Embalming cadaveric upper limbs after freezing and thawing: a novel technique for maximizing body donor usage through fresh frozen and formalin-fixed preservation

Isabella G. Damjanovic*, Madeline M. Damjanovic*, Earl Donaldson, Logan S.W. Bale

Queen's University, Department of Biomedical and Molecular Sciences, 18 Stuart Street, Kingston, Ontario, Canada, K7L 3N6

SUMMARY

Fresh frozen body donors are invaluable for surgical skills training sessions and medical research due to their realistic tissue quality. However, the potential for use as long-term teaching specimens is limited by soft-tissue deterioration following multiple freeze-thaw cycles. Embalming with the use of formalin achieves tissue fixation, thereby preventing tissue deterioration and enabling prolonged use of anatomical specimens. The purpose of this study was to determine whether fresh frozen upper limbs can be successfully embalmed for use as dissection and prosection resources in anatomical sciences education following one or more freeze-thaw cycles, thereby allowing for increased usage of an individual body donor. Four previously frozen left upper limbs were preserved using formalin fixation and were dissected 30 days following arterial embalming to determine whether adequate fixation could be achieved and whether the tissue quality could be maintained. The greatest number of freeze-thaw cycles evaluated in this study was six. To our knowledge, this is the first report in which specimens from fresh frozen human body donors have successfully been embalmed using formalin-fixation techniques following single or multiple freeze-thaw cycles. Following dissection of each upper limb, we conclude that formalin fixation after freezing and thawing is a viable preservation technique that can maintain a level of tissue quality suitable for educational dissection and prosection following use of the fresh frozen cadaver for surgical skills training sessions or medical research.

Key words: Anatomy – Dissection – Embalming – Formaldehyde – Medical education

INTRODUCTION

Anatomy is the foundation of education for students and practising professionals in healthcare related fields, and cadaveric dissection has been regarded as the gold standard for anatomical education since the fourteenth century (Balta et al., 2015; Hildebrandt, 2010; Standring, 2016). It is believed by many anatomists that dissection offers unique insight into the detailed integration of body systems and allows students to gain

Corresponding author:
Isabella Damjanovic. Queen's University, Department of Biomedical and Molecular Sciences, 18 Stuart Street, Kingston, Ontario, Canada, K7L 3N6. Phone: 905-818-0294. E-mail: isabella.damjanovic@queensu.ca
*These authors contributed equally to this work

Submitted: September 6, 2023. **Accepted:** October 5, 2023

https://doi.org/10.52083/SDQC3737

a three-dimensional understanding of anatomy (Ovsenek, 2013).

The use of human donors for teaching and learning is enabled by the preservation of tissues. The introduction of formaldehyde as a fixative in 1893 was an important milestone in the history of embalming for the study of anatomy (Balta et al., 2015). Formaldehyde is gaseous at room temperature, and is termed formalin when converted into a saturated liquid solution. Formaldehyde accomplishes tissue fixation by cross-linking proteins, thus preventing decomposition and microbial action (Brenner, 2014). Embalming is most commonly performed via arterial perfusion, a technique developed in the seventeenth century with the discovery of the circulation of blood (Doomernik et al., 2016). This perfusion method allows for controlled injection of embalming fluid into the arterial system as opposed to embalming methods such as submersion, which involve placing the entire specimen in a tank of embalming fluid (O'Neill et al., 2013).

Donors embalmed with formalin typically do not demonstrate qualities of living tissue with regards to color, texture, and mobility (Balta et al., 2015). However, formalin-fixed donors are most frequently used for gross anatomy dissection and prosection, as the tissue can withstand prolonged use without deterioration and imposes minimal biohazardous risk following the embalming procedure. Routine educational dissection has been used as a hands-on method of teaching anatomy for centuries, with learning outcomes augmented by the experience compared to anatomy instruction without dissection (Iwanaga et al., 2021).

Cadaveric prosections are employed in the teaching and learning of anatomy and can be defined as cadaveric specimens that an anatomist has dissected to demonstrate specific anatomical structures for students (Aziz et al., 2020). Some institutions utilize formalin-fixed prosected specimens to augment the experience of educational dissection, while others rely on prosected specimens to replace student-led dissection. Wet-prosection specimens retain some of the benefits of using real tissue for education, but lack the benefit of the learners performing the dissection themselves (Cornwall, 2011). Prosected specimens can be placed in jars and displayed for decades in medical museums to aid in the teaching and learning of anatomy and observational skills as part of medical education (Marreez et al., 2010).

Fresh frozen donors do not undergo chemical fixation or treatment prior to dissection (Song and Jo, 2022). This preservation method is valuable for clinical and surgical skills training due to the realistic quality of the tissue, with characteristics such as color, texture, and mobility remaining unaltered (Balta et al., 2015). Fresh frozen cadaveric specimens are also used for medical research. Most fresh frozen donors can only be used for an average of four freeze-thaw cycles due to soft tissue deterioration following extensive or prolonged use, limiting the potential for further educational dissection following surgical skills sessions (Jansen et al., 2020). At Queen's University, we find that the regions of the body that are most in demand for surgical skills or medical research (anterior neck, knees, thorax, etc.) will be depleted after a donor is used for two to six sessions. In many cases, one or both upper limbs of these cadavers may be untouched when the donor is prepared for cremation. While untouched upper limbs are routinely harvested and retained frozen for future surgical skills sessions or medical research projects, the University's Human Body Donor Program would benefit from having the option to use these limbs for educational dissection and prosection, thus requiring the need for formalin fixation of the specimens.

Existing literature for embalming methods following the freezing and thawing of body donors is limited. A study by AlShehry et al. (2019) demonstrated successful embalming of previously frozen tissue in a mouse model. The authors used Thiel embalming, a method of soft-tissue preservation, and were able to successfully embalm five fresh frozen mouse cadavers following one freeze-thaw cycle (AlShery et al., 2019, unpublished data). Currently, no publication has demonstrated this embalming technique with formalin as the main tissue fixative or following multiple freeze-thaw cycles, and this method has not yet been demonstrated in human cadavers.

