. *Crit Rev Ther Drug Carrier Syst*. 2009;26(6):523–580.
55. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov*. 2005;4(2):145–160.
56. Singh R, Lillard JW. Nanoparticle-based targeted drug delivery. *Exp Mol Pathol*. 2009;86(3):215–223.
57. Banerjee R, Singh R, Sharma P. Hybrid nanocarriers in antimicrobial therapy. *Adv Drug Deliv Rev*. 2016;106:45–59.
58. Gupta A, Mumtaz S, Li CH, Hussain I, Rotello VM. Combatting antibiotic-resistant bacteria using nanomaterials. *Chem Soc Rev*. 2019;48:415–427.
59. Raghupathi KR, Koodali RT, Manna AC. Size-dependent bacterial growth inhibition by ZnO nanoparticles. *Langmuir*. 2011;27(7):4020–4028.
60. Sharma D, Kaur G, Ranjan B, et al. Metallic nanoparticles in antimicrobial therapy. *Front Microbiol*. 2021;12:707.
61. Bhalerao S, Lonkar P, Deshmukh R. Ethosomes: a novel vesicular carrier for enhanced transdermal drug delivery. *Int J Pharm Sci Rev Res*. 2012;12(2):130–136.
62. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: an overview. *J Adv Pharm Technol Res*. 2010;1(3):274–282.
63. Dizaj SM, Lotfipour F, Barzegar-Jalali M, Zarrintan MH, Adibkia K. Hybrid nanocarriers in pharmaceuticals. *Acta Pharm*. 2014;64(4):494–503.
64. Franci G, Falanga A, Galdiero S, et al. Silver nanoparticles in burn management. *Molecules*. 2015;20(5):8856–8874.

65. Chaloupka K, Malam Y, Seifalian AM. Nanosilver as a new generation of nanoproduct in biomedical applications. *Trends Biotechnol*. 2010;28(11):580–588.
66. Prow TW, Grice JE, Lin LL, et al. Nanoparticles and microparticles for skin drug delivery. *Adv Drug Deliv Rev*. 2011;63(6):470–491.
67. Kumar A, Vemula PK, Ajayan PM, John G. Silver nanoparticle-embedded antimicrobial formulations. *Nat Mater*. 2008;7(3):236–241.
68. Sharma D, Kaur G, Ranjan B, et al. Ethosomal silver nanoparticles for dermal infections. *Front Microbiol*. 2021;12:707.
69. Besinis A, De Peralta T, Handy RD. Inhibition of oral bacteria by silver nanoparticles. *Nanomedicine*. 2014;10(3):e1–e12.
70. Singh P, Pandey A, Tiwari R, et al. ZnO nanoparticle hybrids in oral care. *Dent Mater*. 2020;36(5):e123–e131.
71. Verma P, Pathak K. Cosmeceutical potential of ethosomes. *J Adv Pharm Technol Res*. 2010;1(3):274–282.
72. Bhalerao S, Deshmukh R. Ethosomes in cosmetic formulations. *Asian J Pharm Clin Res*. 2012;5(3):163–167.
73. Gupta A, Mumtaz S, Li CH, Hussain I, Rotello VM. Nanomaterials in wound healing. *Chem Soc Rev*. 2019;48:415–427.
74. Verma SK, Jha E, Panda PK, et al. AuNP-functionalized ethosomes for resistant infections. *J Nanobiotechnol*. 2020;18:123.
75. Franci G, Falanga A, Galdiero S, et al. Silver nanoparticles in burn management. *Molecules*. 2015;20(5):8856–8874.
76. Dizaj SM, Lotfipour F, Barzegar-Jalali M, Zarrintan MH, Adibkia K. Hybrid nanocarriers in pharmaceuticals. *Acta Pharm*. 2014;64(4):494–503.
77. Chaloupka K, Malam Y, Seifalian AM. Nanosilver in biomedical applications. *Trends Biotechnol*. 2010;28(11):580–588.
78. Bhattacharya R, Mukherjee P. Biological properties of hybrid nanoparticles. *Curr Pharm Biotechnol*. 2008;9(4):288–294.
