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Long term potential of a saturated sodium chloride solution for the anatomical preservation of human cadavers

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for the degree of

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Declaration

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Carrie O'Flynn

Abstract

The anatomical world has relied heavily on formaldehyde as an embalming agent since its use began in the 1890s. Efforts to move away from formaldehyde have intensified in recent years, largely in response to health concerns. Another important motivation is to seek out ways to an improved anatomical cadaver. Several new techniques have been investigated for their abilities to provide cadavers with both life-like features and longevity of preservation. A simple saturated salt solution (saturated NaCl solution) was used to embalm 4 cadavers in two phases of study, without the addition of formalin. As “soft-fix” methods are generally viewed as short-term preservatives, the long-term preservative action of the saturated NaCl solution method was assessed. The suitability of this cadaver type for teaching and training was considered; specifically, its utility as a training model for ultrasound-guided regional anaesthesia (USGRA). The saturated NaCl solution method conferred long-lasting preservation of structures with retention of tissue colour and pliability; however, the rapid onset of deterioration occurred when gross dissection began. The cadavers proved to have some utility as simulation models for USGRA training, but lack of vascular circulation limited this suitability.

List of Abbreviations

In alphabetical order

ATEX	atmosphere explosive (in EU safety legislation)
BCE	Before the Common Era (Gregorian Calendar)
CE	Common Era (Gregorian Calendar)
CAHID	The Centre for Anatomy and Human Identification, University of Dundee
CPD	continuous professional development
CUH	Cork University Hospital
DI	deionised (water)
LA	local anaesthetic
PNB	peripheral nerve block
RA	regional anaesthesia
ROM	range of motion
SSS	saturated salt solution
SST	surgical skills training
US	ultrasound
USG	ultrasound-guided
USGRA	ultrasound-guided regional anaesthesia

Chapter 1

Introduction

1.0 Introduction

The acquisition of anatomical knowledge is an essential aspect of the education of medical, dental, health sciences, and science students in third-level institutions throughout the world. The provision of human cadaveric material is central to the ability of medical schools and universities to teach anatomy to their students. Donated bodies in anatomical programs must be stored and dissected over long periods of time, which necessitates preservation with chemicals to ensure tissue degradation does not occur. This has historically been achieved with the use of formalin-based solutions. Numerous concerns exist in relation to the negative effects of and health risks associated with formalin; its use is strongly discouraged by the EU. It is widely acknowledged to cause ocular and nasal irritation, skin irritation, and, more seriously, is a carcinogen which can lead to oropharyngeal cancers and leukaemia in those who are chronically exposed. There are also environmental concerns with regard to the disposal of waste formalin, and to the burial and cremation of bodies treated with formalin or its polymers. These bodies may leach formaldehyde into the air or soil.

Formalin-fixing also affects tissue morphologically, discolouring the tissue and resulting in a hard fix with little pliability which does not bear a close resemblance to living human tissue. This change in texture can aid technicians and students in the basic processes of dissection, but anatomical structures can be difficult for inexperienced dissectors to distinguish between and can lead to errors of identification or accidental removal of structures. These features render formalin-fixed tissue largely useless for any post-graduate training, where the fidelity of

cadaveric tissue to that of live patients is more critical. Despite these disadvantages, formalin-fixation is relied upon globally for its predictable effects.

No method of preservation has yet been achieved which results in an "ideal cadaver" – one with realistic appearance and texture, tissue pliability, joint flexibility, and long-lasting, reliable conservation. Innovative methods have been devised in recent years with many or most of these properties, but visual and textural fidelity generally comes at the cost of longevity, and vice versa. As developments continue, the search for a perfect embalming method may be resolved in the near future.

1.1 Anatomy teaching

The search for anatomical knowledge has preoccupied scientists for millennia. Humans have long sought to understand the nature of our existence, and analysis of the workings of the human body was thought to give insight into the workings of the soul (Aristotle).

The first documentation of formal study in the field of medicine or anatomy is found in Ancient Egypt, with evidence suggesting that these kinds of studies were also found in India and Persia (Owolabi et al., 2017). This was fundamentally practice-based anatomical knowledge, being required for and applied during embalming procedures at the time but not investigated further (Habbal, 2017). The Ancient Greeks elevated anatomy to a science, with a number of well-known figures contributing to the discipline and performing some of the first systematic human dissections (Papa et al., 2019). From the 2nd century CE, due to restrictions on the dissection of humans,

anatomical learning advanced little, with anatomists depending heavily on the work of the ancient physicians and philosophers (Owolabi et al., 2017) and supplemented largely with animal dissection. Clear progress was made in the Arab world, including the description of pulmonary circulation and coronary circulation in the 13th century CE (Siddiquey et al., 2009). The late period of this Islamic golden age coincided with the Early Middle Ages in Europe, and the revival of anatomical investigation there. Contemporaneously, the University of Bologna was establishing its credentials as a medical school. At the time, a dissection of executed criminals was permitted at least every five years in Salerno (Pilcher, 1906). Attendance at the dissection was mandatory for anyone wishing to practice medicine or surgery in the kingdom (Papa et al., 2019), indicating the increasing consideration being given to dissection in the teaching of anatomy.

Bologna remained the centre of dissection-based teaching for a large part of the late 13th and early 14th centuries CE. Dissection-based anatomy teaching continued to grow and develop both in Italian loci and also in more northern areas of Europe from the 14th century on. Official dissections were public affairs, presided over by the professor of anatomy who would customarily sit at the head of proceedings and read aloud from Galen, while an assistant or prosector performed the physical act of dissection and the relevant structures were pointed out (Mayer, 2012).

Andreas Vesalius, the physician who held the chair of anatomy and surgery in Padua, broke with long anatomical tradition and performed dissections himself, encouraging students to participate (Mesquita et al., 2015). His didactic style of encouraging direct

observation and scientific enquiry diverged sharply from the prevalent mode of anatomical teaching at the time. Seven years after taking the post, Vesalius published his magnum opus of human anatomy, *De humani corpora fabrica*, the foundations of which were his own observations of anatomy based on his dissections. The *Fabrica* had an irreversible effect on anatomical teaching and learning, and imparted to cadaveric dissection a supremacy which has hardly lessened up to today. It laid the groundwork for some of the most important anatomical discoveries of the time, such as Harvey's description of the circulatory system.

From the Italian schools of medicine of the 14th century to those across Europe in the 19th, the acquisition and conservation of human bodies for dissection purposes was to prove increasingly problematic. Public dissections were scheduled for cooler months and took place over no more than a few days, rudimentary measures which were taken in an attempt to minimise the deterioration of the corpse during the study period (Papa et al., 2019). The anatomical study period began in October and ended in April/May for this reason. The dissection was also usually performed in an order which would allow the anatomical regions most susceptible to spoiling to be dissected first.

Some enquiries were made into devising a stable means of preservation, with mixed results. More permanent, accurate representations of body structures were required to fill the gaps in anatomical teaching caused by this inconvenient lack of body preservation. From the late 1600s, realistic wax models grew in popularity and use (Ballestriero, 2010). The anatomist Antonio Scarpa believed the waxes were best used

to depict structures which could not be conserved for long due to the lack of preservation techniques (Riva et al., 2010). In fact, many of the waxes show signs of decomposition in the organs that they were moulded from, particularly the earliest ones, being cast from human cadavers which the dissectors were unable to prevent from decaying while they worked (Riva et al., 2010). This inability to prevent degradation to any great degree seriously hampered anatomical studies for centuries, until reliable chemical preservatives came into use.

1.2 Cadaver preservation methods

1.2.1 History of body preservation

The various ways in which body preservation may occur fall into two broad categories: natural means and artificial means. Natural preservation may come about by freezing, dessication, or due to soil chemistry. Artificial means include any method where preservation is deliberately pursued through the application of heat, cold, or chemicals (Brenner, 2014), and is often accompanied by further treatment of the corpse; for example, evisceration, injection, wrapping, or the addition of items such as amulets, masks, or other decorative or protective objects.

The reasons for causing bodies to be preserved historically are not always clear. Culturally or politically significant individuals have often been preserved for commemorative purposes or veneration; the remains of saints, royals, and other persons of note have been conserved in Europe right through the middle ages and into the 20th century. Other, more practical, circumstances have required bodies to be specially preserved, such as transportation of military or diplomatic personages across long distances (Troyer, 2020). Many indigenous cultures around the world still practice

mummification of family members to ensure their continued participation in the community.

The historical record contains numerous cultural exemplars of embalming, the most well-known being that of Ancient Egypt. A 70-day embalming process took place which, in its most basic form, removed internal organs to help prevent decay, after which the body was dehydrated using natron salt. More advanced forms of this process utilised wrappings and unguents, both internal and external. Numerous other cultures have relied on embalming bodies using a variety of materials. Historical accounts mention honey, spices, alcohols, waxes, gum, and pitch among the substances utilised to conserve human remains.

The art of embalming remained largely unchanged through the first 1200-1500 years of the Common Era. Early efforts to conserve bodies in an anatomical setting were not dissimilar to the methods employed above, enhanced by the use of various alcohol solutions and aromatics such as aloes, myrrh, wormwood, rosemary, pumice, marjoram, and coloquintida (colocynth, a bitter plant). Salt, alum, turpentine, and cerecloth (waxed cloth) were also widely used in historic embalming recipes (Mayer, 2012).

It was not until the 17th century that the technique of arterial injection came into use. This provided a leap in efficacy great enough that it has since become the standard technique for funerary and anatomical embalming. Frederick Ruysch is generally credited with perfecting this technique, but it was first used by fellow scientist Jan

Swammerdam and was also used and modified by the anatomist Stephen Blanchard (Mayer, 2012). Swammerdam originated the use of the technique in insects and small animals, and Ruysch applied it to human subjects.

Anatomists in subsequent decades made changes and improvements to the chemical mixes, such as the Scottish anatomist William Hunter, the Italian anatomist Giuseppe Tranchina, French doctor J.P. Suquet , and Cuvier (Brenner, 2014). John Morgan, professor of Anatomy in the University of Dublin, advanced two crucial principles of embalming practice: the use of the largest possible artery for injection, and the use of pressure to increase the transport of preservative through the embalmed body. He is also the earliest known user of a pre-injection solution (Mayer, 2012).

The growth and development of anatomy as a discipline was fundamentally affected by the speed with which unpreserved bodies had to be dissected. The Renaissance period of European history and its emphasis on learning and discovery, particularly in the sciences, accelerated interest in embalming bodies to ensure that fuller study could be made of them. The emergence of long-term methods of embalming was greatly accelerated in the 19th century CE, with the American Civil War in particular precipitating large changes in the practice.

1.2.2 Freezing

Freezing is used in both anatomical and commercial settings to effect long-term storage of cadavers by halting cellular processes and inhibiting microbial growth. It is

important that the tissue is frozen as quickly as possible after death for best results. Freezing equipment is widely available, and no special treatment of the cadaver is required beyond careful wrapping, making it a useful and safe method of preservation that can be maintained almost indefinitely. This method will only preserve tissue for as long as the low temperature is maintained. If the cadaver is removed from cold storage, the natural processes of decay will resume and the tissue will begin to decompose.

Further degradation can come about from the freeze-thaw cycle itself, as ice crystals form in the extracellular matrix and cellular damage occurs through rupturing of the cell membrane and changes to the osmotic pressure of the cell (Li et al., 2018). This can negatively alter the texture and other haptic properties of the tissue. For the most effective cryopreservation, a temperature of -80°C is used, with quick freezing to reduce ice crystal size in order to avoid damage as far as possible. However, many body storage facilities use a more moderate temperature of -20°C and fine control over the speed of freezing and thawing is unachievable. If stored over prolonged periods of time, the tissue can dry out and suffer further damage from freezer burns and oxidation.

1.2.3 Chemical embalming

Embalming is the intentional application of a chemical preparation to a cadaver for the purposes of conserving it. The goal of anatomical embalming (as opposed to funeral embalming) is to maintain the body in a prolonged state of preservation in order to allow extensive dissection to occur.

Fixative substances can be sub-divided into four main chemical groups – aldehyde fixatives, oxidising fixatives, alcohol-based fixatives and metallic-group fixatives. Embalming chemicals can act in a number of ways to fix the tissue. Oxidising agents and aldehydes are part of the larger protein cross-linking group, while alcohol-based chemicals fall into the protein denaturing group. Metallic-group fixatives form insoluble precipitates (Thavarajah et al., 2012). Many fixative chemicals also have an antiseptic effect, which prevents decomposition by inhibiting or ceasing microbial activity within the body. While many fixatives have been identified over the years, few are being used routinely in anatomy facilities.

1.2.3.1 Main Anatomical Fixatives

Formaldehyde was discovered accidentally in 1859 by Alexander Butlerov and isolated in 1868 by the German scientist August Wilhelm von Hofmann. As the century went on, interest in the compound increased and its properties were described further, bringing new industrial applications. In the 1880s it was found to be antiseptic, and in the 1890s was observed by several scientists to harden tissues and cause coagulation of the blood (Musiał et al., 2016). That decade marked the beginning of formaldehyde's use in histological and anatomical preparations. By the end of the 1890s it had largely replaced alcohol as the primary fixative in many laboratory contexts and was already in use as a means for preserving human bodies (Musiał et al., 2016).

The use of formaldehyde as a cadaveric preservative increased in frequency in the 20th century in most Western countries. Alfredo Salafia began working as an embalmer and restorative art practitioner around the turn of the 20th century (Mayer, 2012). It is not understood exactly how he came to embalming, but what is known are the results of his work. He began by experimenting on animals before being given permission to work on unclaimed bodies in Palermo, Sicily (Piombino-Mascali et al., 2009). Salafia did not disclose his methods or formulations while alive. Archival research along with inspection of Salafia's own unpublished notes showed that his preparation was formaldehyde-based, with additions of zinc sulphide and zinc chloride, glycerine, alcohol, and salicylic acid. This is one of the earliest recorded uses of formaldehyde as an embalming chemical seen in Europe (Mayer, 2012).

Formalin use became a standard practice for anatomical embalming, and although studies of its comparative effects were carried out throughout the decades, no other chemical has proven to be as reliable or as cost-effective. For this reason, formaldehyde is the principal fixative in use worldwide. Health concerns did, of course, emerge and increase over time; however, in practice this had little effect on the extent of formalin use until much later in the 20th century (Musiał et al., 2016). Anatomical preparation experienced few significant changes or true innovations until the invention of plastination (von Hagens, 1979). The last four decades have seen increased technical creativity and technological advances, driving the process of anatomical embalming to evolve in a number of significant ways and drastically changing what can be achieved in terms of cadaver appearance, texture, applications and uses.

Formaldehyde (methanal, CH_2O) is a small molecule, occurring as a colourless gas which is highly soluble in water. It is a naturally-occurring organic compound and it spontaneously polymerises, forming solid paraformaldehyde. Industrially, formaldehyde is created by the catalytic oxidation of methanol. Formalin is the aqueous solution of formaldehyde, produced by bubbling the gas through water until saturation has been achieved. Formalin is generally available at a concentration of approximately 37% formaldehyde by mass, which solution may then be diluted by the user to the desired concentration. For example, common anatomical practice is to create a 10% dilution, resulting in approximately 4% formaldehyde concentration. Methanol may be added to the solution in small amounts as a stabiliser, to prevent the formaldehyde from precipitating out of solution or oxidising to formic acid. The hydrated form of formaldehyde is methylene glycol (methanediol, CH_4O_2), and it is the active compound in any formaldehyde solution.

Formaldehyde is classed as a disinfectant due to its broad spectrum antimicrobial action. It reacts easily with a number of functional groups of biological molecules, initially forming cross-linkages between primary amino groups such as amines and thiols (Thavarajah et al., 2012), and subsequently creating covalent methylene bridges between groups of proteins in the cells (Fraenkel-Conrat et al., 1947). This results in fixation of tissue by making cell proteins unavailable for autolytic reactions.

Formaldehyde exhibits what is referred to as the penetration-fixation paradox – it penetrates tissues quickly and easily due to its small molecular size and the great

osmotic pressure it exerts, yet fixation occurs more slowly as the secondary covalent bonding takes time to be established. Once created, the cross-linkages are extremely stable and resistant to breaking down, providing potent and long-lasting fixation. This is one of the primary advantages of formaldehyde as a preservative in an anatomical context. Specimens and cadavers may be successfully stored and utilised in teaching for many years, with only mechanical degradation occurring as the tissue is handled repeatedly. It also results in one of the primary disadvantages of formaldehyde use, which is to say that the tissues of the body display a very different and unnatural texture and colour to those of living tissues. Another disadvantage is that formaldehyde interacts with bilirubin in the skin of jaundiced donors to cause a notable greening reaction. This colour change is likely to be alarming to students and is not conducive to maintaining the dignity of anatomical donors.

Glutaraldehyde is another member of the aldehyde family of chemicals. It was first synthesised in 1908, and was utilised in small quantities as a laboratory reagent for several decades before its recognition as a potent disinfectant and embalming compound in the 1960s (Brenner, 2014). It was shown to have a greater disinfecting effect than formaldehyde, and the development of a reliable industrial method of production precipitated its common use as a medical disinfectant (Bedino, 2003).

Glutaraldehyde (pentanedial, $C_5H_8O_2$) is a larger molecule, occurring as a colourless, oily liquid with a pungent smell, and is completely soluble in both water and ethanol. It is produced industrially by the oxidation of cyclopentane. It is an extremely effective sterilant, being active against spores and viruses in addition to other common

microbes. It typically produces fixation via bridging reactions involving the amine and thiol groups (as with formaldehyde) and extremely strong protein cross-linking.

Glutaraldehyde differs from formaldehyde in a number of significant ways. Rather than the rapid penetration and slow fixation of formaldehyde, glutaraldehyde displays the opposite effects of slow penetration but rapid and permanent fixation of tissue (Bedino, 2003). It is now most often used in the histological preparation of tissue for electron microscopy. Glutaraldehyde has long been suggested as the most obvious replacement for formaldehyde as a cadaver fixative. It does not react with bile salts in cases of jaundice, thereby avoiding the greening reaction seen with formal(Bedino, 2005)dehyde (Bedino, 2005).

Glutaraldehyde, like formaldehyde, is also a strong respiratory irritant and skin sensitiser. In 2021 it was added to the EU's Candidate List of substances of very high concern (SVHC) due to its sensitising effects, considered the first step in the process of a substance being designated as an Authorised substance (having its use restricted). Glutaraldehyde is considered to be toxic, but has not been correlated with carcinogenic effects in the same manner as formaldehyde. Nevertheless, it is generally considered prudent to reduce or eliminate exposure to this chemical in order to mitigate the risks to health.

There are a number of other substances commonly added to embalming mixes which have health implications. Phenol and methanol are routinely used in embalming solutions. Phenol is used as a supplemental preservative and increases the ability of

the fluid to penetrate tissues, and owing to its efficaceous anti-fungal action it also provides an additional disinfecting effect. The bleaching action of phenol also reduces tissue greying which results from formaldehyde fixation. It is a hazardous chemical: highly irritating to skin, eyes, mucous membranes, it can cause chemical burns in cases of skin contact. Chronic exposure can result in other alarming symptoms such as cardiac arrhythmias, vertigo, and anorexia. It is considered to be toxic. Methanol is added to embalming solutions as a stabiliser, to prevent the precipitation of formaldehyde out of solution. It is also highly toxic and a strong CNS depressant; methanol vapours can cause blindness, coma, seizures, and confusion if inhaled.

1.2.4 Modern chemical fixatives

Recent decades have shown a move toward the development of less hazardous and more life-like methods of preservation in anatomical facilities. This has resulted in a proliferation of new soft-fixation methods, some more successful than others. The most desirable properties for any embalming solution are generally considered to be the medium- to long-term preservation of tissues without changes to shape or gross appearance, and the inhibition of microbial growth. To all this, one must add the conservation of pliability, colour, and texture where possible. The primary impetus behind the elucidation of these novel embalming methods has been a rise in demand in the last decade for post-graduate surgical skills education/CPD, and the complete unsuitability of formalin-fixed human tissue for this purpose.

The pre-eminent soft-fixation method is that described by Thiel which is noted for the extreme flexibility and suppleness seen in cadavers. It is achieved with the use of

distinct venous and arterial stem solutions. The colourless embalming solutions include monopropylene glycol, various chemical salts, and boric acid among other chemicals, along with a small amount of formalin. The cadaver is then typically immersed in a third variation of the base solution for a period of time to allow maceration to occur. Originally, the method called for additional perfusion of organs in the body cavities using endotracheal tube, gastric tube, and via the superior sagittal sinus. Anatomical technicians have also made use of intra-orbital injection to further enhance preservation of the brain. Some modifications to this process have taken place since the technique was published, as anatomical departments have begun to experiment with the embalming method to better suit their requirements (Reddy et al., 2017, Hayashi et al., 2014). Thiel-embalmed cadavers retain some colour (a red dye is perfused post-embalming to enhance this) and much flexibility, meaning they will respond to surgical interactions in a more life-like way. However, in practicality the cadavers are hypermobile, and investigations into the biomechanical properties of both human and animal tissues showed altered histology when compared to fresh-frozen tissue, which is the other preservation method most frequently utilised in surgical skills training (Unger et al., 2010).

Other proprietary techniques and embalming solutions have been developed by universities and private companies to achieve similar effects, including Fix 4 Life (van Dam et al., 2015), Genelyn, N-vinyl-2-pyrrolidone (NVP) (Haizuka et al., 2018), and various glycol compounds (Goyri-O'Neill et al., 2013). These are generally not as well-known or commonly-used as the Thiel method. Many, or indeed most, still make use of formalin, albeit in greatly reduced quantities (typically 1% or less); they can

frequently be expensive or require very specific constituents or extra equipment. Current literature has begun to focus on methods which are safer for both the staff member and student, which offer preservation of tissue along with conservation of life-like features, and which are more economical in terms of cost.

Chemical salts feature frequently in many of these modern soft-fix embalming solutions, such as ammonium sulphate, potassium dihydrogen sulphate, various nitrite pickling salts (Janczyk et al., 2011), and sodium salts.

