E12 Sheet Plastination - Techniques and Applications

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ABSTRACT

Introduction: Plastination is an anatomical technique that consists of replacing the liquids and fat of specimens by reactive polymers through forced impregnation in a vacuum. These are then polymerized to achieve the final result. E12 sheet plastination involves epoxy resin impregnation of thin (2-4 mm) and ultra-thin (less than 2 mm) tissue sheets, producing dry, transparent, odorless, non-toxic and long-lasting sheets.

Materials and Methods: E12 sheet plastination techniques were reviewed using MEDLINE, EMBASE and SciELO databases, and manual searches.

Results: After searching, 616 records were found using the on-line and manual searches (MEDLINE, n: 207; EMBASE, n: 346; SciELO, n: 44; Manual search: 23). Finally, 96 records were included in this review (after duplicates and articles unrelated to the subject were excluded).

Conclusions: The aim of this work was to review the E12 sheet plastination technique, searching for articles concerning views of it, identifying the different variants implemented by researchers since its creation by Gunther von Hagens, and to identify its applications from teaching and research in anatomy to morphological sciences.

Key words: Plastination, Sheet, Epoxy resin

INTRODUCTION

Gunther von Hagens invented plastination in 1978 in Heidelberg, Germany. It consists of the forced impregnation, in a vacuum, of biological specimens with reactive polymers (Marks et al., 2008; Sora and Matusz, 2010; Ottone et al., 2015). The technique for sheet plastination to produce

transparent slices in E12 epoxy resins was patented in 1982 (U.S. Patent 4,320,157) (Bickley et al., 1984).

Plastination of biological tissues with E12 epoxy resin allows thin sections of tissue to be preserved for anatomical study (Reed, 2003; Borzooeian and Enteshari, 2006; Sora and Cook, 2007; Sora and Matusz, 2010). During the E12 plastination technique, the water and lipids in the biological tissues are replaced with curable epoxy. The main steps are as follows: preparation of thin slices of the desired sample, cold dehydration in acetone by freeze substitution, degreasing, forced impregnation of epoxy under vacuum, and finally curing in an oven (von Hagens, 1986; Lane, 1990; Weber and Henry, 1993; Cook, 1997; Fasel et al., 1988; An and Zhang, 1999; Sora et al., 2002; Sora et al., 2004; Sora et al., 2007; Ottone et al., 2016) (Fig. 1). The results are dry, transparent, odor-free and durable slices (Sora et al., 2002b; Qiu et al., 2003; Sora et al., 2004; Sora et al., 2007; Sora and Cook, 2007; Marks et al., 2008; Hermans et al., 2009; Sora et al., 2012; Diao et al., 2013). The method prevents decomposition of the sample and encrusts it in transparent epoxy to create an optical interface of high tissue quality (Marks et al., 2008). Furthermore, the slices obtained can easily be used for morphometric processing (Sora, Strobl and Radu, 2004): Didenko et al. (2013) performed a morphometric analysis of the right atrium, Liang et al. (2014) analyzed the morphometry of the transcranial segment of the trigeminal nerve, and Bernal-Mañas et al. (2016) studied the lateral pterygoid muscle and its arrangement in the condyle of the mandible, identifying several muscular fascicles and their insertions and providing morphometric details. Other examples will be mentioned in this article.

The standard method for producing 1-3 mm thick E12-plastinated sections (Sora et al., 2007; Sora and Matusz, 2012), pioneered by von Hagens, is carried out by freezing a fresh tissue sample and cutting sections with a band-saw; the tissue loss is in the order 0.9 mm per slice (von Hagens, 1986) (Figs. 2 and 3A,B).

A different technique is used for plastinating ultra-thin sections, between 0.3 and 1.0 mm (Sora and Matusz, 2012). In general, 3-5 mm sections are used for descriptive and morphometric analysis, and ultra-thin sections (<1 mm) for three-dimensional reconstruction or histological examination (Fritsch and Hegemann, 1991; Cook, 1997; Gruber, 2001; Sora, Strobl and Radu, 2004; Sora et al.; 2012). It is the preferred technique for producing transparent body sections because shrinkage is minimal compared to the sheet plastination technique using polyester (Bickley et al., 1984; Sora, Brugger and Strobl, 2002; Sora and Matusz, 2010). Polyester plastination (P40 plastination technique) was designed to preserve 3-5 mm thick brain sections, enhancing the distinction between gray and white matter, but it can also be used for other anatomical regions. In this sense, the P40 technique allows the transparency of sections to be maintained over time without the tone becoming orange, which is an advantage over the epoxy technique (Henry and Latorre, 2007; Latorre and Henry, 2007) (Fig. 3C,D).

Transparent sections of anatomical regions allow the topography of all body structures to be studied for teaching and research purposes in an uncollapsed state and without displacement (Sasha et al., 1988; Cook, 1997; Skalkos, Williams and Baptista, 1999; Sora et al., 2002b; Sora and Cook, 2007; Soal et al., 2010; Sora and Matusz, 2010; Kürtül et al., 2012; Ottone et al., 2016). They preserve the anatomical positions of all the tissues in situ and can be examined at macro- and micro-scopic levels (Cook and Al-Ali, 1997; Leaper et al., 2005; Lowis et al., 2016).

Furthermore, these specimens are useful for pre-training programs (computed tomography and magnetic resonance imaging). They provide participants of all levels, from student to specialist, with a much clearer understanding of anatomical structures because sections provide a deeper knowledge of anatomy than can be obtained from morphological examination (Lane, 1990; Cook, 1997; Sora et al., 2002b; Sora and Cook, 2007; Sora and Matusz, 2010).

The aim of the present work was to review the techniques for sheet plastination with epoxy resin to identify all the articles that detail the protocol for this technique clearly. This enabled us to identify

the differences among them and the various fields of teaching and research in which they are applied.

MATERIALS AND METHODS

The on-line search of the MEDLINE and EMBASE databases used the terms “plastination”, “plastination” AND “sheet” AND “epoxy”, and “plastination” AND “sheet” AND “E12”. The SciELO database was searched using the terms “plastination” and “plastinacion”. A manual search was also performed. The last search was carried out on March 2nd, 2017.

The articles selected were those that described the technique for sheet plastination with epoxy resin (E12), and its application and scope in the different areas of morphological sciences. Human and animal research articles were included, and some authors reported embalming with 2-15% formalin (Eckel et al., 1993; Sittel et al., 1997; Skalkos, Williams and Baptista, 1999; Qiu et al., 2003, 2004; Sebe et al., 2005a,b; Lunacek et al., 2005; Fritsch et al., 2006; Elnady and Sora, 2009; Koslowsky et al., 2011, 2015; Wegman et al., 2012; Adds & Al-Rekabi, 2014; Konschake & Fritsch, 2014; Ottone et al., 2016). No restrictions were placed on language or date of publication. The initial selection was based on analysis of the title and abstract of the articles; those that were duplicated or proved irrelevant to the subject were eliminated. Once this selection was complete, the full texts were examined.

The following information was extracted from the articles: how the sample block was prepared prior to cutting; the times, equipment and consumables used for cutting the samples; the time and consumables required for dehydration and degreasing; the epoxy polymer mixtures and catalysts used during forced impregnation, and the associated equipment; and the times, epoxy-catalyst mixture and equipment used for curing. In all stages, the researchers looked for information on the temperature at which the various processes were carried out, and on the proportions of epoxy resin and catalyst in the mixtures and the source of the consumables used. They also identified whether

the samples used were human and/or animal. In all the articles, the regions studied were classified, along with the applications and relationship to diagnostic imaging methods, and the possibility of carrying out histological staining and microscopic research on the plastinated sections.

