Topic 2: Acellular Allograft Tissue Engineering

Zoe Goudas, Kshema Karanam, Sharee Cardenas, Alandra DiCicco, Kyrollos Abdelmeseh

Scenario

Our company's allograft, which is used in breast reconstruction and hernia repair, is facing a backorder. We are proposing a change in tissue processing parameters in order to increase production.

Although the proposal is to change the method of processing we must be sure the extracellular matrix is preserved this is because it is necessary to serve its purpose which is provide structure, strength, and support for the tissue. Changes in the existing product specification that have been submitted to regulatory agencies, can affect the cell removal efficiency, the ECM structure and strength and flexibility we will perform construct a plan for senior management to get approval. The 3 studies we will construct for this plan will be

- Biomechanical testing
- Biochemical testing
- DNA/histology testing

Evidence Generation Plan

Biomechanical testing: Uniaxial Tensile Test

- In order to evaluate the effects of processing on the graft's mechanical properties such as strength and elasticity, which directly influence dermal function, samples can undergo uniaxial tensile testing on an Instron before and after processing.

Biochemical testing: ELISA

- Ensuring that the adjusted decellularization process continues to result in an identical extracellular matrix, an ELISA is conducted to measure collagen and elastin content. A sufficient biochemical composition ensures that the mechanical and biocompatible properties remain consistent.

In-Vitro Cellular Repopulation Analysis

- Biocompatibility and opportunity for cell growth are essential to a decellularized allograft to be safe and functional for patients. The original and alternatively processed allografts will be set in-vitro and observed for dermal cell repopulation.

Biomechanical Testing: Uniaxial Tensile Test

OBJECTIVE: Evaluate the effects of processing on the graft's mechanical properties (ex: strength and elasticity), which directly influence dermal function, by placing samples under uniaxial tensile testing on an Instron before and after processing (Jiang et. al, 2025).

Specifically measure:

- Ultimate tensile strength (UTS)
- Elasticity
- Flexibility (Elongation at failure)
- Structural integrity

RATIONALE: The acellular dermal matrix must retain sufficient mechanical strength and flexibility to function properly during implantation and healing

Changes in tissue processing parameters may:

- Damage collagen fiber organization and ECM proteins
- Alter tissue stiffness or elasticity
- Reduce resistance to tearing or deformation

Study design - Biomechanical Testing

GROUPS

1. Control Group (n=10): Standard processing parameters
2. Experimental Group (n=10): Modified processing parameters
3. Matched tissue donors used across both groups, tissue thickness standardized prior to testing, and samples cut into uniform “dog-bone” specimens according to ASTM soft tissue testing guidelines

BLINDING & RANDOMIZATION

1. Randomized sample allocation to groups
2. Biochemical analyses performed blinded to group assignment
3. Coded samples; analyst unaware of processing condition

SAMPLE HANDLING

1. Samples stored at -80°C until analysis
2. Three technical replicates per sample
3. Positive/negative controls included in each assay run

Study Type: In vitro | Design: Parallel-group comparison | Power: $\alpha = 0.05$, $\beta = 0.20$ (80% power)

Materials - Biomechanical Testing

Tissue Samples

- Control allografts (n=10)
- Experimental allografts (n=10)
- Patient-derived dermal tissue samples

Mechanical Testing Equipment

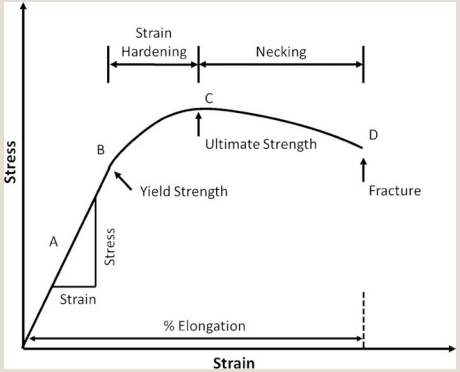
- Instron universal testing machine with 100 N load cell
- Tissue grips
- Digital calipers for thickness measurement
- Hydration chamber or PBS bath

