Controlled-Release Surface Coating to Prevent Ventilator-Associated Pneumonia on Endotracheal Tubes

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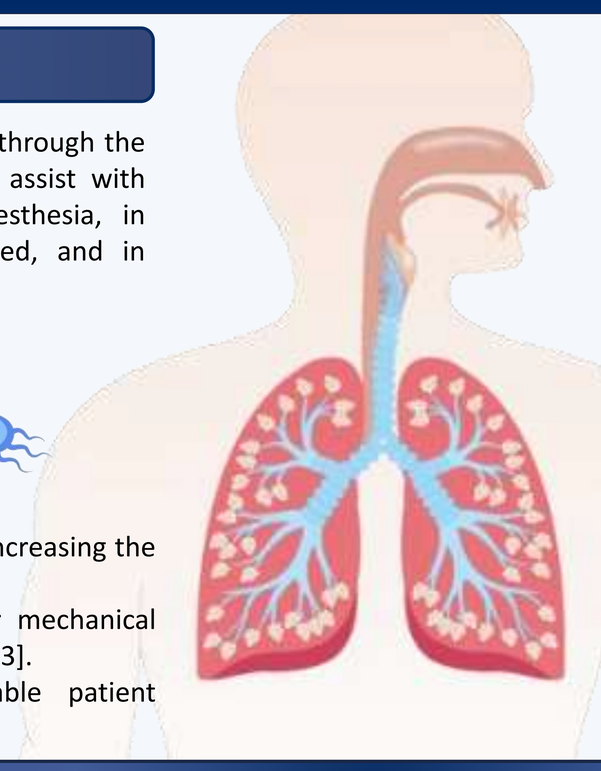
Background

The endotracheal tube (ET tube) is a flexible medical device inserted through the mouth or nose into the trachea to maintain an open airway and assist with mechanical ventilation. It is commonly used during general anesthesia, in emergency situations when spontaneous breathing is compromised, and in intensive care settings.

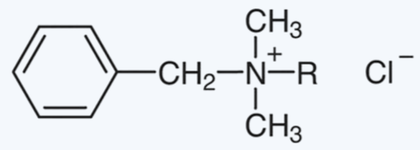
Figure 1. Endotracheal tube (ETT).

- ETs present a foreign surface, promoting bacterial colonization and increasing the risk of VAP [1].
- VAP is defined as pneumonia occurring 48 hours or more after mechanical ventilation and is often linked to the colonization of ETs by bacteria[2,3].
- Mortality rates for VAP can reach up to 76% in vulnerable patient populations[2,3].

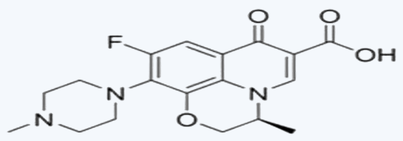


Introduction

This research focuses on developing a multilayered coating on a PVC surface, the primary material used to manufacture endotracheal tubes, enabling the sustained release of levofloxacin and benzalkonium chloride (BKA) [4]. These agents act synergistically to exert broad-spectrum antibacterial and anti-biofilm effects. This approach may offer a promising solution for preventing ventilator-associated pneumonia (VAP).

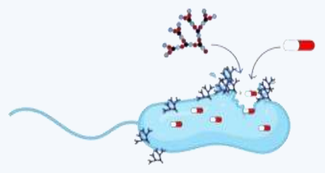


Benzalkonium Chloride (BKA)



Levofloxacin

Disruption of the bacterial membrane by BKA enhances drug uptake and bacterial eradication, while Levofloxacin penetrates the bacterial cell and inhibits DNA replication, leading to bacterial death.



Methods

Polyvinyl chloride (PVC) treatment

- Pyridine Treatment: Surface activation.
- Tin Sensitization: (tin (II) chloride and 37% hydrochloric acid) sensitizes the PVC surface.
- Silver Seeding: silver nitrate (AgNO3), the PVC surface becomes coated with tiny silver particles, acting as catalytic nuclei for further electroless metal deposition.

