## **Review Article**

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# Allograft sterilization and processing: impact on biomechanical strength

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#### ABSTRACT

The allograft used for anterior cruciate ligament reconstruction (ACLR) must posses good biomechanical properties and it should have similar properties to the original tendon. During reconstruction the allograft must undergo proper sterilization and several sterilization methods have been used in the clinical practice. There are varations in the sterilization process and it has significant impact on the allograft tissue performance during ACL reconstruction. It is advisable to refrain from utilising grafts that have been exposed to radiation doses exceeding 15 kGy, as well as grafts that have undergone more than eight freeze-thaw cycles. Gamma radiation has disadvantages when compared to electron beam radiation in term of loss of mechanical strength.

Keywords: ACL, Reconstruction, Allografts, Sterilization, Gamma radiation, Electron beam

#### **INTRODUCTION**

Anterior cruciate ligament reconstruction (ACLR) is a very common and standard orthopedic procedure that can be completed with either autograft or allograft tissue or a hybrid graft (a combination of autograft and allograft). The purpose of ACLR is to restore stability and reduce the risk of future arthritis. In the United States, approximately 175,000 ACL injuries occur annually, and over 100,000 surgeries are performed every year.<sup>1</sup> However, graft choice in primary ACLR remains controversial to date. The utilisation of allograft for ACL repair was first documented in 1986 and has since been widely embraced due to its associated advantages, including less complications at the donor site, decreased duration of surgery, availability of grafts for revision procedures, and a lower risk of arthrofibrosis.<sup>2</sup> The utilisation of allografts in reconstructive procedures has experienced a significant boom in popularity, leading to a substantial increase in their adoption by surgeons. This trend has yielded remarkable improvements in quality of life for patients. Allogenic tissues are acquired from both living and deceased donors. Thorough screening protocols are implemented to ensure that all donors undergo a rigorous evaluation process, so guaranteeing that the collected donor material is devoid of any pathogens capable of transmitting diseases to the receivers of the tissue. Tissue allografts serve as a straightforward and efficient clinical instrument for reconstructive surgery, concurrently circumventing the discomfort, stress, and adverse health effects associated with a subsequent surgical intervention required for obtaining autologous tissue.

The strategies for allograft encompasses grafts isolated from patellar, quadriceps and Achilles tendons and also from soft tissues which includes tendons of hamstring, anterior tibialis, posterior tibialis, and peroneus longus. The long term results of allograft tissue leads to lamentation and resembles original ACL in both macroscopically and microscopically.<sup>3</sup> The advantage of allograft ACLR when compared to autograft is the minimal surgery time, more available, lower rate of postoperative arthrofibrosis lower morbidity risk at donor site and wide graft options.<sup>4</sup> The complications of allograt includes anterior knee pain, loss knee flexion and extension and in addition there is a high graft failure rates and the infections.<sup>5</sup>

The major contributor to the low survival rate of allografts is the graft processing method involved in the preparation of allografts. The tissue used in allograft ACLR is subjected to sterilization by mechanical and chemical methods and it includes use of alcohols, antibiotics and hydrogen peroxide. Post disinfection, a terminal sterilization process is adopted before the implantation of allograft.<sup>6</sup> Fresh Freezing and freeze-drying an sterilization methods are adopted to prepare the allograft with reduced graft antigenicity by destroying class 2 major histocompatibility proteins on the donor cells and without affecting the strength of the graft.<sup>2</sup> Preparation of allograft by this method allows storage of allograft for 6 months and two years respectively. Secondary sterilization includes tissue exposure with ethylene oxide and Y-irradiation technique to eliminate bacterial and viral contamination.<sup>7</sup> However, this series of processes involved in the preparation for allograft results in reduced integrity and bio-mechanical strength of allograft as well as the show higher clinical failure. This exhaustive review article elaborates on the major features of allograft preparation which determines the biomechanical strength of the allograft in ACLR. The purpose of this paper is to provide a comprehensive review of the currently available evidence related to methods of the sterilization and the processing used the allograft tissue for primary reconstruction of the ACL affecting its biomechanical strength and the integrity.