Table 1: comparison of modern (soft) fixative solutions used for anatomical education and/or surgical training

Method	Main fixative chemical	Reported duration of preservation	Source
Thiel	propylene glycol	>1yr	Thiel (1992)
Genelyn	formaldehyde	not reported	Genelyn (2010)
NVP	N-vinyl 2-pyrrolidone	2 yrs	Haizuka et al (2017)
Fix 4 Life	aldehydes (incl formaldehyde)	not reported	Van Dam et al (2015)
Dodge Company	formaldehyde	>2 years	Dodge Co. MSDS info
Universidade Nova de Lisboa	diethylene and monoethylene glycols	6 mo – 1yr	Goyri-O'Neill et al (2013)
Coleman & Kogan	salt, formaldehyde	12 – 15mo	Coleman & Kogan (1998)
SSS	salt, formaldehyde	>14 days	Hayashi et al (2014)
MSSS	salt, formaldehyde	not reported	Kathapillai (2019)
University of Santiago de Compostela	salt	1 – 5yrs	Lombardero et al (2017)

The use of salts and salt solutions in embalming is a practice seen as far back in history as Ancient Egypt, but until relatively recently was overlooked in academia. Embalming with a saturated salt solution is a method of achieving all of the above aims which has come to renewed prominence in scientific literature of late. Studies at both a microcellular level (Olszewski et al., 2005) and a macrocellular level (Al-Saraj, 2010) have shown that fixation with sodium chloride can provide preservation outcomes not-dissimilar temporally to that of formalin-fixation. Tissue structure and morphology remain unchanged and histological analysis supports these findings in many of the investigations. The principle was re-appraised by a Japanese group of colleagues (Hayashi et al., 2014) as a mode of preservation for use in surgical skills training, and it was found that in a surgical-skills lab environment, cadavers embalmed with a low-formaldehyde saturated salt solution performed equally as well as Thiel-embalmed cadavers on a number of points. Further investigations into this principle (Homma et al., 2019, Shirai et al., 2015, Burns et al., 2018, Watanabe et al., 2020) again demonstrated the general utility of SSS-embalmed cadavers for other types of surgical training such as plastic surgery and trauma surgery. It is important to note that these solutions do contain formalin in extremely low concentrations; they are not pure sodium chloride solutions.

The saturated NaCl method has been discussed frequently in the literature in a non-human context, often in low-income nations. Veterinary studies largely use canine cadavers for anatomy, but other small- to medium-sized mammals have also been used, such as rabbits and goats. Lower-income countries are often in need of an effective yet cheap and readily-available embalming solution (Gosomji et al., 2018)

and saturated NaCl solution fills this brief extremely well. The ability to store animal cadavers for prolonged periods of time reduces the number of animals required and allows for the gross dissection of the animal to be undertaken by students over a more consistent and moderate timescale.

1.2.5 Plastination

Plastination is a relatively new method of preservation, developed by Gunther von Hagens in 1977. It involves the replacement of water and lipids in the cells of biological material with resins and silicones which are then hardened (von Hagens, 1979). The result is a robust, fully inert specimen with natural colour, which can be handled safely and will not degrade. Specimens are first fixed (e.g. with formaldehyde) and then dehydrated, before the polymers are forced into the tissue in a vacuum and the resulting specimen is cured to complete the process. Some shrinkage can occur during the process, particularly with fatty organs such as the brain. The equipment required for this procedure is complex and can be expensive, requiring multiple freezers with vacuum chambers, and as acetone is the primary solvent in this method an explosive-resistant area – specifically in Ireland, an ATEX rated area (complying with EU Directives 99/92/EC and 94/9/EC) – is required to safely carry out the polymerisation process. It also creates the requirement for accessory safety features such as chemical storage. The consumables are specialist chemicals provided by a small number of companies, and the considerable amount of free space needed for such an extensive array of equipment can be another obstacle. In an anatomical teaching context, this method of preservation is better employed on smaller prosections or individual organs, which can be stored with little fuss; they can be readily used in class to

demonstrate structures which are often quickly damaged in fixed specimens through frequent (mis)handling, or variations which are not commonly observed. In this way, smaller plastinated specimens may be viewed as a direct successor to the traditional potted specimen.

1.3 Characteristics of embalmed cadavers

The phrase “embalmed cadaver” traditionally elicits mental images of rigid grey flesh and harsh chemical smells. Students very often have strong memories of their experiences in the dissecting room, particularly the impression made on them by the cadavers (Chang et al., 2018, O'Carroll et al., 2002). Anatomical dissection requires the preparation of material for teaching purposes, and as such it is crucial that this material will last as long as is needed to fulfil the teaching objectives of the department in question. At a basic level, cadavers must last at least as long as the teaching period, for gross student-led dissection, and much longer for prosection-based teaching, as the cadaveric material is commonly used for multiple teaching periods across multiple student groups.

Formalin-based embalming agents provide safe, stable, long-term preservation for anatomical education due to their excellent antiseptic properties, but with several notable disadvantages. First, the cadaveric tissue is made harder and largely immobile by the action of the formaldehyde on proteins. This can be useful for novices in terms of developing their dissection skills, as the tissue generally behaves predictably under the scalpel or scissors and musculoskeletal structures are easy to separate; it is especially helpful for the preservation of the brain, as the greatly increased firmness

results in the retention of cerebral shape and features, and the brain can be successfully sectioned. Overall, though, the subtler distinctness of certain tissue types is lost (for example, glandular material can appear extremely like adipose tissue), and the general cadaveric appearance is desaturated and not very like that of a live human. This often leads to misjudgements and errors of identification when dissecting – such as the removal of adrenal glands along with perinephric fat – and increased difficulty in discriminating between visually similar tissues which demonstrators or other teaching staff may show. Difficulties in differentiating tissues may affect students' capacity to positively identify structures. It may require more manual effort in order to dissect, due to tissue resistance and firmness caused by the formalin fixation. Manipulation of the limbs post-fixation is not generally possible, frequently making regions such as the axilla or the palm of the hand inaccessible.

As post-graduate surgical skills training (SST) more commonly becomes a level of instruction provided by anatomy departments and medical schools, the need for an improved cadaveric model increases. The enduring use of hard cadaver fixation can be somewhat justified for undergraduate teaching, despite its drawbacks, as the durability of the material often outweighs other considerations; formalin-fixed tissue, however, is supremely unsuitable for surgical training as it bears no resemblance whatsoever to the textural or visual properties of living human anatomy. Surgeons and other medics frequently rely on haptic cues such as tissue resistance to perform procedures effectively; this is impossible with hard-fixed cadavers.

Soft-embalmed cadavers, on the other hand, generally lack the extreme durability of hard-fixed cadavers but this is compensated for by its many positives. Regardless of the soft-fixation method employed, the cadavers overwhelmingly retain pliability, flexibility, realistic colouring, and accurate texture far in excess of that of hard-fixed bodies. Dissection becomes less arduous as the tissue planes separate with ease, and the need for a scalpel is reduced, resulting in fewer chances for accidental severance of or damage to structures. Studies have investigated the antimicrobial effects of these solutions (Hayashi et al., 2014, Balta et al., 2019a) as a proxy for estimating effectiveness in preventing decay. Formaldehyde performance is unmatched in this respect; however, the antimicrobial effects of soft-fix solutions were generally found to be very good, indicating the potential for satisfactory longevity of preservation.

1.4 Concerns associated with embalming

The foremost concern with formaldehyde-based embalming in recent years has been identified by most of the previous literature in this field as its toxicity. Formaldehyde is classed as a known carcinogen by a number of official bodies such as the International Agency for Research on Cancer (IARC) and the United States' National Toxicology Program (NTP), and is listed as a probable carcinogen by many more. It has been shown in a number of occupational research studies and epidemiological studies that there is a clear link between persistent formalin exposure and increased risk of cancers of the nasal sinuses, the nasopharynx, and leukaemia. At the lower end of the scale, exposure to formaldehyde causes unpleasant nasal and pharyngeal irritation, stinging and watering of the eyes, and skin irritation if splashed or otherwise brought into direct contact with the body. Glutaraldehyde-based embalming solutions are

frequently proposed as a viable alternative, however the ability of glutaraldehyde to cause similar or other adverse reactions in users cannot be ignored.

This potential for harm can be mitigated with good ventilation and the use of adequate PPE when handling formaldehyde- or glutaraldehyde-containing substances, but as many facilities worldwide are located in countries or regions where under-resourcing may be an issue, the ability to successfully reduce this risk is variable. Most of the potential risk is borne by technical staff who are responsible for making up the solutions and perfusing them into the cadavers.

Soft-fix embalming solutions aim to reduce this risk (or, ideally, eliminate it altogether) by radically reducing the amount of formaldehyde required to effect preservation of the cadaver; however, many of these solutions still include harsh chemicals with comparable levels of potential for harm as formaldehyde – diethylene glycol, monoethylene glycol, phenol, NVP are all potentially seriously harmful substances – and the handling of formalin, even in small quantities, carries risk.

1.5 Ultrasonography and the use of cadavers

Ultrasound (US) imaging has become widely used as a way to improve the delivery of regional anaesthesia in the past two decades. Traditionally, techniques such as nerve blocks have been performed using the anatomical knowledge and procedural experience of the practitioner, and rely on haptic feedback to gauge the success of the needle placement. This understandably allows for a number of accidental adverse consequences such as intraneural injection, intravascular injection, and failure to

adequately anaesthetise the target region. The dangers attendant with these could include peripheral nerve damage, cardiovascular events, and seizures. In the case of failed regional anaesthesia, a patient may have to undergo general anaesthesia which brings extra risk.

While nerve stimulation was introduced as an additional safeguarding modality in the 1970s, the adoption of ultrasound imaging has added a means of visually confirming the accuracy of needle placement and block spread. It is generally thought to have improved the patient experience and made the delivery of regional anaesthesia safer and more successful. A 2009 review of literature on the use of US guidance during peripheral nerve blockade (PNB) found that the time taken to perform the block was reduced by several minutes, the practitioner required fewer needle passes to place the block, and the onset of analgesia was achieved more rapidly when compared to traditional methods (Liu et al., 2009). The duration of the anaesthesia was not affected by the earlier onset, and in many cases less anaesthetic was used compared to blind blocks. These conclusions were broadly supported by a second contemporary review (Walker et al., 2009). The reviews in question and a number of other subsequent papers did not find any evidence for a corresponding decrease in anaesthetic failure rates or other positive changes in the occurrence of side-effects. Nevertheless, US imaging has been firmly established as a crucial component in the effective delivery of regional anaesthesia to patients.

As with any medical specialty, adequate training of anaesthesiologists in both ultrasound visualisation and block delivery is extremely important to avoid poor patient experience and reduce risk. Historically, this was achieved by gaining experience in an apprentice-like manner – first by observation, building to assisting before finally being allowed to practice on patients. Nowadays, this training paradigm has fallen out of favour, due in part to greater concern for the patient experience, issues of consent, and the possibility of litigation. It is generally acknowledged that modern procedural training requires repeated, deliberate practice of the relevant skills. Opportunities for this type of repeated practice of skills are no longer easily found in a clinical setting, and labour initiatives such as working time directives further reduce the time in which repeated practice can be accomplished. The need for alternate training opportunities has existed for many years, and a number of simulation modalities are available to trainees.

Simulation phantoms occur in many forms, ranging from the very simple to the complex (Hocking et al., 2011); (Kim, 2016). Water phantoms, gelatin phantoms, rubber phantoms, meat phantoms and cadaveric phantoms present a variety of opportunities for trainees to practice. Virtual reality or high-fidelity mechanical simulators are other ways by which a trainee may gain non-hospital experience in carrying out clinical techniques.

A number of factors contribute to delivering a robust training experience, including replication of key motor skills, durability (which allows for repeated practice), and

visual and haptic feedback which resembles that of live patients. The different types of phantom display varying disadvantages: they can be expensive, difficult to obtain, lack durability, require the euthanasia of animals, be unrepresentative of human anatomy, or only replicate extremely basic aspects of ultrasound visualisation or performance of nerve block. A perfect simulation model is highly desired but as yet has not been developed.

Cadaveric phantoms are understood to represent the training model with the greatest physical fidelity to a clinical setting with live patients. Several studies have been carried out in the past 10 years or more to assess the applicability of both embalmed and unembalmed cadavers as phantoms for verifying technique and for clinical training. Some of the initial studies evaluated the conventional formalin-embalmed cadaver as a training phantom (De Maeseneer et al., 2004); (Tsui et al., 2007); (Schramek et al., 2013, De Maeseneer et al., 2004), followed by investigations into the potentially greater suitability of non-traditional, soft-fixed cadavers. Examination of these newer models was occasionally carried out on imaging potential only (Balta et al., 2017); (Meek et al., 2018), however, the greater proportion of these studies on soft-embalmed cadavers were concerned with clinical aspects of USGRA technique. A recent overview of imaging equipment as used in cadaveric studies noted that the majority of published literature focused on assessing the technical capabilities of US-guided procedure (Simonds et al., 2018). This is particularly noticeable in the 2000s, as the technique was novel and its clinical applicability was routinely assessed for performance of numerous individual nerve blocks. It soon came to be tested as a

clinical training model. Thiel cadavers were among those most frequently assessed (McLeod et al., 2010) (Benkhadra et al., 2009); (Kondrashova et al., 2020) but other soft fixation models were also appraised, such as Anderson light-embalming technique (Wadman et al., 2010); (Adhikari et al., 2014). Fresh cadavers have limited utility as US phantoms due to the frequent presence of tissue gas, which forms due to post-mortem biochemical changes. This produces gas artefacts on the US image, creating dark, hypoechoic areas on screen with potential distortion of structures below. Both formalin-fixed and soft-fixed cadavers produce very clear US images, a quality attributed to the increased volume of fluid introduced during embalming and the excellent echogenicity of water. As shown in the published literature, soft-fixed cadavers strongly outperform hard-fixed cadavers as a phantom in that they can be posed to allow access to more difficult regions to scan such as the axilla. This is almost impossible in traditional hard-fixed cadavers.

Despite growing interest in saturated salt-embalmed cadavers for surgical skills training, and some recent work assessing the suitability of Thiel embalming to produce US training models in the veterinary field (Thanaboonnipat et al., 2021), no work has yet been carried out to establish the potential utility of saturated NaCl cadavers as phantoms for the purposes of training clinicians to perform US-guided regional anaesthetic blocks.

1.6 Aims

The primary aim of this study is to explore the longevity of pure sodium chloride in solution as an agent of preservation in a modern anatomical setting. Soft-fixation methods are generally viewed as having short- to medium-term effectiveness in preserving human and animal tissue. Previous studies have provided encouraging indications of the ability of a saturated NaCl solution to confer extended protection from decay. I will endeavour to demonstrate the long-term effectiveness of sodium chloride-fixation on cadavers which have the potential to be used in a number of educational settings. Particular to this study will be the use of a simple sodium chloride solution, unenhanced by the addition of formaldehyde or any of the common chemical components of other soft-fixation solutions, such as phenol or alcohols. This will provide an opportunity to qualitatively assess the merits of saturated sodium chloride solution on its own. There is much scope for investigation here, as this method is not widely used in human anatomical circles and it merits further enquiry. The resulting cadavers will be considered for their suitability as teaching models for anatomical instruction and clinical training, with particular emphasis on ultrasound-guided regional anaesthesia (USGRA).

Chapter 2

Materials and Methods

2.0 Materials and Methods

2.1 Body donation

The cadavers used in this study were all donors to the University College Cork Anatomical Donation Program. The UCC Department of Anatomy and Neuroscience receives anatomical bequests from the Munster region of Ireland, comprising the counties of Cork, Kerry, Limerick, Tipperary, Waterford and Clare. All donors voluntarily consent in writing, before their deaths, to allow the use of their body for medical education, research and training. The Department receives an average of approximately 25-30 donated bodies per annum, which are most commonly held for a study period of up to three years. At the time their decision is made, donors may elect to bequeath their remains permanently, an option which was first introduced by the Irish Medical Council in 2017, or to limit the period of study. They may also choose to allow the University to retain some body parts indefinitely, to be used in teaching for several years after the remainder of their bodies have been returned to relatives.

2.2 Experimental groups

The experimental design included two groups of two cadavers embalmed at different time points (Figure 1). The cadavers were selected as the first available in a condition suitable for embalming and surplus to teaching needs.

The first group (Group A), consisting of two male donors, was embalmed in January and February of 2018 using the solution prepared in the laboratory (Cadavers A1 and A2, Figure 1). The cadavers were initially prepared for embalming less than 48 hours post-mortem. The embalming solution for this first group was prepared in our

laboratory. 50 litres of salt solution were prepared, consisting of 36g of commercially-available compacted NaCl per 100ml of solution. The salt was broken up as much as possible and dissolved into the appropriate amount of warm deionised (DI) water.

The cadavers for the second group (Group B) were initially prepared for freezer storage, as part of a group of routinely stored cadavers, by being washed with Dodge® cleaning concentrate and sprayed thoroughly with Dodge® Dis-Spray antimicrobial solution (The MazWell Group, UK). Orifices were swabbed and sprayed again. Dodge® Kalon emollient cream was applied thickly to the face, hands, and feet to prevent freezer burn, before the subject was wrapped in a plastic body bag and placed into the freezer at -20°C. The interior of the bag was also sprayed thoroughly with Dodge® Dis-Spray. Both cadavers had been in storage since October and July of 2018, respectively, when they were selected for embalming in April and June of 2019. The second group was embalmed with a NaCl solution which was prepared commercially by KB Scientific Ltd (Cork, Ireland), and was guaranteed to have reached a minimum of 35% NaCl saturation. For this second group, a female and a male subject were embalmed (Cadavers B1 and B2, Figure 1).

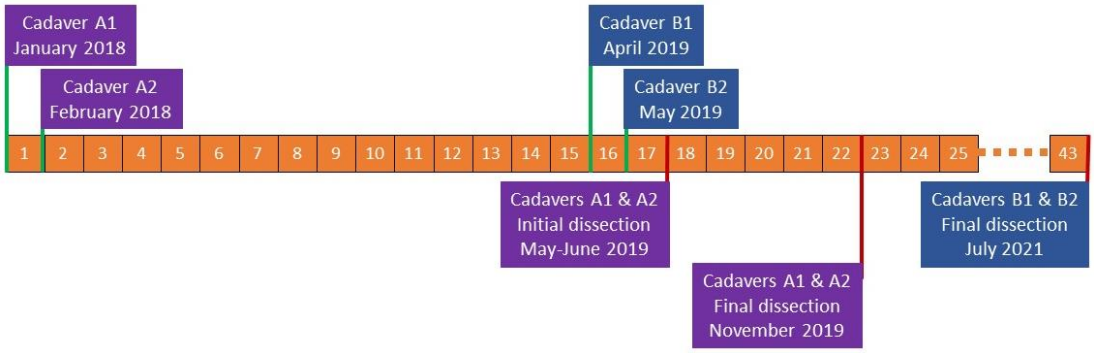


Figure 1: Experimental timeline. The figure shows the main events linked to the anatomical experimental protocol. The orange line identifies the duration of the study in months, starting in January 2018, until completion of the dissections in July 2021. Events displayed above the timeline with a green line are embalming and those displayed below with a red line depict dissection.

2.3 Embalming technique

2.3.1 Group A

For the first group, the cadavers were prepared for embalming as per the University College Cork FLAME Laboratory procedures, less than 48 hours after death. The carotid artery was exposed by dissection and the subject was flushed intra-arterially with 3L of 0.9% saline as a pre-injection solution using a Dodge® Embalming Machine with Automatic Pressure Control. 25L of saturated salt solution was perfused intra-arterially over a period of 2 working days, at a low flow rate, in three distinct phases: two volumes of approx. 10L each were perfused into the thorax via the carotid artery, and the final 4.5L volume was introduced via the femoral artery into the abdomen. Following completion of perfusion, the subject (Cadaver A1) was placed into a plastic body bag with 9L of the same salt solution (consistent with the methodology of Hayashi et al., 2014), wrapped carefully in an additional body bag, and placed into storage at 4°C.

The second subject (Cadaver A2) was embalmed in a similar manner, however the total amount of solution which this cadaver accepted was greatly reduced, being approx. 8L in volume.

Blistering was noted on the limbs of the first subject as a result of increased internal fluid pressure. Some blistering was also noted along the limbs of the second subject. Along with swelling of the eyelids, increased visibility of superficial vasculature, and distension of the abdomen, this was taken by the embalmer as a sign that sufficient perfusion was reached and that the cadaver was adequately embalmed.

2.3.2 Group B

For the second group, the embalming procedure was modified slightly based on the preliminary results of the first group. As the cadavers were removed from the freezer, they were placed on an embalming tray and allowed to thaw gradually over a period of 2-3 days. Goniometric measurements were taken. The femoral artery was exposed by dissection and the subject was perfused intra-arterially with 18L (Cadaver B1) and 16L (Cadaver B2) of the commercially-made saturated NaCl solution at a low flow rate, using a Dodge® Embalming Machine with Automatic Pressure Control. Following completion of perfusion, the subject was placed into a plastic body bag without the addition of extra embalming solution, wrapped carefully in an additional body bag, and placed into storage at 4°C.

Some blistering was noted on the limbs of the third and fourth cadavers; again, most likely as a result of increased internal fluid pressure. The same signs of adequate

perfusion were observed in this group as in Group A and taken as evidence of successful embalming.

For both groups, images were taken of the subjects before storage. Visual checks were carried out at regular intervals to monitor the cadavers for any putrefactive changes or microbial growth with minimal disturbance. At each instance, the first group of cadavers were spot-treated for visible microbial growth, while the second group of cadavers was spot-treated (if required) and sprayed thoroughly with Dodge® Dis-Spray before being placed back into storage. A complete physical inspection was carried out approximately 10 months (Group A) and approximately 8 months (Group B) after embalming to fully assess the extent of preservation.

The first group of subjects was removed from storage at the time of the full inspection and washed thoroughly with Hibiscrub® antimicrobial skin cleanser (Mölnlycke Health Care AB, Sweden) and water to remove any surface debris and minimise potential contamination. Following this, the subjects were placed in a set of fresh plastic body bags with 3L of the embalming solution used for this group as a storage medium and returned to cold storage to await dissection.

The second group of subjects was removed from storage at the time of the ultrasound study (month 34 on Figure 1) and washed with a diluted solution of Dodge® Cleaning Concentrate to remove surface debris and minimise potential contamination. The cadavers were placed in a fresh plastic body bag and sprayed frequently with Dodge®

Dis Spray while the study was ongoing, before being returned to cold storage to await dissection.

