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Effects of different sterilization methods on surface characteristics and biofilm formation on zirconia *in vitro*

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ABSTRACT

Objective. The current laboratory study was to investigate the effect of different sterilization treatments on surface characteristics of zirconia, and biofilm formation on zirconia surface after exposure to these sterilization treatments.

Methods. Commercially available zirconia discs (Cerconbase, Degu-Dent, Hanau, Germany) were prepared and polished to the same value of surface roughness. The discs were treated with one of the following sterilization methods steam autoclave sterilization, dry heat sterilization, ultraviolet C (UVC) irradiation, and gamma (γ) ray irradiation. The characteristics of zirconia surfaces were evaluated by scanning electron microscopy (SEM), surface roughness, surface free energy (SFE), X-ray photoelectron spectroscopy (XPS), and X-ray diffraction (XRD) measurements. Then, *Staphylococcus aureus* (*S.a.*) and *Porphyromonas gingivalis* (*P.g.*) bacteria were used and cultured on the respective sterilized zirconia surfaces. The amount of biofilm formation on zirconia surface was quantified by colony forming unit (CFU) counts.

Results. Significant modifications were detected on the colour and SFE of zirconia. The colour of zirconia samples after UVC irradiation became light yellow whilst dark brown colour was observed after gamma ray irradiation. Moreover, UVC and gamma ray irradiation increased the hydrophilicity of zirconia surface. Overall, dry heat sterilized samples showed the significantly lowest amount of bacteria growth on zirconia, while UVC and gamma ray irradiation resulted in the highest.

Significance. It is evident that various sterilization methods could change the surface which contribute to different biofilm formation and colour on zirconia.

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1. Introduction

Zirconia ceramic is an attractive material for dentistry and considered a material of choice. For example, metal implants may reveal a bluish discoloration of the overlying gingiva when apical bone loss and gingival recession occurred. However, a full zirconia implant avoids this aesthetic problem because of its more tooth-like colour [1]. Moreover, zirconia is also popular due to its high flexural strength and toughness [2].

Conclusions from different studies about zirconia implants application and performance are controversial. A retrospective clinical study by Roehling et al. investigated the clinical performance of zirconia implants up to and after 7 years of loading, and concluded that first-generation zirconia implants showed low overall survival (77.3%) and considered all surviving implants, the success rate was 77.6% [3]. Another study by Hashim et al. concluded that although the overall survival rate was 92% for zirconia implants after 1 year of function, further clinical studies are required to establish long-term results, and to determine the risk of technical and biological complications [4]. However, some studies have reported that zirconia implants have similar osseointegration capability compared to conventional titanium implants [5–7], and they could potentially be the alternative to titanium implants for a non-metallic implant solution. Therefore, zirconia has been increasingly used in implant dentistry due to its aesthetic performance [8], as well as good mechanical properties [9] and biocompatibility [10].

Implants, as surgical components that have intimate contact with bone, need to be properly sterilized prior to the implantation — or during the storage. Sterilization is considered as the final finishing procedure during the manufacturing process, because it may affect the implant surface and its modification, *i.e.*, the physico-chemical properties of implant surface might be changed and thus have an important clinical impact [11,12]. Moreover, sterilization is also applied as an essential step before *in vitro* biological tests, because it is a process enabling the device to be free from viable microorganisms, preventing the proliferation and accumulation of unrelated microorganism that we do not want to focus on [13].

There are many issues to be considered when selecting a sterilization method for a particular application condition, *e.g.*, cost and effectiveness. The ultimate goal is to sterilize implant materials and devices properly without compromising their key surface characteristics that may influence their interaction with surrounding tissue [14]. Various sterilization methods have been used in dentistry, depending on the desired application and material properties [15,16], such as steam autoclaving and γ-irradiation are the most commonly used sterilization processes for implant materials storage since they safe with respect to chemical contamination of the surface [15]. Dry heat and UV sterilization has been for dental implements, *e.g.*, reamer, drill [17–19]. In addition, enhanced osteoblast function has been confirmed on ultraviolet light-irradiated zirconia [20].

The sterilization techniques can be categorized by physical and chemical methods. Physical methods include dry heat sterilization, steam autoclave sterilization, UV radiation, and gamma ray irradiation. Treatments with chemicals such as ethylene oxide (EO), ozone, formaldehyde and phenols belong to chemical sterilization methods [21]. However, cold chemicals for routine sterilization of instruments are not recommend by the American Dental Association, since monitoring the solution can be difficult, and their efficaciousness can be limited by the inability to wrap the instruments in a sterile package [22]. Therefore, physical methods deemed to be the most suitable for sterilizing implant.

Steam autoclaving is a sterilization method commonly used in the dental field, due to its convenience, low cost and reliable sterilization effect [13]. Dry-heat sterilization can be used when the moisture in steam autoclaving would cause corrosion and deterioration of specific material [22]. Indeed, steam autoclave sterilization and dry heat sterilization offer both cost effectiveness and efficacy, but some materials cannot withstand invasive moisture or temperatures above 100 °C [23]. Therefore, other sterilization techniques, such as gamma (γ) irradiation and ultraviolet (UV) irradiation, are also available. Gamma irradiation from a cobalt-60 (Co-60) source is lethal to all forms of microorganism and it has the advantage of sterilizing without high temperature and pressure, chemicals or gases [13]. UV irradiation is divided into four distinct spectral areas according to wavelength, namely: vacuum-UV (100–200 nm), UVC (200–280 nm), UVB (280–315 nm), and UVA (315–400 nm), such that UVC is found to possess a high antimicrobial capability. UV sources, *e.g.*, light-emitting diodes, lasers, and microwave-generated UV plasma, are available for biomedical applications [24].

Cell and bacteria adhesion is sensitive to the surface properties of implant materials [25,26] and different sterilization treatments may influence the surface chemistry and wettability, consequently affecting cellular behavior [14]. A study by Vezeau et al. had explored the effects of sterilization on titanium surface characteristics and fibroblasts attachment *in vitro*. They reported that titanium surface characteristics can be altered by steam autoclave sterilization, and less murine fibroblasts attachment compared to that after UV irradiation [27]. However, there is still no published study investigating the effect of different sterilization treatments on zirconia surface characteristics, as well as the biological responses of biofilm formation.

The aims of this study are twofold: to examine the effects of sterilization methods, *i.e.*, steam autoclave sterilization, dry heat sterilization, UVC irradiation, and gamma ray irradiation, on the surface characteristics of zirconia. Furthermore, after the four different sterilization treatments, the *in vitro* biofilm formation on zirconia surfaces was compared.

2. Materials and methods

2.1. Zirconia sample preparation

Commercially available cylindrical pre-sintered Y-TZP zirconia blocks (Cerconbase, DeguDent, Hanau, Germany) were used in this study. The zirconia blocks were cut into quadrant-shaped specimen (12.5 mm in radius and 1 mm in thickness) using a diamond precision saw (IsoMet^{TM} 5000, Buehler, USA) under cold running water. After being polished with 4000-grit SiC

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