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First Experience of Plastination at 4150 Meters Above Sea Level, at the Altitude of La Paz, Bolivia

Primera Experiencia de Plastinación a 4150 Metros sobre el Nivel del Mar, en la Altura de La Paz, Bolivia

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SUMMARY: Despite attempts to develop the plastination technique in Bolivia, standardized results have not yet been achieved that could be communicated via scientific publications. There is a great deal of misunderstanding around the technique, confusing it with classic techniques of inclusion in different types of resin, such as polyester and epoxy, but these protocols are not plastination. The aim of this work was to communicate the first standardized room-temperature plastination protocol with silicone in Bolivia, with the unique feature of doing so at the altitude of the city of La Paz, thus constituting the first communication of a plastination technique at 4,150 m.a.s.l. sub sede La Paz, La Paz, Bolivia.

KEY WORDS: Plastination; Room temperature; Silicone; Pigmentation; Altitude; Forearm; Hand.

INTRODUCTION

The attempts undertaken worldwide to improve cadaver preservation and conservation were revolutionized with the appearance of plastination, a technique invented by professor Gunther von Hagens in Heidelberg, Germany, in 1977 (von Hagens, 1979; Ottone, 2013; Ottone *et al*., 2016, 2018 a,b; Toaquiza *et al*., 2023). Since its creation, plastination has been applied and innovated in different universities and research centers around the world dedicated to anatomical and morphological study and research. In Bolivia, there are no records of the publication of scientific works applying Gunther von Hagens' plastination protocol.

In 2015, in the Journal of Plastination, Prof. Baptista wrote in the section "Letter from the President" (Baptista, 2015), in which he communicated the existing plastination laboratories in South America up to that time, as there were no plastination laboratories in Bolivia.

In July 2022, Bolivia participated for the first time, in an event of the International Society for Plastination: "20th International Conference on Plastination - 4th International Congress on Anatomical Techniques", which took place online, organized for the first time in South America by the Universidad de La Frontera (UFRO), and where a preliminary communication of this work was presented and in full in this scientific paper (Rodriguez-Torrez & Ottone, 2022). Thus, the first experience of the application of a standardized plastination protocol in La Paz, Bolivia, was presented at a scientific event.

This work presents our experience in the application of room-temperature plastination with silicone modified by Ottone *et al*. (2015), which was adapted to our city, and in this way, we propose the feasibility of its development, emphasizing that this is the first communication of a plastination study, where we describe in detail this modified protocol of room-temperature plastination with silicone at the altitude of La Paz, Bolivia, 4150 meters above sea level (m.a.s.l.).

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MATERIAL AND METHOD

The sample consisted of a human forearm and hand from the Anatomy and Neuroanatomy Theater of the Universidad Privada Del Valle, La Paz campus, weighing approximately 380 grams.

This sample, with the cadaver as a whole, was previously fixed in 10% formaldehyde and submerged for two months in the same preservation solution to obtain its complete embalming prior to the separation of the sample used in this study from the rest of the cadaver. Once separated, an angio-technique was applied to the sample prior to dissection, an anatomical technique that consists, in this case, of injecting pigmented ammonia-based monocomponent semifluid latex arterially and venously. Before its application, the latex was dyed with red and blue resin pigments to identify and differentiate the blood vessels.

Then, the room-temperature plastination technique was applied to the forearm and hand sample, following the protocol modified by Ottone *et al*. (2015), and adapting it to the sample used in this work.

Washing. The sample was submerged in running water for one week, with daily renewal of the water, to eliminate the fixation fluids (mainly formaldehyde) used for its initial preservation.

Dehydration. This stage was meant to replace the formaldehyde with an intermediary and volatile solvent, miscible in water, corresponding to acetone. This intermediary solvent will then be extracted during the forced impregnation stage, allowing the entry of silicone into the sample.