2.4 Goniometric measurements

Goniometric measurements were taken from the second group of cadavers to quantitatively assess the degree of flexibility of the cadavers both before and after embalming. Measurements were taken from the right and left shoulders and the right and left hips. For the shoulders, the first measurements were taken in the prone position, following which the arms were abducted to the maximum extent and measured again. The hips were first measured in the prone position, following which the leg was flexed to the maximum extent and measured again. Depression of the mandible at the maximum extent was measured with a ruler. Measurements were taken as the distance between the front upper and lower incisors, or between the gums at the front of the mouth if the donor was edentulous.

2.5 Assessment of bacterial contamination

Where indicated, bacterial contamination was assessed by completing a culture and plate count. Swabs were taken from discrete regions of the cadaver (oral cavity, hand, groin, and toes) and suspended in 1 ml LB broth. Serial dilutions (1:1,000, 1:10,000 and 1:1,000,000) of the base swab solution were prepared, and 100 µl of each solution plus one direct spread were plated on LB agar Petri plates without antibiotics. A clean swab was used as negative control. The plates were placed in an incubator for 24 hours at 37°C. The number of independent colonies were counted on each plate. This assessment was carried out strictly to establish whether bacteria were present on the

surface of the cadaver or not. No attempt was made to identify the bacterial strains found.

2.6 Anatomical dissection

Subjects A1 and A2 were partially dissected in May and June 2019 as part of an MSc Human Anatomy dissection project (Stasiewicz et al., 2020). Following completion of the project, the cadavers were further dissected in November of 2019 to fully assess the quality and extent of preservation. An investigation of the limbs was made to examine the condition of muscle, tendon, nerve and connective tissue in the gluteal region, the posterior thigh, and the forearm. Visual examination of the previously-dissected areas was also carried out and the dissection was extended in several of these areas to confirm the extent of deterioration, if any. Following this, the thoracic and abdominal cavities were opened and the contents were examined in detail. The calvarium of the first subject was also opened to investigate the level of preservation of the brain.

Subjects B1 and B2 were dissected in June and July of 2021. The calvaria were opened on both subjects to allow dissection of the orbits as part of an MSc Human Anatomy dissection project. On completion of this project, a comprehensive dissection of both subjects was undertaken to fully assess the quality and extent of preservation. An investigation of the posterior thorax and lower limb was made to examine the condition of muscle, tendon, nerve and connective tissue in the shoulders and back, the gluteal region and the posterior thigh. Following this, the thoracic and abdominal cavities were opened and the contents were examined in detail.

2.7 Ultrasound study

In September of 2019 a preliminary investigation was carried out on one individual from the first group of subjects, to assess the feasibility of a formal study on the utility of saturated NaCl solution cadavers as a clinical teaching model. A consultant anaesthetist based in Cork University Hospital (CUH) carried out an initial ultrasound-guided (USG) examination of the subject at the posterior thigh (sciatic nerve), the inguinal region (femoral nerve), and the pectoral region (brachial plexus and median, musculocutaneous, radial and ulnar nerves), and endeavoured to perform a small number of US-guided injections. The needle placement sites were chosen to reflect common injection sites for peripheral anaesthesia in live clinical patients.

The visibility of structures was rated on the Vienna scale of neural visibility (Marhofer, 2008) (Table 2). Simulated nerve blocks were performed using a 22gauge B|Braun Stimuplex Ultra 360-type needle (B. Braun Melsungen AG, Germany) to further assess visibility of structures as seen with the US machine, and to provide rudimentary feedback about the haptic properties of the cadaveric tissue.

Table 2: Vienna Scale of Neural Visibility Point on scale	Visibility
1	Structures within the nerve are visible
2	Only nerve sheath/epineurium visible
3	Nerve not visible, only surrounding structures visible
4	No tissue components visible

A small formal study was subsequently planned for the second group of subjects. This cohort of participants was recruited from a larger group of clinical anaesthetists working in or close to the local area. Due to various restrictions in place as a result of the Covid-19 pandemic and the related increase in demand on the time of medics, the desired number of participants could not be recruited in advance of the completion of this study.

A questionnaire (Appendix 3) was developed to assess the suitability of the cadaveric model as a training tool, based on the impressions of the participants. The study cohort (n=7) were all consultant-level anaesthesiologists. They were asked to examine the cadavers using US in three regions: the neck and axilla (structures relating to infrascalene block and axillary block), the groin (femoral block) and the posterior thigh (sciatic block).

A Terason uSmart 3200T portable ultrasound device (Teratech Corporation, USA) with a small linear transducer was used to visualise the cadaveric anatomy. A single-use probe cover was used to protect the equipment. Simulated nerve blocks were then carried out by placing a 22 gauge B|Braun Stimuplex Ultra 360 needle into the cadaver. All participants carried out three blocks on a single side of both cadavers. It was ultimately decided that the cohort would not introduce any injectable fluid for the purposes of assessing tissue displacement. The procedure was carried out first on one cadaver before being repeated in full on the second cadaver. Questionnaires were completed by the participants after both cadavers had been examined. The questionnaires were filled out anonymously.

2.8 Ethics

Approval was granted for this study by the Social Research Ethics Committee (SREC) of University College Cork (see Appendix 1). The study falls under SREC as capturing clinician responses was the primary focus of the study, rather than testing the cadaveric tissue. Use of bequeathed human cadaveric material in this research is governed by the Anatomy Act, 1832 and Medical Practitioners' Act, 2007, under licence from the Medical Council of Ireland. Consent of participants to publication was sought at the time the study was carried out (see Appendix 2). Consent of the body donors to publication was given at time of donation.

Chapter 3

Results

3.0 Results

3.1 Saturated Salt Solution-Embalmed Cadavers

The experimental design included two groups of cadavers embalmed with aquaeous NaCl solutions. The first group (Group A) comprised two male donors embalmed with an in-house salt solution, while the second group (Group B) comprised a female and a male donor that had been frozen and stored prior to embalming with a saturated salt solution (35% NaCl) (Table 3). At the completion of the embalming process of group A, it was noticed that the full mass of salt did not enter solution; an amount (estimated to be ~20%) of the solute remained undissolved in the container, resulting in a final sodium chloride concentration of approximately 28% in the solution for the first group.

Table 3: Donor characteristics

Group	Donor #	Gender	Age	Length of preservation
A	1161 (A1)	M	97	22 months
A	1162 (A2)	M	78	21 months
B	1176 (B1)	F	90	27 months
B	1169 (B2)	M	75	25 months

3.2 Bacterial and fungal contamination

Subject A1 was assessed for bacterial contamination 8 weeks after embalming by completing a simple culture and plate count. Swabs were taken from the mouth, hand, groin, and toes of the cadaver, and 4 different dilutions of the base swab solution (10^{-2}, 10^{-4}, 10^{-6}, direct spread) were plated on each dish, along with a negative control. These were placed in an incubator for 24 hours at 37°C. The plates were examined and bacterial growth was identified on all of them, irrespective of the anatomical area swabbed (Figure 2), suggesting that some surface bacterial contamination was

present. As it is outside of the scope of the present study, no attempts were made at identifying the bacterial or fungal strains.

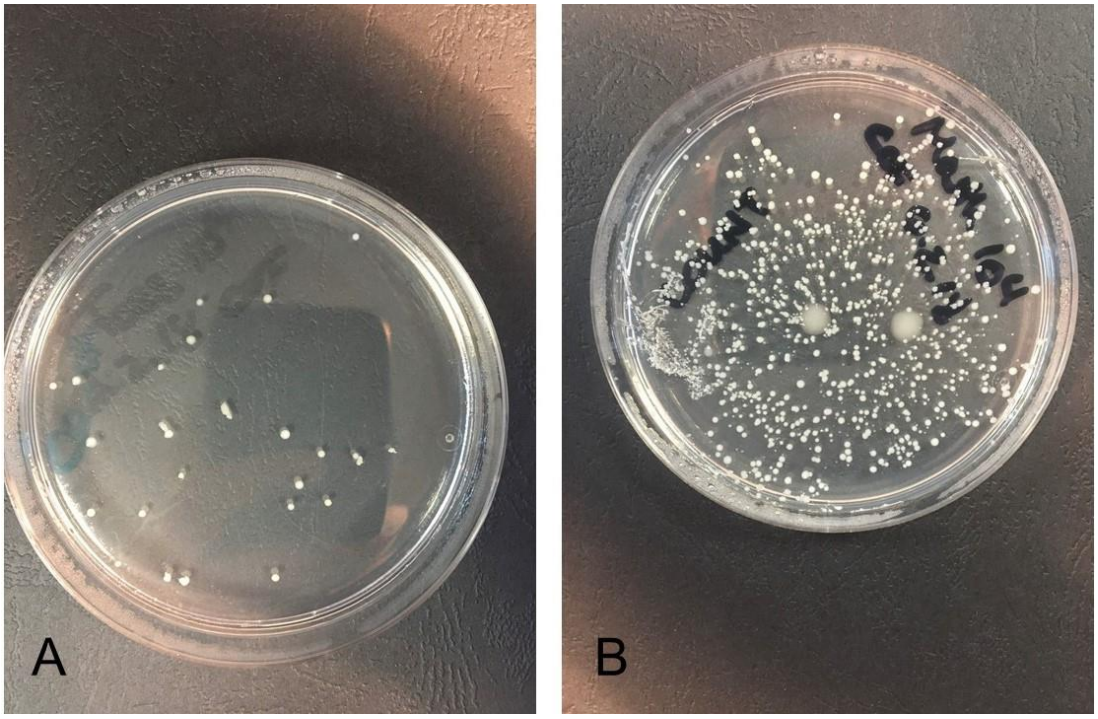


Figure 2: Bacterial spreads were carried out to confirm the presence of bacteria on the cadaver. These plates show the extent of bacterial colonisation at 10^{-6} (A) and 10^{-4} (B) dilutions.

3.3 General visual observations

3.3.1 Group A

The cadavers retained a normal appearance during the first months of the study, with good overall preservation and little physical change. The large blisters present on the limbs and flank of the subjects ruptured and consequently some epidermal slip occurred, giving a shiny appearance to these areas and a somewhat slippery texture (Figure 3). There was leakage of cadaveric body fluids into the storage medium, lending it a dark red colour. The abdomen retained its shape, with no visible discolouration or other sign of putrefaction, and was reasonably firm to the touch

when palpated. The abdomen remained firm throughout the period of study and did not develop obvious signs of decomposition.

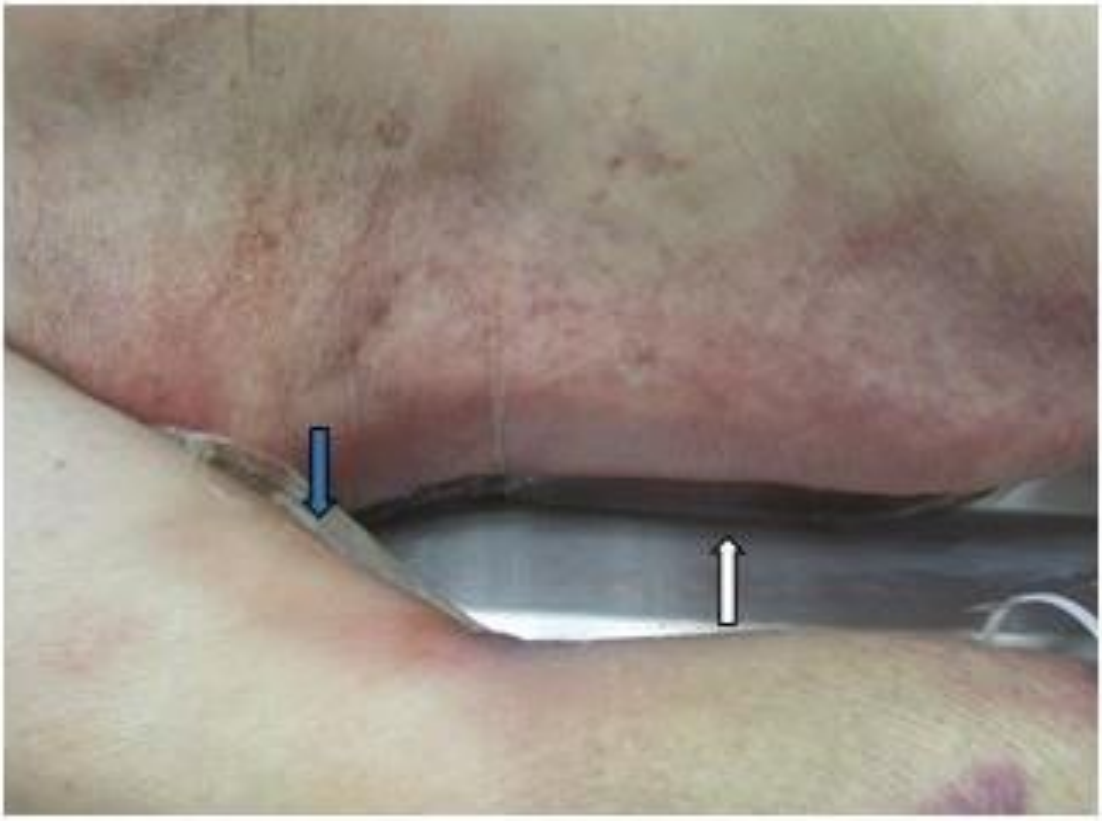


Figure 3: Blistering (white arrow) and some skin slippage (blue arrow) can be seen on the torso and inner arm of this newly-embalmed subject from group A.

The eyes and orbits became sunken after the 5-month visual check, and the surface of the eyeball gained a black appearance. This enophthalmia progressed during the storage period to an extreme degree, far in excess of that usually observed in formalin-embalmed cadavers. In addition to the bacterial growth observed at 8 weeks, it was noted that over time the surface of the cadavers was developing fungi/bacterial growth. Observations at approximately 2 months and 5 months after embalming showed a moderate number of patches of a green-and-white mould (images not included). The full visual inspection approximately 10 months after embalming

demonstrated the presence of areas of mould growth on the anterior surface of the cadaver and on the surface of the storage medium (additional embalming solution). These colonies/mycelia negatively affected the superficial structure of the dermis, giving the skin a discoloured, leathery texture and appearance after the growth was removed (Figures 4 and 5)





Figures 4 and 5: The skin of the first group of cadavers was altered negatively by the action of microbes (images taken approx. 22 months post-embalming). The texture is akin to that of leather and the colour has darkened considerably over time. Desquamation also occurred extensively.

Limited interventions were carried out during the period of storage until the time of the full visual inspection, when the bodies were washed thoroughly with antimicrobial cleansing solution and placed in clean storage bags. This resulted in extremely large amounts of dermal tissue coming free of the cadaver, particularly on the palms of the hands, along with some body hair and finger and toe nails which had loosened. Areas of remaining dermis were toughened and discoloured.

The posterior aspect of the subject was noted to be unusually well-preserved, with tissue colouring retained and the area looking almost as fresh as the day of embalming (Figure 6). A similar level of preservation occurred on the lateral body surfaces where the cadaver was partially-immersed in the embalming solution.

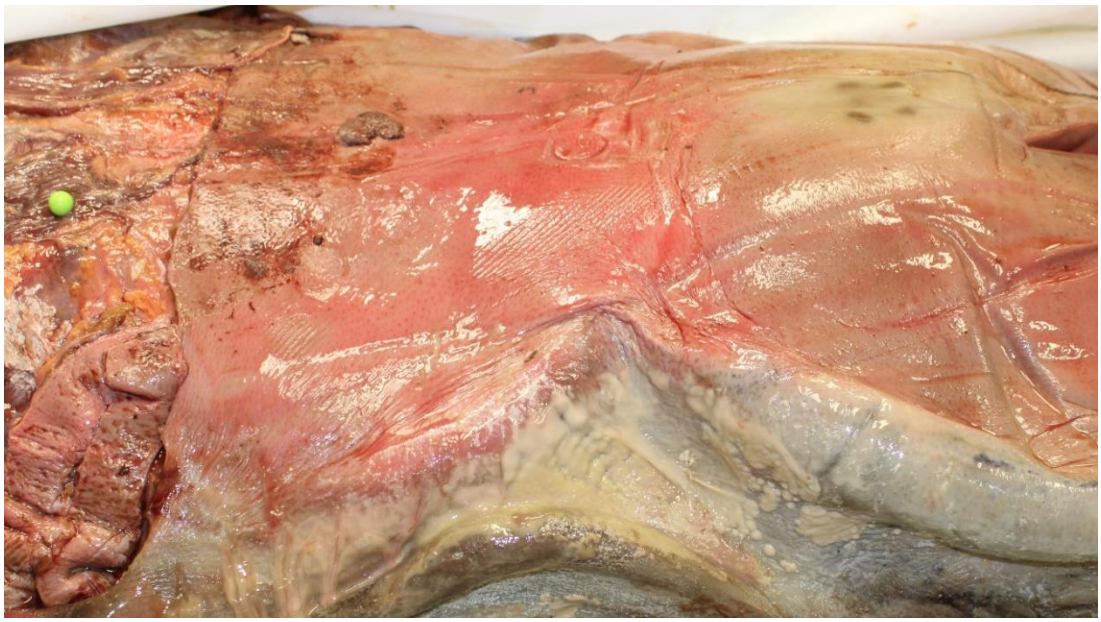


Figure 6: The retention of original colour and texture can easily be observed on the dorsal surface of the cadaver torso, in contrast to the changes observed in other regions (shoulders at left of image, gluteal region at right).

A distinct odour was present in the subject which could be described as somewhat "bread-like", or "yeasty", and not altogether unlike cured meats. This odour grew markedly in strength as the study period advanced, and became very noticeable even when the cadavers were bagged in cold storage, but it was not a smell that is usually associated with putrefaction or tissue decomposition and neither was it especially offensive or overwhelming.

These changes were observed in both cadavers during the study period. The cadavers were held for a period of 30 months in total; the length of time in which they remained in good condition was approx. 21-22 months.

3.3.2 Group B

The second group of subjects also retained a normal appearance during the first months of the study, with good overall preservation and little physical change (Figure 7). The large blisters resulting from the embalming process (which were present on the limbs and flank of the subjects) burst, and consequently some initial epidermal slip was observed in those regions.





Figures 7 and 8: The external appearance of the cadavers following removal from storage approx. 17 months post-embalming. Desquamation can be seen in a number of areas on both cadavers.

There was leakage of cadaveric body fluids into the storage bag, leaving the subjects partially-immersed in a dark red liquid. This group of cadavers experienced minimal mould growth, with only a few very minor patches present on either body. One of the subjects experienced more florid fungal growth around the femoral embalming incision related to a haemostat which was left in the wound in error. This was removed during the first full visual inspection. As a result of this, the muscle tissue directly beside the incision became somewhat discoloured, but the integrity of the tissue did not seem to be affected and no signs of decomposition were observed. The posterior aspect of the cadavers was very soft and pink, similar to the condition of the backs of the first group but with more blistering and epidermal slip occurring, from which the leaching of body fluids mainly appeared to issue.

The abdominal regions of both cadavers remained firm, although the abdomens did sink to a moderate degree, particularly at the pelvic and costal margins (Figure 8). As with the first group of subjects, the two cadavers in Group B also experienced severe enophthalmia, however the black discolouration seen in the previous group was not observed (Figure 9).

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Figure 9: The eyes became sunken to an extreme degree. This was observed in both cadavers in Group B. No other facial features were affected.

Further sloughing of skin occurred while washing the cadavers and during their use in the ultrasound study, as a result of now-extensive epidermal slip. The palms of the hands were particularly affected by this and, accordingly, a number of fingernails were lost during washing. Very little hair loss occurred.

A slight odour was present in both cadavers, similar in quality and character to the previous group, which increased slightly over the three weeks in which the cadavers remained out of cold storage for the ultrasound study. The leakage of fluids from the posterior aspect of the cadavers continued even after washing.

At completion of the study period, the cadavers had been embalmed for a period of 27 and 25 months respectively; both in excellent condition.

3.4 Goniometric measurements

Pre- and post-embalming measurements of the second group of cadavers were taken to assess the general flexibility of the cadavers following the embalming process and to note any changes which may have occurred as a result of the embalming.

Table 4: Pre- and post-embalming goniometry showing the differences in measurements between flexed and extended joints (measured in angular degrees unless otherwise stated).

Cadaver	Jaw (maximal opening)	L shoulder	R shoulder	L hip	R hip
B1 (pre)	1cm	76	65	54	56
B1 (post)	1cm	105	70	62	45
B2 (pre)	2cm	82	77	90	68
B2 (post)	1cm	70	70	60	65

Table 5: Percentage change in joint mobility (post- v. pre-embalming).

Cadaver	Jaw (maximal opening)	L shoulder	R shoulder	L hip	R hip
B1	0%	+38.2%	+7.7%	+14.2%	-19.6%
B2	-50%	-14.6%	-10.0%	-33.3%	-4.4%

3.5 Anatomical dissection

3.5.1 Group A

The cadavers of Group A were dissected in two phases, across several months in 2019. Early in the study period, an investigative window was opened over the anterior aspect of the deltoid muscle, and a small portion of skin and fat was reflected. The skin had increased slightly in toughness and the subcutaneous fat was somewhat drier and less loose when compared to fresh, unembalmed cadavers (an effect frequently seen in

chilled cadavers), but retained its normal colouring and overall texture. When initially exposed, the muscles were brightly-coloured, with firm texture and visible striations (Figure 10). Superficial neural and vascular structures were pliable, and easily identified due to retention of their distinct characteristics. These windowed areas showed minimal alteration throughout the first 18 months or more, with the only notable change being some discolouration of the exposed muscle and fat due to oxidation. Tissues did not appear to lose integrity or visibly deteriorate.

