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Research Protocol

Dentistry Section

Efficiency of the Combination of Amoxicillin and Metronidazole-coated Sutures versus Triclosan-coated Sutures for Antimicrobial and Wound Healing Properties: A Research Protocol

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ABSTRACT

Introduction: Surgical Site Infections (SSIs) pose a significant challenge in various surgical procedures, including periodontal surgery. Sutures used in the oral cavity are constantly exposed to saliva, which creates a wicking effect that can impede the healing process after surgery. To address these issues, modifications to sutures are necessary.

Need for the study: Coated sutures that provide local, sustained release of antibiotics may effectively prevent biofilm formation and reduce bacterial counts in the periodontal surgical area. These innovative sutures combine two drugs, which can help mitigate the wicking effect, promote faster healing, and provide effective antimicrobial action. To date, no clinical studies have evaluated such a combination in suture form, prompting the design of this animal study. Currently, triclosan-coated sutures are the only commercially available option, and the new combination sutures could serve as an alternative. There is a lack of comparative data regarding the antimicrobial and wound-healing potential of alternative antibiotic combinations, particularly in the context of periodontal surgery.

Aim: To evaluate and compare the efficacy of amoxicillin- and metronidazole-coated sutures versus triclosan-coated sutures in terms of antimicrobial activity and wound-healing properties.

Materials and Methods: This in vitro experimental study on rabbits will be conducted in the Department of Pharmacology at the Central Preclinical Research Facility, Datta Meghe College of Pharmacy, Sawangi (Meghe), Wardha, India, from April 2025 to December 2025. The study will be carried out in two phases. In Phase I, amoxicillin-and metronidazole-coated sutures will be prepared and their drug release evaluated. In Phase II, an animal study will be conducted. The study will include 12 New Zealand rabbits of both sexes, with an average weight of 2-4 kg. The rabbits will be categorised into two interventional groups, with six rabbits in each group. Rabbits in Group I will receive 4-0 sutures coated with the combination of amoxicillin and metronidazole in the interdental area after flap elevation. In Group II, 4-0 sutures coated with triclosan will be used to assess antimicrobial activity and wound healing. For inter and intragroup comparisons, unpaired and paired t-tests will be applied, respectively. A p-value of <0.05 will be considered statistically significant.

Keywords: Biofilm, Controlled release, Periodontal surgery, Postoperative complications, Surgical wound infection, Ultraviolet spectrometer

INTRODUCTION

Surgical Site Infections (SSIs) are the second most frequent postoperative complication, following urinary tract infections [1]. The initiation and progression of SSIs are influenced by both patient-related and procedure-related risk factors [2]. Contributing factors include the surgical site, level of wound contamination, quality of pre and postoperative care, and the clinical environment [3]. Sutures can serve as a critical point for infection, as bacteria may adhere to them, enter the wound through capillary action, and form biofilms, ultimately resulting in chronic infections. This process can lead to suture-associated infections driven by the growth of these attached pathogens. Therefore, successful periodontal surgery relies heavily on effective wound closure and, importantly, the prevention of bacterial contamination at the healing site.

Sutures placed in the oral cavity are constantly exposed to saliva containing approximately 7.5×10^8 microorganisms per milliliter, potentially serving as microbial reservoirs and increasing the risk of SSIs [4]. Periodontal surgical procedures inherently carry a risk of complications, including SSIs. According to nosocomial surveillance, the most common pathogens discovered at surgical sites of cutaneous infections are *Staphylococcus aureus*, *E. coli*, Coagulase-Negative Staphylococci (CoNS) and *enterococci* [5]. Additionally, a variety of oral flora, including *streptococci*, *Bacteroides*, *Fusobacteria*, *Prevotella* and *Porphyromonas gingivalis*, can impair oral wound healing and contribute to SSIs [6,7]. Bacterial adherence to sutures varies and is influenced by the suture's structure, the specific microbial species involved, and the chemical composition of the suture material [8]. Antibacterial sutures help prevent infection and promote wound healing, and drug-eluting sutures are particularly effective in this regard. While numerous studies have explored the control of SSIs in skin wound closures using antimicrobial sutures, research focusing on the oral mucosa in dentistry remains limited.

Several studies have investigated enhancing suture effectiveness by incorporating active ingredients that promote wound healing [9-11]. The United States Food and Drug Administration (FDA) approved triclosan-coated sutures in 2002 [12]. Although these sutures are widely used in various surgeries, triclosan has several drawbacks, including potential carcinogenicity, development of antibiotic resistance, and non-specific effects. Triclosan is a

phenoxyphenol compound that has been used in numerous daily products for almost thirty years. It exhibits bacteriostatic properties at low concentrations (0.025-1,000 mg/mL) and bactericidal action at high concentrations (7.5-1,000 mg/mL) [13].

