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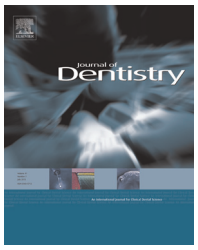

Effect of sterilization techniques prior to antimicrobial testing on physical properties of dental restorative materials

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ABSTRACT

Objectives: The aim of this study was to investigate any changes to the microstructure and surface properties of selected dental materials after sterilization carried out prior to subjecting them to antimicrobial testing. Initial microbial contamination on the material, as well as other possible sources of contamination were also assessed.

Methods: The materials investigated included dentine replacement materials Chemfil Superior®, Ionoseal®, Dyract Extra® and SDR®. The materials were characterized by scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS). The test materials were sterilized using alcohol, steam, ultraviolet light (UV) and ethylene oxide and any changes to these materials were then assessed by SEM, microhardness testing and Fourier transform infrared (FT-IR) spectroscopy. Material microbial levels before treatments were assessed by plate counting technique and turbidity tests. Possible contamination through dispensers was assessed by analysing the CFU/sample.

Results: Ethylene oxide affected the microstructure of the Chemfil, Ionoseal and Dyract, resulting in flattening of the Si–O stretching vibrations and deposition of chlorine and calcium respectively in Chemfil and Dyract. Varied contamination was demonstrated on all materials when incubated in anaerobic conditions.

Conclusions: The different sterilization techniques affected the microstructure of the materials under investigation. Samples of materials produced in sterile conditions could also be contaminated with bacteria, either from the material itself or through the dispensing apparatus.

Clinical significance: Results of antimicrobial studies cannot be extrapolated clinically as the material sterilization treatment results in changes to material chemistry and microstructure, which could in turn affect the materials' antimicrobial activity.

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1. Introduction

Q2 Assessing antimicrobial and anti-biofilm properties of dental materials continues to gain prominence, with over 170 publications on the subject since 1987.[1] Materials with an anti-biofilm effect are thought to improve health and prevent occurrence of diseases such as caries, periodontitis and peri-implantitis, which are all caused by biofilm formations.[1] Different methods have been used to assess antimicrobial properties of dental restorative materials. These include the agar diffusion test,[2–4] changes in optical density,[5–7] determination of colony forming units,[8–10] determination of maximum or minimum inhibitory concentration (MIC),[11–13] minimum bactericidal concentration,[13] direct contact test,[14–16] SEM,[10,17,18] and through viability staining.[13,19,20] The material sample is usually subjected to a disinfection or sterilization treatment prior to antimicrobial testing to a particular organism, to prevent contamination by other organisms.[21] Sterilization methods used include ethylene oxide gas, ultraviolet (UV), gamma irradiation and ethanol.[6,7,11,21–24] Although several investigations have been carried out to assess microbial activity after sterilization, the impact of different sterilization techniques on the dental materials' bulk and surface properties is generally ignored. Also no previous studies document initial contamination levels of dental restorative materials prior to antimicrobial testing and therefore whether the practice of sterilization of the material prior to testing is truly necessary.

Recent work has shown that sterilization methods can affect general material properties, including composites used in medical implants.[25] Steam sterilization has been claimed to cause extensive material degradation because of the high temperatures required for the process. Furthermore, steam has been shown to degrade polyurethane and affect dimensional stability of polyethylenes.[26] High-energy irradiation such as gamma also causes degradation of materials by increasing the temperature of the polymer but also because ionizing γ-radiation has sufficient energy to cause radiolysis damage and break carbon chemical bonds.[27] Use of ethylene oxide has therefore been suggested for temperature, moisture and radiation sensitive products.[27] Ethylene oxide does not affect material properties but was found to be ineffective in sterilizing nano-composites; on the other hand autoclaving appears to be a more suitable technique with no resultant degradation of material and successful sterilization was reported.[25]

Very little work has been published on effects of disinfection or sterilization procedures on dental restorative materials. Exposure to ethanol has been shown to affect physical properties of dental composites composed of both methacrylate and silorane resins.[28] Exposure of dental materials to high temperatures by immersion in hot solution containing ethanol results in higher water sorption, and increased degree of conversion and polymerization of composites.[8] Gamma radiation has also been shown to lead to an increase in the resins' conversion degree, due to γ-radiation's higher penetration power compared to that of visible light. As a result exposure to γ-radiation was found to increase surface hardness and decrease water sorption and solubility.[21]

Sterilization of glass ionomers, resin composite and their hybrids may result in a modification of their microstructure, chemical composition and surface properties. Changes in material properties would result in a different material to what is originally tested and therefore results may not be extrapolated clinically. The aim of this study was to assess whether sterilization methods prior to antimicrobial testing could modify the properties of these material. The need to sterilize the materials prior to antimicrobial testing was also assessed as no documentation was found on whether commercially available materials can be contaminated with bacteria, even when handled in sterile conditions.

2. Materials and methods

The dentine replacement materials investigated in the study included:

Chemfil Superior (Dentsply, Addlesone, UK) – a glass ionomer cement;

Ionoseal (Voco, Briarcliff Manor, NY, USA) – a resin modified glass ionomer cement;

Dyract Extra (Dentsply Caulk, Milford, DE, USA) – a compomer;

Smart Dentine Replacement (SDR; Dentsply Caulk, Milford, DE, USA) – a dentine replacement composite resin.

The composition of the tested material as indicated by the manufacturers is shown in Table 1. The materials were mixed according to manufactures' instructions.

The following sterilization procedures were undertaken in order to sterilize the set materials:

- Sonication with 70% ethanol for 10 min (Clean 35, Ultrasonic Cleaner, Disseptim, Turkey).[11,25]
- Ethylene oxide gas (ACECIL, Campinas, Sao Paolo, Brazil).[7,22,24,29]
- Ultraviolet (UV) light: 256 μm for 60 min (Bio Class I, Contained air solutions, United Kingdom).[23]
- Autoclaving: setting no. 3 – packed samples (Self-Seal sterilization pouches, Technicaland General, London, United Kingdom) 121 °C for 30 min (Domina, Bowie and Dick, London, United Kingdom).[25]

2.1. *Characterization of set materials before and after sterilization*

Materials ($n = 3$ per group) were prepared according to manufacturer instructions and after setting the four materials were sterilized via the four methods described in the methodology for material sterilization section. Characterization was then carried out by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). Infrared spectroscopic analysis was also performed. Unsterilized samples were used as a control.

2.1.1. *Scanning electron microscopy and energy dispersive X-ray spectroscopy*

Cylindrical specimens ($n = 3$ per group) 10 mm in diameter and 2 mm thick were prepared from each material type. The specimens were mounted on aluminium stubs, carbon coated via evaporation of high purity carbon rods and viewed under a scanning electron microscope (Zeiss MERLIN Field Emission

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