The Effect of Gamma Radiation Sterilization on Dental Biomaterials

N.Selcan Türker¹, A.Yekta Özer²*, Burak Kutlu³, Rahime Nohutçu³, Arzu Sungur¹, Hasan Bİlgili¹, Melike Ekizoglu⁶, and Meral Özalp⁶

¹Hacettepe University, Fac Pharmacy, Dep Radiopharmacy. ²MGH-Harvard Medical School, Dep Radiology
³Hacettepe University, Fac Dentistry, Dep Periodontology, 06100, Sihhiye, Ankara
⁴Hacettepe University, Fac Medicine, Department of Pathology, 06100, Sihhiye, Ankara
⁵Ankara University, Faculty of Veterinary Medicine, Department of Orthopaedics and Traumatology, 06110 Diskapi, Ankara
⁶Hacettepe University, Fac Pharmacy, Department of Microbiology, 06100 Sihhiye, Ankara

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Abstract: Biomaterials are used in the field of bone and tissue engineering, orthopaedics and dentistry. Dental biomaterials including commercially available biodegradable materials act as physical barriers to help quicker healing while stimulating the regeneration of periodontal tissues, which is defined as Guided Tissue Regeneration (GTR). Amongst natural and synthetic biomaterials, collagen and aliphatic polyesters, such as polylactic acid (PLA) and poly (lactic-co-glycolic) acid (PLGA) are the most frequently used biomaterials for regenerative therapies due to their excellent biocompatibility and biodegradability. Due to their resorption in the body and interaction with biological systems, the GTR membranes must be sterile and pyrogen free. The sterility and apyrogenicity of the GTR membranes before human use is a regulatory requirement, however the sterilization of biomaterials is challenging due to the physicochemical changes and toxic residues with the commonly used sterilization techniques. The purpose of the present study was to evaluate the effect of gamma radiation and ethylene oxide sterilization on dental biomaterials with analytical, microbiological and histological examinations. PLGA-based GTR dental biomaterial is selected as the most gamma stable membrane according to the FTIR, DSC, TGA, and SEM results. This dental membrane was sterilized with ethylene oxide (EtO) and the effect of sterilization method on PLGA-based membrane was also investigated. Animal experiments were carried out to evaluate the regenerative properties and inflammatory responses of gamma and EtO sterilized PLGA-based GTR membrane after implantation. Histological examinations showed that resorption and bone formation of gamma sterilized PLGA-based GTR membrane was completed in 12 weeks without any inflammatory response; while only 60.095 ± 2.019% of new bone formation was observed with EtO sterilized one. Gamma sterilized PLGA membrane had significantly faster (P < 0.05) resorption and bone formation in comparison with EtO sterilization. In conclusion, the PLGA-based biomaterials could be sterilized safely and time- and cost-effectively with validated radiation doses for the tissue engineering applications.

Key words: Gamma radiation sterilization, Ethylene oxide sterilization, Guided tissue regeneration, Guided Bone Regeneration, Dental biomaterials

1. Introduction

Biomaterials are being used to repair, restore, or replace the functions of living tissues of the human body.¹ During the last decade, various regenerative biomaterials have been examined for the treatment of teeth defects, which presents a significant clinical problem in periodontology.² Among the therapy approaches, periodontal tissue regeneration has considerable success in the treatment of periodontal disease and trauma following the use of grafting biomaterials with improved guided tissue regeneration (GTR).³ The clinical applications of GTR in periodontics involve the placement of a physical barrier membrane to enable the bone and tissue to re-grow and to arrest and control periodontal infection and ultimately to regenerate lost periodontal structures.¹,⁴,⁵

GTR membranes are generally resorbable, which means that they absorbed by the body with no need for a second surgery and allow healing over time.¹,⁶ A wide variety of synthetic and natural
biomaterials are used in dental applications because of their excellent mechanical properties. Poly (lactic acid) (PLA), poly (lactic-co-glycolic acid) (PLGA) and collagenous materials are the most promising bioresorbable GTR membranes and have been widely used during the past several decades.

An ideal GTR membrane should be compatible, safe, non-allergic, non-toxic and have no risk of disease transmission. GTR membranes are continuously in contact with body fluids and tissues that it is necessary to sterilize the whole product before implantation in patients. The mechanical and physicochemical properties of the GTR biomaterials, must be stable after sterilization. Therefore, it is critical and necessary to analyse the compatibility of the selected sterilization method on GTR biomaterials. Among the most widely used sterilization techniques, exposure to the gamma rays is the most commonly used technique to sterilize pharmaceuticals and medical devices because of its high penetrating ability, uniform and time dependent delivery of the required doses without any toxic residue. Furthermore, the dosimeters enable parametric release, which provides cost and sterility assurance advantages of gamma radiation sterilization. Depending on the Pharmacopoeias, a minimum dose of 25 kGy was routinely applied for many medical devices, but now, as recommended by the International Organization for Standardization (ISO), the sterilization dose must be set for each type of product depending on its bioburden.

The purpose of the present study was:

a) to investigate the physicochemical and microbiological effects of gamma radiation sterilization on GTR biomaterials and to select the most gamma stable biomaterial,

b) to compare the physicochemical, regenerative, microbiological properties of gamma and EtO sterilized GTR biomaterials.

2. Materials and Methods

2.1 Materials

The biodegradable GTR biomaterials are listed in Table 1. All the GTR membranes were non-sterile and all the chemicals used in experiments were of analytical grade.

2.2 Methods

Schematic diagram of the experimental protocol is shown in Figure 1. The non-sterile biodegradable GTR membranes were sterilized with gamma radiation with different radiation doses. According to the physicochemical test results, the most gamma stable GTR membrane was selected (coded with M). To compare the effect of the sterilization methods, ‘M’ coded non-sterile GTR membrane was sterilized with EtO (METO). The same analytical tests were done on the selected ‘M’ after EtO sterilization. Animal experiments were carried out with gamma sterilized and EtO sterilized membranes (M and METO) and histopathological analyses were done to compare the regenerative and inflammatory effect of the sterilization methods.

2.3 Sterilization of GTR Membranes

GTR membranes were sterilized with gamma radiation at four different dose levels (5, 10, 25, 50 kGy) under normal conditions (25°C, 60% relative humidity) in dark. 60Co gamma cell (4523 Ci, Hungary) was used to supply an ionizing radiation source with a dose rate of 1.28 kGy.hr\(^{-1}\) at the Sarayköy Gamma Radiation Facility of Turkish Atomic Energy Agency in Ankara. Unirradiated GTR membranes were used as controls to detect physicochemical and antimicrobial activity changes resulting from the action of ionizing radiation.

EtO sterilization (3M Sterivak, USA) was performed at Hacettepe University according to (ISO)10993-7 (Biological Evaluation of MDs-Ethylene Oxide Sterilization Residuals). The gas sterilization process consists of three steps, which the first step was humidity preconditioning at 55°C for 24 h. The