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Cryopreservation Maintains Structural Integrity and Biomechanical Properties of Human Rectus Fascia for up to 1 mo

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Background. Abdominal wall closure after transplantation is crucial to reduce morbidity and to ensure graft and patient survival, but it often remains challenging. Donor nonvascularized rectus fascia (NVRF) allotransplantation offers a valuable solution when primary closure fails. Despite the growing clinical use of NVRF, standardized protocols for NVRF preservation are lacking. This study evaluates the impact of short-term cold storage at 4 °C (8 d) and long-term cryopreservation (1 mo and 6 mo) on human NVRF at the mechanical, histological, and microbiological levels. **Methods.** NVRF was obtained from 10 human donors (6 men; 6 donation after circulatory death III; 4 donation after brain death; age 56 y [26–68 y]; BMI 28 kg/m² [23–31 kg/m²]). Fresh tissue was compared with 3 preservation methods: (1) cold storage in Institute Georges Lopez-1 solution at 4 °C for 8 d; (2) 1 mo cryopreservation; and (3) 6 mo cryopreservation at –80 °C. For each condition, the analysis included uniaxial tensile testing, histological evaluation based on hematoxylin and eosin and picrosirius red staining, and microbiological analysis for yeast and bacteria. **Results.** After 8 d at 4 °C, ultimate strength and rigidity were reduced, whereas histological structure appeared intact. One month of cryopreservation showed no indications for altered mechanical or histological characteristics compared with fresh tissue. After prolonged cryopreservation for 6 mo, extensibility was increased. Yeast contamination was absent in all samples, and commensal bacteria were detected in 17 of 40 fresh and preserved samples, without impact of the preservation method. **Conclusions.** In conclusion, cryopreservation at –80 °C maintains biomechanical characteristics and structural integrity of NVRF for up to 1 mo, offering a promising method for prolonged NVRF preservation in clinical applications.

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INTRODUCTION

Abdominal wall closure after liver, intestinal, or multi-visceral transplantation (Tx) is crucial to minimize post-operative complications including surgical site infections and fistula development, ultimately improving graft and patient survival.^{1,2} Failure to close the abdomen after solid organ Tx increases mortality up to 10%.^{2–4}

Abdominal wall closure often remains a surgical challenge. Primary closure fails in 15%–60% of intestinal or multivisceral Tx, and 33% of patients with successful primary closure ultimately develop secondary dehiscence.^{1,5} Abdominal wall closure post-Tx can be hampered by a donor/recipient size mismatch or a hostile recipient abdomen, with contracted abdominal wall and loss of abdominal domain due to previous abdominal surgery.^{2,5}

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Various synthetic, biological, and biosynthetic meshes have been used to restore the abdominal wall. Synthetic meshes are widely used but are associated with a high risk of infections, adhesions, and fistula formation, and their stiff mechanical characteristics can lead to retraction of remaining abdominal muscles.^{6–8} Biological and biosynthetic meshes were developed to reduce contamination risks. However, outcomes are variable, and hernia recurrence remains high when these meshes are applied in complex and contaminated defects.^{1,6,9–11} Alternatively, abdominal wall vascularized composite allograft Tx can be performed. In this procedure, the abdominal wall is transplanted as a full-thickness, vascularized, myocutaneous free flap containing the rectus abdominis muscles with fascia, subcutaneous tissues, and skin and is anastomosed with the native vasculature. However, this technique is surgically challenging and carries risks of acute rejection and graft-versus-host disease.¹² Donor nonvascularized rectus fascia (NVRF) alloTx provides a promising alternative when primary abdominal wall closure cannot be attained or when herniation occurs.^{1,13} In this technique, the anterior and

posterior rectus fascia with the peritoneum are transplanted as an inlay graft, after removal of the rectus muscles.^{13,14} A recent survey conducted by our group summarized the global experience with NVRF alloTx in 98 patients from 8 transplant centers.¹⁵ Results showed limited clinical or radiographic herniation without signs of rejection and neovascularization in the graft.^{4,16}

Despite the growing clinical use of NVRF Tx, standardized protocols for NVRF preservation are lacking. Our survey revealed substantial variability in preservation practices, with NVRF stored in different solutions at 4 °C for up to 128 h, and 1 center reporting cryopreservation at –60 °C.¹⁵ Comprehensive data on the effects of different preservation methods on the integrity of NVRF are lacking.

This study evaluates the impact of short-term cold storage at 4 °C for 8 d and long-term cryopreservation for 1 and 6 mo on human NVRF on mechanical, histological, and microbiological levels.

MATERIALS AND METHODS

Ethical Approval

No written informed consent was obtained, which was waived by the local Institutional Review Board. Tissue was obtained from donors in Belgium who did not oppose the use of tissue for scientific research in the Belgian National Register (Orgadon). This study was approved by the Ethics Committee Research (S66655) and the Biobank (BB-GEN002-FO03) of University Hospitals Leuven.

Procurement of Rectus Abdominis Muscle

The rectus abdominis muscle was procured from 10 human donors after multiorgan donation (Table 1). Inclusion criteria were: no previous abdominal surgery with incision in the rectus fascia or linea alba, no primary hernia at the rectus muscles, no active smoking, and no diabetes mellitus.

To procure the rectus muscle, a midline incision was made through the skin, subcutaneous tissue, and linea alba. Lateral dissection was performed to remove the skin and subcutaneous tissue up to the lateral muscles. The rectus muscle, containing anterior and posterior fascia and peritoneum, was procured in 2 halves and transported in sterile Institute Georges Lopez-1 (IGL-1) solution on ice.¹⁴ The cranial orientation and umbilical level were indicated for further orientation.

Dissection and Preservation of Rectus Fascia

Within 26 h after procurement (median, 11 h; range, 3–26 h), the fascia was aseptically dissected.¹⁴ As a baseline control, fresh rectus fascia was stored in IGL-1 at 4 °C and analyzed within 39 h after procurement (median, 22 h; range, 18–39 h). Rectus fascia was stored according to 3 preservation methods: (1) 8 d cold stored at 4 °C in IGL-1 (median, 8 d; range, 7–10 d), (2) 1 mo cryopreservation at –80 °C (median, 31 d; range, 30–34 d), and (3) 6 mo cryopreservation at –80 °C (median, 182 d; range, 179–184 d; Table 2, Figure 1).

