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The Antimicrobial Capacity of Embalming Solutions: A Comparative Study

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Running title: Microorganisms on embalmed cadavers

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Abstract

Aims:

Infectious health risks are associated with handling human cadavers and to decrease such risks, cadavers are embalmed using different chemicals. The aim of this study is to quantify the amount of microorganisms present in different regions of human cadavers before embalming, after embalming and over a period of eight months.

Methods and Results:

Human cadavers were embalmed using Thiel, formalin, Genelyn and the Imperial College London soft-preservation (ICL-SP) solution with two cadavers per technique. Sterile swabs were used to collect samples from different regions. Samples were collected every two months. All cadavers had a high number of microbial colonies before embalming. While no colonies were detected on formalin and Genelyn embalmed cadavers post embalming, the number of colonies decreased significantly in Thiel embalmed cadavers and nearly stayed the same in ICL-SP embalmed cadavers.

Conclusions:

Formalin embalmed cadavers showed the strongest disinfecting abilities followed by Thiel embalmed cadavers, then Genelyn embalmed cadavers and finally by ICL-SP cadavers.

Significance and Impact of Study

This study highlights how under researched this area is and the evident variation in the antimicrobial abilities of different embalming solutions on the cadaver as a whole and within different regions of the same cadaver.

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Keywords: Anatomy, microbiology, education, cadaver, embalming, formaldehyde, Thiel, Genelyn.

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Introduction

The human body is a host of complex communities of microorganisms that occupy many surfaces of the human body such as the skin, respiratory, gastrointestinal and urogenital tracts (Costello et al., 2009; Luczynski et al., 2016). These microorganisms play a role in nutrition, resisting pathogens and training our immune system (Wilson, 2008). The collective microorganisms from different areas of the body form the human microbiota that outnumbers our own cells by estimates between 1.3: 1 (Sender et al., 2016) to at least a factor of 10 (Blaser and Webb, 2014). While these microorganisms help our body to nourish and grow when alive, the same organisms are one of the most important factors of decomposition after death (Vass et al., 2002). The decomposition of the human body starts by autolysis where the enzymes within the cells start digesting the cellular membrane (Dent et al., 2004). The subsequent stage is marked by the active breakdown of soft tissue by microorganisms present in the intestines and the respiratory tract (Evans, 1963).

The use of human cadavers to learn about the human body can be traced back to Egyptian civilizations (Loukas et al., 2011), while dissecting a human cadaver to teach anatomy was part of the reform lead by Andreas Vesalius (Silverman, 1991). Nowadays, human cadavers are increasingly being used in education and research to learn human anatomy, practice clinical techniques and experiment new medical equipment (Balta et al., 2015). The work with human cadavers is associated with potential infectious risk due to the normal bacteria present in the body or pathological microorganisms present before death. Several studies have reported the spread of infectious agents to individuals working with human cadavers

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such as Mycobacterium tuberculosis (Sterling et al., 2000). To reduce the risk of infection, human cadavers are treated with chemicals that have antimicrobial abilities. These chemicals are introduced into the body through a process called embalming (Balta et al., 2015).

Embalming of human cadavers have gone through many phases that have been influenced by the development of new equipment and chemicals (Trompette and Lemonnier, 2009). Embalming a human body is common for funeral purposes, as well as academic institutions (Balta et al., 2017a). However, the use of a human cadaver to teach anatomy requires longer periods of preservation compared to the few days needed when embalming for funeral purposes (Balta et al., 2015). Several chemicals are used in different embalming solutions such as formaldehyde which is considered to be the most commonly used chemical in anatomy departments across the five continents (Benkhadra et al., 2011). Formaldehyde has the ability to create cross links between proteins within tissue; preventing the multiplication of microorganisms and therefore preserving them from decay (Fishbein et al., 2007). Several studies have reported the use of formalin embalmed cadavers for over a one year period (Balta et al., 2015). While formalin based solutions are most commonly used in anatomy departments, other solutions exist such as Thiel, Genelyn and Imperial College London soft-preservation (ICL-SP) solution (Thiel, 1992; Barton et al., 2009; Genelyn, 2010; Balta et al., 2017b). Thiel embalming technique was developed over 20 years ago by Walter Thiel and this solution contains a small percentage of formaldehyde along with other salts such as ammonium nitrate, potassium nitrate and sodium sulfite. Cadavers embalmed using Thiel technique can be used for over a year and whilst still retaining good quality of tissue (Eisma et al., 2013). Another available technique is the ICL-SP technique that is used at Imperial College London. This technique is based on alcohol, water, glycerol and phenol and cadavers embalmed by this technique were used for over six months (Barton et al., 2009; Balta et al., 2015). While the first three techniques are freely available for use, Genelyn solution is a

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commercial product manufactured by Genelyn Pty. Ltd, Australia and has relatively high concentrations of formaldehyde (Genelyn, 2010).

