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Micro-Plastination. Technique for Obtaining Slices below 250 µm for the Visualization of Microanatomy in Morphological and Pathological Experimental Protocols

Micro-Plastinación. Técnica para la Obtención de Cortes Inferiores a 250 µm para la Visualización de la Microanatomía en Protocolos Experimentales de Morfología y Patología

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SUMMARY: Plastination has revolutionized the study and research of anatomy, thanks to the biosecurity and indefinite preservation of human and animal bodies and organs. This paper presents the concept of Micro-Plastination, an ultra-thin sheet plastination technique, to obtain ultra-thin slices, of a thickness of less than 250 µm, for the identification and visualization of the microanatomy of any anatomical region in morphological and pathological experimental protocols.

KEY WORDS: Micro-plastination; Ultra thin slices; Experimental morphology; Experimental pathology.

INTRODUCTION

The technique of plastination has radically changed the way of carrying out studies in anatomy, mainly, in relation to the conservation of animal and human bodies and organs, for the practice of anatomy in undergraduate and morphological research. Plastination, invented by Gunther von Hagens in 1977 (von Hagens *et al*., 1987), was disseminated throughout the world from the first moments of its creation, publishing a variety of articles describing the use of the technique in different specimens, such as also details of the technique, with adaptations of it, but only in recent years has it begun to be used for research. Also, in 1986 the International Society for Plastination was created, and since that year, and with the appearance in 1987 of the Journal of Plastination (former Journal of the International Society for Plastination), the dissemination of the technique was further increased, with the particularity of the publication, in 2007, of the standard protocols for the development of all plastination techniques (with silicone at cold and room temperature, and the techniques of sheet plastination, with epoxy and polyester resins).

From our experience in South America, we started at the University of Buenos Aires, in 2006. Since 2014, our knowledge in plastination was deepened, perfecting our plastination protocols at Universidad de La Frontera, setting up the Laboratory of Plastination and Anatomical Techniques of the Faculty of Dentistry, with special support in addition to the Center of Excellence in Morphological and Surgical Studies (CEMyQ).

In this way, not only was a plastination laboratory set up, but also anatomical research was oriented through the use of plastination technique, with new contributions and modifications in the protocols of various plastination techniques [among them, a new plastination technique at room temperature (Ottone *et al*., 2015); and modifications to the sheet plastination techniques with epoxy resin (Ottone *et al*., 2016, 2018b)], and especially, the development of a new application for plastination technique in experimental and pathological morphology protocols (animals and humans, respectively), when studying the morphological

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changes caused by pathologies through plastination technique.

There are very few publications on ultra-thin sheet plastination technique (Fritsch, 1996; Sora *et al*., 2004; Sora, 2007; Soal *et al*., 2010), without guidance, these works, to the visualization of morphological changes, such as the work developed in our Laboratory of Plastination laboratory (Vargas *et al*., 2019). In this sense, we define as a Micro-Plastination technique, the plastination technique oriented to the identification in the macroscopic anatomical material of microscopic characteristics, performing a true microscopic and histological analysis from the macroscopic anatomy, something that could not be achieved before the appearance of plastination. This means that from this Micro-Plastination technique we can obtain ultra-thin slices (less than 0.25 mm/ 250 μm) in which the histological parameters of the tissues can be studied, without having to resort to histological techniques, which only allow the study of small portions of tissue, unlike micro-plastination, where we obtain sections of much larger size, and apply the same histological analyzes, but as indicated, to larger samples.

The aim of this communication was to establish the development of the protocol and the concept of Micro-Plastination, for ultra-thin sheet plastination techniques applied in morphological and pathological experimental research protocols.

RESULTS

Micro-Plastination technique. The microplastination corresponds to ultra-thin sheet plastination techniques, applied in samples corresponding to experimental morphology protocols.

Micro-plastination includes the following steps (Fig. 1):

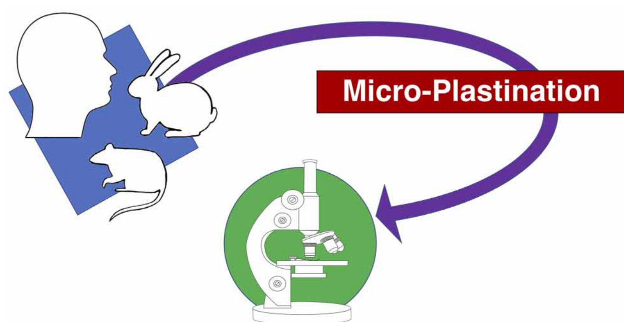

Fig. 1. Schematic representation of Micro-Plastination steps (schemes downloaded and adapted from: es.dreamstime.com).

1. Development of a morphological or pathological experimental research protocol [example: injection of MIA in rat shoulder for the generation of osteoarthritis (Vargas *et al*., 2019)].
2. Microplastination technique (ultra-thin slices, less than 250 μm).
3. Low intensity microscopy, magnifying glass, confocal viewing (Leica® KL 300 LED magnifying glass with Leica® MC 120 HD camera / Leica® DM750 microscope; or similar).