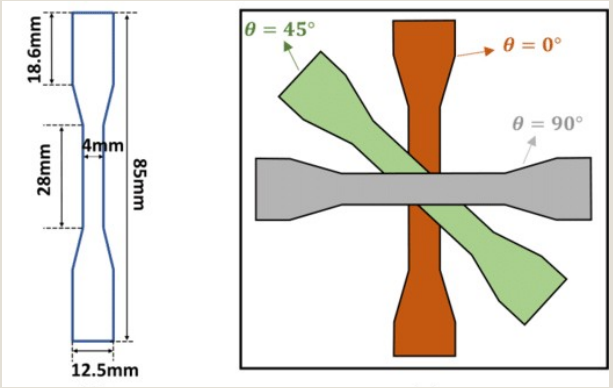
Consumables

- Sterile scalpels, forceps and cutting templates
- Specimen labeling materials

Software & Analysis Tools

- Instron Bluehill software
- Excel
- Image analysis software such as ImageJ





(Lim & Hoag, 2013)

Methods - Biomechanical Testing

Sample Preparation

- Cut allograft specimens into standardized dimensions according to ASTM soft tissue tensile testing recommendations. Measure thickness and width of each specimen using digital calipers to calculate cross-sectional area.

Hydration & Conditioning

- Hydrate samples in PBS for 30 minutes before testing to simulate physiologic conditions and maintain tissue pliability throughout testing. Photograph the specimen from a given angle and distance.

Instron Tensile Testing

- Secure samples between Instron grips while minimizing tissue slippage or preload damage. Apply uniaxial tensile force at a constant strain rate of 10 mm/min until tissue failure occurs.
- The Instron system continuously records force applied (N), displacement (mm), the stress-strain curve, and various mechanical property calculations (UTS, Young's modulus, elongation at break)

Structural Integrity Assessment

- After failure testing, photograph the specimen at the same angle and distance as before. Visually inspect samples for tearing, delamination, fiber disruption, and uneven deformation
- Quantitatively analyze these properties through an image processing software such as ImageJ

Statistical Analysis for Biomechanical Testing

Data Collected:

- Tensile strength
- Elasticity
- Flexibility
- Structural integrity

Analysis:
The average and standard deviation for each biomechanical property will be calculated. The control and experimental groups will be compared to determine whether there is a change in the tensile strength or flexibility of the acellular allograft resulting from the new processing method.

Statistical Test:
An independent t-test for two independent samples can be used to compare the two groups: the original processing method versus the new processing method.

Expected Result:
The new processing method should not have a significant negative impact on the tensile strength, elasticity, or flexibility of the acellular allograft because the allograft must maintain strength, softness, and pliability. If the new processing method has had a significant negative impact on any biomechanical properties of the allograft, it is likely not acceptable.

Expected Outcomes - Biomechanical Testing

PRIMARY HYPOTHESIS: The modified tissue processing parameters will NOT significantly reduce the biomechanical performance of the acellular allograft compared to the currently approved processing method ($p > 0.05$).

✔ ACCEPTABLE RESULT

No statistically significant differences between groups ($p > 0.05$). Tensile strength and elongation remain within acceptable product specification ranges. Failure patterns remain consistent with standard processed grafts. ECM maintains structural integrity without excessive tearing or brittleness

! AMBIGUOUS RESULT

Statistically significant but modest reduction in tensile strength or elasticity in experimental group. Mechanical properties reduced by <15–25%. No extreme structural failure observed. Additional studies recommended, such as cyclic fatigue testing and SEM imaging of collagen architecture before final approval decision.

× UNACCEPTABLE RESULT

Significant decrease in tensile strength, elasticity, or flexibility ($p < 0.05$). Brittle behavior or premature tissue failure. Major disruption of stress-strain behavior. Evidence of ECM structural damage or delamination.

Biochemical Testing: ELISA-Based Biochemical Composition & ECM Integrity Testing

OBJECTIVE: Determine whether the proposed change in tissue processing parameters alters the biochemical composition of the extracellular matrix (ECM) in the acellular allograft.

Specifically measure:

- Collagen content
- Elastin content
- Overall ECM composition

RATIONALE

ELISA (Enzyme-Linked Immunosorbent Assay) enables specific, sensitive, and quantitative detection of collagen and elastin using antibody-based detection — ideal for tissue lysate samples.

