

Antibacterial and Antioxidant Efficacy of Lignocaine-Enhanced Antibiotic-Infused Collagen Membranes: A Comparative in Vitro Evaluation For Oral Wound Healing

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Abstract

Objective:

To comparatively evaluate the antimicrobial and antioxidant properties of collagen membranes infused with antibiotic alone versus antibiotic combined with lignocaine for potential use in enhanced wound healing.

Methods:

Collagen membranes were infused with antibiotic (Group A) or antibiotic with lignocaine (Group B) and evaluated in vitro. Antimicrobial activity was assessed using agar diffusion against *Streptococcus mutans*, *Lactobacillus* spp., *Staphylococcus aureus*, *Candida albicans*, and *Enterococcus faecalis*. Antioxidant activity was determined using DPPH, hydrogen peroxide, FRAP, ABTS, and nitric oxide scavenging assays.

Results:

Both groups demonstrated dose-dependent antimicrobial activity against *S. mutans*, *Lactobacillus* spp., and *S. aureus*, with no significant activity against *C. albicans* or *E. faecalis*. Antioxidant assays revealed strong radical-scavenging activity in both groups, comparable to standard controls, with slightly higher activity observed in Group B.

Conclusion:

Antibiotic-infused collagen membranes, with or without lignocaine, exhibit targeted antimicrobial action along with significant antioxidant properties. The addition of lignocaine enhances overall bioactivity, supporting its potential role in multifunctional biomaterials for oral and periodontal regeneration.

Keywords: Antibiotic-infused collagen membranes, Wound healing, cell adhesion, haemostasis, antimicrobial activity.

Introduction

Wound healing is a dynamic and sequential process involving haemostasis, inflammation, proliferation, and remodelling. Disruption of this cascade by microbial contamination, oxidative stress, or persistent inflammation can impair healing, resulting in chronic wounds or fibrosis. Therefore, an ideal wound-healing biomaterial should possess antimicrobial, antioxidant, properties while supporting tissue regeneration and maintaining biocompatibility.

Collagen is extensively used in regenerative dentistry due to its excellent biocompatibility, biodegradability, and ability to mimic the extracellular matrix, thereby facilitating cell adhesion and tissue repair.

Incorporation of therapeutic agents into collagen scaffolds enhances their functional potential. Antibiotics not only control microbial load but also demonstrate secondary antioxidant and anti-inflammatory effects, including inhibition of reactive oxygen species and modulation of inflammatory mediators. Lignocaine, an amide-type local anaesthetic, has also been shown to exert anti-inflammatory and antioxidant effects by reducing neutrophil activation and nitric oxide production, in addition to providing analgesia.

The combination of these agents within a collagen matrix may offer synergistic benefits by simultaneously addressing infection, oxidative stress, while promoting a favourable healing environment. However, the added value of incorporating lignocaine alongside antibiotics within collagen scaffolds remains insufficiently explored. Hence, this study aims to comparatively evaluate the antimicrobial, antioxidant properties of collagen membranes infused with antibiotic alone versus antibiotic combined with lignocaine through in vitro assays.

Methodology:

The present in vitro study evaluated the antimicrobial, and antioxidant properties of two formulations: Group I (collagen + antibiotic) and Group II (collagen + antibiotic + lignocaine) across standardized concentrations (10–50 µg/mL).

Antimicrobial Activity

Antimicrobial efficacy was evaluated using the agar well diffusion method against common oral pathogens: *Streptococcus mutans*, *Lactobacillus* spp., *Enterococcus faecalis*, *Staphylococcus aureus*, and *Candida albicans*. Sterilized Mueller–Hinton agar plates were inoculated with microbial suspensions using sterile swabs. Wells of 9 mm diameter were prepared and filled with different concentrations (25, 50, and 100 µg/mL) of each formulation. A combination of Augmentin and metronidazole served as the standard. Plates were incubated at 37°C for 24 hours (48 hours for fungal cultures), and antimicrobial activity was determined by measuring the zone of inhibition (in mm).

Antioxidant Activity

Antioxidant potential was assessed using five complementary assays to evaluate radical scavenging and reducing capacity.

DPPH Radical Scavenging Assay:

Test samples (10–50 µg/mL) were mixed with DPPH solution and incubated in the dark for 10 minutes. Absorbance was measured at 517 nm, and scavenging activity was calculated using ascorbic acid as the standard.

Hydrogen Peroxide Scavenging Assay:

Samples were added to hydrogen peroxide solution and incubated for 10 minutes. Absorbance was measured at 230 nm, and percentage inhibition was calculated.

