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Sterilization of Human Tissues for Allo-transplantation

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The use of allografts has been used in clinical practice for many years for the treatment of orthopaedic or cardiovascular disorders. There is always a risk of disease transmission with the use of allograft although it appears to be diminishing through the rigorous screening of donors, and improved harvesting and banking techniques. Whatever method is used, the graft cannot be guaranteed sterile and some centers use methods such as irradiation or ethylene oxide treatment for secondary decontamination of the grafts. These forms of sterilization can influence the biological and/or biomechanical characteristics of the allografts. Another method of reducing the risk of infection is to minimize contamination during the harvesting of the allograft in operating theaters. This contamination, through contact of the graft with the operating room air or with non-sterile surfaces, is also greater from cadaveric donors at the end of multiorgan harvest, than from living donors. Some experimental protocols recommend the rinsing of contaminated grafts with antiseptic or antibiotic solutions. Since antibiotic disinfection procedures were developed to treat heart valve allografts for tissue banking in 1969, several different combinations of antibiotics have been used to treat fresh and/or cryopreserved heart valves. However, antibiotic toxicity can be a problem in situations where cellular viability of the tissues is considered important. In the last part, development of antibiotics with modified composition to improve cellular viability and its practical applications to cryopreserved vascular tissues were described on the basis of our previous studies.

Key Words : Allografts, Sterilization, Contamination, Antibiotics, Cryopreservation, Cellular Viability

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INTRODUCTION

Over the last few decades, the uses of various allogenic and xenogenic grafts have proved to be reliable methods for the reconstruction of orthopaedic and cardiovascular defects. These grafts received from multiorgan donors should be microbiologically sterile and might be either transplanted immediately or cryopreserved for future use. With the use of transplanted graft, however, there is the potential risk of transmission of infective particles. In spite of optimal surgical technique and routine prophylactic treatments, from 3 to 5% of vascular grafts obtained from multiorgan donors have been reported to be contaminated[34]. The incidence of infection associated with the use of allograft bone has also been reported to be between 5 and 13%[29,36,38,61,62] and there have been reports of the implantation of contaminated allografts[29,36,60-62]. As the allograft bone commonly used in orthopaedic surgery is different form other surgical grafts, cellular viability of the tissues is not so much considered important(Table 1). However, microbial infection can be fetal to the viability and/or durability of the post-operative allograft tissues and lead even to death, related to severe inflammatory reaction. To prevent these microbiological contaminations, various sterilization methods have been tried and employed(Table 2). Up to now, the known treatments might be divided into physical methods and chemical treatments. High temperature steam(autoclaving), gas, gamma or ultraviolet ray, plasma, and ultrasound can be used in physical sterilization. Though all chemical compounds with cytotoxicity might belong to antibiotics(Table 3), the antibiotic toxicity can kill tissue cells and modify graft properties, which result in graft failure in a few years post-transplantation[41]. In our previous studies[45],

Table 1. Consideration of cellular viability of tissue graft

cellular viability necessary	cellular viability selective
most grafts except grafts related to orthopaedic surgery	grafts related to orthopaedic surgery (except cartilage)
cardiovascular tissue or organ	cancellous bone
skin	cortical bone
liver	hip joint
lung	
kidney	
pancreas	
esophagus	
nerve	
cornea	
...	
cartilage, marrow etc.	

Table 2. Possible applications to allografts according to sterilization methods

sterilization	advantages	disadvantages	applications	viability consideration
high temperature steam (121℃, 15 min)	elimination of spore-forming bacteria within 30 min	possible contamination by outer air on fast freezing	orthopaedic surgery	×
ethylene oxide gas (55℃)	penetration into bacterial cell wall, inhibition of cell growth	skin irritation burns and haemolysis exposure to air	orthopaedic surgery	×
ultraviolet ray (254 nm)	electro-magnetic breakage of microbial cells, sterilization of surface microbes	ineffectiveness to disinfection of packed or irregular specimen without penetration into opaque materials	tissue disinfection, but less effective	×
gamma irradiation	sterilization of HIV or hepatitis C in human cortical bone grafts	expensive equipments, unsuitable for common hospital facilities	no safety proof for vascular grafts	×
chemical treatments	all chemical compounds with cytotoxicity	lethality to tissue graft, selection of optimal conditions & spectrum	all transplanted grafts	○