While one of the main reasons for embalming is to inhibit the growth of microorganisms hence reducing the risk of infection and preventing the decay of the cadaver; an inadequate amount of data is available in the literature on the antimicrobial abilities of embalming fluids (Demiryurek et al., 2002). Few studies have looked at the disinfecting efficiency of fluids that are used for embalming (Woodburne and Lawrence, 1952; Majewski et al., 2003; Tabaac et al., 2013; Hayashi et al., 2014). Some of these studies reported contradicting information documenting the presence of microorganisms on formalin embalmed cadavers while another more recent study reported the absence of bacteria or yeast- like fungi from cadavers embalmed with the same technique (Tabaac et al., 2013; Hayashi et al., 2014). Meanwhile, Thiel embalmed cadavers showed different results where one cadaver showed a decrease in the amount of microorganisms after embalming, while the other cadaver showed the absence of any after embalming (Hayashi et al., 2014).

To our knowledge, there are no papers comparing the presence of microorganisms on cadavers embalmed using different techniques. The aim of this study is to quantify the amount of bacteria present in different regions of a human cadaver embalmed using Thiel, formalin, Genelyn and ICL-SP before embalming, after embalming and over a period of eight months.

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Materials and Methods

Human Cadavers

The study was carried out under the auspices of the 'License to Practise Anatomy' in University College Cork, granted by the Irish Medical Council to the Chair of Anatomy under the Anatomy Act of 1832. For the purpose of the study human cadavers were embalmed using Thiel, formalin, Genelyn and the Imperial College London soft-preservation (ICL-SP) solution with two cadavers per technique. Appendix 1 includes details on the different embalming solutions. Formalin, Genelyn and ICL-SP solutions are introduced into the body through the femoral artery as per regular embalming procedures. Meanwhile, Thiel technique requires a venous infusion through the superior sagittal sinus, in addition to the arterial infusion through the femoral artery. Donors premorbidly signed written consent to use their bodies for education and research by the Department of Anatomy and Neuroscience.

All cadavers were admitted into the department 24–48 hr after death and stored at -20^0 C from the time of admission until being embalmed. Cadavers were thawed for 5 days after which data was collected and followed by embalming on the same day. All cadavers were embalmed in the same department and by the same embalmer. After embalming, cadavers were stored in a sealed bag at 4^0 C between the start and end of embalming except for Thiel embalmed cadavers that were stored in the tank solution. Table 1 includes information about the background of the cadavers along with the start and end of embalming.

Collection of Samples

Sterile swabs were used to collect samples from the oral cavity, axillary region, inguinal region, rectum and the internal abdominal cavity. Samples were collected every two months +/- 5 days after embalming, with a different timeline for each cadaver. Percutaneous biopsy

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of the liver was performed with an 18G automated core biopsy device to collect samples from the internal abdominal cavity before embalming. After embalming, an incision was performed along the right lower costal margin and along the linea alba down to the umbilicus to gain access to the abdominal cavity. The cadavers were then used for departmental teaching purposes throughout the rest of the data collection period.

