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Comparative Evaluation of Sterilization Methods for Selected Orthodontic Materials: An *In-Vitro* Microbiological Study

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Abstract

Background: Orthodontic auxiliaries are frequently reused and may act as potential sources of cross-infection if not adequately sterilized. Limited comparative data exist regarding the effectiveness of commonly used sterilization methods for orthodontic materials. ***Aim:*** To evaluate and compare the effectiveness of different sterilization and disinfection methods in eliminating microbial contamination from selected orthodontic materials. ***Materials and Methods:*** This in-vitro microbiological study evaluated 48 orthodontic samples including NiTi closed coil springs, pre-formed molar bands with buccal tubes, and Class II elastics. Samples were divided into five groups: control, 70% ethanol, 2% glutaraldehyde, ultraviolet (UV) irradiation, and autoclaving. Following sterilization, all specimens were cultured on tryptic soy agar and incubated at 37°C for 72 hours. Microbial growth was assessed visually. Statistical analysis was performed using chi-square test. ***Results:*** All unsterilized samples demonstrated microbial growth. No microbial growth was observed in any samples treated with 70% ethanol, 2% glutaraldehyde, UV irradiation, or autoclaving. Statistically significant differences were observed between control and treated groups (Chi-square = 8.778, p = 0.003). ***Conclusion:*** All evaluated sterilization methods were effective in eliminating microbial contamination from orthodontic materials. Autoclaving and glutaraldehyde immersion are recommended as primary methods, while UV irradiation and ethanol can serve as adjunctive alternatives.

Keywords: Orthodontic materials, sterilization, UV disinfection, glutaraldehyde, autoclave, infection control.

INTRODUCTION

Effective infection control is essential in orthodontic practice to ensure patient safety and optimal treatment outcomes. Orthodontic procedures involve repeated intraoral use of appliances and instruments, increasing the risk of microbial contamination and cross-infection. Consequently, appropriate sterilization and disinfection of orthodontic materials are critical components of routine clinical practice. [1-3]

Orthodontic components such as brackets, bands, archwires, elastics, and auxiliary instruments are frequently handled and reused during treatment. Inadequate sterilization of these items may lead to transmission of microorganisms between patients and healthcare personnel. Therefore, material-specific sterilization protocols are necessary to minimize cross-contamination in contemporary orthodontics.

Orthodontic appliances become contaminated through exposure to saliva, blood, and dental plaque. Previous studies have demonstrated microbial contamination on both clinically used materials and those obtained directly from manufacturers, indicating contamination may occur before clinical use. [6-8]

Various sterilization methods including steam sterilization, ultraviolet irradiation, and chemical disinfectants such as ethanol and glutaraldehyde have been recommended. However, orthodontic materials

differ in heat tolerance and surface characteristics, making selection of appropriate sterilization methods challenging. [15-24]

Therefore, this study aimed to evaluate and compare commonly used sterilization and disinfection methods for orthodontic materials.

MATERIALS AND METHODS

This in-vitro microbiological study evaluated three orthodontic materials: NiTi closed coil springs, pre-formed molar bands with buccal tubes, and Class II elastics. A total of 48 samples were included. Samples were divided into five groups: control, 70% ethanol, 2% glutaraldehyde, UV irradiation, and autoclave sterilization.

Autoclaving was performed at 121°C and 15 psi for 30 minutes. UV irradiation was applied for 15 minutes on each side. Samples treated with 70% ethanol were immersed for 1–3 minutes, while 2% glutaraldehyde immersion was performed overnight. After treatment, samples were cultured on tryptic soy agar and incubated at 37°C for 72 hours(table-1). Microbial growth was recorded. Statistical analysis was performed using chi-square test with significance level set at $p \leq 0.05$.

METHODOLOGY

The study will use the following technique:

Upon inspection, none of the companies had provided any information regarding sterility in their packaging. Using a sterile method, the packages were unsealed, revealing their contents. NITI coil springs, pre-formed molar bands with a buccal tube, and class II elastics (individually) for the samples before testing. The 3 material samples were split up into the following 5 groups for sterilization and disinfection (Table 2). Institutional Review Boards (IRBs) provided their ethical approval.

Examining Microbial Development with Normal Daylight on Material:

After sterilization, the orthodontic materials were cultured for three days at 37°C on a Tryptic Soy Agar (TSA) plate. TSA is a multipurpose growth medium that is perfect for the growth of gram-positive and gram-negative bacteria. Included are agar, sodium chloride, yeast extract, soybean meal, and a pancreatic digest of casein. Following that, a camera was used to take pictures of the microbial development on the plates.