Micro-Plastination. It consists in the development of an ultra-thin sheet plastination protocol from a block of tissue from the morphological or pathological experimental protocol. The first step of the micro-plastination is the dehydration of the slices, which was performed in 100 % acetone, at -25 °C, for approximately 12 days, with 3 changes of acetone (always of 100 % acetone) being carried out (controlling the concentration of acetone by means of an acetonometer at room temperature, and ending the process upon reaching an acetone concentration greater than 99.5 %, for two days in a row). Subsequently, the degreasing step is continued, fundamental for the removal of fat and obtaining transparency of connective tissue. For this, methylene chloride is used for approximately 4 to 7 days. Due to the high danger of this product, this step was carried out with all safety measures, with facial / eye protection being essential, using gloves, and handling of methylene chloride under a gas extraction hood. Once the fat removal was finished, the forced impregnation process was continued. The same to be carried out at room temperature, and in a vacuum chamber incorporated in a stove (AccuTemp-09s, Across International), with the aim of reaching, during impregnation, temperatures between 30 ºC to 65 ºC. In this way, it was possible to increase the fluidity of the impregnation mixture (epoxy resin plus catalyst), and the penetration thereof into the piece. The impregnation mixture consisted of three components: E12, epoxy resin (100 p.b.w.); E6, hardener (50 p.b.w.); E600, catalyst (0.2 p.b.w.). The samples should be placed in containers according to their size. Before starting the impregnation, the samples were left immersed in the resin mixture for 24 hours at 760 mmHg (without generating vacuum). Subsequently, the forced impregnation process begins at 30 ºC, to continue with two days of impregnation at 60 ºC, ending on the fifth day of impregnation. During forced impregnation, polymerization of the samples occurs. Obtaining samples included inside an epoxy resin block, with the appropriate hardness to be cut at a low speed diamond saw (Buehler Ltd. ISOMET). With this type of saw, you can get cuts below the millimeter thick, in the order of microns (less than 250 μm). Once the ultra-thin sheets have been obtained, they must again be polymerized

in a mixture similar to that used for impregnation, in this way the final transparency is achieved, which allows viewing of the ultra-thin sections under magnifying glass (Leica® KL 300 LED magnifying glass with camera Leica® MC 120 HD), or low intensity microscopy (Leica® DM750 microscope). For this last step, it is again convenient to have the stove with a built-in vacuum chamber, which will allow the final transparency of the samples, together with the elimination of the bubbles that could be left over the slices due to their inclusion in the epoxy resin and catalyst mixture.

DISCUSSION

Some considerations in relation to the microplastination process. As stated by Sora *et al*. (2004), the viscosity of epoxy resin varies with temperature variations. At temperatures below 5 °C, the epoxy resin becomes viscous, but the impregnation time is prolonged, and can be done in two days. However, when impregnated with the epoxy resin, mixed with a special catalyst (Biodur E6) and an accelerator (Biodur E600), and combined with high temperatures (30-60 ºC), the viscosity of the mixture is reduced, allowing this way its arrival to the depths of the anatomical block in question (Ottone *et al*., 2018a). After approximately two days of impregnation at 60 ºC, the resin begins to become increasingly viscous, reaching at lower temperatures, at the end of the impregnation process, the final polymerization and hardening of the block, which may be subsequently subjected to a low speed band saw with diamond blade. The use of the E600 accelerator is essential to achieve polymerization of the block at 60 ºC (Sora *et al*., 2004).

In relation to the transparency of the slices obtained, this starts once the cuts are placed on the resin and catalyst mixture, and then it will be essential to place the cuts (in the flat chamber) in an oven with built-in vacuum chamber, since that in this way polymerization can be achieved with the removal of small bubbles that cannot be extracted with the simple distribution of the resin / catalyst mixture before closing the flat chamber.

CONCLUSION

In this way, we establish the concept of microplastination, with the aim of bringing together under this denomination the techniques of ultra-thin sheet plastination with epoxy resin applied to experimental protocols of morphology and pathology, and through which we seek to obtain ultra-thin slices less than 0.25 mm / 250 µm thick.

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RESUMEN: La plastinación ha revolucionado el estudio y la investigación de la anatomía, gracias a la conservación biosegura y por tiempo indefinido de cadáveres y órganos humanos y animales. En este trabajo se presenta el concepto de Micro-Plastinación, técnica de plastinación de cortes ultrafinos para la obtención de cortes ultradelgados, de un grosor inferior a los 250 µm, para la identificación y visualización de la microanatomía de cualquier región anatómica en protocolos de morfología experimental.

PALABRAS CLAVE: Micro-plastinación; Cortes ultradelgados; Morfología experimental; Patología experimental.

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