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# Irradiation at 11 kGy conserves the biomechanical properties of fascia lata better than irradiation at 25 kGy

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The objective of this study was to determine the biomechanical properties of the fascia lata and the effects of three preservation methods: freezing, cryopreservation with dimethylsulfoxide solution and lyophilization; and to compare the effects of low-dose (11 kGy) and normal-dose (25 kGy) gamma-ray sterilization versus no irradiation.

248 samples from 14 fasciae latae were collected. Freezing samples were frozen at  $-80^{\circ}\text{C}$ . Cryopreservation with dimethylsulfoxide solution samples were frozen with 10 cl dimethylsulfoxide solution at  $-80^{\circ}\text{C}$ . Lyophilization samples were frozen at  $-22^{\circ}\text{C}$  and lyophilized. Each preservation group were then randomly divided into 3 irradiation groups.

The cryopreservation with dimethylsulfoxide solution samples had significantly worse results in all 3 irradiation conditions. Young's modulus was lower for the freezing samples ( $p < 0.001$ ) and lyophilization samples groups ( $p < 0.001$ ). Tear deformation was lower for the freezing samples ( $p = 0.001$ ) and lyophilization samples groups ( $p = 0.003$ ), as was stress at break ( $p < 0.001$  and  $p < 0.001$ ). Taking all preservation methods together, samples irradiated at 25 kGy had worse results than the 0 kGy and 11 kGy groups in terms of Young's modulus ( $p = 0.007$  and  $p = 0.13$ ) and of stress at break ( $p = 0.006$  and  $p = 0.06$ ).

The biomechanical properties of fascia lata allografts were significantly worse under dimethylsulfoxide cryopreservation. The deleterious effects of irradiation were dose-dependent.

## 1. Introduction

Anterior cruciate ligament (ACL) tear is a frequent and serious lesion of the knee. In the United States, 100,000 ACL reconstructions were performed in 2001 and 200,000 in 2010 (Sanders et al., 2016). Iterative failure is frequent. A 2015 prospective multicenter study of 2488 patients found a failure rate of 4.4% at 2 years and 7.7% at 5 years (Kaeding et al., 2015). In the Danish registry, Lind reported  $> 3\%$  failure at 2 years in  $> 4972$  primary ACL reconstructions. (Lind et al., 2012) In case of revision, an allograft is often indicated. In 2013, the American Orthopedic Society for Sports Medicine (AOSSM) found that tendon allografts were used in 27% of primary ACL reconstructions and in 62% of revision procedures. They reduce postoperative pain (Bottoni et al., 2015), avoid the harvesting morbidity and reduce tourniquet

time. Several sizes of grafts are available, enabling adaptation to bone defect left by the primary repair. To be used for ACL reconstruction, they must be stored in such a way that their biomechanical properties are conserved.

Cryopreservation is the maintenance of biological tissues in a state of life and suspended animation at cryogenic temperatures. Chemical reactions, biological processes and intra- and extra-cellular physical interactions are frozen (Bakhach et al., 2007). Certain substances (glycerol and dimethylsulfoxide (DMSO)) theoretically provide cell protection during cryopreservation. These cryoprotective substances form hydrogen bonds with the water molecules to maintain a liquid state at temperatures below their freezing point. Park's team's histological observation of cryopreserved tendons (Park et al., 2009) found many spaces between the fibrillar structures. Their hypothesis was that

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these spaces corresponded to ice crystals, as they disappeared on thawing. Cryopreservation solutions should avoid this phenomenon.

Tendon allografts can also be preserved by freeze-drying (lyophilization), which is more expensive. The freeze-dried product is a stable dry (1% residual moisture) material. In addition to water, oxygen can also influence the stability of the freeze-dried product, and samples must be packed under inert atmosphere or vacuum. Under these conditions, survival of cellular material such as viruses is impossible. Conservation of freeze-dried products is in theory unlimited, but the European standards of the European Association of Musculoskeletal Transplantation limit it to 5 years (Publishing C of E, 2009). Immunologically, lyophilized tissues lose their immunogenicity. Non-rehydrated material has lower resistance and requires 24 hours' rehydration to return to normal (Pelker & Friedlaender, 1987; Pelker et al., 1983).

Irradiation alters graft resistance, dose-dependently (Dziedzic-Goclawska et al., 2005).

Graft sterilization uses gamma or electron beam (E-beam) radiation. It has virucidal and bactericidal action. This modifies their nucleic acids. Irradiation leads to the production of free radicals and causes a breakdown of collagen bonds (Nguyen et al., 2007). The higher the radiation dose, the greater the collagen damage, and the biomechanical properties of the allograft are proportionally disrupted. 25 kGy irradiation is usually recommended to sterilize bone allografts, although several authors have recently investigated the effect of a lower dose. It is recommended to use doses from 25 to 35 kGy (McDermott & Thomas, 2005) for tendon allografts, and few studies have investigated the effects decreasing these doses.

