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CARVACROL AS A VERSATILE THERAPEUTIC MOLECULE: MECHANISMS OF ACTION, ROLE IN WOUND REPAIR, AND NOVEL DRUG DELIVERY APPROACHES

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ABSTRACT

The escalating crisis of antimicrobial resistance (AMR) has catalysed a global search for novel therapeutic agents, particularly for complex clinical challenges like infected wounds. Carvacrol, a natural phenolic monoterpenoid derived from essential oils of the *Lamiaceae* family, has emerged as a compelling candidate due to its remarkable pharmacological versatility. This comprehensive review synthesizes the current body of evidence on carvacrol's therapeutic potential, focusing on its mechanisms of action and applications in wound healing and infection control. We critically examine its potent, broad- spectrum antimicrobial and anti-biofilm activities, which are primarily attributed to its ability to disrupt microbial membrane integrity. Furthermore, we explore its synergistic host- modulating properties, including potent anti-inflammatory and antioxidant effects that mitigate pathological inflammation and oxidative stress in the wound microenvironment, and its demonstrated ability to stimulate fibroblast proliferation and migration. Despite this profound potential, carvacrol's clinical translation is severely hampered by significant physicochemical limitations, including poor aqueous solubility, high volatility, and potential for skin irritation. This review provides a comprehensive overview of advanced drug delivery strategies, including polymeric nanoparticles, nanostructured lipid carriers (NLCs), electro spun nanofibers, stimuli-responsive systems, and hydrogels, that have been developed to overcome these barriers. Special emphasis is placed on the nanoemulgel platform, a hybrid system that effectively enhances carvacrol's bioavailability and topical applicability. By bridging the gap between its intrinsic pharmacological power and practical application, these nanotechnological approaches are paving the way for carvacrol to become a next-generation therapeutic for wound management.

KEYWORDS: Carvacrol, Novel formulations, Biomedical applications, Antimicrobial Resistance, Wound Healing, Drug Delivery, Nanoemulgel, Phytochemicals, Biofilm, Anti- inflammatory.

1. INTRODUCTION

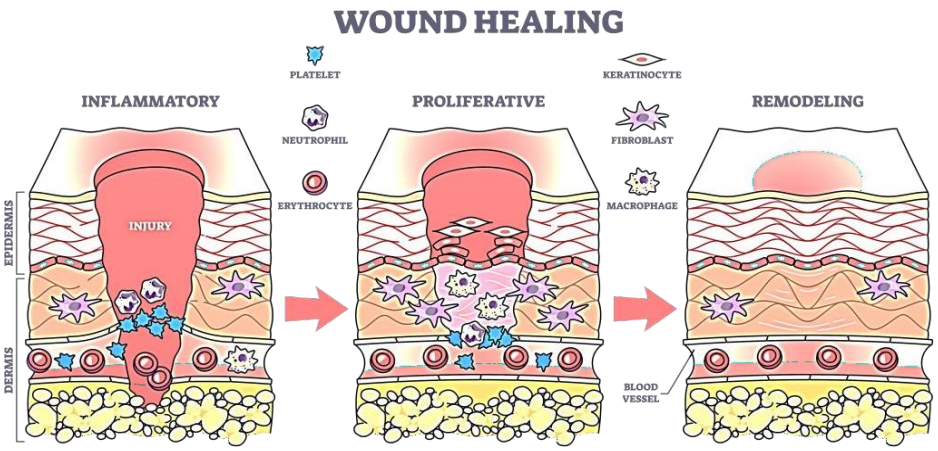
The process of wound healing is a sophisticated and highly orchestrated biological cascade designed to restore the integrity of injured tissue. It unfolds across four distinct yet overlapping phases: hemostasis, inflammation, proliferation, and remodelling.[1, 2] In a healthy individual, this process is remarkably efficient. However, in the presence of systemic comorbidities (e.g., diabetes mellitus, vascular insufficiency) or local factors (e.g., pressure, infection), this cascade can be arrested, leading to the formation of chronic, non-healing wounds.[3] These wounds, such as diabetic foot ulcers and venous leg ulcers, represent a significant and escalating global health crisis, inflicting a devastating burden on patients and healthcare systems alike.[4]

Among the myriad factors that impede healing, microbial infection stands out as a paramount and often decisive challenge.[5] A wound fundamentally represents a breach of the skin's microbial barrier, instantly exposing nutrient-rich subcutaneous tissue to the surrounding environment.[6] This creates an ideal ecological niche for colonization by a wide array of microorganisms, including opportunistic and pathogenic bacteria. Uncontrolled microbial growth subverts the healing process through multiple deleterious mechanisms: direct competition with host cells for oxygen and nutrients, production of cytotoxic virulence factors, and, most critically, the provocation of a persistent and exaggerated inflammatory response that locks the wound in the inflammatory phase.[7]