For tensile testing, 4 dogbone-shaped samples of anterior fascia were punched along the craniocaudal axis for each preservation method (4×4 samples per donor). To minimize the effect of anatomical variation, the 4 samples were

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TABLE 1.
Characteristics of the included donors

Donor	Donor type	Sex	Age, y	Body mass index, kg/m ²
1	DCD III	Male	66	28
2	DCD III	Male	41	24
3	DCD III	Male	43	31
4	DCD III	Male	58	27
5	DCD III	Female	68	27
6	DBD	Female	47	23
7	DBD	Female	67	25
8	DBD	Female	53	28
9	DBD	Male	61	31
10	DCD III	Male	26	28
Median			56	28

DBD, donation after brain death; DCD III, donation after circulatory death type III.

TABLE 2.
Overview of preservation duration of the rectus fascia tissue

Donor	Procurement - start preservation, h	Fresh control, h	Preservation method 1, d	Preservation method 2, d	Preservation method 3, d
1	5	18	10	30	183
2	26	30	8	31	181
3	16	19	8	34	181
4	4	26	6	32	179
5	3	20	8	34	181
6	4	21	7	33	180
7	19	23	7	30	182
8	19	39	7	30	183
9	7	26	9	31	184
10	15	19	7	31	183
Median	11	22	8	31	182

Time between the procurement of the abdominal wall and analysis of the fresh rectus fascia (fresh control), stored in IGL-1 preservation solution at 4 °C. Time between the start of preservation at 4 °C in IGL-1 preservation solution and analysis (preservation method 1). Time between the start of preservation at –80 °C and analysis of the rectus fascia (preservation methods 2 and 3).
IGL-1, Institute Georges Lopez-1.

obtained from 4 distinct regions above the umbilicus (regions A–D), with samples for different preservation methods taken adjacent to each other (Figure 1). For each preservation method, 1 sample (1.5 × 2.5 cm²) containing the anterior and posterior fascia and peritoneum was collected for histological analysis, and 2 small samples (0.5 × 0.5 cm²) were collected for microbiological analysis. Each sample was preserved in separate sterile containers. Cryopreserved samples were frozen at –80 °C and thawed at 4 °C for 18 h in the preservation containers.

Integrity Evaluation at Different Levels

Mechanical Evaluation

To avoid temperature-related influences, samples were tested after equilibration in phosphate-buffered saline (room temperature, 2 h). Sample width and length were assumed to match the puncher dimensions (Figure S1, SDC, <https://links.lww.com/TXD/A885>). Thickness was determined from a side-view image of the sample, covered with a microscopy cover glass next to a reference object, using a Canon EF-S 60 mm f/2.8 Macro USM Reflex camera and GIMP software.

Uniaxial tensile testing was performed in the longitudinal axis of the dogbone punches, corresponding to the

cranio-caudal body axis, using a custom-made device (Supplemental Methods, SDC, <https://links.lww.com/TXD/A889>). Samples were stretched monotonically at a pace of 3 mm/min until mechanical failure. Different mechanical parameters were calculated based on the obtained stress-strain curves. In brief, ultimate strength was defined as the maximal stress observed during a test, and extensibility as the corresponding extension. Rigidity was determined by the equivalent Young’s modulus and the maximal tangent modulus, calculated as the slope of the stress-strain curve at low and high extension, respectively.

Histological Evaluation

Fixed samples (4% formaldehyde, 4 °C, 18 h) were paraffin-embedded, sectioned (5 μm), and stained with hematoxylin and eosin (H&E) and picrosirius red (PSR).

Cellular Features Based on H&E Staining Using QuPath

H&E-stained tissue sections were scanned (NanoZoomer-SQ, Hamamatsu) and analyzed using QuPath (version 5.0.1).¹⁷ Fascia tissue was manually annotated, and cellular features (number of nuclei per annotated region, and nuclear size and circularity) were calculated with the built-in watershed cell detection feature.