The purpose of this project was to investigate a novel technique for maximizing body donor usage

by employing formalin fixation of fresh frozen upper limbs following one or more freeze-thaw cycles. Here we summarize a method that describes successful embalming of upper limbs following multiple freeze-thaw cycles.

MATERIALS AND METHODS

All cadavers used in this study were donated in accordance with the policies of the Human Body Donor Program at Queen's University. Ethical approval for this study was granted by the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board. The authors hereby confirm that every effort was made to comply with all local and international ethical guidelines and laws concerning the use of human cadaveric donors in anatomical research. All fresh frozen cadavers were prepared for 1) surgical training sessions in the fields of emergency medicine, orthopedic surgery, obstetrics and gynecology, and/or anesthesiology, or 2) medical research projects, before undergoing fixation procedures for this study. Photographs were obtained throughout the embalming and dissection procedures using an iPhone XR and exported as JPEG files and stored on Microsoft OneDrive.

Cadaver Information and Arterial Embalming Procedure

The left upper limbs of four fresh frozen cadavers (see Table 1) were embalmed following one or more freeze-thaw cycles. Arterial embalming via the axillary artery was performed using a batch solution containing formalin (see Table 2).

Table 2. List of embalming solution batch ingredients and corresponding volumes. Each upper limb was embalmed with 1.5 L of the batch solution.

Ingredient	Volume
Potassium Acetate	150 mL
Phenol	1 L
Formalin (37% formaldehyde)	1 L
Dettol	600 mL
Glycerol	1 L
Ethanol (95%)	20 L

For the embalming procedure, donors were placed in a supine position and a vertical skin incision was made at the midpoint of the clavicle with an 11-blade scalpel. A small segment of the clavicle, approximately five centimeters in length, was removed using a RIDGID battery-operated saw to provide adequate visualization of the subclavian/axillary artery and vein. Using a probe and forceps, the axillary artery and vein were accessed and isolated using blunt dissection. Two pieces of string were threaded under the axillary artery, and positioned to create a small gap between the strings. The axillary artery was tied off at the proximal end. A small incision in the axillary artery was made parallel to its course, and an embalming trocar was inserted and secured distally with the previously placed string (Fig. 1a). To

Table 1. Characteristics of the four cadavers.

Specimen #	Sex	Age	Cause of Death	# of Freeze-Thaw Cycles	Previous Uses for Surgical Skills and Medical Research
1	Female	92	Congestive heart failure, electrolyte imbalance, dehydration	1	*Emergency Medicine*: surgical airway, chest tubes, thoracotomy *Medical Research:* knees
2	Female	57	Myelodysplastic syndrome and necrotizing pneumonia	2	*Orthopedic Surgery*: feet and ankles *Emergency Medicine*: surgical airway, chest tubes, thoracotomy *Medical Research:* knees
3	Female	106	Advanced dementia, cerebrovascular disease, atrial fibrillation	4	*Emergency Medicine/Anesthesiology*: surgical airway, chest tubes, thoracotomy *Medical Research*: deep back, knees
4	Female	83	Metastatic esophageal cancer	6	*Orthopedic Surgery*: feet and ankles, spine *Emergency Medicine/Anesthesiology*: surgical airway, chest tubes, thoracotomy *Obstetrics and Gynecology*: laparoscopic hysterectomy *Medical research*: knees

