

# Journal of Visualized Experiments

## Detection of vascular pathways of oral mucosa influencing soft- and hard tissue surgeries by latex milk injection.

--Manuscript Draft--

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19<sup>th</sup> November, 2019.

**To:** Journal of Visualized Experiments,

Dear Editorial Board,

We are sending hereby our manuscript entitled “**Detection of vascular pathways of oral mucosa influencing soft- and hard tissue surgeries by latex milk injection**” for your consideration as an *original article* in Journal of Visualized Experiments. This paper is an original work and is not under consideration for publication elsewhere. None of the authors of this manuscript have any potential conflict of interest.

In this paper, we provide the methodological basis to investigate the oral blood supply by implementing the latex milk injection and staining of vessels for detailed macroscopic vascular mapping of the oral mucosa to facilitate the understanding of a proper flap design to prevent from complications and achieve postoperative wound healing.

We do believe that our novel findings presented in the current paper will surely excite both experimental and clinical experts in the field and other readers of Journal of Visualized Experiments. Therefore, we would be delighted if you should be willing to consider this study for publication in your distinguished journal.

We are looking forward to getting your feedback.

Best regards,

Arvin Shahbazi, DMD, PhD  
Corresponding author

**TITLE:**

**Detection of Vascular Pathways of Oral Mucosa Influencing Soft- and Hard Tissue Surgeries by Latex Milk Injection**

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**KEYWORDS:**

radiopaque latex milk injection, oral mucosa, blood vessels, oral surgery, flap design, wound healing

**SUMMARY:**

Here, we describe the staining of blood vessels using latex milk injections. This procedure provides anatomical knowledge of the oral blood supply for detailed macroscopic vascular mapping of the oral mucosa, as well as understanding of proper flap design to prevent complications and promote postoperative wound healing.

**ABSTRACT:**

In this methodological study, the purpose was to visualize the macroscopic vascular pathway of the oral mucosa. Corpses were injected and fixed with Thiel's solution for embalming to keep the natural color, fresh texture, and elasticity of the tissues. Latex milk injection is a technique used to stain blood vessels. A combination of Thiel's embalming method and latex milk injection allows surgeons to work on a raw specimen and identify the distribution and anastomosis of vessels macroscopically in the oral mucosa for flap/incision design in periodontal and maxillofacial surgeries. The latex milk contains radiopaque material that enables clinicians to visualize the pathway of the blood vessels radiographically. A step-by-step protocol is described for the correct adjustment of Thiel embalming and latex milk injection. The combined application of both methods allows the clinician to understand anatomical structures practically. As a result, proper incisions and flaps can be designed, which prevents neurovascular damage, intraoperative bleeding, and postoperative morbidity during patient surgery.

**INTRODUCTION:**

In order to design an appropriate incision or flap in implant, dentoalveolar, and periodontal surgeries, anatomical knowledge is crucial<sup>1,2</sup>. The oral mucosa possesses an intricate pattern of blood vessels arising from the subbranches of the external carotid artery (ECA). The maxilla and the mandible together with their surrounding connective tissues are mainly perfused by the branches of the maxillary artery (MA), the facial artery (FA), and the lingual artery (LA)<sup>3,4,5</sup>. From a clinical point of view, the visualization of the blood vessel pathways is necessary for clinicians to decrease intraoperative bleeding and postoperative morbidity<sup>6</sup>. Clinicians performing oral surgical interventions must be aware of the location of the vascular pathways to establish optimal conditions for wound healing and angiogenesis<sup>7</sup>. A detailed description of the arterial and venous distribution of the oral mucosa would enable oral surgeons and periodontists to minimize the risk of complications during surgery by optimized planning.

There are various methods in the literature to stain the blood vessels of the oral mucosa, such as India ink injection and corrosion casting<sup>8-10</sup>. One of the preferred staining methods uses latex milk<sup>7-12</sup>. As Haenssger<sup>12</sup> defined it, latex milk as a liquid complex emulsion formed by tannins, proteins, resins, sugars, oils, alkaloids, and gums that coagulates when exposed to air<sup>12</sup>. The latex is generally mixed with a red color agent and injected into the common carotid artery (CCA) or ECA to visualize the course of their branches and subbranches. The blood vessels are stained red and become more prominent during dissection. This method provides valuable data for clinicians through the visualization of blood vessels by definitive ex vivo macro- and microscopic analysis establishing a solid anatomical basis. Due to the presence of a radiopaque substance in the latex milk, the method displays excellent visualization in both traditional X-rays and 3D computed tomography (CT)<sup>5</sup>.