The present study compared two gamma irradiation doses: 25 kGy and 11 kGy.

Several grafts are available for ACL reconstruction. Currently, two are more widely used: the patellar tendon and the hamstring tendon (gracilis and semitendinosus tendon). The fascia lata is also used, as originally described by Hey Groves in 1917 (Burnett 2nd & Fowler, 1985). The original technique was later modified by MacIntosh in 1972 (Amirault et al., 1988). It is currently used mainly for lateral extra-articular surgery associated with intra-articular ligamentoplasty, promoting pivot shift control (Lemaire & Combelles, 1980). The fascia lata is a transplant with high biomechanical resistance (Chan et al., 2010). It is easily available during multi-organ harvesting, and we therefore chose to carry out our study on this transplant.

The objective was to compare the biomechanical effects of common preservation methods on fascia lata allografts *ex vivo*. Freezing, lyophilization and cryopreservation using DMSO, low (11 kGy) and medium dose (25 kGy) gamma radiation sterilization were tested.

## 2. Methods

14 fasciae latae were collected from 7 cadaveric donors during multi-organ retrieval between February and April 2017 in our institution. All patients and their families agreed to scientific research on their tissues. There were 2 women and 5 men; mean age was  $48 \pm 20.5$  years (range, 19–69 years). No donors had pathology or trauma that liable to affect fascia lata quality. All fasciae latae were collected under strict aseptic conditions, and prepared immediately following harvesting, by cleaning and cutting into at least 18 segments. Dimensions are presented in Fig. 1.

Each segment was required to be at least 3 cm long and 1 cm wide. Each fragment was required to have an intact macroscopic aspect in order to be preserved. For each fascia, 6 samples were randomized cryopreservation (CRYO DRY group), 6 for cryopreservation with cryopreservation solution (CRYO DMSO group), and 6 for lyophilization (LYOPH group). Fig. 2 shows group and subgroup distribution of samples. When > 18 fragments were obtained from a given patient, the additional fragments were conditioned for cryopreservation for testing before completion of the final tests.

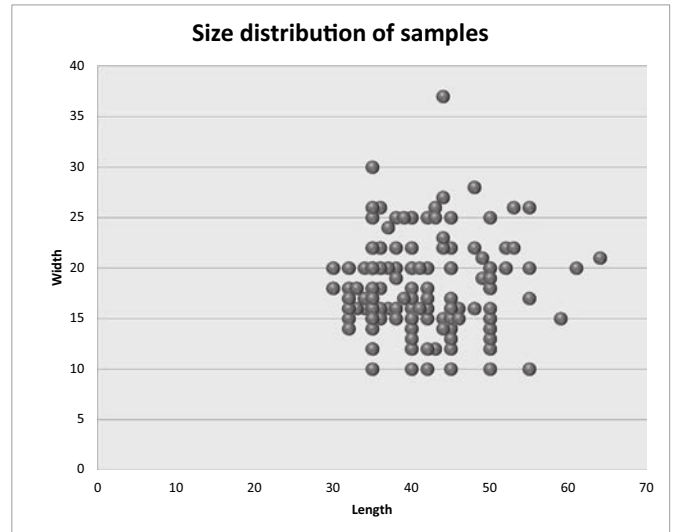


Fig. 1. Size distribution of samples (millimeter).

CRYO DRY samples were packaged in sterile dry containers and stored at 4 °C for up to 48 h before freezing at –80 °C. In this process, the samples were frozen fast because there is no cryopreservation solution (Oswald et al., 2017). CRYO DMSO samples were packaged in sterile containers with 10 cL dimethylsulfoxide (Cryosure DMSO, Wak Chemie Medical GmbH), and frozen within 48 h at –80 °C. In this process, the samples were frozen slowly in the DMSO (Oswald et al., 2017). LYOPH samples were stored in sterile containers in a refrigerator at 4 °C and freeze-dried within 72 h; samples were frozen at –22 °C at the start of lyophilization. Then, two desiccation phases were carried out in a desiccator (Alpha 2–4 LSC, Christ). Primary drying was carried out at 0.1 mbar for 24 h and secondary drying at 0.0047 mbar for 48 h. Grafts were stored in the Ostéobanque tissue bank.

Irradiation was carried out by the Ionisos company (Dagneux, France), with verified irradiation dose (exact dose for 11 kGy = 11.3 kGy; exact dose for 25 kGy = 26.7 kGy). Samples were stored at 4 °C during this procedure.