#### STERILIZATION AND CHEMICAL PROCESSING

During ACLR, to decrease the risk of host site immune response and infectious disease transmission such as bacterial, viral, or fungal, the allograft intended must be sterilized.7 In earlier times, ethylene oxide or peratic acid was used for bone sterilization but currently, their use is not in favor due to high failure rates, complications, and incidences of graft removal resulting from chronic synovitis or immune responses.<sup>8</sup> The utilisation of ethylene oxide sterilisation was found to be linked with a reduction in maximal force (29% of the untreated group) and decreased graft stiffness (43%) in goats during the 6 and 12-month periods following BPTB allograft restoration.9 The literature has documented that peracetic acid demonstrated a 39% reduction in load to failure (LTF) in sheep 12 weeks following ACL reconstruction.<sup>10</sup> Conversely, no significant differences were observed in stiffness or LTF in cadaveric BPTB grafts.<sup>11</sup> In contrast, rabbits exhibited a 48% rise in LTF 12 weeks after ACL reconstruction when treated with peracetic acid.<sup>12</sup>

Recently, novel sterilization methods have been used for graft sterilization during ACLR. The newer methods include a mixture of detergents, alcohol, antibiotics and peroxides for the tissue disinfection and also reduces the disease transmission risk and also minifies the damage to graft mechanical properties. BioCleanse (Regeneration Technologies, Alachua, FL) is a complete automated device which encompasses a combination of mechanical and chemical processes. In this process there is an alternate cycles of vaccum and pressure which eliminates donor debris like blood and lipids and also perfuse the entire tissue with chemical agents to remove the bacteria, viruses and fungal spores.<sup>13</sup> In a laboratory study conducted on central third or hemi-BPTB units from both knees of 17 cadaveric tissue donors showed that treatment with BioCleanse showed no significant difference in the preimplantation allograft mechanical properties as compared to untreated allografts. The in vitro study concluded that the preimplantation mechanical properties of BPTB allografts treated with are not significantly different from those of untreated controls.14 In a randomized study conducted on 67 patients undergoing ACLR and divided into two groups BioCleanse-sterilized or aseptic BTB allografts. BioCleanse treatment did not offer any significant difference in the clinical outcome at 2 years of follow up when compared to aseptically processed allograft tissue.15

#### **IRRADIATION**

Radiation sterilisation stands as a widely implemented and highly effective utilisation of radiation. The efficacy of eliminating bacteria is contingent upon the ionising radiation's capacity to induce lethality. The recognition of the lethal effects of ionising radiation on micro-organisms dates back to 1896, in close proximity to the initial discovery of X-rays. In the year 1899, Pierre and Maria Curie conducted an observation of the effects of beta and gamma rays emitted by natural isotopes on various materials and tissues. Sterilisation is conducted through two primary methods: gamma irradiation utilising <sup>60</sup>Co, and electron-beam irradiation facilitated by a range of electron accelerators. The sterilization of allograft primarily uses ionizing radiation such as Y-irradiation. The measurement of Y-radiation dosage is quantified in units of kilogray (kGy). The initial proposal for the sterilisation of medicinal products with a dose of 25 kGy (2.5 Mrad) was put up in 1959 by Artandli and Van Winkle. The proposed dose was determined by considering the minimal lethal dose for about 150 different microbial species. The dose of 25 kGy was chosen for sterilisation because to its 40% higher value compared to the minimal dose necessary for eliminating the resilient microorganisms.16