Several types of fixative solutions that can be used for embalming head specimens<sup>13,14,15</sup> are explained and compared in the literature. Walter Thiel introduced Thiel's solution in 1992<sup>16,17</sup>. This solution conserves the natural color of tissues and vessels<sup>16-20</sup>. It has no detectable odor and maintains tissue elasticity of body parts as well as antimicrobial preservation of cadavers, and it can be useful for the development of new surgical devices or evaluation of a surgeon's skill<sup>16-20</sup>.

## **PROTOCOL:**

The method of latex milk injection was used bilaterally on human cadavers, which were donated to the Department of Macroscopic and Clinical Anatomy of the Medical University of Graz, Austria, complying with the Department's Donation Program and according to Styrian burial law. 10 fresh cadavers (5 male-5 female, age 59–72) were selected. The path of the blood vessels in oral mucosa, utilized in combination with a layer-by-layer dissection protocol and CT, were analyzed.

### **1. Latex milk injection together with the examination of the blood vessels in the oral mucosa (Figure 1 and Figure 2)**

1.1. Penetrate the center of the sagittal suture between the parietal bones with a drill (see **Table of Materials**) to reach into the superior sagittal sinus.

1.2. Design an incision in the carotid triangles parallel to the sternocleidomastoid muscle by Nr. 20 or Nr. 15 surgical blades. Then, separate the edges of the skin, adipose tissue, and the muscles with a Weitlaner retractor (140 mm, 3/4 teeth) to expose the surgical site.

1.3. Gently reveal the common carotid arteries (CCAs) using Nr. 15 surgical blades and block the end using serrated dressing forceps.

1.4. After that, cannulate the CCAs or ECAs and superior sagittal sinus carefully with a metal catheter.

1.5. Isolate the catheter using a ligature with alternating knots on each side of the CCA. Fix the catheter very securely to prevent any leakage of Thiel's intravascular solution or latex milk when injected under pressure.

1.6. Subsequently, perfuse the corpse tissues via the CCAs and superior sagittal sinus simultaneously with an intravascular solution (e.g., Thiel's or phosphate buffer solution) using an air pressure pump system (0.25–0.5 bar) for ~10–15 min.

NOTE: The main components of the intravascular and embalming Thiel's solution are composed of ammonium nitrate (fixative), potassium nitrate (fixative), 4-chloro-3-methylphenol (fixative), sodium sulfite (fixative), boric acid (disinfection), ethylene glycol (preservation of tissue plasticity), hot water (softening of the tissues), as well as formalin (disinfection, tissue fixative, and embalming)<sup>16-20</sup>.

1.7. (Optional) Before injection of the latex milk, inject 20–30 mL of diluted ammonia to clear the vessels.

1.8. Carefully mix a red color agent with 150 mL of latex milk (see **Table of Materials**).

1.9. Inject the mixture using an air pressure pump system for about 10–15 min into the CCAs.

NOTE: Take care during the mixing and injection of the latex milk to keep it as bubble-free as possible. If a bubble is present, a gap develops inside the vessel, which might result in rupture of the artery during surgical intervention performed on the cadaver.

1.10. During the injection, use the same pressure or slightly higher to completely distribute the latex milk into the finer vessels in the orofacial region.

1.11. After complete filling and staining of the CCAs and the subbranches, obstruct them with surgical vessel clamps to prevent the exiting of the injected latex milk.

1.12. Embalm the specimens for approximately 6–8 months in Thiel's solution inside chrome vanadium corrosion resistant steel tanks.

1.13. Keep the corpses in zipper polyethylene plastic bags for another 6 months with antifungal and preservative agents such as chlorocresol. During this period, the excess amount of extracellular liquid is pushed out of the body, and the specimens become ready for dissection.

NOTE: Following the completion of the fixation period, the tissues and the blood vessels of the maxillofacial region are ready and capable of withstanding the rigidity of dissection. The stained arteries can be analyzed by CT scans. Some of the subbranches in the palate, vestibule, tongue, and maxillofacial region are observable without removal of the mucosa. Finally, specimens should be dissected using 2.5x magnification loupes using Nr. 15C surgical blades. Mucosal elevation can be performed by dissecting the stained arteries layer by layer, and the course of the vessels and their variations can be macroscopically assessed.