In this work, five consecutive acetone changes occurred during the dehydration phase, using nail polish remover in the first dehydration bath, whereas in the four following changes, 100% acetone was used. Each acetone bath lasted seven days, with the following sequence of acetone changes and post-stabilization monitoring to achieve a final purity equal to or greater than 99.5% acetone. Next, the process is detailed:

- 1st acetone change (nail polish remover) post-stabilization: acetonometer indicated 49% acetone purity (Fig. 1).

- 2ne acetone change post-stabilization: acetonometer marked 89% acetone purity (Fig. 2).
- 3rd acetone change post-stabilization: acetonometer marked 96% acetone purity.
- 4th acetone change post-stabilization: acetonometer marked 97% acetone purity (Fig. 3A).
- 5th acetone change post-stabilization: acetonometer marked 99.5% acetone purity (Fig. 3B).

Forced impregnation. In this phase, the most important in the plastination technique, the acetone (intermediary and volatile solvent) is replaced by the impregnation mixture (silicone/catalyst). For this, the sample must be submerged in silicone/catalyst and placed in a vacuum chamber

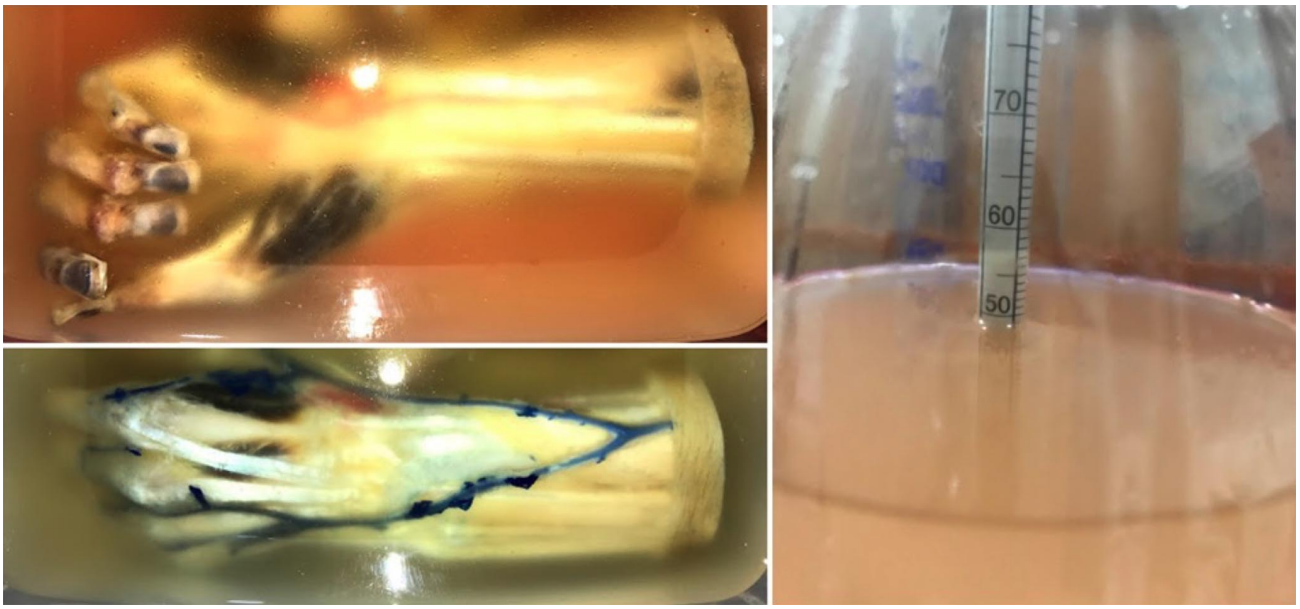

Fig. 1. Dehydration. First refill of acetone (nail polish remover) after the acetometer gave us 49% acetone purity.

