Determination of gamma radiation sterilization dose on bioceramic BCP-Sr-Ag as bone graft according to ISO 11137 standards

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ABSTRACT

Background: BCP-Sr-Ag as a bone graft needs to be sterilized. One way of sterilization is to use gamma radiation. Sterilization by gamma radiation requires the correct sterilization dose. Based on ISO 11137 the determination of the sterilization dose through 3 steps: determination bioburden, determination of verification dose, and determination of sterilization dose. Purpose: Determination of gamma radiation sterilization dose on BCP-Sr-Ag bioceramic as bone graft based on ISO 11137 through determination bioburden, determination verification dose, and determination of sterilization dose. Methods: A total 30 samples of 3 different batch BCP-Sr-Ag were determined for bioburden using TSA media. A total 100 samples of BCP-Sr-Ag were irradiated with a verification dose using cobalt-60 gamma source. The 100 samples then tested for sterilization using TSA media to determine the sterilization dose. Results: The average bioburden values of batches 1, 2, and 3 were 56.8; 61.8; and 60.5 CFU. The average value of the whole batch is 59.7 CFU. Based on ISO 11137, the verification dose is 7.4 kGy and the sterilization dose is 20.5 kGy. Conclusion: The average bioburden value of the entire batch was 59.7 CFU. The bioburden value used for verification dose determination is 64.22 CFU so the the gamma radiation sterilization dose is 20.5 kGy.

Keywords: BCP-Sr-Ag; bone graft; gamma radiation; sterilization dose

INTRODUCTION

Bone defects in the oral cavity can occur due to various factors such as: tooth loss, periodontal disease, systemic pathological conditions, trauma, cysts, tumors, and infections.¹ Currently, replace or restore damaged bone can use bone graft. Bone graft will induce the formation of new bone and interact to increase macrophage activity to help the bone recovery process.²

Bone grafting is a surgical procedure to repair damaged bone with materials from the patient’s own body, synthetic, or natural substitutes.³ Bone grafting aims to strengthen, heal, and improve the function of the bone. New bone regeneration is the expected end result of the bone grafting process.²

The ideal bone graft has osteogenic, osteoinductive, and osteoconductive properties. Osteogenesis means living osteoblasts derived from the graft contribute to the production of new bone cells. Osteoinduction means the stimulation of osteoprogenitor cells that differentiate into osteoblasts, usually influenced by bone morphogenic proteins released from the graft. Osteoconduction means that the graft provides a ‘skeleton’ that helps capillaries and precursor bone cells to develop.²

The types of bone grafts that are often used are autograft, allograft, xenograft, and alloplastic. Autograft is a bone graft material that comes from the patient’s own donor. Allograft is a type of bone graft that comes from another donor of the same species. Xenografts obtained from different species and alloplastic obtained from synthetic materials can be ceramic, hydroxyapatite, tricalcium phosphate, or calcium phosphate.¹

Autograft is the gold standard and is considered the best compared to other types of bone graft.⁴ However, donor sources are limited and the potential for complications in the donor area is a problem.³ Therefore, alloplastic was developed as an alternative choice for bone restoration. Alloplastic that are often used are Hydroxyapatite (HA), β-Tricalcium Phosphate (β-TCP), and Biphasic Calcium Phosphate (BCP).

Biphasic Calcium Phosphate (BCP) is a combination of the osteoconductive properties of HA and the resorbability of β-TCP, resulting in the advantages of these two materials.⁶ BCP bioactivity can be modified by adding ionic materials as
Sterilization is important to produce sterile products in health product and medicine. Sterilization is a process carried out to remove or inactive living microbes from a product.\(^9\) Sterilization methods are heat sterilization, radiation sterilization, filtration sterilization, and chemical sterilization.\(^1\) Improper sterilization of health products can cause infection, mortality, and morbidity problems in patients.\(^2\)

One method that can be used to sterilize bone grafts is using gamma radiation. The advantages of sterilization using gamma irradiation are that it does not produce excessive temperature rise and does not leave toxic residues.\(^3\) The weakness of sterilization using gamma irradiation is that chemical breakdown can occur in chemical bonds that affect the mechanical properties of the bone graft.\(^4\)