#### REPRESENTATIVE RESULTS:

Using Thiel's solution as a fixative for human corpses, training in surgical techniques and invasive clinical procedures becomes possible. One of the critical advantages of Thiel's solution is the maintenance of the natural color of soft and hard tissues. Thiel's solution is less irritating compared to many other fixatives, such as formaldehyde. Its use results in flexibility of the temporomandibular joints and the masticatory and suprahyoid muscles, which facilitates dentoalveolar cadaver surgeries. Also, the texture of the neurovascular bundles is kept unspoiled and helps make practicing surgical techniques more convenient for the surgeon. Combining Thiel's embalming method and subsequent latex milk injection is a clear, straightforward approach to prepare odor-free, fresh cadavers with natural consistency, in which the arteries of the maxillofacial region, especially the oral vestibule and the palate, are injected with a red color latex (**Figure 3, Figure 4**). The presence of radiopaque material in the latex milk allows the clinician to perform radiographical (CT) analysis (**Figure 5**) of the head specimen. Subsequently, the pathway of the vessels can be observed macroscopically to understand where and how to create an incision or a flap during implant placement, sinus floor elevation, or periodontal and maxillofacial surgeries. As a result of understanding the basic solid anatomy, acceptable postoperative angiogenesis and primary wound healing might be achieved when performing surgery.

#### FIGURE LEGENDS:

**Figure 1: Step by step protocol for Thiel's solution and latex milk injection.** (A) Catheter placement in the superior sagittal sinus for injection of Thiel's solution. (B) Localization of the carotid trigone. (C) Designing an incision parallel to the sternocleidomastoid muscle. (D) Separation of skin contacts and removal of cervical adipose tissue. (E) Dissection of the carotid sheath and cervical fascia between the sternocleidomastoid muscle (laterally) and inferior belly of the omohyoid muscle (medially). (F) Preparation of a dissected common carotid artery. (G) Adjustment of the catheter in a common carotid artery. (H) Fixation and isolation of the catheter. (I) Isolated catheter ready for injection of the Thiel's solution and red color latex milk injection.

**Figure 2: Demonstration of hardened red colored latex milk in the external carotid artery (\*) with its subbranches at the upper part of the carotid trigone.**

**Figure 3: Vascular survey of the palate.** Palatal mucosal distribution of the greater palatine and nasopalatine branches are visible macroscopically.

**Figure 4: Vascular survey of the oral vestibule.** (A) Horizontal path of superior labial (\*) and posterior superior alveolar (\*\*) arteries with their subbranches supplying the upper vestibular mucosa. (B) Vertical orientation of the arteries in the maxillary vestibule.

**Figure 5: CT images of the various arterial pathways injected with the red colored latex milk in the maxillofacial region.**

#### **DISCUSSION:**

The unique combination of Thiel's embalming and latex milk injection methods enable the oral-maxillo-facial surgeons and periodontists to practice on fresh specimens while observing the directions of different vessels<sup>4,19,20</sup>. However, there might be some limitations with the staining of small-diameter vessels due to blockage by clots or not using enough pressure to push the material into the small arteries during the procedure.

The Thiel embalming method contains crucial vital advantages. For example, the cadavers are preserved in their natural color<sup>16,17,20</sup>. Due to the maintenance of resilience and elasticity of tissues<sup>20,21</sup>, this is an excellent method to demonstrate surgery and oral surgical interventions<sup>19,22,23</sup>. Because the Thiel method is odorless and less toxic compared to other fixation methods such as formaldehyde, it makes specimen preparation more convenient<sup>16-18,20</sup>. In 2011, Benkhedra investigated the histological features of unfixed and fixed skeletal muscle fibers comparing Thiel's solution and formalin<sup>24</sup>. With the Thiel method, the arrangement of the collagen fibers and the integrity of the muscles remained aligned compared to the other method<sup>24</sup>. Furthermore, ultrasound-guided research has reported that the Thiel embalming method gives more realistic results for radiologists and anesthesiologists<sup>25</sup>.

There is insufficient information regarding the precise route and anastomosis of the vessels in the oral mucosa, which may produce an improper flap design in surgery. The latex milk injection stains the blood vessels, and as a result, they become visible<sup>4,11</sup>. This facilitates the dissection of soft and hard tissues by discovering their anatomical and physiological relation to the vessels. Another essential characteristic of latex milk is that it is radiopaque due to the presence of lead(II,IV) oxide. Therefore, the pathway of the vessels can be observed three-dimensionally by CT analysis, which allows the operator to plan surgery according to an anatomical and vascular survey. As a consequence, an appropriate incision can be designed that prevents nerve damage, excessive bleeding<sup>26</sup>, and postoperative complications such as tissue necrosis and delayed primary wound healing<sup>27</sup> in different oral surgical interventions.

#### **ACKNOWLEDGMENTS:**

The authors would like to specially thank to all co-workers of the Department of Macroscopical and Clinical Anatomy of Graz University; the co-workers of the Department of Anatomy, Histology and Embryology, Semmelweis University; as well as Prof. Dr. Péter Windisch at the Department

of Periodontology, Semmelweis University for their work and support.