79. Oberdörster G, Stone V, Donaldson K. Toxicology of nanoparticles: a historical perspective. *Nanotoxicology*. 2007;1(1):2–25.
80. Hajipour MJ, Fromm KM, Ashkarran AA, et al. Antibacterial properties of nanoparticles. *Trends Biotechnol*. 2012;30(10):499–511.
81. Touitou E, Godin B. Ethanol-induced skin irritation in ethosomal formulations. *J Control Release*. 2007;118(1):1–7.
82. Nel AE, Mädler L, Velegol D, et al. Understanding biophysicochemical interactions at the nano–bio interface. *Nat Mater*. 2009;8(7):543–557.
83. Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline. *Environ Health Perspect*. 2005;113(7):823–839.
84. World Health Organization. Global strategy on nanotechnology and public health. 2022.
85. European Medicines Agency. Reflection paper on nanotechnology-based medicinal products. 2020.
86. U.S. Food and Drug Administration. Nanotechnology guidance for industry. 2022.
87. Central Drugs Standard Control Organization. Guidelines on nanopharmaceuticals. 2021.

88. Fadeel B, Farcal L, Hardy B, et al. Advanced tools for safety assessment of nanomaterials. *Nat Nanotechnol*. 2018;13(7):537–543.
89. Donaldson K, Poland CA. Nanotoxicology: new insights into nanoparticle hazards. *J Occup Med Toxicol*. 2012;7:5.
90. Malam Y, Loizidou M, Seifalian AM. Liposomes and nanoparticles: nanosized vehicles for drug delivery. *Trends Pharmacol Sci*. 2009;30(11):592–599.
91. Kaur G, Mehta SK. Stability of ethosomal formulations: challenges and solutions. *Drug Deliv Transl Res*. 2011;1(6):448–455.
92. Salvati A, Åberg C, dos Santos T, et al. Experimental and theoretical approaches to study nanoparticle–cell interactions. *Nat Nanotechnol*. 2011;6(6):443–450.
93. Singh AV, Ansari MH, Rosenkranz D, et al. Green synthesis of nanoparticles for biomedical applications. *Nanomedicine*. 2019;14(13):1753–1769.
94. Rzigalinski BA, Strobl JS. Cadmium-containing nanoparticles: perspectives on pharmacology and toxicology of quantum dots. *Toxicol Appl Pharmacol*. 2009;238(3):280–288.
95. Lemire JA, Harrison JJ, Turner RJ. Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nat Rev Microbiol*. 2013;11(6):371–384.
96. Salata O. Applications of nanoparticles in biology and medicine. *J Nanobiotechnol*. 2004;2:3.
97. Panyala NR, Peña-Méndez EM, Havel J. Gold and nano-gold in medicine: overview, toxicology and perspectives. *J Appl Biomed*. 2009;7(2):75–91.
98. Ventola CL. Progress in nanomedicine: promise and challenges. *Pharm Ther*. 2012;37(9):512–525.
99. Lansdown AB. Silver in health care: antimicrobial effects and safety in use. *Curr Probl Dermatol*. 2006;33:17–34
100. Thomas S, McCubbin P. An in vitro analysis of the antimicrobial properties of 10 silver-containing dressings. *J Wound Care*. 2003;12(8):305–308..
101. Nohynek GJ, Lademann J, Ribaud C, Roberts MS. Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. *Crit Rev Toxicol*. 2007;37(3):251–277.
102. Popov AP, Priezzhev AV, Lademann J, Myllylä R. Biophotonics of ZnO nanoparticles for sunscreen and cosmetic applications. *J Biomed Opt*. 2005;10(6):064037.
103. Jain S, Tiwary AK, Sapra B. Nanotechnology in cosmetics and cosmeceuticals: a review. *J Pharm Sci*. 2017;106(9):2527–2540.
104. Bhalerao SS, Raje DV. Ethosomes: a novel vesicular carrier for enhanced transdermal delivery of drugs. *Int J Pharm Sci Res*. 2013;4(2):175–182.
105. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: an overview. *J Adv Pharm Technol Res*. 2010;1(3):274–282.