For biomechanical testing, grafts were thawed by keeping them in their compact at room temperature for 4 h, and tests were performed immediately after thawing. The lyophilized samples were rehydrated with 10 cl 0.9% NaCl solution for 24 h. Tensile tests were carried out, as shown in Fig. 3, on a traction device (MTS 20 M) equipped with a force sensor of 500 daN. The gripping system was tightened by screws. Gripping was standardized at 30 N.m. For the alignment in the jaws, we first fix the graft in the upper part and then the gravity places the graft vertically and aligned and then we fix the graft in the lower part.

The system was driven by software that managed the entire test procedure and processing of results. Deformation was measured from the recording of the vertical displacement (100 mm resolution). Displacement speed was fixed for all tests at 10 mm/min: i.e., strain rate slightly  $< 0.01 \text{ s}^{-1}$ . The tensile test up to breakage provided a stress/strain curve characteristic of the mechanical behavior of the particular specimen tested. Testing provided typical load-deformation curves, as in Fig. 4. The separation of the phases is done by the software and can be adjusted manually. The dimensions of the specimens were used to determine the material properties.

In order to facilitate analysis and enable comparison of mechanical responses between the various specimens, results were converted in conventional stresses and deformations, calculated from the apparent dimensions of the samples. These dimensions had the following mean values: length about 40 mm, width 18 mm, and thickness 0.49 mm (i.e., cross-section slightly  $< 9 \text{ mm}^2$ ). The aspect of the curves corresponded to viscoelastic behavior.



Fig. 2. Distribution of samples.

Statistical analysis used Stata v12 software. All tests were bilateral, and a p-value < 5% was considered statistically significant. Data were reported as numbers and percentages for qualitative variables and as means  $\pm$  standard deviation and medians [interquartile range] (IQR) for quantitative variables. All tests were age-matched. Young's modulus, deformation and tensile stress were compared between preservation methods (CRYO DRY, CRYO DMSO and LYOPH) and radiation doses (0, 11 and 25 kGy) on non-parametric Kruskal-Wallis analysis of variance, given the non-normal data distributions (checked graphically and tested on Shapiro-Wilk test). Post-hoc Dunn tests were performed in case of statistical significance, with Bonferroni adjustment. These analyses were performed in subgroups (preservation method and radiation dose) using the same methods. Multivariate analyses were performed for each criterion (Young's modulus, deformation and breaking stress), using a mixed linear regression model, taking into account the "subject" random effect and adjusting for fixed effects method (CRYO DRY, CRYO

DMSO, LYOPH) and radiation dose (0, 11 and 25 kGy), with interaction tests.

### 3. Results

The samples showed viscoelastic properties, as seen in Fig. 4. Test results are presented in Table 1. The medians and inter-sample intervals of Young's modulus, deformation at break and tensile stress are respectively represented in Figs. 5, 6 and 7.

The effects of preservation method and of radiation dose on biomechanical properties were compared and combined effects of the two parameters were studied on multivariate analysis. Table 2 shows significant findings.

In the balance sheet, the results relating to the effect of the preservation method show that, for all doses combined and for all mechanical characteristics tested (Young's modulus, deformation and

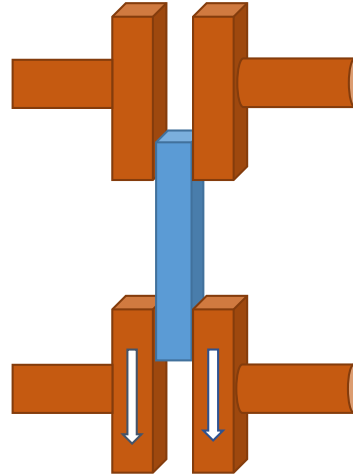


Fig. 3. MTS 20 M traction device and explanatory diagram of the traction system.

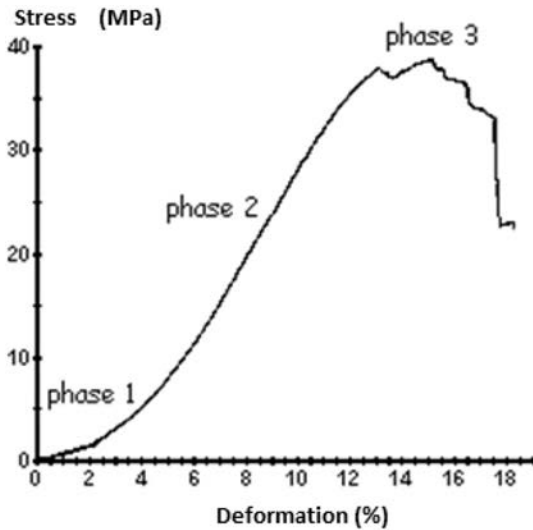


Fig. 4. Stress/strain curve of a non-irradiated lyophilized sample in traction (example: test n°16).