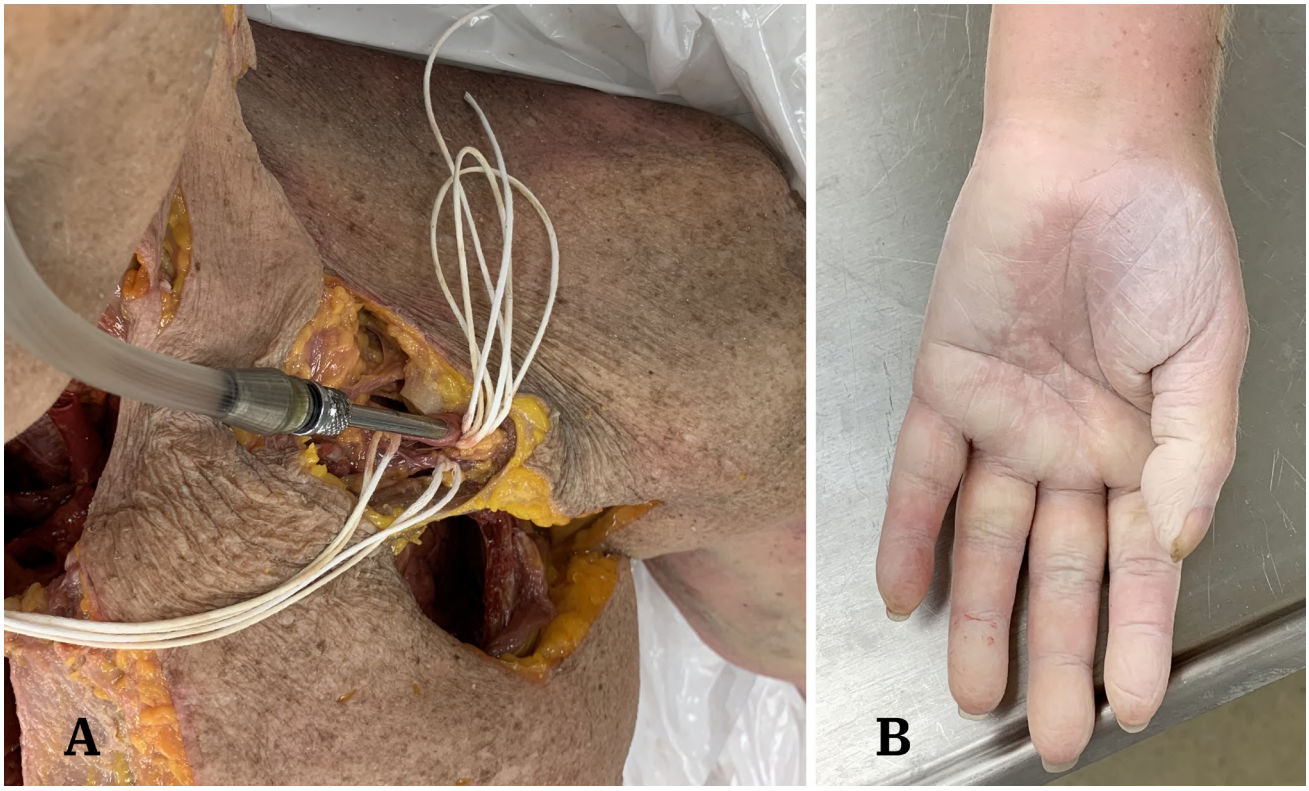

Fig. 1a.- Superior view of left axilla and proximal upper limb with cadaver in supine position to show the embalming site prior to injection of fluid. **1b:** The upper limb was monitored throughout the procedure for signs of tissue perfusion, such as skin mottling (skin appears white in some areas).

increase pressure and minimize retrograde flow of embalming fluid into the axilla and thorax, the axillary vein was tied off at the same level as the axillary artery. One and a half liters of embalming fluid were pumped into each limb at a minimal pressure of 6-7 pounds per square inch, and any small blood vessels that continued to leak fluid under pressure were clamped using hemostats. The embalming fluid was pumped to perfuse each upper limb using a PORTI-BOY Mark V embalming machine. Throughout the embalming procedure, the upper limb was monitored for signs of tissue perfusion, such as skin mottling (Fig. 1b). The embalming line was disconnected when the procedure was complete. The trocar was left in the axillary artery and all hemostats remained clamped. The donor cadavers were placed in a refrigerator at four degrees Celsius for 24 hours following embalming.

Disarticulation and Storage Procedures

Following the 24-hour period, each donor was removed from the refrigerator and an incision was made along the cut end of the clavicle and through the axilla using a 22-blade scalpel. The incision was extended posteriorly along the external surface of the ribs to facilitate the removal of the upper limb from the body at the scapulothoracic joint. The disarticulated limb was placed in a polyethylene bag that measured 20 x 30 inches and 6 mil in thickness. The elbow joint was bent at approximately 90 degrees so that the proximal part of the limb could be submerged in the embalming fluid that drained from the limb during storage (Fig. 2a). Each bag was labelled with the donor's identification number and sealed with a zip tie. The bagged upper limbs were positioned in metal trays to maintain an upright position and the trays were placed in a refrigerator at four degrees Celsius. Three days after disarticulation, a wooden block (7.5 cm x 7.5 cm x 6 cm tall) was placed under each elbow to elevate the limb, assisting in draining excess fluid towards the exposed shoulder region (Fig. 2b). Seven days following elevation of the elbow (ten days after initial disarticulation of the limb) the blocks were removed and the limbs were stored in a flat position in the refrigerator. All upper limbs were allowed to undergo fixation for a total of 30 days following the arterial embalming procedure.

Dissection Procedure

Following storage for 30 days to allow for fixation, limbs were dissected to evaluate the degree of fixation and the quality of the tissue to determine the potential for use as an educational resource. Each limb was photographed prior to the dissection process for documentation. Excess embalming fluid that was pooled in the bottom of the polyethylene bags was disposed according to standard procedures. The upper limbs were dissected by three of the contributing authors, all of whom have considerable experience dissecting formalin-fixed specimens. Standard dissection procedures, as outlined in Appendix A, were followed for the shoulder, arm, forearm, and hand. Each limb was dissected over a two-day period, with two days of storage between the first and second dissection sessions. The upper limbs were wrapped in linen saturated with moistening solution (Table 3) after the first dissection day, and stored in a refrigerator at four degrees Celsius in the original polyethylene bag that corresponded to each specimen. Photos were taken of the completed specimens.

Table 3. List of moistening solution batch ingredients and corresponding volumes.

Ingredient	Volume
Potassium Acetate	150 mL
Ethanol (95%)	250 mL
Dettol	750 mL
Glycerol	1500 mL

RESULTS

All four upper limbs demonstrated adequate tissue fixation 30 days following the arterial embalming procedure. The fixation methods provided suitable conditions for educational dissection and the preparation of prosected specimens. Local areas of unfixed tissue were not identified.

Within each upper limb specimen there were slight differences in texture, such that certain areas of the limb were softer and more edematous compared to other areas, which were firmer. Minimal skin slippage was observed on the wrists and hands prior to dissection of the upper limbs. Additionally, the anterior arm of some specimens had tissue integrity that was delicate, and therefore careful handling was required to avoid unwanted tearing of structures.