Any processing change that degrades these proteins could compromise:

- Mechanical performance
- Regulatory compliance
- Clinical safety of the product

Study design - Biochemical Testing

Controlled Comparative Testing — Control vs. Experimental Processing

GROUPS

1. Control Group (n=10): Standard processing parameters
2. Experimental Group (n=10): Modified processing parameters
3. Matched tissue donors used across both groups to reduce variability

BLINDING & RANDOMIZATION

1. Randomized sample allocation to groups
2. Biochemical analyses performed blinded to group assignment
3. Coded samples; analyst unaware of processing condition

SAMPLE HANDLING

1. Samples stored at -80°C until analysis
2. Three technical replicates per sample
3. Positive/negative controls included in each assay run

Study Type: In vitro | Design: Two-group parallel comparison | Power: $\alpha = 0.05$, $\beta = 0.20$ (80% power)

Materials - Biochemical Testing

TISSUE SAMPLES

- Control allograft (n=10, standard processing)
- Experimental allograft (n=10, modified processing)
- Matched donor tissue pairs

REAGENTS & CONSUMABLES

- PBS + protease inhibitors (tissue lysis buffer)
- ELISA wash buffer, stop solution (H_2SO_4), TMB substrate
- 96-well ELISA plates, pipette tips, microcentrifuge tubes

ELISA KITS

- Human Collagen Type I ELISA Kit (e.g., Abcam ab210966)
- Human Elastin ELISA Kit (e.g., Abcam ab212080)
- Total ECM Protein ELISA / BCA Protein Assay Kit

EQUIPMENT

- Microplate ELISA reader (450 nm, 570 nm reference)
- Tissue homogenizer & analytical balance
- Microcentrifuge, plate washer, incubator (37°C)

Methods - Biochemical Testing

ELISA-Based Biochemical Analysis Protocol

1. **Sample Preparation**
   Homogenize allograft in PBS + protease inhibitors. Centrifuge at 10,000×g for 10 min at 4°C. Collect supernatant; quantify total protein via BCA assay.

2. **ELISA: Collagen Type I**
   Dilute samples per kit instructions. Add to pre-coated collagen ELISA plate; incubate 2 hrs. Wash, add detection antibody, then HRP conjugate. Add TMB substrate; stop with H_2SO_4. Read at 450 nm.

3. **ELISA: Elastin**
   Apply diluted lysate to elastin-coated wells; incubate. Wash and add biotinylated detection antibody, then streptavidin-HRP. Develop with TMB substrate. Read absorbance at 450 nm (ref. 570 nm).

4. **Standard Curve & Quantification**
   Run recombinant collagen and elastin standards (8-point curve) on each plate. Interpolate sample concentrations. Normalize to total protein (ng analyte / µg total protein).

5. **Data Collection & QC**
   Run samples in triplicate per plate. Include inter-assay controls across plates. Accept CV < 15% for technical replicates; repeat any outlier wells.

Statistical Analysis - Biochemical Testing

STATISTICAL APPROACH
Test: Two-sample independent t-test (two-tailed)
Variables: Collagen Type I (ng/µg protein), Elastin (ng/µg protein), ECM composition (% total protein)
Descriptive Stats: Mean ± SD and % difference (control vs. experimental)
Significance Level: $\alpha = 0.05$
Software: GraphPad Prism or SPSS

ASSUMPTIONS & NOTES

- Normality tested via Shapiro-Wilk test prior to t-test
- Levene's test for equality of variances; Welch's t-test used if variances unequal
- If normality assumption violated: Mann-Whitney U test (non-parametric alternative)
- Bonferroni correction applied for multiple comparisons (3 analytes)
- Data presented as mean ± SD with individual data points shown in figures

Expected Outcomes - Biochemical Testing

Anticipated Results & Decision Criteria

PRIMARY HYPOTHESIS: The modified processing parameters will NOT significantly alter collagen content, elastin content, or overall ECM composition ($p > 0.05$), demonstrating biochemical equivalence.

✓ ACCEPTABLE RESULT

No significant difference in collagen, elastin, or total ECM composition between control and experimental groups ($p > 0.05$). Values within ±15% of control mean. Processing change deemed acceptable.

! AMBIGUOUS RESULT

Statistically significant but small % difference (<20%) in one analyte. Senior management and regulatory team review required. Additional studies (e.g., mechanical testing) to inform decision.

× UNACCEPTABLE RESULT

Significant reduction in collagen or elastin ($p < 0.05$, >20% decrease). Processing change rejected. R&D to investigate alternative parameters that preserve ECM integrity.

In vitro Cellular Repopulation & Fluorescence Histology

Objective:

To determine whether the proposed change in tissue processing parameters affects the ability of the acellular allograft to support dermal cell repopulation in vitro.