The examination of tissue allografts' biological characteristics, including immunogenicity, resorption rate, ability to stimulate regeneration processes, such as the osteoinductive capacity of bone grafts, and occasionally their mechanical properties, holds significant clinical significance. The impact of Y-radiation from cobalt-60 sources on the mechanical and biological characteristics of bone allografts has been reported to be dose-dependent. These changes occur during the process of irradiation and affect the properties of the allografts. According to research findings, it has been observed that the mechanical characteristics of cortical bone experience a notable decline when exposed to gamma irradiation at doses over 25 kGy. Similarly, for cancellous bone, a considerable drop in mechanical properties is documented at doses surpassing 60 kGy.<sup>17</sup> The successful integration of transplant bone and the rate of recovery can be influenced by several crucial aspects, including biocompatibility, osteogenic capacity, biomechanical strength, and architectural considerations. The application of gamma irradiation for sterilisation purposes has been shown to result in a decrease in the osteogenic capacity of bone. This drop is attributed to a reduction in biocompatibility caused by the synthesis of peroxidized lipids.<sup>18</sup>Additionally, the biomechanical stability of the bone is compromised as a result of this sterilisation method.19

The impact of low-dose gamma irradiation ( $\leq 20$  kGy) on biomechanical qualities was found to be varied. For instance, stiffness was seen to decrease by 20% when exposed to 10 to 12 kGy, while LTF decreased by 20% when exposed to 20 kGy.<sup>20</sup> However, no significant differences in biomechanical parameters were observed when treated with 12 to 18 kGy.<sup>21</sup> A correlation was found between the dosage of gamma irradiation and the levels of LTF, with larger doses (20-40 kGy) consistently associated with reduced LTF (54%-74% of nonirradiated tissue).<sup>22,23</sup> The stiffness of the tissue was found to be reduced in five out of six studies that examined various amounts of irradiation. The reduction in stiffness ranged from 54% to 85% when compared to nonirradiated tissue.<sup>24,25</sup>

The most widely used allograft in the clinical scenario is the fresh-frozen, and Y irradiated BPTB.<sup>26</sup> Earlier reports showed that there is no marked differences in the clinical outcome for irradiated and nonirradiated BPTB allografts and additional studies reported that Y-irradiated Achilles allograft displayed more failure rates as compared to nonirradiated graft.<sup>27,28</sup> In a study done by Guo et al patients reconstructed with Y-irradiated allograft displayed marked knee loosening as compared to other sterilization methods based on KT-1000, Lachman and pivot-shift evaluations for a follow up period of 6 years. Meanwhile, they also reported higher incidence of graft failure in the  $\Upsilon$ -irradiated allograft groups.<sup>29,29</sup> Kan et al conducted a meta-analysis and he reported that autograft is more efficient than irradiated allografts and also there is no significant in the clinical outcome autografts and nonirradiated allografts.<sup>30</sup> In a study done by Maletis et al the allografts prepared from soft tissue and irradiated with 1.8 Mrad displayed increased risk of revision as that of BPTB autografts and hamstring autografts.<sup>31</sup> Thus the above study findings reveals that Y-irradiated allograft is not a

suitable option for ACLR and it needs routine examination at frequent follow-ups.

#### **ELECTRON BEAM**

The electron beam (E-beam) is a suitable replacement for irradiation in the sterilization of graft tissue. The E-beam offers significant advantage over gamma irradiation with more accuracy, wide range of dose and low processing time.<sup>31</sup> The main disadvantage of E-beam is decreased penetration depth as compared to gamma rays.<sup>32</sup> The decreased depth of penetration is mainly evident in allograft with thickness of 5 cm and so this method is not patellar tendon suitable for thinner allografts like allografts which are routinely used in the ACL reconstruction.<sup>33</sup> In Hoburg et al study they evaluated the human patellar tendon allografts mechanical strength after variuous sterilization process. In their study 3 doses of electron beam radiation are used 15, 25, or 34 kGy respectively.<sup>33</sup>

In another study done by Hoburg et al reported that biomechanical properties of Ebeam irradiated BPTB grafts is higher than gamma rays' method.<sup>33</sup> In this study two doses of Ebeam and Gamma were used 25 kGy and 34 kGy respectively and grafts without radiation is used as a control. The study showed that Gamma-irradiated grafts displayed significant reduction in stiffness, failure loads and increased creep.