RESULTS

In total, 616 records were found using the online and manual searches. Of these, 553 were found in the MEDLINE (n: 207) and EMBASE (n: 346) databases, and 44 in SciELO (plastination: 23 / plastinacion: 21). The remaining 20 records were found by manual searching (not included in the databases used). Of these, 18 were articles in the Journal of Plastination, published by the International Society for Plastination. Since 1987, this journal has published 195 articles; these 18 refer to sheet plastination in epoxy resin. The other article obtained by manual search was published by the journal Curator. The researchers then eliminated articles not related to the subject, duplicates, and those excluded by the article selection criteria. The selection criteria considered: how the sample block was prepared prior to sawing (temperature, process times, type of band saw); the time and materials required for dehydration and degreasing; proportions of epoxy polymer and catalysts; and equipment used during forced impregnation and curing. In all stages we looked for information on the temperature at which the various processes were carried out, the proportions of epoxy resin and catalyst in the mixtures, and the source of the supplies used. We also identified whether the samples were human and/or animal, and the applications in relation to morphological teaching and research, imaging diagnosis, and the possibility of carrying out histological staining and microscopic research on plastinated sections. The Heidelberg plastination folder by von Hagens (1986) (in English) was also included. In addition, three articles from the Journal of Plastination, referring to the polyester sheet plastination technique, were found by manual searching and included. Finally, 96 records were included in this review. In relation to the specimens used in the articles, 68 articles used humans, 12 used animals, and two both humans and animals; eight articles

detailed only the methods and did not specify the sample type. Finally, two were letters to the editor.

- **Analysis of Protocols for Sheet Plastination with Epoxy Resin**

Table 1 contains descriptions of three plastination processes using epoxy resin (E12): the classic method, developed by von Hagens et al. (1987), and two methods developed by one of the researchers with the most publications on the technique, Professor Constantin Sora. He described two protocols, one for 3-5 mm sections (Sora and Cook, 2007) and one for ultra-thin sections (<1 mm) (Sora, 2007). Fifty-one articles described the technique for sheet plastination with epoxy resin in detail (Supplementary Material).

The steps in the techniques described differed among protocols, as summarized below.

Preparing and cutting the sample block

The first difference found in executing the plastination technique, requiring a correct decision by the researchers, is whether to cut the sections at the start of the process or to plastinate the whole sample first and then cut the sections; i.e., whether to cut the sections and then plastinate them, or plastinate the whole sample to convert it into a block impregnated with epoxy resin, cut the block, and finally cure the sections. The answer to this question is directly related to the thickness of the sections required. When ultra-thin sections are required (less than 1.5 mm), the whole piece is plastinated and the ultra-thin sections are cut with a diamond saw (Eckel et al., 1993; Sha et al., 2001; Zhang et al., 2002; Qiu et al., 2003; Qiu et al., 2004; Sebe et al., 2005a,b; Lunacek et al., 2005; Fritsch et al., 2006; Sora et al., 2007; Kürtül et al., 2012; Konschake and Fritsch, 2014). If sections 1.5-4.0 mm thick are desired, the piece is frozen at a very low temperature (generally -80ºC) and then cut. The individual sections are then plastinated (Fritsch, 1996; Cook, 1997; Sora et al., 2002a,b; Zhang and Lee, 2002; Sora et al., 2004; Leaper et al., 2005; Nash et al. 2005a,b; Sora and Genser-Strobl, 2005; Fritsch et al., 2006; Sora et al., 2008; Al-Ali et al., 2009; Elnady and Sora, 2009; Arredondo et al., 2012; Chen et al., 2012; Wegman et al., 2012; Liu et al., 2013; Konschake

and Fritsch, 2014; Liang et al., 2014; Bernal-Mañas et al., 2016; Lowis et al., 2016; Ottone et al., 2016).

The use of a polystyrene box to fix the frozen specimen is one of the best options for handling the frozen block during slicing, ensuring a straight cut. The polyurethane foam is obtained by mixing two components in equal parts (50:50); the first component consists of a diisocyanate and a polyol, and the second is the activating agent. The activating agent is released together with the heat generated in the reaction, giving the foam a volume close to four times that in the liquid state. The polyurethane foam is also used already-mixed, applied as a spray (Ottone et al., 2016).

A diamond saw can be used to obtain plastinated sections around 400-500 μm thickness with no decalcification; this is the E12 ultra-thin technique (Sora, 2007; Soal et al., 2010). With this diamond saw it is possible to obtain sections that include metal or ceramic implants to study the interface of these biomaterials.

von Hagens et al. (1987) indicated that specimens should be frozen to be cut, adding that the saw guide must be cooled to 0°C. Different researchers cut the sections at different temperatures: Steinke (2001), -35ºC; Porzionato et al. (2005), -20ºC; Sora and Cook (2007), -75ºC; Macchi et al. (2008) and Ottone et al. (2016), -20ºC; Scali et al. (2015a, 2015b), -85ºC; Bernal-Mañas et al. (2016), -20ºC and -80ºC. Different materials can be used to make the block in which the samples to be cut are frozen; Soal et al. (2010) described the use of polyurethane foam. Liang et al. (2014) embedded human heads in 20% gelatin and produced a gelatin block frozen at -30ºC; after that, the gelatin block was frozen for 24 h at -80ºC.

The slices depend mainly on the type of saw-blade used for cutting. Alston et al. (1997) analyzed the type of blade used for different sections. They compared standard saw blades (10 teeth per inch) with the "Shark Band" blade, which has fewer teeth (three per inch) and deeper separations between them. Thus, when the "Shark Band" is used, the sawdust is more easily eliminated from the surface of the cut, which is also smoother and cleaner than with a standard blade. However, the authors

found that the better quality of cut achieved with the "Shark Band" comes at a cost: the blade has a shorter useful life than a standard blade.

Fasel et al. (1988) modified the saw to obtain thin slices (2-3 mm), adding liquid nitrogen to cool the saw-blade and the stop guide on which the block rests for cutting; this was performed for approximately 2 h before cutting. They also recommended using acetone at -25ºC to clean the sawdust from the sections and placing the sections in dehydration liquid immediately. Liquid nitrogen is necessary to prevent the sample from thawing while it is being cut.

The tissue loss during cutting is related to the thickness of the saw blade. Several authors have reported various levels of tissue loss during cutting: 0.4 mm (Sora et al. 2004; Sora & Genser-Strobl, 2005; Sora, 2007; Sora et al., 2007; Sora et al., 2008), 0.5 mm (Cook, 1997), 1 mm (Sora et al., 2002a,b; Elnady & Sora, 2009), 1.6 mm (Nash et al., 2005a,b); and 2 mm (Zhang & Lee, 2002).

Dehydration

There are two reasons for using acetone during dehydration: first, acetone is the dehydration medium used; second, it has the physicochemical characteristics of an intermediary solvent to be exchanged by the polymer during impregnation. The acetone concentration is monitored with an acetonometer. It has to be measured before the specimen is transferred to a new acetone bath. When the acetone concentration exceeds 99% and remains stable for two days, dehydration can be stopped. The acetone:tissue ratio for dehydration is 10:1 volume per volume (von Hagens, 1986).

Most authors carried out dehydration at -25ºC (Eckel et al., 1993; Fritsch, 1996; Sha et al., 2001; Johnson and Zhang, 2002; Sora et al., 2002a,b; Qiu et al., 2003, 2004; Sora et al., 2004; Lunacek et al., 2005; Nash et al. 2005a,b; Porzionato et al., 2005; Sora and Genser-Strobl, 2005; Fritsch et al., 2006; Sora et al., 2007; Sora and Cook, 2007; Macchi et al., 2008; Sora et al., 2008; Elnady and Sora, 2009; Soal et al., 2010; Koslowsky et al., 2011; Arredondo et al., 2012; Koslowsky et al., 2015; Kürtül et al., 2012; Sora et al., 2012; Wegman et al., 2012; Konschake and Fritsch, 2014; Scali et al., 2015a,b; Ottone et al., 2016), except for Zhang and Lee (2002), Leaper et al. (2005),

Chen et al. (2012), Liu et al. (2013), Liang et al. (2014) and Lowis et al. (2016), who used a temperature of -30ºC. In all cases, the volumes of acetone necessary for dehydration started at over 90% and reached 100% in the final acetone bath. Very few authors failed to state the dehydration temperature (Cook, 1997; Sittel et al., 1997; Zhang et al., 2002; Sebe et al., 2005a,b; Al-Ali et al., 2009; Bernal-Mañas et al., 2016); in accordance with the references cited in their publications, especially the publication of Professor von Hagens, we suppose that the temperature was -25ºC.