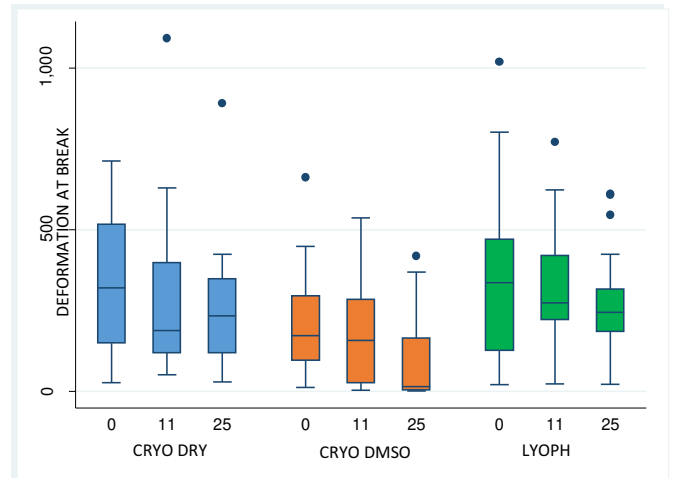


Fig. 5. Median, quartiles and range of deformation at break for each preservation method and radiation dose (MPa).

Table 1  
R7results of all biomechanical tests.

Method		Radiation 0 kGy	11 kGy	25 kGy	All
CRYO DRY	Young's modulus (MPa)	319.7 ± 206.9[148.7–516.6]	187.6 ± 236.3[118.7–398.2]	272.8 ± 169.7[221.65–420.3]	236.9 ± 205.9[134.3–386.5]
	Ultimate Deformation (%)	16.9 ± 11.2[10.5–24.7]	17.4 ± 12.2[14.2–27.8]	15.9 ± 9[11.7–24.2]	16.9 ± 10.8[11.1–25.4]
	Stress at break (MPa)	26.2 ± 14.5[17.7–40.1]	20.2 ± 16[12.9–33.2]	20.9 ± 10.9[12.7–29.1]	22.6 ± 14[13.4–31.8]
DRYO DMSO	Young's modulus (MPa)	171.65 ± 151.2[96.6–294.9]	156.95 ± 155.3[24.75–284.6]	14.2 ± 126.1[3.1–164.9]	144.7 ± 151.8[18.4–27.2]
	Ultimate Deformation (%)	19.9 ± 23.2[14.8–31.9]	16.7 ± 55.2[13.6–44.1]	37.3 ± 32.3[21.7–76.7]	23 ± 39.9[14.5–44.6]
	Stress at break (MPa)	16.1 ± 12.6[12.8–24.2]	14.1 ± 15.4[5.9–28]	3.9 ± 14.2[0.7–21.5]	14 ± 14.5[4.3–23]
LYOPH	Young's modulus (MPa)	335.4 ± 250[127.3–470.3]	233.15 ± 171.9[118.65–348.05]	243.85 ± 145.8[184.65–316.4]	265.8 ± 194.4[167.4–393.6]
	Ultimate Deformation (%)	18.5 ± 22.5[12.5–25.6]	15.8 ± 11.2[12.5–18.7]	16.6 ± 9.4[13.2–19.5]	16.7 ± 15.6[12.8–19.8]
	Stress at break (MPa)	28.3 ± 16.2[14.6–36.9]	26.3 ± 11.9[20.8–34.5]	22.8 ± 9.9[14.3–31.7]	25.2 ± 13[17.6–33]
All	Young's modulus (MPa)	296.5 ± 213.1[138.7–440.4]	256.5 ± 195.2[114.1–361.5]	205 ± 166.4[76.2–297.1]	227.1 ± 195.5[99.6–368.1]
	Ultimate Deformation (%)	18.6 ± 19.8[12.1–28.2]	16.9 ± 35.6[12.8–23]	18.7 ± 23.5[13.2–30]	18.1 ± 27[12.8–26.9]
	Stress at break (MPa)	22.6 ± 14.9[14.4–36.7]	22.1 ± 14.8[12.6–32.5]	19.3 ± 12.8[8.1–28.8]	20.9 ± 14.4[11.9–31.7]

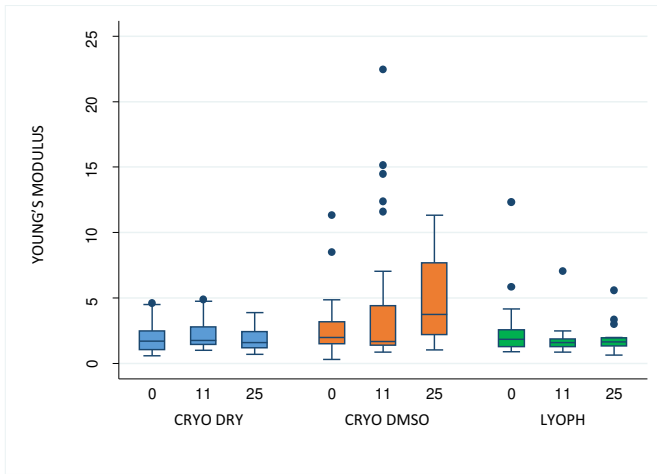


Fig. 6. Median, quartiles and range of Young's Modulus for each preservation method and radiation dose (MPa).