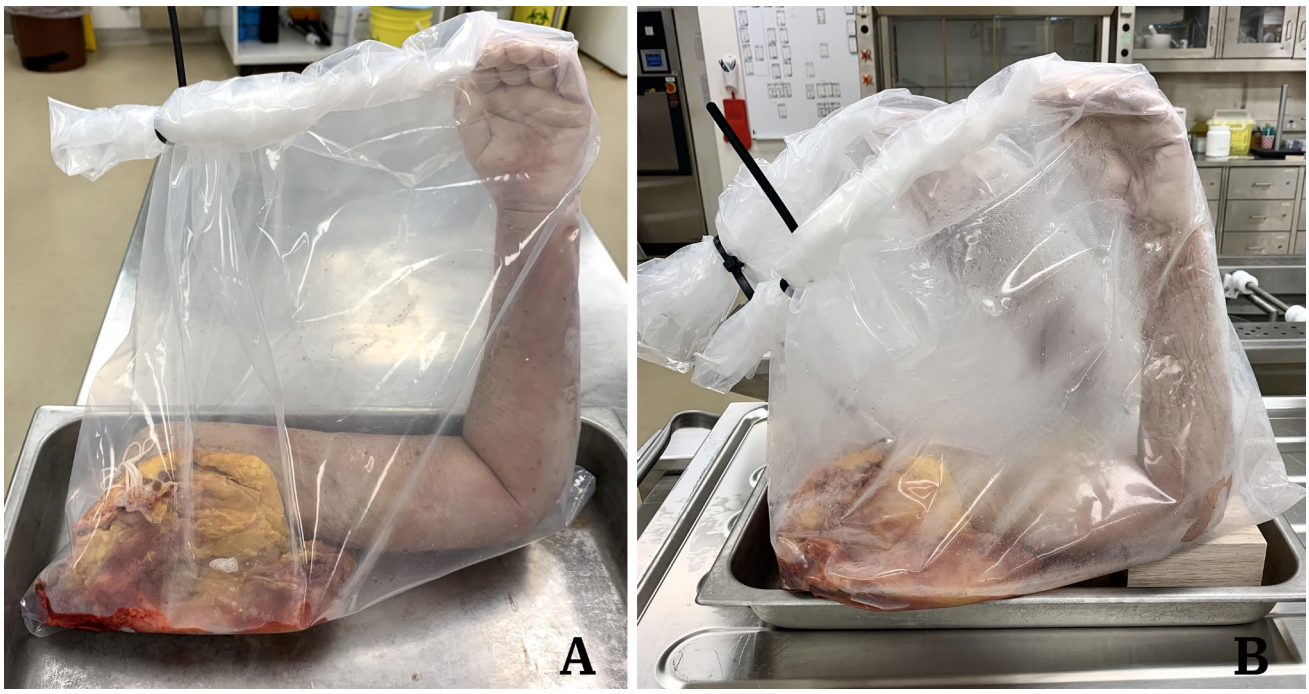

Fig. 2a.- Image of a left upper limb in a polyethylene bag for storage following disarticulation. The upper limb was positioned with the elbow bent at a 90-degree angle to aid in the drainage of additional embalming fluid for partial immersion. **2b:** The elbow was positioned on a wooden block three days following disarticulation to gather fluid at the exposed shoulder region.

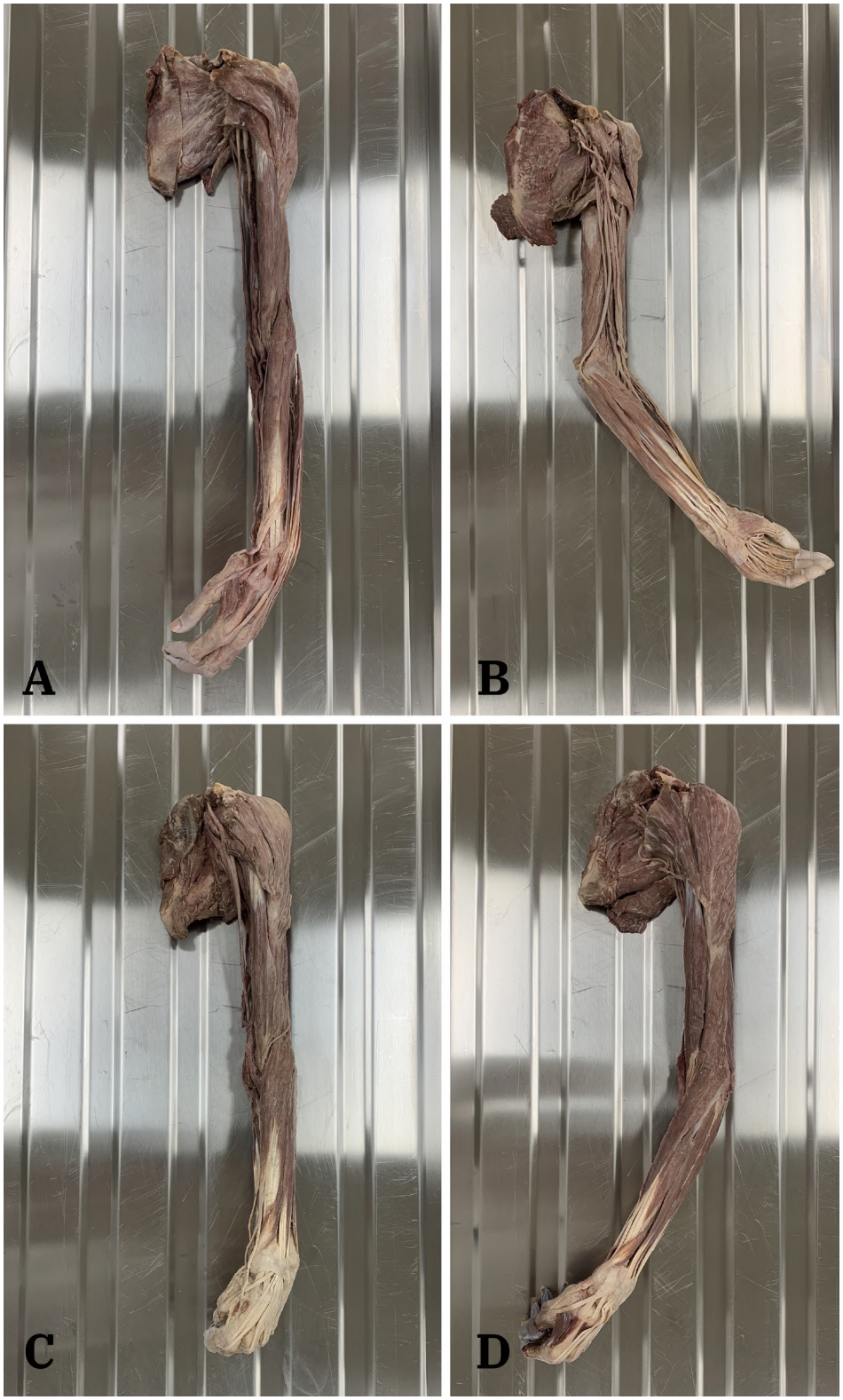

Fig. 3.- Images of four dissected left upper limbs in order of number of freeze-thaw cycles. **3a:** One freeze-thaw cycle. **3b:** Two freeze-thaw cycles. **3c:** Four freeze-thaw cycles. **3d:** Six freeze-thaw cycles.