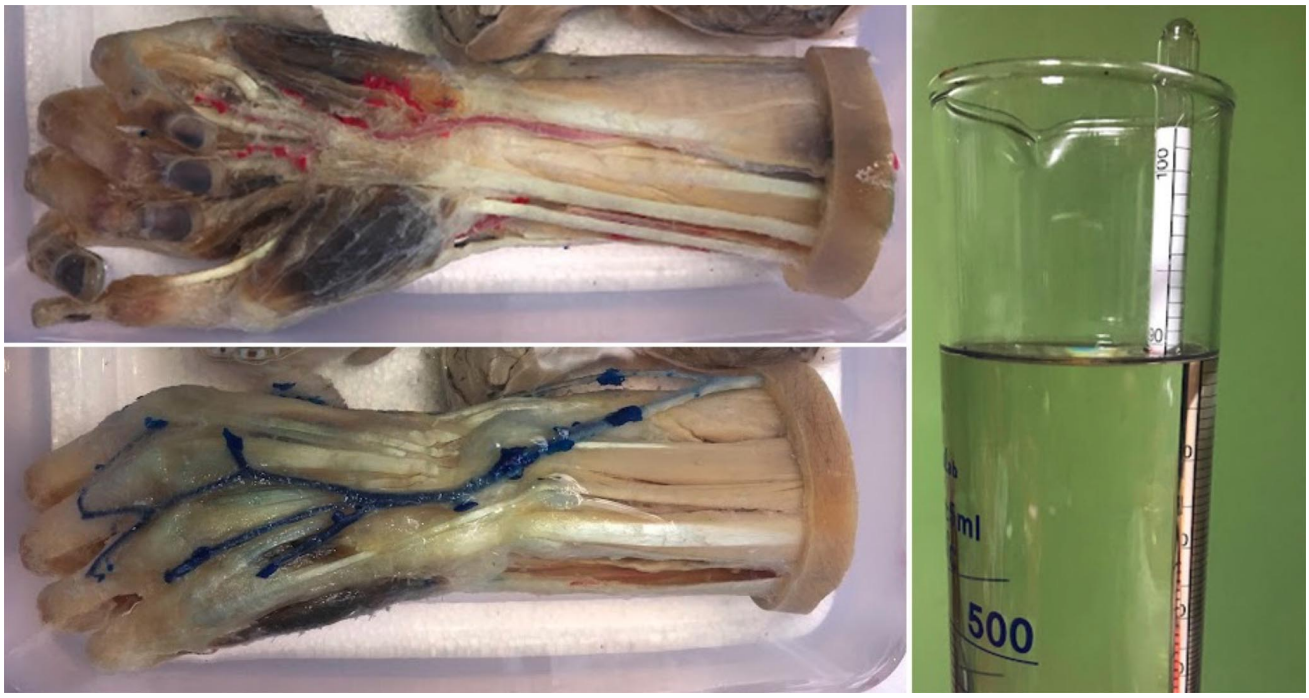

Fig. 2. Dehydration. Second replacement of acetone post stabilization, the acetometer marked 89% purity.

Fig. 3. Dehydration. A. Fourth replacement of acetone after stabilization, the acetometer marked 97% purity. B. Fifth replacement of acetone after stabilization, the acetometer marked 99.5% purity.

connected to a vacuum pump that generates a vacuum inside the chamber, ensuring the development of the forced impregnation stage. Thus, in this step, generating a vacuum, the acetone is extracted from the cell structure and the sample space thanks to its change of state, liquid to gas (which occurs between 180-185 mmHg), and ensuring the entry of the silicone/catalyst mixture (impregnation polymer) to permit impregnation. This process is developed thanks to the increased vapor pressure of the acetone in comparison with the low vapor pressure of the silicone/catalyst mixture; hence, being so volatile, the acetone is removed by the action of the vacuum pump, using two stages: "active" and "passive" according to the modified room-temperature technique proposed by Ottone *et al.* (2015). The pressure difference produced causes the polymer to enter the sample.