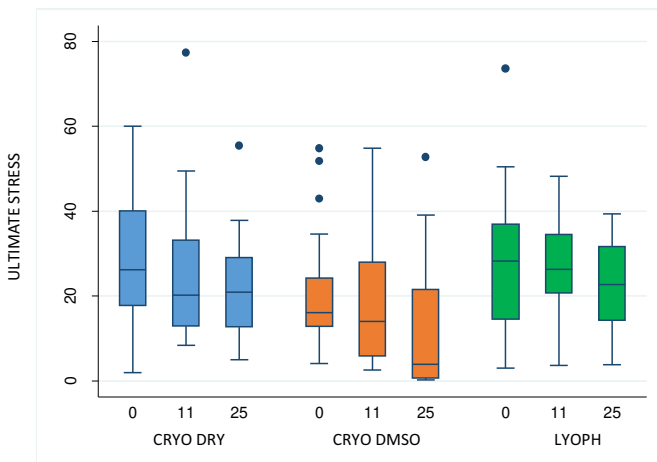


Fig. 7. Median, quartiles and range of ultimate stress for each preservation method and radiation dose (MPa).

tensile strength), cryopreservation with DMSO (CRYO DMSO) was different from dry cryopreservation (CRYO DRY) and lyophilization (LYOPH). Taking the CRYO DMSO method as reference and comparing it versus the CRYO DRY and LYOPH methods revealed a decrease in Young's modulus and breaking stress and an increase in breaking strain. This indicates lower sample rigidity (elastic modulus and the breaking stress) with the CRYO DMSO method, associated with greater ductility (deformation at break). Considering preservation method impact according to radiation dose, the above difference in mechanical properties with the CRYO DMSO method was accentuated with the highest radiation dose (25 kGy).

Comparing all preservation methods, radiation dose effects on mechanical characteristics were found only for Young's modulus and stress at break, with lower values for both with increasing radiation dose. Analyzing radiation dose according to preservation method showed a pattern similar to that of the preservation method effect, confirming the reduction in Young's modulus and tensile stress and increase in breakage strain with increasing radiation dose using the CRYO DMSO method.

Multivariate statistical analysis only reinforced the conclusion that emerged from the study: for all of the mechanical characteristics tested (Young's modulus, tensile stress and breakage strain), an interaction exists between radiation dose and the CRYO DMSO method such that the radiation dose effect the 3 mechanical characteristics exists only

when the CRYO DMSO method is used.

#### 4. Discussion

Preservation method and radiation dose influence the biomechanical properties of fascia lata allografts. Cryopreservation by DMSO gave poorer results than freezing or lyophilization for Young's modulus, deformation and breaking stress. We found no significant difference between dry cryopreserved and freeze-dried samples. Young's modulus and stress-resistance (i.e., rigidity) were lower with higher radiation dose.

Concerning preservation method, results found in the literature are divergent. The use of a cryopreservation agent such as glycerol for cryopreservation of tendon allografts has been the subject of several studies, with discordant results. In 2008, Kaminski et al. studied 3 different preservation methods (dry cryopreservation, cryopreservation with glycerol, and freeze-drying) on 35 kGy-irradiated bone-tendon allografts. (Kamiński et al., 2009). They found significantly higher tensile strength for dry cryopreserved samples than cryopreserved samples with glycerol. This is agreement with the present results, but Kaminski et al. did not study a low radiation dose such as 11 kGy. However, in 2011, Suhodolčan et al., studying the biomechanical characteristics of 70 patellar bone-tendon allografts conserved for 9 months (Suhodolčan et al., 2012), found better resistance of samples preserved with 10% glycerol at  $-80^{\circ}\text{C}$  than for dry cryopreservation at the same temperature. This difference with regard to the present findings may be explained by the fact that we used a different cryopreservation solution, DMSO, which demonstrated a positive effect on angiogenesis and immune tolerance of osteochondral allografts in mice (Wingenfeld et al., 2002). We have not found any clinical studies of its use as a cryopreservation agent for allografts, and our results in vitro do not encourage its clinical use.