Despite these observations, all four limbs were deemed to be valuable for use during educational dissection and for prosection purposes. The variety of educational resources that can be created and utilized from formalin-fixed tissue that has previously been frozen and thawed allows for maximal use of body donors for surgical skills training, medical research, and anatomical sciences education.

Educational Dissection

The tissue demonstrated acceptable quality for use as an educational dissection resource, re-

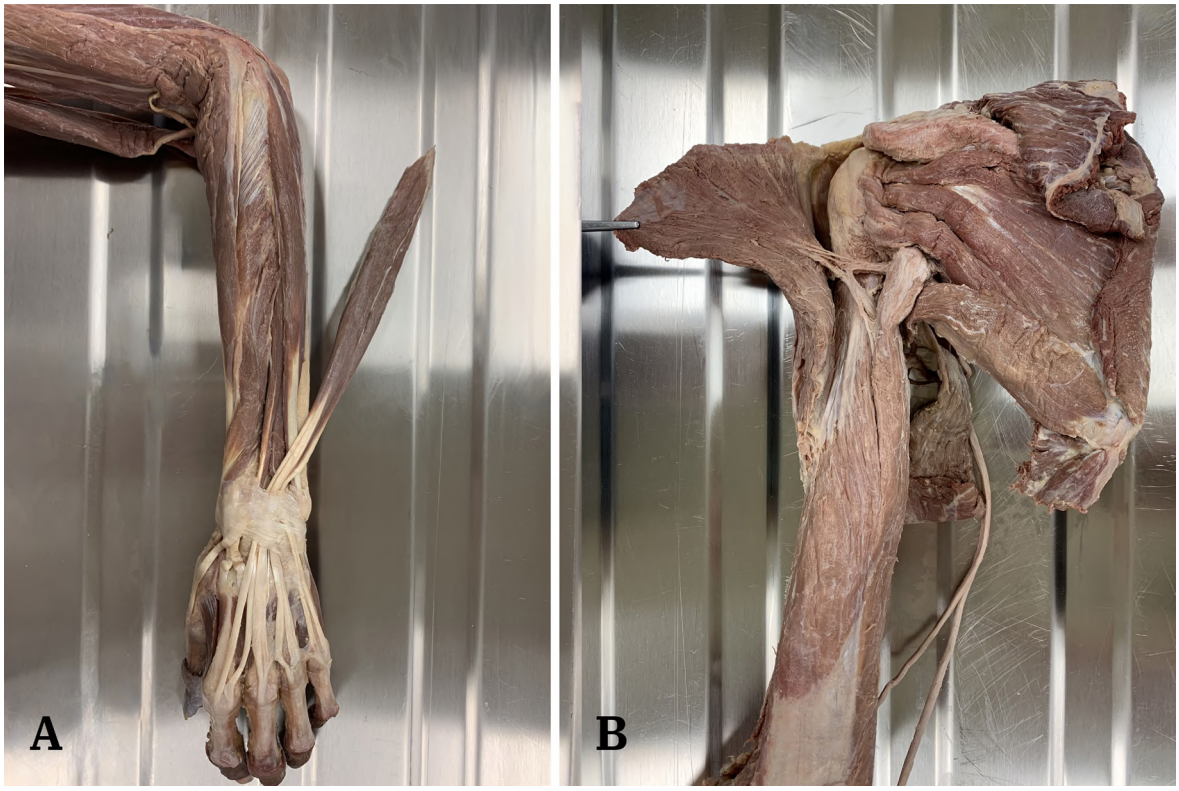

Fig. 4.- Images representing muscle reflections to demonstrate deep structures of the arm and forearm. **4a:** Reflection of extensor digitorum. **4b:** Reflection of deltoid.

gardless of the number of freeze-thaw cycles. The soft tissues were flexible and easy to manipulate, while still maintaining proper form. A superficial dissection demonstrated good muscle integrity, allowing for the definition and mobilization of muscle borders and isolation of neurovascular structures (Fig. 3). A deep dissection following reflection of deltoid, pronator teres, palmaris longus, and extensor digitorum demonstrated adequate tissue integrity, even to the deepest layers of muscle (Fig. 4). The tissue quality allowed for detailed dissection of delicate neurovascular structures, such as the common and proper palmar digital arteries, arising from the superficial palmar arch, and the common and proper palmar digital nerves, arising from the median and ulnar nerves, in the palmar hand (Fig. 5).

Prosection

Two upper limbs from this project were added to the collection of wet prosected specimens at Queen's University, which are used to facilitate the teaching and learning of gross anatomy of the upper limb during laboratory sessions without requiring the time-consuming dissection process.

Additionally, two upper limbs were processed further and added to the collection of specimens displayed in glass jars in the upper limb section of the Anatomy Museum at Queen's University (Fig. 6). The prosections that were produced during this project will be maintained for long-term preservation, and will be beneficial for learners from a wide range of degree programs and educational levels.

DISCUSSION

In this study, four fresh frozen left upper limbs were successfully embalmed using formalin-fixation methods following one or more freeze-thaw cycles. The fixation was deemed to provide sufficient conditions for specimen preparation through educational dissection and prosection, the latter including both wet specimens that can be handled, and specimens preserved in glass jars for long term display. The upper limbs maintained adequate form and tissue integrity while still allowing for the mobilization of muscular and neurovascular structures during dissection. The highest number of freeze-thaw cycles evaluated in this study was six. This limb demonstrat-

