

Different sterilization methods used for human tendons – A systematic review

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Abstract

Background

It is important to know the biomechanical properties of an allograft. This is because when looking to do a transplant of a tendon, the tendon must have very similar biomechanical properties to the original tendon. To use tendon allografts, it is critical to properly sterilize the tendon before implantation. In past decades, several sterilization procedures have been used. This study aimed to systematically evaluate the existing literature to compare the values of failure load/ultimate strength and Young's modulus of elasticity of different sterilization methods on commonly used tendon allografts. Five major scientific literature databases (Web of Science, Science Direct, Scopus, PLOS ONE, Hindawi) and additional sources were used.

Results

Studies used had to show a particular sterilization method. Studies were identified to meet the following inclusion criteria: is a controlled laboratory study, gamma irradiation (dose reported), and other sterilization methods. Search for publications dated between 1991 and March 31st, 2020. A total of 54 articles have been included. Data collected from the full-text assessment included: a) author and date, b) type of tendon, c) type of sterilization, d) sterilization dose, and g) measured and calculated parameters. Different types of sterilization methods used before tendon durability testing have been identified and categorized.

Conclusions

Identified sterilization methods (gamma irradiation, dry ice, supercritical carbon dioxide (SCCO₂), BioCleanse, Electron Beam) are offered as a catalog of potential methods.

As a result of the broadness of the present research, it provides an overview of sterilization methods. It does not stand for the state-of-the-art of any single process.

1. Background

In orthopedic reconstruction, the use of tendon allografts has become more widely accepted in recent years, especially in the anterior cruciate ligament reconstruction. There have been multiple studies conducted which back the use of tendon allografts. The benefits include reduced operation time, reduced donor site morbidity, and unaltered mechanics secondary to harvesting. Additionally, studies conducted on animals and humans have demonstrated that soft tissue allografts are statistically comparable to autografts on an anatomical and biomechanical basis. [1]–[10]

Anterior cruciate ligament (ACL) reconstruction is a common procedure in orthopedic practice. A critical decision to be made is the choice of graft. Despite autografts proving capable and displaying good outcomes, graft harvest can cause continual pain at the site of harvest and limit the range of motion. For

this reason, a significant increase in allograft use can be seen in the last decade, and despite the higher costs, it remains a feasible choice, specifically in revision cases. In young patients, an increased rate of failure of allografts has been recorded. This mostly seems to be caused by the sterilization process, as some studies also reported increased failure rates when comparing fresh-frozen allografts and autografts. A couple of studies displayed more inferior results in sterilization compared with fresh-frozen allografts. A major risk of using allografts is disease transmission. Septic arthritis after ACL reconstruction has been reported as a risk factor for unsterilized allografts. Also, higher rates of postoperative infections have been reported when allografts were aseptically processed rather than irradiated allografts or autografts. [11]–[21]

A potential complication of using allografts is the risk of disease transmission. Possible diseases include both infections from HIV, Hepatitis C, and bacterial infection from organisms. Whilst the prevalence of the transmission of the disease via allograft tissue is minimal; the potential impact is high. After multiple documented cases of sepsis and infection following ACL reconstruction using allografts, the bacterial transmission of diseases has come to the forefront. These cases were associated with non-irradiated tissue grafts. For this reason, an effort has been made to reduce the scarce but possibly disastrous occurrences of bacterial infection by using enhanced sterilization techniques. [10], [22]–[25]

1.1. Aim of the study

The present systematic literature review aims to identify and to categorize existing sterilization methods and their effects on the mechanical properties (failure load/ultimate tensile strength and Young's modulus of elasticity) of the tendons.

Combining the collected materials should allow us to investigate how different methods can differentiate between participant groups and which methods are more encouraging in research or clinical practice.

