Determination Of Gamma Radiation Sterilization Dose on Bioceramic BCP-Sr-Ag as Bone Graft According to ISO 11137 Standards

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Abstract

One type of bone graft being developed is alloplastic. The alloplastic that is used is Biphasic Calcium Phosphate (BCP). Bioactivity of BCP can be modified by adding ionic material as doping. Doping BCP with Strontium (Sr2+) and Silver (Ag+) becomes bioceramic which has better characteristics and degradability. BCP-Sr-Ag as a health product 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.

The aim of this study is to determinate 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.

This research was an in vitro laboratory. A total 30 samples of 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.

The results of the research showed that 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. The verification dose of bioceramic BCP-Sr-Ag as bone graft is acceptable so that the gamma radiation sterilization dose can be determined according to ISO 11137.

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Introduction

Bone grafting is a surgical procedure to repair damaged bones with materials from the patient's body, *artificial*, synthetic, or *natural substitutes*.[1] *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 *bone grafts* that are often used are *autograft, allograft, xenograft*, and *alloplastic*.[3] *Autograft* is the *gold standard* and is considered superior to other types of bone graft.[4] Limited donor sources and potential complications in the donor area are the main weaknesses of autografts[5] so that *alloplastic* was developed as an alternative choice as a *bone graft* material. *Alloplastics* 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

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materials as *doping*.[7] *Single doping* can use strontium ions (Sr^{2+}) which help cell proliferation and osteogenesis activity.[8] *Multi-layer doping* can use antimicrobial materials, namely silver ions (Ag^{+}).[9] *Doping* BCP with Sr^{2+} and Ag^{+} ions to *bioceramic* BCP-Sr-Ag increases osteogenesis and antimicrobial activity. *Bioceramic* BCP-Sr-Ag has better characteristics and degradability when compared to a single material as a substitute for *bone graft*.

According to standard procedures for health products, *bone grafts* need to be sterilized to produce sterile products. Sterilization is a process carried out to remove or inactivate microbes from the product so that an improper sterilization process can cause problems when it comes to *bone grafts*.[10,11] The sterilization method consists of: heat, radiation, filtration, and chemical sterilization.[12] One way to sterilize *bone grafts* is by using gamma radiation. The advantages of sterilization using gamma irradiation are that it does not produce excessive temperature increases and does not leave toxic residues.[13] The weakness of sterilization using gamma-ray radiation is that *chemical breakdown* can occur in chemical bonds that affect the *mechanical properties* of the *bone graft*.[11]

Gamma radiation sterilization requires the right radiation dose as recommended by *The International Atomic Energy Agency* (IAEA). The IAEA recommends a radiation dose for medical product sterilization of 25 kGy. The radiation dose can be smaller than a dose of 25 kGy which depends on the bioburden value based on the *International Organization for Standardization* (ISO) 11137.[11] 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} based on ISO 11137. SAL 10^{-2} is acceptable if out of 100 samples sterilized with the 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.[14]

The sterilization dose is determined based on the ISO 11137 table using SAL 10^{-6}. This means that out of one million products that are sterilized, only a maximum of 2 products are allowed that are not sterile.[14] The purpose of determining the sterilization dose is to determine the minimum dose needed to achieve the specified SAL.[15]

Materials and methods

Research Samples

The sample size was calculated based on ISO 11137, namely: the bioburden test was carried out using *3 batches*, each *batch* consisting of 10 samples and gamma radiation sterilization using 100 samples from one *batch*.[16] The research instrument used in this study was the radioisotope *cobalt-60* as the main source of gamma radiation.

Research methods

1. Sample preparation

BCP-Sr-Ag was taken as many as 10 samples from each of the 3 different production batches and put into a sterile *micro tube*. Each sample was put into 4 ml of 0.1% bacto peptone in a test tube and shaken. Each sample was poured into a petri dish containing ± 8 ml of *Tryptic Soy Agar* (TSA) and incubated at 37^{0}C for 4 days. For gamma-ray radiation, 100 samples were taken from 1 production batch which were put into sterile *micro tubes*.[17]

2. Bioburden test

The test was carried out by observing the number of colonies found on TSA media. After knowing the amount of bioburden for each sample from 1 production *batch*, the average bioburden for each *batch* is calculated. Next determine the average of the overall bioburden. If the average bioburden value for each *batch* is less than twice the average bioburden for the entire *batch*, then the average value for the entire *batch* is used to determine the verification dose. If the average bioburden value for each *batch* is twice as large as the average bioburden for the entire *batch*, then the average *batch* value used is the highest average *batch* value.[17]

