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The effect of sterilization and storage on the viscoelastic properties of human tendon allografts – Continued: Storage for 0 to 4 months

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ABSTRACT

The role of donor-derived tendons, also known as allografts, in anterior cruciate ligament replacement surgeries is steadily increasing. Before surgery, temporary storage and, in most cases, sterilization are essential. It is, thus, crucial to determine how these procedures alter the grafts' biomechanical properties. The purpose of this research was to analyze the effect of different sterilization methods (native, frozen, frozen + 21 kGy gamma irradiation, frozen + 21 kGy electron beam irradiation) and storage durations (0 to 4 months) on the deformation and creep of two tendon types (tibialis anterior, peroneus longus). 80 tibialis anterior and 83 peroneus longus tendons from 51 human cadavers were included. The samples were removed, placed in a radio-cryoprotectant solution, then slowly cooled, sterilized and stored at −80 °C. All groups were subject to 60 s static creep test with 250 N load. Deformation during the loading phase, creep during static loading, and the ratio of these two were evaluated.

Deformation at the end of the loading phase and creep consistently exhibited significantly smaller values in the tibialis anterior compared to the peroneus longus type, as well as in electron beam-sterilized grafts as opposed to gamma beam-sterilized ones. Prolonged storage periods (within 0 to 4 months) resulted in a notable increase in these values, particularly in deformation. Based on the experimental data, the tibialis anterior tendon type and sterilization by gamma beam irradiation are better choices for anterior cruciate ligament reconstruction than the peroneus longus and sterilization by electron beam. Increased storage time affects negatively the evaluated mechanical properties.

1. Introduction

The standard surgical treatment for anterior cruciate ligament (ACL) tear is reconstruction. The selection between autograft and allograft continues to be a topic of discussion not only in scientific circles but also in clinical settings, where decisions may be influenced by various factors. ACL reconstruction involves the replacement of a ligament. Due to their characteristics, often tendon-grafts are used for this purpose. While autografts are the most popular choice, allografts, with their numerous advantages, are increasingly favored for ACL reconstruction. Allografts allow shorter operation time and smaller surgical scars with no donor-site morbidity, as well as the ability to provide a more controllable graft size (Barrett et al., 2005; Beer et al., 2019; Brown and Carter, 2018; Edgar et al., 2008; Malinin et al., 2002; Mascarenhas et al., 2010; Sun et al., 2009), leading to faster rehabilitation (Tavaszi et al., 2022). Nevertheless, before implantation, allografts must undergo sterilization to prevent the transmission of viral and/or bacterial infections, a process that compromises the mechanical characteristics of the tissues (Azar, 2009; Wang et al., 2018).

Identifying the least detrimental sterilizing approach to tissue properties is crucial, as the use of tendons closely resembling the original ACL is essential for the long-term success of the surgery. The most

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Table 1
Number of samples in each group.

Months	T0	TF	TG	TE	P0	PF	PG	PE
0	5	–	–	–	8	–	–	–
1	–	5	6	5	–	5	5	5
2	–	6	7	7	–	8	8	5
3	–	7	8	6	–	8	8	6
4	–	5	7	6	–	5	6	6
	Σ 80				Σ 83			

Abbreviations: Tendon type: T - tibialis anterior, P - peroneus longus; Sterilization method: 0 - none, F - deep freezing, G - deep freezing + 21 kGy gamma irradiation, E - deep freezing + 21 kGy electron beam irradiation; Storage duration: 0–0 months, 1–1 month, 2–2 months, 3–3 months, 4–4 months.

Fig. 1. Measurement arrangement: doubled tendon clamped, frozen with dry ice, and connected to the material measuring device.

common methods are chemical and irradiation sterilization, with gamma and electron beam irradiation being two of the most widely used ones, due to being easy to conduct, safe and effective (Singh et al., 2016). These two forms of radiation target the pathogens' nucleic acid components, therefore allowing the sterilization of heat-sensitive biological materials. Both are effective at room temperature and even below 0 °C, due to being able to pass through materials' interiors and inactivate microorganisms without encountering issues with heat exchange, pressure differences or diffusion barriers (Azar, 2009; Dziedzic-Goclawska et al., 2005; Yang et al., 2019). To avoid radiation-caused declines in mechanical strength, it has been recommended to protect allografts against ionizing radiation via a radio-protectant solution (Seto et al., 2013a; Singh et al., 2016). These solutions do not alter the biomechanical characteristics of the tendons or add unneeded chemicals to them (Grieb et al., 2006; Hangody et al., 2017; Seto et al., 2013b; Singh et al., 2016). Allografts frequently need to be safely stored prior to surgery. This can be performed by deep freezing, lyophilization or cryopreservation. The simplest, most affordable, and most popular

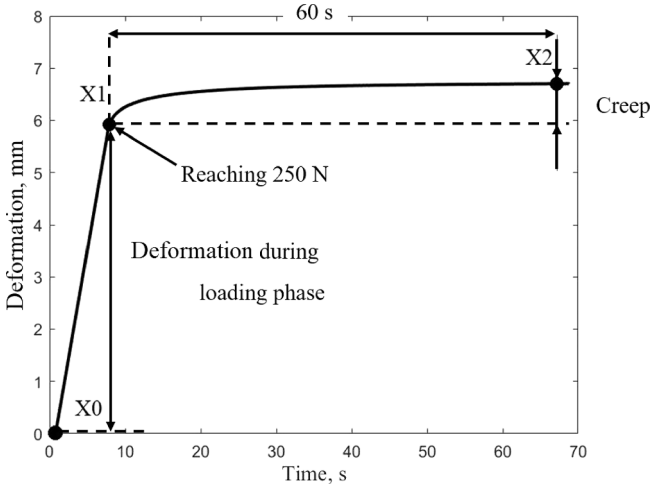

Fig. 2. Representative creep curve to define parameters;Deformation between X0 and X1: deformation during the loading phase; X1: deformation when 250 N force is reached; Deformation between X1 and X2: creep during 60 s of static loading.