The sample was submerged in a mixture of silicone and catalyst (polydimethylsiloxane and dibutyltin dilaurate, in a 100:1 ratio) in a plastic container inside the vacuum chamber at room temperature. The classic description (von Hagens, 1979, 1986; von Hagens *et al*., 1987; Ottone *et al*., 2015) indicates that the pressure is reduced from 760 mmHg (atmospheric pressure at sea level) to 20 mmHg. In the case of the work by Ottone *et al*. (2015), this reduction in pressure was achieved in 5 days, applying active/passive forced impregnation periods (Ottone *et al*., 2015).

Acetone extraction is visualized as bubbling, representing the acetone vapor extracted from inside the sample. Once the final impregnation pressure is reached (10-20 mmHg) and there are no bubbles (acetone vapor), the forced impregnation stage is complete, understanding that all the acetone has been extracted and replaced by the silicone/catalyst mixture.

However, we must emphasize that in this study, the forced impregnation phase began at 462 mmHg, the barometric pressure of La Paz, Bolivia (4150 m.a.s.l.). Thus, in only 3 days, a pressure of 20 mmHg was reached with the application of active and passive forced impregnation, where the characteristics of the bubbling appeared throughout this phase (Fig. 4). **Table I** details the forced impregnation phase.

Drying. With the forced impregnation stage complete, the sample was drained with a paper towel to favor the extraction of the excess silicone and thus prepare the sample for the nextpigmentation stage.

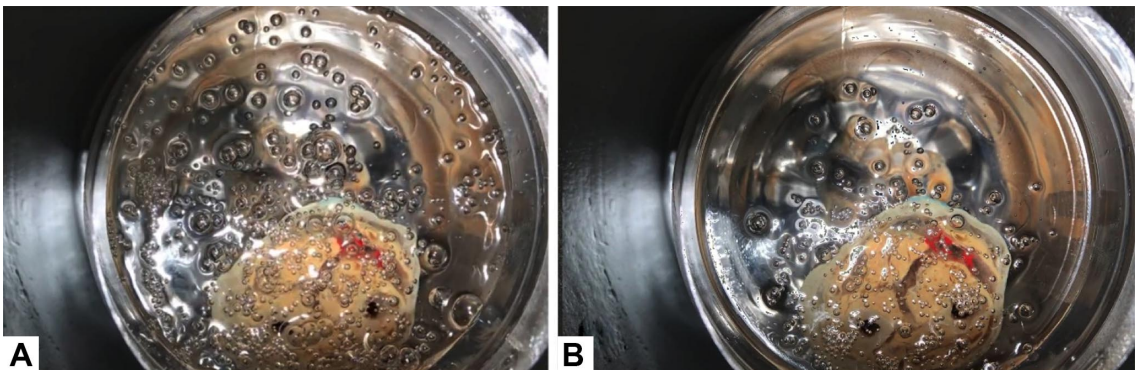

Fig. 4. Forced impregnation at room temperature. A. Active bubbling. B. Slow bubbling.

Fig. 5. Surface treatment through the use of silicone pigments from the Smooth-On, Inc. line. Applied by dilution in

Table I. In this three-day process, active and passive forced impregnation periods were combined, with varying periods of time in the day, while ensuring the integrity of the structures. The pressure inside the vacuum chamber was gradually increased from 462 mmHg to 20 mmHg. Once the bubbling disappeared, it indicated that the forced impregnation process had ended.

Días	Forced Impregnation	Pressure	Bubbling characteristics
1st day	Active 2 hours Pasive 22 hours	462 mmHg to 260 mmHg 260 mmHg	Active bubbling Slow bubbling
2nd day	Active 4 hours Pasive 20 hours	260 mmHg to 60 mmHg 60 mmHg	Slow bubbling Little bubbling
3rd day	Active 4 hours Pasive 8 hours	60 mmHg to 20 mmHg 20 mmHg	Slow bubbling Little bubbling

Pigmentation. The dry sample underwent a surface treatment using pigments for silicone from the Smooth-On, Inc. product line. The same was applied using dilution in solvents, an airbrush, and sable-hair brushes to define the fine details (Fig. 5).