Gamma radiation sterilization requires the right dose of radiation. The International Atomic Energy Agency (IAEA) has recommended that the radiation dose for sterilizing health products is 25 kGy, however, the radiation dose can be smaller depending on the bioburden value. Determination of the right radiation dose with the bioburden value based on the International Organization for Standardization (ISO) 11137.\(^5\) Bioburden is the number of microbes present in a product before being sterilized.\(^6\) The bioburden value can be known in the bioburden test. The bioburden test was carried out to obtain the average bioburden value which is used to determine the verification dose and the sterilization dose.

The verification dose is a gamma-ray radiation dose determined using the average bioburden value to obtain a sterility assurance level (SAL) and is used in determining the sterility dose. The SAL used for the verification dose is \(10^{-2}\) according to ISO 11137. In this case, SAL \(10^{-2}\) can be accepted if out of 100 samples sterilized with a verification dose, only a maximum of 2 samples are not sterile. If the verification dose based on SAL \(10^{-2}\) is acceptable, then the sterilization dose can be determined.\(^7\)

The sterilization dose is determined based on the ISO 11137 table using SAL \(10^{-6}\). SAL \(10^{-6}\) means that out of one million products that are sterilized, only a maximum of 2 products are not sterile.\(^8\) The purpose of determining the sterilization dose is to determine the minimum doses needed to achieve the specified SAL.\(^9\)

Excessive doses of gamma-ray radiation can cause changes in mechanical properties, thereby reducing the efficacy of bone grafts.\(^10\) A dose of gamma-ray radiation that is too small will result in the bone graft not being sterile enough to be used in the body. Therefore, it is necessary to determine the dose of gamma radiation correctly to obtain a product that is sterile and does not damage the mechanical properties of the BCP-Sr-Ag biomaterial. The method used for gamma sterilization on BCP-Sr-Ag is based on ISO 11137.\(^11\) In this study, gamma sterilization was carried out using the radioisotope cobalt-60 as the main source of gamma-ray radiation.

### MATERIALS AND METHODS

Ethical permission was obtained from the Health Research Ethics Licensing Commission, Faculty of Dental Medicine, Airlangga University with number 586/HRECC.FODM/VIII/2022 for this research. BCP-Sr-Ag was taken as many as 10 samples from each of the 3 different production batches and put into sterile micro tubes. Each sample was put into 4 ml of 0.1% bacto pepton in a test tube and shaken. Each sample was poured into a petri dish containing Tryptic Soy Agar (TSA) media of ± 8 ml and incubated at 37°C for 4 days. The total number of colonies growing TSA media were recorded.\(^17\) For gamma-ray radiation as many as 100 samples were taken from 1 production batch put into sterile micro tube.\(^18\)

Bioburden test was carried out by observing the number of colonies contained in the TSA media. After knowing the amount of bioburden of each sample from 1 production batch, the average bioburden of each batch is calculated. Furthermore, it determines the average of the overall bioburden. If the average bioburden value of each batch is smaller than twice the average bioburden of the entire batch, then the average value of the entire batch is used to determine the verification dose. If the average bioburden value of each batch has a result greater than twice the average bioburden of the entire batch, then the average batch value used is the highest batch average value.\(^19\)

The average bioburden value obtained was used to determine the verification dose based on SAL \(10^{-2}\) in the ISO 11137 table.\(^10\) Verification doses are used in the establishment of sterilization doses.\(^10\) A total of 100 samples from 1 production batch were put into sterilized sterile micro tubes and then irradiated using cobalt-60 radioisotopes according to the verification dose that had been obtained.\(^18\)

100 samples that had been irradiated at the verification dose were put into a 0.1% bacto pepton solution and poured into TSA media. Furthermore, TSA media was incubated at 37°C for 14 days. The presence of growing microbes is observed. If the sterility test results give a positive result of microbes ≤ 2 then the verification dose is accepted. Sterilization doses are established based on ISO 11137 tables using SAL \(10^{-6}\).\(^18\)