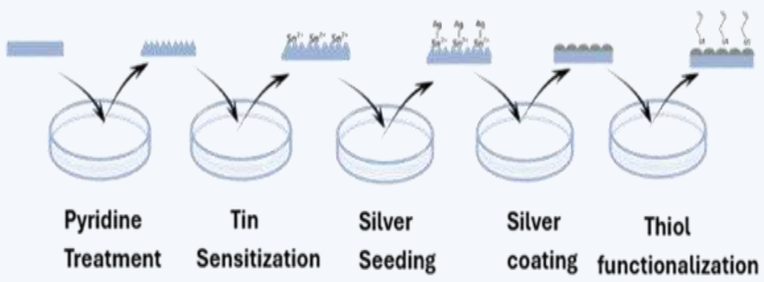


Figure 2: Surface Treatment of PVC: Silver Coating and Thiol Functionalization

- To create a silver layer on the pretreated PVC surface, samples were placed in freshly prepared Tollens' reagent for 15 minutes.
- To functionalize the Ag-PVC surface with a copolymerisable thiol, samples were placed in an ethanolic solution of allyl mercaptan (AM) for 24 hours.

Note: All procedures were performed in a fume hood

Hydrogel coating

Table 1. Formulations of hydrogel coatings.

Component /type	2-(HEMA)	MMA	(EGDMA)	P.I
P[HEMA]	9.6 g	0 g	0.1 g	0.1 g
P[HEMA-co-MMA(10%)]	8.6 g	1 g	0.1 g	0.1 g
P[HEMA-co-MMA(25%)]	7.3g	2.5 g	0.1 g	0.1 g

2-hydroxyethyl methacrylate (HEMA)

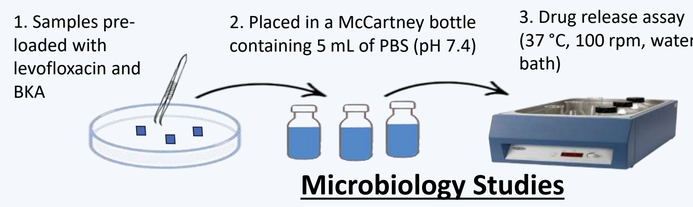
Methyl Methacrylate (MMA)

Figure 2. UV-Curing Chamber.

Ag-AM-PVC samples (1 cm x 1 cm) were each coated with 100 µl of hydrogel solution by dropping the solution onto the surface of the samples. Coated samples were then cured in a UV chamber for 4 hours to ensure complete polymerization.

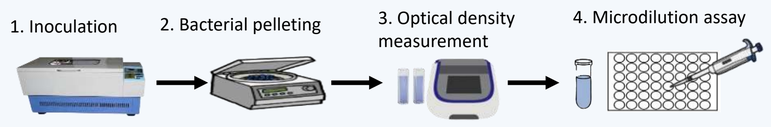
Drug Release Studies

Drug release from hydrogel-coated AM-Ag-PVC substrates was evaluated for levofloxacin and BAK, individually and combined, using a post-loading method with 5 mg/mL solutions in PBS (pH 7.4). Samples were incubated in PBS (pH 7.4) at 37 °C with shaking at 100 rpm. Drug release was quantified using UV-Vis spectrophotometry

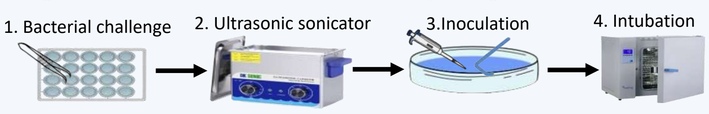


Microbiology Studies

The antimicrobial activity of levofloxacin and BAK against *S. aureus* and *P. aeruginosa* was evaluated via MIC, MBC, and MBEC assays. MIC and MBC were determined using broth microdilution.



Bacterial adhesion of *S. aureus* and *P. aeruginosa* on unmodified and surface-modified PVC was assessed by incubating hydrogel-coated samples with 1×10^6 CFU/mL suspensions at 37 °C for 4 and 24 h, followed by PBS rinsing, QSRS sonication, and viable count plating.



Results and Discussion

In Vitro Drug Release Studies

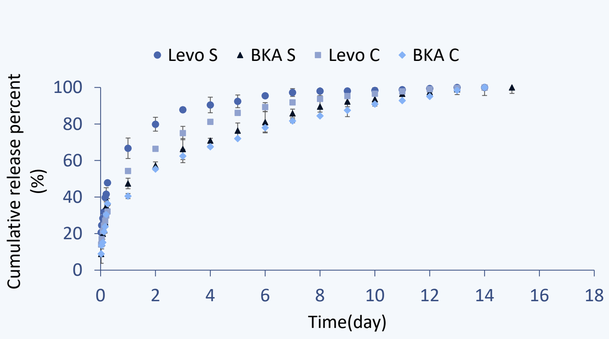


Figure 3. Release Profile of Levofloxacin and Benzalkonium Chloride as single and combination from p[HEMA]-AM-Ag-PVC, (Mean ± SD).