Table No. 1: Sterilization instruments

Sterilization/Disinfection Method	Manufacture	Description
Steam autoclave	UNICLAV C-79B	Every sample underwent a 30-minute autoclave at 121°C and 15 PSI of pressure.
UV sterilization	UV cabinet	The samples underwent a 15-minute UV treatment in the chamber. (both sides)
70% ethyl alcohol	SLA chemicals	Items were submerged in 70% ethanol for one to three minutes, then cleaned with PBS and dried with sterile filter paper.
2% glutaraldehyde	SLA chemicals	Samples were submerged in 2% glutaraldehyde for an entire night. dried with sterile filter paper after being cleaned with PBS.

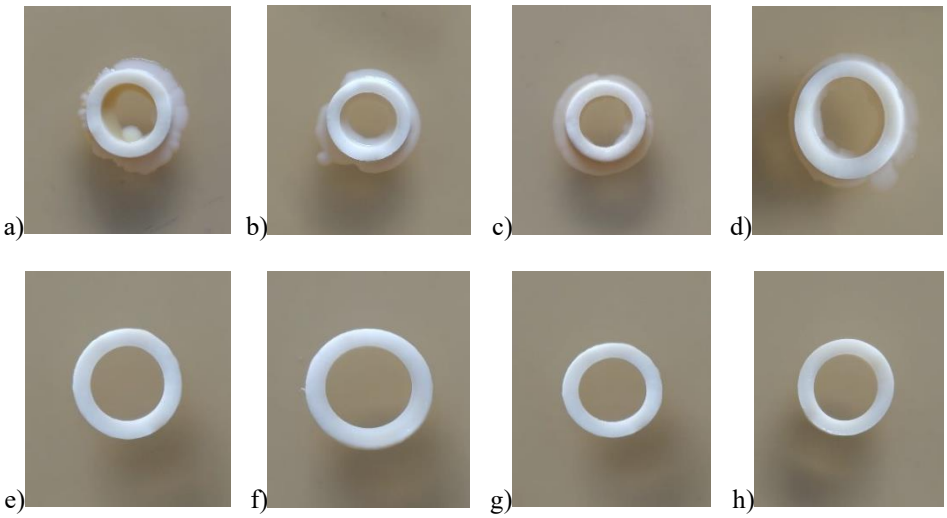

Figure 1: Determination of the bacterial contamination of class II elastics

The class II elastics were sterilized with different methods as described in the materials and methods, and control was placed on the TSA agar plate and incubated at 37°C for 3 days. The class II elastics

were then photographed using a camera. (a-d) Representative class II elastics without sterilization; bacterial growth was seen. (d-i) The non-sterilized class II elastics showed bacterial contamination.

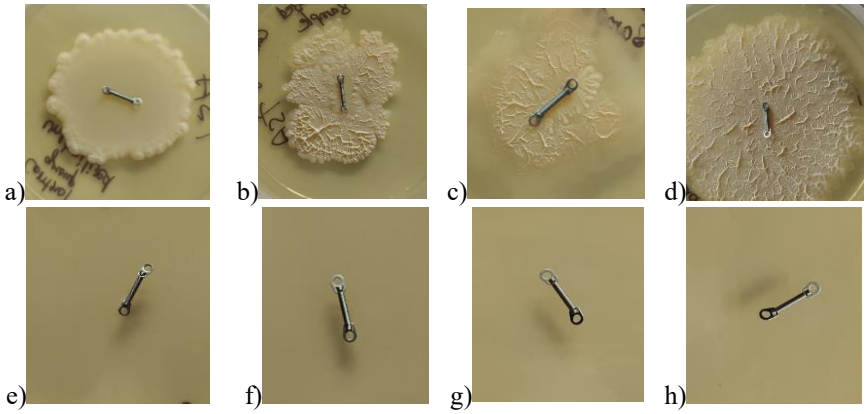

Figure 2: Determination of the bacterial contamination of the closed coil spring

The closed coil spring was sterilized with different methods as described in the materials and methods, and control was placed on the TSA agar plate and incubated at 37°C for 3 days. The closed coil spring was then photographed. (e-h) Representative of the closed coil spring of different sterilization methods; no bacterial growth was seen. (a-d) The non-sterilized closed coil spring showed bacterial contamination.