### RESULTS

BCP-Sr-Ag bioburden values were performed on 10 samples from 3 different batches (Table 1). The average value of bioburden batch 1 is 56.8 CFU, batch 2 is 61.8 CFU, batch 3 is 60.5 CFU. The average value of the entire batch indicates a value of 59.7 CFU.
Based on the average value of the bioburden of the entire batch, the bioburden value is not shown in Table 2, so the bioburden value at the level above it is used as a verification dose. After radiation was carried out based on the verification dose, 100 of the samples were tested for sterility to determine whether the verification dose given was acceptable. Sterility test results show that there are no samples are not sterile (Table 3).

**DISCUSSION**

BCP-Sr-Ag as a bone graft can be used as an option to repair bone damage. However, microbial contamination in BCP-Sr-Ag can be the cause of morbidity and mortality in patients. Contamination can arise from laboratory equipment, operators, and bone graft making environments. Aseptic bone graft manufacturing procedures can reduce contamination but cannot eliminate microbial contamination. Therefore, BCP-Sr-Ag must be sterilized to inactivate microbes so as to prevent risks to patients.

### Table 1. The average of bioburden value of BCP-Sr-Ag from three different batches

<table>
<thead>
<tr>
<th>No. sample</th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
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</thead>
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<tr>
<td>1</td>
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<td>60</td>
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<tr>
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</tr>
<tr>
<td>10</td>
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<td>63</td>
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</tbody>
</table>

Batch average: 56.8, Average of the entire batch: 59.7

*CFU=colony forming unit

### Table 2. The radiation dose in kGy units required to achieve a given SAL for an average bioburden

<table>
<thead>
<tr>
<th>Average bioburden (CFU)</th>
<th>Sterility Assurance Level (kGy)</th>
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<tr>
<td></td>
<td>$10^{-2}$</td>
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<tr>
<td>59.20</td>
<td>7.3</td>
</tr>
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<td>64.22</td>
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<td>69.65</td>
<td>7.5</td>
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<td>75.51</td>
<td>7.6</td>
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|                         | $10^{-3}$                       |
| 59.20                   | 10.3                            |
| 64.22                   | 10.4                            |
| 69.65                   | 10.5                            |
| 75.51                   | 10.6                            |

|                         | $10^{-4}$                       |
| 59.20                   | 13.5                            |
| 64.22                   | 13.6                            |
| 69.65                   | 13.7                            |
| 75.51                   | 13.9                            |

|                         | $10^{-5}$                       |
| 59.20                   | 16.9                            |
| 64.22                   | 17.0                            |
| 69.65                   | 17.1                            |
| 75.51                   | 17.3                            |

|                         | $10^{-6}$                       |
| 59.20                   | 20.4                            |
| 64.22                   | 20.5                            |
| 69.65                   | 20.7                            |
| 75.51                   | 20.8                            |

### Table 3. The results of sterility test of 100 samples of BCP-Sr-Ag

<table>
<thead>
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<th>No. Sample</th>
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<th>Value</th>
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Zero value (0) = BCP-Sr-Ag sterile (no microbes)
One of the sterilization methods that can be used is gamma radiation sterilization. The number of microbes is important in the application of gamma radiation sterilization technology. The number of microbes surviving after radiation decreases with increased doses. However, excessive doses can damage the structure of the product. Determination of the sterilization dose of bioceramic BCP-Sr-Ag is carried out in order to obtain the right sterilization dose so that the bioceramic becomes sterile and does not damage the mechanical properties of the BCP-Sr-Ag bioceramic. Based on ISO 11137, the gamma radiation sterilization dose is determined through several stages, namely bioburden testing on BCP-Sr-Ag, verification dose determination, and sterilization dose determination.

In this study, a bioburden test was carried out to obtain the value of microbial contamination (bioburden) in BCP-Sr-Ag. The results of the bioburden test on the BCP-Sr-Ag bioceramic showed that the average bioburden of each batch of the three BCP-Sr-Ag batches used in a row was 56.8; 61.8; and 60.5 cell forming units (CFU) and the average bioburden value of the entire batch was 59.7 CFU.