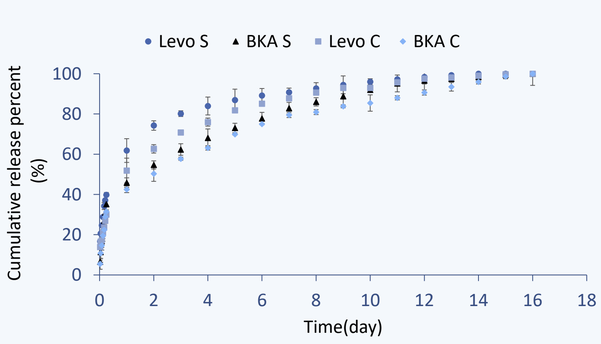


Figure 4. Release Profile of Levofloxacin and Benzalkonium Chloride as single and combination from p[HEMA-co-MMA(10%)]-AM-Ag-PVC, (Mean ± SD).

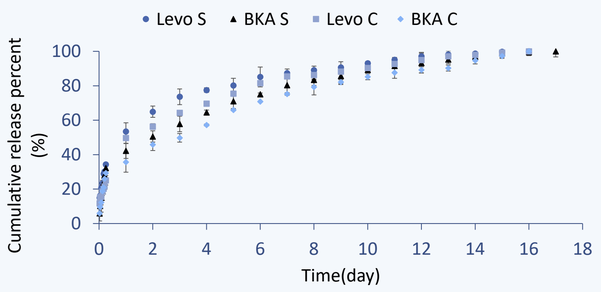


Figure 5. Release Profile of Levofloxacin and Benzalkonium Chloride as single and combination from p[HEMA-co-MMA(25%)]-AM-Ag-PVC, (Mean ± SD).

- Zwitterionic nature of levofloxacin enhances molecular interactions and hydration.
- Electrostatic interaction between levofloxacin (COO^-) and BKA (R_4N^+) forms temporary complexes.
- Dense hydration shell around complexes increases size and slows diffusion.

Biofilm Eradication Concentration Determination (MBEC)

- Levofloxacin exhibited significantly lower MBEC values than BKA, indicating greater biofilm eradication efficacy.
- Biofilm resistance was higher in *P. aeruginosa* due to its outer membrane and efflux pumps.
- BKA's reduced efficacy is linked to its electrostatic binding to biofilm matrix components (e.g., alginate, eDNA), which limits its availability to bactericidal effect.

Table 2. MBEC results of Levofloxacin and BAK against S. aureus and P. aeruginosa.

Bacterial species	Drug	MBEC (µg/mL)
S. aureus	Levofloxacin	1.46
	BKA	15.23
P. aeruginosa	Levofloxacin	5.8
	BKA	243.73

Evaluation of synergistic effects: Checkerboard assay

Table 3. FICI values for combinations of Levofloxacin and BKA.

Bacterial species	Antibacterial combination	MICs alone (µg mL^{-1})	MICs in combination (µg mL^{-1})	FICI	Outcome
S. aureus	Levofloxacin/BKA	0.18/1.904	0.011/0.01	0.066	Synergistic
P. aeruginosa	Levofloxacin/ BKA	0.73/60.9	0.04/0.115	0.056	Synergistic

- The Fractional Inhibitory Concentration Index (FICI) is a quantitative method to assess the interaction between two antimicrobial agents
- A FICI value less than 0.5 indicates a synergistic effect between the two compounds, while a value greater than 4 suggests antagonism. Values ranging from 0.5 to 4 imply an additive effect or no significant change in antibacterial activity.

Bacterial adherence assay

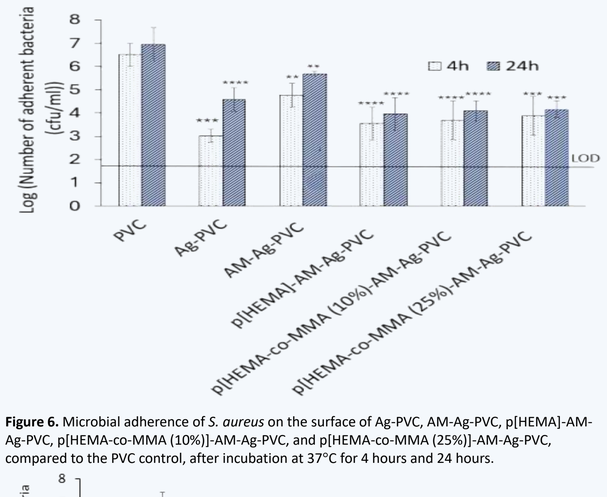


Figure 6. Microbial adherence of *S. aureus* on the surface of Ag-PVC, AM-Ag-PVC, p[HEMA]-AM-Ag-PVC, p[HEMA-co-MMA (10%)]-AM-Ag-PVC, and p[HEMA-co-MMA (25%)]-AM-Ag-PVC, compared to the PVC control, after incubation at 37°C for 4 hours and 24 hours.