2. Materials And Methods

2.1. Search strategy

2.1.1. Identification of materials

This systematic review was executed according to the PRISMA guidelines [26]. Five electronic databases were used (Web of Science, Science Direct, Scopus, PubMed, PloS ONE, and Hindawi) to search for publications dated between 1991 and March 31st, 2020. Key search terms used with Boolean conjunction included: tendon, allograft tendon, tendon sterilization, biomechanical testing, mechanical properties, and synonyms of these terms. The expressions were matched to each database. Other sources included reference lists of formerly cited articles containing our published works on similar topics. (Table 1)

For example, a search strategy used for the Science Direct database is as follows. In the Advanced search, the following phrases were added in All fields: (allograft tendon OR tendon) AND (tendon

sterilization OR ligament sterilization OR sterilization AND biomechanical properties OR mechanical properties OR modulus). The search was refined to journal publications. Publication dates were set from 2008 to the Present, with the search performed on March 31st, 2020. This search in the Science Direct database yielded 82 records. Key search terms were identified and agreed upon by DF and RMK; electronic search and downloading of results were conducted by DF. Screening, eligibility check of materials, and data extraction were executed by DF, BK, and BS.

2.1.2. Screening materials

The associated materials were screened based on title and abstract, removing duplicate entries. The material of unrelated topics, aims, or completely theoretical work was excluded. Proof of concept articles was not omitted.

2.1.3. Eligibility check of materials

Inclusion and exclusion criteria were determined to check for eligibility. Studies had to meet all the inclusion criteria to be included in the final synthesis (Table 1). Studies that either met an exclusion criterion or otherwise failed to meet inclusion criteria were excluded. These criteria were created to provide a quality assessment to a certain extent, i.e., the methods applied had to be well communicated, and the evaluation of the measurement results had to be objective. No additional quality assessment was carried out on the materials included.

2.1.4. Data extraction

In compliance with the objective of this research, the final overview of the types of sterilization was to extract relevant information on the assessment of mechanical properties. The data collected from the articles included a) author and date, b) type of tendon, c) type of sterilization, d) sterilization dose, and g) measured and calculated parameters such as failure load/ultimate tensile strength and Young's modulus of elasticity.

Table 1
Viewpoints and their inclusion criteria and exclusion criteria

Viewpoints	Inclusion criteria	Exclusion criteria
Tendon irradiation and mechanical test	Studies that included tendon sterilization and mechanical tests in their experimental processes.	Studies which only included tendon irradiation method without any type of mechanical tests.
Description of tendon irradiation and mechanical test	Studies with a detailed explanation of the tendon irradiation and mechanical test and the experimental procedure that was completed.	Studies without detail or incomplete descriptions of the tendon irradiation and mechanical test and the experimental procedures that were completed.
Assessment of results	Studies with unbiased results based on measurable parameters.	Studies with biased scoring/assessments of results, not (fully) based on measurable parameters.

3. Results

The database search and additional sources resulted in 284 records (Fig. 1, in supplementary file). 152 records remained after the removal of duplicates and material with missing or unavailable abstracts. The title and abstract screening eliminated 60 records because of being unfit articles. The 92 items which remained went through a full-text eligibility check. 38 publications were eliminated from the 92 publications for various reasons, including clinical case reports, publications not written in English, conference abstract, and book chapters. Eight papers were literature review articles related to sterilization methods. The review articles found had an aim and scope different from the present study. The number of articles used in the final synthesis was 54 (n = 54).

The following were the reasons for exclusion: several studies applied criteria outside of the scope of our definition of tendon irradiation and mechanical properties, e.g., effects of gamma radiation if the tendon was infected with HIV. If multiple studies described similar tendon irradiation with an equal measurement method and similar instrumentation for assessment, the newest publication was used (30 pieces). Several theoretical articles did not detail a specific measurement setup and were therefore excluded (32 pieces). Some articles used a biased scoring assessment (6 pieces), and a few articles had an unrelated aim of the study, e.g., clinical case (7 pieces).

3.1. Type of sterilization

These studies evaluated the effect of sterilization's type: non-sterilized and non-frozen tendons (fresh tendons) [27, 28, 29, 30; Table 2.], gamma irradiation [27, 31–41; Table 3.], Supercritical carbon dioxide (SCCO₂) [27; Table 4.], Ethylene Oxide [42; Table 5.], BioCleanse [8, 43–45; Table 6.], Electron Beam (E-beam) [30, 46–49; Table 7.], Peracetic acid-ethanol in combination with low-dose gamma irradiation (PE-

R) [50; Table 8.], Hydrogen peroxide [51; Table 9.] and chlorhexidine [52; Table 10.]. The structure of the tendons may be destroyed by sterilization, so the method used is important. The following subsections detailed these sterilization methods, and the section of conclusion compared the failure load and Young's modulus of elasticity results found in the literature.