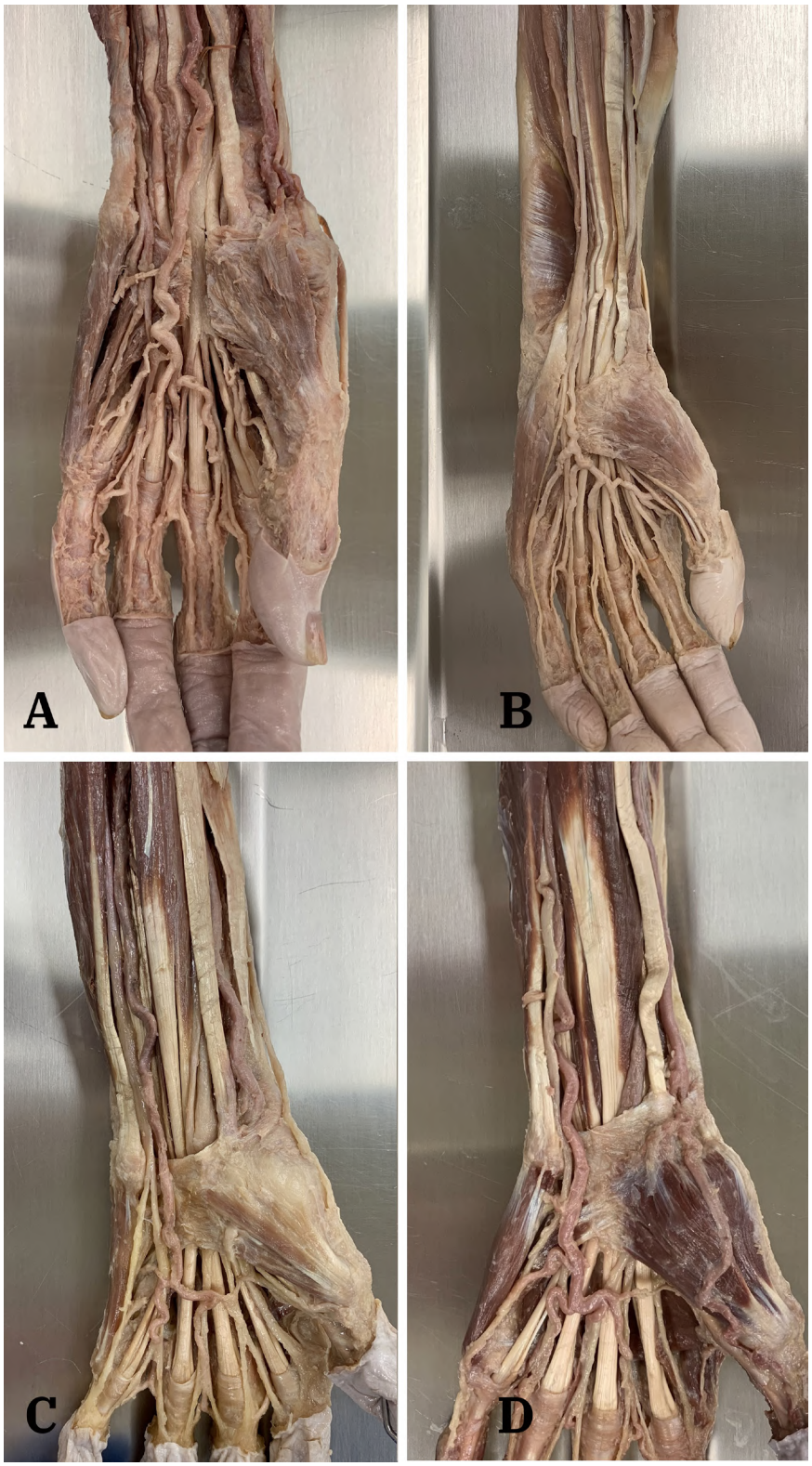

Fig. 5.- Images of the distal anterior forearms and palmar hands of four dissected upper limbs in order of number of freeze-thaw cycles. **5a:** One freeze-thaw cycle. **5b:** Two freeze-thaw cycles. **5c:** Four freeze-thaw cycles. **5d:** Six freeze-thaw cycles.

ed similar tissue characteristics compared to the other three limbs included in the study and was suitable for use as a museum specimen.

Although all four upper limbs were successfully embalmed and demonstrated adequate tissue fixation and tissue quality, some observations that were noted by the dissectors during specimen preparation include: local areas that varied in texture (softer, firmer, edematous, etc.), skin slippage on the wrists and hands, and delicate tissue integrity within the anterior arm of some of the limbs. These factors were not considered to be major impediments with respect to the overall tissue quality

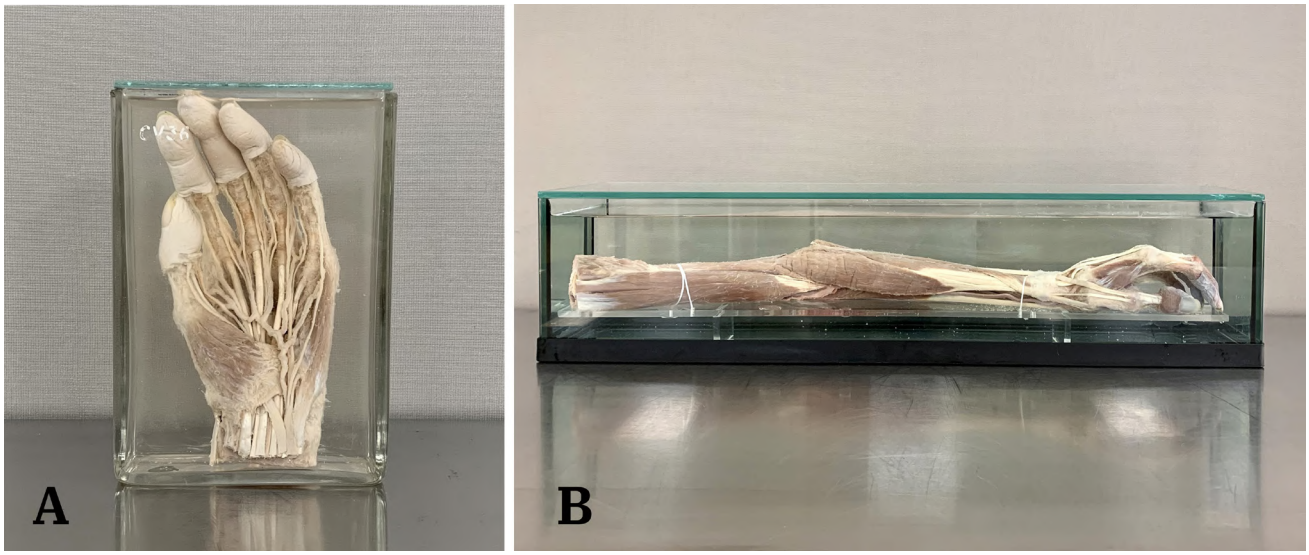

Fig. 6.- Image of two jarred specimens. **6a:** Two freeze-thaw cycles. **6b:** Six freeze-thaw cycles.

of the specimens and the potential use of the specimens for educational dissection and prosection.