Ferric Reducing Antioxidant Power (FRAP) Assay:

Test samples were mixed with freshly prepared FRAP reagent and incubated at 37°C for 30 minutes. Absorbance was recorded at 593 nm to determine reducing power.

ABTS Radical Cation Assay:

ABTS radical solution was mixed with test samples and incubated for 10 minutes in the dark. Absorbance was measured at 734 nm, and percentage inhibition was calculated.

Nitric Oxide Scavenging Assay:

Samples were incubated with sodium nitroprusside, followed by reaction with Griess reagent to form a chromophore. Absorbance was measured at 540 nm, and nitric oxide inhibition was calculated.

Results

Antimicrobial Activity

Both formulations demonstrated selective and concentration-dependent antimicrobial activity. No inhibitory effect was observed against *Candida albicans* and *Enterococcus faecalis* at any concentration (25, 50, or 100 µg/mL), with zone diameters consistently measuring 9 mm, comparable to the standard control.

Against *Streptococcus mutans*, Group I (collagen + antibiotic) showed a dose-dependent increase in inhibition zones from 18 mm to 26 mm, while Group II (collagen + antibiotic + lignocaine) exhibited slightly higher values ranging from 20 mm to 28 mm.

Lactobacillus spp. demonstrated the highest susceptibility, with Group I showing inhibition zones of 31–46 mm and Group II showing enhanced activity ranging from 40–49 mm.

Similarly, *Staphylococcus aureus* showed marked sensitivity, with Group I exhibiting inhibition zones of 30–35 mm and Group II demonstrating comparable or slightly improved inhibition. Overall, Group II showed a marginal enhancement in antimicrobial activity compared to Group I.

Antioxidant Activity

Both formulations demonstrated consistent, dose-dependent antioxidant activity across all assays.

In the DPPH assay, both groups showed strong radical scavenging activity, though slightly lower than the standard, with minimal differences between groups.

Hydrogen peroxide scavenging revealed moderate activity in both groups, again slightly below the standard.

In the FRAP assay, both formulations exhibited strong reducing power, approaching standard values at higher concentrations (40–50 µg/mL).

Similarly, ABTS radical scavenging and nitric oxide inhibition assays demonstrated concentration-dependent increases in activity, with both groups showing comparable performance and maintaining high antioxidant potential.

Discussion

Antimicrobial Properties

The present study demonstrates that both formulations exhibit selective and concentration-dependent antimicrobial activity. The absence of inhibition against *Candida albicans* and *Enterococcus faecalis* suggests either intrinsic microbial resistance or limited diffusion of the incorporated antibiotic within the collagen matrix, particularly for biofilm-forming organisms. In contrast, significant activity against *Streptococcus mutans*, *Lactobacillus* spp., and *Staphylococcus aureus* highlights the effectiveness of collagen-based drug delivery systems against key oral pathogens.

The enhanced inhibition observed with increasing concentrations supports the concept of sustained local drug release from collagen scaffolds, which maintains effective antimicrobial levels at the target site. The slightly improved performance of the lignocaine-containing group suggests a possible adjunctive effect, although its primary role remains non-antimicrobial. These findings align with current biomaterial strategies where collagen serves not only as a structural scaffold but also as a localized therapeutic delivery system.

Antioxidant Potential

Both formulations exhibited strong antioxidant activity across all assays (DPPH, H_2O_2 , FRAP, ABTS, and nitric oxide), with a clear dose-dependent trend. The results confirm that collagen retains its intrinsic free radical scavenging ability even after incorporation of therapeutic agents. This is likely due to the presence of amino acids such as glycine, proline, and hydroxyproline, which facilitate electron donation and stabilization of reactive species.

The addition of antibiotics appeared to contribute modestly to antioxidant activity, consistent with reports of their ability to reduce reactive oxygen species and lipid peroxidation. Importantly, the inclusion of lignocaine did not compromise antioxidant performance, as both groups demonstrated comparable activity across assays. The observed nitric oxide scavenging further supports the anti-inflammatory potential of these formulations, given the role of nitric oxide in mediating inflammatory responses.

Limitations and Future Scope

A key strength of this study is the comprehensive in vitro evaluation using multiple complementary assays. However, in vitro findings may not fully replicate the complex oral environment, which involves immune responses, saliva, mechanical forces, and polymicrobial biofilms. Additionally, cytocompatibility, degradation behavior, and long-term clinical performance were not assessed.

Further in vivo and clinical studies are necessary to validate the safety, efficacy, and translational potential of these multifunctional collagen-based scaffolds in oral and periodontal regeneration.