Curing or polymerization. Once the pigmentation was finished, the sample was placed in a sealed chamber and subjected to a chain extension fluid, tetraethyl orthosilicate (TEOS or S6), which is vaporized on the sample to accelerate the polymerization of the silicone/catalyst mixture. This TEOS allows the "side to side" connection of the silicone molecules, contributing to the drying of the preparation. Once the curing is done, the sample is dry and nontoxic.

To achieve vaporization, the sample is introduced in the curing chamber, where the TEOS is placed in a container, and using an air pump, the TEOS bubbles and gasifies, accelerating the superficial polymerization of the sample to obtain superficial drying of the sample (Fig. 6). There are two stages of curing: the fast or superficial curing, done in 4 to 5 days, subjecting the sample to TEOS vapor. This achieves the superficial drying of the sample. On the other hand, slow curing is the process that occurs within 3 or 4 months from the completion of the rapid curing process, characterized as a process of prolonged and internal polymerization, ensuring the completion of the process inside the sample. The sample must remain in a sealed plastic bag to achieve final polymerization.

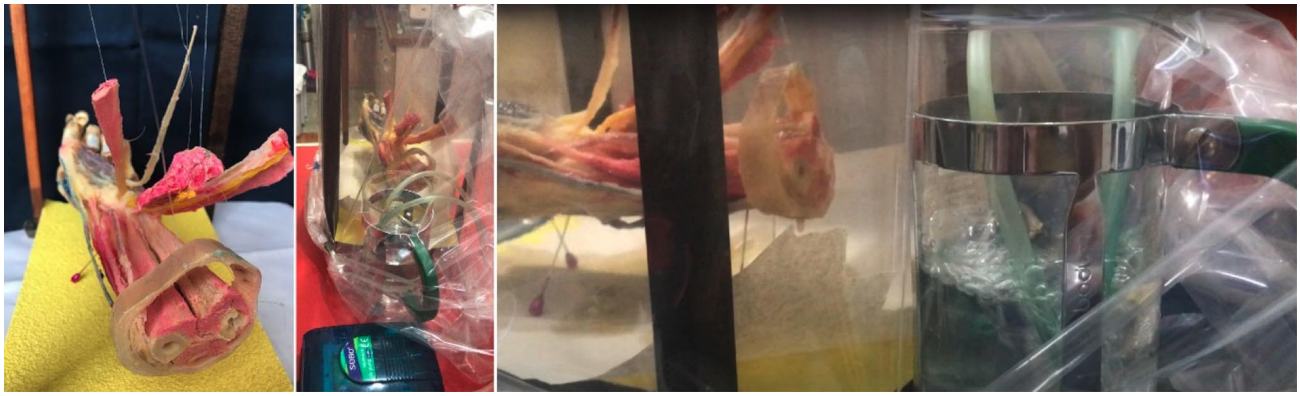

Fig. 6. Gasification of the TEOS, placed inside a container and through the use of an aquarium oxygenator aerator motor, it is bubbled and vaporized, to accelerate the superficial polymerization of the sample, achieving the final hardening and drying of the anatomical preparation.

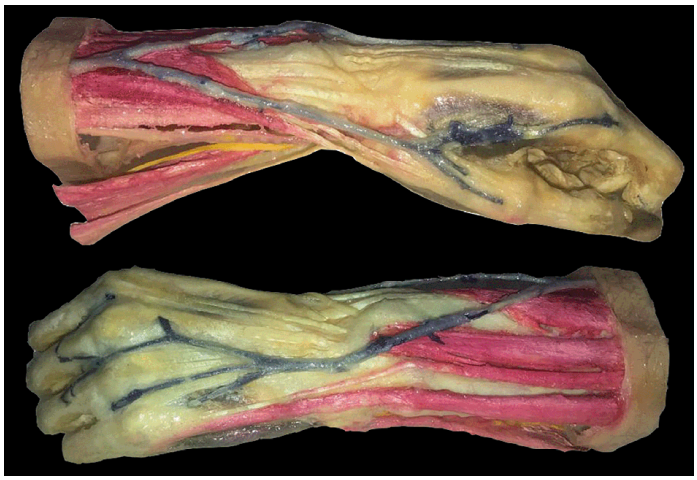

Fig. 7. Final result of the platination process, with subsequent pigmentation, developed in the heights of La Paz, Bolivia.