3. Dosage verification

The average value of the bioburden obtained is used to determine the verification dose based on SAL 10^{-2} in the ISO 11137 table. The verification dose is used in determining the sterilization dose.[10]

4. Gamma radiation

A total of 100 samples from 1 production *batch* were put into a sterile *micro tube* and then

irradiated using radioisotope *cobalt-60* according to the verification dose that had been obtained.[17]

5. Sterility test

100 samples that have been irradiated at the verification dose were put into a 0,1% bacto peptone solution and put into TSA media. Then the TSA media was incubated at 37^0C for 14 days. The presence of growing microbes was counted. If the results of the sterility test give positive results for microbes ≤ 2 then the verification dose is accepted. The sterilization dose is determined based on the ISO 11137 table using SAL 10-*6*.[17]

Results

Repetition	Bioburden (CFU*)		
	Batch 1	*Batch* 2	*Batch* 3
1	48	67	60
2	58	77	63
3	56	73	64
4	49	50	60
5	45	57	55
6	48	68	58
7	58	64	76
8	67	69	60
9	69	44	46
10	70	49	63
Batch Average	56,8	61,8	60,5
All *Batch* Average	59,7		

Table 1. BCP-Sr-Ag bioburden values in 3 different batches. *CFU = *colony forming unit.*

Based on the average bioburden value of the entire *batch* (from table 1), the bioburden value is not listed in the ISO 11137 table, so the bioburden value at the level above is used as a verification dose. The verification dose used was 7,4 kGy based on SAL 10^{-2} at an average bioburden of 64,22 CFU.

Average bioburden (CFU)	*Sterility Assurance Level*	
	10^{-2}	10^{-6}
64,22	7,4	20,5

Table 2. Radiation dose (kGy) required to achieve SAL at bioburden values based on ISO 11137 table.

In this study, the first data processing used the normality test using the *Shapiro Wilk* test with a significance level of 0,05. This test aims to determine whether the data population is normally distributed or not and to determine the appropriate statistical test to be used in this study. If the significance value is > 0,05 the data is normally distributed. In the *Shapiro Wilk* test, the p value from each batch was 0,191 in batch 1; 0,491 in batch 2; 0,291 in *batch* 3. The conclusion shows that the data for each group is normally distributed. Furthermore, the homogeneity test was carried out using the *Levene test*. If the significance > 0,05 means the data is homogeneous. The results of the calculations show a significance of 0,149 which means the data is homogeneous.

Then a *one way ANOVA* test was carried out to see significant differences between groups. The results showed a significance of > 0,05 (p=0.482) which indicated that there was no significant difference between groups.

Sterility Test Results

No. Sample	Value	No. Sample	Value	No. Sample	Value	No. Sample	Value	No. Sample	Value
1	0	21	0	41	0	61	0	81	0
2	0	22	0	42	0	62	0	82	0
3	0	23	0	43	0	63	0	83	0
4	0	24	0	44	0	64	0	84	0
5	0	25	0	45	0	65	0	85	0
6	0	26	0	46	0	66	0	86	0
7	0	27	0	47	0	67	0	87	0
8	0	28	0	48	0	68	0	88	0
9	0	29	0	49	0	69	0	89	0
10	0	30	0	50	0	70	0	90	0
11	0	31	0	51	0	71	0	91	0
12	0	32	0	52	0	72	0	92	0
13	0	33	0	53	0	73	0	93	0
14	0	34	0	54	0	74	0	94	0
15	0	35	0	55	0	75	0	95	0
16	0	36	0	56	0	76	0	96	0
17	0	37	0	57	0	77	0	97	0
18	0	38	0	58	0	78	0	98	0
19	0	39	0	59	0	79	0	99	0
20	0	40	0	60	0	80	0	100	0

Table 3. Results of the sterility test for 100 BCP-Sr-Ag samples after being irradiated with a verification dose (7,4 kGy). Zero value (0) = sterile BCP-Sr-Ag (no microbes).

100 samples from one of the *batches* were irradiated based on the verification dose. After irradiation based on the verification dose, the 100 samples were subjected to a sterility test to determine whether the given verification dose was acceptable. The results of the sterility test are presented in table 3.

Discussion

BCP-Sr-Ag as a *bone graft* can be used as an option to repair bone damage. Microbial contamination of BCP-Sr-Ag can be a cause of morbidity and mortality in patients which can arise from laboratory equipment, operators, and the *bone graft* manufacturing environment.[11] The aseptic procedure for making *bone grafts* can reduce contamination but cannot eliminate microbial contamination.[18] BCP-Sr-Ag needs to

be sterilized to inactivate microbes so as to prevent risks to patients.