Table 3. Comparison of chemical sterilization methods

chemical agent	properties	application examples
all antibiotics penicillin, tetracycline, clindamycin, ampotericin B etc.	inhibition of bacterial cell wall and protein synthesis, detrimental to mammalian cells resistance to specific chemical	reported in many literatures (skin[16], vein[13,51,53], aorta[13,33] etc.)
peracetic acid -ethanol	used in disinfection of open wounds with chlorhexidine no histological injury/low toxicity	femoral head from hip replacement[55], cornea[49]
10% povidone -iodine solution	bactericidal, fungicidal, virucidal and sporicidal effects	defatted human spongiosa[48]
ethylene oxide gas	penetration into inner side of tissue grafts elimination bacteria and virus	cancellous bone[31,68], cortical bone[32,50,63,66], allodermis[10]

modified antibiotic composition was developed to improve cellular viability, to maximize sterilizing power and to trigger synergistic effect, and then applied to cryopreserved vascular tissues.

The general purpose of a tissue bank is to provide safe and effective allografts for patients[19]. Sterile tissue recovery in an

Table 4. Conventional combination of antibiotics

antibiotics[a)]	concentration (μg/ml)
cefoxitin	240
lincomycin	120
vancomycin	100
polymixin B	50

a) used at Toronto Tissue Bank[42-44,53)]

Table 5. Modified combination of antibiotics

antibiotics[b)]	concentration (μg/ml)
liposomal cefoxitin	625
clindamycin	8
vancomycin	250
polymixin B	250

b) used at Yonsei Cardiovascular Tissue Bank[45)]

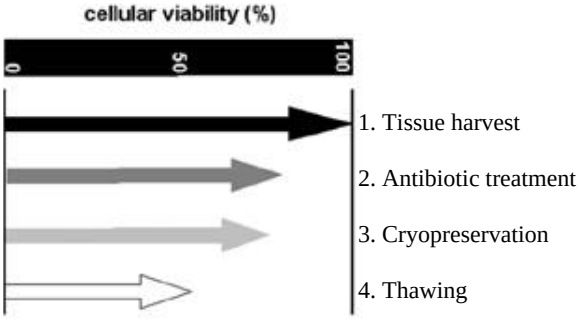

Fig. 1. Cellular viability changes according to cryopreservation process.

operating theatre is required for the collection of allografts transplanted without undergoing secondary sterilization[40)]. Cultures from all collected tissues must be taken, and contaminated allografts discarded[9,40)]. In contrast to living donors, multiorgan and tissue donors allow recovery of long bones(femurs, tibiae, fibulae, humeri, radii, ulnae), as well as bone-tendon-bone complexes such as patella-patellar-tendon-tibial tubercle grafts, Achilles tendons with or without bone blocks, tibial tendons, and tissues obtained for special purposes[54,62)]. With increasing demand for allograft bone, infection of implanted allograft bones is of increasing concern. However, a universal consensus on how a bone bank should be operated has not been reached, and the experience with graft-associated infection has not been made widely available[35)]. Therefore, the goals of this review are to analyze the relationship between previous organ and tissue procurements with allograft contamination and to introduce the efficiency of antibiotic sterilization and its practical applications on the basis of our previous studies.

EO gas sterilization

Allografts have been used in orthopedic surgery for many years. Autoclaving is known to be unsuitable for the sterilization of bone allografts, because it may denature structural and bioactive proteins, including collagen and bone morphogenetic proteins. Another method of sterilization of medical supplies, with ethylene oxide(EO) gas, is widely used in hospitals and in the medical products industry; although freeze-dried bone is not used much for allografts in Japan[30)], EO gas sterilization is now frequently used in the manufacturing process of this bone. Zhang et al.[68)] have reported that bone induction was little affected clinically by EO gas sterilization at 40℃. Chip bone allografts defatted with chloroform and methanol, freeze-dried, and sterilized with EO gas have been used in various procedures of orthopedic surgery since 1994[14,15,31)]. It was evident that repeated

pre-operative aeration and more than 2 weeks' preservation were recommended before use for reducing the residual EO concentration and that intra-operative rinsing with 500 ml of physiological saline for 10 min reduced the EO residual level[31].

Exposure to EO gas is accepted as an effective sterilization method for bone transplants, because EO completely penetrates into bone and destroys bacteria and viruses. The sterilization effect of EO gas is based on the acylation of amino acids. Because of the potential carcinogenicity of EO and its products, the reduction of the EO residual concentration in allografts is an urgent problem. EO gas is very soluble in both water and fat. Defatting and freeze-drying before EO exposure result in lower residual concentrations than freeze-drying after EO exposure. However, exposure to EO residuals has been reported to cause adverse changes in the human body[3,18,20].