The challenge is critically compounded by the formation of biofilms and the global crisis of antimicrobial resistance (AMR).[8] Biofilms are highly organized microbial communities encased in a self-produced matrix of extracellular polymeric substances (EPS), which renders them notoriously resistant to conventional antibiotics and host immune defences.[9,10] The convergence of this resilient phenotype with the genetics of multidrug-resistant (MDR) pathogens, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and MDR *Pseudomonas aeruginosa*, creates a perfect storm, rendering many chronic wound infections virtually untreatable.[11,12]

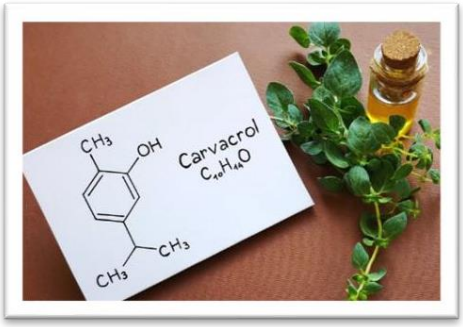
This dire situation has spurred intensive research into alternative therapeutic strategies, with a particular focus on phytochemicals derived from medicinal plants.[13] Unlike conventional antibiotics that often target a single bacterial enzyme, many natural compounds exert their antimicrobial effects through multiple, synergistic mechanisms, making the evolution of resistance a far more complex and statistically less likely event.[14] Carvacrol (5-isopropyl- 2-methylphenol), a phenolic monoterpenoid and the major active constituent of essential oils from oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*), has emerged as an exceptionally promising agent in this context.[15] Its therapeutic potential extends far beyond its antimicrobial action, encompassing a suite of properties that are highly relevant to wound care.

This review provides a comprehensive analysis of the scientific literature surrounding carvacrol's therapeutic utility. We will first dissect its multifaceted pharmacological mechanisms, then address the significant physicochemical barriers that have hindered its clinical application, and finally, provide a critical overview of the advanced drug delivery systems being developed to unlock its full potential as a next-generation agent for wound management.



2. Diverse Pharmacological Actions of Carvacrol

Carvacrol's value in wound care is defined by its unique ability to simultaneously address multiple pathological facets of a non-healing wound: it combats microbial invaders, resolves pathological inflammation, mitigates oxidative stress, and actively stimulates the host's own regenerative processes.



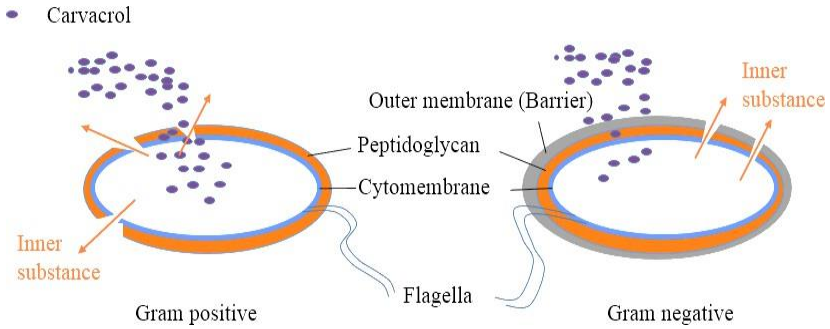
2.1. Strong Antimicrobial and Biofilm-Inhibiting Potential

The cornerstone of carvacrol's therapeutic profile is its powerful, broad-spectrum antimicrobial activity. It has demonstrated efficacy against a wide range of Gram-positive and Gram-negative bacteria, fungi, and yeasts, including clinically significant MDR strains such as MRSA and Vancomycin-resistant *Enterococci* (VRE).[12,14]

The principal antimicrobial mechanism is the disruption of the microbial cytoplasmic membrane. As a highly lipophilic molecule, carvacrol readily partitions into the lipid bilayer of the microbial membrane. Its presence disorganizes the acyl chains of the membrane phospholipids, thereby increasing the membrane's fluidity and permeability[16] This structural damage leads to a cascade of catastrophic and irreversible events for the microbial cell:

- **Leakage of Intracellular Components:** The compromised membrane can no longer maintain ionic gradients, leading to the uncontrolled efflux of vital intracellular components like potassium ions (K^+), protons (H^+), and ATP.[17]
- **Dissipation of Proton Motive Force (PMF):** The loss of the proton gradient across the membrane dissipates the PMF, which is the primary energy source for essential cellular processes like ATP synthesis, solute transport, and motility.
- **Cellular Lysis:** The structural failure of the membrane ultimately leads to cell lysis and death.

This membrane-disruptive mechanism is a physical mode of action, making it difficult for bacteria to develop resistance through common genetic modifications of target sites. This has been proven effective even against recalcitrant, biofilm-forming bacteria.[19] Furthermore, carvacrol has been shown to effectively penetrate the EPS matrix of established biofilms, acting on the dormant, antibiotic-tolerant persisted cells within, making it a promising agent for treating biofilm-associated chronic infections.[10, 20]



2.2. Adjustment of Host Immune-Inflammatory Pathways

Chronic wounds are often trapped in a vicious cycle of persistent inflammation, characterized by an excessive influx of neutrophils and macrophages and high levels of pro-inflammatory cytokines and destructive proteases (e.g., matrix metalloproteinases).[2] This environment is hostile to the cells responsible for tissue regeneration. Carvacrol exerts potent anti- inflammatory effects that help to break this cycle. Studies have demonstrated that carvacrol can significantly downregulate the production of key pro-inflammatory cytokines, including Tumour Necrosis Factor-alpha (TNF-α) and Interleukin-1 beta (IL-1β).[6] By tempering this excessive inflammatory response, carvacrol helps to shift the wound microenvironment from a destructive, catabolic state to a constructive, anabolic state, paving the way for the proliferative phase of healing.