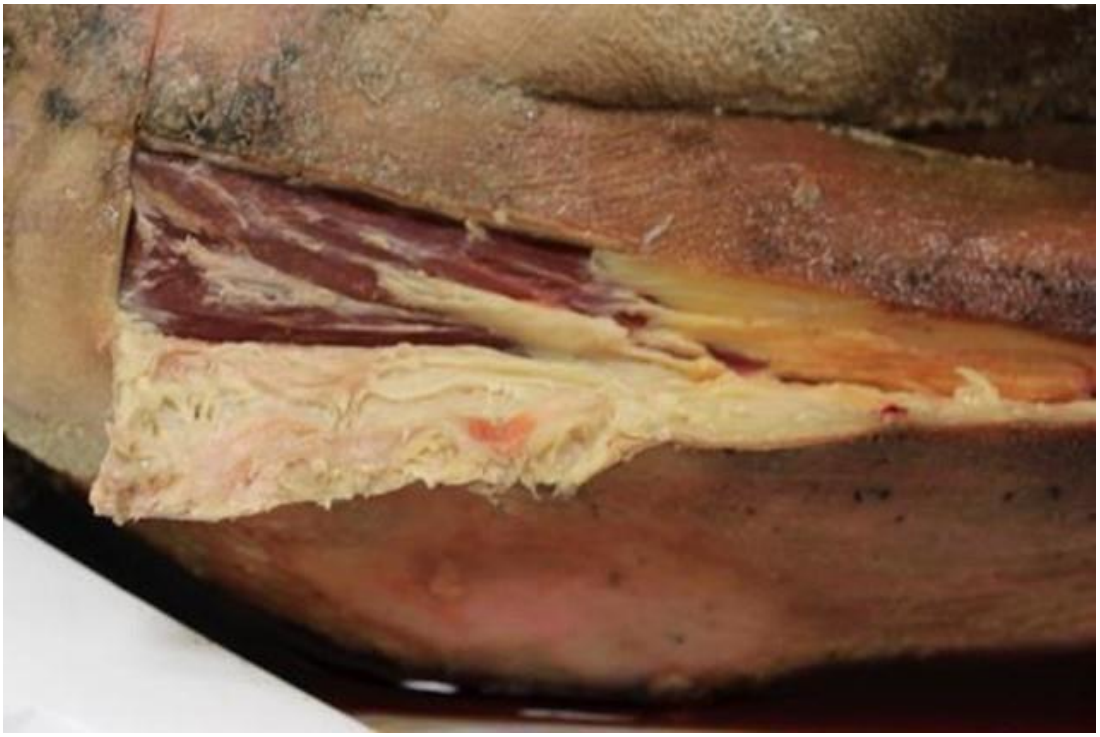


Figure 10: An exploratory dissection window on the cadaveric arm of this Group A subject show good retention of colour and structure (image taken approx. 8 months post-embalming). Note the areas of textural change on the skin above the incision, resulting from microbial action.

More extensive dissection began at a much later stage of the study period, when the cadavers had been held for approx. 18 months. The first phase was carried out by one of the students undertaking an MSc in Human Anatomy in the Department of Anatomy

and Neuroscience, UCC., as part of a dissection project required to complete the program of study. The project comprised a detailed investigation into the course of the accessory nerve (CNXI) in the neck and shoulder, and resulted in a paper describing a previously unreported accessory muscle of the trapezius (Stasiewicz et al., 2020).

The external structure of the cadavers had altered (as described in Section 3.3.1), however the musculature and subcutaneous tissues, both superficial and deep, were unaffected. Muscles were soft and supple, with vivid colouration, distinguishable fibre bundles and pliable fascial coverings. The limbs were readily mobilised and the cadaver could be placed into different postures to aid dissection of less accessible regions, an advantage which is notably absent from formalin-fixed cadavers. The appearance of muscles had darkened somewhat in the peripheral areas, where the skin was thinner, but the overall consistency and colouring of tissues was excellent.

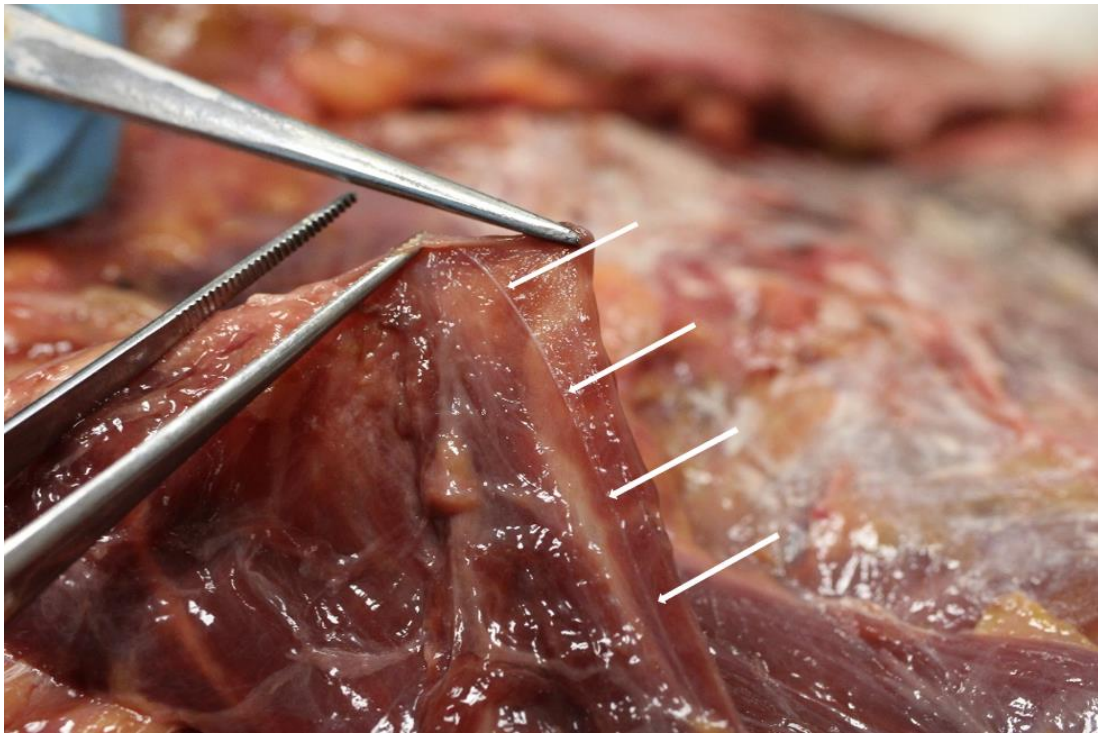


Figure 11: Fine detail is conserved well, as seen in this close-up image of the dorsum of cadaver A1– even very small nerves are visible (white arrows) and can be dissected away from the surrounding tissue.

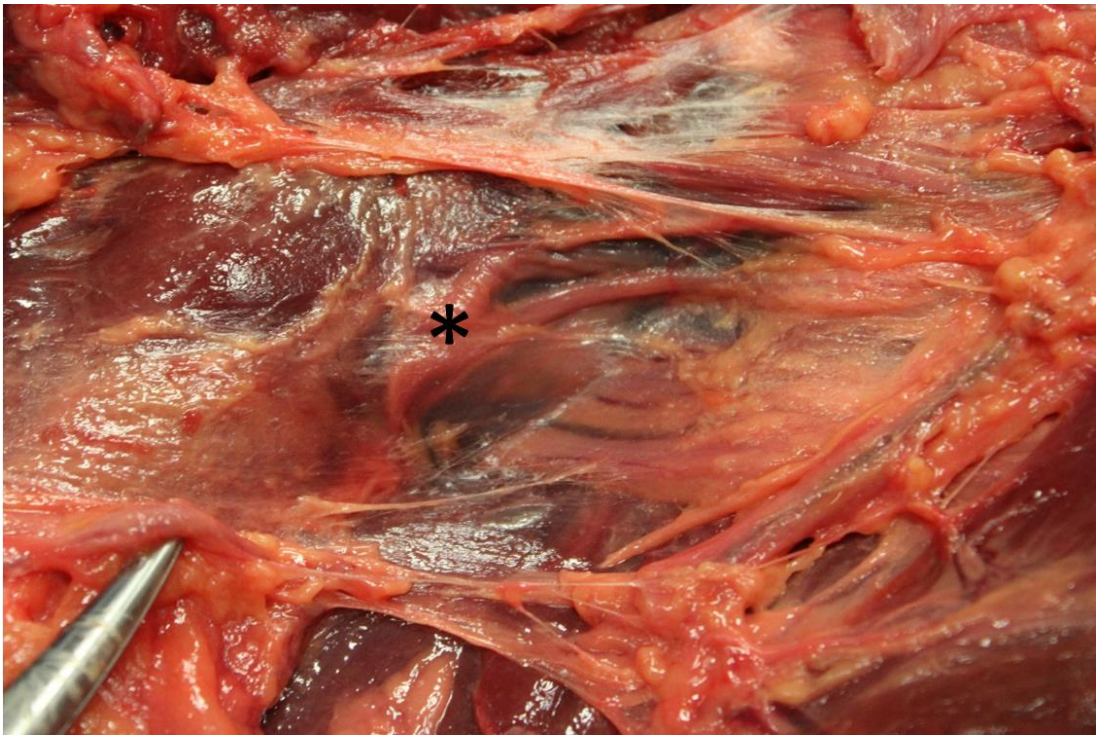


Figure 12: Connective tissue looks and behaves much more like living tissue, with vascular structures (*) clearly differentiated and visible through connective tissue membranes in the sub-gluteal area (approx. 22 months post-embalming).

The student reported that the tissue was very different to that of formalin-embalmed cadavers, displaying softness, pliability and colouration not observed in hard-fixed tissue. The cadavers were easier to dissect due to conservation of the distinct characteristics of the tissue types and layers, allowing tissue planes to be separated with less difficulty than usual for formalin-fixed cadavers. It was noted that these factors greatly aided precise working and allowed the dissection to proceed at a good speed.

As the dissection project progressed, the dermis of the cadavers began to degrade, observed by further darkening of the subjects and also, notably, in changes to the texture and integrity of the skin. The cervical region had been dissected before work advanced down the torso of the subject, and it could clearly be seen that the previously-exposed muscular structures had lost the bright red colouring of the more recently-dissected tissues (Figure 13). The texture of those structures had also deteriorated markedly in a matter of weeks, and were much less firm and more prone to disintegration when handled. By the completion of the dissection project, the appearance and haptic qualities of the affected areas gave every indication that decomposition had begun to occur.



Figure 13: This view of cadaver A1 displays markedly deteriorated tissue in the cervical and occipital regions, while the newly-dissected areas of the trapezius muscles (on either side of the spine) are fresh and vivid.

A second, final phase of dissection was undertaken approx. 2 months after the completion of the MSc project. The cadavers were rigorously examined in this phase, with the major cavities of the body opened and investigated in addition to dissection of the trunk and limbs. By this point, the areas dissected during the project had deteriorated considerably and the regions immediately adjacent to them had also undergone changes. Muscles in the affected areas were frangible and tore easily, appeared much darker and more discoloured, and the general appearance of these areas and the related structures was unpleasant and did not give any impression of good preservation (Figures 14-16).





Figures 14 and 15: Superficial structures and previously-exposed areas displayed extremely fragile muscles, discoloured tissues, and a generally unpleasant appearance, as seen in these images of the lower limb of Cadaver A1 (posterior aspect at top of image).



Figure 16: The palm of the hand of cadaver A2 was in a very poor condition. The palmar aponeurosis (A) is visible here, but surrounding structures are degraded and cannot be readily identified.

By contrast, the untouched regions of the cadavers remained in excellent condition (Figures 17-18). The gluteal region demonstrated bright red muscles and yellow adipose tissue, with blood still liquid in the larger vessels. The separation of layers was achieved with relative ease, in contrast to hard-fixed cadavers, where the great change to tissue texture can make it more difficult to identify subtle changes in structure. Undamaged tendons in the limbs were highly elastic; arteries were firm and springy with faithful colouration.

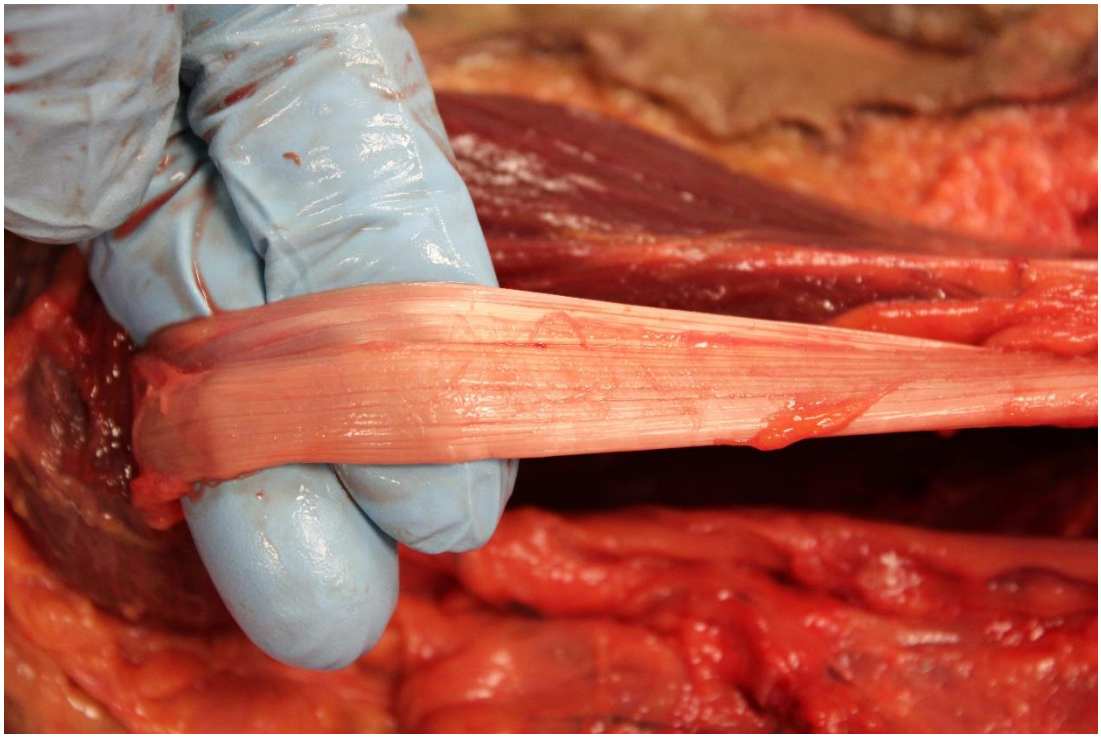


Figure 17: Tendinous structures in unexposed regions retained strength and the surround musculature is brightly-coloured, with full conservation of pliability.

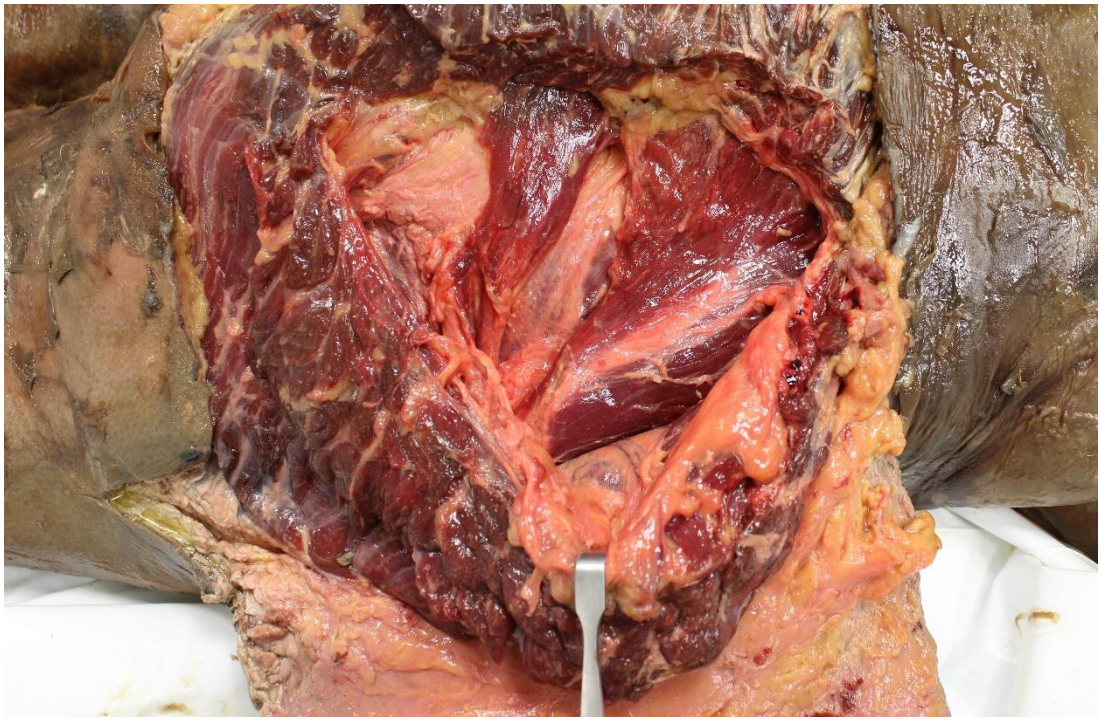


Figure 18: Gluteus maximus has been retracted to show piriformis and gluteus medius. It can be seen that despite the altered appearance of the skin surrounding the region, structures below remain in a good state of preservation.

The calvarium was opened and the dura mater and brain were found intact and in good condition, with pale pink colouring. Surface vasculature of the brain could be observed and gyri and sulci were apparent, although the organ itself had lost its shape as a result of settling due to gravity.

The thoracic cavity was exposed and the lungs were found to be intact, albeit reduced somewhat in size, and the heart had experienced a notable dissipation of form, appearing flaccid and shapeless (Figure 19). Internal cardiac architecture was discernible and structures such as the chordae tendinae and valves were well-preserved.

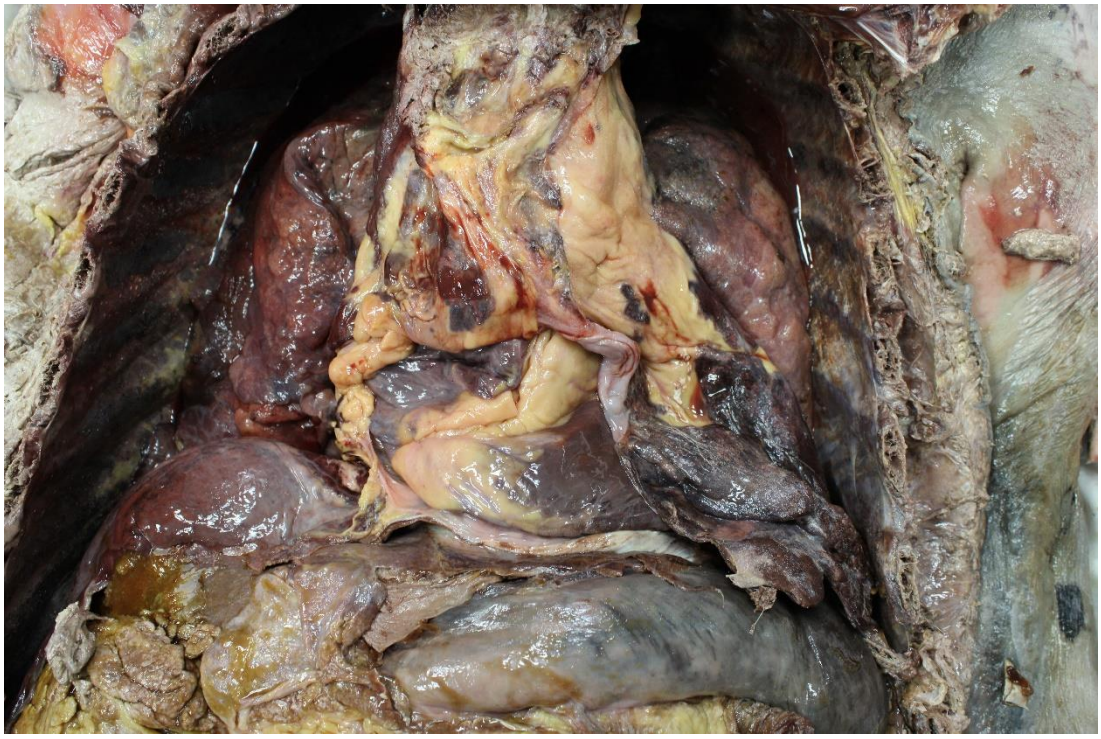
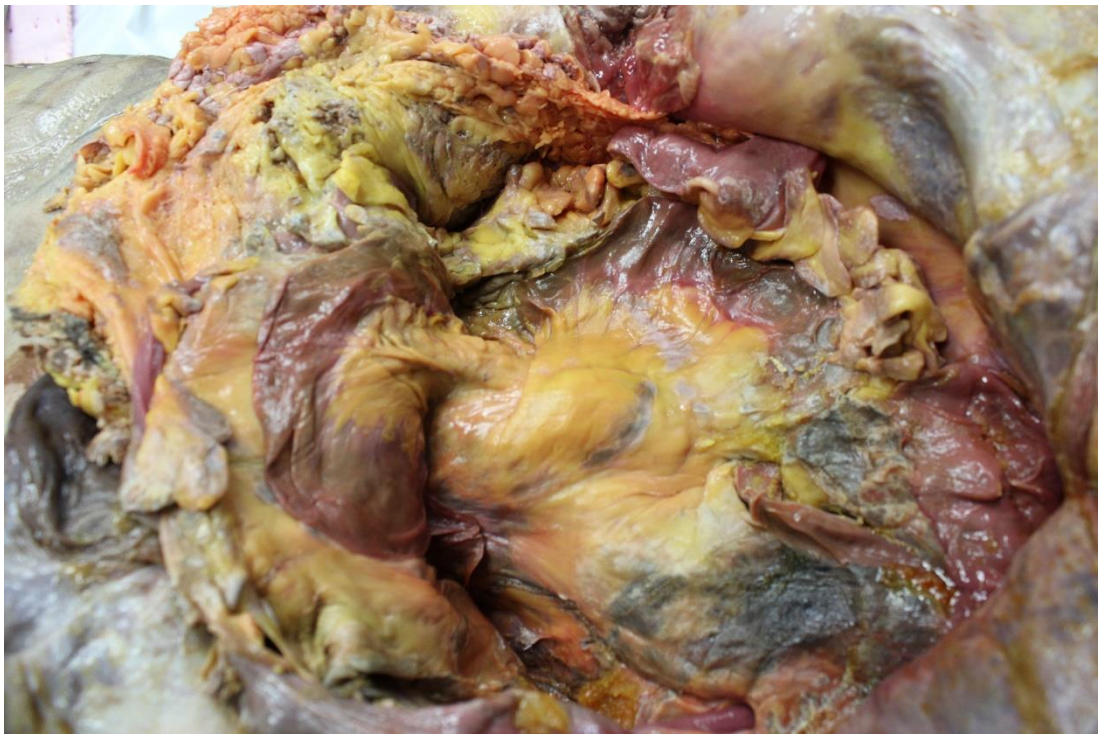


Figure 19: The viscera of the thoracic cavity remained intact, although they lost their shape and became extremely flaccid.