#### DISCLOSURES:

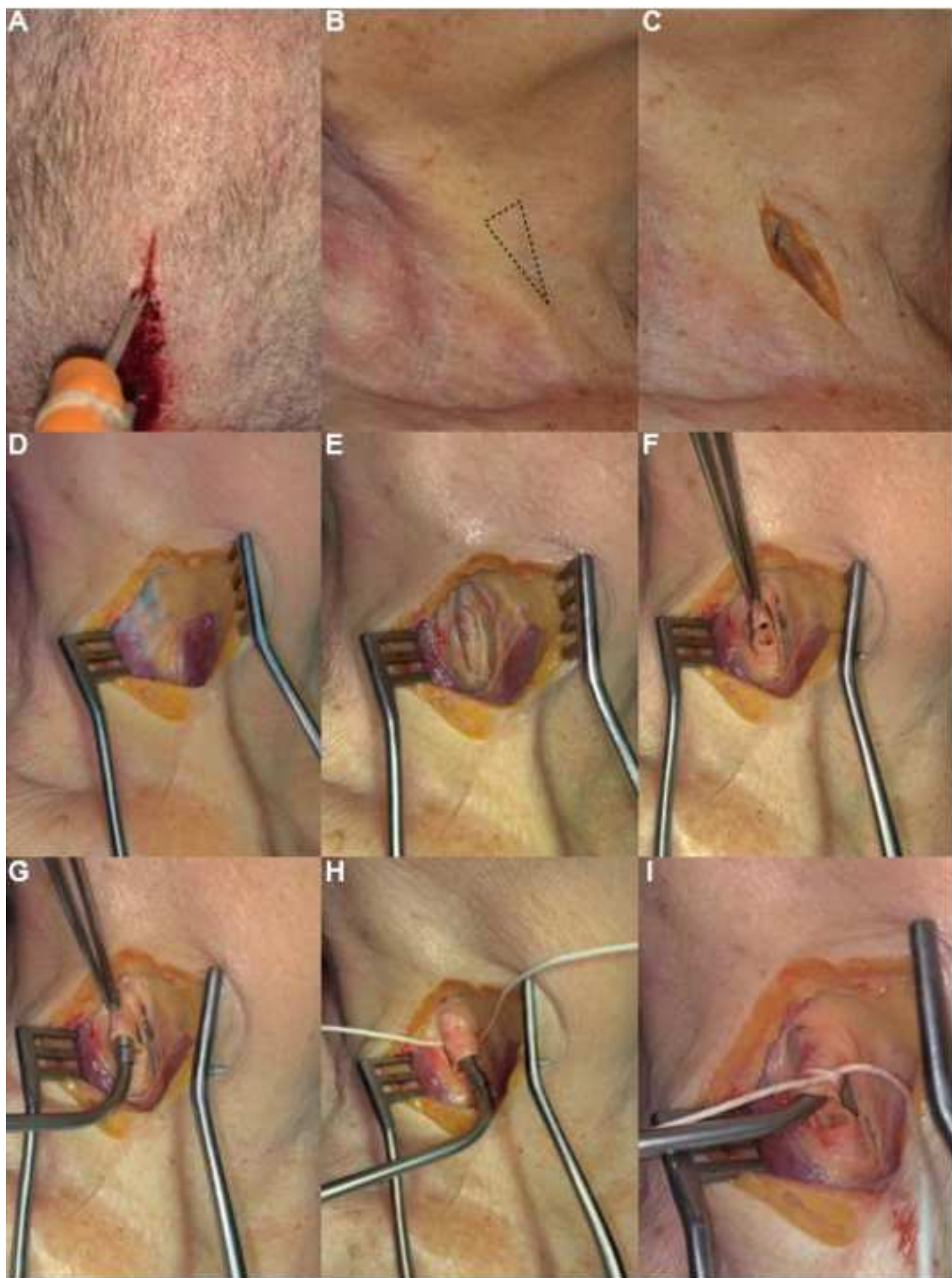
The authors have no conflict of interest.