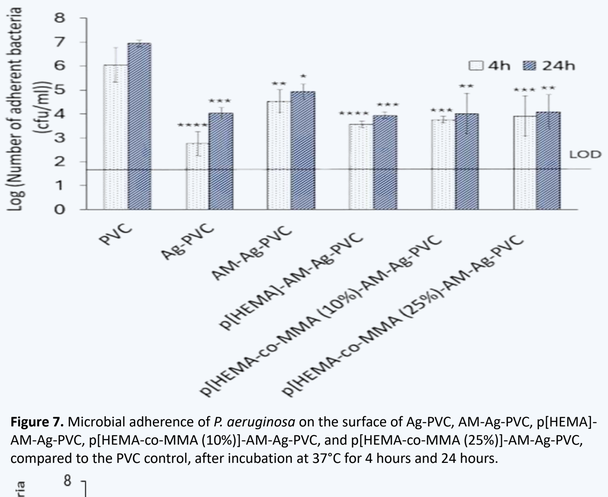


Figure 7. Microbial adherence of *P. aeruginosa* on the surface of Ag-PVC, AM-Ag-PVC, p[HEMA]-AM-Ag-PVC, p[HEMA-co-MMA (10%)]-AM-Ag-PVC, and p[HEMA-co-MMA (25%)]-AM-Ag-PVC, compared to the PVC control, after incubation at 37°C for 4 hours and 24 hours.

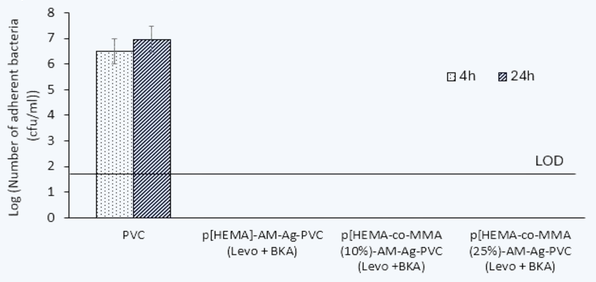


Figure 8. Microbial adherence of *S. aureus* on the surface of P[HEMA]-AM-Ag-PVC, P[HEMA-co-MMA (10%)]-AM-Ag-PVC, and P[HEMA-co-MMA (25%)]-AM-Ag-PVC, loaded with a combination of levofloxacin and BKA, compared to the PVC control, after incubation at 37°C for 4 hours and 24 hours.

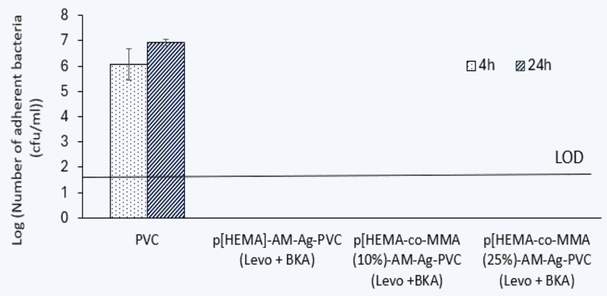


Figure 9. Microbial adherence of *P. aeruginosa* on the surface of P[HEMA]-AM-Ag-PVC, P[HEMA-co-MMA (10%)]-AM-Ag-PVC, and P[HEMA-co-MMA (25%)]-AM-Ag-PVC, loaded with a combination of levofloxacin and BKA, compared to the PVC control, after incubation at 37°C for 4 hours and 24 hours.

Conclusions

The multi-layered endotracheal tube coating demonstrates promising potential for preventing VAP through sustained drug delivery from a durable surface coating. AM-Ag-PVC exhibited superior adhesion and structural stability, while copolymerization with HEMA-MMA provided extended drug release profiles lasting up to 16 days, aligning with typical clinical intubation durations.

References

(1)Fernandez, Juan F., et al. *Chest*, 2012. https://doi.org/10.1378/chest.11-2420
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(3)Howroyd, Fiona, et al. *Nature Communications*,2024. https://doi.org/10.1038/s41467-024-50805-z
(4)Brodsky, Martin B., et al. *Critical Care Medicine*,2018. https://doi.org/10.1097/ccm.0000000000003368