The abdominal cavity was extremely well-conserved, with no conspicuous changes to form or individual structures (Figures 20 and 21). Intestines could be lifted and moved, the greater omentum was reflected with ease, and other viscera could be identified without difficulty and were all equally well-preserved. Small inclusions of salt were observed in some membraneous tissues, a feature also noted in some of the literature reviewed for this study. These inclusions did not negatively affect the appearance or texture of the viscera. Internally, no obvious signs of decomposition were noted, such as visible changes to the texture or appearance of structures, or by the presence of any unpleasant odour.





Figures 20 and 21: The abdominal cavity was shown to have good preservation of structures such as the greater omentum, and viscera were pliable and could be mobilised to expose deeper structures. Mesentery and intestines retained their colouration.

3.5.2 Group B

The second group of cadavers were dissected in a single phase mid-2021. While the dissections were not performed in the same order as Group A due to the different requirements of the MSc projects each year, the same structures were investigated. The calvaria of both cadavers were opened, inspected, and the brains removed to facilitate dissection of the orbits as part of a dissection project required to complete the program of study for an MSc in Human Anatomy, run by the Department of Anatomy and Neuroscience, UCC.

The brain was in an excellent state of preservation in both cadavers. Great care was taken not to damage the organ when removing the calvarium - brain tissue is extremely prone to damage as it is very soft and pulpous. When the calvarium was lifted, the brain appeared in an exceptional state of preservation. External features were retained and even the smallest surface vessels were present (Figure 22). Cranial nerves were identifiable and membranes were intact. Minor discolouration was seen in discrete areas, but as the integrity of the tissue was unaffected, this appeared more likely to be staining rather than any change related to deterioration.

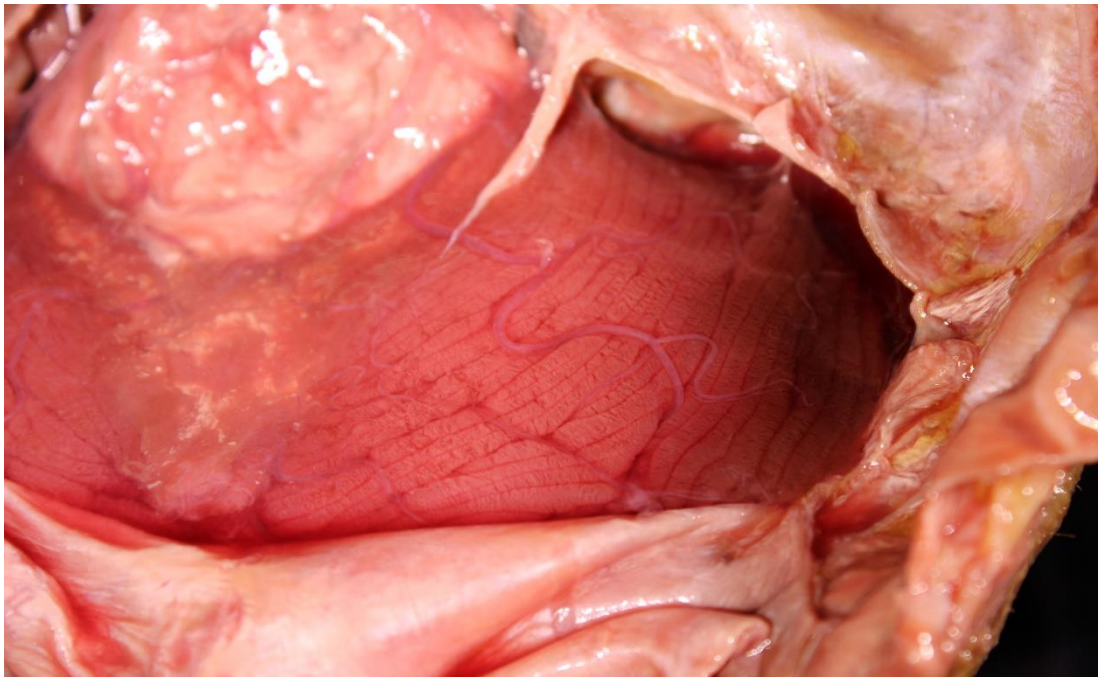


Figure 22: This close-up image of the cerebellum demonstrates the effectiveness of the NaCl solution at conserving cerebellar architecture and surface vasculature.

Two weeks later, upon completion of the MSc project, the cadavers were dissected in full. The skin of the back was reflected and the musculature exposed. All structures looked to be in excellent condition and retained full pliability and lifelike colouration. The superficial layer of musculature of the female cadaver had a slightly darker appearance than that of the male cadaver, but this had no discernible effect on the quality of preservation. Fascial layers, tendinous attachments, adipose tissue and cutaneous nerves were generally comparable in appearance and feel to those of fresh cadavers. Preservation of structures uncovered in dissection of the gluteal region and posterior thigh of both cadavers was as good – if not better – than that seen in the back. Colouration of tissues was intense and very true to life.



Figure 23: The musculature of the back has been exposed in cadaver B2. Structures remain in excellent condition.



Figure 24: The musculature of the back of cadaver B1. Note the slight darkening of the most superficial muscles, trapezius and latissimus dorsi. The thin dorsal skin of this donor did not confer complete protection from oxidative changes.



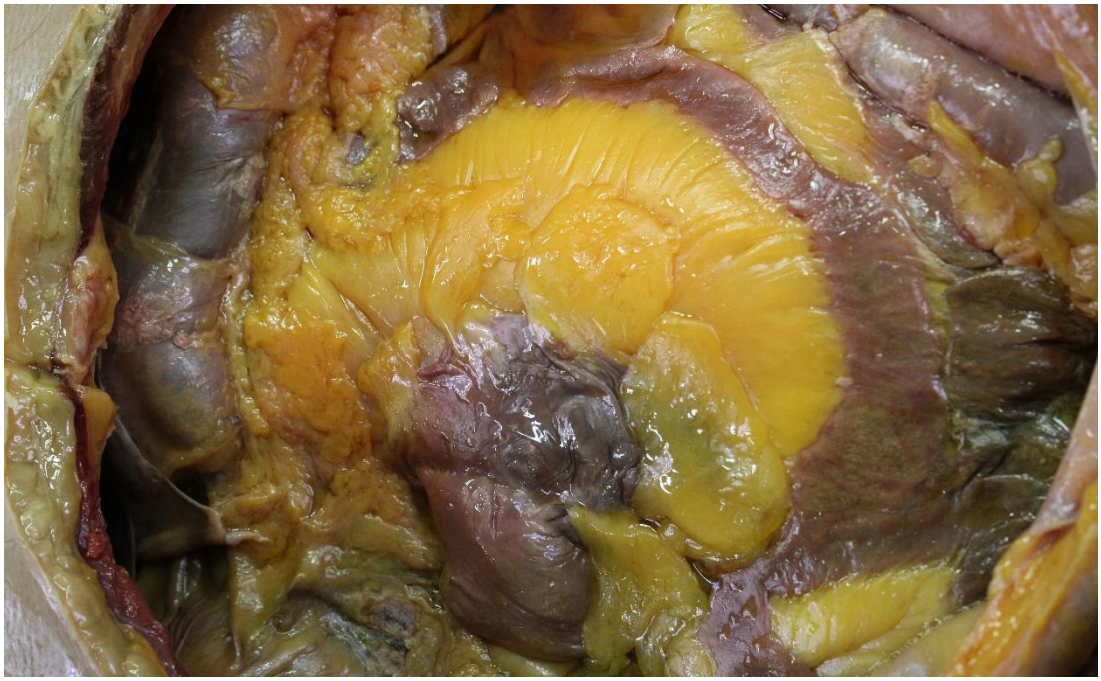


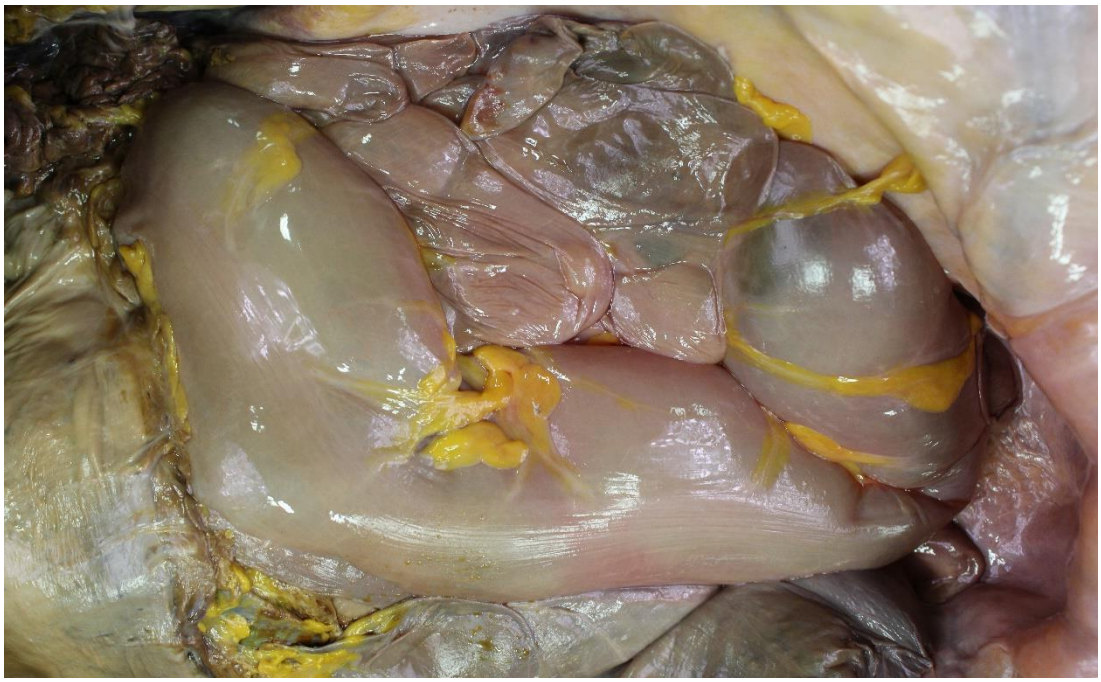
Figures 25 and 26: The posterior thigh region dissected in donor B2 (top) and B1 (bottom). Both donors display vivid colouration of muscles and subcutaneous fat. Structural integrity of all anatomical structures was excellent and the tissues showed little difference from fresh tissue.



Figure 27: Detailed view of the surface of gluteus maximus. The interlaced pattern of fascial fibres can be clearly observed due to the preservation method. This level of differentiation is not usually seen with formalin fixation.

The abdominal cavity was opened and the viscera examined. All organs were found intact and in superb condition. Mesentery had vivid yellow colouration; peritoneum was pink with a life-like sheen to it. The viscera could be mobilised easily, and unlike the cadavers in Group A, no crystalline inclusions were noted. The liver was jelly-like in consistency, in complete opposition to that of formalin-fixed livers, which become excessively dense and hardened.





Figures 28 and 29: The intestines of the cadaver B2 (top) and B1 (bottom) when the abdominal cavity was opened (left side of image is rostral, right side is caudal). Organs are well-preserved and retain their shape.

The paracolic gutters of the male cadaver contained large quantities of a clear, brown, oily liquid which had an intense odour. This was deduced to be a by-product of the NaCl embalming process, as although strong-smelling, neither the odour nor the appearance of the oily substance were reminiscent of putrefaction in any way, and no visible indications of putrefactive activity were seen in the contents of the abdominal cavity.

The thoracic cavity was then opened and investigated. The colouration of the lungs was a little less saturated than might be seen in fresh cadavers or live patients, however the surface texture and overall appearance was extremely close to lifelike. The great vessels were in a good state of preservation within the mediastinum. As noted in the cadavers of Group A, the heart was the organ most adversely affected by the embalming process, albeit not to the same extent in Group B. The pericardial fat was visible and the general shape of the heart was conserved. When the pericardial sac was excised, the heart appeared somewhat deflated and lacked a good deal of muscle tone. Internal cardiac anatomy was unchanged, with well-preserved chordae tendinae, papillary and pectinate muscles, and trabeculae carnae.





Figures 30 and 31: The thoracic cavities of cadaver B2 (top) and B1 (bottom) with exposed viscera. It can be seen that the heart has become slightly flaccid within the cavity, but integrity of the tissue itself was not affected.

Overall, both cadavers were in a remarkable state of preservation given the lengthy period of study (27 and 25 months, respectively), with few adverse effects and with the majority of anatomical structures in excellent condition.

3.6 Ultrasound study

The responses of the cohort (n=7) to the cadaveric model were mixed, with participants occasionally having very divergent responses to the same question. The cadavers generally demonstrated a good physical resemblance to living patients when viewed under the US probe; 5 of 7 respondents rated the appearance of the cadaver under the probe as being moderately or very like a live patient. Different tissue types (nerve, muscle, fascia, bone, etc.) could be distinguished from each other, and the haptic sensations experienced when introducing a needle into the cadaver were considered to be very like those experienced when carrying out the peripheral blocks in live patients. Specifically, fascial transfixion results in a very distinctive “pop” and the sensation is an important cue in the procedural training of anaesthetists. This was rated as very good in the cadaveric model (Figure 32), with a number of written comments emphasising this.

The visibility of the needle on the ultrasound image was rated highest of all the qualities assessed in this study, with 6 of 7 respondents describing it as very like or exactly like a clinical scenario with a live patient. The cadaver could be posed to allow performance of the various blocks, although a small number of the surveyed clinicians

did not rate this quality as being comparable to live patients and the median score for this quality was “moderately like” (Figure 32).

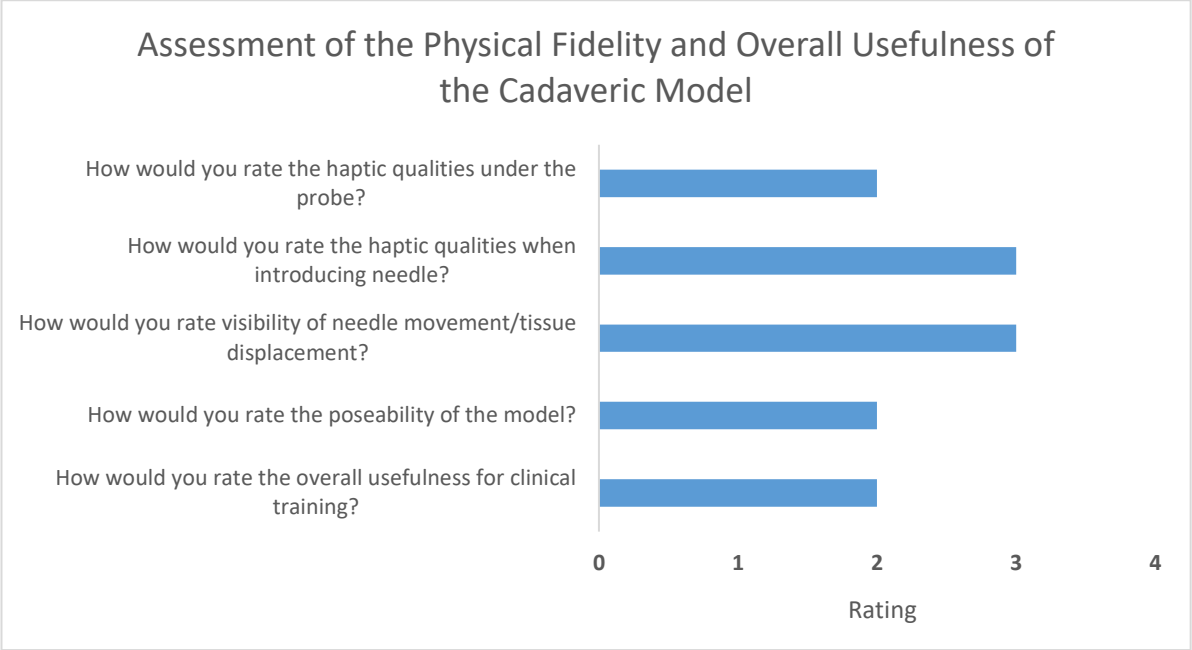


Figure 32: Median scores for the overall fidelity of the cadaveric model compared to a live patient, where 0= nothing like and 4= exactly like. For the final question in this group relating to usefulness, 0= not at all useful and 4= extremely useful.

The clinicians were also asked to rate on a Likert-scale the visibility of structures and ultrasonography landmarks under the ultrasound probe. The regions assessed included the shoulder and cervical region, the inguinal region and the posterior thigh. The responses (Figures 33 to 38) indicate a variability in the visibility of structures with some muscles described as easy to identify while others were moderately visible. Certain components of the brachial plexus, such as the nerves of the upper arm, were noted to be easier to visualise than others due to their anatomical positions. (Figure 34).

Vascular visibility was rated as extremely poor by all respondents (Figures 33, 35, and 37). This caused issues with locating and/or identifying structures with absolute certainty for a number of participants. Vascular visibility is an extremely important visual clue, relied on for orientation when identifying anatomical structures in the ultrasound image. Arteries and veins present as large, round, dark areas on the US view, and these were notably absent from almost all of the cadaveric US images. Written comments from participants included, “One of the major drawbacks was the absence of a pulsating artery to identify the nerve structures (in my practice, this is a critical step)” and “Arteries are poorly visualized, veins not at all”.

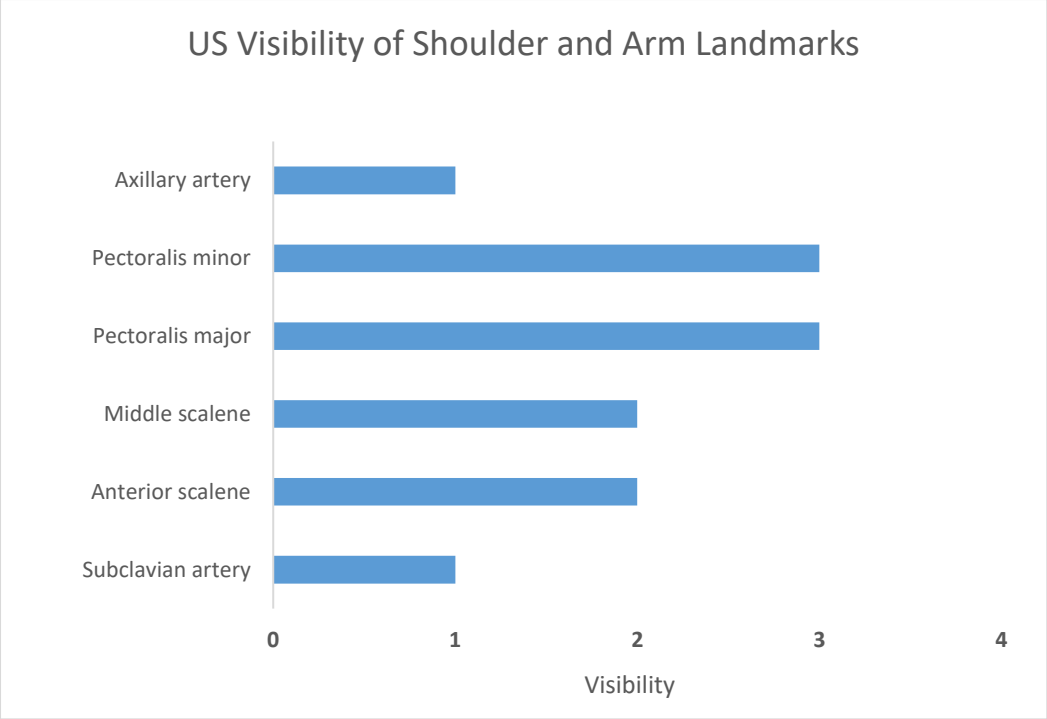


Figure 33: Median scores for the visibility of relevant anatomical landmarks in the supraclavicular, pectoral and axillary regions of the cadavers, where 0= cannot be visualised and 4= excellent visualisation.

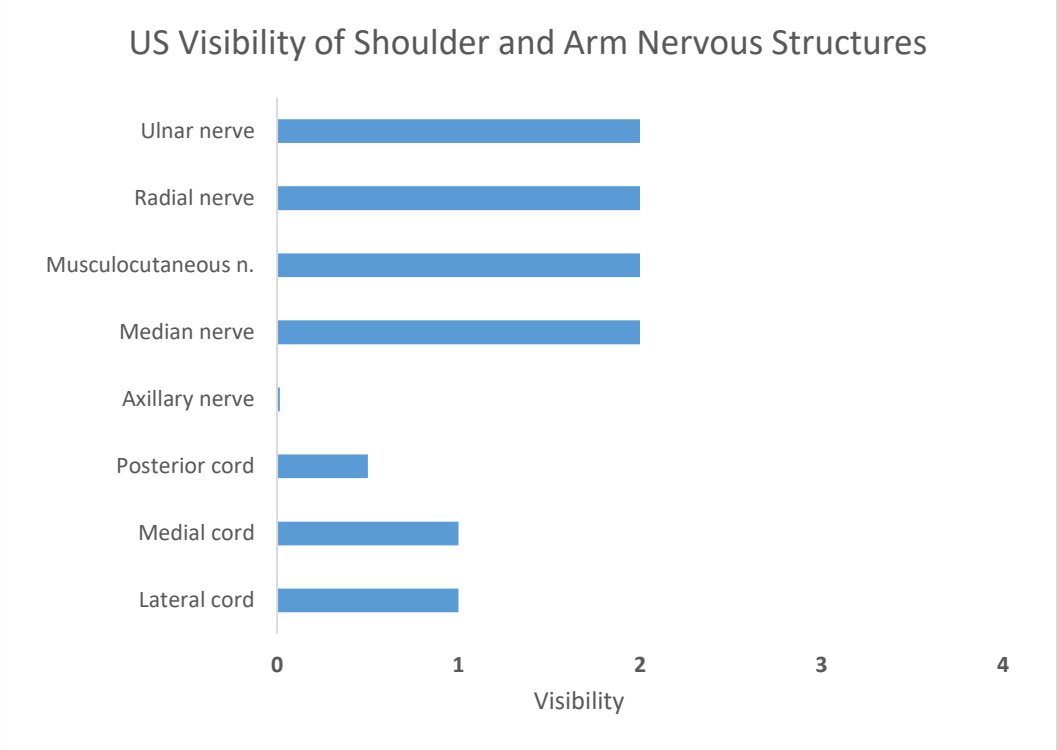


Figure 34: Median scores given by respondents for the visibility of nerves in the supraclavicular, pectoral and axillary regions of the cadavers, where 0= cannot be visualised and 4= excellent visualisation.