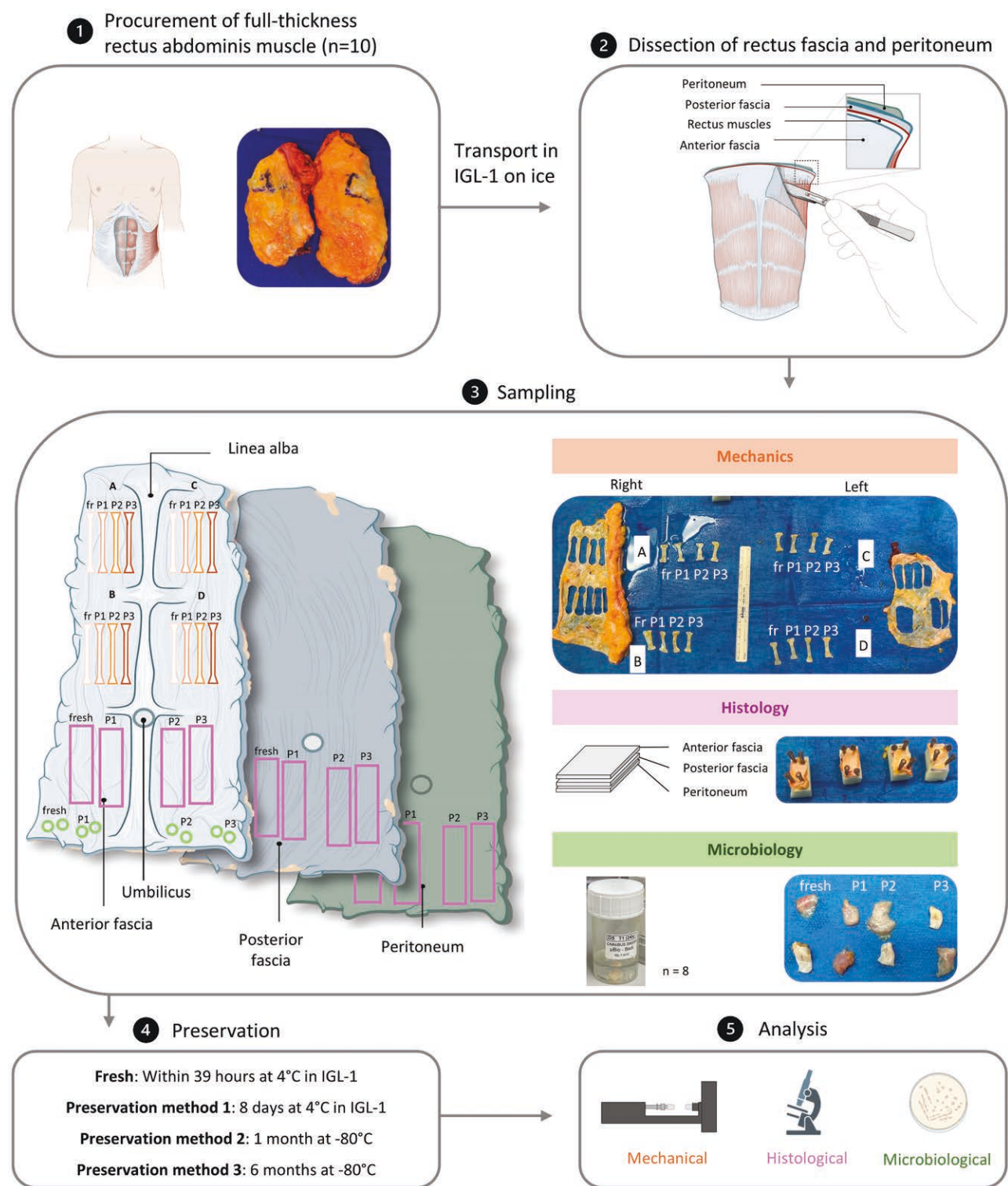


FIGURE 1. Experimental setup of the study. 1—Procurement of the rectus abdominis muscle from 10 human organ donors. 2—Dissection of the rectus fascia and peritoneum from the muscle. 3—Sample collection for fresh (fr) and preserved (P1–3) samples. 4—Analysis of fresh samples and preservation according to 3 methods: 8 d in IGL-1 preservation solution at 4 °C (P1), 1 mo at –80 °C (P2), and 6 mo at –80 °C (P3). Performed for every donor. 5—Mechanical, histological, and microbiological analysis. IGL-1, Institute Georges Lopez-1.

Fiber Alignment Using Histogram of Oriented Gradients

Fiber alignment was assessed on H&E-stained tissue sections where individual longitudinal fibers were clearly visible. For every donor, at least 2 longitudinal views were

exported from NDPI files at 20X magnification (NDP.view2, Hamamatsu). Images were processed using an in-house developed computer vision approach based on the Histogram of Oriented Gradients method.¹⁸ This tool computes an angular distribution of fiber directions across the image, from which

the most common fiber direction and dispersion relative to it were quantified.

Collagen Histoarchitecture Based on PSR Staining

Tissue sections were stained with PSR to evaluate collagen fiber organization. The PSR dye molecules bind along the longitudinal axis of the collagen fibers, enhancing their natural birefringence under polarized light. With this staining, thin, less packed fibers ($\leq 0.8 \mu\text{m}$) exhibit weak birefringence and appear green under polarized light, whereas thick ($1.6\text{--}2.4 \mu\text{m}$), packed fibers appear red, and intermediate fibers appear orange under polarized light. As such, the color of PSR staining under polarized light can be correlated to the thickness and organization of the collagen fibers.¹⁹

PSR-stained tissue was visualized using an Axioscan7 slide scanner (Zeiss) equipped with an Orca Flash 4.0 V3 camera (Hamamatsu) and a 10X objective, and postprocessed with ZEN blue (3.6, Carl Zeiss Microscopy GmbH). Images were analyzed in FIJI²⁰ to determine the areas of red, orange, and green pixels based on Hue Saturation Brightness thresholding.

Microbiological Evaluation

For every preservation method, 2 separate tissue samples were analyzed for bacterial and yeast contamination, respectively. In case of bacterial growth in Wilkens-Chalgren Broth (7 d, 36 °C), the bacterial isolate was identified by Gram staining and MALDI-TOF Mass Spectrometry. Yeast contamination was detected by incubation in Sabouraud agar (21 d, 36 °C). Samples showing no bacterial or yeast growth were considered negative.

Statistical Analysis

Every data point represents 1 donor. Absolute values are visualized with overall mean $\pm$ SD. Preserved samples were normalized to fresh samples of the same donor. Logarithmic relative values were averaged, and the exponent of the mean was calculated to obtain the normalized value for each preservation method. Relative results are reported as overall mean $\pm$ 95% confidence interval (95% CI). For mechanical analysis, tissue from 4 locations per donor was analyzed. Normalization was performed to the corresponding fresh sample from the same donor and location, and the donor mean was used for analysis. For histology, 1 section was analyzed, and fiber alignment was determined by ≥ 2 images per donor and condition.

Similarity between preserved and fresh tissue was assessed using 95% CIs for relative values. If the 95% CI included 1, there was no indication of changes compared with fresh tissue, and no significant difference was assumed. If the 95% CI did not include 1, a 1-sample *t* test or nonparametric Wilcoxon signed-rank test was performed to assess the difference from 1. Graphical illustrations and statistical analysis were performed using GraphPad Prism version 8.0.1.

RESULTS

Donor Demographics

In 4 cases, NVRF tissue was procured from donation after brain death (DBD), and in 6 cases from donation after circulatory death III (DCD III) donors. Four donors were women,

6 were men, with a median age of 56 y (range, 26–68 y) and BMI of 28 kg/m² (range, 23–31 kg/m²; Table 1).

Variable Baseline Mechanical Characteristics of Fresh Rectus Fascia Tissue

Uniaxial tensile testing revealed biological variation in baseline mechanical characteristics of fresh anterior rectus fascia of different donors, with extensibility ranging between 0.21 and 0.56 mm/mm, ultimate strength between 296 and 851 kPa, Young's modulus between 244 and 660 kPa, and maximal tangent modulus between 1947 and 5406 kPa (Figure S2, SDC, <https://links.lww.com/TXD/A886>). Moreover, mechanical characteristics varied across different locations within the anterior fascia, indicating the importance of normalization by location (Figure S3A, SDC, <https://links.lww.com/TXD/A887>). Baseline mechanical characteristics of fresh NVRF were not significantly different between male versus female donors, nor between DCD III and DBD donors (Figure S3B and C, SDC, <https://links.lww.com/TXD/A887>).

Tissue Thickening After 8 d at 4 °C, With no Impact of Cryopreservation

After 8 d at 4 °C, the thickness of the anterior fascia was increased by 15% (95% CI, 1.04–1.28; *P* = 0.0137), observed in 8 donors. No significant changes in thickness were observed after 1 mo of cryopreservation. After 6 mo of cryopreservation, a tendency of decreasing thickness was observed, although not significant (95% CI, 0.77–1.06; Figure 2).