In general, the thinner the section, the shorter the required dehydration time. Soal et al. (2010) cut 1 mm sections (in specimens more than 150 mm long), for which dehydration took 10 days. Sha et al. (2001) cut sections of the ankle 1.2 mm thick that required dehydration for 35 days. In the work of Qiu et al. (2003, 2004), dehydration of sections of 1.0 mm and 700 µm took four weeks. However, Porzionato et al. (2005) cut sections of 2-3 mm and completed dehydration in two weeks. In the other works studied, dehydration took between four weeks and four months. Sittel et al. (1997) cut sections of the larynx at thicknesses between four and 80 µm, but these sections came from epoxy blocks and were already dehydrated and impregnated.

Steinke (2001) added peracetic acid to the acetone (8%) to produce gentle bleaching during substitution by freezing (following a personal recommendation by von Hagens).

Degreasing

In the degreasing stage, the fat in the specimens is eliminated at ambient temperature. Some authors use 100% acetone for this stage, exploiting its degreasing power (Steinke, 2001; Zhang and Lee, 2002; Leaper et al., 2005; Nash et al. 2005a,b; Macchi et al., 2008; Soal et al., 2010; Kürtül et al., 2012; Chen et al., 2012; Liu et al., 2013; Wegman et al., 2012; Liang et al., 2014; Lowis et al., 2016; Ottone et al., 2016). Other authors use MeCl as the degreasing agent, known for its powerful action but more toxic than acetone (Eckel et al., 1993; Fritsch, 1996; Sittel et al., 1997; Sora et al., 2002a,b; Sora et al., 2004; Lunacek et al., 2005; Sora and Genser-Strobl, 2005; Fritsch et al., 2006; Fritsch et al., 2006; Sora et al., 2007; Sora et al., 2008; Al-Ali et al., 2009; Elnady and Sora, 2009;

Koslowsky et al., 2011, 2015; Sora et al., 2012; Konschake and Fritsch, 2014). In one work, a combination of acetone and MeCl is used, always starting with acetone as a continuation of the dehydration process (Cook, 1997). Some authors used either acetone or MeCl indifferently, without indications (Porzionato et al., 2005; Sora and Cook, 2007). In all cases, degreasing was carried out between 15ºC and 24ºC. Cook and Al-Ali (1997) indicate that sections with the best optical quality were obtained when degreasing was performed at ambient temperature, first with acetone and then with MeCl to ensure that more lipids were extracted.

Forced impregnation

This is the crucial stage in all plastination techniques, when the resin is incorporated into the samples as the acetone is extracted as vapor by a vacuum pump, reducing the pressure from 760 to 30 mmHg. Forced impregnation was performed at room temperature (20-22ºC) in a vacuum chamber (Ottone et al., 2015). The vacuum chamber pressure was carefully adjusted and reduced (760 to 30 mmHg), a bypass valve being used to control the admission of air into the chamber. Bubbling indicated that acetone was being extracted, so the pressure was maintained at that value until the bubbling stopped again, then reduced until bubbling recommenced. Once the final pressure was reached (30 mmHg) and the bubbling stopped completely, the forced impregnation phase was finished. The samples are submerged in different mixtures of epoxy resins, catalysts and accelerators, mostly produced by Biodur (see Table 2), in a vacuum chamber. In nine articles, the authors mentioned the use of epoxy resins with their catalysts and accelerators, but without naming the commercial brand (Cook, 1997; Sebe et al., 2005a,b; Lunacek et al., 2005; Koslowsky et al., 2011, 2015; Wegman et al., 2012; Scali et al., 2015a,b; Ottone et al., 2016). The time allowed for the forced impregnation differed depending on whether the regular epoxy technique (24-48 h) or ultrathin section technique (several days) was used. In the regular epoxy technique, the process most frequently lasts for 24 h (Sittel et al., 1997; Zhang and Lee, 2002; Leaper et al., 2005; Nash et al. 2005a,b; Soal et al., 2010; Konschake and Fritsch, 2014; Liang et al., 2014); the next most

frequent period, used by authors who follow the classic description of von Hagens (1986; 1987), is 48 h (Cook, 1997; Elnady and Sora, 2009; Kürtül et al., 2012; Scali et al., 2015a,b; Lowis et al., 2016). Other authors continue the process over two weeks (Lunacek et al., 2005; Fritsch et al., 2006); these are investigations using fetal samples from the pelvic region, with ultra-thin sections of 300 to 700 µm cut with a diamond saw. The forced impregnation process needs to be slower than usual in these cases to avoid shrinkage of the ultra-thin sections, as in fetal tissues. Eckel et al. (1993) made a similar recommendation for forced impregnation over 10 days for 0.8 mm sections of the adult larynx. Again, the recommendation reflects the processing of ultra-thin sections and the need to avoid tissue shrinkage. Wegman et al. (2012) applied forced impregnation for only 10 h for lower limb sections 3-4 mm thick. According to the classic descriptions of von Hagens (von Hagens, 1986; von Hagens et al., 1987), this process is carried out at room temperature. However, Mircea-Constantin Sora in his various works (Sora et al., 2002a,b; Sora et al., 2004; Sora and Genser-Strobl, 2005; Sora and Cook, 2007; Elnady and Sora, 2009) carried out forced impregnations at 5ºC on sections of 1.6 to 3.8 mm. Other authors have used an even lower temperature of 0ºC (Zhang and Lee, 2002; Leaper et al., 2005; Nash et al. 2005a,b; Chen et al., 2012; Liu et al., 2013; Liang et al., 2014; Lowis et al., 2016). Scali et al. (2015a,b) used temperatures between -8ºC and 0ºC. Sora et al. (2007) cut ultra-thin sections (1 mm) with a diamond saw-blade from an ankle plastinated in a block. Forced impregnation was performed in a drying oven at 30ºC under a vacuum; the sections were cut after curing.

Latorre et al. (2002) carried out interesting research into this technique. They proposed forced impregnation with epoxy resin alone with no hardener. The objective was to determine whether this modification in the impregnation mixture could diminish the yellowing of the plastinated sections that usually occurs a few days after production. The authors also wanted to test whether the resin alone (with no hardener) would remain for longer without being catalyzed, i.e., in a liquid state that would allow it to be re-used. Finally, they hoped to show whether the casting time of the sections

could be extended indefinitely after impregnation with epoxy resin to obtain better-quality sections. They managed to show a reduction in the yellowing of the plastinated sections and to extend the usable time of the epoxy resin by not mixing it with hardener; this also extended the time it took to complete the cured sections, giving the researchers longer to finish setting up the curing chambers to complete the plastination process.

Curing

In the curing stage, the epoxy resin mixture is polymerized with its catalyst for final hardening to produce the sample laminas. The classic method of curing is in a 45ºC oven (von Hagens, 1986; von Hagens et al., 1987); the sections are introduced into curing chambers made from glass plates lined with sheets of acetate on which the mixture of epoxy resin-catalyst (E12-E1, according to von Hagens, 1986) is placed. The chambers are placed in the oven to harden and dry. The curing chambers can be set up as flat chambers, i.e., with a gasket of silicon between two glass plates held in place with binder clips to form a hermetic compartment. The epoxy resin and catalyst mixture is placed in the chamber with the section of tissue already completely impregnated; the entire mixture is polymerized during this stage (Fritsch, 1996; Cook, 1997; Sittel et al., 1997; Sora et al., 2002a,b; Sora et al., 2004; Sora and Genser-Strobl, 2005; Fritsch et al., 2006; Sora et al., 2008; Al-Ali et al., 2009; Elnady and Sora, 2009; Konschake and Fritsch, 2014; Ottone et al., 2016). The other curing technique is the sandwich method, in which the sample sections are placed one on top of the other, separated by sheets of acetate, the whole sandwich being contained between two glass plates. This method allows several sections of tissue to be cured at once (Steinke, 2001; Zhang and Lee, 2002; Leaper et al., 2005; Nash et al. 2005a,b; Sora and Cook, 2007; Soal et al., 2010; Liang et al., 2014; Scali et al., 2015a,b). The temperatures are approximately 30ºC to 65ºC.

Borzooeian and Enteshari (2006) proposed a silicone gasket with a steel center covered by 1.5 mm of silicone. This produces sections with smooth, square edges and a rectangular shape, making

casting easier. The absence of glass means that the specimens can be positioned precisely, and bubbles can be removed.