Table 2
Numerical results (median, 25th and 75th percentiles) of the deformation during the loading phase, mm.

	Months	0	1	2	3	4
T0	Median	3.6035	–	–	–	–
	25 %	2.9827	–	–	–	–
	75 %	3.9890	–	–	–	–
TF	Median	–	3.7737	5.8097	6.0097	5.7223
	25 %	–	2.6557	5.6890	4.6180	5.6807
	75 %	–	4.0138	6.7931	6.6013	7.2012
TG	Median	–	7.0616	4.9431	4.7890	5.1867
	25 %	–	5.8242	4.1930	4.5828	4.8742
	75 %	–	7.5117	6.2182	5.1014	6.7410
TE	Median	–	4.6776	6.2098	5.1617	7.3097
	25 %	–	3.9450	5.7957	4.9181	5.8028
	75 %	–	5.6453	6.7098	5.5744	8.0556
P0	Median	4.8952	–	–	–	–
	25 %	4.7118	–	–	–	–
	75 %	4.9421	–	–	–	–
PF	Median	–	4.6224	5.2348	4.8098	7.5555
	25 %	–	4.4806	3.2928	4.6807	5.8222
	75 %	–	5.0492	5.6231	5.3868	8.0640
PG	Median	–	9.3119	5.4598	5.0418	8.0202
	25 %	–	7.8952	3.7765	3.7972	6.6742
	75 %	–	9.6035	6.7972	5.4713	8.5542
PE	Median	–	4.4951	5.6534	5.7577	7.7054
	25 %	–	3.6453	4.7433	4.4428	5.9806
	75 %	–	5.7452	6.3265	6.3736	7.7720

Abbreviations: Tendon type: T - tibialis anterior, P - peroneus longus; Sterilization method: 0 - none, F - deep freezing, G - deep freezing + 21 kGy gamma irradiation, E - deep freezing + 21 kGy electron beam irradiation; Storage duration: 0–0 months, 1–1 month, 2–2 months, 3–3 months, 4–4 months.

approach is deep freezing (Azar, 2009; Beer et al., 2019; Hulet et al., 2019), which, according to previous studies, is possible with a minimal effect on the structural and mechanical characteristics of the tissues (Clavert et al., 2001; Dietrich-Zagonel et al., 2021; Moon et al., 2006).

For ACL reconstruction, a wide range of allografts are provided by tissue banks, which can be divided into soft tissue allografts (fascia lata, hamstrings, tibialis anterior and posterior, peroneal tendons) and bone-tendon allografts (bone-patellar tendon-bone, quadriceps tendon, Achilles tendon) (Beer et al., 2019; Edgar et al., 2008; Furio et al., 2017; Hoburg et al., 2010; Hulet et al., 2019; Robertson et al., 2006). In our previous study, five tendon types (quadriceps, semitendinosus, gracilis, tibialis anterior (TA), peroneus longus (PL), and Achilles tendon) were

Table 3
Numerical results (median, 25th and 75th percentiles) of creep during 60 s load, mm.

	Months	0	1	2	3	4
T0	Median	0.5782	–	–	–	–
	25 %	0.4734	–	–	–	–
	75 %	0.7579	–	–	–	–
TF	Median	–	0.9847	0.9953	1.0879	0.8030
	25 %	–	0.9406	0.9048	0.9368	0.7992
	75 %	–	1.0344	1.0642	1.1291	0.8940
TG	Median	–	0.8499	0.8330	1.0946	0.8483
	25 %	–	0.6999	0.7477	0.9297	0.8062
	75 %	–	0.8965	0.9867	1.2185	0.9601
TE	Median	–	0.8295	1.3019	1.1394	0.9229
	25 %	–	0.8288	1.1376	1.0217	0.8972
	75 %	–	0.8476	1.5280	1.2755	0.9762
P0	Median	0.9563	–	–	–	–
	25 %	0.9408	–	–	–	–
	75 %	1.1318	–	–	–	–
PF	Median	–	1.1339	1.1459	1.1174	0.9413
	25 %	–	0.9824	0.9175	0.9383	0.9102
	75 %	–	1.4715	1.3471	1.3361	0.9707
PG	Median	–	0.8510	1.0126	1.1725	0.8752
	25 %	–	0.8483	0.8829	1.0227	0.8270
	75 %	–	1.0019	1.2614	1.3044	0.9944
PE	Median	–	1.0769	1.1329	0.9750	0.8098
	25 %	–	0.8596	1.0222	0.8335	0.7504
	75 %	–	1.0997	1.3023	1.1449	0.9015

Abbreviations: Tendon type: T - tibialis anterior, P - peroneus longus; Sterilization method: 0 - none, F - deep freezing, G - deep freezing + 21 kGy gamma irradiation, E - deep freezing + 21 kGy electron beam irradiation; Storage duration: 0–0 months, 1–1 month, 2–2 months, 3–3 months, 4–4 months.

examined after 21 kGy or 42 kGy gamma irradiation. The results of the mechanical tests indicated that the TA and the PL suffered the least adverse effects (Hangody et al., 2017). Therefore, the focus was placed on these two tendon types in the present study. In the present research, the bactericide dose (21 kGy) was employed, as opposed to the virucide dose (42 kGy), because it still sterilizes the tendons sufficiently (Farago et al., 2021; Greaves et al., 2008; Vopat et al., 2020), while having less adverse impact on the mechanical properties in the majority of the graft types than the irradiation with 42 kGy (Gomes et al., 2015; Hangody et al., 2017; Lansdown et al., 2017; Ng et al., 2013).