Reduced Ultimate Strength, Young's Modulus, and Maximal Tangent Modulus After 8 d at 4 °C

After 8 d at 4 °C, ultimate strength was reduced by 30% compared with fresh tissue (95% CI, 0.55–0.89; *P* = 0.016), observed in 9 donors (Figure 3A). Moreover, Young's modulus was decreased by 34% (95% CI, 0.51–0.84; *P* = 0.0014) and the maximal tangent modulus was reduced by 34% compared with fresh tissue (95% CI, 0.54–0.81; *P* = 0.002; Figure 3A). These changes were observed across all donors. There were no indications of altered extensibility after 8 d at 4 °C compared with fresh tissue (Figure 3A).

No Mechanical Changes After 1 mo but Increased Extensibility After 6 mo Cryopreservation

After 1 mo of cryopreservation, there were no indications of altered mechanical characteristics compared with fresh tissue, including extensibility (95% CI, 0.97–1.26), ultimate strength (95% CI, 0.70–1.17), Young's modulus (95% CI, 0.63–1.24), and maximal tangent modulus (95% CI, 0.6–1.13; Figure 3A).

After 6 mo of cryopreservation, extensibility was increased by 15% compared with fresh tissue (95% CI, 0.98–1.34; *P* = 0.042), observed in 8 donors. No changes were detected in ultimate strength, Young's modulus, or maximal tangent modulus after 6 mo preservation at -80 °C compared with fresh tissue (Figure 3A).

Increased Duration of Cryopreservation Impacts Mechanical Characteristics

To isolate the effect of prolonged cryopreservation beyond the initial freezing, mechanical parameters after 6 mo at -80

Thickness of tissue absolute and relative to fresh

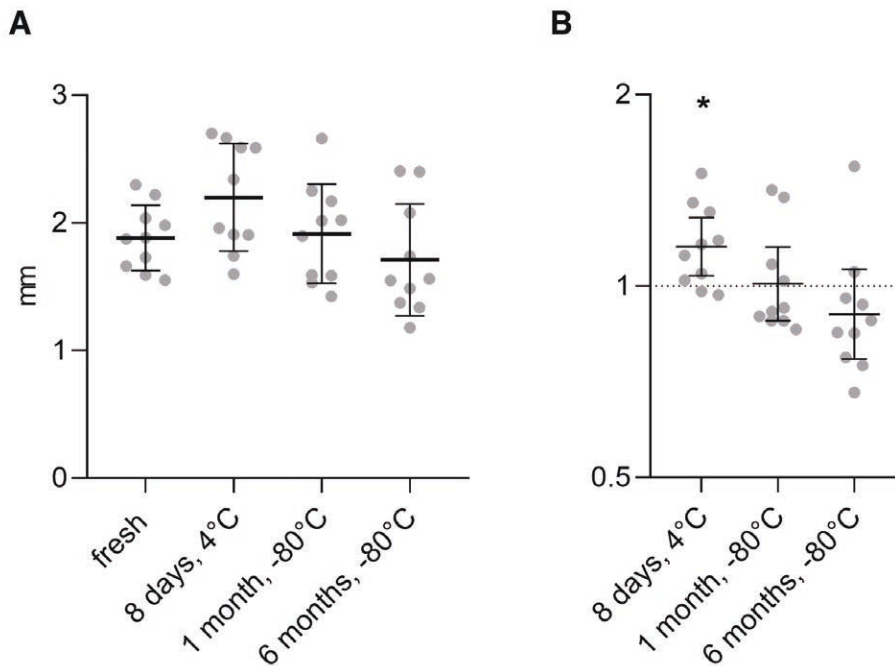


FIGURE 2. Impact of preservation on tissue thickness. A, Absolute thickness of fresh and preserved anterior rectus fascia samples. Individual data points for 10 different donors, obtained by the mean of 4 different locations. Data represented as mean $\pm$ SD. B, Tissue thickness after preservation normalized to fresh tissue represented as mean $\pm$ 95% CI. One-sample Wilcoxon test to test difference from 1, * $P < 0.05$. CI, confidence interval.

°C were normalized to those obtained after 1 mo at -80°C . After 6 mo of cryopreservation, ultimate strength increased by 34% in 8 donors (95% CI, 1.09-1.65; $P = 0.013$), and the maximal tangent modulus increased by 27% (95% CI, 1.00-1.61; $P = 0.037$) in 7 donors, compared with 1 mo at -80°C (Figure 3B).

Tendency of Cellular and Tissue Damage After Cryopreservation for 6 mo

General tissue microstructure and nuclear features were evaluated on H&E staining (Figure 4A). The extracellular matrix, stained with eosin, appeared intact after 8 d at 4°C and after 1 mo of cryopreservation. However, 6-mo cryopreserved tissue appeared overall disorganized, with multiple small holes visible throughout the tissue (Figure 4A).

In general, preservation did not impact nuclear density (Figure 4B). Nuclear size tended to decrease from $19.2 \pm 2.8 \mu\text{m}^2$ in fresh tissue to $17.8 \pm 1.2 \mu\text{m}^2$ after 8 d at 4°C , $18.3 \pm 1.8 \mu\text{m}^2$ after 1 mo of cryopreservation, and $18.0 \pm 1.1 \mu\text{m}^2$ after 6 mo of cryopreservation. When normalized to fresh tissue, the nucleus size after preservation showed a decreasing tendency (8 d at 4°C : 95% CI, 0.85-1.04; 1 mo -80°C : 95% CI, 0.86-1.07; and 6 mo -80°C : 95% CI, 0.84-1.08; Figure 4B). Finally, nuclear circularity was analyzed, with 1 representing a perfectly circular nucleus and values <1 indicating a less circular, more elongated nucleus. Compared with fresh tissue (0.69 ± 0.04), nuclear circularity did not change after 8 d at 4°C (0.70 ± 0.26) and 1 mo at -80°C (0.69 ± 0.03). However, after 6 mo of cryopreservation, nuclei

tended to have increased circularity (0.72 ± 0.02). When normalizing circularity after preservation to fresh tissue, a tendency for increased circularity after 6 mo of cryopreservation was observed (8 d at 4°C : 95% CI, 0.96-1.07; 1 mo -80°C : 95% CI, 0.95-1.05; and 6 mo -80°C : 95% CI, 0.98-1.10; Figure 4B).

Tendency of Reduced Fiber Alignment Upon 6 mo of Cryopreservation

The main fiber directionality and the angular deviation of the main directionality were determined from H&E-stained longitudinal tissue sections. In fresh rectus fascia, the deviation from the main direction ranged from 35° to 42° , showing a baseline variation due to donor differences and the wavy tissue structure (Figure 4C). No changes in fiber alignment were observed after 8 d at 4°C and 1 mo of cryopreservation compared with fresh tissue. However, after 6 mo of cryopreservation, deviation from the main direction tended to increase compared with fresh tissue, although not significantly (95% CI, 1.00-1.156; $P = 0.055$; Figure 4C).