Polyglycolic acid (Vicryl) is a commercially available, woven, absorbable synthetic suture material. While triclosan-coated sutures have proven effective against a wide range of infections, particularly those caused by *Staphylococcus aureus*, concerns remain regarding the development of triclosan-resistant bacteria [14]. Triclosan has target-specific activity, raises toxicity concerns, and can trigger allergic reactions.

Among the eight principal antibiotic groups used in periodontology, amoxicillin and metronidazole have been extensively evaluated and shown to yield excellent clinical outcomes with a significant reduction in pathogenic microbial flora [15]. To the best of our knowledge, no studies to date have investigated the efficacy of sutures coated with a combination of amoxicillin and metronidazole in comparison to triclosan-coated sutures for promoting wound healing and reducing microbial load to prevent postoperative SSIs.

REVIEW OF LITERATURE

Antibacterial-coated sutures and local drug delivery systems are gaining attention in periodontal therapy for their role in reducing infection and enhancing healing. Studies have shown that coated sutures lower bacterial load, while nanofiber-based antibiotic delivery improves clinical outcomes. The choice of suture material also impacts patient comfort and promotes faster recovery.

A study conducted by Mirzaeei S et al., on metronidazole- and amoxicillin-loaded Poly Lactic-co-Glycolic Acid (PLGA) and Polycaprolactone (PCL) nanofibers as potential drug delivery systems for the treatment of periodontitis (in-vitro and in-vivo evaluation) concluded that SE Scanning Electron Microscope (SEM) analysis of PLGA and PCL nanofibers loaded with drugs demonstrated uniform, bead-free morphology and good tensile strength. Cytotoxicity tests showed high cell viability (>80%), and High-Performance Liquid Chromatography (HPLC) analysis revealed nearly complete drug release within 7-9 days in vitro and 14 days in vivo. These findings suggest that nanofiber-based drug delivery systems significantly improve healing, reduce infection rates, and increase patient satisfaction across all time points ($p<0.05$). Patients also reported less pain and greater comfort. Vicryl Plus sutures demonstrated superior clinical and patient-centered outcomes and are suggested as a preferable option for Free Gingival Graft (FGG) fixation [16].

A comparative study evaluated the effectiveness of two antibacterial-coated resorbable sutures- triclosan-coated and chlorhexidine-coated- against non-coated resorbable sutures in a periodontal flap surgical procedure. While improvements in healing index and pain scores were statistically insignificant, a significant reduction in anaerobic bacterial concentration was observed in the antibacterial-coated groups compared to the non-coated group. Confocal laser scanning microscopy revealed a higher presence of viable bacteria on non-coated sutures, reinforcing the antimicrobial benefit of coated variants [17].

A study conducted by El Sharaki A et al. in 2023 compared the clinical outcomes of FGGs stabilised using silk sutures versus Vicryl Plus sutures in 60 patients with gingival recession. Graft healing, infection rates, and patient-reported outcomes were assessed at one week, one month, and three months postoperatively [18]. The results showed that at the three-month follow-up, the Vicryl Plus sutures group demonstrated significantly better graft healing compared to the silk sutures group. Additionally, a study in rats investigating the efficacy of a local drug delivery system for experimental periodontitis demonstrated significantly improved periodontal clinical parameters with a controlled-release gel containing a novel combination of amoxicillin and metronidazole nanoparticles [19].

This study aims to evaluate and compare the efficiency of amoxicillin- and metronidazole-coated sutures with triclosan-coated sutures in terms of antimicrobial properties and wound healing.

Primary objectives:

- To formulate an amoxicillin-metronidazole combination suture using an immersion method.
- To investigate drug release under static conditions using a UV-Visible spectrophotometer.
- To assess the antimicrobial activity of amoxicillin-metronidazole-coated sutures at baseline and on the 7th day using the blood agar diffusion test.
- To evaluate the antimicrobial activity of triclosan-coated sutures at baseline and on the 7th day using the blood agar diffusion test.

Secondary objective:

- To compare the antimicrobial activity and wound healing potential of amoxicillin-metronidazole-coated sutures with triclosan-coated sutures at baseline and on the 7th day using the blood agar diffusion test.