The structural characteristics of allografts and autografts should resemble those of the native ACL. Young's modulus of elasticity, maximal force, stiffness, relative elongation, and elongation at break are the graft parameters that have received the greatest attention in research so far (Almqvist et al., 2007; Hangody et al., 2017; Pearsall et al., 2003). However, it is also important to point out that while grafts must be flexible to allow natural movement, it is not recommended to use tendons that respond to mechanical stress with high elongation. Joints need to maintain stability and resist applied stress, making creep tests crucial for evaluating how a sample responds to a steady mechanical stress load.

Tendinous tissues deform nonlinearly in a time-dependent manner when they are subjected to constant mechanical stress, due to their viscoelastic properties (Gomes et al., 2015; Maganaris, 2002; Montanheiro et al., 2022; Pearson et al., 2007; Ries et al., 2022). The purpose of the creep test is to record this phenomenon. Determining the exact cross-sectional area of human tendons poses significant challenges. A pragmatic solution in creep tests frequently involves the assumption of a constant cross-sectional area, resulting in a consistent force level maintained throughout the tests, rather than adjusting for stress variations. Following a brief loading phase, the test sample is subjected to a steady load for a specified amount of time while the measurement outcomes are recorded continuously (Vas and Bakonyi, 2012).

In the present study, two measures were used to compare the specimens' characteristics: deformation during the loading phase (between X0 and X1 in Fig. 2) and creep during 60 s of static loading (between X1 and X2 in Fig. 2). The initial linear part in the deformation-time diagrams represents the loading phase, where the deformation reflects the momentary elastic component. Creep, occurring over the 60 s under static load, results from the combined effects of delayed elastic deformation and viscous flow components, represented by the nearly horizontal portion in the diagrams (Fig. 2). In practice, finding an allograft that does not undergo changes in length over time under constant mechanical stress is not achievable due to creep. If a graft responds to mechanical stress with high elongation, the joint may lose its stability and become incapable of effectively withstanding applied impacts. Hence, it is recommended to select tendons for knee ligament reconstruction surgeries that closely resemble the native ACL. In an earlier research, the creep of non-sterilized ACL was measured by applying a consistent 200 N load to the grafts for 5 min, with recorded creep at 20 ± 20 % (43 ± 8 mm) of the initial length of 35 mm (Delcroix et al., 2013).

In most cases, soft tissues are kept in storage for no longer than 6 months before being used in surgery (Suhodolčan et al., 2013). The mechanical performance of the above-mentioned two tendon types, sterilized by gamma or electron beam irradiation, stored for 5 and 6 months have already been examined in our previous research, suggesting that the PL sterilized by electron beam and stored deep frozen for 5 months is a better choice for ACL reconstruction than TA, sterilization by gamma irradiation, and storage for 6 months (Gökler et al., 2021). However, the prior study did not address tendons stored for less than 5 months. The present study, thus, covers the 0 to 4 months timeframe to ascertain the safe storage duration for tendon allografts.

The objective of the present study was to mechanically assess the effect on the deformation and creep of various sterilization methods (gamma and electron beam irradiation) and storage durations (0 to 4 months) in two types of tendon allografts (PL and TA) used in knee ligament reconstruction. The null hypothesis was that there is no significant difference between the deformation and creep of the allografts based on these factors. Furthermore, the initial deformation and creep values of native (non-treated, freshly harvested) allografts are presented as a benchmark. The outcomes of this study are expected to be beneficial in assisting in the selection of the best methods for sterilizing and preserving tendon allografts, considering the short-term (0 to 4 months) storage effects, with a special emphasis on durability aspects.

2. Materials and methods

Using sterile surgical techniques, the tendons were retrieved from 51 human donor cadavers in at a pathology facility (male/female ratio: 25/26; age range: 42–96 years; average age: 79 ± 11 years) within 24 h of the death. The tests were authorized by the Research Ethics Committee of Uzsoki Utcai and Péterffy Sándor Hospital (number: 02/2022). After removing the soft tissues that surrounded the tendons, the allografts were examined for degenerative lesions, yielding negative results. The donors included in the study had no documented history of tendon-related illnesses or injuries. Samples from individuals with prior tendon-related injuries or harvesting injuries were excluded, leading to the examination of a total of 83 PL and 80 TA tendons (Table 1).

The samples were categorized into groups based on storage duration, treatment type, and graft type. Each group's name consists of three characters: type (T-tibialis anterior, P-peroneus longus), treatment (0-none, F-deep freezing, G-deep freezing + 21 kGy gamma irradiation, E-deep freezing + 21 kGy electron beam irradiation), and storage duration (0–0 months, 1–1 month, 2–2 months, 3–3 months, 4–4 months).

The samples were instantly placed in a pre-mixed radio-protectant solution containing 16.7 % 1,2-propanediol, 24.2 % dimethyl-sulfoxide, 3.8 % D-trehalose, and 2.7 % D-mannitol all w/w (Sigma-Aldrich, Saint Louis, USA) (Grieb et al., 2006; Hangody et al., 2017). A deep freeze of −80 °C was applied to each tendon, after which the sterilization process was performed in a frozen state.

On the test day, the grafts were thawed at room temperature and then at 37 °C for 20 min right before the measurements. The free ends of