Specifically evaluate:

- Density of dermal cells colonizing the allograft scaffold
- Residual host DNA content (decellularization efficacy)

Rationale:

- Incomplete cell removal is a primary cause of immune rejection in allograft implants
  - Must minimize residual DNA and cellular antigens
- Acellular allograft must function as a scaffold for host cell infiltration after implantation
- Altered processing may disrupt ECM architecture needed for cell attachment and growth
- Fluorescent microscopy with DAPI staining allows quantification of nuclear density
  - Direct and validated measure of cellular repopulation
- DNA quantification confirms complete decellularization prior to in vitro study
  - Must meet industry standards - decellularization defined ass < 50 ng dsDNA per mg of dry tissue (Crapo et al. 2011)

Study Design - Histology Testing

GROUPS & SEEDING

1. Control Group (n=10): Standard processing allograft seeded with patient dermal fibroblasts
2. Experimental Group (n=10): Modified processing allograft seeded with same donor fibroblast batch
3. Cell passage ≤ 5
   Seeding density standardized at 50,000 cells/cm^2

INCUBATION

1. 14 day in vitro incubation at 37 C, 5% CO_2 in standard DMEM/10% FBS medium (Wu et al. 2019)
2. Medium changed every 48 hours, volume standardization across all samples
3. All samples processed simultaneously to minimize run to run variability

BLINDING & CONTROLS

1. Analyst blinded to group assignment during imaging and cell counting
2. Unseeded (cell free) graft controls included to assess background fluorescence
3. Cell only controls (no scaffold) to confirm fibroblast viability

Materials - Histology Testing

Tissue and Cells

- Control and experimental allografts (n = 10 per group)
- Patient derived dermal fibroblasts (passage ≤ 5)
- Cryopreserved cell stocks from a single donor lot

Cell Culture Consumables

- DMEM + 10% FBS + 1% penicillin/streptomycin
- PBS, 4% paraformaldehyde (fixative), Triton X - 100
- 6 well tissue culture plates, sterile forceps, scalpel

Staining and Imaging Reagents

- DAPI (4′, 6-diamino-2-phenylindole): nuclear stain (Karg & Golic 2018)
- Phallodin-Alexa Fluor 488: F-actin/cytoskeletal stain (Abcam 2021)
- Mounting medium, glass coverslips, slides

Equipment

- Fluorescence microscope: DAPI and FITC filter sets
- Digital camera and image analysis software: ImageJ
- Cryostat or vibratome for thin section preparation

Methods - Histology Testing

1. **Pre study Quality Control**
    - Confirms decellularization; digest allograft sections in proteinase K; extract DNA and quantify via PicoGreen assay (Ban & Kim, 2024)
        - Threshold: < 50 ng dsDNA/mg dry tissue (ISO standard)
1. **Cell Seeding**
    - Cut allografts into standardized 1×1 cm sections. Place dermis side up in 6 well plates. Seed 50,000 dermal fibroblasts/cm^2 onto each graft. Incubate for 14 days, change medium every 48 hours
1. **Fixation and Sectioning**
    - After day 14, rinse grafts in PBS and fix in 4% paraformaldehyde for 24 hours. Cut thin cross sections using cryostat/vibratome. Mount sections onto glass slides
1. **Fluorescent staining**
    - Permeable sections with Triton X-100. Apply DAPI and Phalloidin-Alexa 488 to stain nucleus blue and actin/cytoskeleton green, respectively. Cover with anti fade mounting medium, cure overnight. (Ooi et al. 2025)
1. **Imaging and Quantification**
    - Capture fluorescent images at 10X and 20X magnification (≥ 5 fields/section). Use ImageJ to threshold DAPI channel and count nuclei per mm^2. Calculate mean cell density per group.

Statistical Analysis - Histology Testing

Statistical Approach

- Primary test: two sample independent t-test (two tailed) for cell density (nuclei/mm^2)
- DNA data: descriptive statistics (mean ± SD) compared to 50 ng/mg threshold
- Descriptive statistics: mean ± SD, % difference between control and experimental groups
- Significance: p= 0.05
- Software: ImageJ for quantification, Excel for statistics

Assumptions and Notes

- Data presented as mean ± SD with individual data points overlaid on bar graphs
- ≥ 5 fields/section analyzed
    - Field means that it is averaged per sample prior to group comparison

Expected Outcomes - Histology Testing

PRIMARY HYPOTHESIS: The modified processing parameters will NOT significantly alter dermal cell repopulation density compared to the control group ($p>0.05$), and both groups will confirm complete decellularization (<50 ng DNA/mg dry tissue).

