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STERILIZATION OF BONE ALLOGRAFTS IN THE BONE BANK, AHMEDABAD: A SYSTEMATIC REVIEW AND ITS EFFECTS

Sakchi Bhushan*, Jagdish Gulabrai Buch

*Cognoscent Organ Management Private Limited, Ahmedabad, Gujarat 380051.

***Corresponding Author: Sakchi Bhushan**

Cognoscent Organ Management Private Limited, Ahmedabad, Gujarat 380051.

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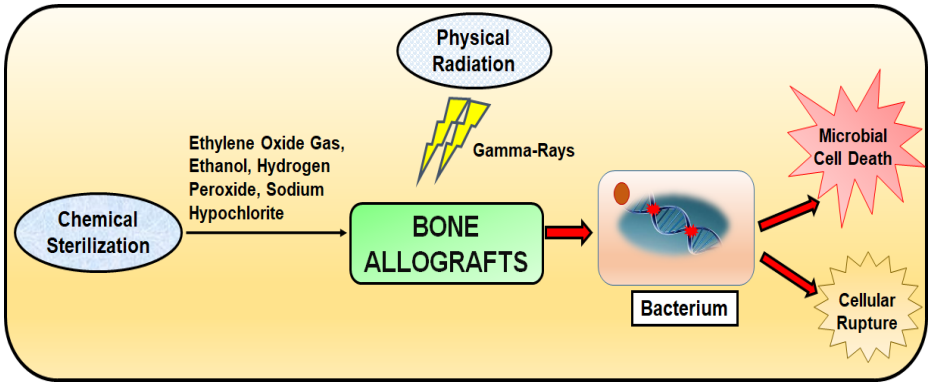
ABSTRACT

There is an increased requirement for bone substitutes in orthopaedic applications, spinal fusion, dental applications and craniofacial reconstruction surgeries. Bone allografts are used to repair bone tissue due to their excellent osteoconductivity and ease of availability. Still, the transmission of infectious disease from the donor tissue is an aspect influencing its assurance. Therefore, bone allografts should be sterilized for safe implantation in the recipient to achieve better clinical outcomes. This review summarizes the importance of sterilization and its types, advantages, and disadvantages. In addition to that, for the commercialization of bone allografts, the association of medically approved sterilization techniques is of vital importance. Overall, we summarize the review in which the use of chemical sterilants followed by physical radiation as terminal sterilization in The Bone Bank is well discussed.

KEYWORDS: Bone allografts, Infectious diseases, Chemical sterilants, Physical agents, Terminal sterilization.



INTRODUCTION

The use of bone grafts in orthopaedic applications, including dental applications, trauma and spinal fusion surgeries has increased in recent years. The selection of bone graft material is the most critical task for the surgeon, supporting the bone repair.[1] Autograft is the gold standard, in which biologically-matched graft material with osteogenic property is retrieved and implanted into the same patient supporting *neo*-bone formation due to the presence of multiple growth factors and signaling molecules in the graft material.[2] But certain factors, such as multiple surgeries and dose requirement to fill the bone defect limit its application.[3] So, allografts are an alternative graft material which is easily available and shows much similar properties to autograft.[4] Furthermore, the requirement of microbial screening of the donor before bone harvesting, as well as the maintenance of aseptic conditions from processing to

packaging of bone allografts is a key factor for its guaranteed application in the healthcare sector.[5] For this reason, efforts are being made to reduce the microbial load of the bone allograft from the time of the retrieval to the packaging of sterile products by opting various sterilization agents.[6] Sterilization is a vital factor in the tissue bank to ensure recipient safety with no compromise on the quality and efficacy of the allograft.[7] So, the types and parameters of sterilization are decided prior to processing in order to validate the sterility of bone allograft before commercialization. In past decades, various procedures were performed to sterilize bone allografts.[6] The chemicals used in the bone bank disinfect the surface of the bone tissue during processing. But these chemicals do not penetrate the bone tissue, which allows the pathogens to remain viable in the center, especially femoral head.[8] So, physical sterilization by the use of high-energy radiation is used after bone processing to inactivate the microbial cells up to a permissible limit without affecting the osteogenic property and mechanical stability of the bone allografts.[7] As per the standard of the American Association of Tissue Banks (AATB), the tissue banks are standardized to various chemicals and sterilizing agents for allograft safety and sterility prior to use.[9] The different types of chemical and physical agents for allograft sterilization in The Bone Bank, Ahmedabad, India, and their mechanisms has not been discussed so far. **Fig. 1** depicts the aim of the review is to highlight the effects of sterilization on allograft from processing to packaging for the reduction of microbial load in the bone allograft for successful implantation in patients.