The recommended curing times in the oven range from only 2-3 days (Konschake and Fritsch, 2014) to two weeks (Sha et al., 2001; Zhang and Lee, 2002; Qiu et al., 2003, 2004; Leaper et al., 2005; Liang et al., 2014).

Another alternative is to plastinate the whole sample with epoxy resin, making a resin block that is then cut (Eckel et al., 1993; Sha et al., 2001; Qiu et al., 2003, 2004; Koslowsky et al., 2011, 2015; Kürtül et al., 2012; Sora et al., 2012). Sora et al. (2007) described this modification of the technique, in conjunction with forced impregnation in a 30ºC oven under vacuum. This enabled them to obtain ultra-thin sections (<1 mm) as described below.

- **Details of the Technique for Ultra-thin Sections**

Fritsch (1996) used a saw with a diamond blade (Well@, Mannheim, Alemania) to obtain 300-500 µm sections of the feet of neonates. He then examined the sections histologically, staining them with methylene blue and IU blue and counter-coloring with basic fuchsin (Fritsch et al., 1996, 2006). Steinke (2001) cooled the diamond saw-blade with liquid CO_2 and cleaned it continuously during cutting. A nozzle was fitted underneath the saw bench, connected to a bottle containing CO_2 and liquid nitrogen. By this means, he made 800 µm sections of human tissue.

Soal et al. (2010) developed a technique for plastinating ultra-thin sections by making a block of tissue enveloped in polyurethane foam. This produced 1 mm sections with less than 2% tissue loss. With this technique, tissue shrinkage did not exceed 2% in each linear dimension, producing a maximum potential volume loss of 6% (Sora et al. 2002; Soal et al., 2010). However, the technique described by Soal et al. (2010) requires decalcification of the bone for application with commercial saws, otherwise the blade will become dull; in any case it will need regular sharpening.

Thin slices are easier to impregnate than large blocks of tissue, which also require higher temperatures (30-60ºC) during treatment (Sora et al., 2004, 2007). However, to obtain sections thinner than 1 mm, the whole sample block must be impregnated before cutting, as described above, at a high temperature (for the first four days of impregnation the temperature is 30ºC; on the fifth day it is increased to 60ºC). At these temperatures, the polymer becomes sufficiently thin to penetrate deeply into the sample block (Sora, Strobl and Radu, 2004).

One-millimeter sections can be a basic tool for proper histological examinations, with or without staining (Gruber et al., 2001; Sora, Strobl and Radu, 2004); they are also valuable for high-quality three-dimensional reconstructions (Sora, Strobl and Radu, 2004). In general, the techniques for plastinating ultra-thin sections require initial impregnation of the whole sample, to produce a block of resin that can then be sliced. However, some authors slice the tissue first, as with traditional sections (3-5 mm) (Sittel et al., 1997; Soal et al., 2010; Koslowsky et al., 2011, 2015).

- **Anatomical Regions in which the Technique for Sheet Plastination with Epoxy Resin can be used**

Head and neck

Sittel et al. (1997) investigated the human larynx to develop a plastination technique that allows histological examination to be conducted with no need for prior decalcification, as in traditional histological techniques.

Johnson et al. (2000) studied the arrangement of the connective tissue of muscle tendons and ligamentous tissue in the posterior region of the cervical column. They examined the plastinated sections under low and high magnifications using a Leica MZ8 stereoscopic microscope. They compared the plastinated sections with histological sections, detecting advantages of plastination over paraffin. The anatomical structures and their relationships to surrounding tissues were better maintained in the plastinated samples, with no tissue disruption. Although the sections were larger, it was possible to distinguish between connective tissue and loose adipose.

Nash et al. (2005a) studied the configuration of the connective tissue in the posterior atlanto-occipital interspace, indicating that as a result of plastination the connective tissue, especially collagen, is endogenously autofluorescent if excited at 488 nm (Phillips et al., 2002; Nash et al., 2004, 2005a,b; Liang et al., 2014). These authors made sections 107 µm thick, which they observed under a Leica MZ8 microscope for stereoscopic dissection (Leica, Heerbrugg, Switzerland) from 1.25-5; the images were recorded electronically. From their studies they concluded that the deep cervical fascia does not exist in humans (Nash et al., 2005b). Phillips et al. (2002) and Nash et al. (2004) determined that tissues can be viewed under confocal microscopy and that three-dimensional images can be constructed from soft-tissue structures.

Leaper et al. (2005) studied the protrusion of the posterior hypopharyngeal wall in adult human cadavers; this technique also allowed them to study the relationship between the structures and surrounding tissues such as the retropharyngeal spaces, prevertebral fascia and vertebral fascia.

Scali et al. (2015a) used plastination for the first study of the alar fascia and potential adjacent spaces, producing results that allow pre-surgery planning of retropharyngeal lymphadenectomy operations to be more exact. In a second article, Scali et al. (2015b) studied the intervertebral epidural spaces at the level of C3 by viewing the sections obtained through a Leica MZ8 stereoscopic microscope (Johnson et al., 2000; Johnson and Zhang, 2002; Chen et al., 2012; Diao et al., 2013; Liu et al., 2013; Liang et al., 2014; Scali et al., 2015b); they could observe the upper cervical region without compromising the morphological arrangement.

Zhang and Lee (2002) identified the difficulties involved in distinguishing the membranous (or fibrous) part of the subcutaneous tissue, deep fascia, epimysium and epitendineum during routine dissection. Although these structures can be distinguished histologically, the standard histological technique is limited by the small area of the sample. Therefore, Zhang and Lee (2002) considered that sheet plastination offers a new approach to revealing the detailed structure of connective tissue at both the macro- and micro-scopic levels. An important finding in this research was that there was

no aggregation of fibrous connective tissue connecting the sternocleidomastoid and trapezius muscles, the space between them being completely occupied by fatty tissue indistinguishable from subcutaneous cell tissue.

Sora et al. (2002b) plastinated a human sample that presented compression of the optic nerve due to hypertrophy and degeneration of the extra-ocular muscles close to the apex of the orbit. They then applied histological techniques to the plastinated sections, with the advantage that they did not have to decalcify the tissue. Zhang et al. (2002) used sheet plastination in epoxy resin to study the course of the oculomotor nerve and blood vessels, and the differences between plastinated sections and MR images. Liang et al. (2014) studied the trigeminal nerve, particularly mechanical compression of it and the appearance of trigeminal neuralgia. They were able to study the nerve together with surrounding structures. Qiu et al. (2004) investigated the lateral base of the cranium and its relationship to adjacent structures at microscopic resolution and showed better differentiation than was obtainable with computed tomography images.

Nash et al. (2005b) affirmed the importance of anatomical knowledge obtained from sections plastinated in epoxy resin. This knowledge is useful for anesthesia applied by blocking the cervical plexus, and for surgeons planning endoscopic approaches to the regions of the anterior and posterior cervical triangles, thyroid gland and lymphatic ganglia in the same region.

Chen et al. (2012) used plastination for a pioneering study of the fibrous configuration of the capsule of the cricothyroid joint and its relationship to the articular cavity at the macro- and microscopic levels. Liu et al. (2013) investigated the cricoarytenoid joint and its relationship to its own capsule. In both investigations, the authors studied the biomechanics of rotation in the two joints, especially the movements of the vocal fold and surgical correlation in cases of joint dysfunction (Liu et al., 2013).

Lowis et al. (2016) studied the detailed configuration in situ of a complete type II cyst and its relationship to surrounding anatomical structures for the first time. The authors proposed that the

difficulty of dissecting the cyst wall, and the limitations imposed on histological study by the size of the sample areas, indicated that plastination provided better anatomical information.

Arredondo et al. (2012) applied plastination to the study of the temporomandibular joint in the cat. To color the arterial vascular structures, they injected a colored epoxy resin before plastination and then froze the samples at -30ºC for 48 h. In the plastinated sample, the proximity of the TMJ to the mandibular nerve, maxillary artery and middle ear was clear, and was correlated with images from tomographic diagnosis techniques such as CT and magnetic resonance.

Diao et al. (2013) investigated the medial wall of the cavernous sinus, which is important for determining the direction of growth of pituitary adenomas when hypophyseal surgery is planned. They sought to establish the existence of a dural wall between the pituitary gland and cavernous sinus.