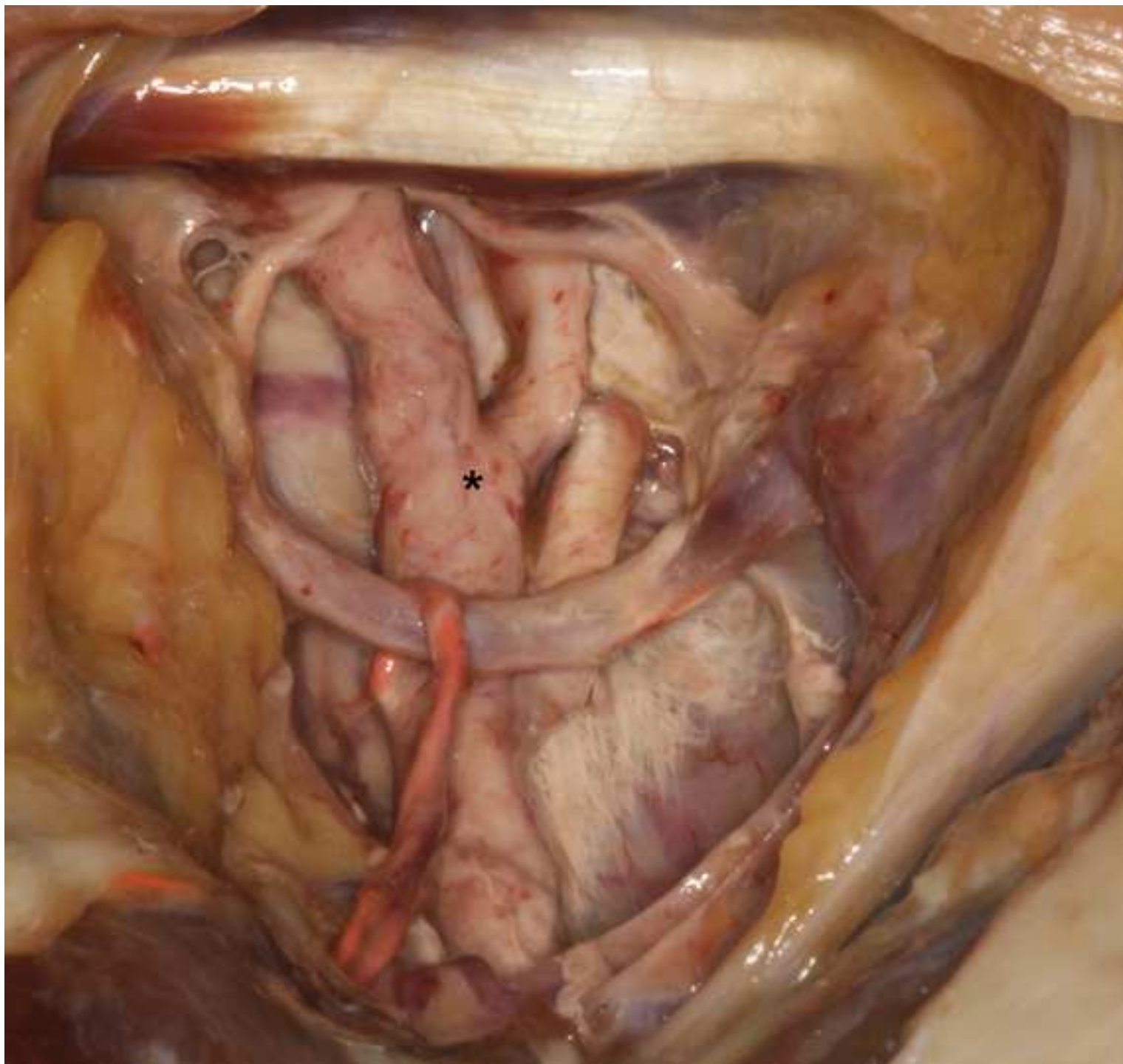
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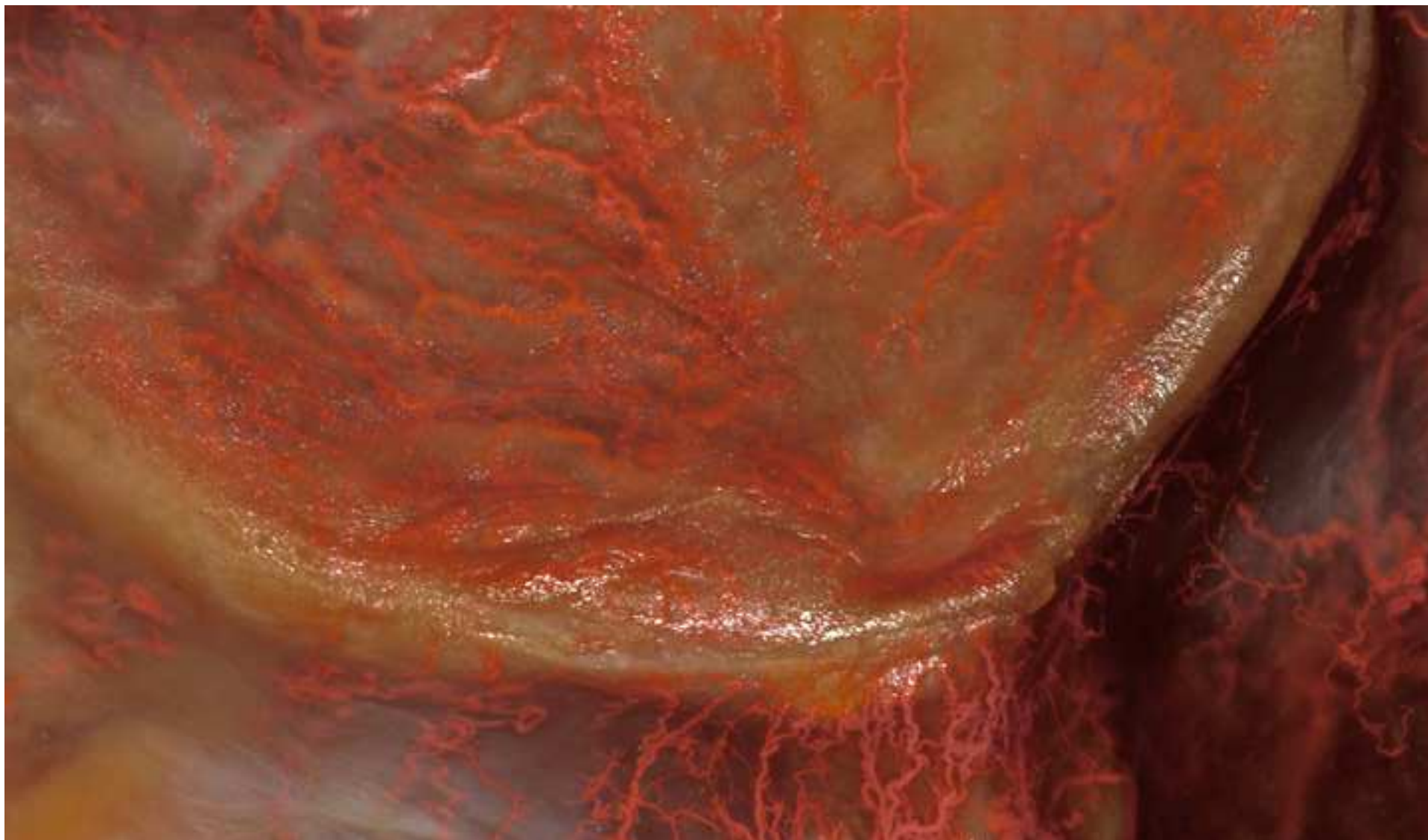