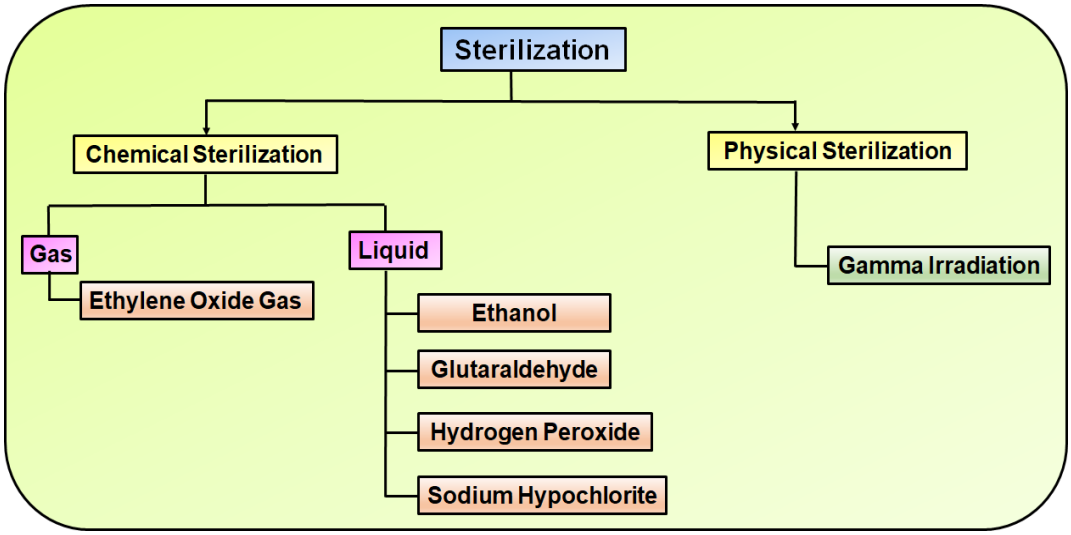


Figure 1: Types of Sterilization performed in The Bone Bank.

TYPES OF STERILIZATION IN THE BONE BANK, AHMEDABAD

There are various chemicals used for sterilization in bone processing, such as ethylene oxide in gaseous form and ethanol, glutaraldehyde, hydrogen peroxide, and sodium hypochlorite in liquid form. In addition to that, there is a radiation-based *i.e.*, gamma irradiation, a terminal sterilization method used for value-added certainty of sterile allografts.

(A) CHEMICAL AGENTS FOR STERILIZATION

The chemical agents in sterilants or in disinfectants form are used to treat heat-sensitive items. As bone allografts come in direct contact with the recipient's body. So, the focus of The Bone Bank is to use the microbes-free bone allografts. The list of chemical sterilants with is described as follows

- **Ethylene oxide gas**

Ethylene oxide gas (ETO) is an inexpensive chemical sterilant for allograft sterilization, which was in high demand at the time of World War II.[10] It is used to sterilize heat-sensitive products such as medical implants or human tissues. Ethylene oxide is a strong alkylating agent. The alkyl group of ETO interacts with the protein and nucleic acids present in the micro-organisms through their –OH, -NH_2, -SH, and –COOH groups, affecting their cellular physiology, resulting in non-viable cells.[11] The ETO sterilize the implants in a highly reactive manner attributed to its high energy with high diffusibility.[12] ETO is flushed and aerated properly to minimize the threat of exposure to the patients. Still, the workers working in the sterilization chambers get affected due to ETO exposure.[13] The application of ETO as a gaseous chemical sterilant is limited in bone bank due to the release of toxic by-products such as ethylene chlorohydrin and ethylene glycol, resulting in carcinogenicity and graft failure.[14] So, the strict OSHA standards are regulated in order to minimize the work exposure.[15] Moreover, the regulatory agency such as AATB (American Association of Tissue Banks) do not recommend ETO as a terminal sterilant for bone allografts. The reduction in bioburden by ETO sterilization is mainly dependent on the ETO concentration and time of exposure.[16] Previous year work reported that the sterilization temperature and exposed aeration time of ETO gas negatively affect osteoinduction property of the bone allografts.[17] The