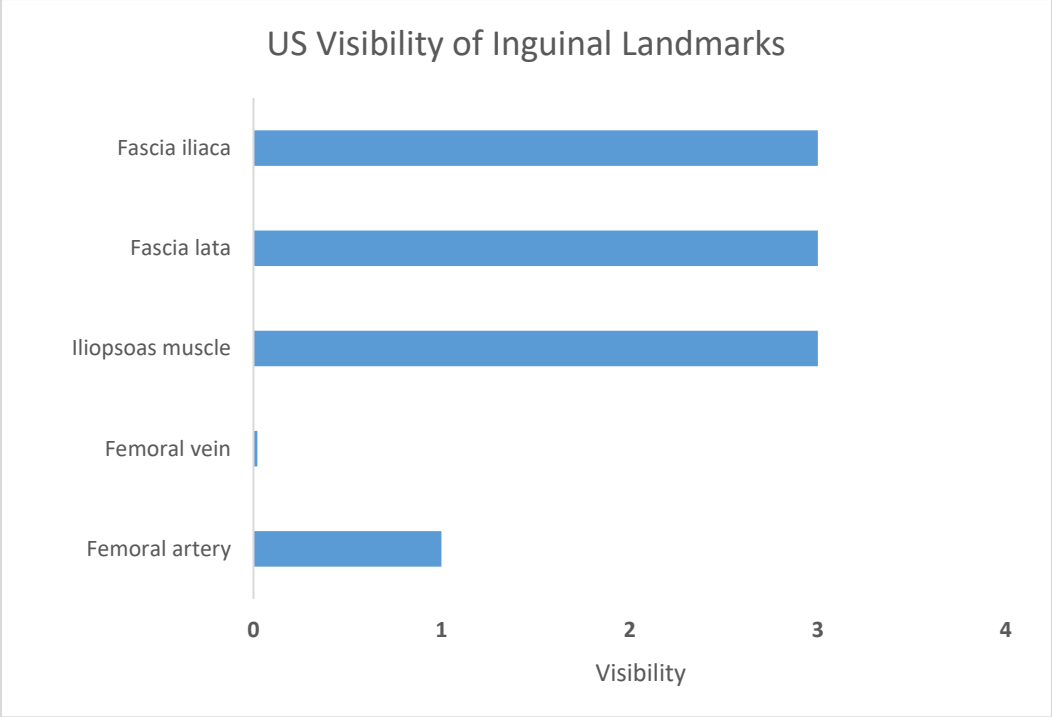


Figure 35: Median scores given by respondents for visibility of relevant anatomical landmarks in the inguinal region, where 0= cannot be visualised and 4 = excellent visualisation.

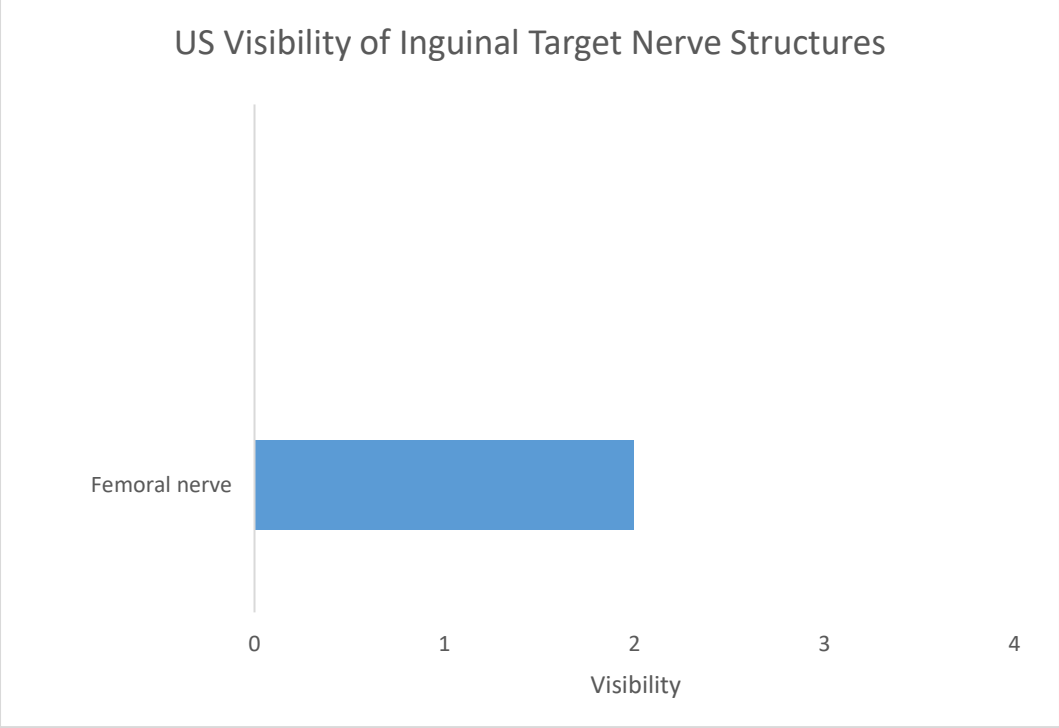


Figure 36: Median scores given for the visibility of nerves in the inguinal region of the cadavers, where 0= cannot be visualised and 4= excellent visualisation.

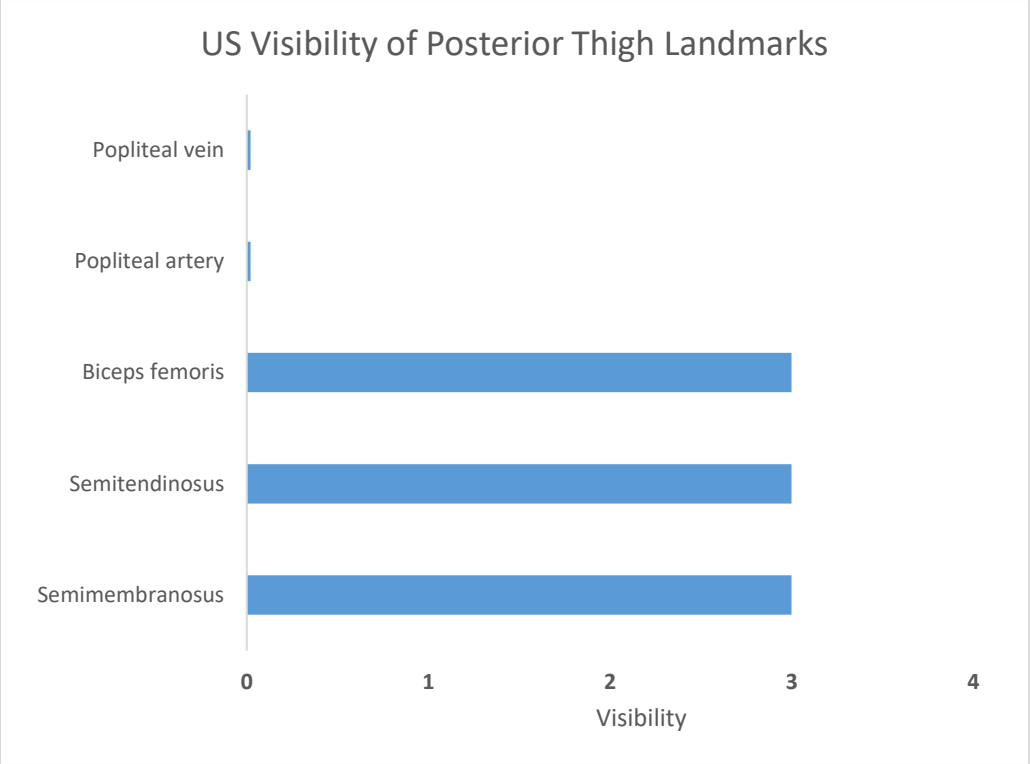


Figure 37: Median scores given by respondents for visibility of relevant anatomical landmarks in the posterior thigh region, where 0= cannot be visualised and 4 = excellent visualisation.

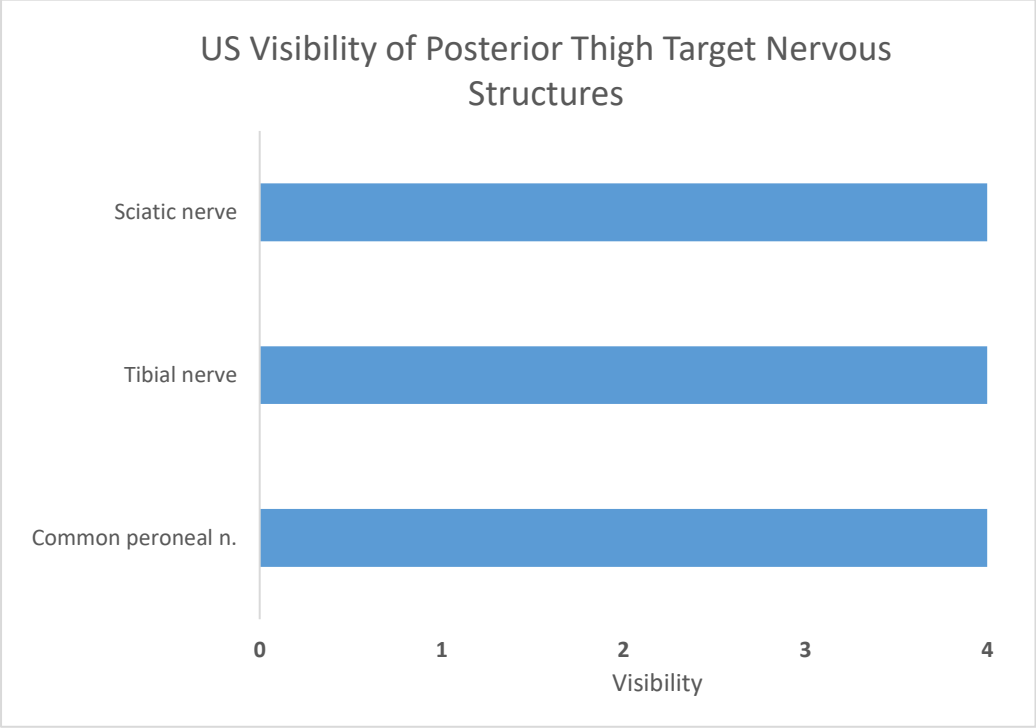


Figure 38: Median scores given for the visibility of nerves in the posterior thigh region of the cadavers, where 0= cannot be visualised and 4= excellent visualisation.

The femoral block (Figure 39) was considered the most difficult due to poor visualisation of the nerve, however, this can often present similar challenges in live patients (Munirama and McLeod, 2013). The sciatic nerve and its branches (Figures 40 and 41) were easiest for all participants to visualise during the study, and consequently these nerves scored highest of all the structures examined (Figure 38).

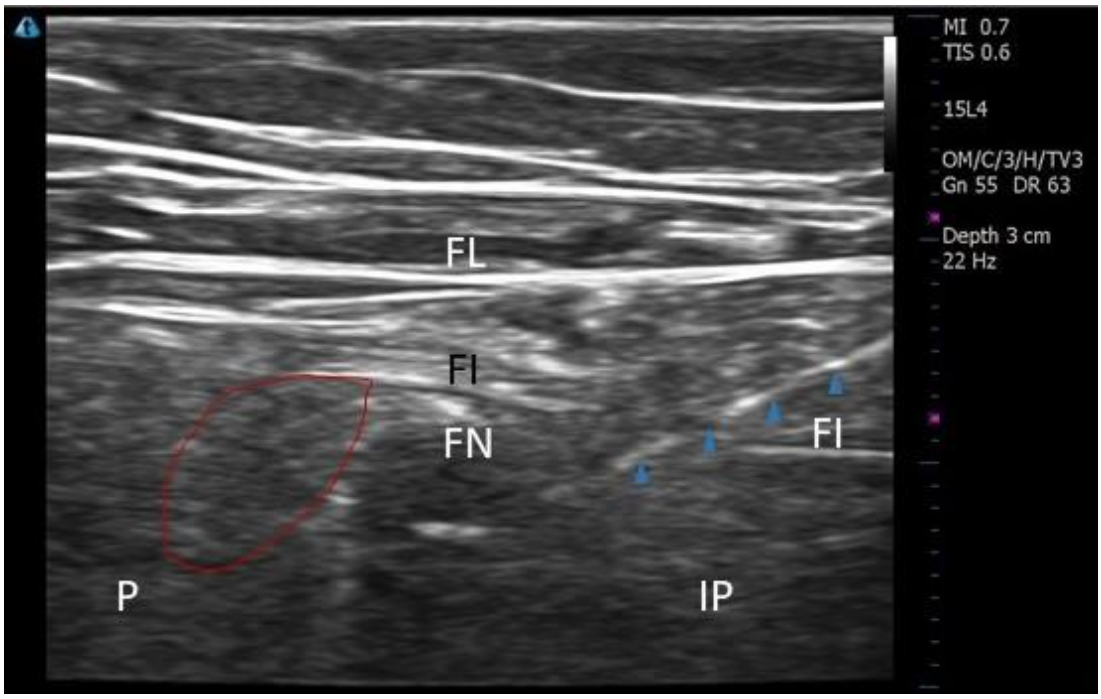
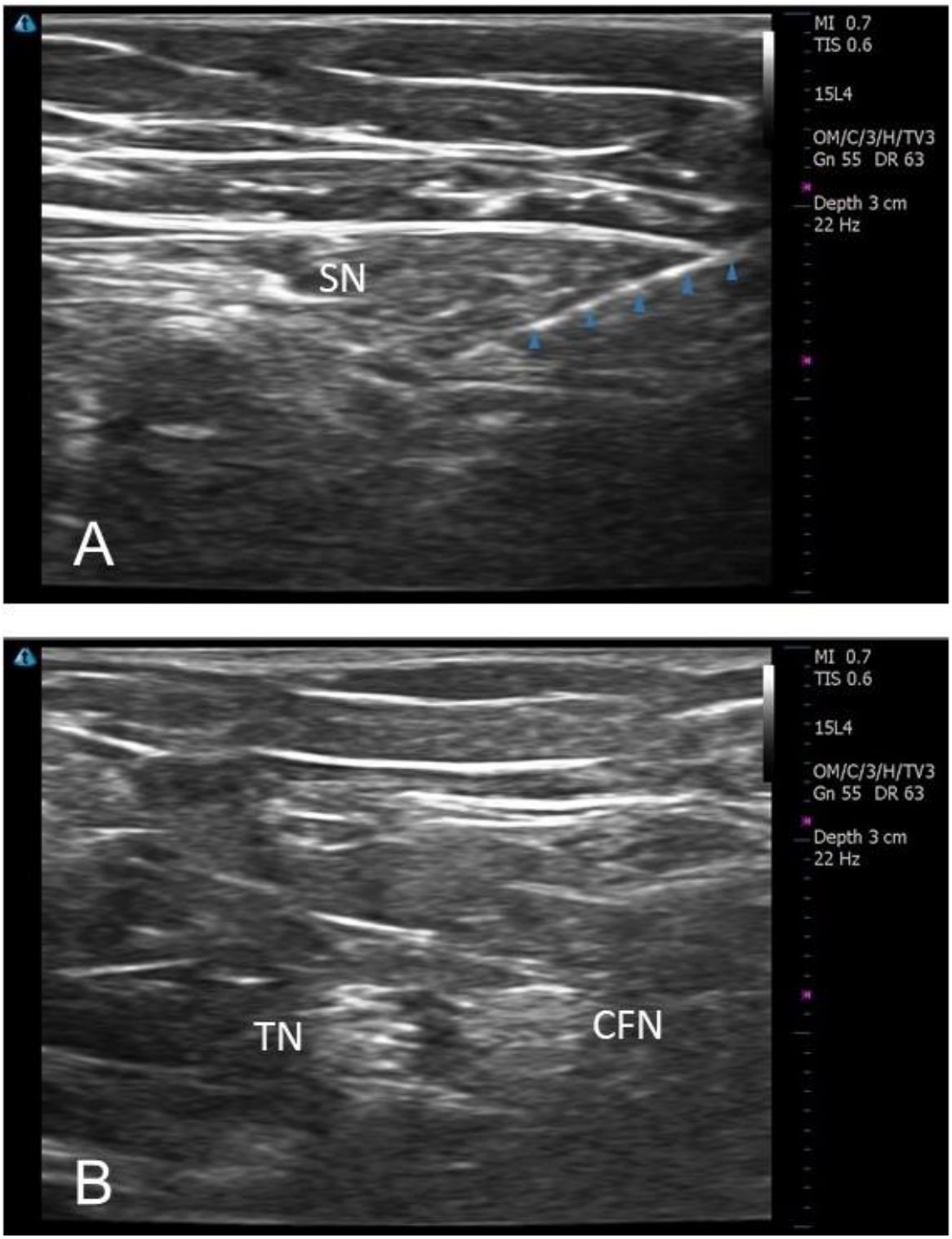


Figure 39: Simulated femoral block with needle inserted (blue arrows). FL fascia lata; FI fascia iliaca; FN femoral nerve; P pectineus muscle; IP iliopsoas muscle. The outlined area (red) indicates approximately where the anaesthesiologist would expect to see evidence of vascular structures.



Figures 40 and 41: simulated sciatic block. SN sciatic nerve; TN tibial nerve; CFN common fibular nerve. In Figure 40, the probe is placed proximally on the posterior thigh. The anaesthetic needle (blue arrows) can be seen lateral to and just below the sciatic nerve. Figure 41 is distal to this and shows the division of the sciatic nerve into the tibial and common fibular nerves, a feature which aids positive identification of the sciatic nerve. Note: for this block the top of the image is the posterior surface of the thigh.

Chapter 4

Discussion

4.0 Discussion

The volume of literature on the subject of cadaver preservation methods, and particularly that concerning novel fixation techniques, has increased dramatically in the previous decade. Anatomical facilities are beginning to move away from formalin fixation and investigate alternative methodologies, due in part to long-term concerns for staff and student health. At worst, formaldehyde is recognised as carcinogenic, and at best it is a chronic irritant of eyes, nose, throat, and skin. Good ventilation (mechanical or otherwise) and appropriate PPE can minimise the immediate adverse effects of formalin exposure, but the ability to provide this can vary from facility to facility.

Another consideration for anatomy laboratories is the increasing unsuitability of formalin fixation to the delivery of learning outcomes, especially for institutes which have become centres of surgical training. Formalin-fixed cadavers have an undeniable advantage in the longevity of their preservation, and this is surely the primary reason why medical schools and other anatomical facilities have historically been unable or unwilling to move away from using formaldehyde in their embalming preparations. This is a more acute concern for facilities which may have a small pool of cadavers available to them annually. Formalin fixation ensures that body donors may be utilised over a long period of study, with little fear that the tissue will degrade in that time. However, the lack of visual and textural fidelity found in hard-fixed cadavers can become an issue in the later years of medical education. Specialised training courses are becoming a more common part of surgical skills acquisition, and accurate

cadaveric models are necessary to ensure the maximum benefit is gained by attendees.

Fresh frozen bodies and live animal models are possibly the most commonly-used patient substitutes, but there are notable disadvantages. Animal models are often useful when resources are scarce, but the comparative anatomy is not ideal and ethical concerns must be taken into account; fresh frozen cadavers may bring infection risks and can be difficult to maintain in optimum condition for the full duration of longer training modules. Soft-fixation methods thus present an appealing alternative. Cadavers preserved this way can be held for longer periods of time than fresh frozen bodies, present a lesser infection risk due to the anti-microbial effect of the embalming mixes, and are highly comparable to fresh frozen cadavers in terms of appearance and tactility.

The development of the Thiel soft embalming method (1992, 2002) was followed by a number of studies published in the ensuing years which examined the method from multiple angles: biomechanical studies (Wilke et al., 2011, Ling et al., 2016, Fessel et al., 2011), investigations into the educational uses (Ottone et al., 2016, Balta et al., 2015), and, inevitably, numerous explorations of its utility as a surgical training model (Prasad Rai et al., 2012, Holzle et al., 2012, Healy et al., 2015, Guo et al., 2012, Wolff et al., 2008). The Centre for Anatomy and Human Identification (CAHID) at the University of Dundee, Scotland was an early adopter of the method for their medical educational programs (Eisma et al., 2013) and currently employs the Thiel method as the sole method of cadaver preservation. Several other novel soft-fixation techniques were subsequently developed, including Genelyn, Fix 4 Life (van Dam et al., 2015), and

those of Imperial College London and the Dodge Company, with varying degrees of cadaveric flexibility achieved (Jaung et al., 2011, Balta et al., 2019b).

It is in this context that the use of saturated salt fixation has seen a resurgence of interest. A short account was given to the Journal of Anatomy by Coleman and Kogan in 1998, detailing their initial forays into the use of an enhanced saturated salt solution to give a low-formaldehyde, longer-lasting soft fix. The method was remarkably successful, providing good preservation of cadavers with a high degree of visual fidelity and near-lifelike tissue texture. Flexibility of the joints was retained, there was minimal distortion of anatomical structures, and no fungal growth for the duration of the study period (12-15 months) despite frequent handling during the dissection-based teaching term. Literature on this resurgence of salt embalming is surprisingly sparse, with a number of the existing studies originating from the African continent, SWANA regions, and the Indian subcontinent. The early literature concerns veterinary facilities alone, with almost no studies investigating the applications of saturated NaCl solution embalming in human cadavers aside from the original 1998 correspondence. Interest began to increase with the publication of Hayashi et al (2014) whereby the modified saturated salt solution was first explored as a suitable embalming fluid to prepare cadavers for surgical skills training. Since then, further investigations have been made into the utility of the saturated NaCl solution cadaveric model for various surgical and medical applications, including ultrasound training and catherisation (Homma et al., 2019, Kathapillai, 2019, Shirai et al., 2015, Burns et al., 2018, Watanabe et al., 2020). In these studies, the saturated NaCl solution cadavers were viewed extremely favourably as a surgical training model, both on their own merits and when

directly compared with other embalming techniques. Metrics commonly examined by investigators included odour, appearance, joint flexion, and tissue feel. All of the above embalming solutions contained a small amount of formaldehyde, typically below 1% (Table 7), along with other chemicals frequently used in more traditional embalming solutions, such as alcohols, glycerin, and phenol.