Impacted Collagen Organization After Cryopreservation for 6 mo

PSR-stained tissue sections were analyzed with polarized light to assess collagen fiber thickness and organization (Figure 5A). In fresh tissue, 32% of the detected tissue appeared red, indicating thick and packed fibers; 29% was green, indicating thin and less packed fibers; and 39% consisted of intermediate fibers, appearing in orange (Figure 5B).

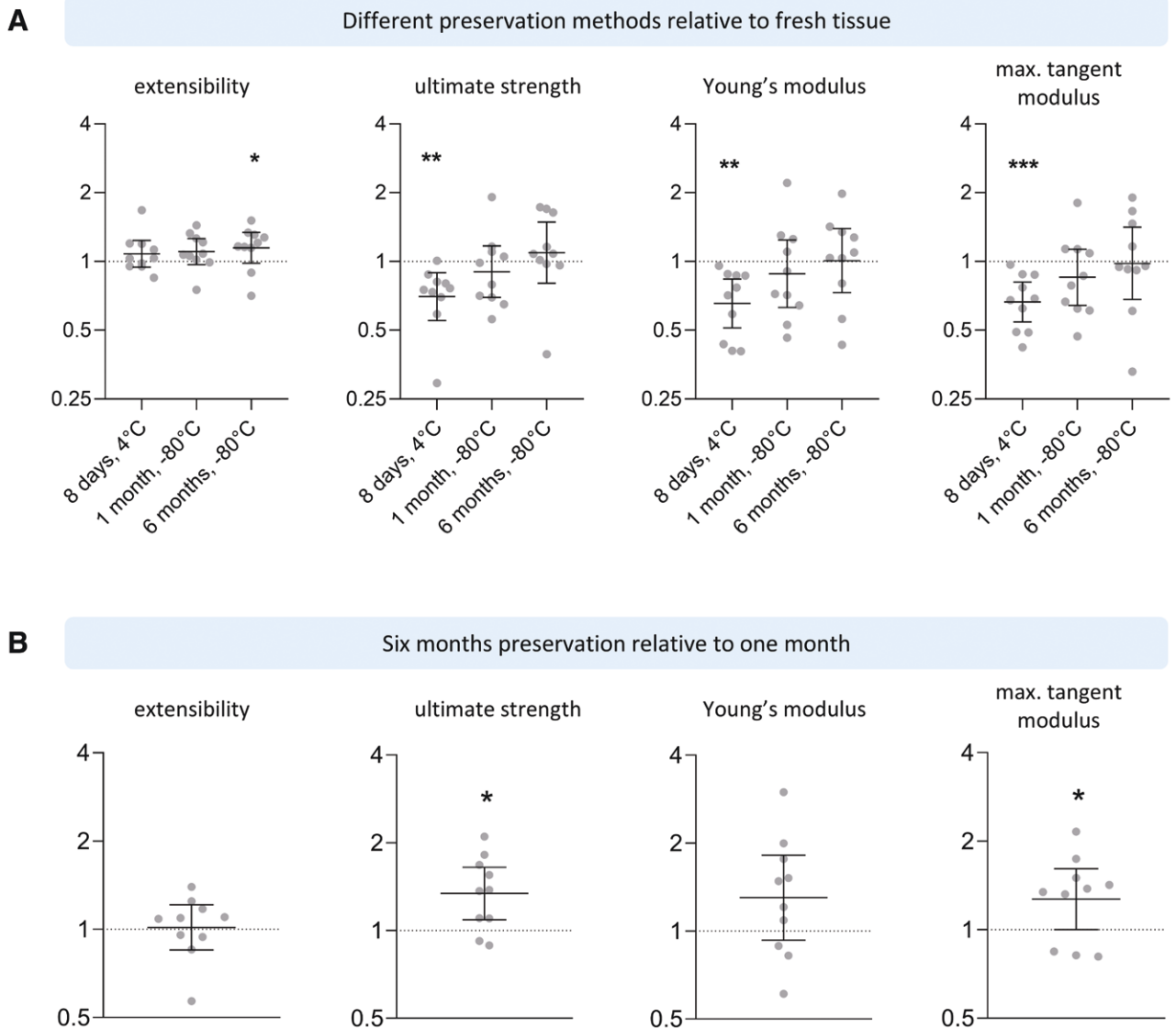


FIGURE 3. Impact of preservation on mechanical characteristics. Mechanical parameters were obtained from stress-strain curves of uniaxial tensile tests. A, Values after preservation normalized to fresh tissue. Data points represent the mean of 4 different locations per donor, shown with mean $\pm$ 95% CI. B, Values after 6 mo at -80°C normalized to 1 mo at -80°C . Data points represent the mean of 4 different locations per donor, shown with mean $\pm$ 95% CI. One-sample t tests were used to assess differences from 1, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. CI, confidence interval.

Preservation for 8 d at 4°C did not alter the relative distribution of red/orange/green fibers, and there were no indications of altered collagen organization compared with fresh fascia tissue (Figure 5B). In contrast, prolonged cryopreservation for 6 mo resulted in a tendency toward decreased proportions of thick red fibers (25%), whereas the proportion of thin green fibers tended to increase (36%) compared with fresh tissue (Figure 5B).

Absence of Yeast but Presence of Commensal Bacteria in Fresh and Preserved Tissue

All fresh and preserved samples were free of yeast contamination, but commensal bacteria were detected in 17 of 40 analyzed samples (Table 3), including *Micrococcus luteus*, *Staphylococcus epidermidis*, *Cutibacterium acnes*, *Streptococcus mitis*, *Staphylococcus hominis*, *Kocuria kristinae*, *Staphylococcus pasteurii*, *Streptococcus viridans*,

Staphylococcus capitis, and *Escherichia coli*. Bacteria were detected in 4 fresh samples. After 8 d at 4°C , 3 samples were contaminated; after 1 mo of cryopreservation, 6 samples were contaminated; and after 6 mo of cryopreservation, bacterial contamination was detected in 4 samples.

For 2 donors, all fresh and preserved samples were free of contamination. For 4 donors, fresh samples were not contaminated, but commensal bacteria were detected after preservation. For 1 donor, bacterial contamination was detected in fresh tissue, but not after preservation. Moreover, different bacterial strains were detected within a single donor, depending on the preservation method (Table 3).