Processing of Samples

After the collection of samples, the tip of the swab was transferred to a sterile tube containing 1 mL of a diluting solution using aseptic techniques. The tube was then Vortexed for 30 seconds to homogenise the solution. A 10 fold serial dilution was performed to establish a 1/10,000 dilution and then 0.1mL of the diluted solutions was transferred to two counting agar plates. The plates were incubated at 37°C overnight. Colonies were counted on both plates and averaged and then the number of colony forming unit per swab was calculated using the below formula:

$$\text{CFU/swab} = \left\{ \frac{\overline{X} \times 10}{10^{\text{dilution factor}}} \right\} \times 2$$

Results

Oral Cavity

To standardize the data, a ratio was computed using the number of colonies before embalming (relative to each cadaver) as denominator. Swabs from the oral cavity showed that all cadavers had a relatively high number of microbial colonies in this region before embalming. Figure 1 shows the average amount of CFU before and after embalming along with the four swabs every two months for each embalming technique. After six months,

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samples could not be taken from cadaver eight which was embalmed using ICL-SP technique as the cadaver showed signs of late decomposition with microbial colonies covering the liver. This was considered as a hazard and hence the cadaver was taken out of the study. The results of cadavers embalmed using the ICL-SP split after the second sample as cadaver ICL-SP 2 was assumed to have an infinite amount of microorganisms after four months of embalming. Meanwhile cadaver ICL-SP 1 had a value of 13.97 after eight months of embalming.

Axillary and Inguinal Regions

Results from these two regions varied as some cadavers did not have any microbial colonies before embalming while others did. No microbial colonies were observed after embalming. Cadavers embalmed using Formalin, Thiel and Genelyn did not show any growth throughout the eight months period in the axillary regions. Meanwhile, cadavers embalmed using ICL-SP showed growth after four months on cadaver ICL-SP 2 in the axillary region while ICL-SP 1 after six months.

Results from the inguinal region showed that all cadavers had microbial colonies before embalming. Thiel, formalin, and Genelyn embalmed cadavers did not grow any colonies post embalming nor throughout the eight months period, except for a small amount present on Thiel 2 (284.5 colonies). Meanwhile, cadavers embalmed using ICL-SP showed growth after four months on cadaver ICL-SP 2 and after six months on cadaver ICL-SP 1.

Rectal Region

All cadavers had a high number of microbial colonies before embalming. While no colonies were detected on formalin and Genelyn embalmed cadavers post embalming, the amount of colonies decreased significantly in Thiel embalmed cadavers and nearly stayed the same in ICL-SP embalmed cadavers. Formalin and Genelyn embalmed cadavers did not grow any

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colonies over the eight months period. The number of colonies in Thiel 1 cadaver increased slowly over the eight months period, while a quicker increase was observed after four months on Thiel 2. Both ICL-SP cadavers showed no microbial growth after two months, while ICL-SP 1 gradually increased and ICL-SP 2 shows a fivefold increase after four months and was taken out of the study after six months as demonstrated in Table 2.

Internal Organs

One out of the two Thiel, formalin and Genelyn cadavers had microbial colonies in the liver before embalming. Meanwhile, both ICL-SP cadavers had microbial colonies before being embalmed. Cadavers embalmed using Thiel, Formalin and Genelyn did not show any microbial growth after embalming nor after eight months. ICL-SP cadavers both showed a positive growth of microbial colonies after embalming, which disappeared after two months. While ICL-SP 1 cadaver did not show any microbial growth from liver samples till the eighth month, ICL-SP 2 hosted a positive growth after four months.

Discussion

There is a paucity of data on the comparative antimicrobial ability of embalming fluids. Here we show that different regions within the same embalmed cadaver react differently with the introduced chemicals. Moreover, the influence of these chemicals on the consistency of the tissue has an impact on the flourishing of microorganisms.

Body Regions

Microorganisms were detected in different parts of the body in variable concentrations, and the highest amounts were present in the oral cavity and rectal region. While the presence of microorganisms in the rectal region could be due to the gut microbiota, different reasons

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could explain the high abundance of microbial colonies in the oral cavity in comparison with other regions. In Irish culture, the human body after death is viewed as part of 'The Wake' (Grainger, 1998) after being washed and prepared for the event (Trompette and Lemonnier, 2009). Disinfectants are used to wash specific body surfaces that do could be a potential health hazard when touched by family and friends excluding the oral cavity. This could explain the high amounts of microorganisms in the oral cavity in comparison with other body surfaces.

Assessing Embalming techniques

Different chemicals could have different antimicrobial abilities when used in an embalming solution. Both Formalin embalmed cadavers showed no microbial colonies post embalming in any of the body regions and during the eight month period. This shows that chemicals used in the solution has high disinfecting qualities especially the relatively high concentrations of formaldehyde which is considered as a disinfectant (Fox et al., 1985). These results align with those by Hayashi et al. 2014.