Bernal-Mañas et al. (2016) studied the arrangement of the muscle fibers of the lateral pterygoid muscle in human specimens using sufficiently transparent 2-3 mm thick sections. They represented the topography of the anatomical structures correctly and could study different tissues with a stereoscopic microscope.

In veterinary science, Elnady and Sora (2009) plastinated the head of a dicephalic kid, observing numerous macro- and micro-anatomical details. Rodríguez et al. (2006) studied the temporomandibular joint of the horse through gross dissection, vascular injection and sheet plastination. Following these studies, Rodríguez et al. (2007) analyzed the anatomy of the same joint in a pure-bred Spanish horse by ultrasonography and compared it with sheet plastination. Finally, Rodríguez et al. (2008) studied the temporomandibular joint in the young horse with epoxy sheet plastination and computed tomography imaging.

Thorax

Didenko et al. (2013) plastinated sections of 84 hearts in epoxy resin, studying the arrangement of Koch's triangle and its application in clinical practice. Skalkos, Williams and Baptista (1999) analyzed the arrangement of the myocardial fibers in sections plastinated in epoxy resin and compared them with magnetic resonance images obtained prior to plastination. They could see details of the heart in three dimensions in the plastinated sections.

Abdominal cavity

Párraga et al. (2013) published the only work related to the abdominal cavity and used sheet plastination to study the pneumoperitoneum effect. They compared the plastinated sections with MRI images.

Pelvis

Porzionato et al. (2005) studied the morphology and topography of the rectourethral muscle and its application in surgery for muscle insertions, along with its histology and immunohistochemistry. Macchi et al. (2008) analyzed the characteristics of the longitudinal anal muscle. Al-Ali et al. (2009) used coronal and axial sections to investigate the structure and dimensions of the anal sphincter, combining plastination with conventional histological microscopy.

Sebe et al. (2005a) also studied the rectourethral muscle, considering the term inappropriate in the urological literature because there is no developed muscular structure connecting the rectum to the membranous urethra. They stained their samples histologically (azure II / methylene blue in alkaline solution and counter-coloring with basic fuchsin). In a second article, Sebe et al. (2005b) investigated the fetal development of the external urinary sphincter and striated sphincter in females, especially the growth and organization of the muscle fibers around the urethra and morphological modifications due to the development of the vagina. They used the same histological stains as in the previous work (Sebe et al., 2005a).

Lunacek et al. (2005) studied the cavernous nerves and their topographical relationship to surrounding anatomical structures such as the seminal vesicles, prostate and urethra, so this new knowledge could be exploited to modify the surgical technique for radical prostatectomy, preserving the nerves and their functioning.

Fritsch et al. (2006) investigated the anatomy of the connective tissue structures around the urethra in females to determine the anatomical structures that support the urethra and rhabdosphincter. This knowledge is important for the surgical treatment of urinary incontinence in females.

Sora et al. (2012) made a 3D reproduction of a pelvis by plastinating sections in epoxy resin. They concluded that the reconstructed model could be used for education during residency and that it provided more precise information about the pelvic floor (useful for gynecologists, radiologists, surgeons, urologists, and physiotherapists). The information could help in planning unusual, complex surgery and in developing new surgical approaches, providing a stereoscopic view with which to study the anatomical relationships of adjacent structures and the arrangement of the various sections of the pelvis. Beyersdorff et al. (2001) used plastination to view the structures of the muscles of the pelvic floor and their relationship to adjacent structures at the microscopic level.

Kaulhausen et al. (2012) analyzed the supraspinous and interspinous ligaments and the position of a device to decompress the interspinous process, particularly after injury caused by surgical implantation.

Limbs

Sora and Genser-Strobl (2005) examined the morphology of the carpal tunnel and its neurovascular structures. Koslowsky et al. (2011) studied the peri- and intra-osseous vascular architecture of the radial head and vascular interaction with surgical implants. In another study from 2015, Koslowsky et al. investigated the peri- and intra-osseous vascular supply proximal to the ulna.

Fritsch (1996) used sheet plastination combined with histology to obtain sections 300-500 µm thick of eight feet from fetuses. He established that the topographical relationships among the bone, cartilage and connective tissue structures are almost undisturbed in plastinated preparations; thus, they closely resemble the live state and can be compared with magnetic resonance planes.

Sora et al. (2007) described a method for developing a computerized model of the human ankle using plastinated sections. The reconstructed model can be used for education during residency, surgery planning and the development of new surgical approaches. In 2008, Sora et al. (2008) studied the topography of the posteromedial neurovascular bundle in the ankle in view of its importance in planning minimally invasive surgery. Then, in 2012, Sora et al. (2012) used sections plastinated in epoxy resin to obtain complete data on syndesmotic ankle injuries, especially the length, breadth and inclination of the fibular incision, because a reduction in the inclination of the incision could signal anatomical predisposition to syndesmotic lesions.

Wegman et al. (2012) studied human legs to demonstrate the consequences of incorrect posterior positioning of the plates for the security of medio-lateral distal blocking screws and considered the options available.

Rath et al. (2009) studied the arterial supply to the metatarsal bones in relation to possible arterial lesions after surgery to the point of the foot. Windisch and Weiglein (2001) studied the tendon sheaths in the talocrural region, finding that they are not cylindrical as in classical descriptions. Faymonville et al. (2012) studied the compartments of the foot. Oppermann et al. (2014) considered that plastination is an excellent technique for mapping the intra-osseous vascular network of the talus.

Villamonte-Chevalier et al. (2012, 2015) studied the elbow joint in the dog, correlating ultrasound images with transparent plastinated anatomical sections. Soler et al. (2007) studied the stifle joint in dogs with sheet plastination, comparing the results with ultrasonography, computed tomography and magnetic resonance images.

- **Improving Diagnostic Image Analysis using Plastinated Specimens**

Image analysis, from X-rays to MRI, requires a new view of anatomy (Elnady and Sora, 2009). Thus, cross-section anatomy — as part of topographical anatomy — is becoming more important, resulting in better interpretation and diagnostic image analysis (Lane, 1990; Steinke, 2001; Elnady and Sora, 2009). These specimens are useful in advanced training programs for sectional topography and training during residency in CT and NMR (Cook and Al-Ali, 1997; Beyersdorff et al., 2001, Steinke, 2001; Sora, Brugger and Strobl, 2002; Thomas et al., 2003; Sora and Cook, 2007; Elnady and Sora, 2009; Soal et al., 2010; Steinke et al., 2010; Sora et al., 2012; Bernal-Mañas et al., 2016). The reconstructed 3D model can also be used for training during residency, to carry out unusual surgery and develop new surgical approaches (Sha et al., 2001; Qiu et al., 2003; Sora et al., 2012). Sheet plastination in epoxy resin provides a better-quality image than two-dimensional echography and would be very useful for complementing this technique in teaching human and veterinary anatomy (Elnady and Sora, 2009).

Hermans et al. (2009) studied six frozen legs that were injected with gadolinium and subjected to magnetic resonance arthrography. They then impregnated the whole legs with epoxy and viewed them by magnetic resonance again. Finally, they cut 3 mm sections and completed the plastination process. These researchers showed it was possible to mix a polymer with a gadolinium solution, producing a signal sufficiently intense for the plastinated pieces to be scanned effectively. When a polymer is added to the gadolinium solution, the intensity of the signal diminishes over time because of the mixture becomes more solid as the polymer is cured. The mixture that gave the strongest signal over the longest time was a slow-curing polymer with a gadolinium solution diluted 1:50.

- **Studies oriented toward Three-Dimensional Reconstruction using Plastinated Specimens**

Sha et al. (2001) made a 3D reconstruction of the lateral structures of the ankle and subtalar joints using plastination to obtain a series of equidistant sections 1.2 mm thick. Qiu et al. (2003) generated a 3D computed reconstruction of the temporal bone and intratemporal structures by first carrying out plastination to obtain a series of equidistant 1.2 mm thick sections without prior decalcification.

Steinke et al. (2010) examined the ligaments of the human sacroiliac joint, carrying out magnetic resonance studies of the specimens and obtaining plastinated sections. They subsequently performed a three-dimensional reconstruction of the anatomical structures studied. These authors concluded that the combination of high resolution MR and plastination of thin sections permitted the ligaments of the sacroiliac joint to be reconstructed precisely, making anatomical viewing in situ and morphometric description possible.