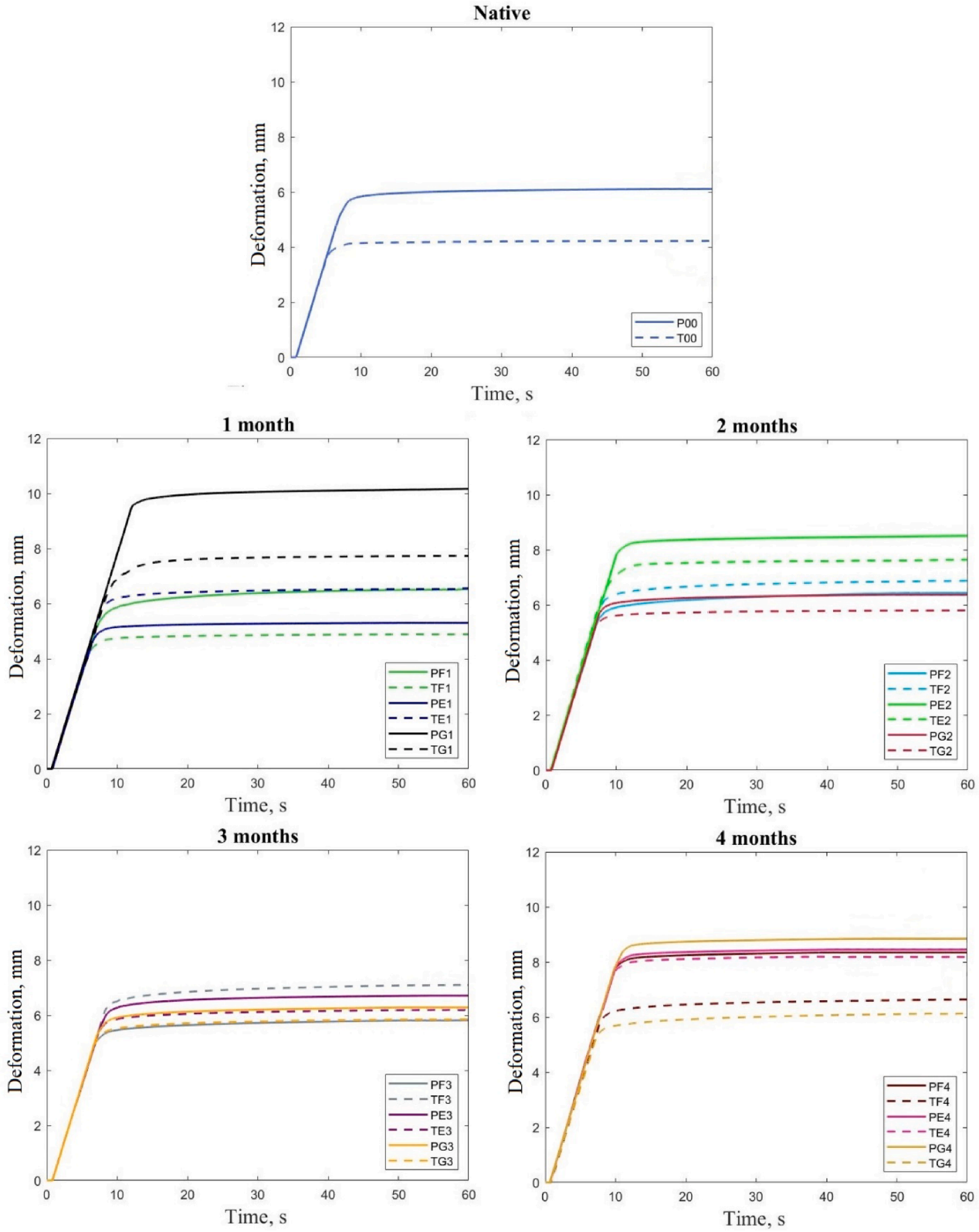

Fig. 3. Deformation-time curves of the groups of tendons, grouped by storage time; Abbreviations: Tendon type: T - tibialis anterior, P - peroneus longus; Sterilization method: 0 - none, F - deep freezing, G - deep freezing + 21 kGy gamma irradiation, E - deep freezing + 21 kGy electron beam irradiation; Storage duration: 0–0 months, 1–1 month, 2–2 months, 3–3 months, 4–4 months.

Table 4
P-values of the statistically significant differences between groups based on deformation at the end of the loading phase.

	T00	TF1	TF2	TF3	TF4	TG1	TG2	TG3	TG4	TE1	TE2	TE3	TE4	P00	PF1	PF2	PF3	PF4	PG1	PG2	PG3	PG4	PE1	PE2	PE3	PE4
T00														0.0105												
TF1			0.0080			0.0121																				
TF2		0.0080																								
TF3																										
TF4																										
TG1		0.0121																								
TG2																										
TG3																										
TG4																						0.0385				
TE1																										
TE2																										
TE3																										
TE4																										
P00	0.0105																									
PF1																										
PF2																										
PF3																										
PF4																										
PG1																										
PG2																										
PG3																						0.0080				
PG4									0.0385												0.0080					
PE1																										
PE2																										
PE3																										
PE4																										

p-values below 0.05 are considered statistically significant; Gray: significant difference between the effects of storage times; Yellow: significant difference between the effects of sterilization methods; Blue: significant difference between the effects of tendon types; Abbreviations: Tendon type: T - tibialis anterior, P - peroneus longus; Sterilization method: 0 - none, F - deep freezing, G - deep freezing + 21 kGy gamma irradiation, E - deep freezing + 21 kGy electron beam irradiation; Storage duration: 0–0 months, 1–1 month, 2–2 months, 3–3 months, 4–4 months.

each graft were stitched together, allowing for testing of doubled tendons, a configuration commonly employed in ACL reconstruction due to the similar cross-section to the original ligament. In addition, the loop arrangement contributed to the stability of the measurement setup (Hangody et al., 2016). The measurements were performed with a Zwick Z020 (ZwickRoell GmbH&Co. KG, Ulm, Germany) computer-controlled universal tensile tester with a 25 kN load capacity load cell and a freezer clamp structure at the accredited materials testing laboratory of the Budapest University of Technology and Economics Department of Polymer Engineering. The samples were clamped and connected to the material measuring device (Fig. 1). The space between the clamps, and thus, the original length of the tendons was 60 mm in each case. To provide a more secure fixation, the clamp was frozen in dry ice for 3 min. It was reported that this duration of freezing time does not lead to the freezing of the allografts (Hangody et al., 2016). A modified creep test was performed, assuming that the cross-sectional area of the allograft remains unchanged, thus, the force was kept constant. The control program, testXpert 11.00 (ZwickRoell GmbH&Co. KG, Ulm, Germany), was configured with the following parameters: samples were preloaded at a speed of 20 mm/min up to 2 N, then loaded at a speed of 50 mm/min up to 250 N (Gökler et al., 2021; Haut Donahue et al., 2002). The samples were held under load for 60 s after reaching 250 N.