3.1.1. Non-sterilized tendon (fresh/control tendon)

Four articles mention the fresh grafts, which did not receive any sterilization [8, 27–30, 43–45, 50, 52; Table 2.]. After they were procured from human cadavers, they were placed in a saline solution and cooled until the measurement. In comparative studies, non-sterilized tendons are measured as a standard to be able to tell to what extent the sterilization method and dose change its properties. Baldini et al. [27] analyzed three groups of anterior and posterior tibialis tendons. The results showed that the failure load and Young's modulus of elasticity of the fresh group are weaker than the gamma-irradiated and SCCO2 sterilized groups [27; Table 2.]. Aguila et al. [28] found that no significant differences were between the results of the failure load and Young's modulus of elasticity of the non-sterilized and the gamma-irradiated groups in peroneus brevis and peroneus longus [28, Table 2.]. Suhodolcan et al. [29] also compared three groups (fresh, frozen, and cryopreserved) of the patellar tendon; they compared the results of the fresh group to the frozen and the cryopreserved [29, Table 2.]. Wei et al. [30] found that the mechanical properties (failure load and Young's modulus of elasticity) of the fresh group were better than the gamma-irradiated and the fractionated gamma-irradiated group [30; Table 2.]. From the above results, it can be stated that the values of the failure load and Young's modulus of elasticity of sterilized tendons either did not change or decrease as a result of gamma and fractioned gamma-irradiated [8, 27–30, 43–45, 50, 52; Table 2.].

Table 2
Average result of failure load and Young's modulus of elasticity of non-sterilized and non-frozen tendons
[8, 27–30, 43–45, 50, 52]

Type of tendon	Pieces	Failure load (N)	Young's modulus of elasticity (MPa)	Authors
Peroneus longus	14	2091.6 (148.7)	90.3 (11.3)	Aguila et al.
Peroneus brevis	20	1485.7 (209.3)	82.4 (19.0)	
Anterior or posterior tibialis	15	2427.3 (682.8)	75.4 (30.0)	Baldini et al.
Patellar tendons	10	-	25.0 (9.0)	Suhodolcan et al.
Human flexor digitorum superficialis	10	402.5 (38.4)	400.5 (58.5)	Wei et al.
Patellar tendon	14	-	158.0 (49)	Bechtold et al.
Achilles	10	3032 (677)	292.04 (123.15)	Conrad et al.
Tibialis anterior	12	1665 (291.3)	19.9 (4.7)	Schimizzi et al.
Tibialis tendon	10	606.73 (283.52)	213.13 (98.86)	Elenes et al.
Bone – patellar tendon – bone	10	1741 (304)	-	Hoburg et al. Hoburg et al.
Human flexor tendons	-	360.01 (88.17)	221.55 (73.06)	Zhou et al.
Patellar tendon	8	1878 (613)	-	Sobel et al.

3.1.2. Gamma irradiation

Gamma radiation is one of the most common sterilization methods. This sterilization method is processed by ten articles [8, 28, 31–41, 44–46, 48, 53; Table 3.]. During radiation, two doses are distinguished, the low dose (bacterial dose) of 15–26 kGy and the high dose (viricidal dose) of 30–45 kGy [38, 41]. The dosage unit is Mrad or kGy, 1 Mrad = 10 kGy

The articles were split into two groups; in the first group, multiple doses were used [31, 34, 40; Table 3.]. In the second group, only one dose was used [28, 32–33, 35–37, 39; Table 3.]. Balsly et al. [31] used two irradiation doses, where the absorbed dose is 18.3–21.8 kGy and 24–28.5 kGy. Four tendons (patellar, anterior tibialis, semitendinosus, and fascia lata) were examined. Based on the results, it could not be

declared that the increasing dose value diminished or increased the failure load and Young's modulus of elasticity of the tendons [31]. Gut et al. [34] used four doses (25, 35, 50, 100 kGy) for the patellar tendon. The failure load of the irradiated tendons deteriorated relative to the control group. With an increase in dose, the mechanical properties of the tendon improved to 35 kGy, and after that, they deteriorated [34]. In the article by Hangody et al. [40], the target doses were 21 and 42 kGy, and five different types of tendons were tested. In the case of the 21 kGy dose, the failure load and Young's modulus of elasticity of tendons improved versus the frozen group. In the case of the 42 kGy dose, the mechanical properties of some grafts improved, and others deteriorated [40].s