tendon grafts exposed to 12% ethylene oxide for 15 h at 32 °C before freeze drying results in deterioration of the mechanical strength of the tendon allograft.[18]

- **Ethanol**

Ethanol is a colorless liquid, widely used in healthcare to disinfect surgical tools, gloves and skin antisepsis [19]. It reveals broad-range antimicrobial property against bacteria, fungi and viruses due to the presence of –OH groups in their chemical structure.[20] These hydroxyl groups interact with proteins of the micro-organisms *via* hydrogen bonds and disrupt their structure, resulting in denaturation of protein.[21] In past decades, the use of ethanol in bone bank is focused as it minimizes the risk of infectious disease with no residue after treatment.[22] Moreover, the concentration of ethanol is also a crucial factor for the determination of antimicrobial properties. Ethanol at lower concentration *i.e.* 30-50% possess lower bactericidal property. On the other hand, the use of 100% ethanol does not inhibit bacteria, as it dehydrates the microbial cell leading to the protective shell, which inhibits the denaturation of protein. So, ethanol at 70-80% is optimal for usage, as 30% water with 70% ethanol plays a dual role in cell penetration as well as protein denaturation.[23] (**Fig. 2**). It is reported that the ethanol (70%) immersion for 3 h result in the inactivation of HIV (Human Immunodeficiency Virus) up to the deep level in human tendons.[24] However, the use of ethanol requires other chemical agents for effective sterilization. The use of ethanol with peracetic acid in human cancellous bone grafts (CBG) has proved to be the most effective sterilization agent for the removal of microorganisms such as bacteria, fungi, viruses, and even spores.[25] The combined chemical effect *via* ultrasonication in the bone allografts helps to clear residual DNA more effectively from the empty cavities, a rough surface of bone tissues, and a trabecular structure of bones *i.e.* femoral head.[26]

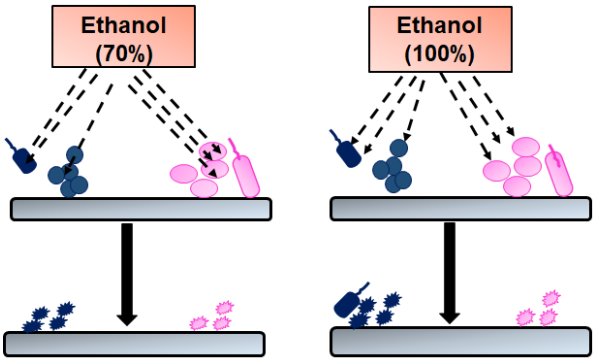


Figure 2: Effect of ethanol at 70% and 100% on bacterial inhibition.

- **Hydrogen Peroxide**

The use of H_2O_2 (hydrogen peroxide) as an antiseptic is widely accepted due to its biocidal property, with elimination of water and oxygen as residual products.[27] However, the use of hydrogen peroxide as an oxidizing agent is explored in the bone bank, as it generates reactive oxygen species, which attack the biomolecules such as nucleic acids, protein and lipid, maintaining the cellular and viral integrity of the cell.[28] The occurrence of H_2O_2 in animal and human tissues plays a significant role in cell signaling, inflammation and aging. Hydrogen peroxide possesses deantigenation with antimicrobial activities against bacteria, fungi, spores, and viruses. Few studies regarding H_2O_2 usage have examined its cytotoxicity without affecting the osteoconductivity of allografts. Generally, 3 to 6% is recommended for bone processing, eliminating donor cells and pathogens. In addition to that, there are no alterations in the mechanical property of cortical bone allografts due to H_2O_2 treatment.[29] H_2O_2 at 3% decreased osteoinductivity of demineralized bone matrix obtained from bovine femur, attributed to its strong oxidant property resulting in the apoptosis of cells.[30] However, high exposure of H_2O_2 may affect the BMP (bone morphogenetic proteins), resulting in decreased osteoinductivity. Also, the hydrogen exposure decreases the bioburden to extremely low level, but not to negligible.