Arterial perfusion embalming was selected for the work reported here because body donors designated for formalin-fixation are embalmed using arterial perfusion at Queen's University. Arterial perfusion was considered advantageous compared to simply submerging the upper limbs in fixative. While most often discussed as a component of Thiel embalming (Thiel, 1992), submersion was not considered a viable fixation method for the upper limb specimens. Submersion was predicted to require a greater amount of embalming fluid and fixation time compared to arterial perfusion, and would have complicated storage during the fixation process because of the need for vessels large enough to submerge the specimens for an extended period. The storage procedures for the method described in this work were simple, whereby each specimen was bagged and the limb positioned so that the exposed shoulder was in contact with the excess embalming fluid that pooled in the bag. Fixation of the shoulder region was expected to require partial immersion for two reasons: firstly, the cut end of the specimen was not fully covered by skin and therefore would not retain fluid well after embalming; and secondly, the site the embalming trocar was placed into the axillary artery was distal to the proximal end of the specimen, and therefore it was difficult to predict how well the embalming fluid would travel to the blood vessels in the shoulder region.

Anatomical education with the use of cadaveric dissection as a learning resource is dependent on the ability of body donation programs to maintain a sustainable supply of body donors, a process which requires balancing the number of donations with requests for use (McCumber et al., 2021). Fresh frozen donors are typically reserved for use in surgical skills training sessions or medical research projects, and are prone to tissue deterioration over time, thus limiting their potential use for educational dissection and prosection following the initial sessions. This study has demonstrated a novel approach to maximize body donor usage through formalin fixation of previously frozen and thawed upper limbs. This method allows for the use of fresh frozen donors for surgical skills training sessions and medical research, as well as educational dissection, prosection, and long-term preservation following the formalin-fixation embalming procedure.

We recognize that our study has limitations, such as the small sample size of four left upper limbs and the lack of an objective assessment tool for evaluating the quality of the specimens after fixation is complete. Future methodology may consider assessing the tissue fixation and tissue quality by surveying the users of the specimens. This approach has been used to evaluate the quality of cadavers used in surgical skills training, and could be applied to formalin-fixed specimens (Wang, et al. 2023).

It is likely that untouched lower limbs could be preserved using the method described here by perfusing the femoral artery after a donor has completed fresh frozen usage. Likewise, we believe that formalin-fixed head and neck specimens could be produced by perfusing the common carotid artery, although the brain would not likely be viable, as significant deterioration is expected as a result of freezing and thawing.

As adequate fixation was achieved 30 days following the embalming procedure, we predict that a shorter fixation window could be feasible in achieving adequate results using this technique. However, in estimating the number of days required for fixation it is predicated that the size of the upper limb is an important consideration, as a large upper limb from an overweight donor would likely require a greater number of days for fixation compared to a small upper limb from an emaciated donor.

In conclusion, formalin fixation of four upper limbs following freezing and thawing resulted in tissue fixation and tissue quality suitable for use as educational resources, maximizing the potential usage of human body donors for education and research in anatomical sciences.

ACKNOWLEDGEMENTS

The authors sincerely thank those who donated their bodies to science so that anatomical research and teaching could be performed. Results from such research can potentially increase scientific knowledge and can improve patient care. Therefore, these donors and their families deserve our highest respect. We also thank Dr. William Borman and the Department of Basic Sciences at University of Western States for providing use of the dissection instructions.

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APPENDIX A. DISSECTION INSTRUCTIONS

Modified from Gross Anatomy I Dissector, University of Western States Original author Dr. William Borman

Initial Dissection

Remove all skin from the shoulder to the middle phalanges. It is not necessary to preserve superficial neurovascular structures. Veins, particularly large and obstructive veins, should be removed to facilitate an efficient dissection.

Posterior Shoulder

1. **Find, clean, and reflect the deltoid muscle**
   Remove the deep fascia from the deltoid muscle so the directionality and borders of the muscle fibers are clearly visible.
   Reflect the posterior fibers of the deltoid muscle from the scapular spine and acromion process.
   Locate and identify the axillary nerve and posterior humeral circumflex artery passing through the quadrangular space.
2. **Locate and identify the posterior scapular muscles**
   Remove the infraspinous deep fascia and supraspinous deep fascia to reveal the infraspinatus and supraspinatus muscles.
   Locate the slender teres minor muscle and larger teres major muscle.
   Follow and clean the tendon of latissimus dorsi muscle to its insertion.
3. **Locate and identify two of the heads of the triceps brachii muscle**
   Clean and isolate the long head and lateral head of the triceps brachii muscle.

Anterior Shoulder & Arm

1. **Reflect the rest of the deltoid muscle and identify the anterior arm muscles**
   Remove the deep fascia from the deltoid muscle so the directionality of the muscle fibers and its borders are clearly visible.
   Reflect the anterior fibers of the deltoid muscle from the clavicle and acromion process.
   When completed, the deltoid muscle will only be attached to the humerus.
   Confirm that pectoralis minor, coracobrachialis, and short head of biceps brachii each attach to the coracoid process of the scapula.
2. **Locate and identify the subscapularis muscle insertion**
   Locate the insertion of the subscapularis muscle on the lesser tubercle of the humerus.
3. **Trace the terminal branches of the brachial plexus through the arm**
   Musculocutaneous nerve (terminates as lateral antebrachial cutaneous nerve)
   Median nerve
   Ulnar nerve
   Medial antebrachial cutaneous nerve
   Radial nerve
   Axillary nerve
4. **Trace the vascular elements through the anterior arm**
   Brachial artery
   Brachial veins
   Profunda brachii artery is an early branch of the brachial artery that passes with the radial nerve toward the triangular interval to the posterior arm.
   Several muscular arteries branch from the brachial artery to supply the anterior arm muscles.