In the second group, the effect of gamma irradiation was examined compared to the control group. The values between 14.6–40 kGy radiation dose were examined. In two cases [33, 36], the gamma radiation improved the failure load and Young's modulus of elasticity of the tendons. Weber et al. used a 15–25 kGy irradiation dose for the Achilles tendon [36]. Curran et al. [33] used a 20 kGy irradiation dose for the patellar tendon. The gamma irradiation caused a deterioration in failure load, and Young's modulus of elasticity [28, 35, 37, 39] Aguila et al. [28] used 15–25 kGy for peroneus longus and peroneus brevis, Deyne and Haut [35] used 20 kGy for patellar tendons, Ren et al. [37] used 25 kGy for flexor digitorum superficialis, and Rasmussen et al. [39] used 40 kGy for the patellar tendon. Greaves et al. [32] compared three age groups ("young", "middle", "old"). Deterioration can be observed in the "middle" group and the "old" group. There are values of failure load, and Young's modulus of elasticity, no noticeable change in the "young" group [32].

Table 3
Average result of failure load and Young's modulus of elasticity of gamma-irradiated tendons [8, 28, 31–41, 44–46, 48, 53]

Type of tendon	Irradiation dose	Pieces	Failure load (N)	Young's modulus of elasticity (MPa)	Authors
Peroneus longus	1.5–2.5 Mrad	14	2122.8 (380.0)	94.8 (21.0)	Aguila et al.
Peroneus brevis	1.5–2.5 Mrad	20	1318.4 (296.9)	72.5 (16.6)	
Patellar tendon	18.3–21.8 kGy	9	2410 (1100)	88.11 (26.9)	Balsly et al.
Patellar tendon	24.0-28.5 kGy	9	2410 (930)	72.44 (21.30)	
Anterior tibialis	18.3–21.8 kGy	10	2890 (720)	328.47 (37.12)	
Anterior tibialis	24.0-28.5 kGy	10	2420 (330)	309.66 (56.67)	
Semitendinosus	18.3–21.8 kGy	8	1010 (360)	369.08 (122.47)	
Semitendinosus	24.0-28.5 kGy	10	1230 (380)	410.08 (98.86)	
Fascia latta	18.3–21.8 kGy	10	460 (140)	366.27 (87.38)	Greaves et al.
Fascia latta	24.0-28.5 kGy	10	420 (190)	238.51 (113.43)	
Anterior or posterior tibialis (young)	1.48–1.80 Mrad	10	3062 (699)	-	
Anterior or posterior tibialis (middle)	1.48–1.80 Mrad	13	2729 (995)	-	
Anterior or posterior tibialis (old)	1.48–1.80 Mrad	10	3004 (603)	-	
Flexor digitorum superficialis	25 kGy	10	335.96 (28.32)	357.72 (43.97)	Ren et al.
Flexor digitorum superficialis	25 kGy (freeze thaw)	10	287.41 (23.20)	346.95 (69.09)	
Achilles	18–24 kGy	10	3572.54 (393.57)	181.7 (24.05)	Hangody et al.

Type of tendon	Irradiation dose	Pieces	Failure load (N)	Young's modulus of elasticity (MPa)	Authors
Achilles	38–46 kGy	10	392.01 (180.91)	134.75 (15.07)	
Quadriceps	18–24 kGy	10	3184.32 (101.62)	302.96 (45.71)	
Quadriceps	38–46 kGy	10	3464.35 (462.45)	192.28 (51.88)	
Semitendinosus + gracilis	18–24 kGy	10	2310.32 (561.2)	248.93 (14.43)	
Semitendinosus + gracilis	38–46 kGy	10	2271.96 (651.12)	213.88 (20.28)	
Tibialis anterior	18–24 kGy	10	3107.76 (606.41)	327.93 (44.31)	
Tibialis anterior	38–46 kGy	10	2678.96 (181.45)	375.15 (67.84)	
Peroneus longus	18–24 kGy	10	2631.81 (297.94)	284.84 (16.03)	
Peroneus longus	38–46 kGy	10	2291.71 (523.76)	333.11 (79.11)	
Patellar tendon	4 Mrad	16	1884 (330)	-	Rasmussen et al.
Achilles	1.5–2.5 Mrad	10	1972 (558)	129.48 (53.22)	Conrad et al.
Tibialis anterior	20–26 kGy	12	1671.9 (290.2)	22.6 (5.9)	Schimizzi et al.
Tibialis tendon	17.1–21 kGy	10	597.09 (280.32)	179.02 (73.13)	Elenes et al.
Bone – patellar tendon – bone	25 kGy	10	1009 (400)	-	Hoburg et al.
Bone – patellar tendon – bone	34 kGy	10	1073 (617)	-	Hoburg et al.