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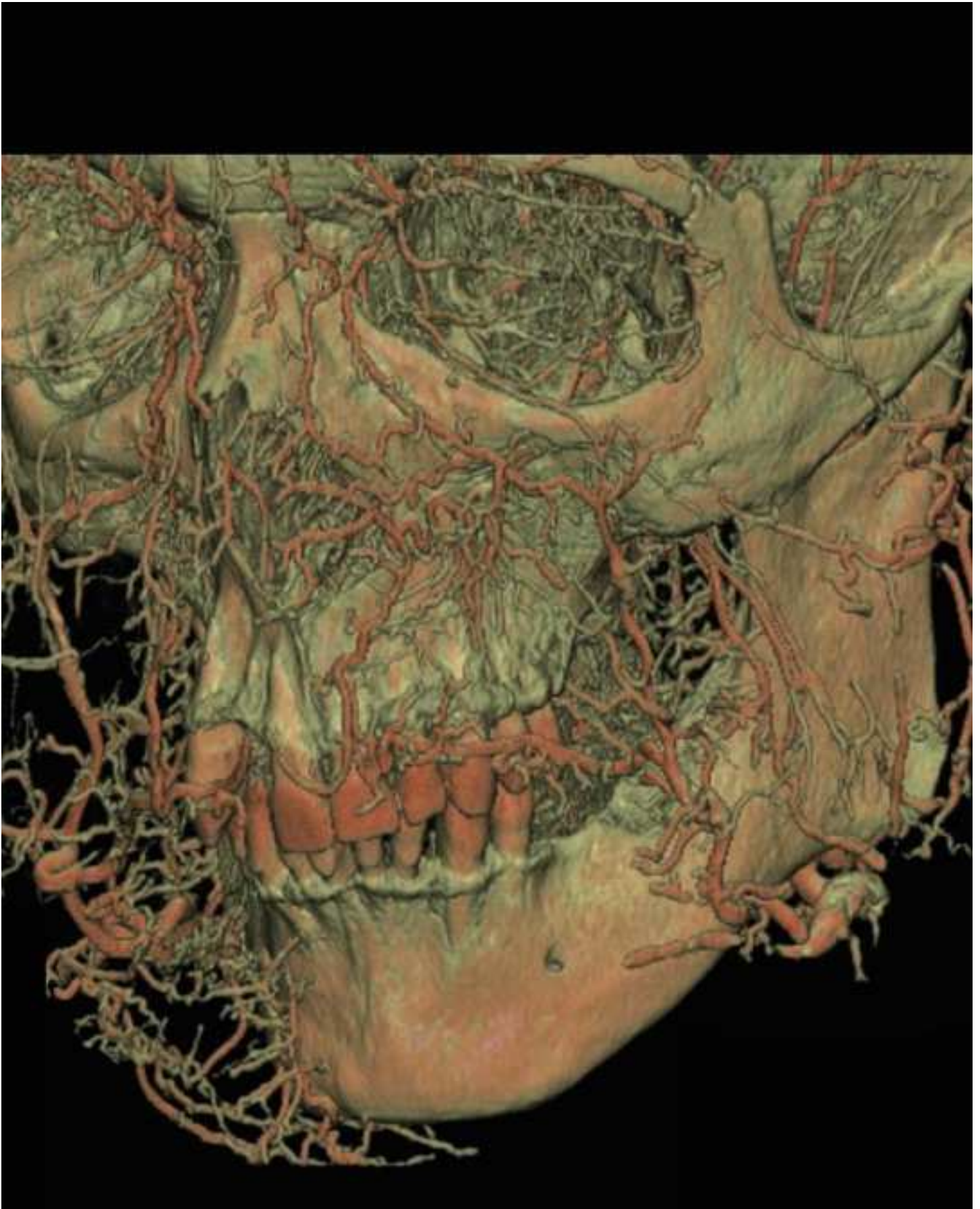












