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# Effect of Electron Beam Irradiation on Biomechanical Properties of Patellar Tendon Allografts in Anterior Cruciate Ligament Reconstruction

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**Background:** Sterilization of anterior cruciate ligament (ACL) allografts is an important prerequisite to prevent disease transmission. However, mechanical tissue properties are compromised by most current sterilization procedures, so that uncompromised sterilization of allografts is difficult to achieve.

**Hypothesis/Purpose:** The aim of this study was to evaluate the effect of the novel electron beam sterilization procedure on the biomechanical properties of human patellar tendon allografts at various irradiation dosages. Electron beam sterilization may be an appropriate alternative to gamma sterilization.

**Study Design:** Controlled laboratory study.

**Methods:** Thirty-two human 10-mm wide bone-patellar tendon-bone grafts were randomized into 4 groups of sterilization with 15, 25, or 34 kGy of electron beam irradiation, respectively. The grafts' biomechanical properties were evaluated at time zero. Unsterilized grafts functioned as controls. Biomechanical properties were analyzed during cyclic and load-to-failure testing.

**Results:** Strain and cyclic elongation response showed no significant differences between the groups. Electron beam irradiation had no significant effect on stiffness and failure load with the exception of 34 kGy, which resulted in a significant decrease in failure load ( $1300.6 \pm 229.2$  N) compared with unsterilized grafts ( $1630.5 \pm 331.1$  N).

**Conclusion:** This study showed that electron beam might be an appropriate alternative in sterilization of patellar tendon allografts with minimal effect on mechanical properties of tendon grafts in vitro. Future studies will have to evaluate the effect of the process on the biological properties of allografts in vitro and in vivo.

**Clinical Relevance:** Terminal sterilization of patellar tendon allografts with electron beam irradiation can ensure higher safety of transplanted grafts and hence improve patient safety and acceptance.

**Keywords:** anterior cruciate ligament; allograft; sterilization; electron beam irradiation; human bone-patellar tendon-bone graft

Tears of the ACL are one of the most common injuries in young and active humans. Anterior cruciate ligament

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reconstruction has been recommended to restore knee stability and preserve the patient's activity level. In today's surgical practice, it is common to use autologous graft tissue for ACL reconstruction.<sup>20</sup> However, autologous tissue harvest may be associated with graft site morbidity, persisting anterior knee pain, loss of muscle strength, and compromised cosmesis.<sup>1,2,8,19,23</sup> Therefore, efforts have been made to find alternative graft sources, such as artificial ligaments and, increasingly, allograft tissue. Especially in revision surgeries as well as multiligament injuries, allografts are often the only remaining option for ligamentous reconstruction. Allografts have recently seen

a significant surge in popularity in ACL reconstruction, reflected by a 30% increase over the past 2.5 years.<sup>5</sup> Today, they are not only exclusively used for revision surgery, but increasingly for primary ACL reconstruction as well.<sup>5</sup>

However, questions about graft sterility and reported cases of disease transmission have raised concerns and prompted investigations of new sterilization techniques.<sup>22</sup> Several sterilization methods exist. Although ethylene oxide and peracetic acid have been shown to work effectively in bone sterilization, they showed significant complications after patellar tendon sterilization.<sup>29</sup> These include substantial inflammatory responses or impairment of biological healing, which cause significantly increased failure rates. Therefore, these techniques have been abandoned for patellar tendon sterilization.<sup>27</sup> The most frequently used type of sterilization of patellar tendon grafts uses ionizing radiation. The effect of ionizing irradiation is based on input of energy-generating excited atoms or molecules and ions for subsequent radical induced chemical reactions (cross-linking, branching, chain-scission, grafting, elimination). These reactions are induced in both tissue and pathogens. The induced changes in pathogens result in their destruction; however, tissue damage is also observed.<sup>32,36</sup> Several irradiation methods exist; gamma irradiation, especially Co<sup>60</sup>, is the most frequently used one. Gamma irradiation has a high penetrability and is effective for bulk sterilization. However, doses higher than 20 to 25 kGy cause significant deterioration of structural and mechanical properties of such treated patellar tendon grafts.<sup>13,28</sup> Bacterial pathogens can be safely eliminated with irradiation dosages of 15 kGy,<sup>24</sup> which has been shown not to affect material and structural properties of patellar tendon grafts<sup>15</sup> (and is therefore predominantly used in sterilization procedures by most of the tissue banks around the world). However, terminal sterilization of grafts cannot be achieved with low absorbed dose because the required sterilization dose also depends on the number and species of micro-organisms and/or viruses present on the product to be sterilized. Small viruses particularly require higher dosages for obtaining the sterility assurance level, which requires a reduction of the respective pathogen by 6 log<sub>10</sub>. Pruss et al<sup>25</sup> reported that a reduction of at least 4 log<sub>10</sub> for parvovirus B19 (which is the most resistant pathogen to irradiation) can be achieved only at a dose of 34 kGy, which might compromise mechanical strength of such treated patellar tendon.<sup>26,28</sup>