3.1.3. Supercritical carbon dioxide (SCCO₂)

Supercritical carbon dioxide is an alternative sterilization method [27, Table 4.]. The allografts were secured in their casing and placed in a chamber with heated and pressurized CO₂, which forms a solvent that sterilizes the allograft. Baldini et al. [27] compared the SCCO₂ method with gamma irradiation in

anterior and posterior tibialis. Based on the measurements, the SCCO₂ treated grafts show better values of failure load and Young's modulus of elasticity than the gamma irradiation group [27, Table 4.].

Table 4

Average result of failure load and Young's modulus of elasticity of sterilized tendons with supercritical carbon dioxide [27]

Type of tendon	Pieces	Failure load (N)	Young's modulus of elasticity (MPa)	Authors
Anterior or posterior tibialis	11	2450.3 (576.8)	91.9 (30.2)	Baldini et al.

3.1.4. Ethylene oxide

Bechtold et al. [42] used ethylene oxide for sterilization [42, Table 5.]. The grafts are assigned to freeze-drying and sterilization. The tendon is exposed to 12% ethylene oxide gas at 32°C for 15 hours, with a relative humidity of approximately 80% before being freeze-dried. The study presented two types of freezing as a control group. This alternative procedure does not improve the failure load and Young's modulus of elasticity of the tendons [42, Table 5.].

Table 5

Average result of failure load and Young's modulus of elasticity of sterilized tendons with ethylene oxide [42]

Type of tendon	Pieces	Failure load (N)	Young's modulus of elasticity (MPa)	Authors
Patellar tendon	14	-	143.0 (67)	Bechtold et al.

3.1.5. BioCleanse

Following the BioCleanse protocol, all tendons were closed in a chamber and were exposed to a solvent at differing pressures and vacuum cycles [8, 43–45, 54; Table 6.]. The treatment group was exposed to the chemical solutions for twice as long as the standard time of exposure. This was designed to portray the worst outcome for degrading the structural and mechanical characteristics of the donor material [54]. Based on the results, the failure load and Young's modulus of elasticity showed no significant differences between gamma irradiation and BioCleanse groups [8, 43–45, 54; Table 6.].

Table 6

Average result of failure load and Young's modulus of elasticity of sterilized tendons with BioCleanse [8, 43–45, 54]

Type of tendon	Pieces	Failure load (N)	Young's modulus of elasticity (MPa)	Authors
Achilles	10	2472 (701)	154.06 (104.54)	Conrad et al.
Tibialis anterior	12	1559 (176)	484.2 (99.1)	Colaco et al.
Tibialis anterior	12	1651.6 (377.4)	19.7 (5.4)	Schimizzi et al.

3.1.6. Electron Beam (E-beam)

The sterilization method did not differ much from the traditional gamma irradiation method [30, 46–49; Table 7.]. The tendon grafts were encased in a specially created Styrofoam box, packed with dry ice to preserve the approximate temperature of -40°C to -50°C throughout the complete irradiation process. Better mechanical properties are achieved by the E-beam method [30, 46–49; Table 7.] compared to the method with gamma irradiation, but the differences were not significant.