One aspect of the examination of this method which is absent from the literature is a reliable measure of how long these cadavers may be preserved for. Coleman and Kogan demonstrated a preservation period of a year or longer; successive studies utilised the human cadavers for shorter periods of time – typically no more than a few months. Animal studies also showed the potential for a longer period of retention than had been demonstrated previously. No human cadaveric studies had been conducted to demonstrate the longevity of preservative effect of a simple saturated NaCl solution to date.

The original embalming solution used in this study was prepared in house, utilising commercially-available compacted salt and deionised water. The method as described by Coleman and Kogan did not specify that distilled/DI water was to be used, nor any particular grade of salt. The salt was manually crushed to reduce the size of the pieces, before being added to the DI water. This was used as it is readily available on tap in UCC laboratories, and it was hoped that it would improve the concentration of the final solution, given the coarse quality of the salt being utilised. It was noted that a significant portion of the salt did not enter solution; this may be as a result of the salt particles not being adequately crushed, or due to other experimental conditions such

as water temperature or salt purity. The amount of solute remaining at the end of the container was estimated to be approx. 20% of the total salt added. This gave the salt solution an estimated concentration of 28%. Salt saturates a solution at 36-37%, so this estimate is appreciably lower than the hoped-for concentration. Nevertheless, it was felt that an appropriate saturation level had been reached to enable full preservation of the cadavers, and this was borne out by the prolonged period for which the cadavers were conserved.

No other substances were added to the embalming solution used in this experiment. This differs markedly from the majority of papers which were referenced in preparation for this study, wherein a small amount of formaldehyde was added to the embalming solutions. Coleman and Kogan (1998), the foundational paper of this study, used a saturated NaCl solution with a final formaldehyde concentration ≤0.75%, and the other papers examined in the course of this study had varying levels of formaldehyde ranging from 0.6% to 2.85% (Table 6). A notable exception to this is the veterinary study completed by Lombardero et al (2017), being the sole paper reviewed which did not make use of any components beyond water and salt. The authors of that article did, however, find that open abdominal access was required in order to effect optimal preservation of the viscera, and additionally recommended cyclical perfusion as a means to reduce the volume of embalming fluid needed.

Table 6: Formaldehyde concentration of embalming solutions, given in %. The concentrations are as directly quoted by the authors, or were calculated based on information given in the materials and methods.

Embalming method	Main preservative component	Formaldehyde concentration
Thiel	Propylene glycol	0.6%
Coleman and Kogan	Sodium chloride	≤0.75%
Kathapillai	Sodium chloride	0.7%
Hayashi et al.	Sodium chloride	0.8%
Gosomji et al.	Sodium chloride	1.48%, 2.85%
Lombardero et al.	Sodium chloride	0.0%
O'Flynn (this study)	Sodium chloride	0.0%

This study fell into the same methodological group as Coleman and Kogan (1998) and Hayashi et al (2014) in the use of large volumes of salt solution, which were infused into the cadavers and allowed to remain there to effect preservation. Differences in volume of embalming fluid are difficult to control for – the total amount of fluid a cadaver will accept varies on body size, composition, and other intrinsic factors which may not be perceptible to the embalmer. This resulted in some variation in the total amounts of fluid perfused into each of the 4 cadavers studied here.

The method of Coleman and Kogan called for the embalming solution to be placed in the bag in which the cadavers were stored. This element of the authors' method was retained as part of the initial methodology for this study. It was not made clear in the paper whether the storage bag was sealed in some way or not. The cadavers in this study were simply placed in a central-opening plastic body bag (purchased from Barber Medical, UK), and the leaves were overlapped as far as possible and tucked under the cadaver to secure.

It was found that the benefits of the embalming fluid being used as a storage medium were limited in this case to the areas of the cadaver that were submerged in the fluid. This particular aspect of the methodology proved to be very difficult to neatly contain in storage, easily spilling out of the bags; and it gave no considerable additional benefits in terms of preservation. The original authors gave no reasoning for the addition of a storage medium; neither did they elaborate further on the practicalities of same, for example, ensuring all parts of the cadaver were immersed in the storage solution, or whether this was necessary or advised. Conjecturally, it may be a form of replacement for an immersion medium, given that many anatomical facilities use this mode of storage. However, full coverage of the cadaver with the solution could not be achieved simply by adding the recommended volume to the storage tray, and so based on this assessment and on the results of the first study group, the decision was made to remove the storage fluid from the methodology in the second part of the study. It was demonstrated effectively that the preservation of the second group of cadavers was not adversely affected by this change.

Goniometric measurements were taken from the cadavers in Group B of, both pre- and post-embalming. As noted in the literature (Beger et al., 2020, Benkhadra et al., 2011a), Thiel embalming leads to hyperflexibility in cadavers. This is posited to be as a result of “mincing” or fragmentation of the muscle fibres within the intact collagen sheath (Benkhadra et al, 2011a) and/or the alteration of collagenous networks (Fessel et al., 2011). This is generally believed to be due to the boric acid content of the embalming solutions, although recent investigation has shown that it is as likely to be one or more of the chemical salts used in the solution (McDougall et al., 2020).

The exact mechanism of preservation of saturated NaCl solution embalming has not been described in any of the papers reviewed in advance of this study, a fact also noted by Gosomji et al (2018). One of the modes by which embalming results in conservation of tissues is by halting microbial propagation and thus preventing the natural process of bacterial-driven decomposition. Due to the hypertonic nature of salt, any microbes present become dehydrated and die as a result of osmotic pressure, a mechanism long exploited historically to avoid food spoilage and to effect body preservation. High concentrations of salt can denature proteins by removing the essential layer of water molecules which is present on the surface of proteins (Sinha and Khare, 2014). This is generally caused by ionic interactions which interfere with hydrogen bonding. The possibility of protein denaturation may occur as a mode of tissue preservation in parallel with the antibiotic effect of the salt, but this remains completely untested in the literature as well as in this study. It is clear, however, that the inaccessibility of water molecules as a result of the altered osmotic gradient has a wide-ranging and comprehensive preservative effect on human tissues.

A comparison was made between the pre- and post-embalming goniometric measurements to discover whether any significant changes occurred in the flexibility of the cadavers as a result of the preservation process. There is some variation expected within cadavers and across joint types, but no consistent or statistically significant increase or decrease can be seen which would indicate a notable change in the overall flexibility or range of motion (ROM) of the joints of the cadavers as a result of the saturated NaCl solution. The cadavers retained excellent joint movement and

could be manipulated and posed for dissection and for clinical purposes with little effort.

Some bacterial sampling was carried out early in the study to establish the presence or absence of microbes on the cadavers. Swabs were taken from discrete areas of one cadaver, plated, and propagated overnight. The results of the plate counts show that there was some bacterial presence on the skin and nasal mucosa, but this may have been due to pre-embalming conditions or other contamination. The cadavers were washed with an antimicrobial solution before embalming, and the possibility exists that areas such as the nostrils, the groin, or between the toes may have been less thoroughly sanitised than other parts of the body. The embalming solution may have been effective at reducing or removing surface microbes initially, but that level of decontamination was not maintained, potentially due to contact with the storage bag. It is likely that the non-sterile bag carried microbial contaminants which were transferred to the skin of the subjects and flourished in subsequent weeks.

The overall quality of preservation of the first group of cadavers was mixed. Notably, portions of the anterior surface of both cadavers was colonised by microorganisms, including fungi, which significantly affected the superficial structure and appearance of the subjects. This colonisation was limited to areas of the cadaver which had been exposed to the air. The lack of mould growth caused by the contact of the posterior surface of the cadaver with the tray kept the skin intact and prevented the type of changes which happened anteriorly; a similar effect was seen where the partially-

submerged areas of the flank and legs were not subjected to microbial growth and the skin was largely intact.

The visible presence of bacteria and fungi on the surface of the cadavers while subcutaneous tissues were adequately conserved appears to confirm that the contamination was merely superficial. Preparation of the donors did not include intestinal flushing via the use of an enema, and the lack of visceral deterioration in the cadavers even while faecal matter was still present in the large intestine demonstrates the effectiveness of the embalming method in conserving some of the least resilient organs in the body.

It is important to note that although microbial sampling was carried out, no attempt was made to formally identify which strains of bacteria and/or fungi which constituted areas of growth seen on the cadavers. It can be surmised that any bacteria present are aerobes, given that they did not appear to propagate beneath the surface of the storage fluid.

The frequent employment of Dodge® Dis Spray during the study period of Group B had a very real impact on the superficial condition of the cadavers. These cadavers were sprayed each time they underwent a visual check, and every day while they remained out of storage while the ultrasound study was ongoing. The storage bag was also thoroughly sprayed at these points in time to discourage any microbial growth which could result from contamination of the non-sterile plastic surfaces. At no point

during the study did the subjects show any noticeable signs of microbial growth. Both groups of subjects were stored for similarly considerable lengths of time before being dissected, meaning that the lack of microbial growth on the Group B cadavers cannot solely be ascribed to the infrequent amount of handling they received. Rather, the regular spraying of the cadavers and their storage bags undeniably contributed to the exemplary level of surface conservation observed in this group.

The skin slippage observed in all the cadavers to differing degrees was not an unexpected occurrence. Cadavers embalmed using the Thiel method undergo extensive desquamation while they are stored in macerating tanks. The immersion fluid, when removed, typically contains quantities of hair, nails, and sloughed-off skin. Lombardero et al noted the loss of body hair from saturated NaCl animal cadavers while stored in immersion tanks. An element of conjecture must be employed here, as a search of scientific literature returned no viable mechanistic explanation for the occurrence of desquamation in cadavers, apart from attributing it to the general process of putrefaction. It may be presumed that skin slippage in Thiel cadavers is related to the suggested alteration of collagen networks that allows hyperflexibility of the muscles and joints (Fessel et al., 2011). Other connective tissues of the body are likely to be affected by this process, including type VII collagen which is present at the dermal-epidermal junction. Disruption of this connective tissue may lead to loss of adhesion of the epidermis to the dermis and result in desquamation, and in hair and nail loss as a result of a decrease in integrity of the connective tissue stuctures of hair follicles and of the nail matrix. This process seems less likely to affect the saturated NaCl solution cadavers; rather, the mechanism of desquamation there may be simple

mechanical disruption of the layers of the skin by fluid or osmotic pressure, particularly given the large volumes of solution introduced during embalming.

Dissection of the cadavers presented several aspects to consider. The durability of tissues following larger-scale dissection was extremely poor in Group A, as structures quickly lost integrity and the appearance and textures changed very much for the worse. The cadavers of Group B were not held for as long as Group A post-dissection (being released to families within days of completion of the dissection), however the structures dissected weeks earlier showed no signs of adverse change besides the oxidative browning of some muscles due to exposure to the environment. The degradation seen in Group A from approximately the 20-month mark was not observed in Group B, and the cadavers of Group B were held in exceptional condition for several months longer. There was no indication that these subjects were likely to begin deteriorating if held for a longer period of time.

The studies by Coleman and Kogan and Gosomji et al. demonstrated the ability to dissect saturated NaCl solution cadavers, both human and animal, over periods of time marked in months rather than weeks, with minimal visual change or other indications of degradation. Gosomji et al stored animal cadavers for a week before commencing dissection, while Coleman and Kogan recommended no less than 3 months but ideally up to a year before dissecting. The four cadavers in this study were dissected after approximately 20 months (Group A) and approximately 26 months (Group B). It may be that storing the Group A cadavers for such a long period before dissection led to a quicker deterioration when dissection began. If the cadavers were

approaching the limit of efficacy of the preservation period, they may have been more susceptible to putrefaction when conditions were changed. This could not be investigated fully with Group B as by the time dissection occurred, the subjects had reached the maximum length of time for which they could be held by the Department of Anatomy and they were released immediately afterwards.

It must be noted that full saturation of the solution with salt was not achieved with the original methodology. The problem of an under-saturated embalming solution was not restricted to this study, being an issue also experienced by Hayashi et al. when using the Coleman and Kogan protocol (personal communication, February 8, 2017). The estimated 28% concentration was demonstrably sufficient to preserve the Group A cadavers for a significant period of time, but the deviation from full saturation may very well have compromised the potential length of time for which the cadavers could be held in peak condition. The cadavers preserved with industrially-prepared saturated NaCl solution (Group B) were maintained in near-perfect condition for over 2 years; given the differences in microbial colonisation between groups it is difficult to assert that the reduced salt concentration is the sole cause of the rapid deterioration of the first cadaver group, but it is certainly an important distinction and deserves consideration.

Another important difference between previous medium- to long-term studies and this study is that the embalming solutions used in Gosomji et al and Coleman and Kogan both contained a small amount of formaldehyde. While feasible, it is not possible to contend that this conferred a substantially greater advantage over a simple

saturated NaCl solution in providing the ability to dissect without danger of tissue degradation. A comparative study would have to be carried out to evaluate this more fully.

As mentioned in previous literature, one of the most compelling reasons to consider adopting saturated NaCl solution embalming is the overall high returns on the low cost of the materials. Some of the obstacles barring conversion to Thiel cadaver use are the mortuary infrastructure required, which must be invested in before embalming commences, and the cost and sourcing of the numerous chemical components of the stem solutions (Benkhadra et al., 2011b, Eisma et al., 2013).

An additional consideration is space – immersion tanks for whole cadavers are of considerable size and require sufficient mortuary space so as not to impact other activities. These concerns are even more relevant for smaller, less well-resourced facilities. Tap water and consumer-grade salt are almost all that is required for saturated NaCl solution, and no special equipment is needed. The industrially-made saturated NaCl solution utilised for the second group of cadavers had a base cost of €16.00/5L; embalming each body incurred an average cost of under €60 for the solution.

One negative aspect of the embalming procedure encountered during this study was the corrosion caused to parts of the Dodge® embalming pump, despite extensive flushing with DI water following use. If equipment is not well-maintained, the additional costs of purchasing new equipment or replacing multiple parts could

negate any financial gains made using a cheaper embalming technique. This could potentially be avoided by using a gravity feed system in the place of mechanical pump systems, or a detergent to more effectively cleanse the pump.

4.1 Saturated NaCl solution cadavers as anatomical teaching models

The first group of cadavers (Group A) were not deemed to be useful as anatomical teaching models. The microbial colonisation which occurred meant that the skin texture was drastically altered by the action of the fungi, greatly reducing its tactile fidelity. The external appearance of cadavers - particularly in the later part of the study - was unsightly and would likely prove difficult for undergraduate medical students to view, potentially distracting them from their learning objectives. The Department of Anatomy and Neuroscience, UCC teaching strategy is centred on using prosected body parts in the delivery of anatomical knowledge, which allows material from a limited number of donors to be used across multiple modules and student groups. Based on the results of this study, the lack of durability of the Group A cadavers once dissection had commenced would be a serious barrier to the provision of this type of instruction.

The second group of cadavers had much greater potential for use as a teaching model. The external appearance was not dissimilar to that of hard-fixed cadavers, with what could be described as “normal” colouration and feel. Based on the visibly good condition of the cadavers, integrity was maintained in all regions with pliable muscular structures and easily distinguishable tissue types. The thoracic cavity would potentially be less suitable for anatomical instruction given the altered structural configuration

of the heart, but the peritoneal cavity and retroperitoneal structures would be excellent for use in instruction.

If the period of time for which the cadavers could be continually dissected were to be reliably established and/or improved upon, the UCC teaching program which could benefit the most from the use of saturated NaCl solution cadavers would be the MSc Human Anatomy. These students dissect whole cadavers in small groups of two or three, completing a full-body anatomical examination over the course of two semesters. Previous years have utilised formalin-fixed cadavers only, while the 2020-21 academic year saw the first use of bodies embalmed using a Dodge® proprietary soft-fix embalming mix. The initial impressions of staff of the Dodge® technique is that there is a tendency for some of the cadavers to dry out in the less fatty areas of the body, such as the head and neck. The amount of adipose tissue and subcutaneous fat varies from cadaver to cadaver, and so no firm conclusions can be drawn as yet. The UCC anatomy laboratory makes use of high-air flow dissection tables, drawing air over and away from the cadaveric material. This is a formaldehyde-reducing measure, but it can negatively affect prosections and full cadavers by dehydrating exposed tissues. The first group of saturated NaCl solution cadavers did not show obvious signs of this dehydration despite spending numerous weeks on the ventilated tables, a characteristic also noted in the 1998 study by Coleman and Kogan, who stated that the high salt content would prevent dessication by holding onto water. Saturated NaCl solution cadavers could potentially remain in a good state of hydration over the course of a year's instruction, and, given the favourable impression of the Group A student dissector concerning the ease with which dissection could be carried out and the

visual and textural distinctness of the different tissues, it could provide a much more rewarding experience for students. Any institution with a similarly-structured teaching programme should experience comparable benefits.

4.2 Saturated NaCl solution cadavers as a clinical training model

Given the volume of recent studies demonstrating this principle, it should not have to be re-emphasised that the saturated NaCl solution cadavers would be good models for surgical training. A number of different surgical modalities have been investigated by other authors, and the saturated NaCl cadavers were found to be at least as useful as Thiel-embalmed cadavers for laparoscopy, trauma, orthopaedics, and other specialities. The area of medical imaging has seen some attention paid to it, however, many of the relevant papers referenced for this study focused on Thiel cadavers (Benkhadra et al., 2009, Munirama et al., 2016, Kondrashova et al., 2020). The comparative results of Hayashi et al (2014) indicate that it would be expected that saturated NaCl solution cadavers would perform similarly to Thiel cadavers as an ultrasound phantom for use in clinical training. Initial findings from that study indicated that the visibility of structures was very good, and that the particular consistency of the saturated NaCl solution tissue allowed catheterisation of the saturated NaCl solution cadavers, a procedure which was more difficult to achieve in Thiel cadavers.

As with other medical specialities, clinical skills training is an important part of the education of medics. There are certain risks directly associated with peripheral nerve block (PNB), namely vascular puncture, neural puncture, intravascular/intraneural

injection of local anaesthetic, and resulting peripheral nerve injury and local anaesthetic toxicity (Jeng et al., 2010). Ultrasound imaging has been used to guide insertion of PNB needles and catheters since the early 2000s, and is generally believed to improve the success of the procedure, aiding the prevention of inaccurate needle placement and reducing the time spent performing the block (Walker et al., 2009). It is essential that novice anaesthesiologists are exposed to adequate simulated training opportunities in order to build up their skills without harming patients.

The preliminary investigation carried out to assess the viability of a cadaveric US study as part of this thesis gave very encouraging results. The structures were rated by the anaesthesiologist as very visible, with numerous target structures positively identified. This led to the carrying out of a formal study to test the cadavers as a potential training model. The target neural and muscular structures were, on the whole, identifiable; some with more difficulty than others. The tactile feedback was felt to be comparable to that of live patients, providing the vital fascial “pop”. The cadaver could be manipulated into different poses to mimic clinical practice. In these ways, the cadaveric model possessed a good physical resemblance to a live patient and was rated a mixture “moderately like” and “very like” for these metrics.

There were some notable disadvantages, however. The extreme lack of visibility of vessels created problems for a number of the clinicians, and this proved to be an overwhelming disadvantage for the training model. This deficiency has been noted in some previous studies, both in veterinary (Thanaboonnipat et al., 2021) and human contexts. Hayashi et al observed much better results for jugular vein catheterisation

of SSS cadavers than that of Thiel cadavers. Vascular contents were present and could be aspirated to confirm correct placement of the catheter. It is not clear from the published study how quickly the cadavers were used after embalming, but recently-embalmed cadavers may potentially have intravascular contents which persist for a short period and which dematerialise over time.

The absence of these crucial landmarks led to a reduction in confidence with regard to the positive identification of nerves, even for experienced US users. Participants stated that they were quite certain of the identification, but that the presence of vessels would put the matter beyond doubt. All were confident enough to perform the block on the cadaveric model, given the low stakes, but perhaps would not be so comfortable performing the block on a live patient with comparable gaps in the visual assessment. In some blocks, the needle may be passing relatively close to the vessels, and the participants could not be certain that they would not transfix the vessels without visual confirmation of their location. In general, participants felt there were some merits to the model, but the lack of clear vessel visualisation very negatively impacted its utility as an ultrasound training model.

The durability of the cadaver model was not directly assessed as part of this study. One respondant noted persistent needle tracks in one region of one cadaver while they performed the block, but this was not observed or mentioned by other respondants. Due to Covid-19, there were large lapses of time between some study sessions, giving more time for tissue recovery, but on the occasion where study sessions were conducted on the same day, no comment was made by participants

regarding the presence of any previous needle marks. In this regard, the durability of the model would appear to be adequate.

One obvious limitation of this study is the structure of the questionnaire such that participants were asked to assess the cadaveric model based on a direct comparison with their experiences of live patients. The accuracy of evaluator recall may be variable, and cannot be controlled for in the experimental protocol. It is important to note, however, that all but one of the evaluators were engaged in current clinical practice and regularly performing these blocks, and as such their recall should be considered reasonably accurate.

It must be clearly established for any training model what the overall aim is to be. What educational goals or learning outcomes are desired? Recent thought on the subject has proposed that concepts such as “fidelity” can be vague and unhelpful, and that simulation-based training should instead place more emphasis on functional task alignment (Hamstra et al., 2014). In the case of the saturated NaCl solution cadaveric model, there is a reasonable physical resemblance, but the functional alignment with the complex task of performing a PNB is not wholly present.

The way forward in this regard could be to break down the learning objectives a little more. The haptic fidelity of the model was good, with similar needle feel and strong replication of the sensations of fascial transfixion. The cadavers could be utilised best as training for early-career anaesthesiologists, allowing trainees to become familiar with the tactile feedback, different sensations and physical cues of needle insertion

which are central to the successful performance of PNBs. It would enable novices to practice probe handling on irregular body surfaces, to gain greater familiarity with the appearance of different anatomical structures under US visualisation, and to learn to coordinate real-time physical manipulation of the PNB needle with the images as seen on screen. The overall usefulness of the cadaveric model was rated as either moderately useful or extremely useful by the majority of respondants in this study.

Chapter 5

Conclusions

5.0 Conclusions

The study provided some useful information on the applicability of saturated NaCl solution embalming for long-term anatomical preservation. The results were encouraging, showing that cadavers could be held in good condition for longer periods of time than previously documented. It also provided some needed context for the suitability of the cadavers as a clinical training model.

Both groups were held for extended periods of study with little deterioration taking place. The first study group were subject to extensive microbial growth due to surface contamination while in storage. This altered the texture and appearance of external features, but musculoskeletal and visceral structures remained unaffected throughout the long storage period. These cadavers experienced a sudden and rapid loss of tissue stability once dissection commenced. This was generally limited to exposed areas, however - when the final dissection phase took place, large regions of intact tissue were found in excellent condition alongside the clearly degraded previously-dissected areas.