Electron beam is an alternative irradiation technique. High-energy electrons cause chemical changes similar to gamma irradiation.<sup>37</sup> Electron beam offers certain advantages compared with gamma irradiation, such as much improved control and accuracy of applied range of dosage and substantially reduced processing time (seconds for electron beam vs several hours for gamma irradiation).<sup>37</sup> It has also been shown that the addition of CO<sub>2</sub> provided a tissue-protective effect in the sterilization of nonhuman materials.<sup>17</sup> This effect has not yet been evaluated for human tissue. One of the disadvantages of electron beam irradiation is the reduced penetration depth compared with gamma rays.<sup>37</sup> This limitation only becomes evident for allografts with a thickness of about 5 cm (for 10 MeV electrons) and is therefore irrelevant for the much thinner



**Figure 1.** Human bone-patellar tendon-bone transplant before mechanical testing.

patellar tendon allografts that are typically used in ACL reconstruction.<sup>11</sup>

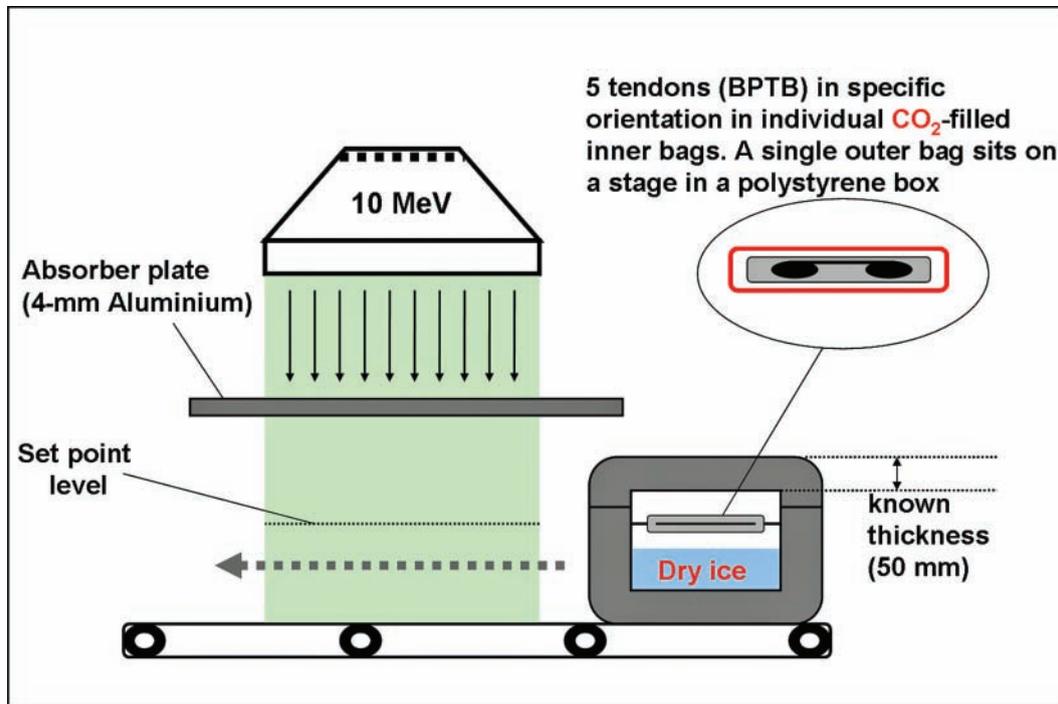
In a preliminary study we found that the biomechanical properties at a dosage of 34 kGy were significantly less affected with electron beam compared with gamma irradiation of 10 mm-wide bone-patellar tendon-bone grafts.<sup>29</sup> Several studies exist that have examined the effect of various doses of gamma irradiation from 10 to 50 kGy<sup>4,10,13,14,26,28,36</sup> and found a direct relationship between increasing dosage and decreasing biomechanical properties of such treated patellar tendon grafts. No such information is available for electron beam irradiation. Therefore, it was the aim of this study to analyze the effect of the novel electron beam sterilization procedure incorporating tissue-protective measures on the biomechanical properties of human patellar tendon allografts at various dosages between 15 and 34 kGy.