Sora et al. (2012) established that thin plastinated sections offered many advantages over other current methods of raw anatomical preparation for generating images for computed reconstruction. These authors considered that reconstruction of anatomical structures is only possible because the plastinated sections are transparent. The sectional anatomy obtained by plastination confers a great advantage because the bone tissue does not need to be decalcified and the spatial relationships between contiguous features of different composition are maintained.

Adds and Al-Rekabi (2014) made a three-dimensional reconstruction of the ethmoidal arteries after plastination with E12 in the lateral wall of the orbit. They stained the plastinated sections with Miller's elastin stain to make the vascular structures more visible. This highlighted the arterial walls and facilitated 3D reconstruction.

Arredondo et al. (2016) studied the elbow joint of the dog. The elbow block was plastinated using the E12-E6-E600 technique (Sora, 2007). They then scanned the ultrathin slices and recreated a 3D model of the elbow joint using the software Winsurf®. They revealed the anatomical structures precisely without losing the topographical relationships among them. The authors said that the model could be manipulated digitally to reveal the anatomical details from different perspectives,

which can be very useful in planning conventional approaches or developing new surgical strategies.

- **Studies related to Histological Analysis from Plastinated Specimens**

Eckel et al. (1993) plastinated ultra-thin sections of the human larynx to develop histological techniques that could be applied to samples after deplastination (with sodium methylate).

Fritsch (1996) showed that there is less contraction in plastinated sections than in conventional histological sections (von Hagens et al., 1987); this is an outstanding advantage for studying fetal tissues, which tend to contract markedly owing to their high water content.

Konschake and Fritsch (2014) developed a technique for histological plastination, in which the samples were first hardened completely and then cut into horizontal, frontal and sagittal sections of 500 µm thickness with a diamond saw-blade (WellVR, Ebner, Mannheim, Germany). The sections were placed on plates, wet sanded and polished and then stained with azure II/methylene blue in sodium bicarbonate for microscopic investigation. They were then counter-colored with basic fuchsin (Fritsch et al., 1996; Lunacek et al., 2005; Fritsch et al., 2006; Konschake and Fritsch, 2014). This staining method colored the connective tissue violet blue, the muscles greenish blue, the adipose tissue brilliant pink, the bones blackish brown and cartilage violet. The samples were from the nasal mucus.

According to Sebe et al. (2005b), plastination offers the advantage of analyzing much thicker tissue sections (50:1) than conventional staining techniques. Thus, the in situ morphology of the anatomical structures can be magnified.

- **Brief comparison with a new sheet plastination technique with polyester.**

Although it is not part of the objective of this work in relation to the polyester technique (Henry and Latorre, 2007; Latorre and Henry, 2007), we can establish a brief comparison based on an article

published by Sui and Henry (2015), which make improvements to the standard polyester technique. In this article, they establish a new polyester cutting plastination technique called Hoffen P45, in which they use polyester resin and other components from the company Hoffen. This technique is developed to obtain cuts of between 2 to 3 mm of brain and any body region. The fundamental improvements in relation to the steps of the standard technique are the inclusion of the fabric cuts in a glass chamber (flat chamber) to carry out the forced impregnation for eight hours. Subsequently, these same chambers are used for curing, which involves immersing them in warm water at 40ºC for three days. Another outstanding feature of this new technique is that the use of the flat chambers from the impregnation step reduces the amount of polyester used throughout the process. In total, the technique has a duration of 30 days. However, there is no mention of tissue shrinkage in the article. It would be interesting to see this technique developed by more researchers, especially for comparison with E12 in relation to tissue shrinkage.

DISCUSSION

The plastination technique is still novel in both application and development. It allows structures and adjacent elements to be fixed in situ in their anatomical position, allowing the topography of the human and animal body to be studied without alteration, in an uncollapsed and undisplaced state, as in the live state (Johnson et al., 2000; Beyersdorff et al., 2001; Windisch and Weiglein, 2001; Sora et al., 2002a; Sora et al., 2002b; Sora, Brugger and Strobl, 2002; Zhang et al., 2002; Sora et al., 2004; Sora, Strobl and Radu, 2004; Leaper et al., 2005; Fritsch et al., 2006; Sora and Cook, 2007; Sora et al., 2008; Sora et al., 2012; Adds and Al-Rekabi, 2014; Scali et al., 2015a,b; Arredondo et al., 2016; Lowis et al., 2016; Ottone et al., 2016). This makes plastination a valuable tool for clarifying anatomical relationships (Lane, 1990; Sora et al., 2008).

The sections also present anatomical proportions with minimal shrinkage (Eckel et al., 1993; Sora et al., 2002b; Zhang et al., 2002) owing to the low temperatures used in the process (-25ºC) (von

Hagens, 1986). According to Sora et al. (2002a), this property of the technique makes it easy to take morphological measurements. Sora, Brugger and Strobl (2002) found that the sections shrunk by 6.65% (± 1.123 SD): the sum of the shrinkage of 1.33% in the first acetone bath (96% acetone), 0.8% in the second bath (99% acetone), and 4.52% during the degreasing with MeCl and the remainder of the plastination process (forced impregnation and curing). This shrinkage results from the shrinkage of the epoxy resin itself and of the sections during the process (Sora, Brugger and Strobl, 2002).

Another advantage of plastination is that sections can be prepared without decalcification (Eckel et al., 1993; Johnson et al., 2000; Sora et al., 2002b; Qiu et al., 2003; Sora et al., 2007; Sora et al., 2012; Adds and Al-Rekabi, 2014; Lowis et al., 2016).

Plastinated sections are transparent, dry, resistant, odor free, of unlimited durability, and easy to maintain (Fasel et al., 1988; Eckel et al., 1993; Weber and Henry, 1993; Cook and Al-Ali, 1997; Sora et al., 2002a; Sora et al., 2002b; Sora, Brugger and Strobl, 2002; Qiu et al., 2003; Qiu et al., 2004; Sora et al., 2004; Sora and Genser-Strobl, 2005; Fritsch et al., 2006; Sora and Cook, 2007; Marks et al., 2008; Elnady and Sora, 2009; Soal et al., 2010; Bernal-Mañas et al., 2016; Ottone et al., 2016). Their transparency, especially of the connective and lipid tissue, means that the arteries and veins can be seen and measured easily (Windisch and Weiglein, 2001; Sora et al., 2002a). These characteristics of plastinated sections are directly related to their ease of handling, storage at ambient temperature and maintenance (Eckel et al., 1993; Sora et al., 2002a; Sora et al., 2002b; Sora and Cook, 2007).

Plastinated sections conserve the microscopic characteristics of the tissues (Johnson et al., 2000; Marks et al., 2008). Marks et al. (2008) noted that the infusion of cured epoxy polymers into tissues is also used in microscopy of the ultrastructure of electrons **[AUTHORS, IS THIS CORRECT?]** to preserve details at the nanometric scale, suggesting that incrustation with polymers could also be useful for studying the microstructures of tissues. Accordingly, sheet plastination specimens can be

viewed under low-powered magnification without compromising the morphological arrangement (Johnson et al., 2000; Sora et al., 2002b; Sora et al., 2007; Scali et al., 2015b). Leaper et al. (2005) considered that histology only allows very small areas of a sample to be examined; however, sheet plastination is an excellent method for investigating the fine architecture of muscle and connective tissues in large pieces, at both the macro- and micro-scopic levels (Cook and Al-Ali, 1997; Sora et al., 2002b; Qiu et al., 2004; Sora and Genser-Strobl, 2005; Soal et al., 2010). It is also ideal for histological staining (Gruber et al., 2001). Adds and Al-Rekabi (2014) stated that the advantage of plastinated sections soaked in epoxy resin is their capacity for staining without the need to rehydrate the samples before the stain is applied.

Anatomical dissection or the appearance of artifacts in conventional histological treatment can change the topographical anatomy; however, this does not occur with sheet plastination in epoxy resin because all the tissues and structures, including bone and cartilage, can be studied in the sections (Fritsch et al., 2006).

They can be used for three-dimensional reconstruction of any anatomical region (Sha et al., 2001; Sora et al., 2002b; Sora et al., 2004; Sora et al., 2012), with a higher resolution than photographs obtained from frozen samples (Qiu et al., 2003).