2.1. Statistical analysis

The StatSoft Statistica 13.3 (StatSoft Inc., Tulsa, OK, USA) software was used to conduct the statistical analysis. For each category, the median and associated interquartile range (25th and 75th percentiles) are presented. Although values obtained from mechanical research usually exhibit a normal distribution, this requirement is not fulfilled for small sample sizes, which were used in the present study. Thus, non-parametric methods were used to compare the categories in terms of graft type, sterilizing technique, and storage period. First, the Kruskal-Wallis test was applied to determine whether there is at least one sample stochastically dominating another sample in a given category. Then pairwise Mann-Whitney *U* test was performed as a post hoc analysis. A p-value of less than 0.05 was considered statistically significant (Fidy and Makara, 2005). To correct for multiple statistical tests, the Benjamini-Hochberg procedure was employed (Benjamini and Hochberg, 1995).

3. Results

The samples were first compared according to the storage duration. The groups were paired in a way that only the storage time was different, the graft type and the treatment method were the same. Our previous research – using the same tendon types and treatments as in the present tests –, suggested that increasing storage time in the 5 to 6 months range causes increased creep and deformation during the loading phase (Gökler et al., 2021). The present results (Tables 2-3) revealed a similar tendency between the storage duration in the 0 to 4 months range and the extent of deformation during the loading phase, as well as creep, with the only exception of groups PE4 and PE2 (Fig. 3). TF1 underwent significantly less elongation during the loading phase than TF2 ($p = 0.0080$) and PG3 significantly less than PG4 ($p = 0.0080$) (Table 4 and Fig. 4). Groups TE1 and TE4 underwent significantly less creep during the loading phase than group TE2 ($p = 0.0058$, $p = 0.0053$) (Table 5 and Fig. 5). Furthermore, the previous study indicated that when comparing the effect of storage durations of 5 and 6 months, deformation during the loading phase is more significantly impacted compared to creep. In the current study, groups PF1-PF4 and PE1-PE4 display a similar pattern (Fig. 5). Thus, the current results point towards a significant impact on the deterioration of tendon mechanical properties with increased storage time in the 0 to 4 months range.

The comparison of allograft types was carried out with pairs of groups only differing in type. It was found that TA tendons are significantly less prone to creep and deformation during the loading phase than PL in the investigated storage time range. The deformation during loading phase was significantly smaller for TG4 than for PG4 ($p = 0.0385$) (Table 4 and Fig. 4 a). The creep of group TF4 was significantly smaller than that of group PF4 ($p = 0.0472$) (Table 5 and Fig. 4 b). The