Null hypothesis: There is no difference in the efficacy of amoxicillin-metronidazole-coated sutures and triclosan-coated sutures on tissue healing and bacterial load reduction to prevent SSIs.

Alternative hypothesis: Amoxicillin-metronidazole-coated sutures may be a preferable choice over triclosan-coated sutures for reducing bacterial load and enhancing intraoral tissue healing at the surgical site after periodontal flap surgery.

MATERIALS AND METHODS

An experimental animal study will be conducted at the Central Preclinical Research Facility, Datta Meghe College, Maharashtra, India, from April 2025 to December 2025. The study has been approved by the Institutional Ethical Committee (Ethical approval number: DMIHER/IAEC/24-25/46). References to the Guide to the Care and Use of Experimental Animals will be followed, as set forth by the Committee for Control and Supervision of Experiments on Animals (CCSEA) [20].

The preparation of amoxicillin-metronidazole-coated sutures in vitro will be carried out in the Department of Pharmacy at Datta Meghe College (Sawangi), Wardha. Polyglactin 910 suture material (Vicryl Plus or triclosan suture) will be purchased from Ethicon (Johnson & Johnson), and metronidazole (Zeta, scientific code Z003394) will be procured from Shree Sai Enterprises, Thakurli.

Polyglactin material will be treated with 1% (w/w) sodium hydroxide to remove surface impurities, followed by thorough washing with deionised water. Polymers coating on of antibiotic s75mg (Trimox) and metronidazole (Flagyl) suture materials will be done, followed by the incorporation 60mg per gram of suture material.

The polyglactin material will be treated with 1% (w/w) sodium hydroxide to remove surface impurities, followed by thorough washing with deionised water. Antibiotic coating will be applied using amoxicillin (Trimox) and metronidazole (Flagyl) at a concentration of 60 mg per gram of suture material. Drug add-on percentage will be calculated and charted. To assess drug release, coated sutures will be placed in sample tubes and analysed for drug concentration using a UV-Visible spectrophotometer (Shimadzu UV 1900). Sample tubes will be replaced with fresh water, which will be tested for drug concentration the following day. This procedure will continue for a total of seven days to evaluate static drug release [21]. The prepared coated sutures will then be sterilised using gamma radiation and packed in sterile pouches.

Inclusion criteria:

- Healthy rabbits of both genders with a body weight of 2-4 kg.

Exclusion criteria:

- Sick or underweight rabbits.
- Pregnant rabbits.

Study Procedure

A total of 12 rabbits will be randomly divided into two groups, each containing six rabbits. The rabbits will be housed individually, with a regulated light/dark cycle, temperature of 16-22°C, and humidity levels of 30-70%. All animals will be provided with a standard diet and allowed to acclimatise for at least seven days before the experiment. All animals will be weighed, and identification marks will be assigned to each rabbit.

Animals will be anesthetised, and an incision will be performed between two teeth in the upper or lower jaw area. The flap will be carefully elevated to confirm separation of the underlying tissue. The incision will then be closed using amoxicillin-metronidazole-coated sutures in Group I and triclosan-coated sutures in Group II. Soft, easily digestible food and appropriate medications will be provided to ensure the health and well-being of the rabbits.

Outcomes: The wound healing process will be evaluated at 7, 14, and 21 days, based on the wound healing criteria described by Landry RG et al., [22]:

1. **Very Poor:** Tissue abnormality (≥50% red gingiva), bleeding on palpation, granulation tissue present, incisional edge non-epithelialised with loss of epithelial coverage, and purulence present.
2. **Poor:** Tissue color ≥50% red, bleeding on palpation, granulation present, edge non-epithelialised with exposed connective tissue.
3. **Good:** Tissue redness <25%, no bleeding on palpation, no granulation, no connective tissue exposure.
4. **Very Good:** Tissue redness <25%, no bleeding, no granulation, incisional edge intact without connective tissue exposure.
5. **Excellent:** Pink tissue, no bleeding, no granulation, incisional edge intact with no connective tissue exposure.

The wound healing healing assessment will be done on 7,14, and 21 days.

Microbiological assessment: On the 7th day, one part of the suture with maximum deposit will be cut and transported in a thioglycolate carrier medium to the Department of Microbiology for analysis of *Staphylococcus aureus* and *E. coli*. Microbiological parameters will be determined using the agar diffusion test.

STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS) Version 27 will be used for data analysis. Mean and standard deviation values will be calculated for all parameters at a 95% confidence interval. For inter and intragroup comparisons, unpaired and paired t-tests will be applied. A p-value of less than 0.05 will be considered statistically significant.

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