Relatively similar results could be observed on Thiel cadavers as no microorganisms were detected after embalming on different body regions except for the rectal region. The lack of hardening of tissue in Thiel cadavers in comparison with that of formalin cadavers could explain these high concentrations. The fixing ability of formaldehyde leads to the constriction of the anal sphincter which does not occur in Thiel embalmed cadavers; leading to the discharge of faecal material from the rectum causing the high amount of microorganisms. While no microorganisms were detected on Thiel 1 cadaver after eight months except in the rectal region, Thiel 2 cadaver showed a high number of microbial colonies in the oral cavity after eight months a small number in the inguinal region. The discrepancy in the embalming

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process could explain the differences in the results. Thiel 1 was fully embalmed after three months of being submerged in the tank, which is the average period needed to complete the process (Eisma et al., 2013), while Thiel 2 took six months. These results also align with those by Hayashi et al. 2014.

A previous study looked at the fungicidal properties of the Genelyn solution (Jaung et al., 2011), but no literature was found on the antimicrobial abilities of both Genelyn and ICL-SP embalmed cadavers. Similar results are observed from both cadavers embalmed by the Genelyn solution. Both cadavers did not show the growth of microbes in the inguinal region after eight months, but microorganisms were detected in the mouth and rectal region mainly after six months. Meanwhile, results varied between ICL-SP 1 and ICL-SP 2. Different reasons could explain the faster decomposition in cadaver 2, such as the larger burden of disease from metastatic cancer which could speed up the decomposition process as described in literature (Khuhawar et al., 1999). ICL-SP 1 died from respiratory infection, whereas ICL-SP 2 had pancreatic cancer with gall bladder metastasis indicating a more widespread systemic involvement of disease. Another reason could be problems with the embalming process where chemicals did not perfuse into the tissue to prevent its decay. ICL-SP embalmed cadavers do not contain formaldehyde, hence there is no need for the three months storage period performed in the other three embalming techniques. This could be true for preserving the quality of tissue, but results from this study shows that lower amounts of microbial colonies could be observed after two months.

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It is worthwhile to acknowledge that there are limitations specific to the current study many of which generalise to cadaver-based studies. With an average of 20 donations per year admitted into the anatomy department at our institution, it was difficult to recruit a larger number of donated bodies for the study. For this reason cadavers had to be frozen to allow consistent timing for embalming, which is another limitation as it could have an influence on microbial growth. Another limitation was a lack of knowledge regarding the donors' medical history. Having access to the full medical background of the donors would help interpret the experimental results and explain any problems with the embalming process that lead to the difference in embalming time. This was not provided in this study as the Anatomy Act of 1832 does not give anatomy departments any access to donors' medical history.

While decreasing any potential health risks is the main reason for embalming a human cadaver, few studies have looked at the antimicrobial abilities of these solutions. Further research is required to look at the disinfecting abilities of each chemical used within an embalming solution taking into consideration the medical background and the cause of death of each body donor. It would also be important to identify these microorganisms as that would help in implementing better health and safety procedures.

The method used in this study enables testing the antimicrobial capacity of the embalming solutions. It also takes into account the limitations associated with the interaction between these chemicals and the tissue of the human cadavers. Meanwhile, other procedures such as the tube dilution method are used where the embalming solution is added to a nutrient rich broth containing a specific type of microorganism that has been incubated for a period of time under certain conditions. Similar methods fail to consider any possible interaction

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between the tested chemicals and the tissue of the human cadaver, hence reporting inaccurate antimicrobial properties of these chemicals.

Formalin embalmed cadavers showed the strongest disinfecting abilities followed by Thiel embalmed cadavers, then Genelyn embalmed cadavers and finally by ICL-SP cadavers. The antimicrobial properties are an important factor when considering an embalming solution, but other properties such as quality of tissue and decomposition rate should also be considered. Some embalming techniques are used to preserve cadavers for a short period of time to conduct surgical courses, while others are used for longer periods such as dissecting courses. A higher number of samples is needed for the purpose of validating the findings of the study.

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Conflict of Interest

No conflict of interest declared.