We found only two studies in the literature focusing on the biomechanical properties of lyophilized tendon allografts. In 2016, Negrín et al. studied the biomechanical properties of porcine gracilis tendon in a randomized biomechanical and histological experimental study (Negrín et al., 2016). They found no difference in biomechanical properties between lyophilized and dry cryopreserved tendons. In his 2008 study, Kaminski found lower tensile strength in freeze-dried allografts irradiated at 35 kGy than in dry cryopreserved allografts irradiated at the same dose (Kamiński et al., 2009). The present study found no significant difference between these two methods. This difference between studies may be explained by a combined adverse effect of lyophilization and irradiation at 35 kGy that was not observed in the present study for lower irradiation doses of 11 kGy and 25 kGy. Theoretically, as reported by Kempner (Kempner, 2001), irradiation in a water-poor environment causes breaks in the covalent bonds of the macromolecules essential to the biomechanical properties of collagenous tissues, whereas the free radical  $\cdot\text{OH}$  produced by radiolysis of water (indirect effect of irradiation) promotes bonds between collagen fibers. Only one study reported the in-vivo use of lyophilized fascia lata allograft in orthopedics (Givissis et al., 2017). It was used as a metatarsophalangeal arthroplasty interposition graft for hallux rigidus, with no specific complications in the 18 patients. Freeze-dried grafts have the advantage of not requiring a cold chain, and can be stored for a long time at room temperature.

Regarding irradiation, numerous studies have shown that gamma ray sterilization alters the biomechanical properties of tendons, and the present study confirmed a dose-dependent influence of irradiation, including a significant impact on Young's modulus. This quantifies the stiffness of a material, and decreased after irradiation, by 14% (from 297 MPa to 256 MPa) for 11 kGy, and 31% (from 297 MPa to 205 MPa) for 25 kGy. Yanke et al. also demonstrated a change in the biomechanical properties of patellar bone-tendon allografts, even when irradiated at low doses (Yanke et al., 2013); samples were either non-irradiated or irradiated at 10–12 kGy and then matched; the rigidity of

**Table 2**  
Effects of preservation method and radiation dose on biomechanical properties.

Comparison	Parameter studied	p value	Comparison	Parameter studied	p value	Comparison	Parameter studied	p value	Comparison	Parameter studied	p value
All radiation doses											
All methods			0 kGy			11 kGy			25 kGy		
CRYO DRY	Young's modulus	<b>p = 0.001</b>	All Methods	Young's modulus	<b>p = 0.06</b>	All Methods	Young's modulus	<b>p = 0.01</b>	All Methods	Young's modulus	<b>p &lt; 0.001</b>
DMSO	Young's modulus	<b>p &lt; 0.001</b>	CRYO DRY	Young's modulus	<b>p = 0.03</b>	CRYO DRY	Young's modulus	<b>p = 0.26</b>	CRYO DRY	Young's modulus	<b>p &lt; 0.001</b>
LYOPH	Young's modulus	<b>p = 0.56</b>	LYOPH	Young's modulus	<b>p = 1</b>	LYOPH	Young's modulus	<b>p = 0.19</b>	LYOPH	Young's modulus	<b>p = 1</b>
CRYO DMSO	Young's modulus	<b>p &lt; 0.001</b>	CRYO	Young's modulus	<b>p = 0.09</b>	CRYO	Young's modulus	<b>p = 0.004</b>	CRYO	Young's modulus	<b>p &lt; 0.001</b>
All Methods	Deformation	<b>p = 0.002</b>	DMSO	Deformation	<b>p = 0.26</b>	DMSO	Deformation	<b>p = 0.34</b>	DMSO	Deformation	<b>p &lt; 0.001</b>
CRYO DRY	Deformation	<b>p = 0.001</b>	All Methods	Deformation	<b>p = 0.16</b>	All Methods	Deformation	<b>p = 1</b>	All Methods	Deformation	<b>p &lt; 0.001</b>
DMSO	Deformation	<b>p = 1</b>	CRYO DRY	Deformation	<b>p = 0.74</b>	CRYO DRY	Deformation	<b>p = 0.47</b>	CRYO DRY	Deformation	<b>p = 1</b>
LYOPH	Deformation	<b>p = 0.003</b>	CRYO	Deformation	<b>p = 0.52</b>	CRYO	Deformation	<b>p = 0.23</b>	CRYO	Deformation	<b>p &lt; 0.001</b>
LYOPH	Deformation	<b>p = 0.001</b>	DMSO	Deformation	<b>p = 0.05</b>	DMSO	Deformation	<b>p = 0.02</b>	DMSO	Deformation	<b>p &lt; 0.001</b>
All Methods	Stress at break	<b>p &lt; 0.001</b>	All Methods	Stress at break	<b>p = 0.04</b>	All Methods	Stress at break	<b>p = 0.11</b>	All Methods	Stress at break	<b>p = 0.002</b>
CRYO DRY	Stress at break	<b>p &lt; 0.001</b>	CRYO DRY	Stress at break	<b>p = 1</b>	CRYO DRY	Stress at break	<b>p = 0.57</b>	CRYO DRY	Stress at break	<b>p = 0.87</b>
DMSO	Stress at break	<b>p &lt; 0.001</b>	DMSO	Stress at break	<b>p = 0.07</b>	DMSO	Stress at break	<b>p = 0.009</b>	DMSO	Stress at break	<b>p &lt; 0.001</b>
CRYO DRY	Stress at break	<b>p = 0.71</b>	LYOPH	Stress at break	<b>p = 0.21</b>	LYOPH	Stress at break	<b>p = 0.71</b>	LYOPH	Stress at break	<b>p = 0.49</b>
CRYO DMSO	Stress at break	<b>p &lt; 0.001</b>	CRYO	Stress at break	<b>p = 0.19</b>	CRYO	Stress at break	<b>p &lt; 0.001</b>	CRYO	Stress at break	<b>p = 1</b>
All Methods of preservation			LYOPH	Stress at break	<b>p = 0.65</b>	LYOPH	Stress at break	<b>p = 0.07</b>	LYOPH	Stress at break	<b>p = 0.45</b>
All Doses (kGy)			CRYO DRY	Stress at break	<b>p = 0.52</b>	CRYO DRY	Stress at break	<b>p = 0.07</b>	CRYO DRY	Stress at break	<b>p = 0.48</b>
0	Young's modulus	<b>p = 0.02</b>	All Doses (kGy)	Stress at break	<b>p = 1</b>	All Doses (kGy)	Stress at break	<b>p = 0.001</b>	All Doses (kGy)	Stress at break	<b>p = 0.35</b>
11	Young's modulus	<b>p = 0.41</b>	0	Stress at break	<b>p = 0.67</b>	0	Stress at break	<b>p = 0.06</b>	0	Stress at break	<b>p = 0.66</b>
25	Young's modulus	<b>p = 0.007</b>	11	Stress at break	<b>p = 0.39</b>	11	Stress at break	<b>p = 0.07</b>	11	Stress at break	<b>p = 1</b>
0	Deformation	<b>p = 0.13</b>	All Doses (kGy)	Stress at break	<b>p = 0.01</b>	All Doses (kGy)	Stress at break	<b>p = 0.003</b>	All Doses (kGy)	Stress at break	<b>p = 0.26</b>
11	Deformation	<b>p = 0.52</b>	0	Stress at break	<b>p = 0.65</b>	0	Stress at break	<b>p = 0.5</b>	0	Stress at break	<b>p = 1</b>
25	Deformation	<b>p = 1</b>	11	Stress at break	<b>p = 0.15</b>	11	Stress at break	<b>p = 0.001</b>	11	Stress at break	<b>p = 0.26</b>
0	Stress at break	<b>p = 0.67</b>	0	Stress at break	<b>p = 0.91</b>	0	Stress at break	<b>p = 0.03</b>	0	Stress at break	<b>p = 0.21</b>
25	Stress at break	<b>p = 0.39</b>	11	Stress at break	<b>p = 0.06</b>	11	Stress at break	<b>p = 0.03</b>	11	Stress at break	<b>p = 0.21</b>
All Doses (kGy)											
0	Young's modulus	<b>p = 0.01</b>									
11	Young's modulus	<b>p = 0.65</b>									
25	Young's modulus	<b>p = 0.006</b>									
0	Stress at break	<b>p = 0.06</b>									
11	Stress at break	<b>p = 0.06</b>									
25	Stress at break	<b>p = 0.06</b>									