2.3. Strong Antioxidant Potential

The wound bed is a site of high metabolic activity and immune cell function, leading to the generation of significant amounts of reactive oxygen species (ROS).[13] While ROS play a role in signalling and host defense, their overproduction leads to oxidative stress, which causes damage to the lipids, proteins, and DNA of fragile, regenerating host cells, thereby impairing healing. As a phenolic compound, carvacrol possesses a hydroxyl group attached to an aromatic ring, which makes it an excellent hydrogen donor and a powerful antioxidant.[11] It can effectively scavenge free radicals like the superoxide anion and hydroxyl radical, thus protecting the wound tissue from oxidative damage and supporting the viability of fibroblasts and keratinocytes. This antioxidant activity is synergistic with its anti-inflammatory effects, contributing to a healthier and more balanced wound microenvironment.

2.4. Activation of Regenerative Cellular Processes.

Beyond simply creating a more favourable environment for healing, carvacrol appears to actively stimulate the key cellular processes involved in tissue reconstruction. The proliferative phase of healing is critically dependent on the function of dermal fibroblasts, which migrate into the wound defect, proliferate to repopulate the area, and synthesize the new extracellular matrix (ECM) that forms the scaffold for new tissue.[1]

Multiple *in vitro* studies have provided direct evidence of carvacrol's pro-regenerative effects. Tabatabaei et al. (2023) demonstrated that carvacrol promotes both the migration and proliferation of human dermal fibroblasts in a scratch-

wound assay, even under the challenging conditions of high-glucose stress designed to mimic a diabetic state.[17] This suggests a particular therapeutic potential for the treatment of diabetic foot ulcers, a major clinical challenge.

These cellular effects have been robustly corroborated in numerous *in vivo* animal models. Topical application of carvacrol-containing formulations has been shown to significantly accelerate the rate of wound closure, enhance the deposition and organization of collagen fibers, and promote angiogenesis (the formation of new blood vessels to supply the regenerating tissue with oxygen and nutrients) when compared to control groups.[18, 21] This multifaceted ability to simultaneously target pathogens, quell inflammation, and stimulate regeneration makes carvacrol a uniquely holistic therapeutic agent for wound care.

3. The Formulation Conundrum: Physicochemical Barriers to Clinical Translation

Despite the wealth of compelling pre-clinical data highlighting its therapeutic promise, the translation of pure carvacrol from the laboratory bench to the patient bedside has been severely impeded by a series of formidable physicochemical challenges. These practical hurdles are not related to a lack of biological potency but to the inherent difficulty of formulating this molecule into a stable, effective, and patient-compliant product.

3.1. Poor Aqueous Solubility and High Lipophilicity

Carvacrol is an oily liquid with a Log P value of approximately 3.6, which signifies its high lipophilicity and, consequently, its negligible solubility in water (≈0.11 mg/mL).[12] This property makes its incorporation into conventional, water-based topical vehicles—such as aqueous gels, creams, and lotions—extremely problematic. Formulating a therapeutically relevant concentration would require high levels of organic co-solvents (e.g., ethanol) or synthetic surfactants, which can themselves cause skin irritation, disrupt the skin barrier, and are often undesirable in a wound care product intended for compromised tissue.

3.2. High Volatility and Instability

As a primary component of aromatic essential oils, carvacrol is inherently volatile. When applied topically in a simple formulation, it rapidly evaporates from the skin surface. This leads to several negative consequences:

- **Loss of Active Ingredient:** A significant portion of the applied dose is lost to the atmosphere before it can exert a therapeutic effect.
- **Transient and Uncontrolled Dosing:** The concentration of the drug at the target site spikes and then rapidly declines, failing to provide the sustained therapeutic levels necessary for effective antimicrobial action and promotion of healing over time.
- **Instability:** Carvacrol is also susceptible to degradation upon exposure to light and air (oxidation), further reducing the stability and shelf-life of simple formulations.[12]

3.3. Potential for Skin Irritation and Cytotoxicity

To achieve a robust antimicrobial effect, carvacrol must often be used at concentrations that can be irritating or even cytotoxic to host cells. While it demonstrates selectivity for microbial membranes over mammalian cell membranes to some extent, high local concentrations can disrupt the membranes of keratinocytes and fibroblasts, leading to irritation, erythema, and impaired healing.[22] A successful delivery system must therefore be able to deliver a therapeutically effective dose without causing these high, transient local concentrations that could be detrimental to the delicate wound tissue.