Name of Material/Equipment	Company
2.5x magnification loupes	Harmonycom
4-chloro-3-methylphenol	Jäckle Chemie Austria GmbH, Vienna Austria Lactan Chemicals, Graz Austria Brenntag Austria GmbH, Vienna, Austria
Air pressure pump system	Heiz-Hofstätter, Graz, Austria
Ammonium nitrate	Jäckle Chemie Austria GmbH, Vienna Austria Lactan Chemicals, Graz Austria Brenntag Austria GmbH, Vienna, Austria
Block End Serrated Dressing Forceps	VETisco, Chesterfield, England
Boric acid	Jäckle Chemie Austria GmbH, Vienna Austria Lactan Chemicals, Graz Austria Brenntag Austria GmbH, Vienna, Austria
Chlorocresol	Jäckle Chemie Austria GmbH, Vienna Austria Lactan Chemicals, Graz Austria Brenntag Austria GmbH, Vienna, Austria
Chrome Vanadium Corrosion Resistant Steel tanks	
Computed tomography (CT)	Siemens, Emotion, Munich, Germany
Diluted ammonia	Jäckle Chemie Austria GmbH, Vienna Austria Lactan Chemicals, Graz Austria Brenntag Austria GmbH, Vienna, Austria
Drill (PSR, 10.8,LI-2)	Bosch AG, Gerlingen, Germany
Ethylene glycol	Jäckle Chemie Austria GmbH, Vienna Austria Lactan Chemicals, Graz Austria Brenntag Austria GmbH, Vienna, Austria
Formalin	Jäckle Chemie Austria GmbH, Vienna Austria Lactan Chemicals, Graz Austria Brenntag Austria GmbH, Vienna, Austria
Hot water	-----
Latexmilch (latex milk)	Creato, Zitzmann Zentrale, Baden, Germany
Metal catheter	Grall Enterprise, Graz, Austria
Nr. 15 surgical blade	Braun, Tuttlingen, Germany
Nr. 15C surgical blade	Braun, Tuttlingen, Germany

Pintasol red E-L3 mix paste

Potassium nitrate

Sodium sulfite

Surgical vessel clamp

Weitlaner Retractor (Self-Retaining) 140mm 3/4 Teeth

Zipper polyethylene bags (plastics)

Mixol-products Diebold GmbH, Kirchheim, Germany

Jäckle Chemie Austria GmbH, Vienna Austria Lactan

Chemicals, Graz Austria

Brenntag Austria

GmbH, Vienna, Austria

Jäckle Chemie Austria GmbH, Vienna Austria Lactan

Chemicals, Graz Austria

Brenntag Austria

GmbH, Vienna, Austria

Grall, Graz Austria

VETisco, Chesterfield, England

Wettlinger, Kunststoffe, Vienna, Austria



**Catalog Number**

**Comments/Description**

Color agent

15<sup>th</sup> January, 2020.