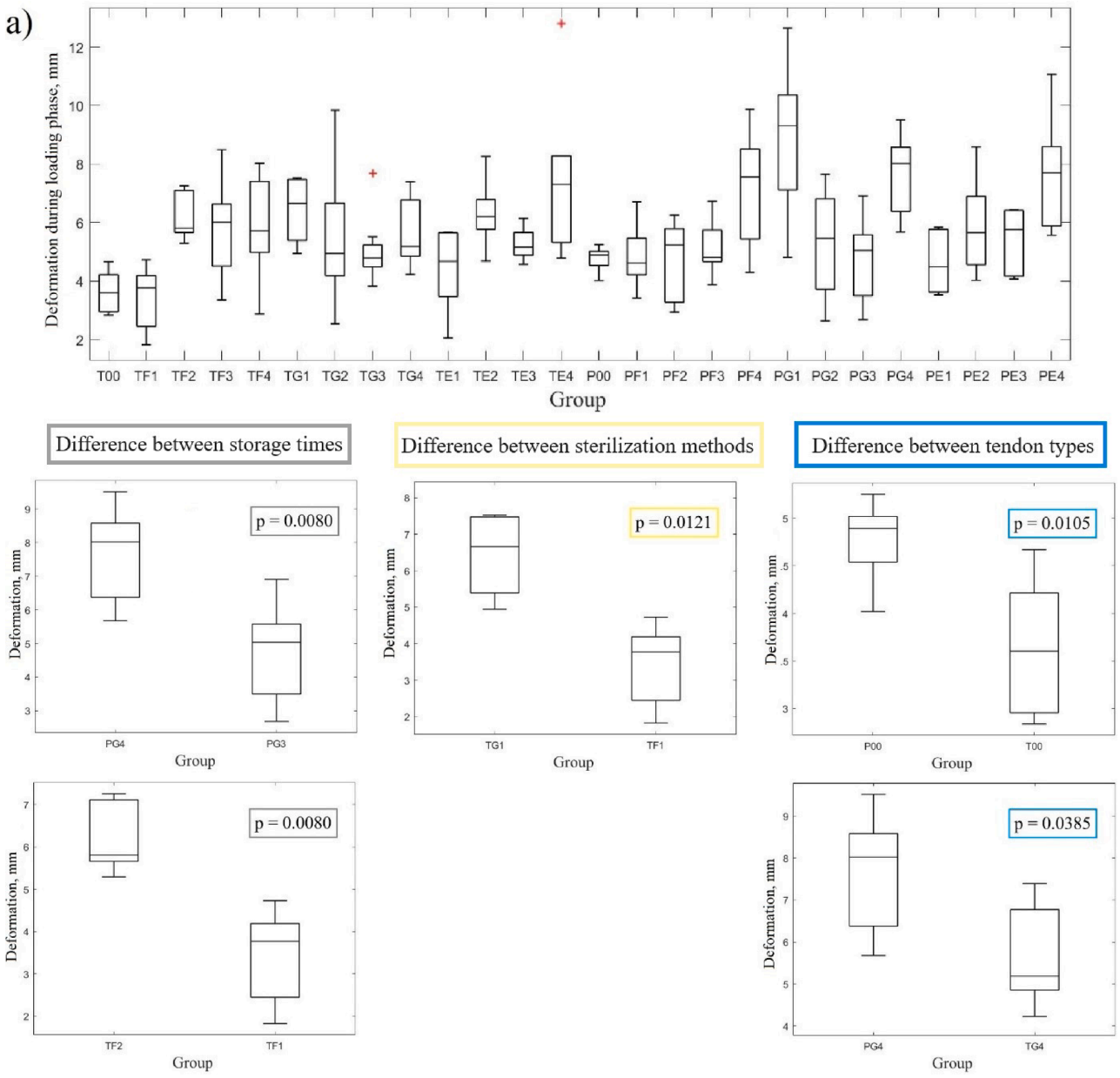

Fig. 4. (a). Median, 25th and 75th percentiles, minimum and maximum values of the deformation during loading phase; Top: all groups illustrated together; Bottom: only the group pairings with significant difference are presented; Bottom Left (gray): difference between the effect of storage time; Bottom Middle (yellow): difference between the effect of sterilization methods; Bottom Right (blue): difference between the effect of tendon type (b). Median, 25th and 75th percentiles, minimum and maximum values of creep; Top: all groups illustrated together; Bottom: only the group pairings with significant difference are presented; Bottom Left (gray): difference between storage time; Bottom Middle (yellow): difference between treatment; Bottom Right (blue): difference between tendon type; Abbreviations: Tendon type: T - tibialis anterior, P - peroneus longus; Sterilization method: 0 - none, F - deep freezing, G - deep freezing + 21 kGy gamma irradiation, E - deep freezing + 21 kGy electron beam irradiation; Storage duration: 0–0 months, 1–1 month, 2–2 months, 3–3 months, 4–4 months. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

results of our earlier tests – comparing the same tendon types and treatments as in the current tests – showed that after a storage period of 5 or 6 months, the PL suffered less creep and deformation during loading phase than the TA (Gökler et al., 2021). These results indicate that the TA is more susceptible to the effects of extended storage compared to the PL, resulting in a more pronounced decline in its properties after the fourth month compared to the PL. The results of the native grafts can be used as a benchmark for comparison (Fig. 3). The deformation during loading phase of the native group T00 was 6.01 ± 0.3 % of the initial length, a significantly smaller value compared to that of P00, which was 8.16 ± 0.2 % ($p = 0.0105$). The creep of group T00 (0.91 ± 0.2 %) was also significantly smaller than that of P00, which was 1.47 ± 0.2 % of the initial length ($p = 0.0232$).

The effect of the sterilization methods was also compared, with groups differing only in the sterilization method, while the tendon type and the storage period were the same. The tests showed a significant difference in the following cases: The deformation during the loading phase was significantly lower for TF1 than for TG1 ($p = 0.0121$) (Table 4 and Fig. 4 a). The creep of groups TF2 and TG2 was significantly lower than that of group TE2 ($p = 0.0124$, $p = 0.0151$) (Table 5 and Fig. 4 b). This implies that sterilization with electron beam is significantly more detrimental on the creep of the grafts than with gamma irradiation.