3.4. Patient Compliance Issues

Carvacrol possesses very strong, pungent, and characteristic oregano-like Odor. While acceptable in culinary applications, this potent aroma can be unpleasant for patients in a medical context, potentially leading to poor compliance, especially for chronic wounds requiring long-term treatment. To harness the full clinical potential of carvacrol, it is therefore imperative to move beyond simple formulations and utilize advanced drug delivery systems designed specifically to overcome these formidable challenges.

4. Advanced Drug Delivery Systems for Unlocking Carvacrol's Potential

The efficacy of a therapeutic agent is inextricably linked to its delivery system. For a challenging molecule like carvacrol, an advanced vehicle must perform multiple functions: enhance solubility, improve stability, control the release profile to provide sustained action, and reduce irritancy. Nanotechnology has provided a powerful and versatile toolkit to achieve these goals, and numerous platforms have been investigated for the delivery of carvacrol.

4.1. The Rationale for Nanoscale Encapsulation

Encapsulating carvacrol within a nanocarrier (typically with a size range of 20-500 nm) offers several distinct advantages over conventional formulations:

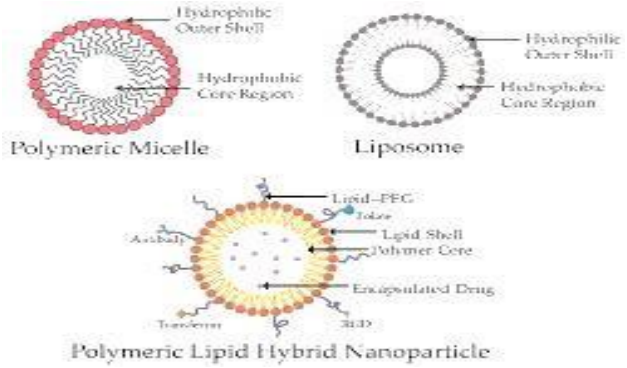
- **Enhanced Solubility:** By entrapping the lipophilic carvacrol within the core of a nanocarrier, it can be effectively dispersed in an aqueous medium, creating a macroscopically homogenous system.
- **Improved Stability:** The carrier matrix protects carvacrol from premature degradation due to light and oxidation, and significantly reduces its evaporative loss, thereby increasing its residence time at the application site.
- **Controlled Release:** The nanocarrier can be engineered to release carvacrol in a sustained manner over an extended period, maintaining therapeutic concentrations in the wound bed while avoiding the high initial concentration spikes that can cause irritation.
- **Reduced Irritancy:** By preventing direct contact of high concentrations of free carvacrol with the skin surface, encapsulation can significantly reduce its potential for irritation and cytotoxicity to host cells.
- **Masking of Odor:** Encapsulation within the core of a nanoparticle can effectively mask the pungent Odor of carvacrol, improving patient compliance.

4.2. Particulate and Fibrous Carrier Systems

A wide variety of nanocarrier architectures have been explored for carvacrol delivery.

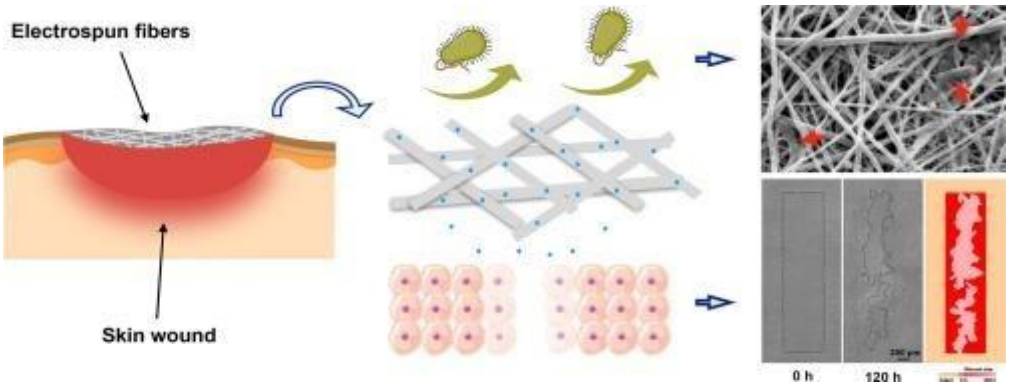
4.2.1. Polymeric and Lipid-Based Nanoparticles

Polymeric nanoparticles, fabricated from biodegradable polymers like poly (lactic acid) (PLA), can effectively encapsulate carvacrol. Niza et al. (2020) demonstrated that coating PLA- carvacrol nanoparticles with polyethyleneimine (PEI), a cationic polymer, enhanced their antimicrobial activity five-fold, likely by promoting electrostatic adhesion to the negatively charged bacterial surface.[10] Nanostructured lipid carriers (NLCs), which are composed of a blend of solid and liquid lipids, offer high drug loading and improved stability. The work by Fauzian et al. (2024) showed that carvacrol-loaded NLCs significantly improved the rate of wound contraction in diabetic rat models, showcasing the platform's ability to provide both occlusion and sustained release.[22] Another innovative approach utilizes phytosomes, where carvacrol is complexed with phospholipids to form highly bioavailable, lipid-compatible nanostructures. This approach has been shown to lead to superior collagen synthesis and epidermal thickening in wound models.[23, 24]