Results:

Antimicrobial activity

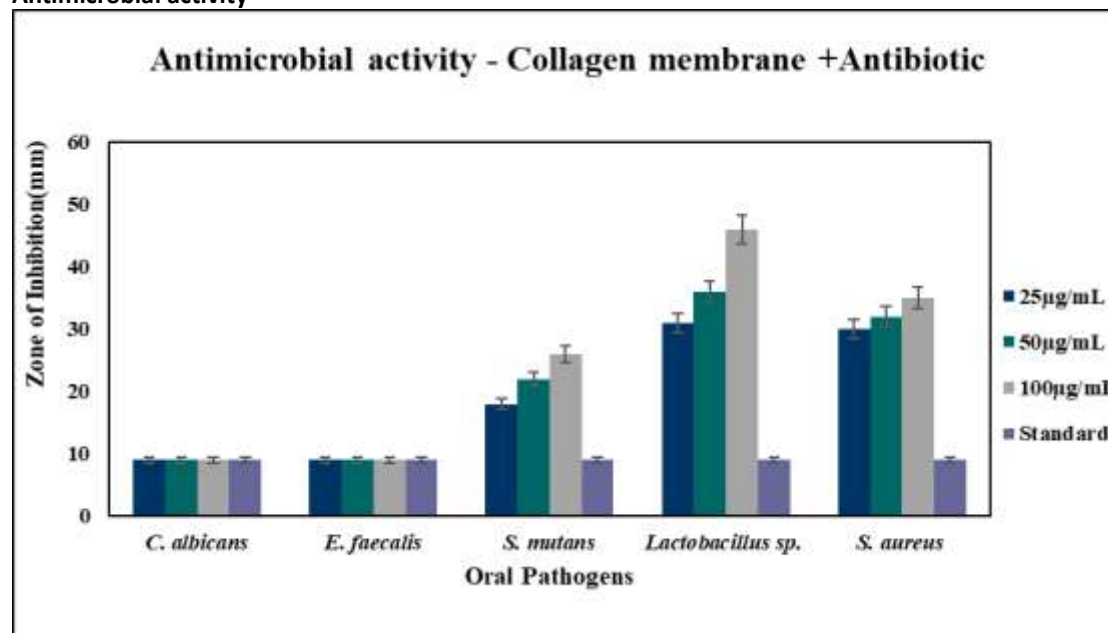


Figure 1: Graph representing antimicrobial efficacy of Collagen +Antibiotic

Table 1: Data of antimicrobial efficacy of Collagen +Antibiotic

Collagen +Antibiotic	25µg/mL	50µg/mL	100µg/mL	Standard
<i>C. albicans</i>	9	9	9	9
<i>E. faecalis</i>	9	9	9	9
<i>S. mutans</i>	18	22	26	9
<i>Lactobacillus</i> sp.	31	36	46	9
<i>S. aureus</i>	30	32	35	9

Collagen+Antibiotic+ Lignocaine:

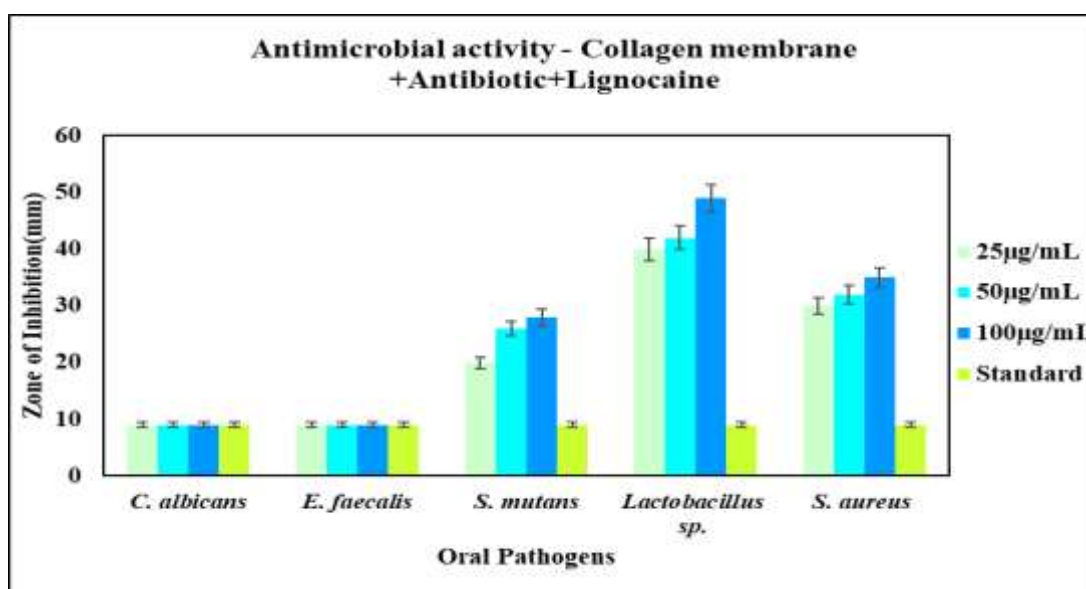


Figure 2: Graph representing antimicrobial efficacy of Collagen +Antibiotic+Lignocaine

Table 2: Data of antimicrobial efficacy of Collagen +Antibiotic +Lignocaine

Collagen +Antibiotic+Lignocaine	25µg/mL	50µg/mL	100µg/mL	Standard
<i>C. albicans</i>	9	9	9	9
<i>E. faecalis</i>	9	9	9	9
<i>S. mutans</i>	20	26	28	9

Lactobacillus sp.	40	42	49	9
S. aureus	30	32	35	9

Antioxidant activity:

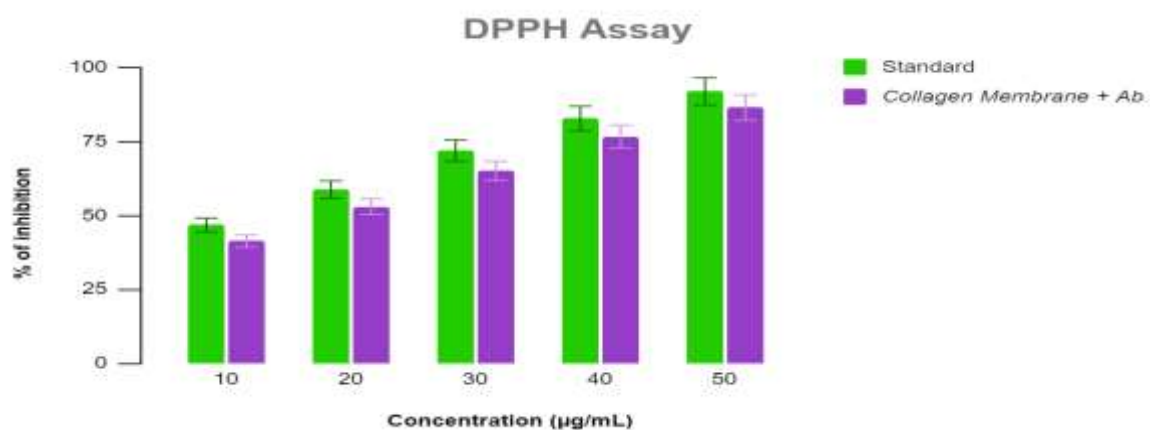


Figure 3: Graphical representation of DPPH Assay

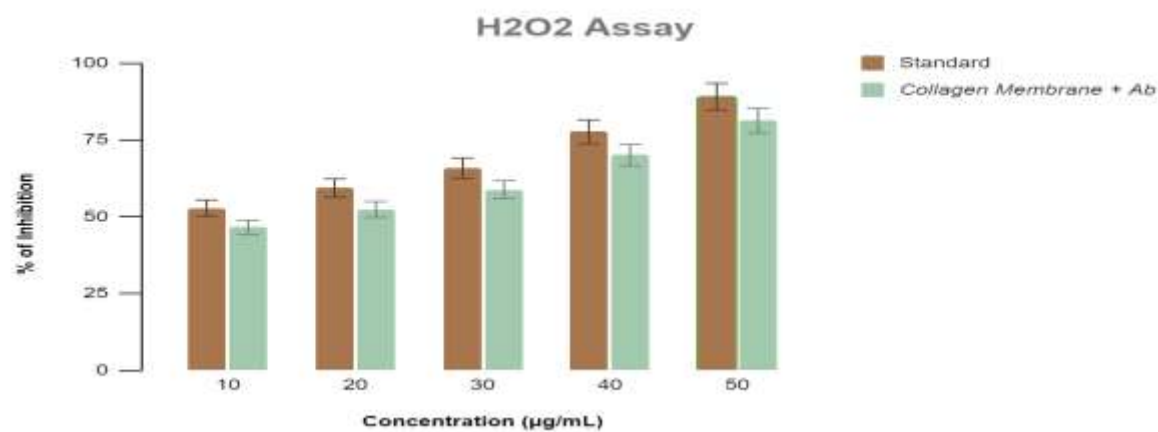


Figure 4: Graph representation of H2O2 Assay

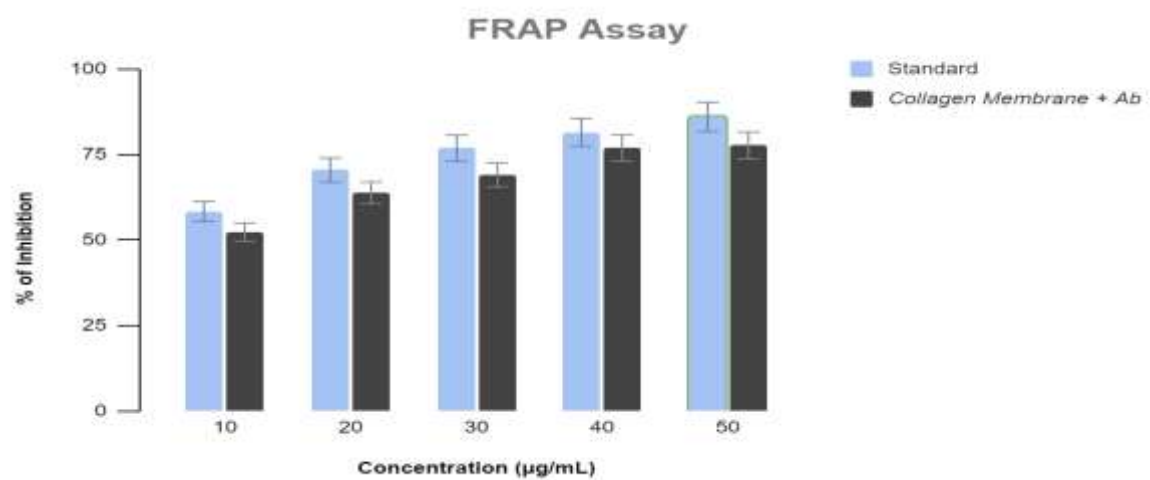


Figure 5: Graph representation of FRAP Assay

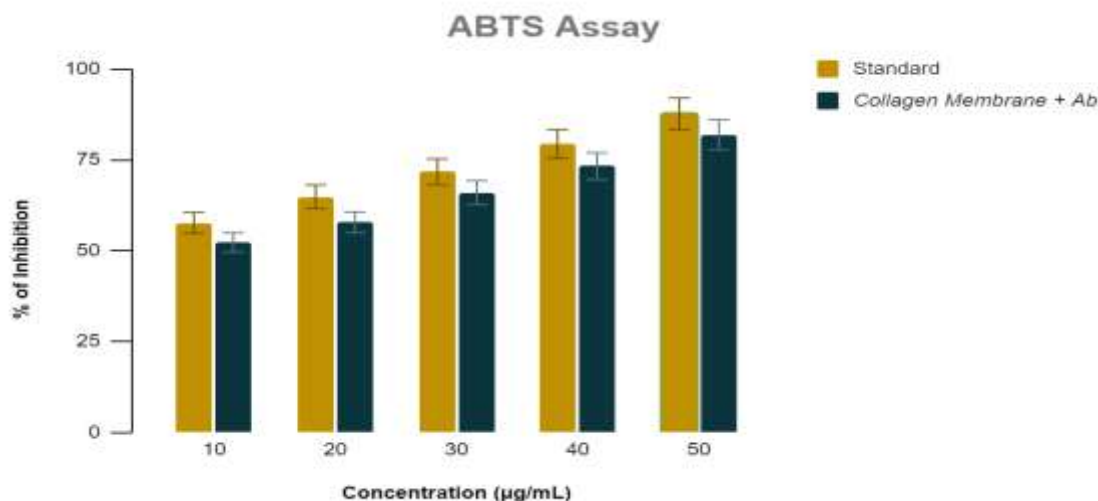


Figure 6: Graph representation of ABTS Assay

Conclusion

The modified collagen membranes showed specific antimicrobial effects against major oral pathogens like *Streptococcus mutans*, *Lactobacillus* species, and *Staphylococcus aureus*, with a lasting bactericidal impact, suggesting their ability to inhibit early wound colonisation. Both formulations demonstrated significant antioxidant capacity in various assays, with dose-dependent activity nearing that of standard antioxidants at elevated concentrations. These findings indicate that antibiotic- and lignocaine-infused collagen membranes could be multifunctional biomaterials for use in oral and periodontal regenerative procedures.

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