RESULTS AND DISCUSSION

The classic cold plastination technique with silicone (S10) developed by Prof. Gunther von Hagens (von Hagens, 1979; von Hagens *et al*., 1986, 1987) makes it possible to produce resistant and opaque samples of differing hardness (from rigid to flexible). After the dehydration with acetone, the samples are impregnated at -25 ºC in a vacuum chamber, submerged in a mixture of silicone (S10) and catalyst (called S3) (100:1, respectively), that have a low vapor pressure (high boiling point). A vacuum pump constantly removes the volatile intermediary (acetone) found in the specimen. Once the acetone is removed, a pressure difference will determine that the polymer enters the specimen. Forced impregnation must be carried out slowly as the polymer enters the specimen, where the acetone changes from a liquid to a gaseous state and is removed. The impregnation speed is carefully adjusted by the controlled addition of air inside the vacuum pump using a bypass valve. The duration of the forced impregnation stage will depend mainly on the size (and amount) of the specimen, the density of the tissue, and the viscosity of the polymer used. During this period, the vacuum must be intensified to a pressure of 760 mmHg, according to the desired formation of bubbles (intermediate), at a pressure of approximately 10 mmHg, where the tiny bubbles will

rise to the surface (bubbles indicate the acetone is exiting the interior of the specimen).

On the other hand, Zheng *et al*. (1998), published the basic foundations for the development of the room-temperature plastination technique in the journal of the International Society for Plastination, i.e., using vacuum chambers for forced impregnation without a freezer at 20 ºC. This was implemented initially by Roy Glover in the United States, seeking an alternative method to the plastination technique created by Gunther von Hagens. Moreover, in 1998, the abstracts of the 9th International Plastination were published in the journal of the International Society for Plastination, including the work by Roy Glover (Glover *et al*., 1998). The technique consists of combining the components differently: in the forced impregnation, they mixed the silicone (S10 from von Hagens) with the curing agent (S6 from von Hagens), and as a curing agent, they used the catalyst (S3 from von Hagens), but they did not gas it on the preparation, but rather the preparation was sprayed or brushed with the S3.

In this sense, in 2014, Ottone *et al*. (2014) implemented a room-temperature plastination technique with silicone, but with substantial modifications in the forced impregnation process and in the way the polymer, catalyst, and curing agent were used. This technique was applied on laboratory rats, indicating the need for the plastination technique to use such specimens dedicated to the practice of surgical approach techniques and the possibility of respecting the 3 Rs, with the reduction in animal use for this type of training, ensuring ethicalhandling of the animals (Ottone *et al*., 2014). Later, Ottone *et al*. (2015) published the contributions of this new room-temperature plastination technique, which consisted of modifying the forced impregnation, combining active and passive periods related to turning the vacuum pump on and off, respectively, throughout the forced impregnation process. In addition, the silicone, catalyst, and curing agent were combined in the same way as the cold plastination technique (von Hagens, 1979) but performing the forced impregnation at room temperature.

In relation to our study, we must first emphasize that it is the first study to describe the development of a plastination technique at an altitude of 4150 m.a.s.l., thereby beginning the forced impregnation process at a pressure of 462 mmHg and reaching the final 20 mmHg in only 3 days due to the development of the modified room-temperature plastination protocol with silicone (Ottone *et al*., 2015). This way, the reduction in impregnation time represented an advantage due to the reduction in oxygenation at 4150 m.a.s.l.