**To:** Journal of Visualized Experiments,

Dear Editorial board and Reviewers,

Thank you for the valuable comments and guidances, please find our response accordingly. We are sending hereby our revised manuscript entitled “**Detection of vascular pathways of oral mucosa influencing soft- and hard tissue surgeries by latex milk injection**” for your consideration as an *original article* in Journal of Visualized Experiments.

Our replies;

**Editorial comments:**

1. Please take this opportunity to again thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

**The manuscript was controlled and the spelling and grammar mistakes were corrected.**

2. The protocol is still not in the imperative (e.g., “Do this,” “Ensure that,” etc.); please rephrase.

**The protocol was converted to imperative version.**

3. Please include more information in the discussion to help researchers replicate your protocol; i.e., critical steps, troubleshooting, and modifications.

**In the protocol part some of the critical steps are explained and since it is a straight forward method, we could not add anything else to (as modification, critical steps) the discussion part.**

4. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al.

**We have controlled the discussion part, and we believe the references are organized according to the JOVE journal requirements. Please let us know specifically if there is missing information.**

## **Reviewers' comments:**

### **Reviewer #1: Manuscript Summary:**

This manuscript describes the utilization of radiopaque latex injection to dissect and CT-image vasculature of the face and oral mucosa of Thiel-embalmed human cadavers.

#### **Major**

I have no major concerns.

#### **Concerns:**

#### **Minor**

Please identify the "radiopaque material" that is in the latex milk-- e.g. is it barium?

#### **Concerns:**

**Thank you for your precious comment. We did not add any other material in the latex milk. One of the most important characteristics of latex milk that we use in Graz is to be radiopaque. This is due to the substance named as Menninge (lead-oxide) which makes it radiopaque. We have added this information in the Discussion part.**

Please identify the brand / manufacturer of the latex milk so that the procedure can be replicated. If the latex milk was bespoke, please include a replicable method / formula for its mixture.

**Thank you for your comment. In the previous version of the manuscript we mentioned the brand name but the Editorial board of the Journal asked us to remove it. It is in the table of the material (Latexmilch, Creato, Zitzmann Zentrale, Baden, Germany).**

Consider referring to "latex milk injection" as "radiopaque latex milk injection" throughout. 'Radiopaque latex injection' is a common technique and might be picked up by more keyword searches.

**Thank you for your valuable comment, It is changed in the key words as a “radiopaque latex milk injection”.**

Radiopaque latex injection is common in natural history / zoological studies, but relatively (and unfortunately) rare in medical studies. I commend the authors for promoting the utility of these methods to a more medically-oriented audience. I would encourage the authors to consider adding references to some of the foundational methodological literature for radiopaque vascular injection and X-Ray tomography:

Early use of cadaveric radiopaque vascular injection (combining barium and gelatin in X-ray imaging): Ganey T, Ogden J, Olsen J. 1990. Development of the giraffe horn and its blood supply. Anat Rec 227:497-507.  
Revision of Ganey's method to include liquid latex and barium (in X-ray stereoangiography): Sedlmayr JC, Witmer LM. 2002. Rapid technique for imaging the blood vascular system using stereoangiography. Anat Rec 267:330-336.

Expansion of Sedlmayr's work to include CT scanning and 3D analysis: Holliday CM, Ridgely RC, Balanoff AM, Witmer LM. 2006. Cephalic vascular anatomy in flamingos (*Phoenicopterus ruber*) based on novel vascular injection and computed tomographic imaging analyses. *Anat Rec* 288A:1031-1041.

Expansion of Holliday's work to include video fluoroscopy: O'Brien HD, Williams SH. 2014. Using biplanar fluoroscopy to guide radiopaque vascular injections: A new method for vascular imaging. *PLoS ONE* 9(5): e97940.

**Thank you for the information. There are many different studies regarding radiopaque material. We have selected an article which was published by one of coauthors:**

**Pils, U., Anderhuber, F., & Neugebauer, S. The Facial Artery-The Main Blood Vessel for the Anterior Face? *Dermatologic surgery*. 42 (2), 203-8 (2016).**

<b>Reviewer</b>	<b>#2:</b>	<b>Manuscript</b>	<b>Summary:</b>
We commend the Authors for describing the technical aspects of preparing these specimen that can help surgeons better understand the vascular anatomy of this region. It would have been nice to inject the veins in blue at the same time as they already had access at both the neck and cranial sinuses.			

**Thank you for your valuable comment. We are planning for the future studies to inject both of common carotid artery and the internal jugular vein.**

<b>Major</b>	<b>Concerns:</b>	none
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**Minor Concerns:** none

We are looking forward to getting your feedback.  
Best regards,

Arvin Shahbazi, DMD, PhD  
Corresponding author