irradiated samples was reduced by 20%, but without change in other biomechanical properties. The present results are in agreement with these findings. In 2011, McGivray et al., studying the effects of irradiation on the biomechanical properties of bone-tendon-bone ovine allografts (McGivray et al., 2011), found that low-dose 15 kGy radiation did not alter biomechanical properties, in contrast to average dose irradiation of 25 kGy, which reduced breaking stress by 26.9%; this figure was 27.1% in the present study. These results encourage an in-vitro study to evaluate the microbiological efficacy of low-dose radiation sterilization on tendon allografts. Nguyen et al. studied this parameter for bone allografts (Nguyen et al., 2011), and found optimal sterilization safety with irradiation at 11 kGy, which they recommended to preserve the resistance of bone allografts.

The strengths of our study are the large number of samples tested, and the comparisons between several storage methods and radiation doses. All previous biomechanical studies focused on results with allografts other than fascia lata. A study conducted by Palmer in 2017 focused in particular on the biomechanical properties of several types of non-irradiated dry cryopreserved allografts (tibialis anterior, tibialis posterior, peroneus longus and semitendinosus tendons) (Palmer et al., 2017). The results encouraged the use of all these allografts, with no particular preference for any one of them. The Young's modulus and tensile stress values (respectively, 600 MPa and 60 MPa) were significantly higher than reported here for non-irradiated dry cryopreserved samples (339 MPa elastic modulus and 28 MPa breaking stress); the value for deformation at break, on the other hand, (approximately 20%) was similar to the present finding (19.5%). However, Palmer used samples with much greater cross-section than in the present study (on average, 35 mm<sup>2</sup> vs 9 mm<sup>2</sup>). Values of mechanical variables (elastic modulus, 450 MPa; stress and strain at break, about 45 MPa and 20%) reported elsewhere for other samples with greater cross-sectional area (about 55 mm<sup>2</sup>) were closer to our results.