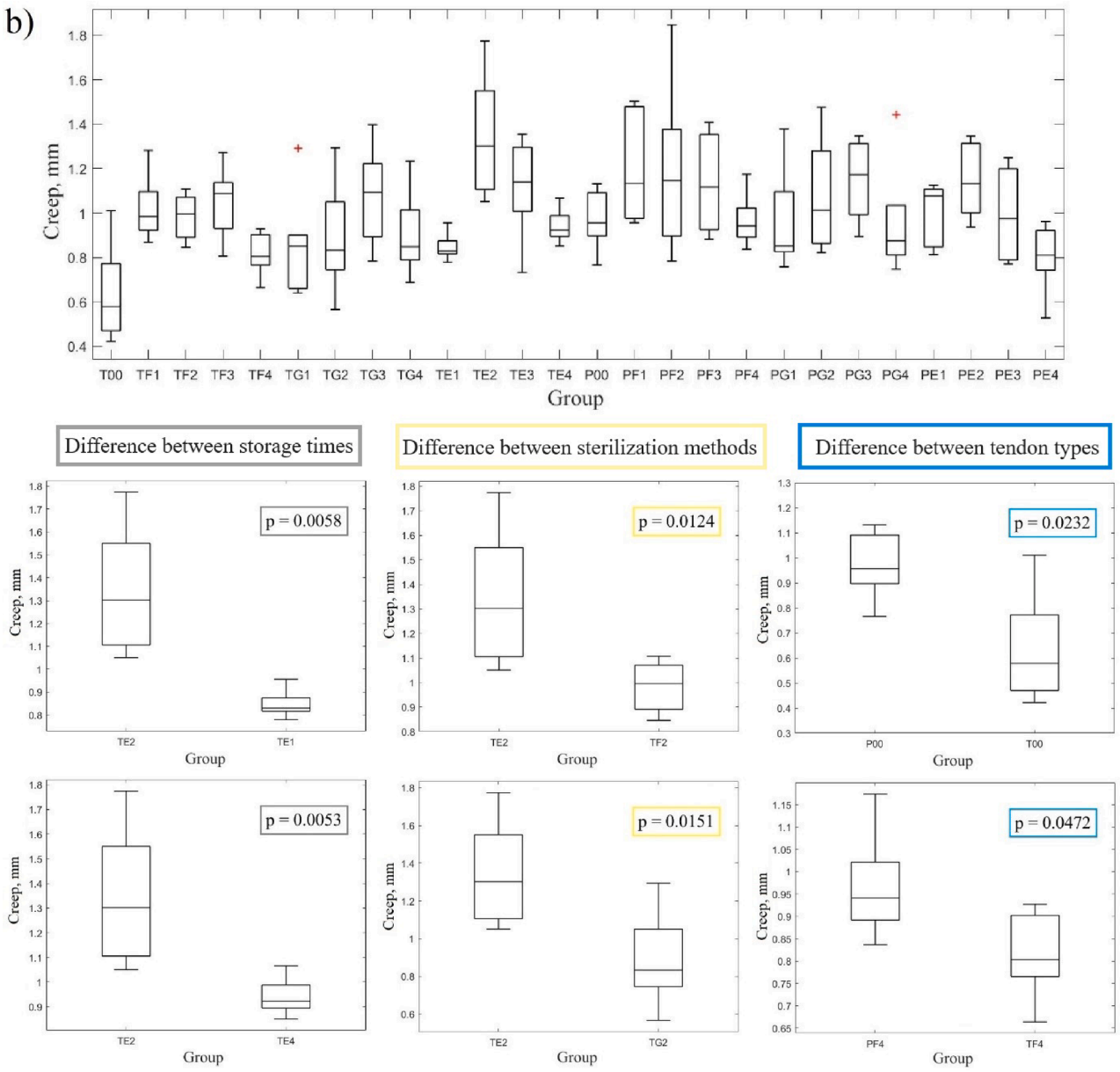

Fig. 4. (*continued*).

The original null hypothesis of no significant difference between the allograft groups based on tendon type, sterilization method, or storage duration was accepted in all other combinations.

4. Discussion

Allografts can be sterilized before surgery to decrease the possibility of disease transmission and, in most cases, they must be safely stored. The mechanical characteristics of the grafts can be affected in varying degrees by the type of graft, treatment method, and storage duration (Azar, 2009; Wang et al., 2018; Yang et al., 2019). The purpose of the present study was to evaluate the effects of different tendon types (PL and TA), sterilization methods (gamma and electron beam irradiation), and storage times (0 to 4 months) on the deformation and creep behavior of tendon allografts to find the parameters resulting in the values most similar to the native ACL. Based on the statistical analysis of the measured data, it can be established that the TA tendons sterilized by gamma beam irradiation are the optimal choice as an allograft if they are safely stored for 0 to 4 months. These groups were the least likely to deform during the load-increasing period and under constant load, making them ideal for ACL restoration procedures. In addition, shorter storage durations are recommended to prevent the effects of prolonged storage on deformation and creep.

Several static and dynamic analyses have already been conducted concerning the impact of sterilization on important mechanical properties of tendons, including Young's modulus, maximal force, stiffness, relative elongation and elongation at break (Giannini et al., 2008; Hangody et al., 2017; Hashemi et al., 2005; Kamiński et al., 2009; Seto et al., 2008). Nevertheless, an essential aspect is the creep behavior, which is unfortunately underrepresented in the current literature. The creep of ACL without any sterilization was previously measured as 20 ± 20 % (43 ± 8 mm) of the initial length (35 mm) under a 200 N static load for 5 min (Delcroix et al., 2013). In the present study, carried out with 250 N static load during 60 s, the creep of group T00 resulted in 0.91 ± 0.2 % of the initial length, while the creep of P00 was 1.47 ± 0.2 % of the initial length. Both values are notably lower than the previously mentioned ACL creep, even when accounting for the difference in measurement duration.

Table 5
P-values of the statistically significant differences between groups based on creep; p-values below 0.05 are considered statistically significant.

	T00	TF1	TF2	TF3	TF4	TG1	TG2	TG3	TG4	TE1	TE2	TE3	TE4	P00	PF1	PF2	PF3	PF4	PG1	PG2	PG3	PG4	PE1	PE2	PE3	PE4
T00														0.0232												
TF1																										
TF2											0.0124															
TF3																										
TF4																		0.0472								
TG1																										
TG2											0.0151															
TG3																										
TG4																										
TE1											0.0058															
TE2			0.0124				0.0151			0.0058			0.0053													
TE3																										
TE4											0.0053															
P00	0.0232																									
PF1																										
PF2																										
PF3																										
PF4					0.0472																					
PG1																										
PG2																										
PG3																										
PG4																										
PE1																										
PE2																										
PE3																										
PE4																										

Gray: significant difference between the effects of storage times; Yellow: significant difference between the effects of sterilization methods; Blue: significant difference between the effects of tendon types; Abbreviations: Tendon type: T - tibialis anterior, P - peroneus longus; Sterilization method: 0 - none, F - deep freezing, G - deep freezing + 21 kGy gamma irradiation, E - deep freezing + 21 kGy electron beam irradiation; Storage duration: 0–0 months, 1–1 month, 2–2 months, 3–3 months, 4–4 months.