## MATERIALS AND METHODS

Thirty-two 10 mm-wide human bone-patellar tendon-bone grafts were prepared from 10 donors between 19 and 65 years (mean, 48 years) (Figure 1) and randomized into 4 groups (n = 8 per group): A, no sterilization (0 kGy); B, 15 kGy; C, 25 kGy; and D, 34 kGy electron beam irradiation.

All grafts were individually packed in CO<sub>2</sub>-filled gas-impermeable sterilization bags at an authorized sterilization facility (DIZG, Berlin, Germany) and deep-frozen at -70°C. Electron beam sterilization was performed at Gamma Service Produktbestrahlung GmbH (Radeberg, Germany). Grafts were placed on a height-adjusted stage in a dry ice-filled polystyrene box such that the sterilization process was conducted at very low temperatures, which were maintained by dry ice at approximately -70°C (Figure 2). During the sterilization procedure, the applied dosage was very tightly controlled with a mean dose deviation of ±1.65 kGy. Average time duration of the sterilization procedure was 30 seconds. After sterilization, all grafts were stored in polystyrene boxes at -70°C until the day of mechanical testing. Maximum time of storage was 10 days.

For biomechanical testing, grafts were thawed at room temperature and both the patella and tibia were potted in polymethyl methacrylate and mounted on aluminum clamps for fixation on a materials testing machine (model



**Figure 2.** Schematic overview of the sterilization process.

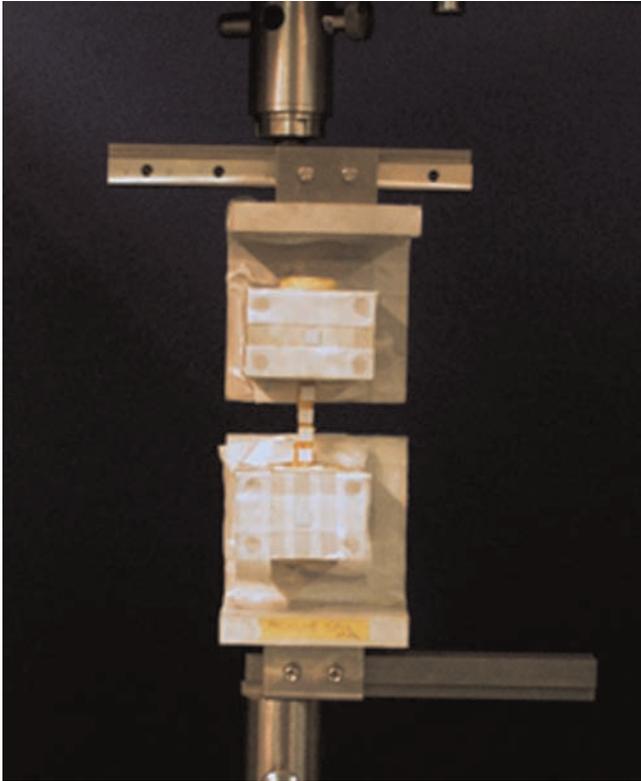


**Figure 3.** Testing setup with the graft embedded in polymethylmethacrylate and fixed in aluminum clamps for mechanical testing.

1455; Zwick GmbH, Ulm, Germany) (Figure 3). The biomechanical testing procedure included preconditioning (10 cycles, 0-20 N), cyclic loading (200 cycles, 20-200 N), and a load-to-failure test. The strain rate was 150 mm/min. Graft motion during cyclic loading was tracked with an infrared motion analysis system (MacReflex, Qualisys Inc, Partille, Sweden) (Figures 4 and 5).

For analysis of graft motion, 4 circular reflective tape markers were glued to the tendon surface: 2 in the mid-substance and 2 at the bony insertion sites of the grafts.