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 decreased with increasing dose.[19] However, excessive dosage may damage the product structure. Determination of the sterilizing dose of BCP-Sr-Ag bioceramic 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.[11] Based on ISO 11137, the dose of gamma radiation sterilization is determined through several stages, namely the bioburden test on BCP-Sr-Ag, determination of the verification dose, and determination of the sterilization dose.[10]

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 BCP-Sr-Ag bioceramics showed that the average bioburden of each *batch* of three *batches* of BCP-Sr-Ag used was 56,8; 61,8; and 60,5 *cell forming units* (CFU) and the average bioburden value of the whole *batch* is 59.7 CFU. According to ISO 11137, if the average bioburden value for each batch is less than twice the average bioburden value for the entire *batch*, the average value for the entire *batch* is used to determine the verification dose.[17] This study showed that the average bioburden value of *batch* 1, *batch* 2, and *batch* 3 was less than twice the average of the entire *batch* (2 x 59,7 = 119,4). The bioburden value used to determine the verification dose is the average bioburden value for the entire *batch*. If the average bioburden value is not listed in the ISO 11137 table, then the bioburden value used to determine the verification dose is the bioburden value at the level above.[17] In the ISO 11137 table there is no bioburden value of 59,7, so the bioburden value at the level above, namely 64.22 is used to determine the verification dose. Using Table ISO 11137, at a bioburden value of 64,22 and SAL 10^{-2}, the verification dose is 7,4 kGy.

In this study, 100 BCP-Sr-Ag samples were irradiated using the radioisotope *cobalt-60* as the main source of gamma radiation based on a verification dose of 7,4 kGy. Gamma radiation dose eliminates microbes by two mechanisms. The primary mechanism produces an immediate effect. Direct effects occur when radiation interacts with biological molecules causing excitation, lesion and cutting of the polymer structure. The high energy photons of ionizing radiation produced by the ionization process can damage DNA. The changes that occur are *single-stranded* DNA *breaks*, namely the breaking of the phosphate sugar chain from each DNA *strand*, *double-strand* DNA *breaks*, namely the breaking of adjacent chains on both DNA *strands*, and the formation of *intramolecular crosslinks* or *intermolecular crosslinks*. Damage to this DNA structure inhibits DNA synthesis, causes errors in protein synthesis, and results in cell death.[20]

Secondary mechanisms produce indirect effects. Indirect effects are caused by the formation of water free radicals due to radiolosis of water in microorganisms. These water free radicals play a role in the transfer of radiation to DNA. Radiation interacts with water to produce free radicals that damage DNA and inactivate microbial reproduction processes resulting in microbial death.[20] 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 subjected to a sterility test. The results of the sterility test showed that out of 100 samples there were no samples that were not sterile. According to ISO 11137 the verification dose can be accepted if out of 100 samples irradiated with the verification dose there can only be a maximum of 2 samples that are not sterile.[10] In this study there were no samples that were not sterile, so the verification dose could be accepted and used to determine the sterilization dose based on Table ISO 11137.

Sterilization is performed 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 required SAL value is 10^{-6} for materials used in direct contact with body tissues. SAL 10^{-6} means that the product must receive a sterilizing dose that ensures that the probability of the microbes surviving the dose is no greater than two in one million products being sterilized.[19] BCP-Sr-Ag as a *bone graft* material is a product that is used in direct contact with body tissues. Therefore, BCP-Sr-Ag should receive a sterilizing dose based on SAL 10^{-6}.

The recommended dose for radiation sterilization for health products is 25 kGy, but the dose given can be smaller depending on the bioburden value of the product.[11] In this study, the BCP-Sr-Ag bioburden value was 64,22, so the sterilization dose given was 20,5 kGy based on SAL 10^{-6}. With a sterilization dose of 20,5 kGy it can ensure that BCP-Sr-Ag is sterile and safe for clinical use.

Conclusions

The verification dose of bioceramic BCP-Sr-Ag as bone graft is acceptable so that the gamma radiation sterilization dose can be determined according to ISO 11137.

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Ethical policy and institutional review board

Ethical clearance had been obtained from the Ethics Commission of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya (No.586/HRECC.FODM/VIII/2022) on 19 August 2022.

Declaration of Interest

The authors declare that there are no conflicts of interest.