Cortical bone transplantation is often performed to fill cortical bone defects following bone fractures or bone tumours[22]. It is not necessary that these cortical grafts contain viable cells, since they are mainly used for mechanical support and as a scaffold for new bone formation. Therefore, dead cortical bone can be used as graft[32]. Several preservation methods are used, such as freezing lyophilization or drying at room temperature. Contamination of the preserved grafts due to infectious diseases or as a result of manipulation is a risk that can be overcome by sterilizing the graft. The most commonly used sterilization procedures in man and animals are high-dose irradiation or EO-sterilization[21]. However, sterilization and preservation can change the properties of the grafts. High-dose irradiation can decrease the biomechanical properties[51,58], the antigenicity and/or the osteoinductive capacity of the grafts[8], as has been described for human transplants. The current EO-sterilization procedures at temperatures of 43.3~64℃ and pressures of 7.584 bar decrease the biomechanical properties of dog grafts[50,66]. According to Aspenberg et al.[3], EO destroys the dose-dependent bone-induction properties, as is seen for human transplants. There are no data on the effect of EO-sterilization on the antigenicity of the bone grafts, either in man or in animals. Optimally sterilized and preserved cortical bone allografts should retain their biomechanical and osteoinductive properties and have a reduced antigenicity. Tshamala et al.[64] described changes in the EO-sterilization procedure that resulted in preservation of the biomechanical properties of dog bone allografts. It was also demonstrated that the antigenicity of dog cortical bone allografts was very weak and the EO-sterilization on dog grafts had no effect on osteoinduction, but decreased bone resorption[63].

Gamma irradiation

There are presently various techniques for preparing allografts. The most frequently used is cryopreservation in deep freezers(-40 to -80℃) or liquid nitrogen(-196℃). Cryopreservation has been reported to reduce immunogenicity as the

donor's cells are destroyed but it has no effect on bacterial and viral contamination[24,52]. A drastic selection of donors, the use of a surgical theater with aseptic conditions during harvesting and a serological survey are necessary[6]. An additional 3 week antibiotic therapy is also recommended by the American Association of Orthopedic Surgery and Traumatology[23]. Lyophilization is another method that implies complete dehydration of bone samples under vacuum. It provides materials that can be stored at room temperature. However, the method works poorly on large allografts, suffers from prohibitive cost and does not exhibit sterilizing properties[59].

Gamma(or beta) irradiation is presently a widely accepted procedure and a 25,000 gray dosage is usually recommended. Irradiation is active on bacteria but its effects on virus are questionable. Irradiation was also reported to alter bone mechanical properties[37]. One should note that bone pieces that will be used for filling purposes(e.g., femoral heads) are generally cleaned of articular cartilage by grinding before sterilization. However, the organic components present in the marrow cavities are not removed. These are mainly composed of fatty cells that can account for a very important fraction of the total weight for an adult femoral head: 60~70%. Adipocytes are specialized cells responsible for lipid synthesis and storage. When exposed to oxidizing conditions, lipids are known to be altered by peroxidation.

It has been shown that extensive defatting was a prerequisite for preparing xenogenic bone and that incompletely processed materials induced an inflammatory reaction associated with Langhans-like giant cells[12]. Gamma irradiation is a powerful oxidizing process and its effect on medullary lipids has never been investigated. Defatted slices that had been sterilized by gamma radiations or UV did not induce cell death, and defatting procedures should be added when preparing bone allografts in human bone banks[39].

Chemical treatments

The use of allograft bone in orthopaedic surgery is now commonplace. With the use of transplanted bone, however, there is potential risk of transmission of infective particles. The contamination of allograft material is not an uncommon event, and the incidences reported in the literature range from 1 to 37%[5,11,29,36,59,62]. The higher figure was reported from cadaveric bone, and there is a lower incidence of contamination of bone from live donors. Some experimental protocols[54,65] recommend the rinsing of contaminated grafts with antiseptic or antibiotic solutions, but few studies have analyzed the clinical benefit of these allograft decontaminations[7]. Several methods have described in literature that offer the possibility of peroperative decontamination of autografts that have been accidentally polluted by falling on the floor. In the most of these cases, there are experimental studies of bacteriological efficiency of antibiotic or antiseptic solutions on bone-tendon grafts during ligament replacements[7,27,47,57]. For some years, 10% povidone-iodine solution

has been used to decontaminate wounds. It was revealed that a 10% povidone-iodine solution could decontaminate inoculated bone grafts, but a sufficient time of exposure according to the level of contamination must be allowed[55].