DISCUSSION

NVRF alloTx provides an emerging tool to close the abdominal wall post-Tx. However, standardized guidelines on

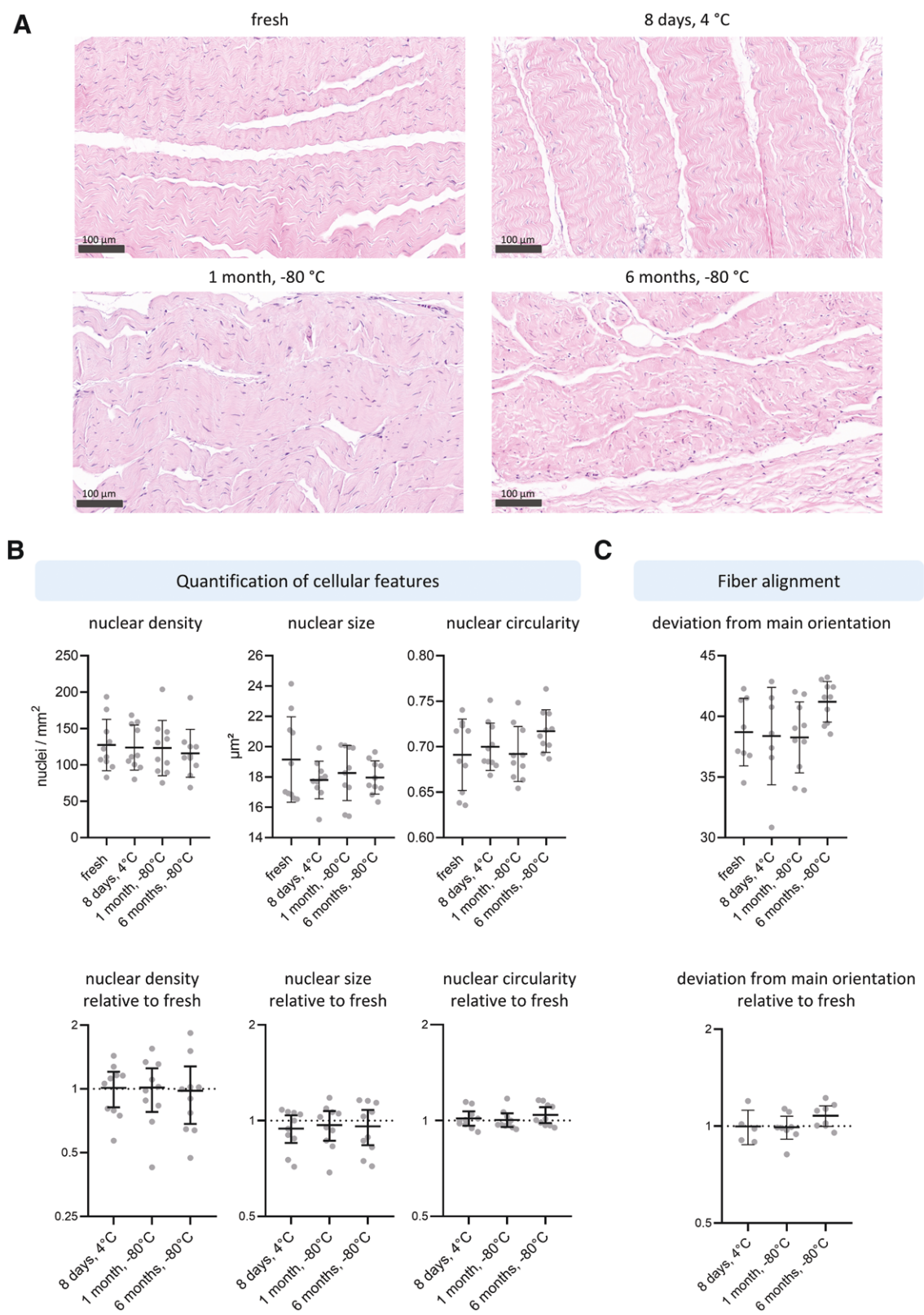


FIGURE 4. Impact of preservation on cellular features. A, Hematoxylin and eosin staining of human rectus fascia, showing nuclei in purple and cytoplasm in pink. B, Quantification of nuclear features (density, size, and circularity) showing absolute values per donor with mean ± SD and values normalized to fresh tissue (mean ± 95% CI). C, Deviation of fiber orientation from the main direction, shown as absolute values and normalized to fresh tissue. Individual data points represent the mean of at least 2 images of longitudinally oriented fascia per donor, and data are represented as mean ± 95% CI. CI, confidence interval.