✓ ACCEPTABLE RESULT

No significant difference in cell density (nuclei/mm^2) between groups ($p>0.05$). Both groups pass DNA threshold (<50 ng/mg). Scaffold architecture supports equivalent cell repopulation, meaning the processing change is deemed acceptable.

! AMBIGUOUS RESULT

Statistically significant but modest reduction (<25%) in cell density in experimental group. DNA threshold still met. Additional testing (longer culture duration, SEM imaging) recommended before final decision.

× UNACCEPTABLE RESULT

Significant reduction in cell density ($p<0.05$, 25% decrease) or failure to meet DNA decellularization threshold (>50 ng/mg). Processing change rejected. ECM scaffold integrity likely compromised.

Closing Remarks

With all assays and tests passed, our experimental approaches comprehensively evaluate the effectiveness of modified processing to increase acellular allograft production without compromising its quality.

- Prioritize preserving ECM integrity, functionality, and biocompatibility
- Biomechanical, biochemical, and histological testing provide quantitative and qualitative values for industry standards
- Further studies, or altered versions of the aforementioned approaches, may be pursued to ensure quality

References

Thomas W. Gilbert, Tiffany L. Sellaro, Stephen F. Badylak. (2006). Decellularization of tissues and organs, Biomaterials, Volume 27, Issue 19, Pages 3675-3683, ISSN 0142-9612, https://doi.org/10.1016/j.biomaterials.2006.02.014

Zienowicz, R. J., & Karacaoglu, E. (2007). Implant-based breast reconstruction with allograft. Plastic and reconstructive surgery, 120(2), 373–381. https://doi.org/10.1097/01.prs.0000267340.31742.1

Crapo et al. (2011) — "An Overview of Tissue and Whole Organ Decellularization Processes" Biomaterials, 32(12), 3233–3243 https://pmc.ncbi.nlm.nih.gov/articles/PMC3084613/

Abcam ab210966 — Human Pro-Collagen Type I ELISA Kit
https://www.abcam.com/en-us/products/elisa-kits/human-pro-collagen-i-alpha-1-elisa-kit-ab210966

"Decellularization in Tissue Engineering and Regenerative Medicine: Evaluation, Modification, and Application Methods" (PMC, 2022)
https://pmc.ncbi.nlm.nih.gov/articles/PMC9081537/

Lim, H., & Hoag, S. W. (2013, May 21). Plasticizer effects on physical–mechanical properties of solvent cast Soluplus® films - AAPS pharmscitech. SpringerLink.

References

Ban, E., & Kim, A. (2024). PicoGreen assay for nucleic acid quantification - Applications, challenges, and solutions. Analytical biochemistry, 692, 115577. https://doi.org/10.1016/j.ab.2024.115577

Ooi, J. Y. Y., Tsang, Y. S., McMahon, L. P., & Wong, L. (2025). Protocol for in vitro immunofluorescence staining in a Transwell co-culture system. STAR protocols, 6(4), 104162. https://doi.org/10.1016/j.xpro.2025.104162

Wu, Y. D., Li, M., Liao, X., Li, S. H., Yan, J. X., Fan, L., She, W. L., Song, J. X., & Liu, H. W. (2019). Effects of storage culture media, temperature and duration on human adipose-derived stem cell viability for clinical use. Molecular medicine reports, 19(3), 2189–2201. https://doi.org/10.3892/mmr.2019.9842

Karg, T. J., & Golic, K. G. (2018). Photoconversion of DAPI and Hoechst dyes to green and red-emitting forms after exposure to UV excitation. Chromosoma, 127(2), 235–245. https://doi.org/10.1007/s00412-017-0654-5

Abcam. (2021). Phalloidin staining. Abcam.com. https://www.abcam.com/en-us/technical-resources/protocols/phalloidin-staining

Jiang, F., Tohgasaki, T., Kami, M., Sanuki, R., Nakata, Y., Kondo, S., & Chen, X. (2025). Influence of aging on dermal elastin fiber architecture and skin firmness assessed by finite element modeling. Scientific reports, 15(1), 28598. https://doi.org/10.1038/s41598-025-14393-2