In relation to the sample, in particular, a piece was obtained that could show its internal anatomical makeup, which improved its appearance and presentation using pigmentation. The modified plastination protocol proposed by Ottone *et al*. (2015), was highly suited to the final preservation, enabling its correct adaptation to the elevation (4,150 m.a.s.l.) of La Paz, Bolivia.

It is important to emphasize that our protocol made three modifications to the previously suggested plastination technique (Ottone *et al*., 2014, 2015). The first modification was developed during the dehydration stage, in which an intermediary dissolvent known as “nail polish remover” was used as the first dehydration bath. This was due to the difficulty in obtaining pure acetone locally, and this component, free to purchase, was used, but it contained acetone, ethyl acetate, ethanol, water, and glycerin.

The second modification was associated with the geographic characteristics of the city where our laboratory is located, corresponding then to the beginning of the forced impregnation phase, where the atmospheric pressure of La Paz is 462 mm Hg, and this made it possible to reach the final 20 mmHg in only 3 days. This factor determined a reduction in the forced impregnation time, expediting this protocol.

Finally, the third modification occurred at the curing or polymerization stage, where pigments were used to give suitable color to the plastinated sample. This way, Smooth-On Inc. brand silicone-based pigments were used, which were first diluted with solvents (xylene) and then applied on the sample using an airbrush, and they were also applied with brushes to color the fine details and thus gave the plastinated sample a more realistic appearance.

CONCLUSION

The development of the plastination technique proposed in this study yielded an odorless, durable, biosafe human sample that does not require a great deal of maintenance and does not deteriorate over time (von Hagens, 1979; Bickley *et al*., 1981; Ottone, 2013; Ottone *et al*., 2014, 2015; Ottone *et al*., 2018b; Toaquiza *et al*., 2023). Hence, specimens can be handled without needing primary biosafety measures such as gloves. This technique solves the problem of formalin, its need for maintenance, and the toxicity that its use entails (Ottone *et al*., 2015, 2018c). In addition, the impregnation times were remarkably reduced, favored by the altitude of La Paz, achieving results similar to the previously suggested original techniques by von Hagens (1979) and Ottone *et al*. (2015). The development of novel techniques for the preservation of the human body is fundamental, since, with these types of plastinated samples, the visual impact of death on first-year

students is less shocking and friendlier (von Hagens *et al*., 1987; Cook, 1997; Ottone *et al*., 2014, 2018b,c). Finally, we draw the conclusion that the findings of this study enable the preservation of specimens in their true state for inspection, analysis, and comparative anatomical study, with all the benefits that the plastination technique offers, providing a substitute for the drawbacks associated with the use of formaldehyde as a traditional preservation method.

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RODRÍGUEZ-TORREZ, V. H. & OTTONE, N. E. Primera experiencia de plastinación a 4150 metros sobre el nivel del mar, en la altura de La Paz, Bolivia. *Int. J. Morphol., 41(6)*:1603-1609, 2023.

RESUMEN: En Bolivia, a pesar de los intentos en el desarrollo de la técnica de Plastinación, aún no se han alcanzado resultados estandarizados que pudieran ser comunicados por medio de publicaciones científicas. Existe una gran confusión al momento de desarrollar la técnica, confundiéndola con técnicas clásicas de inclusión en distintos tipos de reina, como poliéster y epoxy, pero no correspondiendo estos protocolos desarrollados a la técnica de plastinación. En este sentido, el objetivo de esta trabajo consistió en comunicar el primer protocolo estandarizado de plastinación a temperatura ambiente con silicona de Bolivia, con la particularidad de desarrollarlo en la altura de la ciudad de La Paz, constituyéndose, de esta manera, en la primera comunicación de una técnica de plastinación a 4.150 metros sobre el nivel del mar.

PALABRAS CLAVE: Plastinación; Temperatura ambiente; Silicona; Pigmentación; Altura; Antebrazo; Mano.

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