References

1. Titsinides S, Agrogiannis G, Karatzas T. Bone grafting materials in dentoalveolar reconstruction: A comprehensive review. Japanese dental science review. 2019; 55(1):26-32.
2. Soulissa AG, Nathania I. The efficacy of fish scales as bone graft alternative materials. Scientific Dental Journal. 2018; 2(1): 9-17.
3. Gulia SK, Nagarathna PJ, Qayyum MA, et al. Basic knowledge about bone grafting amongst dental professionals: a qualitative analysis. Journal of Advanced Medical and Dental Sciences Research. 2020; 8(4):32-5.
4. Simanjuntak R, Amalia M. Dentin sebagai bahan cangkok tulang dalam perawatan regenerasi jaringan periodontal: sebuah tinjauan. B-Dent: Jurnal Kedokteran Gigi Universitas Baiturrahmah. 2021; 8(2):185-92.
5. Rahmawati D, Sunarso S, Irawan B. Aplikasi hidroksiapatit sebagai bone filler pasca pencabutan gigi. Jurnal Material Kedokteran Gigi. 2021;9(2):39-46.
6. Zhao R. Yang R, Cooper PR, et al. 2021 Bone grafts and bone substitutes in dentistry: a review of current trends and developments. Journal of Oral Research and Review. 2021; 26(10): 1–27.
7. Hanif MHM, Lee MG, Mohamad HM, Kasim SR. Effect of magnesium to the properties of sintered biphasic calcium phosphate (BCP) for bone regeneration. AIP Conference Proceedings. 2020; 2267(1): 020019-1 – 020019-4.
8. Khan PK, Mahato A, Kundu B, et al. Influence of single and binary doping of strontium and lithium on in vivo biological properties of bioactive glass scaffolds. Scientific reports. 2016; 6(1):1-18.
9. Jacobs A, Gaulier M, Duval A, Renaudin G. Silver doping mechanism in bioceramics—from Ag^{+}: doped HAp to Ag°/BCP nanocomposite. Crystals. 2019;9(7): 326.
10. Radwan Mahmoud, Bashandy Abd, El-Ghamry Abbas, Fouda Amr. Evaluation and microbiological investigations of radiation sterilization of re-usable polysulfone membrane. International Journal of Advanced Research. 2016;4(1):559 – 70.
11. Gomes AD, de Oliveira AA, Houmard M, Nunes EH. Gamma sterilization of collagen/hydroxyapatite composites: validation and radiation effects. Applied Radiation and Isotopes. 2021; 174(109758):1-7.
12. Sandle T. Sterility, sterilisation and sterility Assurance for Pharmaceuticals.1st ed. India: Woodhead Publishing; 2013: 143-55.
13. Antebi U, Mathor MB, Silva AFD, Guimarães RP, Honda EK. Effects of ionizing radiation on proteins in lyophilized or frozen demineralized human bone. Revista Brasileira de Ortopedia. 2016; 51:224-30.
14. Fitriana R, Yasmine H, Yunus AL, et al. 2021 Penentuan dosis iradiasi gamma untuk sterilisasi baju hazmat bekas pakai. Jurnal Ilmiah Aplikasi Isotop dan Radiasi. 2021; 17(1): 35-40.
15. Kátia Aparecida da Silva Aquino. Sterilization by gamma irradiation, gamma radiation. Prof. Feriz Adrovic Ed; Brazil: Federal University of Pernambuco-Department of Nuclear Energy; 2012: 172 – 205.
16. Fajar L, Indrani, Decky J, Darmawan. Pengaruh iradiasi sinar gamma terhadap permeabilitas membran pembalut luka kitosan/kolagen. Jurnal Teknik Biomedis Indonesia. 2016; 2(2):32-6.
17. Darwis D, Warastuti Y, Hardiningsih L. Penentuan dosis sterilisasi membran selulosa mikroba dengan iradiasi berkas elektron berdasarkan ISO 11137. Jurnal Ilmiah Aplikasi Isotop dan Radiasi. 2013; 5(2):165-76.
18. Harrell CR, Djonov V, Fellabaum C, Volarevic V. Risks of using sterilization by gamma radiation: the other side of the coin. International journal of medical sciences. 2018; 15(3):274.
19. Singh R, Singh D. Gamma irradiated bone allografts processed from femoral heads. Frontiers in Science. 2012; 2(5):119-26.
20. Singh R, Singh D, Singh A. Radiation sterilization of tissue allografts: a review. World J Radiol. 2016; 8(4):335-69.