4.2.2. Electro spun Nanofibers and Therapeutic Films

Electrospinning is a versatile technique used to produce non-woven mats of nanofibers that can serve as advanced wound dressings. These mats possess a high surface-area-to-volume ratio and a porous architecture that mimics the natural ECM, which is conducive to cell infiltration and tissue regeneration.[25] Several research groups have successfully incorporated carvacrol into electro spun nanofibers for sustained delivery. Wang et al. (2024) developed carvacrol-loaded polyacrylonitrile/poly (ethylene oxide) nanofibers that provided drug release over 72 hours and achieved a 99% kill rate against MRSA.[21] Similarly, carvacrol has been integrated into nanofibers made from PVP/lanolin.[25] and solution blow-spun fish-skin gelatin.[14] Beyond nanofibers, simpler platforms like chitosan films containing carvacrol have also proven effective, significantly accelerating the healing of full-thickness wounds in rats compared to blank films.[18]

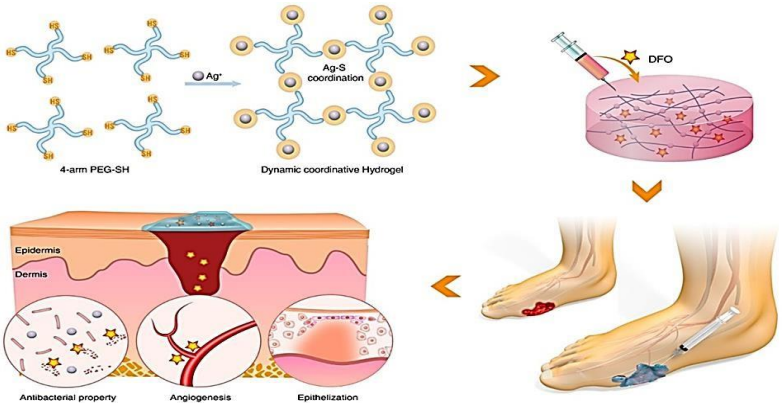


4.3. Hydrogel-Based Delivery Platforms

Hydrogels are three-dimensional networks of hydrophilic polymers that can hold large amounts of water, making them ideal for creating the moist environment known to facilitate wound healing.[26] Their soft, pliable nature is also well-suited for application to sensitive wound beds.

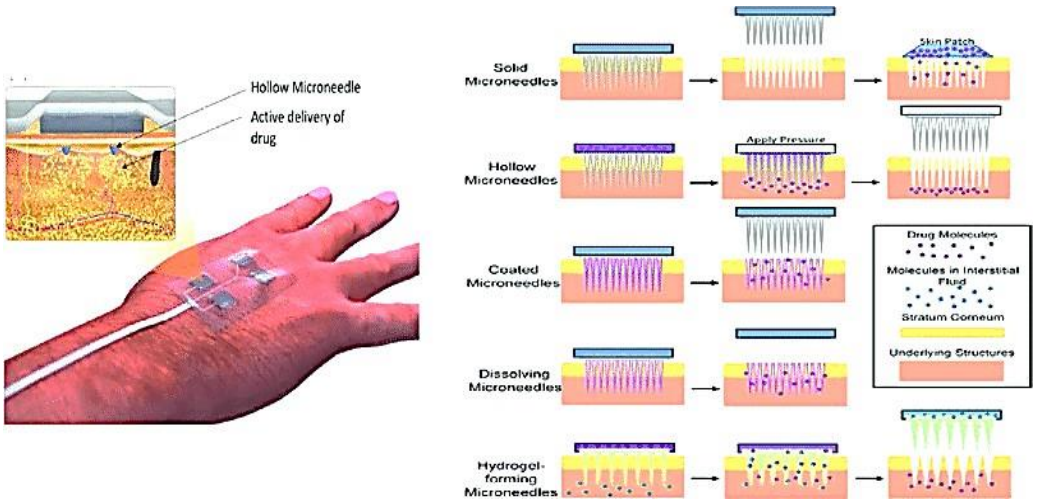
4.3.1. Self-Assembling and Injectable Hydrogels

Recent innovations have led to the development of "carrier-free" hydrogel systems. In a groundbreaking study, Cui et al. (2024) created an injectable and self-assembling hydrogel based on non-covalent hydrogen bonding between carvacrol and glycyrrhizin. This system formed a hydrogel upon injection into the wound, where it eradicated MRSA infection and significantly enhanced the healing process without the need for any additional synthetic polymers.[8] Other research has focused on green starch-based hydrogels that exhibit excellent injectability, self-healing, and photothermal properties, in addition to antibacterial activity, for promoting wound healing.[26] These systems offer the promise of minimally invasive application and conformal wound coverage.