Table 7
Average result of failure load and Young's modulus of elasticity of sterilized tendons with Electron Beam
[30, 46–49]

Type of tendon	Irradiation dose	Pieces	Failure load (N)	Young's modulus of elasticity (MPa)	Authors
Tibialis tendon	9.2–12.2 kGy	10	876.38 (310.73)	206.71 (88.87)	Elenes et al.
Tibialis tendon	17.1–21.0 kGy	10	660.24 (312.12)	152.64 (75.10)	
Human flexor digitorum superficialis	50 kGy	10	282.3 (53.0)	291.6 (50.8)	Wei et al.
Human flexor digitorum superficialis	50 kGy, Fractionation	10	360.9 (49.3)	354.3 (49.1)	
Human flexor digitorum superficialis	50 kGy, Fractionation + Ascorbate	10	390.6 (42.1)	390.4 (50.6)	
Bone – patellar tendon – bone	34 kGy	11	1024 (204)	-	Hoburg et al.
Bone – patellar tendon – bone	34 kGy, fractionated	11	1327 (305)	-	
Bone – patellar tendon – bone	25 kGy	10	1177 (512)	-	Hoburg et al.
Bone – patellar tendon – bone	34 kGy	10	1139 (445)	-	

3.1.7. Peracetic acid-ethanol in combination with low-dose gamma irradiation (PE-R)

This hybrid method consists of chemical sterilization and gamma radiation [50; Table 8]. The PAA/ethanol (PE) solution was prepared using distilled water and absolute ethanol. It consisted of 0.2% PAA and 24% ethanol. Tendons were submerged in the PE solution for 30 minutes and later washed with a saline solution until the concentration of PAA was lower than 1 ppm. The refined human flexor tendons were then closed and subjected to gamma irradiation at an average dose of 15 kGy. The sterilized muscles were stowed at -80°C [50], and they showed better mechanical results than the control group. This hybrid procedure has not been compared with other sterilization methods [50; Table 8].

Table 8

Average result of failure load and Young's modulus of elasticity of sterilized tendons with PE-R [50]

Type of tendon	Pieces	Failure load (N)	Young's modulus of elasticity (MPa)	Authors
Human flexor tendons	-	306.96 (61.52)	235.78 (96.13)	Zhou et al.

3.1.8. Hydrogen peroxide

This chemical process requires that 3% hydrogen peroxide be added to the solution [51; Table 9.]. The tissues should be left in the solution for 5 minutes. This is the time needed to kill the bacteria. Based on the results of the study, there is no change in the mechanical properties of treated and untreated tendons. Gardner et al. did not compare this method with other sterilization methods. This process does not kill viruses [51; Table 9.].

Table 9

Average result of failure load and Young's modulus of elasticity of sterilized tendons with Hydrogen peroxide [51]

Type of tendon	Pieces	Ultimate tensile strength [MPa]	Young's modulus of elasticity (MPa)	Authors
Tibialis posterior	5	2366 (447)	-	Gardner et al.
Tibialis anterior	2	2308 (806)	-	
Extensor digitorum longus	6	1574 (116)	-	
Extensor hallucis longus	5	588 (16)	-	
Flexor digitorum longus	1	1087	-	
Flexor hallucis longus	3	1210 (155)	-	

3.1.9. Chlorhexidine

In this chemical process, the experimental group is immersed in a 4% chlorhexidine gluconate solution for 30 minutes, while the control group was kept moist in saline gauze. The results of patellar tendons were not compared with other sterilization procedures [52]. The experimental group had better mechanical properties than the control group [52; Table 10].

Table 10
Average result of failure load and Young's modulus of elasticity of sterilized tendons with Chlorhexidine [52]

Type of tendon	Pieces	Failure load (N)	Young modulus of elasticity (MPa)	Authors
Patellar tendon	8	2219 (808.1)	-	Sobel et al.

4. Discussion

The two factors that can affect the mechanical properties of a tendon are the type of tendon and the sterilization methods. Tendon grafts show to be promising for transplants. It is important to sterilize the tendons to ensure no bacteria is transmitted that could cause infections. We reviewed peroneus longus, peroneus brevis, tibialis anterior, tibialis posterior, patellar tendon, human flexor digitorum superficial, Achilles, bone-patellar tendon-bone, semitendinosus, semitendinosus + gracilis, fascia latta types of tendon and gamma irradiation, SCCO₂, ethylene oxide, BioCleanse, E-Beam, PE-R, hydrogen peroxide, chlorhexidine sterilization methods.