- **Sodium hypochlorite**

Sodium hypochlorite (NaOCl) in hospitals is explored as disinfectant, or a bleaching agent attributed to its non-toxicity, low-cost, easy availability, water-soluble property with long-term stability both in concentrated and dilute solutions.[31] Sodium hypochlorite is an antimicrobial agent that inhibits the bacterial enzymes by disrupting the cytoplasmic membrane and phospholipid, with inhibition in the active transport of energy sources of bacteria.[32] In tissue banks, during sterilization, when NaOCl solution comes in direct contact with the biological tissues, then chlorine is released from hypochlorous acid (HOCl), which is present in NaOCl solution.[33] This released chlorine interact with the -NH_2 group present in protein forming chloramines (R-NHCl), a potent strong oxidant disrupts the bacterial cell membrane by oxidizing the cytoplasmic proteins and nucleic acid.[34] 1% NaOCl has a moistening capacity of 1 h 27 min, *i.e.* suitable for long term effect of this disinfecting solvent.[35] The use of 1% sodium hypochlorite is suitable for biological compatibility to be used as a disinfectant.[36]

William et al., 2013 classified the chemical agents as critical, semi-critical, and non-critical for its application in hospitals, in which there is an inherent drawback that it does not maintain its sterility following processing and product packing.[37] Similarly, chemical sterilants in bone allografts preparation does not maintain its guaranteed sterility during bone processing and bone allograft packing. In that case, bone allografts should be subjected to terminal sterilization after sealed product packaging.[38]

(B) PHYSICAL AGENTS FOR STERILIZATION

- **Gamma Radiation**

In past decades, the bone bank mainly relied on the use of terminal sterilization for guaranteed application in orthopaedic applications in various dental applications, trauma and spinal fusion surgeries.[39] Gamma irradiation, a cold sterilization method, is used for terminal sterilization of the bone allografts at a commercial scale. It uses high energy gamma photons sources from radioactive isotopes, Cobalt-60.[40] Gamma irradiation kills the micro-organisms by two mechanisms, *i.e.*, by directly altering the nucleic acids resulting in genome dysfunction and destruction with the generation of free radicals.[41] These gamma rays penetrate deeply in the bone tissue, destruct microbial DNA, resulting in the inactivation of microbial cells on the biological construct to obtain sterilized products. The unique deep penetration power of gamma rays is not restricted only to sterilize bone allografts, but also other healthcare products, such as medical devices and pharmaceuticals.[42]

Few studies reported 25 kGy as a radiation dose to detect the efficiency for sterilization on high bioburden level on the artificially micro-organism infected material. In 1973, 25 kGy was used as a standard dose to sterilize medical products such as syringes and sutures.[43] Finally, in 1990 IAEA (International Atomic Energy Agency) recommended 25 kGy as the reference dose to sterilize the allograft tissue, with the main focus on maintaining the biological entity of the tissue.[44] The dose of 25 kGy sterilization dose of gamma irradiation is recommended for medical devices such as bone allografts, maintaining the sterility assurance level (SAL) of 10^{-6}.[45] Previously, the choice of irradiation dose to sterilize the biological tissue depends on the number of micro-organisms present, *i.e.*, bioburden prior to sterilization. The microbial reduction was much higher at 25 kGy than at 15 kGy, making this radiation dose suitable in joint surgeries, such as decreased complication in fracture of bone allograft, nonunion and prosthetic loosening.[46]

CONCLUSION

The use of chemical sterilants in bone tissue processing, followed by terminal sterilization for sterile allograft packaging, is a vital task of The Bone bank to provide satisfactory outcomes for orthopaedic applications. On the basis of this review, we recommend the use of chemicals followed by gamma sterilization for the commercialization of bone allografts. These procedures of sterilization improve the efficacy of bone allografts with preserving the biological integrity of the bone.

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