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Figure Legends

Figure 1. The average amount of CFU in the oral cavity before and after embalming along with the value every 2 months with Thiel, Formalin, Genelyn, ICL-SP 1, ICL-SP 2 (ICL-SP= Imperial College London soft-preservation).

Tables

Table1. Information on donors used in this study.

Donor	Gender	Race	Age	Cause of Death	Date of Admission	Start of Embalming	End of Embalming
Thiel 1	M	Caucasian	83	Pneumonia	30/05/2014	23/07/2014	13/11/2014
Thiel 2	M	Caucasian	69	Uraemia, kidney cancer	30/09/2013	23/07/2014	24/03/2015
Formalin 1	M	Caucasian	82	Cardiac failure	31/07/2014	02/09/2014	21/12/2014
Formalin 2	F	Caucasian	64	breast cancer, secondary in the spine	24/12/2014	13/01/2015	24/03/2015
Genelyn 1	F	Caucasian	84	Pneumonia, myasthenia gravis and heart disease	14/12/2014	13/01/2015	16/04/2015
Genelyn 2	M	Caucasian	69	Bladder cancer	2/1/2015	21/01/2015	16/04/2015
ICL-SP* 1	M	Caucasian	88	Respiratory infection	31/12/2014	30/03/2015	12/04/2015
ICL-SP* 2	M	Caucasian	74	Pancreatic cancer with gall bladder Metastasis	9/4/2015	28/04/2015	08/05/2015

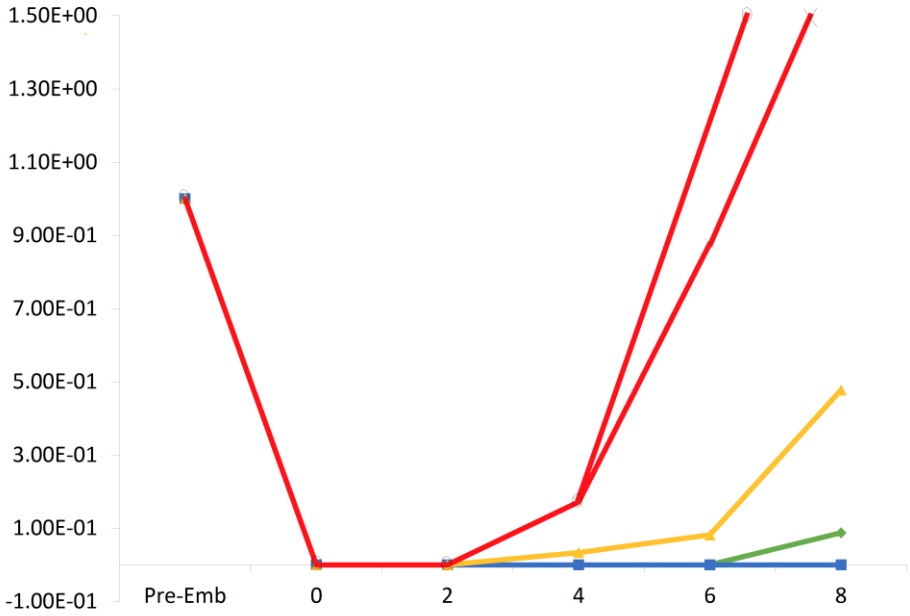
*Imperial College London soft-preservation

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Table 2. The ratio of bacterial colonies present in the rectal region of the 8 embalmed cadavers.

	Rectal Region					
Cadavers	Pre-Emb	Post-Emb	2nd Month	4th Month	6th Month	8th Month
Thiel 1	1	1.39×10^{-4}	2.65×10^{-4}	3.02×10^{-3}	8.17×10^{-3}	2.55×10^{-2}
Thiel 2	1	5.78×10^{-5}	7.16×10^{-5}	6.21×10^{-2}	8.97×10^{-1}	1.31
Formalin 1	1	0	0	0	0	0
Formalin 2	1	0	0	0	0	0
Genelyn 1	1	0	0	0	1.23×10^{-3}	8.6×10^{-3}
Genelyn 2	1	0	0	0	2×10^{-2}	5.13×10^{-2}
ICL-SP* 1	1	8.98×10^{-1}	0	5.96×10^{-3}	1.64×10^{-1}	2.23
ICL-SP* 2	1	1.93	0	5.08	Full	Full

*Imperial College London soft-preservation



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