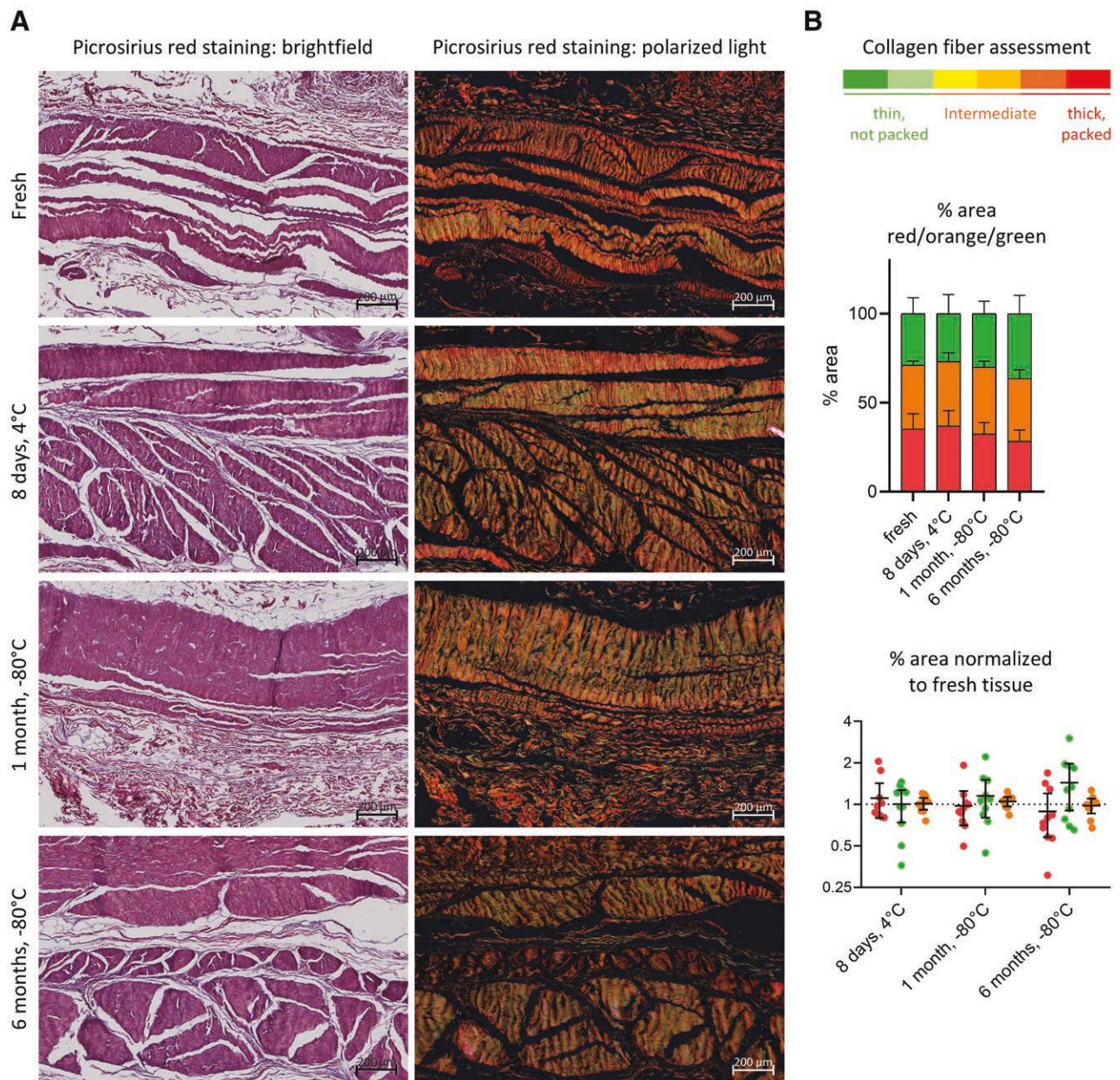


FIGURE 5. Impact of preservation on collagen histoarchitecture. A, Picrosirius red staining of fresh and preserved NVRF with brightfield and polarized light. Red: thick, packed fibers; green: thin, less packed fibers. B, Quantification of percentage red, orange, and green stained fibers on polarized light microscopy scans from 10 donors, showing absolute percentages (mean $\pm$ SD) and percentages normalized to fresh tissue (mean $\pm$ 95% CI). CI, confidence interval; NVRF, nonvascularized rectus fascia.

NVRF preservation are lacking. Therefore, this study investigates the impact of short-term cold storage at 4 °C for 8 d and long-term cryopreservation for 1 mo and 6 mo of NVRF on mechanical, histological, and microbiological levels.

NVRF was obtained from human donors with varying demographics after multiorgan donation. Consistent with the literature, no mechanical differences were observed in anterior rectus fascia from male versus female donors²¹ or between DBD versus DCD III donors. These findings support the use of NVRF from a broad donor pool, offering a reliable graft source for abdominal wall reconstruction.

The abdominal wall is a multilayered structure of muscle and connective tissue, functioning as a mechanically complex composite system.⁸ The mechanical behavior of the rectus

fascia is highly anisotropic and depends on direction and anatomical location.^{8,21} Therefore, consistent sampling for mechanical analysis is crucial. In this study, the anterior rectus fascia was used for mechanical characterization, which is an important layer in the structural response to mechanical loading.²² In accordance with the literature, we used the anterior fascia above the umbilicus.^{8,21,23,24} To minimize anatomical variability, samples were collected from 4 regions, and post-preservation mechanical parameters were normalized to fresh tissue from the same donor and location, allowing direct comparison of mechanical characteristics after different preservation methods.

Data on the preservation of fascial tissue is sparse and predominantly derived from a nonclinical setting. Kriener et al²⁴ reported indications for altered biomechanical properties

TABLE 3.
Assessment of bacterial contamination in fresh and preserved rectus fascia samples

	Fresh	8 d, 4 °C	1 mo, −80 °C	6 mo, −80 °C
Donor 1	<i>Micrococcus luteus</i>	—	<i>Kocuria kristinae</i>	—
Donor 2	—	<i>Staphylococcus hominis</i>	<i>Cutibacterium acnes</i>	—
Donor 3	—	<i>Escherichia coli</i>	<i>Micrococcus luteus</i>	<i>Escherichia coli</i>
Donor 4	<i>Staphylococcus epidermidis</i>	—	<i>Staphylococcus epidermidis</i> , <i>pasteuri</i>	<i>Micrococcus luteus</i>
Donor 5	—	—	—	—
Donor 6	<i>Cutibacterium acnes</i>	—	—	—
Donor 7	<i>Streptococcus mitis</i>	<i>Streptococcus mitis</i>	<i>Streptococcus viridans</i> , <i>Staphylococcus hominis</i>	<i>Streptococcus mitis</i>
Donor 8	—	—	<i>Staphylococcus capitis</i>	—
Donor 9	—	—	—	<i>Staphylococcus capitis</i>
Donor 10	—	—	—	—
Total	4/10	3/10	6/10	4/10

from rectus abdominis muscles from frozen (≥ 1 freeze-thaw cycle) compared with fresh (within 72 h postmortem) human cadavers, whereas our results showed no changes after 1 mo of cryopreservation.²⁴ The altered mechanical properties in this study might be attributed to the number of freeze-thaw cycles, preservation duration, or donor variability, which are controlled for with our sampling strategy.

Park et al²⁵ preserved decellularized rabbit hindlimb fascia for 1 mo under different conditions: at room temperature in glycerol/ethanol or manuka honey, frozen (-80 °C), lyophilized, and in liquid nitrogen (-196 °C). Consistent with our findings, stiffness and extensibility of decellularized fascia were not altered after freezing (-80 °C and -196 °C), but ultimate strength was reduced.²⁵ Caution should be taken when comparing these results, because decellularization may impact mechanical behavior, and cryopreservation effects may differ between decellularized and native fascia.