Gamma radiation is the most common method of sterilization (Table 3). The 14.8–28.5 kGy (1.48–2.85 Mrad) radiation proved best because this radiation rate killed bacteria. It also kept the failure load (e.g., failure load of Achilles sterilized at 18–24 kGy is 3572.54 N vs. failure load of Achilles sterilized at 38–46 kGy is 392.01 N, tibialis anterior sterilized at 18–24 kGy: 3107.76N, failure load of tibialis anterior sterilized at 38–46 kGy: 2678.96N. [39–40]. Comparing with Sect. 3.1.1 (non-sterilized tendon), the results showed that failure load improved (Achilles: 3032 N, tibialis anterior: 2427.3 N). It can be observed that the failure load's tendency of quadriceps was different (18–24 kGy: 3184.32 N, 38–46 kGy: 3464.35 N). At 38–46 kGy (3.8–4.6 Mrad), the radiation killed viruses as well, but the biomechanical properties deteriorate [Tables 2 and 3.]. Viruses can be tested for in labs prior to a transplant [28, 31–41].

The supercritical carbon dioxide (SCCO₂) sterilization method has been discussed only by Baldini et al. [27]. Baldini et al. discussed anterior or posterior tibial tendon treated with SCCO₂. Based on the results, there was a negligible improvement in the failure load and Young's modulus of elasticity of the tendons (non-sterilized: 2427.3 N, 75.4 MPa vs. SCCO₂: 2450.3 N, 91.9 MPa) [27; Table 2. and 3.]. The origin of samples and measurement methods are different; the result could not be compared to the results of different sterilization methods. Therefore, we cannot accurately conclude.

The Ethylene oxide procedure does not yield better mechanical properties than other methods [42; Table 5.]. Tendons sterilized with this method generate connective tissue growth. During this tissue growth, cancerous cells can develop. Bechtold et al. [46] examined the patellar tendon and its Young's modulus of elasticity. After sterilization, Young's modulus of the tendon deteriorated slightly (non-sterilized: 158.0 MPa, sterilized: 143.0 MPa). This sterilization method is also not comparable to other sterilization methods, but the method impaired Young's modulus of the patellar tendon [42].

The BioCleanse wash poses the same issues as the hydrogen peroxide and chlorhexidine method [43–45, 54; Table 6.]. Since this is a wash, only the outside of the tendon is sterilized. Dangerous bacteria might still be found in the tendon. The BioCleanse solution is not as effective as gamma irradiation. The sterilization method is addressed in three studies [Table 6.]. Conrad et al. [8] examined the failure load and Young's modulus of the Achilles. Non-sterilized BioCleanse and gamma irradiation sterilization methods are investigated. In the case of failure load, BioCleanse shows worse results than non-sterilized, but even worse are the results of the gamma irradiation sterilization method (non-sterilized: 3032 N, BioCleanse: 2472 N, gamma irradiation: 1972 N). The values of the modulus of elasticity showed a similar tendency (292.04-154.06-129.48 MPa) [8]. Colaco et al. [43] and Schimizzi et al. [44], the tibialis anterior was examined. Colaco et al. [43] compared different BioCleanse mixtures. However, no comparison was made with other sterilization methods. Schimizzi et al. [44] compared the method used BioCleanse to non-sterilized and gamma irradiation methods. Based on the results, BioCleanse did not achieve significantly worse results (failure load and Young's modulus) compared to the other two groups (non-sterilized: 1665 N and 19.9 MPa, gamma-irradiated: 1671.9 N and 22.6 MPa, BioCleanse: 1651.6 N and 19.7 MPa) [43–45].