Choice of fascia lata was a strong point, but this structure also has limitations. Fascia lata is a heterogeneous structure, and samples from the most proximal part of the fascia appeared macroscopically slightly different from samples from the distal part. Matching by location could have been helpful.

Another weak point was that storage time was short, with a minimum of 3 months and we don't have temperature curve to assess the decreasing temperature. The use of 10 cL DMSO is questionable because this product is diluted when it is used for cell culture because of its toxicity. In our case, there is no survey of the cells, that's why we choose this concentration, maybe another study with diluted DMSO would be useful and could show less mechanical loss.

The present study population ranged from 19 to 69 years of age, and age-related impairment of the biomechanical qualities of the tendons can be suspected. To remedy this, all tests were age-matched. Kamiński's study suggested diminished tendon resistance after 60 years of age (Kamiński et al., 2009). However, Gagliano's results in 2017, specifically studying the influence of age on morphological and molecular properties, were not in agreement (Gagliano et al., 2017), finding equivalent tendon structure in semimembranosus and gracilis tendons taken from middle-aged (41.8 ± 13.3 years) and older subjects (72.7 ± 7.03 years); these results were explained by the ability of tenocytes to remodel the extracellular matrix.

The present study did not deal with an innovative conservation technique: decellularization. Dong's animal model in 2015 suggested very promising results (Qi et al., 2015): semimembranosus allografts taken from rabbits and reimplanted in other rabbits in ACL reconstruction showed greater resistance when conserved by decellularization than by dry cryopreservation. The results of this study encourage performing a biomechanical study on human tendon allografts conserved by decellularization.

The present study could also have investigated the effect of sterilization by another irradiation method: electron beam irradiation is an alternative to gamma irradiation. Hoburg et al.'s study suggested that

the adverse effects of irradiation are less severe with this method (Hoburg et al., 2015). They compared the biomechanical properties of electron-beam sterilized bone-tendon-bone graft versus medium- (25 kGy) and high-dose gamma radiation (34 kGy). The biomechanical properties of the tendons were significantly less impaired in medium-dose gamma irradiated and electron-beam irradiated grafts. Electron-beam irradiation also has the advantage of taking just a few seconds, unlike gamma irradiation, which takes several hours; however, access is limited and costs are higher. In addition, we did not find any studies on its effectiveness in graft sterilization. It would be interesting to carry out an in-vitro study on this subject before proceeding to a clinical trial. Finally, the interest of graft irradiation is still debated and many surgeons prefer non-irradiated allografts. A meta-analysis conducted in 2014 by Park et al. included results of ACL reconstruction using low-dose irradiated or non-irradiated allografts in 1453 patients (Park et al., 2015). They found a lower recovery rate for the low-dose radiation group than for the non-irradiated group. On the other hand, there were 2 cases of allograft infection in the non-irradiated group versus none in the irradiated group.

A single study by Pritchard et al. in 1995 reported results of fascia lata allograft for ACL reconstruction (Pritchard et al., 1995). The study included 62 patients who had ACL reconstruction associated to extra-articular external reconstruction. Grafts were conserved under dry cryopreservation; the sterilization method was not specified. They found good or excellent functional outcomes (IKDC a and b) in 87% of patients at 10 years follow-up and no evidence of graft rejection. We found no subsequent clinical studies of fascial allografts in knee surgery. However, the present study suggests that fascia lata allograft is an interesting option in view of the results of the biomechanical tests performed. In addition, we observed in our study that conservation of fascia lata allografts by cryopreservation or lyophilization should be preferred over cryopreservation with DMSO solution. Finally, the present study confirmed that medium-dose irradiation alters the biomechanical properties of fascia lata allografts.

## 5. Conclusion

Dry cryopreserved fascia lata allografts showed biomechanical properties similar to those lyophilized fascia lata allografts. Cryopreserved fascia lata allografts with DMSO solution showed lower stiffness and greater ductility.

Sterilization of fascia lata allografts by gamma radiation alters their biomechanical properties dose-dependently. However, the microbiological efficacy of low-dose radiation (11 kGy) on fascia allografts needs to be evaluated in further studies.

The present results are similar to those found in the literature for other types of tendon allograft. This biomechanical study is only a preliminary step in the evaluation of allografts.

## Author contribution statement

Writing: RE, SA, GV; manipulation: RE, SA, RC; statistics: AM; review: SD, SB

## Conflict of interest

S. Boisgard is a consultant for Zimmer-Biomet, outside of this study. All the authors participate in Ostéobanque on a voluntary basis, without compensation.

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