4.3.2. "Smart" Hydrogels and Microneedle Systems

The next frontier in delivery involves creating "smart" systems that respond to specific triggers within the wound microenvironment. Mir et al. (2019, 2020) have pioneered the use of microneedles for carvacrol delivery. In one system, enzyme-responsive nanoparticles loaded with carvacrol were embedded in dissolving microneedles. Upon application, these microneedles painlessly breach the stratum corneum. In the presence of infection, bacterial- secreted lipases degrade the nanoparticles, triggering a targeted, on-demand release of carvacrol precisely where it is needed most.[15,27] A hybrid microneedle system incorporating carvacrol, cyclodextrin (for solubilization), and ceria nanoparticles (for antioxidant activity) has also been developed specifically to modulate the microenvironment of diabetic wounds and promote healing.[21]



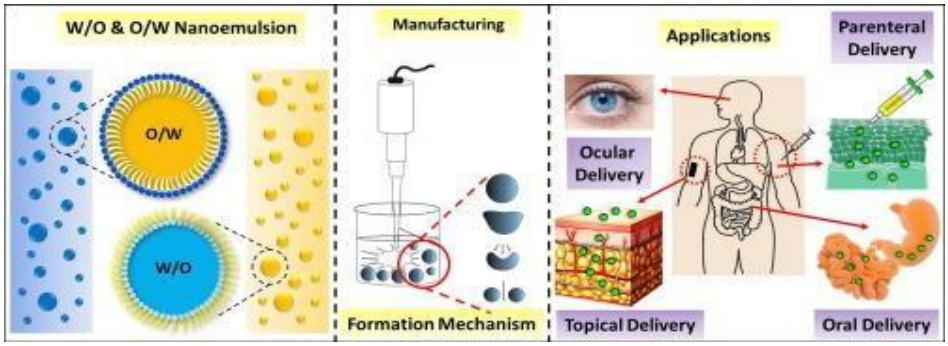
4.4. The Nanoemulgel Platform: A Synergistic and Highly Pragmatic Approach

While each of the previously mentioned systems offers unique and innovative advantages, the nanoemulgel platform has emerged as a particularly effective, scalable, and pragmatic strategy for the topical delivery of challenging lipophilic agents like carvacrol.[28,29] A nanoemulgel is a sophisticated hybrid system that rationally combines the superior delivery characteristics of an oil-in-water (O/W) nano emulsion with the desirable application properties of a hydrogel.

4.4.1. The Internal Phase: The Optimized Nanoemulsion

The core of the nanoemulgel is a carefully optimized nano emulsion, which serves as the high- performance drug carrier. The development of a stable and effective nano emulsion is the critical first step and depends on a delicate balance of several formulation variables.

- **Oil Phase Composition:** The oil phase solubilizes the lipophilic drug. In the case of carvacrol, which is itself an oil, a biocompatible carrier oil such as isopropyl myristate (IPM) is often included. IPM not only acts as a solvent but also serves as a skin permeation enhancer, facilitating the delivery of carvacrol into the deeper layers of the skin.[33] The total concentration of the oil phase is a crucial parameter; typically, a moderate concentration of 5-10% (w/w) is optimal to ensure both adequate drug loading and the ability of the surfactant system to form stable nanodroplets.[34,35]



- **Surfactant/Co-surfactant (Smix) System:** The formation of nanodroplets is energetically unfavourable and requires the input of energy (e.g., ultrasonication) and the presence of emulsifiers to reduce the interfacial tension between the oil and water phases. A combination of a primary surfactant and a co-surfactant (often referred to as a Smix) is typically most effective. Non-ionic surfactants with a high Hydrophile- Lipophile Balance (HLB) value, such as Tween 80 (HLB ≈ 15), are ideal for stabilizing O/W nano emulsions.[36] A short-chain alcohol or glycol, such as propylene glycol (PG), is often used as a co-surfactant. The co-surfactant penetrates the surfactant monolayer at the oil-water interface, increasing its fluidity and flexibility, which facilitates the formation of smaller, more stable droplets during the emulsification process.[37] The mass ratio of surfactant to co-surfactant is a critical variable that must be empirically optimized. An optimal Smix ratio (e.g., 2:1 Tween 80:PG) can reduce interfacial tension to the ultra-low levels required for efficient nano-droplet formation and long-term kinetic stability.
- **Key Characterization Parameters:** The quality of the nano emulsion is defined by three key parameters:

1. **Droplet Size:** Must be in the nano meter range (typically < 200 nm) to provide a large surface area for drug release.
2. **Polydispersity Index (PDI):** A measure of the uniformity of the droplet size distribution. A PDI value below 0.3 indicates a monodisperse and homogenous system, which is essential for consistent performance and stability.[38]
3. **Zeta Potential:** A measure of the magnitude of the electrostatic charge on the droplet surface. A high absolute zeta

potential value (typically > |30| mV) indicates strong electrostatic repulsion between droplets, which prevents them from aggregating and ensures long-term physical stability.[39]

The work underpinning this review successfully optimized a Nano emulsion (F5) with a mean droplet size of 143.8 nm, a PDI of 0.219, and a zeta potential of -32.5 mV, representing a high- quality and stable internal phase.