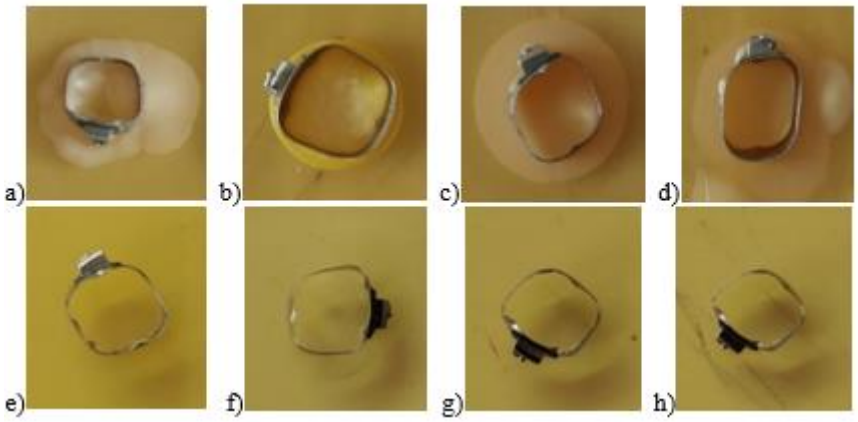

Figure 3: Determination of the bacterial contamination of the pre-formed molar bands with the buccal tube

The pre-formed molar bands with the buccal tube of sterile and non-sterile samples cultured using TSA agar then incubated for 3 days and then photographed. (e-h) Representative of the pre-formed molar bands with buccal tube of different sterilization methods; no bacterial growth was seen. (a-d) The non-sterilized pre-formed molar band with buccal tube showed bacterial contamination.

Table No. 2: Master Sheet Or Observation Sheet

Materials used	**Company**	**Sample size**	**Sample tested**	**Sterilization method**	**Growth Or no growth**
Pre-formed molar bands with buccal tube	Koden	Individual	4 3 3 3 3	None 70% ethanol 2%glutaraldehyd UV light Auto clave	G NG NG NG NG
Class II elastics	Orthometric elastomers Intraoral elastics	Individual Medium 1/4	4 3 3 3 3	None 70% ethanol 2%glutaraldehyd UV light Auto clave	G NG NG NG NG
Niti coil springs	U orthodontics	Length 9mm Diameter: 0.010	4 3	None 70% ethanol	G NG

Materials used	Company	Sample size	Sample tested	Sterilization method	Growth Or no growth
		individual	3	2%glutaraldehyd	NG
			3	UV light	NG
			3	Auto clave	NG

RESULTS

All unsterilized samples demonstrated microbial growth. In contrast, no microbial growth was observed in samples treated with 70% ethanol, 2% glutaraldehyde, UV irradiation, or autoclaving(table-2) [Fig. 1(a-h),2(a-h),3(a-h)].

For pre-formed molar bands, Class II elastics, and NiTi closed coil springs, identical patterns were observed, with complete elimination of microbial contamination in all treated groups. Statistical analysis revealed significant differences between control and sterilized groups (Chi-square = 8.778, p = 0.003).

DISCUSSION

Reusable orthodontic auxiliaries are exposed to contamination during manufacturing, packaging, and clinical handling. Previous studies have reported microbial contamination on orthodontic materials received directly from manufacturers [2,3,4,14,17,20,44]

The present study demonstrated that autoclaving, UV irradiation, 70% ethanol, and 2% glutaraldehyde were equally effective in eliminating microbial contamination. These findings are consistent with previous investigations reporting effective decontamination using chemical and physical sterilization methods. [1,9,11]

Autoclaving remains the gold standard for sterilization of heat-resistant materials, while glutaraldehyde provides an effective alternative for heat-sensitive components. UV irradiation offers a rapid non-contact method with minimal effect on material properties. [1,32,39,41]

Limitations of the study include small sample size, in-vitro design, and absence of quantitative microbial analysis. Further studies evaluating repeated sterilization cycles and material property changes are recommended.

CONCLUSION

All sterilization methods evaluated—70% ethanol, 2% glutaraldehyde, UV irradiation, and autoclaving—successfully eliminated microbial contamination from orthodontic materials. Autoclaving and glutaraldehyde immersion are recommended as primary methods, while UV irradiation and ethanol may serve as adjunctive alternatives. Adoption of standardized sterilization protocols can reduce cross-infection risk and improve patient safety in orthodontic practice.

Authors Contribution:

Dr. K. Loganathan, Dr. Atul Kumar Singh, Dr. Omkar Singh Yadav: Critical review and Experimental design; Dr. Anbarasu, Dr. Tiapongamri, Dr. Apoorv Tomar, Dr. Taruna Pratap Singh, Dr. Ankita Sarkar: Literature search and manuscript writing.

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