Some authors describe limitations such as the possible shrinkage of certain soft tissues, e.g., muscle and connective or adipose tissues (Sora et al., 2008; Scali et al., 2015a,b); however, Scali et al. (2015a,b) indicate that this limitation could be turned to advantage because, in the case of the alar fascia and its adjacent spaces (the main object of their investigation), the shrinkage made investigation easier owing to the separation of the fascial planes and better delineation of the aponeurotic structures. For histological processing, a longer time is required for plastination than for other histological techniques involving soaking in paraffin (Eckel et al., 1993). Another limiting characteristic is that, with time, the plastinated sections can lose their transparency, taking on a yellowish hue (Cook and Al-Ali, 1997).

Nevertheless, a very important aspect regarding teaching and research with the plastination epoxy technique is that the low refractive index of the epoxy resin E12, combined with its minimal shrinkage during polymerization, makes it the method of choice for studying different tissues in different planes of cutting from the macroscopic to microscopic levels (Phillips et al., 2002; Liang et al., 2014).

CONCLUSION

Plastination is a tissue-preserving method that has proven applicable in different fields of teaching and research in the morphological sciences and in normal and pathological anatomy. The specimens are dry and odor free, easy to handle and maintain, making them useful tools for teaching anatomy at the macro- and micro-scopic levels. Plastination of sections in epoxy resin can be used as an alternative to standard histological techniques (Windisch and Weiglein, 2001), and histological staining can be applied to sections of macroscopic samples. According to Marks et al. (2008), the transparency resulting from impregnation with epoxy resin is an advantage for microscopic analysis, while the rigidity can help to stabilize the microstructure in the same way as the epoxy-impregnated samples for the microscopic study of electron ultrastructure. Because of the excellent preservation of the sectioned tissues, it is a promising research tool, which in the near future can be further improved with new polymers and catalysts.

Sheet plastination is an anatomical technique that allows anatomical structures to be preserved in situ, without modification of their arrangement, permitting detailed observation of their macro- and micro-scopic morphological characteristics. Therefore, the specimens are of great value not only for undergraduate teaching but also for advanced training programs in sectional topography and for training specialists in computed tomography and magnetic resonance imaging (Sora and Cook, 2007; Sora et al. (2012). Finally, it is an aid to surgical planning because it allows for excellent viewing of human and animal anatomy.

Conflict of Interest: The authors declare that they have no conflict of interest.

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TABLES

Table 1. E12 sheet plastination technique general protocols (von Hagens et al., 1987; Sora and Cook, 2007; Sora, 2007).

Sheet plastination of transparent slices, filling method with epoxy resin (E 12) (von Hagens et al., 1987)		General protocol for epoxy slices (2-3 mm) (Sora and Cook, 2007)		Ultra-thin epoxy slice (<1 mm) protocol (Sora, 2007)	
Day 0	Freeze unfixed or fixed specimens.	Day 0	Freeze specimen -75ºC.	Day 1	Immerse specimens in nº1 -25ºC acetone bath (> 90ºC).
Day 2	Saw into 2.5 mm slices	Day 1	Slice and clean sawdust from slices.	Day 14	Check and record purity of acetone bath nº1, Immerse in nº2, -25ºC acetone (100%).
Day 2	Stack between plastic nettings and grids, avoid thawing and drying.	Day 2	Immerse specimens in nº1 -25ºC acetone bath (> 90ºC).	Day 28	Check and record purity of acetone bath nº2, Immerse in nº3, -25ºC acetone (100%).
Day 2	Dehydrate by freeze substitution with acetone followed by degreasing in methylene chloride.	Day 5	Immerse in nº2 -25ºC acetone bath (100ºC). Check and record purity of bath nº1.	Day 42	Degrease slices in MeCl at room temperature (RT) (minimum two weeks).
Day 10	Immerse in E 12/E 1-reaction mixture.	Day 8	Immerse in nº3 -25ºC acetone bath (100ºC). Check and record purity of bath nº2.	Day 56	Immerse in E12 resin-mix (+30ºC).
Day 11	Impregnate in vacuum to under 2 mm Hg	Day 11	Degrease slices in acetone or MeCl at room temperature (RT).	Day 57	Impregnate in E12 resin-mix (+30ºC).
Day 12	Place slices on glass plate, assemble flat chamber and fill.	Day 18	Impregnate in E12 resin-mix (+5ºC or RT).	Day 61	Impregnate in E12 resin-mix and increase temperature to +60ºC.
Day 13	Cure at room temperature or at + 50ºC.	Day 20 or 27	Cast or sandwich slices. Lay cast 15º from horizontal at RT.	Day 62	Cure specimen +65ºC oven.
Day 14	Open flat chamber.	Day 21 or 28	Cure upright in +45ºC oven.	Day 66	Remove block and slice when convenient.
		Day 25 or 33	Open flat chamber or the sandwich, cover slice with foil, saw and sand.		

Table 2. Forced impregnation mixtures [E12, E50: epoxy resin; E1, E6, E7: catalyst; AE3: glass separator (for separating the cured specimen from the glass walls of the flat chamber); AE10: plasticizer (for epoxy resins); E500, E600, E700: epoxy accelerators].

Products of mixture	Mixture proportions	Authors
Biodur® E12/E6/E600	100:70 pbw, 0.15%	Eckel et al., 1993
	100:50 pbw, 0.2%	Sora, 2007; Sora et al., 2007; Sora et al., 2012; Kürtül et al., 2012; Adds and Al-Rekabi, 2014;
	no information	Zhang et al., 2002*[4]; Chen et al., 2012*[1]; Liu et al., 2013*[1]; Lowis et al., 2016
Biodur® E12/AE30/AE10/E1	95:5:20:26 pbw	Cook and Al-Ali, 1997; Sora and Cook, 2007
Biodur® E50/E7/AE10/E700	20:16:5 pbw; 0.1%	Sittel et al., 1997;
Biodur® E12/E6/E500	100:50 pbw, 0.1%	Sha et al., 2001; Qiu et al., 2003; Qiu et al., 2004
Biodur® E12/E6	72% - 18%	Windisch and Weiglein, 2001
Biodur® E12/E1/AE10 (von Hagens, 1986)	no information	Sora et al., 2002a; Sora et al., 2002b; Sora et al., 2004; Sora and Genser-Strobl, 2005
	95:26:10 pbw	Sora, Brugger and Strobl, 2002; Elnady and Sora, 2009
Biodur® E12/E1/AE10/AE30	100:28:20:5, pbw	Zhang and Lee, 2002; Leaper et al., 2005; Nash et al. 2005a; Nash et al. 2005b
	69.6:19.4:13.9:3.4 pbw	Soal et al., 2010; Liang et al., 2014

Biodur® E12	no information	Fritsch, 1996; Fritsch et al., 2006; Macchi et al., 2008; Sora et al., 2008; Al-Ali et al., 2009; Konschake and Fritsch, 2014
Epoxy resin + catalyst	no information	Cook, 1997; Sebe et al., 2005a, 2005b; Lunacek et al., 2005; Koslowsky et al., 2011, 2015; Scali et al., 2015a, 2015b
Commercial epoxy resin pre-accelerated (without hardener)		Ottone et al., 2016

FIGURE LEGENDS

Figure 1. Flow diagram of epoxy sheet plastination technique.

Figure 2. Epoxy sheet plastination of a human foot (Dr. Carlos Baptista collection).

Figure 3.A. Horizontal plastinated sheet of a pig vertebral column (epoxy). B. Horizontal plastinated sheet of a dog sacroiliac joint (epoxy). C. Horizontal plastinated sheet of a human thorax (polyester). D. Magnification of the vertebral column area of figure 3C. Figures 3A and 3B are from Dr. Rafael Latorre´s collection. Figure 3C is from Dr. Carlos Baptista´s collection.