4.4.2. The External Phase: The Hydrogel Matrix

Once the optimized nano emulsion is formed, it is dispersed within an aqueous hydrogel matrix to create the final semi-solid nanoemulgel. The choice of gelling agent is critical to the final product's performance and acceptability.

- **Gelling Agent Selection:** High-molecular-weight, cross-linked poly(acrylic acid) polymers, such as Carbopol 940, are widely used and highly effective gelling agents.[40] In their un-neutralized state, the polymer chains are coiled. Upon neutralization with a suitable base (e.g., triethanolamine) to a pH above ~5.5, the carboxylic acid groups become ionized, leading to electrostatic repulsion between adjacent groups. This causes the polymer chains to uncoil and swell, trapping large amounts of water and the dispersed nano emulsion droplets within a three-dimensional network, thereby dramatically increasing the viscosity of the system.[41]
- **Desirable Rheological Properties:** A well-formulated Carbopol-based nanoemulgel exhibits ideal pseudoplastic, or shear-thinning, behaviour. At rest (low shear), it has a high viscosity, which prevents it from running off the application site. However, during application (rubbing, high shear), the viscosity drops dramatically, allowing it to be spread easily and smoothly over the delicate wound surface. Once the shear force is removed, the viscosity rapidly recovers, ensuring the formulation remains in place.
- **Biocompatibility and Hydration:** The final nanoemulgel should have a pH that is compatible with the skin's natural acidic mantle (pH 4.5-6.0) to minimize the risk of irritation.[42] The high-water content of the hydrogel also provides a soothing and hydrating effect to the wound bed, which is known to be beneficial for the healing process by preventing desiccation and facilitating cell migration. Other excipients, such as humectants like glycerine, can be added to further improve patient comfort and prevent the gel from drying out.[43]

4.4.3. The Synergistic Advantage of the Nanoemulgel Platform

The nanoemulgel platform synergistically combines the advantages of its constituent parts to create a superior delivery system:

- It effectively **solubilizes and stabilizes** carvacrol within the oily nanodroplets.
- It provides **sustained drug release**, driven by the large surface area of the nanodroplets.
- It has the **ideal semi-solid consistency** for topical application, ensuring ease of use and prolonged residence time.
- It provides a **soothing, hydrating environment** to the wound.

This rational combination of properties makes the nanoemulgel a highly promising and pragmatic platform for transforming carvacrol from a challenging natural compound into a potent and clinically viable therapeutic agent for wound healing. As demonstrated in the work underpinning this review, this platform resulted in a threefold increase in carvacrol release and a dramatic acceleration of *in vitro* wound closure compared to a conventional emulsion.

5. In Vitro and In Vivo Evidence of Efficacy

The development of advanced carvacrol formulations has been paralleled by a growing body of evidence demonstrating their enhanced efficacy in relevant biological models.

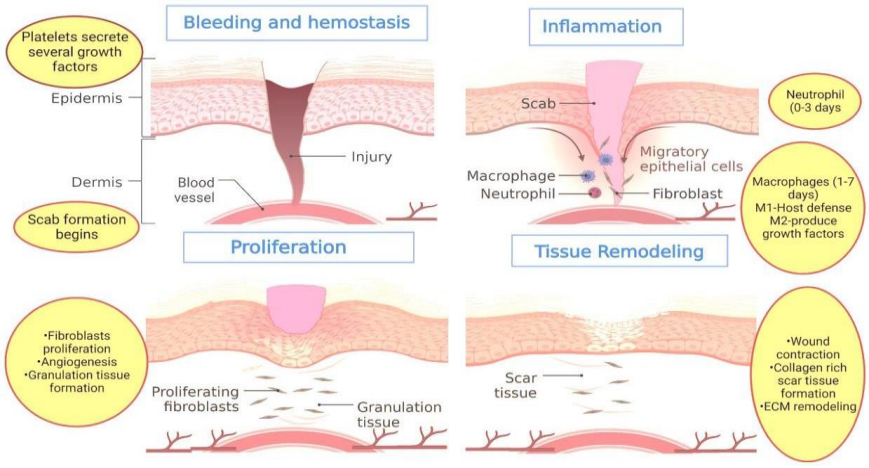
5.1. Enhanced Antimicrobial Activity

Encapsulation can significantly boost carvacrol's antimicrobial power. The aforementioned study by Niza et al. (2020) is a prime example, where encapsulating carvacrol in PEI-coated PLA nanoparticles resulted in a five-fold increase in bactericidal activity against *E. coli* compared to free carvacrol.[10] This enhancement is likely due to the cationic PEI promoting close association and fusion of the nanoparticles with the negatively charged bacterial membrane, leading to more efficient delivery of the carvacrol payload directly to its site of action. The high kill rates (99%) against MRSA observed with carvacrol-loaded nanofibers also underscore the efficacy of providing a sustained, localized release of the agent directly at the site of potential infection.[21]