According to ISO 11137, if the average bioburden value of each batch is smaller than twice the average bioburden value of the entire batch, the average value of the entire batch is used to determine the verification dose. This study showed that the average bioburden value of batch 1, batch 2, and batch 3 was smaller than twice the average of the entire batch (2 x 59.7 = 119.4). So that the bioburden value used to determine the verification dose is the average bioburden value of the entire batch. If the average bioburden value is not contained in the ISO 11137 table, then the bioburden value used to determine the verification dose is the average bioburden value of the entire batch. If the average bioburden value of BCP-Sr-Ag was 64.22 so that the average bioburden value is 59.7 CFU.

In the ISO 11137 table, the bioburden value of 59.7 is missing, so the bioburden value at the level above it. In the ISO 11137 table, the bioburden value of 64.22 is used to determine the verification dose. Using ISO Table 11137, at bioburden values of 64.22 and SAL 10^-2, the verification dose is 7.4 kGy.

In this study, as many as 100 BCP-Sr-Ag samples were irradiated using cobalt-60 radioisotopes as the main source of gamma radiation based on a verification dose of 7.4 kGy. Gamma radiation doses eliminate microbes by two mechanisms. The primary mechanism produces an immediate effect. Direct effects occur when radiation interacts with biological molecules causing excitation, lesions, and cutting of polymer structures. High-energy photons from ionizing radiation produced by the ionization process can damage DNA. The changes that occur are single stranded DNA break, namely the breaking of the sugar phosphate chain from each strand of DNA, double-strand DNA break, which is the breaking of adjacent chains on both DNA strands, and the formation of intramolecular crosslinks or intermolecular crosslinks. This damage to DNA structures inhibits DNA synthesis, causes errors in protein synthesis, and results in cell death.

Secondary mechanisms produce indirect effects. The indirect effect is caused by the formation of water free radicals due to the radiolysis of water in microorganisms. These water free radicals play a role in the transfer of radiation to DNA. Radiation interacting with water produces free radicals that damage DNA and inactivate the microbial reproduction process resulting in microbial death. Both primary and secondary mechanisms cause microbial death resulting in a sterile product.

After the sample is irradiated using a verification dose, the sample is performed a sterility test. The results of sterility testing showed that out of 100 samples there were no non-sterile samples. According to ISO 11137 the verification dose is acceptable if out of 100 samples irradiated with the verification dose there can only be a maximum of 2 non-sterile samples. In this study, there were no non-sterile samples, so the verification dose was acceptable and used to determine the sterilization dose based on ISO Table 11137.

Sterilization is carried out to provide the required sterility assurance level (SAL). SAL is the possibility of microbes that live on the product after sterilization. In its application, the material whose use is in direct contact with body tissues the required SAL value is 10^-6. SAL 10^-6 means that the product must receive a sterilization dose that ensures that the probability of microbes surviving the dose is no greater than two in a million sterilized products. BCP-Sr-Ag as a bone graft material is a product whose use is in direct contact with body tissues. Therefore, BCP-Sr-Ag should receive a sterilization dose based on SAL 10^-6.

The recommended radiation sterilization dose of health products is 25 kGy, but the dose given can be smaller depending on the bioburden value of the product. In this study, the bioburden value of BCP-Sr-Ag was 64.22 so that the sterilization dose given was 20.5 kGy based on SAL 10^-6. With a sterilization dose of 20.5 kGy it can ensure BCP-Sr-Ag is sterile and safe for clinical use.

In conclusion, the average bioburden value of the entire batch is 59.7 CFU. The bioburden value used for verification dose determination is 64.22 CFU so the verification dose is 7.4 kGy and the gamma radiation sterilization dose is 20.5 kGy.

ACKNOWLEDGMENTS

Authors are thankful to Dental Research Center, Faculty of Dental Medicine, Universitas Airlangga for providing necessary instrumental facilities and also thankful to BRIN for providing the gamma radiation facilities for the bone graft.

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