An additional marker was attached to each clamp. The infrared motion analysis system consisted of 2 high-speed cameras (Figures 4 and 5) that measured motion along all 3 axes. Our preliminary testing showed that out-of-plane motion was minimal; therefore, we only recorded motion along the longitudinal axis of the testing machine between the reflective tape markers attached to the graft's insertion sites. Before each test, a special calibration frame was used to calibrate the system, which provided a precision of 0.1 mm or less for longitudinal graft motion. Customized software (MacReflex) was used to record and transfer these data to Microsoft Excel software (Microsoft, Redmond, Washington) for final data processing. This setting allowed for analysis of overall graft motion during the cyclic loading test. Overall strain and cyclic elongation response were calculated from data obtained with the motion analysis system during cyclic loading testing. We reported strain difference, which was defined as the difference in length at 200 N between the 200th and 1st cycle divided by the initial length of the graft in percent (strain difference:  $[L_{200} - L_1] / L_0 \times 100$ ). Overall strain was measured at the last cycle as follows:  $((L_{200} - L_0) / L_0) \times 100$ .  $L_0$  was measured as the distance between both markers attached to the respective insertion sites of the patellar tendon at the last cycle of preconditioning at 20 N. Cyclic elongation response was calculated as the difference in elongation at 200 N between the 200th and 1st cycle (Cyclic elongation response:  $\text{Elong}_{200} - \text{Elong}_1$ ). Structural properties such as failure load and displacement at failure were recorded, and stiffness was derived from these values. Failure loads were defined as the highest load of the linear load-elongation curve, which always corresponded to



**Figure 4.** Testing setup for the MacReflex optical measurement system. The graft is fixed in aluminum clamps and 6 markers (white squares) are attached to the graft and clamp for optical measurement of graft motion.

intratendinous rupture or tendon peel off from the tibial insertion site, which was considered graft failure. A more detailed description of the biomechanical testing setup has been previously published.<sup>29</sup>

One-way analysis of variance and the Bonferroni post hoc test were used to test for significant differences in biomechanical properties between the groups. The level of significance was set at  $P < .05$ .

## RESULTS

We did not detect any significant differences in strain behavior between the nonsterilized ( $4.02 \pm 0.83$ ) and the sterilized grafts with 15 kGy ( $3.28 \pm 0.96$ ), 25 kGy ( $4.02 \pm 1.12$ ), or 34 kGy ( $4.29 \pm 1.79$ ;  $P = .342$ ). Cyclic elongation response derived from the markers during the cyclic loading phase showed a similar pattern with  $0.08 \pm 0.15$  mm for the nonirradiated grafts and  $0.06 \pm 0.11$  mm (15 kGy),  $0.17 \pm 0.24$  mm (25 kGy), and  $0.09 \pm 0.12$  mm (34 kGy;  $P = .578$ ) for the sterilized grafts, respectively (Table 1). Structural properties showed no significant differences among the respective groups (Table 2). Stiffness and failure loads were not significantly different, with the exception of the 34 kGy group, which had significantly lower failure loads ( $1300.6 \pm 229.2$  N) than the nonirradiated controls

( $1630.5 \pm 331.1$  N;  $P = .036$ , Bonferroni post hoc test). All grafts failed by either intratendinous rupture or peel off from the tibial insertion site in all groups.