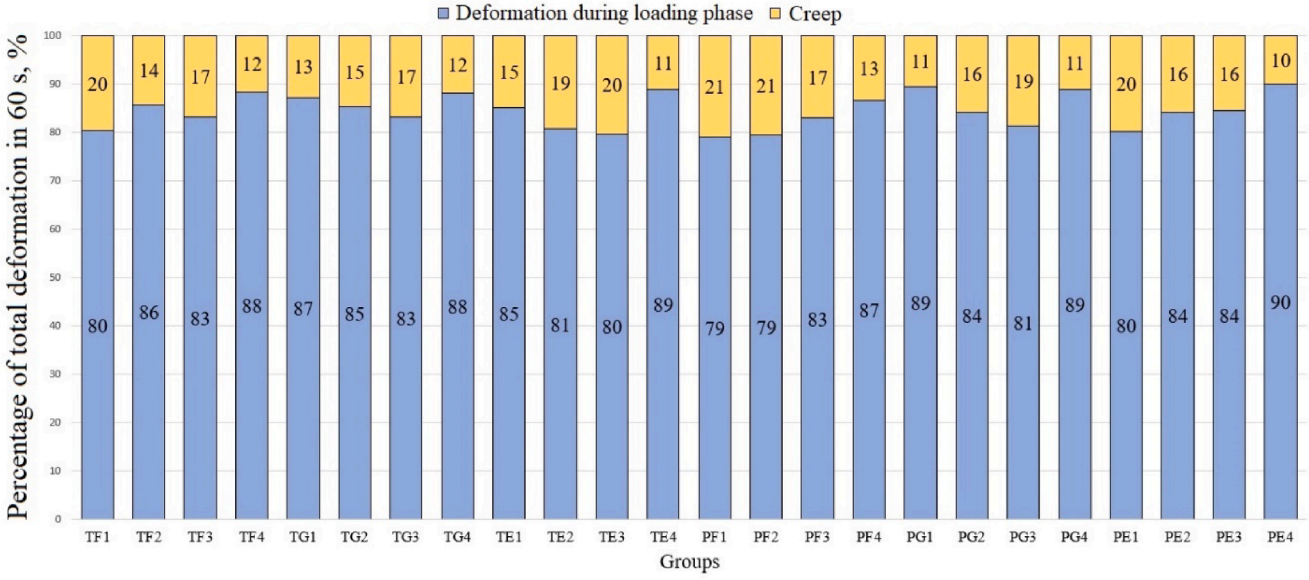

Fig. 5. Creep and deformation during the loading phase as percentages of the total deformation of the tendon during the measurement; Abbreviations: Tendon type: T - tibialis anterior, P - peroneus longus; Sterilization method: 0 - none, F - deep freezing, G - deep freezing + 21 kGy gamma irradiation, E - deep freezing + 21 kGy electron beam irradiation; Storage duration: 0–0 months, 1–1 month, 2–2 months, 3–3 months, 4–4 months.

The creep of the native TA was documented as 4 ± 2 % (0.4 ± 0.2 mm) of the initial length (10 mm) under a 10 N static load for 2 min (0.3 ± 0.1 mm after gamma irradiation between 20 and 26 kGy) (Schimizzi et al., 2007). These values are notably higher than the present values of T00 (0.91 ± 0.2 %) and P00 (1.47 ± 0.2 %). This discrepancy can be attributed to the absence of a specified loading phase in the referenced article; instead, the creep was measured from the initiation of applying any force to the tendons.

On the other hand, Haut Donahue et al. (2002) reported the creep value of the native TA as 0.3 ± 0.1 % of the initial length under a 250 N load for 15 min. This value is lower than the ones corresponding to T00 (0.91 ± 0.2 %) and P00 (1.47 ± 0.2 %), potentially attributable to the preconditioning procedure they carried out 15 min before the creep test. The present study did not include a preconditioning procedure.

The findings of the present study suggest that both low-dose (21 kGy) gamma and electron beam irradiation significantly increase the

deformation and creep of the allografts (Tables 4 and 5). It is important to highlight that if the surgical team has sufficient information about the expected deformation, the tendons can be preloaded before surgery. On the other hand, the creep behavior cannot be prevented, making it a more crucial parameter in this context. In contrast to Hoburg et al. (2015), who found that electron beam irradiation of 25 kGy and 34 kGy caused less creep after short-term storage (maximum 10 days) than gamma irradiation of the same doses, our study showed that the impact of low-dose electron beam irradiation, leading to higher creep, is more harmful than that of gamma irradiation within the investigated storage duration of 0 to 4 months. This may be due to the different tendon types (human bone-patellar tendon-bone vs. TA and PL), as well as the different radiation doses of 25 and 34 kGy versus 21 and 50 kGy used.

Notably, our prior studies drew contrasting conclusions, indicating that after 5 or 6 months of storage, the effect of electron beam irradiation was less detrimental than that of gamma (Gökler et al., 2021). Thus, it can be inferred that the effect of gamma irradiation is less damaging in the short term (0 to 4 months), but becomes more destructive over the long term (after 4 months) compared to electron beam irradiation.

While the present study offers valuable insights into the deformation and creep behavior of tendon allografts, it acknowledges the limitation of focusing exclusively on these properties. To fully map the mechanical changes induced by sterilization, storage, and treatment methods, future research is suggested to incorporate additional mechanical tests, including tensile tests. Tensile tests can reveal crucial parameters such as maximum force tolerance and deformation under maximum stress, providing a more comprehensive understanding of graft biomechanics.

The present study carried out a 60 s long static creep test using a 250 N load to simulate conditions associated with ACL reconstruction. It is essential to assess the presented findings in conjunction with dynamic test outcomes, as the mechanical responses of tendon grafts in real-world scenarios encompass a range of loading conditions. Future investigations could enhance understanding by examining different load magnitudes and dynamic loading to provide a more comprehensive evaluation of graft behavior.

In addition, while the study identifies recommended tendon types and sterilization methods for allografts based on mechanical properties, the direct translation of these findings to clinical outcomes is a crucial consideration. Future research could explore clinical trials and patient outcomes to strengthen the evidence supporting the practical implications of the study's recommendations within the context of ACL reconstruction surgeries.

In conclusion, the present study revealed a novel scientific finding: considering the deformation and creep in the 0 to 4 months storage duration range, the recommended tendon type and sterilization method for allografts used in ACL reconstruction are TA over PL and gamma irradiation over electron beam irradiation, while prolonged storage duration causes increased deterioration of these properties.

CRediT authorship contribution statement

Daniella Judit Gökler: Writing – original draft, Investigation. **Atilla Ferenc Karácsony:** Writing – original draft, Investigation. **Dénes Faragó:** Writing – original draft, Investigation. **Gábor Szebényi:** Conceptualization, Investigation, Writing – review & editing, Methodology. **Rita Mária Kiss:** Conceptualization, Writing – review & editing, Methodology. **Károly Pap:** Writing – review & editing, Supervision, Resources, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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