In Germany about 25,000 allogenic bone graft transplantations are performed annually. Human bone tissue serves as source material for the production of these transplants. Accordingly, special requirements have to be made on the biological safety of the tissues. Apart from a validated clinical effectiveness of the transplants, the quality assurance measures must focus on the validation of the sterilization process. In accordance with the guidelines for managing a bone tissue bank issued by the Federal Medical Society, sterilization is particularly indicated if a second testing of the bone tissue donor after an appropriate time cannot be performed, because the tissue was obtained from a cadaveric source. In addition, these validations are required standards in Germany, where all transplants manufactured in national tissue banks are regarded as medicinal products.

So far, no normative specifications exist regarding validation of the disinfection or sterilization of bone tissue intended to be used for transplantation. At present, several procedures are used for inactivating bacteria and fungi, including their spores, as well as viruses in the context of the production of bone tissue transplants. Gamma irradiation, thermal treatment as well as peracetic acid-ethanol(PES) treatment under negative pressure conditions, are applied. Pruss et al.[48] demonstrated that the PES procedure proved to be a reliable method for the sterilization of human bone transplants derived from spongiosa. However, ethylene oxide as well as beta-propiolactone and formaldehyde treatment are no longer considered as suitable methods for sterilization in Germany, due to their carcinogenic and mutagenic effect. Additionally, the latter clearly reduces the osteoinductive effect. Therefore, for as long as the last 20 years, ethanol and peracetic acid have been increasingly used for tissue sterilization. However, the diffusion of these substances into the tissue is limited. Penetration-inhibiting fat barriers must be removed by treating the spongiosa with a chloroform-methanol mixture or by an equivalent validated procedure.

Antibiotic treatments

The use of allograft tissues, saphenous veins, and heart valves for cardiovascular reconstruction has increased considerably during the last decade. Despite the use of appropriate systemic antibiotic prophylaxis, graft infection occurs after 1~6% of all vascular reconstructions[46]. To prevent this microbial contamination, sterilization of the graft by using antibiotics has been employed for further clinical use[9]. Sterilization with antibiotics affects the molecular structure of the graft, and cellular viability is related to its subsequent durability[51]. Traditional chemical methods of sterilization can kill all heart valve cells and modify valve material properties. Some investigators reported that antibiotic treatments caused morphological

changes[26], inhibited invasion of tissues by fibroblasts[25], and affected viability of heart valve grafts[1]. Hu et al.[28] showed that the amphotericin B component of these antibiotic disinfection mixtures reduced cellular viability of porcine heart valves by 100% after 12 h storage at 4℃. The effect of antibiotics on cells and tissues are dependent on the condition of treatment, and the optimal time and temperature of treatment are necessary to maintain cellular viability and sterilization effect[28,42,53]. Therefore, reducing damage of antibiotics to cells and tissues can provide the basis to improve cellular viability and function in the grafted tissue. In our previous study, it was shown that antibiotic treatment for 4 h at 4℃ was the optimal condition for antibacterial activity with maintaining cellular viability in canine veins[43,44].

In recent years, researchers and surgeons have been investigating the use of cryopreserved allograft veins for tissue transplantation. The cryopreservation of tissue at -70℃ to -196℃ offers the prospect of indefinite storage. The preservation method of an acquired tissue influences the viability of cells such as fibroblasts and endothelial cells. The harvesting, an interval between the arrest of donor's heart and preservation of valves in refrigeration, sterilization(antibiotic treatment), cryopreservation, and thawing are primary factors which affect the cellular physiology[2,4,67]. According to the previous study, the viability of cells in the harvested veins was gradually reduced during cryopreservation and then rapidly decreased after thawing(Fig. 1)[42,43]. To overcome these problems, the conventional combination of antibiotics used at Toronto Tissue Bank(Table 4) was changed into the new one. It has been developed to improve cellular viability, to maximize sterilizing power, and to trigger synergistic effect and then employed at Yonsei Cardiovascular Tissue Bank(Table 5)[45]. Lincomycin was substituted with clindamycin in order to extend limited antimicrobial spectrum only for Gram-positive bacteria. Moreover, cefoxitin was entrapped with liposome to give the possibility to improve the cellular viability by controlling the excess presentation of drug in tissue. In terms of sterilization effect, liposomal cefoxitin showed the antibacterial effect comparable to the free cefoxitin, indicating that liposomal entrapment of cefoxitin could not affected the original antibiotic effect.

In conclusion, when pretreated to human saphenous vein, liposomal entrapment of cefoxitin could improve the viability and function of endothelial cells and maintain the original sterilization effect. The antibiotics described here would also be useful for sterilization of other grafts for transplantation in which cellular viability and function should be considered important.

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