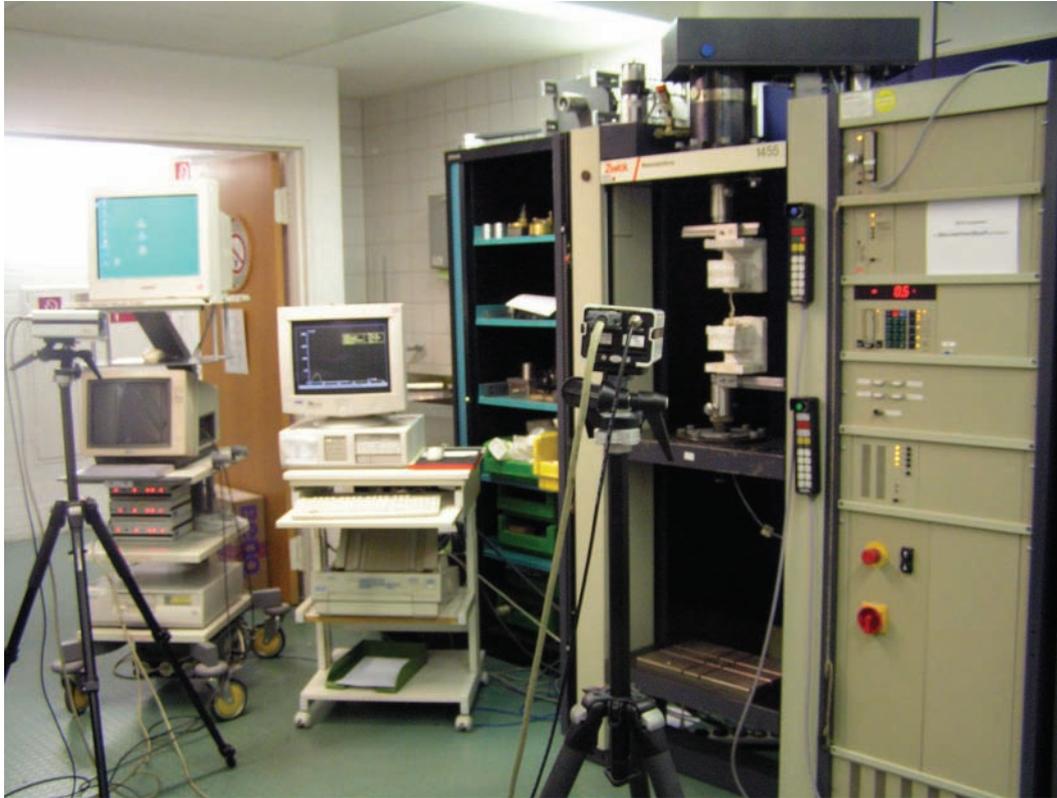
## DISCUSSION

The use of allografts for ACL reconstruction has become an accepted method because various preservation methods as well as an intensive donor screening have minimized infection and related adverse effects of allogenic tissue. However, it must be understood that these procedures can only reduce the risk of disease transmission to a certain point and not deliver sterile products, as is mandated by governmental restrictions in many countries of the world.

To overcome these shortcomings, various sterilization methods were developed and are currently used. Deep freezing and freeze-drying of allografts have been shown to reduce antigenicity of grafts and minimize the damaging effect of free radicals because they are less active at these temperatures.<sup>9,21,32,35</sup> These procedures cannot achieve terminal sterilization and thus eliminate the risk of disease transmission, but they may contribute to a higher safety of grafts by reducing risk of graft-to-host reactions without compromising graft integrity.

Antibiotic soaks can effectively reduce bacterial contamination on the surface of grafts, but they are unable to penetrate the whole tissue and have no effect on viral inactivation.<sup>12</sup> Another problem is that a contamination during final packing can occur, even when conducted under aseptic conditions. Hence, the current standard procedure is ionizing irradiation because it has a high penetration depth, good effectiveness, and can be performed in final packings. Because gamma irradiation has been shown to cause a dose-dependent deterioration of the biomechanical properties of patellar tendon allografts and might predispose them to premature failure,<sup>28</sup> efforts have been made to further reduce tissue damage by ionizing irradiation.

Different studies have evaluated the protective effect of radical scavengers. It is difficult to compare the results of these studies because different methods and techniques were used for radical scavenging, but mostly a protective effect was found on the structural integrity of the grafts.<sup>3,16,38</sup> Our study shows that the novel electron beam sterilization process may be appropriate for the sterilization of patellar tendon allografts. We found only a small adverse effect on the failure loads after high-dose irradiation with 34 kGy, while the remaining mechanical properties were not significantly affected. This is in contrast to Seto et al,<sup>31</sup> who reported a weakening effect of electron beam irradiation on mechanical properties similar to gamma irradiation. However, this might be due to the different procedures used in their study. We used CO<sub>2</sub> to avoid undesired reactions in water as well as in tissue, while Seto et al used radical scavengers like mannitol, riboflavin, and ascorbate. The addition of CO<sub>2</sub> in our study is based on experiences of its use in electron beam curing of lacquers and coatings.<sup>30</sup> It has been shown in electron



**Figure 5.** Photo of the testing setup. The Zwick testing machine can be observed on the right. Cameras and computer are part of the optical measurement system (MacReflex).