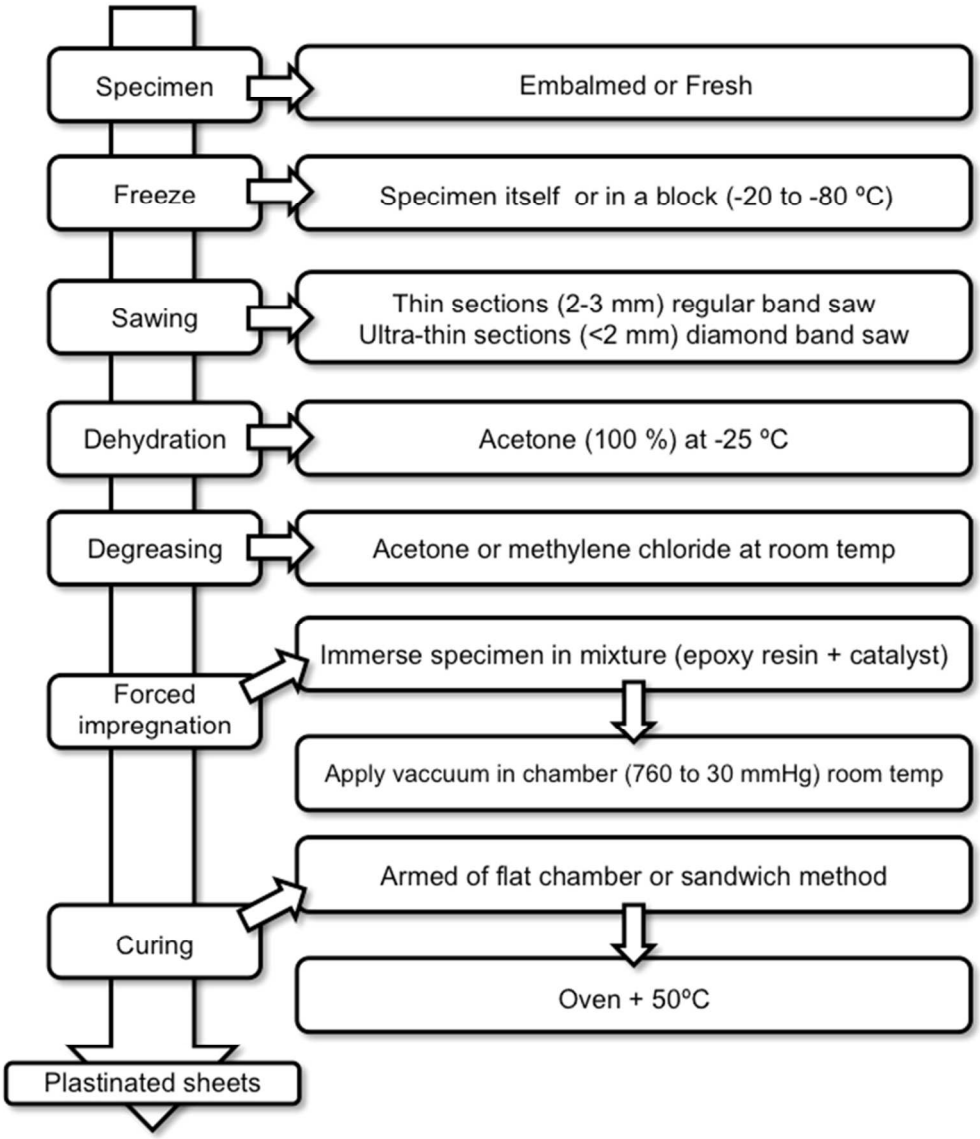

Figure 1. Flow diagram of epoxy sheet plastination technique.

99x115mm (300 x 300 DPI)

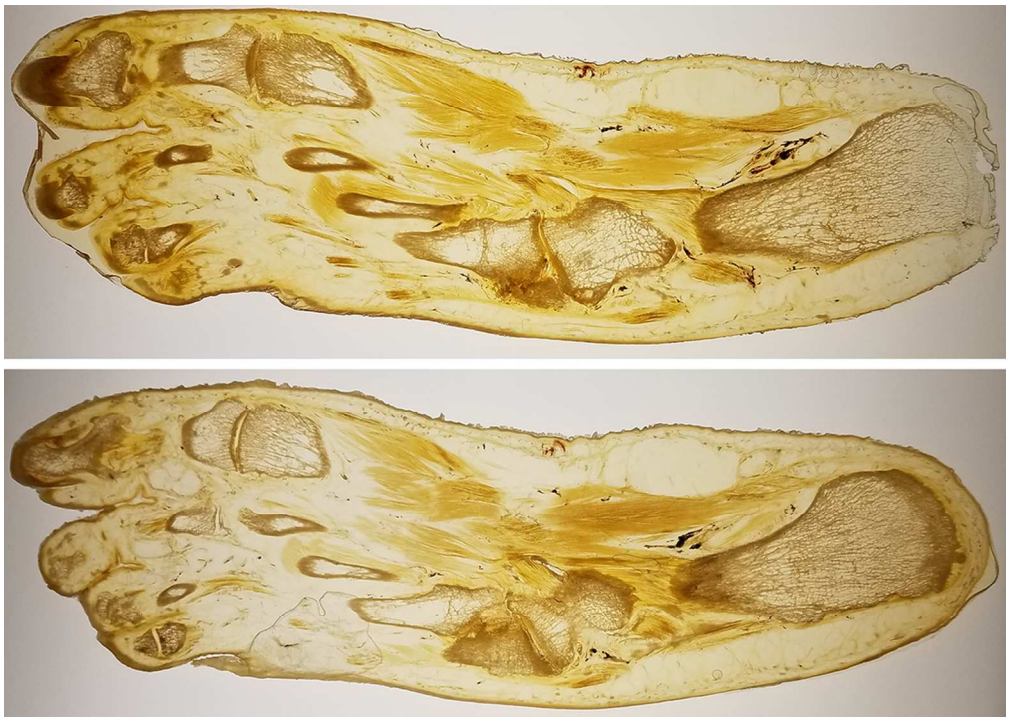

Figure 2. Epoxy sheet plastination of a human foot (Dr. Carlos Baptista collection).

99x71mm (300 x 300 DPI)

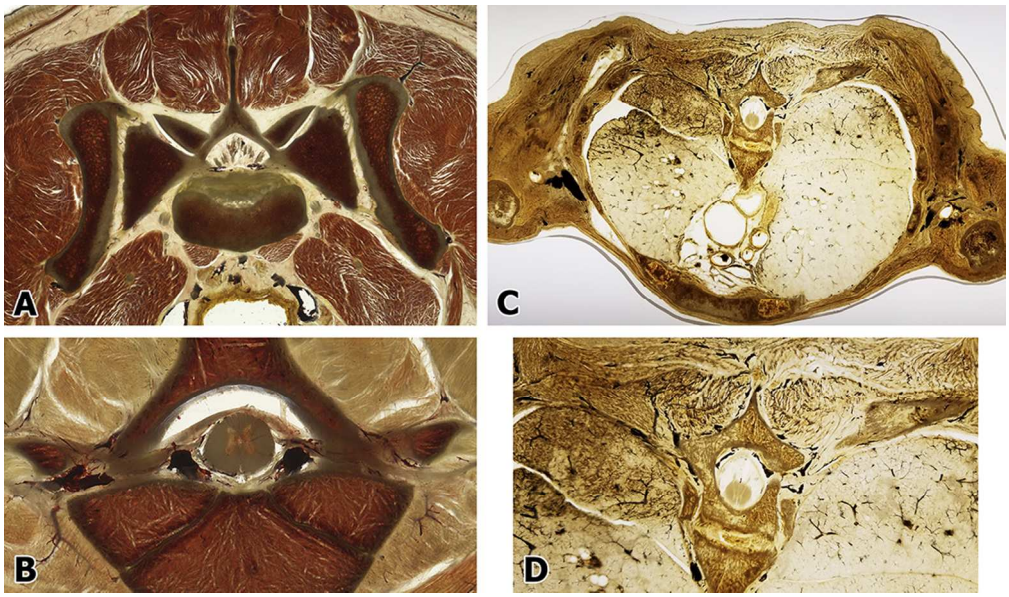

Figure 3. A. Horizontal plastinated sheet of a pig vertebral column (epoxy). B. Horizontal plastinated sheet of a dog sacroiliac joint (epoxy). C. Horizontal plastinated sheet of a human thorax (polyester). D. Magnification of the vertebral column area of figure 3C. Figures 3A and 3B are from Dr. Rafael Latorre´s collection. Figure 3C is from Dr. Carlos Baptista´s collection.

99x58mm (300 x 300 DPI)