Cubital Fossa & Anterior Forearm

1. **Reflect the bicipital aponeurosis**
   Identify and incise the bicipital aponeurosis to expose the brachial artery and median nerve in the cubital fossa.
2. **Identify the muscular elements of the cubital fossa and reflect pronator teres**
   Brachioradialis muscle forms the lateral border of the cubital fossa.
   Pronator teres muscle (with its deep and superficial heads) forms the medial border of the cubital fossa.
   Brachialis muscle forms the floor of the cubital fossa medially.
   Locate the median nerve and trace it between the superficial and deep heads of the pronator teres muscle.
   Reflect the pronator teres muscle from its distal attachment on the radius.
3. **Identify the forearm flexor muscles**
   Identify and remove the deep fascia from the anterior forearm.
   Identify the three remaining superficial forearm flexor muscles: flexor carpi ulnaris, flexor carpi radialis, and palmaris longus.
   Identify the ulnar nerve and artery as they run deep to the flexor carpi ulnaris muscle and become superficial at the wrist.
   Flexor digitorum superficialis muscle is the large muscle in the intermediate layer.
   Flex the hand at the wrist and move the tendons of flexor digitorum superficialis to the side to expose the flexor digitorum profundus muscle in the deep layer.
   Locate and identify the flexor pollicis longus muscle in the deep layer, lateral to the flexor digitorum profundus muscle.
   Pronator quadratus muscle is located deep to the tendons of flexor digitorum profundus.
4. **Locate and identify the radial nerve and its two branches**
   Locate the radial nerve deep in the tissue plane between the brachioradialis muscle and the brachialis muscle.
   Identify the bifurcation of the radial nerve into the superficial radial nerve and deep radial nerve.

5. **Locate the radial and ulnar arteries in the cubital fossa**
   Brachial artery bifurcates in the cubital fossa to form the radial artery (laterally) and ulnar artery (medially)

Posterior Forearm & Dorsum Hand

1. **Identify the superficial forearm extensor muscles**
   Identify and remove the deep fascia from the posterior forearm.
   Brachioradialis muscle forms the lateral border of the cubital fossa.
   Identify the extensor carpi radialis longus muscle on the lateral aspect of the forearm and trace it to its attachment on the base of the 2nd metacarpal.
   Identify the extensor carpi radialis brevis muscle on the lateral aspect of the forearm and trace it to its attachment on the base of the 3rd metacarpal.
   Extensor digitorum muscle is the centrally located multicaudal muscle. Note the expansion of these tendons on the dorsum of the digits.
   Extensor digiti minimi muscle is often blended with the medial aspect of the extensor digitorum muscle.
   Extensor carpi ulnaris muscle is located on the medial aspect of the forearm.
   Locate and identify the extensor retinaculum.

2. **Identify the deep and outcropping forearm extensor muscles**
   Extending the hand at the wrist will facilitate separating and identifying these muscles.
   Three outcropping muscles act on the thumb: abductor pollicis longus, extensor pollicis brevis, and extensor pollicis longus.
   One of the deep muscles acts on the index finger: extensor indicis.
   Expose the supinator muscle and the outcropping muscles more fully by reflecting the extensor digitorum muscle from its proximal attachment.

3. **Identify the neurovascular elements of the posterior forearm**
   Locate and identify the deep radial nerve in the anterolateral elbow.
   Locate and identify the posterior interosseous nerve as it emerges from the supinator muscle.
   Observe the tendinous borders of the anatomical snuffbox: abductor pollicis longus tendon and extensor pollicis brevis tendon laterally and the extensor pollicis longus tendon medially.
   Identify the radial artery in the anatomical snuffbox

Palmar Hand

1. **Carefully reflect the skin from the palmar surface of the hand and digits**
   Palmaris brevis muscle will be in the superficial palmar fascia near the proximal region of the hypothenar eminence.

2. **Find, clean, and reflect the palmar aponeurosis**
   Expose the palmar aponeurosis and note it is a relatively thick, tough sheet of dense fascia with fibrous cords extending longitudinally to the digits and deep to the metacarpal bones.
   Carefully reflect the palmar aponeurosis from its distal attachments and its metacarpal attachments leaving it connected only to the palmaris longus tendon and palmaris brevis muscle.
   The palmar carpal ligament is under the palmaris longus tendon.

3. **Follow the ulnar artery into the palm**
   Trace the ulnar artery superficial to the flexor retinaculum to its bifurcation into the deep and superficial branches.
   The deep branch of the ulnar artery contributes to the formation of the deep palmar arterial arch.
   The superficial branch of the ulnar artery is the predominant contributor to the superficial palmar arterial arch.
   Common palmar digital arteries branch from the superficial palmar arterial arch and subsequently bifurcate into proper palmar digital arteries.

4. **Follow the ulnar nerve and median nerve into the hand**
   Trace the ulnar nerve superficial to the flexor retinaculum.
   Deep ulnar nerve enters the hypothenar eminence and continues to several deep muscles in the palm.
   Superficial ulnar nerve branches to form common palmar digital nerves which subsequently branch to form proper palmar digital nerves to the medial 1 ½ digits.
   Just distal to the flexor retinaculum, identify the recurrent branch of the median nerve entering the thenar eminence.
   Identify the common palmar digital nerves as they arise from the median nerve and subsequently branch to form proper palmar digital nerves to the lateral 3 ½ digits.

5. **Separate and identify muscles of the palm**
   Blunt dissect and separate the three muscles of the thenar eminence: abductor pollicis brevis, flexor pollicis brevis, and opponens pollicis.
   Blunt dissect and separate the three muscles of the hypothenar eminence: abductor digiti minimi, flexor digiti minimi brevis, and opponens digiti minimi
   Separate and identify the four lumbrical muscles.
   Clean and identify the fibrous digital sheaths surrounding the tendons of the flexor digitorum superficialis muscle and flexor digitorum profundus muscle.