**TABLE 1**  
Differences in Strain and Cyclic Elongation Behavior Among the Different Groups

| Irradiation Doses, kGy | Strain, %   | Strain Difference, % | Cyclic Elongation, mm |
|------------------------|-------------|----------------------|-----------------------|
| 0                      | 4.47 ± 1.71 | 4.02 ± 0.83          | 0.08 ± 0.15           |
| 15                     | 3.25 ± 0.96 | 3.28 ± 0.96          | 0.06 ± 0.11           |
| 25                     | 4.54 ± 1.32 | 4.02 ± 1.12          | 0.17 ± 0.24           |
| 34                     | 5.96 ± 3.30 | 4.29 ± 1.79          | 0.09 ± 0.12           |

**TABLE 2**  
Structural Properties of Electron Beam-Irradiated Bone-Patellar Tendon-Bone Allografts

| Irradiation Doses, kGy | Stiffness, N/mm | Failure load, N             |
|------------------------|-----------------|-----------------------------|
| 0                      | 239.4 ± 24.9    | 1630.5 ± 331.1              |
| 15                     | 230.5 ± 60.4    | 1523.7 ± 374.8              |
| 25                     | 228.1 ± 58.8    | 1441.9 ± 356.8              |
| 34                     | 217.5 ± 25.8    | 1300.6 ± 229.2 <sup>a</sup> |

<sup>a</sup>Significantly lower failure loads were only found at 34 kGy compared with nonirradiated controls ( $P < .05$ ).

beam curing that the addition of CO<sub>2</sub> can reduce the unwanted by-reactions of air oxygen radicals.<sup>6</sup> Because irradiation of patellar tendon grafts is always carried out in the presence of oxygen, we assumed that the addition of CO<sub>2</sub> would have a similar protecting effect as in electron beam curing. In addition, we did not evaluate the irradiation dose of 50 kGy, which was used in the study of Seto et al<sup>31</sup> and might explain the differences between both studies. These differences suggest that future studies are warranted to find radical scavengers so that irradiation at very high dosages could still be carried out without compromising biomechanical properties of such treated patellar tendon grafts.

Viscoelastic properties were not affected in our study in any of the treatment groups. This is consistent with work by Seto et al,<sup>31</sup> who showed that viscoelastic properties

were minimally affected by irradiation. Rasmussen et al<sup>26</sup> used 40 kGy of gamma irradiation and found that stiffness values were significantly reduced. This suggests that either the lower dose applied in our study or the electron beam procedure itself might have had a less detrimental effect compared with gamma treatment. Another important advantage of the electron beam procedure is the precise dosage application due to higher reproducibility of treatment conditions compared with gamma irradiation, where large dose variations are inherent in current application techniques.

Also, the time that tissue is exposed to electron beam irradiation is only seconds compared with several hours for gamma irradiation. This factor might not only reduce tissue damage but may also provide an economical

advantage, reducing the costs of allografts and hence facilitating their acceptance.

We still found a significant reduction of failure loads caused by high doses of 34-kGy electron beam irradiation. However, failure loads at 34 kGy were still comparable with values (maximum failure load of  $1503 \pm 83$  N) reported by Woo et al<sup>34</sup> for native ACLs in a similar age group (40-50 years) as used in this study (mean, 48 years). Failure loads for nonsterilized bone-patellar tendon-bone grafts of this study are comparable with values reported in the literature, such as by Wilson et al<sup>33</sup> of around 1700 N.

Also, it has been shown that the in situ forces of the ACL during daily living activities are only 20% of its failure capacity.<sup>7</sup> Further, gamma-irradiated grafts improved their biomechanical properties during the ensuing remodeling process to values of nonirradiated grafts at mid- and long-term healing after ACL reconstruction.<sup>18</sup> Based on this information, the biomechanical properties of electron beam-treated grafts in our study at all irradiation levels can be considered sufficient for successful ACL reconstruction when compared with current alternatives.

There are certain limitations to this study that have to be mentioned. We only evaluated the graft integrity at time zero after sterilization and could not predict the in vivo behavior of these grafts. Also, we only evaluated CO<sub>2</sub> and did not compare different preservation methods. However, we believe that despite these limitations, our study is important in finding an optimal sterilization procedure and contributes to a better understanding of the effects of electron beam irradiation. Future studies will include analyses of the immediate effect of this procedure on the biological composition of patellar tendon grafts in vitro and in vivo studies to evaluate the remodeling behavior of electron beam-sterilized grafts.

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