In line with clinical practice in our center, fascia was stored in IGL-1 preservation solution at 4 °C.^{16,26} After 8 d in IGL-1 solution at 4 °C, ultimate strength and rigidity were reduced compared with fresh tissue, whereas the histological appearance remained intact. This might relate to the increased tissue thickness, potentially driven by osmotic imbalances induced by the solution. The rectus fascia is rich in collagen and glycosaminoglycans, which are highly interactive with water.^{27,28} Minor differences in ion concentrations between the tissue and surrounding preservation solution can generate osmotic pressure, driving water influx into the extracellular matrix, and leading to tissue swelling.²⁹ Future studies might evaluate other preservation solutions for cold storage, such as the University of Wisconsin solution, histidine-tryptophan-ketoglutarate solution, or Celsior.^{4,30,31}

Cryopreservation is used to store different cells and tissues, including musculoskeletal tissue, ovarian tissue, and cardiovascular tissues.³²⁻³⁴ In our study, 1 mo of cryopreservation of NVRF at -80 °C did not alter mechanical characteristics. The histological architecture appeared intact, and the alignment of collagen fibers was maintained, supporting that this method is suitable for prolonged preservation of NVRF. Silan et al successfully used cryopreserved fascia lata from human deceased donor material for fascial reanimation.³⁵ These grafts were

cryopreserved at -160 °C in 10% dimethyl sulfoxide and can be stored at -80 °C for up to 90 d, demonstrating clinical feasibility.

In contrast, prolonged cryopreservation for 6 mo impacted mechanical characteristics and tended to disturb histological integrity compared with fresh tissue. When comparing 1 mo versus 6 mo of cryopreservation, ultimate strength and rigidity increased with longer preservation, which could be explained by the reduction in thick, aligned collagen fibers after 6 mo of preservation. In addition, nuclei tended to lose their native elongated phenotype and become more rounded after 6 mo of cryopreservation, and the extracellular matrix appeared damaged, with small holes. These changes could be caused by extracellular ice formation, which increases extracellular osmolarity, induces cellular dehydration, and disrupts the extracellular matrix.^{36,37} Optimizing strategies for long-term cryopreservation may include the use of cryoprotectants such as dimethyl sulfoxide to prevent ice crystal formation, applying controlled freezing and thawing rates and optimizing storage temperature.^{36,38,39}

In general, reduced ultimate strength may increase herniation risk. The reduced ultimate strength observed after 8 d at 4 °C remained >220 kPa, the accepted peak maximum tensile strength of the abdominal wall, suggesting limited clinical impact.^{40,41} Moreover, the extensibility of preserved fascia was 20%–40%, matching the elongation required for meshes for abdominal wall repair.⁴⁰ It is challenging to predict the impact of rigidity changes. Increased rigidity may cause discomfort, foreign body sensation, and might hamper regeneration due to stress shielding.⁴² In contrast, reduced rigidity can cause bulging of the abdominal content or reherniation.⁸ In vivo studies are required to assess the clinical impact of the observed mechanical and histological changes after preservation.

In addition to mechanical and histological analyses, microbiological analyses were performed to assess the impact of preservation on contamination. Importantly, yeast was absent in all fresh and preserved tissues. However, different commensal bacteria were detected in fresh and preserved tissues from different donors, without a clear impact of the preservation method. Similar bacterial contamination is reported in musculoskeletal tissue banking,

where 35%–55% of the grafts were contaminated. After 2 decontamination cycles (ceftazidime, lincomycin, polymyxin B, and vancomycin), the degree of contamination decreased to 0.4%.³⁴ Variation in bacterial strains within the same donors suggests that contamination was highly sample-dependent and likely introduced during tissue handling, caused by the extensive sampling in this study, which increased the likelihood of contamination compared with clinical practice. *Escherichia coli* was detected in 1 DCD III donor, likely resulting from postmortem translocation of intestinal bacteria on bowel movement arrest.^{43,44} No similar translocation-derived contaminations were observed in other DCD III donors. Although the clinical implications of commensal bacterial contamination might be limited,⁴⁵ caution is warranted in an immunosuppressed, vulnerable patient population. In clinical NVRF alloTx, additional measures are taken to limit bacterial contamination. NVRF is stored in preservation solution with antibiotics such as gentamicin, vancomycin, and meropenem and is rinsed with povidone-iodine before Tx.^{14,16,35} Moreover, patients receive prophylactic antibiotics upon implantation and postoperatively based on the standard procedures in the hospital. Taken together, preservation did not impact commensal bacterial contamination, but the addition of antibiotics to preservation solutions and upon implantation is crucial for clinical safety.

Our findings demonstrate that NVRF maintains its histological and mechanical integrity after cryopreservation, supporting the feasibility of storing these grafts for at least 1 mo until Tx. This has important clinical implications. Cryopreservation enables the creation of a readily available tissue bank, allowing NVRF grafts to be procured from multiple donors and accessed when needed. As a result, dependence on single-donor availability is reduced, the number of NVRF Tx can increase, and NVRF becomes available in urgent, unplanned surgical settings. Moreover, the practical feasibility of preservation methods in clinical practice should be considered. Cold storage at 4 °C is easy to perform without specialized equipment, and stored tissues are immediately available. However, the cold storage time remains limited, with indications for mechanical alterations after 8 d of storage. Cryopreservation allows longer preservation duration, with no indication of alterations after 1 mo of cryopreservation. These results were obtained using straightforward freezing at –80 °C and overnight thawing at 4 °C. However, the addition of cryoprotectants or controlled freezing rates might be required for long-term cryopreservation beyond 1 mo, increasing the complexity of the cryopreservation procedure. Moreover, faster thawing at 37 °C would allow more flexible timing for surgery.

Limitations of this study include the selection of the anterior rectus fascia for mechanical characterization, given the technical challenges to analyze the complex multilayered system of NVRF. Multilevel mechanical characterization could include multidirectional tensile testing, compression, shear, and indentation tests.⁸ Further research might include a comparison of different preservation solutions for cold storage and the optimization of cryopreservation methods for prolonged storage beyond 1 mo in collaboration with tissue banks. Moreover, the potential of (cryo)preserved NVRF should be evaluated in vivo, assessing integration, remodeling, vascularization, and immunological response.

CONCLUSIONS

This study provides a comprehensive evaluation of the impact of short-term cold storage at 4 °C (8 d) and long-term cryopreservation (1 mo and 6 mo) methods on human NVRF at the mechanical, histological, and microbiological levels in a clinically relevant setting. Preservation for 8 d in IGL-1 solution at 4 °C reduced rigidity and ultimate strength, whereas the histological structure remained intact. Cryopreservation at –80 °C for 1 mo preserved both mechanical and histological properties, whereas extended cryopreservation for 6 mo resulted in increased extensibility compared with fresh tissue. Microbiological analysis showed no preservation-related increase in contamination but highlighted the need for antibiotics in preservation solutions. This study indicates that cryopreservation is a promising method for prolonged NVRF preservation in clinical applications. Further preclinical and clinical research is needed to confirm functional performance and regenerative capacity of (cryo)preserved NVRF grafts in abdominal wall reconstruction.

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