This sudden deterioration may be suggested as resulting from the limit of effectiveness of the saturated NaCl solution being reached around the time that dissection commenced; or from the complete exclusion of formaldehyde in the embalming solution, in contrast to similar studies. As the scope of the study was not extended to allow for further, more comprehensive investigation, the exact cause cannot be determined from the results of this thesis.

The second group of cadavers were treated specifically to prevent microbial colonisation, a goal which was successfully achieved by the liberal application of a commercial anti-microbial spray solution at regular intervals. Aside from a relatively minor level of desquamation, the cadavers maintained an exemplary external appearance for almost 2 years.

The outcomes of the two study groups were distinct from each other, having some divergence in methodology, thus creating differences which presented themselves as the study progressed. Choices made regarding the initial methodology for the first group of cadavers which gave undesirable results were rectified in the second group, and the impeccable external condition of the second group of cadavers demonstrated the effectiveness of the improvements made to the process.

Due to constraints on the length of time the donors could be retained for anatomical study (maximum of 3 years in the case of Group B) and the timing of the donor release period, the comparative ability of Group B cadavers to avoid the rapid deterioration of tissues post-dissection as observed in Group A could unfortunately not be examined. Further limitations occurred with the small number of participants recruited to the US study, due primarily to local and national Covid-19 restrictions which were put in place throughout 2020 and 2021.

Overall, the saturated NaCl solution method of embalming undoubtedly permits long-term conservation of human cadavers for anatomical study and clinical training, and has promise as a method of introducing PNB delivery to anaesthesiology trainees, but

some finer details must be investigated and improved in order to present a robust and reliable alternative to either formalin-fixation or to the better-established soft-fixation methods known today.

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Appendices

Appendix 1: Ethics Application

UCC University College Cork, Ireland Coláiste na hOllscoile Corcaigh	**Social Research Ethics Committee (SREC)** **ETHICS APPROVAL FORM** srec@ucc.ie https://www.ucc.ie/en/research/about/ethics/

Introduction

UCC academic staff and postgraduate research students who are seeking ethical approval should complete this approval form. Ethical review by the Social Research Ethics Committee (SREC)[1] is required where the methodology is not clinical or therapeutic in nature and proposes to involve:

- direct interaction with human participants for the purpose of data collection using research methods such as questionnaires, interviews, observations, focus groups etc.;
- indirect observation with human participants for example using observation, web surveys etc.;
- access to, or utilisation of, anonymised datasets;
- access to, or utilisation of, data or case files/records concerning identifiable individuals;
- conducting Internet Research or research online.

SREC @ UCC considers itself an enabling committee, promoting strong research ethics amongst UCC's community of staff and student researchers. We are open to all types of research in the social research domain and if your research approach does not readily fit into this research form, do not be discouraged. Please add additional relevant notes to convey what you think is pertinent about the ethical aspects of your study.

Application Checklist

This checklist includes all of the items that are required for an application to be deemed complete. In the event that any of these are not present, the application will be returned to the applicant ***without*** having been sent for review. Please complete the checklist underneath, and ensure that your application includes all of these prior to submission. Thank you and best of luck with your research. If you require additional guidance, click here.

	Delete as applicable
All relevant files are combined into one PDF file (SREC application form, consent/assent forms, information sheets, data collection instruments, permission letters, etc.)	Yes
Completed SREC Application Form	Yes
Information Sheet(s) / Information Statement (i.e. at the beginning of an electronic survey) included	Yes
Consent Sheet(s) / Consent Statement (i.e. at the beginning of an electronic survey) included	Yes
Data Collection Instrument: Psychometric Instruments / Interview Guide / Focus Group Schedule / Survey Questionnaire / etc. included	Yes
Copy of permission letters to undertake research from relevant agencies/services included (if available)	NA
If you are under academic supervision, your supervisor(s) have approved the wording of and co-signed this application prior to submission	Yes
If this is a resubmission, all the revised and new text is highlighted in yellow	NA
Have you applied for ethical approval for this project from another UCC ethics committee?	No

APPLICANT(S) DETAILS

Name of UCC applicant(s)	CARRIE O'FLYNN	**Date**	07/02/2020
Name of Department / School / Research Institute / Centre / Unit / College	DEPARTMENT OF ANATOMY AND NEUROSCIENCE	**Contact No.**	ext: 5461
Correspondence Address	DEPARTMENT OF ANATOMY AND NEUROSCIENCE	**Email Address**	carrie.oflynn@ucc.ie
Name and year of course (students only)	MSc Human Anatomy (part-time) Year 2	**Name of supervisor(s)** (students only)	ANDRÉ TOULOUSE
Is this a resubmission?	No	SREC Log No. (if a resubmission):	
Obtaining ethical approval from SREC does not free you from securing permissions and approvals from other institutional decision-makers and agency ethical review bodies. These bodies may accept the SREC approval, but researchers are responsible for ensuring they are compliant in advance of collecting data.			

Project working title	DEVELOPMENT OF A CADAVERIC TEACHING MODEL

If this is a collaborative project / community-based participatory research project / *joint* application with another agency, please complete this additional section:

Names of research partners / civil society organisations collaborating on this project (this section must be completed for participatory / community-based participatory research studies)	Not applicable
Agency contact person and position	
Agency address	
Details of the partnership (Please identify clearly the roles and responsibilities held by each party in the partnership in relation to the different aspects of the research).	

ETHICAL APPROVAL SELF-EVALUATION

If your answer falls into any of the shaded boxes below, please address each point later on in the application form

		YES	NO
1	Do you consider that this project has significant ethical implications?		x
2	Will you describe the main research procedures to participants in advance, so that they are informed about what to expect?	x	
3	Will participation be voluntary?	x	
4	Will you obtain informed consent in writing from participants?	x	
5	Will you tell participants that they may withdraw from the research at any time and for any reason, and (where relevant) omit questionnaire items / questions to which they do not wish to respond?	x	
6a	Will data be treated with full confidentiality / anonymity (as appropriate)?	x	
6b	Does your project require you to carry out a Data Protection Impact Assessment (DPIA) in compliance with UCC Data Protection Policy?		x
7	Will data be securely held for a minimum period of ten years after the completion of a research project, in line with the University's *Code of Research Conduct* (2016)?	x	
8	If results are published, will anonymity be maintained and participants not identified? (see Q. 30 below regarding open data considerations, if relevant)	x	
9	Will you debrief participants at the end of their participation (i.e. give them a brief explanation of the study)?	x	
10	Will your project involve deliberately misleading participants in any way?		x
11	Will your participants include children / young persons (under 18 years of age)?		x
12	If yes to question 11, is your research in compliance with the UCC *Child Safeguarding Statement* which sets out the legal requirements under the Children First Act 2015?		
13	Will your project require you to carry out "relevant work"[2] as defined in the National Vetting Bureau (Children and Vulnerable Persons) Acts 2012 to 2016?		x
14	Do you require official Garda Vetting through UCC before collecting data from children or vulnerable adults? (Please note that having a Garda Vetting through another body is not sufficient; a separate UCC Garda Vetting is always required.)		x
15	Will your participants include people with learning or communication difficulties?		x
16	Will your participants include patients / service users / clients?		x
17	Will your participants include people in custody?		x
18	Will your participants include people engaged in illegal activities (e.g. drug taking, illegal internet behaviour, crime, etc.)?		x
19a	Is there a realistic risk of participants experiencing either physical or psychological distress?		x
19b	Is there a realistic risk of the researcher experiencing either physical or psychological distress?		x
20	If yes to question 19a, has a proposed procedure for linking the participants to an appropriate support, including the name of a contact person, been given? (see Q. 33)		
21	If yes to question 19b, has a proposed procedure/support structure been identified?		
22	Are your research participants students with whom you have some current/previous connection (module coordinator, research supervisor, professional tutor, etc.)?		x
23	Will your study participants receive payment / gifts / voucher / etc. for participating in this study?		x
24	If your research is conducted on the internet, does it involve human participants? (e.g. through web surveys, social media, accessing or utilising data (information) generated by or about the participant/s; or involve observing human participants in their online interactions/behaviour). If yes, please review and utilise the UCC policy for conducting Internet Research.		x

DESCRIPTION OF THE PROJECT

*Ethical review requires that you **reflect** and seek to **anticipate** ethical issues that may arise, rather than reproduce copious text from existing research proposals into these boxes. Entries should be **concise** and relevant to the point / question.*

25. Very brief description of your study (15-25 words max.)
[e.g. This is a qualitative study of primary school teachers' attitudes towards religious teaching using focus groups to collect original data]

A quantitative survey of clinicians' appreciation of a novel cadaveric model for clinical training.

26. What is your study about? (100-200 words max.)

Standard cadaveric preservation alters the quality of biological tissue, leading to a hardened, discoloured and less pliable body. These models cannot be used for clinical teaching in a number of disciplines including anaesthesiology. Nerve blocks are commonly applied using ultrasound to guide the needles around sensitive structures such as the spinal cord, but in standard cadaveric models, the hardened tissue reduces the visibility on ultrasound and does not allow needle placement.

Two cadavers were embalmed with a novel embalming solution preserving life-like features. These cadavers have been stored to test the long-term preservation properties and we now wish to assess the quality of the tissue after preservation.

We aim to assess the quality of the two preserved cadavers by asking clinicians to perform a series of clinical techniques (ultrasound, nerve block, body positioning) and relate their appreciation to their experience in the clinical setting with real patients.

A group of anaesthesiologists will be recruited to examine the cadavers under ultrasound and perform a nerve block in three discrete areas. These clinicians will be asked to provide feedback relating to the visibility of anatomical structures on ultrasound, the haptic qualities of the tissue while performing the nerve block, and rate the preserved cadavers and their usefulness as a clinical training model.

The present ethics application relates to the survey part of the study. The use of cadaveric material for teaching and research in anatomy is covered by the legislation governing the practice of anatomy in the Republic of Ireland (Anatomy Act 1832, Medical Practitioners Act 2007). The license holder is the Professor of Anatomy.

27. What are your research questions?[3]

The aim of the study is to assess the usefulness of a novel cadaver-embalming method for anesthesiology training.

The questions will target three main themes
Does the novel model allow satisfactory visual acuity under ultrasound imaging?

Does the model offer satisfactory haptic properties during needle placement?
Haptic properties refer to the tactile feeling, resistance and elasticity of the tissue.

Is the model sufficiently movable to allow the clinicians to position it for clinical intervention simulation?

Following the completion of a series of clinical tasks, the participants will be asked to relate their impressions of the novel cadaveric model to their clinical experience with live patients

28. Who are the participants in your study? (recruitment methods, number, age, gender, exclusion/inclusion criteria, detail permissions to be sought / secured already, and how will you recruit participants?)

We are planning to recruit 10 participants from the CUH Department of Anaesthesiology.

Participants will be practising anaesthesiologists at all stages of training and practice. They must have clinical experience with the techniques that will be employed in this study. This is the only inclusion / exclusion criterion.

Age and gender will not be recorded or used as selection criteria.

29. Concise statement of *anticipated* ethical issues raised by your project. How do you intend to deal with them? Please address *all* items where your answers fell into a shaded box in the self-evaluation above. (350 words max.)

There are no anticipated ethical issues.

The use of cadaveric material for teaching and research in anatomy is covered by the legislation governing the practice of anatomy in the Republic of Ireland (Anatomy Act 1832, Medical Practitioners Act 2007).

30. Data.
(a) How will you collect your data? Brief description and justification of methods and data collection measures to be used.
(b) If you are creating audio/video recordings, who will perform the transcription? (If transcription is being outsourced the transcription service used needs to be trustworthy, reliable and confidential and ensure that data transfer is done securely).
(c) What type of data will you be storing?
(d) How and where will you store your data?[4] (provide details for both physical *and* electronic documents . See page 7, Electronic Data Storage for guidance around data storage).
(e) For how long will you store the data? (A minimum storage period of 10 years is required)
(f) Who will you share the data with? (*Sample* prompts: If you plan to make your raw research dataset available publicly as part of the open data movement, or if you are required to do so as part of funding/journal requirements, please address your protocol here (make explicit links to Q. 32 below and show that you have addressed this in your consent form and information sheet). For collaborative/community-based participatory research, please address issues such as shared ownership of data, publication of findings, etc. If your funder contractually requires you to give them access to the 'raw' dataset, examine relevant implications, including appropriate anonymisation, protocols for secure access to the dataset, etc.).
(g) If you are planning to analyse an existing dataset, please outline how the original consent process allows for your data analysis.
(h) If you are planning to request access to health/case files/personal records that were not created for research purposes, please address Data Protection considerations, provide a strong rationale and comprehensively address associated ethical issues.
(i) If you ticked yes to Q.6 above, have you submitted your DPIA?

(a) Data will be collected by asking the participants to complete paper copies of the attached survey instrument

(b) n/a

(c) Anonymous responses to the attached survey questions only. The participants are anonymous. Their position in the order of use of the model will be recorded on the survey to assess for potential deterioration of anatomical structure after repeated needle placement.

(d) Physical responses will be stored in a locked filing cabinet in the Anatomy Flame Lab office (room 3.59, Western Gateway Building, UCC) until they are converted to e-documents after which they will be sent for confidential shredding.

Candidates' consent forms will also be stored here. Electronic documents will be stored in secure UCC One Drive storage. All surveys are anonymous therefore there will be no identifiable information apart from the consent forms.

(e) 10 years

(f) The dataset will only be used by myself and my academic supervisor in the course of this research. Once findings are compiled and analysed, the compiled results will be submitted for publication. Consent for this will have been sought at the time of the study. All information will remain anonymous.

(g) n/a

(h) n/a

(i) n/a

31. Arrangements for informing participants about the nature of the study (e.g. information sheets, letters of invitation, social media information, participant recruitment, focus group welcome/schedule, withdrawal, etc.)

A comprehensive information sheet (see attached) will be circulated before the study commences. Participants will also be briefed on this at the time of the study.

32. How you will ensure that participants provide informed consent? (cf. Question 4 - attach relevant form(s); address special considerations in terms of children / young people / vulnerable persons / adults who have difficulty in making decisions unaided)

Participants will be informed of the nature of the study before commencing, and given the opportunity to opt-out at any point.
Signed written informed consent forms will be collected before commencement of the study (please find the informed consent form attached with the application).

33. Outline of debriefing process at the end of the data collection process (cf. Question 9)**. If you answered Yes to Questions 19a or 19b, give details here. State what you will advise participants to do if they should experience problems (e.g. who to contact for help).**

There will be no formal debriefing process as we do not anticipate any ethical or physical/psychological issues arising from this study. However, any question(s)/quer(ies) will be addressed; before and after the study. For additional questions/comments later, participants will have the option of contacting the researcher at the following email address: carrie.oflynn@ucc.ie

34. Estimated start date and duration of project

March 2020
Estimated duration no more than 4-6 weeks of data collection. These exact dates will depend on the availability of clinicians to attend study sessions.

Appendix 2: US Study Information and Consent Form

Department of Anatomy and Neuroscience, UCC

Project information for research participants

Investigators: Ms Carrie O'Flynn, Dr André Toulouse

Study Location: Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland

Purpose of the study: This study is concerned with the development and assessment of a novel cadaveric clinical training model. The aim of the study is to determine the utility of the cadaveric model to train clinical anaesthetists to perform 3 types of ultrasound-guided nerve blocks.

What will the study involve? Before the start of the study, you will be asked to sign the attached informed consent form. You can retain this information section. When inside the reception area of the anatomy suite, you will be briefed about the study and given a randomised number which will be used to track the order in which you perform the blocks on each of the two cadavers.

In the anatomy laboratory, you will perform three (3) nerve blocks guided by ultrasound, first on one cadaver and immediately afterwards on the second. Following this, you will be asked to complete an anonymous survey in which you rate the cadaveric model on several metrics related to your performance of the block. The feedback obtained from your answers will be used to make a determination of the utility of the cadaveric model in future clinical training.

Your participation is entirely voluntary and we greatly value your input. You have the option of withdrawing before the study commences or to discontinue your involvement within 2 weeks of data collection.

The data will be kept confidential for the duration of the study. On completion of the experiment, they will be retained for ten years and then destroyed. The results will be presented in an MSc thesis and may be published in a research journal. No clues to your identity will appear in the thesis. Any extracts from your survey response that may be quoted in the thesis will be entirely anonymous.

I don't envisage any negative consequences for you in taking part. At the end of the procedure, I will discuss with you how you found the experience and whether you have any questions or comments you would like to share with me. Any further questions/queries will be addressed by contacting the researcher, Carrie O'Flynn (carrie.oflynn@ucc.ie).

This study has been reviewed and approved by the Social Research Ethics Committee at University College Cork.

If you agree to take part in the study, please sign the consent form overleaf.

We would like to sincerely thank you for your participation in this study.



Consent Form

Development and Assessment of a Novel Cadaveric Clinical Training Model

I, ... (BLOCK PRINT), agree to participate in this research study.

I confirm that:

- The purpose and nature of the study has been explained to me in writing.
- I am participating voluntarily;
- I give permission for my data to be used for further analysis and research;
- I understand that I can withdraw from the study without repercussions, at any time, whether before it starts or while I am participating;
- I understand that I can withdraw permission to use the data within two weeks of data collection, in which case the information will be deleted;
- I understand that anonymity will be ensured throughout the course of the study;
- I understand that anonymous extracts from my survey responses may be quoted in the thesis and any subsequent publications.

PRINT NAME: _____

Signed _____ Date: _____

Appendix 3: US Questionnaire

ULTRASOUND-GUIDED NERVE BLOCK CADAVERIC MODEL

1. Please choose your current level of specialised professional experience:

   Under 2 years ☐ 2-5 years ☐ 5-10 years ☐ Over 10 years ☐

2. Did your anatomy education or clinical training make use of human cadaveric material?

   YES ☐ NO ☐

   If yes, was this material: formalin (hard) fixed ☐

   Soft fixed ☐

   Fresh frozen ☐

3. Did your anatomical education or clinical training make use of other training models?

   YES ☐ NO ☐

   If yes, please describe below:

4. BRACHIAL PLEXUS
   Please rate the visibility of the following landmark structures on the ultrasound image, where 0 = cannot be visualised and 4 = can be fully visualised:

	0 = cannot be visualised	1 = poor visualisation	2 = adequate visualisation	3 = good visualisation	4 = excellent visualisation
Subclavian artery					
Anterior scalene					
Middle scalene					
Pectoralis major					
Pectoralis minor					
Axillary artery					

5. Please rate the visibility of the following neural structures specifically, using the below scale:
   0 = cannot visualise the nerve
   1 = visualisation is poor (can locate the nerve but attempting block would be extremely difficult)
   2 = visualisation is adequate (performing block would be challenging but achievable)
   3 = good visualisation (performing block would not be difficult)
   4 = excellent visualisation (there would be no problems performing the block)

	0 = cannot visualise nerve	1 = poor visualisation	2 = adequate visualisation	3 = good visualisation	4 = excellent visualisation
Lateral cord					
Medial cord					
Posterior cord					
Axillary nerve					
Medial nerve					
Musculocutaneous n.					
Radial nerve					
Ulnar nerve					

6. INGUINAL REGION
   Please rate the visibility of the following landmark structures on the ultrasound image, where 0 = cannot be visualised and 4 = can be fully visualised:

	0 = cannot be visualised	1 = poor visualisation	2 = adequate visualisation	3 = good visualisation	4 = excellent visualisation
Femoral artery					
Femoral vein					
Iliopsoas muscle					
Fascia lata					
Fascia iliaca					

7. Please rate the visibility of the following neural structures specifically, using the below scale:
   0 = cannot visualise the nerve
   1 = visualisation is poor (can locate the nerve but attempting block would be extremely difficult)
   2 = visualisation is adequate (performing block would be challenging but achievable)
   3 = good visualisation (performing block would not be difficult)
   4 = excellent visualisation (there should be no problems performing the block)

	0 = cannot visualise nerve	1 = poor visualisation	2 = adequate visualisation	3 = good visualisation	4 = excellent visualisation
Femoral nerve					

8. POPLITEAL REGION
   Please rate the visibility of the following landmark structures on the ultrasound image, where 0 = cannot be visualised and 3 = can be fully visualised:

	0 = cannot be visualised	1 = poor visualisation	2 = adequate visualisation	3 = good visualisation	4 = excellent visualisation
Semimembranosus					
Semitendinosus					
Biceps femoris					
Popliteal artery					
Popliteal vein					

9. Please rate the visibility of the following neural structures specifically, using the below scale:
   0 = cannot visualise the nerve
   1 = visualisation is poor (can locate the nerve but attempting block would be extremely difficult)
   2 = visualisation is adequate (performing block would be challenging but achievable)
   3 = good visualisation (performing block would not be difficult)
   4 = excellent visualisation (there should be no problems performing the block)

	0 = cannot be visualised	1 = poor visualisation	2 = adequate visualisation	3 = good visualisation	4 = excellent visualisation
Common peroneal n.					
Tibial nerve					
Sciatic nerve					

10. How would you rate the haptic qualities of the model under the probe, compared to a live patient?

0 = nothing like	1 = a little like	2 = moderately like	3 = very like	4 = exactly like

11. How would you rate the haptic qualities of the model when introducing the needle, compared to a live patient?

0 = nothing like	1 = a little like	2 = moderately like	3 = very like	4 = exactly like

12. How would you rate the visibility of the needle movement/tissue displacement in the model, compared to a live patient?

0 = nothing like	1 = a little like	2 = moderately like	3 = very like	4 = exactly like

13. How would you rate the haptic qualities of the model when injecting anaesthetic/substitute medium, when compared to a live patient?

0 = nothing like	1 = a little like	2 = moderately like	3 = very like	4 = exactly like

14. How would you rate the dispersal of anaesthetic/substitute medium in the model, compared to a live patient?

0 = nothing like	1 = a little like	2 = moderately like	3 = very like	4 = exactly like

15. How would you rate the poseability of the model, compared to a live patient?

0 = nothing like	1 = a little like	2 = moderately like	3 = very like	4 = exactly like

16. How would you rate the overall usefulness of the model for this type of clinical training?

0 = not at all useful	1 = a little useful	2 = neither useful nor useless	3 = very useful	4 = extremely useful

17. Please add any comments you may have below:

THANK YOU FOR YOUR PARTICIPATION