The effect of sterilization by Electron Beam (E-beam) was analyzed in three articles [30, 46–47, Table 7]. Elenes et al.[46] compared failure load of the tibialis anterior with non-sterilized, gamma irradiation, and E-beam sterilization methods. It uses two types of E-beam doses, one low (9.2–12.2 kGy) and one high (17.1–21.0 kGy). Based on the maximum load results [47], the low-dose E-beam performed significantly better compared to the other three categories (non-sterilized: 606.73 N, E-beam low: 876.38 N, E-beam high: 660.24 N, gamma: 597.09 N). In comparison to the values of modulus of elasticity, it can be observed that the values decrease for all three sterilization methods compared to non-sterilized ones. Of the three sterilization methods, the smallest deterioration is shown by E-beam low (non-sterilized: 213.13 MPa, E-beam low: 206.71 MPa, E-beam high: 152.64 MPa, gamma: 179.02 MPa) [46]. Wei et al. [30] compared three types of E-beam doses to the non-sterilized group. Based on the results, all three doses of E-beam performed worse than the non-sterilized human flexor digitorum superficialis (fresh: 402.5 N and 400.5 MPa, 50 kGy: 282.3 N and 291.6 MPa, 50 kGy (Fr.): 360.9 N and 354.3 MPa, 50 kGy (Fr. + As.): 390.6 N and 390.4 MPa) [30]. Hoburg et al. [48] examined the E-beam in the most recent of his studies. Bone-patellar tendon-bone treated with two doses of E-beam radiation (25 and 34 kGy) was compared with two different doses of gamma radiation (25 and 34 kGy) and non-sterilized. Based on the results, all four sterilizations significantly worsened the maximum force values; however, the values of E-beam are better than the values of the same dose of gamma radiation (non-sterilized: 1741 N, gamma 25 kGy: 1009 N, E-beam 25 kGy: 1177 N, gamma 34 kGy: 1073 N, E-beam 34 kGy: 1139 N) [48]. Zhou et al. [50] examined human flexor tendon sterilized with Peracetic acid - ethanol in combination with low-dose gamma irradiation (PE-R). The results showed a worse failure load compared to the non-sterilized tendon (non-sterilized: 360.01 N and 221.55 MPa, PE-R: 306.96 N, and 235.78 MPa). PE-R is a hybrid method consisting of immersing the tendon in a chemical solution and then using gamma irradiation. This method yielded a worse failure load than the Electron beam sterilization and gamma sterilization. [50].

The hydrogen peroxide method is just a wash; the inside of the tendon is not properly sterilized, which can lead to infections. Gardner et al. [51] examined the effect of hydrogen peroxide on several different types of tendons. The ultimate tensile strength was not compared to other sterilization methods or non-sterilized tendons [Table 9.]. Comparing the results of the ultimate tensile strength of previous studies with the results, hydrogen peroxide is not a suitable method for sterilizing tendons [51].

The Chlorhexidine chemical solution is also a wash, the same as the hydrogen peroxide method [52]. While it does pose better results of failure load for the tendon, the inside of the tendon is not properly sterilized. According to the method of Sobel et al. [52] showed that the value of the failure load increases as a result of sterilization (non-sterilized: 1876 N, Chlorhexidine: 2219 N) [Table 10.].

4.1. Limitation

This review analyzed and classified multiple existing sterilization methods used in determining the biomechanical properties of tendons. Since this review looked at different techniques, a meta-analysis could not be performed. Limitations can also surface because only three major scientific databases were utilized in the search for papers. Because of the full range of this research, it only provides an overview of multiple sterilization processes. It does not discuss the advancement of any single process.

5. Conclusion

The purpose of this literature review was to systematically evaluate existing literature to compare the biomechanical effects of different sterilization methods on commonly used tendon allografts. The biomechanical properties of tendons that were determined included failure load and Young's modulus of elasticity.

Based on a systematic literature review, it can be said that the most common method of sterilization is gamma radiation. However, after comparing the different literature, it is established that mechanical properties are improved, compared to non-sterilized tendons, with the E-beam sterilization method. The method is not widespread, due to the lack of laboratories and instruments suitable for conducting this treatment. The procedure using Chlorhexidine has yielded similar results to the E-beam. Only a few studies are available on the subject, so further research is needed.

Based on the above-mentioned data, we recommend freezing and gamma irradiation or electron beam at 14.8–28.5 kGy [Table 2–3, 7]. This method is effective at keeping or improving the biomechanical properties, while fully sterilizing the inside and the outside of the tendon. [8, 28, 30–41, 45–46, 48, 53].

6. List Of Abbreviations

ACL

Anterior cruciate ligament

SCCO2

supercritical carbon dioxide

E-beam

Electron Beam

PE-R

Peracetic acid-ethanol in combination with low-dose gamma irradiation

7. Declarations

Ethics approval and consent to participate – Not applicable

Consent for publication – Not applicable

Availability of data and materials – The data that support the findings of this study are available from authors of not open access journals but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from Denes Farago upon reasonable request and with permission of authors of not open access journals. All data generated or analysed during this study are included in this published review.

Competing interests - The authors declare that they have no competing interests

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