5.2. Accelerated Wound Closure and Tissue Regeneration

The ultimate goal of a wound healing therapy is to accelerate the restoration of tissue integrity. Numerous studies using *in vivo* animal models have confirmed the superior efficacy of carvacrol delivered via advanced formulations. Barreto et al. (2015) showed that a simple chitosan film containing carvacrol was able to close full-thickness wounds in rats significantly faster than a blank chitosan film, highlighting the pro-regenerative activity of the released carvacrol.[18]

More sophisticated nanocarrier systems have shown even more dramatic results. The carvacrol- loaded NLCs developed by Fauzian et al. (2024) improved diabetic wound contraction by 2.8- fold versus unencapsulated drug.[22] The phytosomes formulation developed by Tafish et al. (2023) led to superior collagen synthesis and a thicker, more well-formed epidermis in a rat wound model.[24]



The *in vitro* scratch assay is a widely used and reliable model for assessing the ability of a compound to stimulate the key cellular processes of migration and proliferation that underpin wound closure. The near-complete (97.3%) wound closure observed within 24 hours with the optimized carvacrol nanoemulgel stands in stark contrast to the modest effect of the conventional emulsion (41.3%). This result provides powerful, direct evidence at the cellular level that enhancing the bioavailability of carvacrol through an advanced delivery system dramatically amplifies its inherent pro-healing biological activity.

5.3. Efficacy in Specialized and Challenging Models

The versatility and potency of carvacrol are further underscored by its success in highly challenging and clinically relevant models that go beyond simple dermal wounds. Recent research has explored its use in treating infected bone defects, a notoriously difficult clinical problem. Cui et al. (2024) loaded carvacrol into a premixed calcium phosphate

bone cement and demonstrated that the composite material exhibited exceptional osteogenic (bone-forming) and antibacterial properties, effectively healing infected bone defects.[19] Similarly, Dahiya et al. (2024) have shown that carvacrol nanoparticles on 3D printed scaffolds can promote bone healing.[20] These studies highlight the broad applicability of carvacrol and the critical role of the delivery system in adapting it for specific and challenging therapeutic applications.

6. Conclusion and Future Perspectives

The scientific evidence accumulated over the past decade paints a clear and compelling picture: carvacrol is a multifunctional phytochemical with profound therapeutic potential for wound healing and antimicrobial applications. Its ability to simultaneously exert potent antimicrobial action, resolve pathological inflammation, mitigate oxidative stress, and directly stimulate the cellular machinery of tissue regeneration makes it a uniquely holistic agent. It addresses not just one, but multiple key impediments to the healing process, a profile that is highly desirable for the complex pathophysiology of chronic wounds.

However, it is equally clear that this intrinsic biological power is fundamentally locked behind a wall of formidable physicochemical challenges. The poor aqueous solubility, high volatility, and potential for irritation have, for many years, prevented its successful translation into a viable clinical product. This review has highlighted that the key to unlocking this potential lies in the rational design of advanced drug delivery systems.

A diverse array of innovative platforms—from polymeric nanoparticles and nanofibers to smart hydrogels and microneedles—have been successfully developed to encapsulate carvacrol. These systems are not merely passive carriers; they are enabling technologies that transform carvacrol into a stable, effective, and clinically relevant therapeutic. They enhance its solubility, protect it from degradation, control its release to provide sustained therapeutic action, and reduce its irritancy, thereby bridging the critical gap between its inherent potency and its practical application.

Among these platforms, the nanoemulgel has emerged as a particularly robust, versatile, and pragmatic solution for topical delivery. By synergistically combining the superior bioavailability of a nano emulsion with the ideal application properties of a hydrogel, it creates a final product that is both highly effective and user-friendly.

While the *in vitro* and pre-clinical data are overwhelmingly positive, the journey from laboratory to clinic is not yet complete. The immediate future of carvacrol research should focus on several key areas:

1. ***In Vivo* Validation in Clinically Relevant Models:** The promising results from *in vitro* assays and simple animal models must be validated in more challenging *in vivo* models, such as infected wounds in diabetic or immunocompromised animals, which more closely mimic the human clinical condition.
2. **Mechanistic Studies:** While the broad mechanisms of action are known, further research using molecular biology techniques (e.g., Western blot, qRT-PCR) is needed to elucidate the specific cellular signalling pathways (e.g., MAPK, PI3K/Akt) that are modulated by carvacrol to promote healing.[31]
3. **Long-Term Stability and Scale-Up:** Comprehensive long-term stability studies, conducted according to ICH guidelines, are essential for any formulation intended for clinical use. Furthermore, the manufacturing processes for the most promising delivery systems must be optimized for potential industrial scale-up.
4. **Combinatorial Therapies:** Future studies could explore the potential of combining carvacrol with other

therapeutic agents (e.g., other phytochemicals, growth factors, or even conventional antibiotics) to achieve synergistic effects.[44,51]

In conclusion, carvacrol stands as a powerful testament to the therapeutic potential held within nature's pharmacopeia. Through the continued application of rational formulation design and advanced drug delivery science, carvacrol is well-positioned to be developed into a next- generation topical therapy, offering a new and effective weapon in the fight against complex wounds and antimicrobial resistance.

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