The above studies showed that Ebeam is a suitable alternative for Gamma rays for graft sterilization with less effects on mechanical properties.

#### **PRESERVATION METHODS**

Allografts are conventionally preserved using freezing or freeze-drying techniques. Frozen grafts necessitate specific transportation and storage conditions, in addition to the requirement of thawing the graft prior to its utilisation. Prior to use, freeze-dried grafts necessitate rehydration, a process that may not fully reinstate the inherent characteristics of the bone.<sup>34</sup> The biomechanical integrity of an implant may be impaired when it is partially hydrated. The preservation techniques employed for allografts are crucial in ensuring the viability, structural integrity, and safety of tissues and organs procured from a donor and intended for transplantation into a recipient. The selection of the preservation strategy is contingent upon several aspects, including as the nature of the allograft, the anticipated duration of storage, and the specific demands of the transplantation procedure.35

The researchers developed a preservation method known as glycerol-based preservation (GBP), which utilises the unique properties of glycerol to safeguard tissue integrity and maintain its hydration levels. Glycerol, a liquid substance classified by the FDA as 'generally recognised as safe' (GRAS), is characterised by its non-toxicity and biodegradability. This approach eliminates the need for freeze-drying, thereby avoiding the expenses and tissue modifications associated with this technique. Furthermore, GBP enables the convenience of shipping and storage at ambient temperatures, offering reduced costs compared to the conventional methods of freezing or refrigerating tissue. GBP offers a technique for conserving bone and soft tissue transplants by the substitution of water molecules in the tissue with glycerol.<sup>36</sup> Glycerol exhibits a relatively low molecular weight, enabling it to effectively displace water molecules by occupying vacant regions within the tissue architecture. Glycerol facilitates the preservation of bone and dermis allografts at ambient temperature by maintaining their moisture content, hence preventing desiccation.

The application of glycerolization and lyophilization techniques prior to irradiation resulted in a significant reduction of approximately 40% to 50% in LTF.37 Furthermore, the treatment of allografts with propylene glycol and glycerol monolaurate or chloroform-methanol extraction led to a decrease in both peak load and stiffness, with values reaching approximately 30% and 43% of those observed in a normal ACL, respectively.<sup>38</sup> The application of a cryoprotectant for a duration of 2 to 8 hours led to a reduction in stiffness ranging from 17% to 19%, while it did not have any significant effect on LTF.39 The utilisation of glycerol as a cryoprotectant for the preservation of cadaveric bone-patellar tendon-bone (BPTB) grafts for a period ranging from 3 to 9 months did not yield any significant disparities in terms of ultimate stress (ranging from 112% to 121% of fresh allograft) and ultimate stiffness (ranging from 104% to 115%) when compared to fresh allografts.40

Thus, findings from investigations have indicated that the preservation of bone using glycerol results in a material that retains its biomechanical strength, hence obviating the necessity for prolonged rehydration or thawing periods when employed in clinical settings. Furthermore, experimental findings conducted in living organisms indicate that the preservation of bone grafts using glycerol does not have any detrimental effects on their capacity to effectively contribute to the process of new bone production and fusion.

#### CONCLUSION

In summary, a multitude of factors have a role in influencing the biomechanical characteristics of allograft tissue when utilised for ACL replacement. Surgeons' decision-making about the choice of allografts and their preferences for specific graft qualities can be influenced by their understanding of these criteria. Consequently, this knowledge may assist surgeons in effectively communicating their requirements to local tissue banks when ordering grafts. In order to enhance the biomechanical characteristics of allografts, it is recommended that surgeons employ looped soft tissue grafts or central third patellar tendon. It is advisable to refrain from utilising grafts that have been exposed to radiation doses exceeding 15 kGy, as well as grafts that have undergone more than eight freeze-thaw cycles. In order to enhance clinical care with allograft tissue, surgeons are required to familiarise themselves with the processing and sterilisation techniques employed by their tissue bank.

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