seven days, since when the body and head blocks started to unfreeze, it was necessary to interrupt and repeat the freezing procedure for another 24 hours in order to maintain the quality and integrity of the slices cut. During the step, the cuts already made were stored in the freezer (-25º) inside a plastic container in the order of slicing. At the time of slicing the left leg, it was suspected that it had an intramedullary metallic rod in the tibia, since there was resistance to cut. This hypothesis was later confirmed with x-ray examination and, therefore, this segment could not be sliced, and a dissection preparation was performed to show the metal rod. To amplify the use and the anatomical content of the slices, the hands and feet were cut in different planes, without prior PU packing. One hand was cut in half in the frontal plane (coronal) and the other in the middle of the fingers in the sagittal plane. On the feet, one was cut in the transverse plane, following the body, and the other in the sagittal plane, having as reference the middle of the toes.

Figure 3. Band saw process of slicing the frozen body embedded in polyurethane for plastination.

Once the slicing step was completed, all slices were labeled with a numerical identification to locate the position of the slices. In addition to the number, the upper and lower limbs were identified by the letters D or E for right or left (direita and esquerda, in Portuguese), respectively. After identified, the cuts were sequentially and carefully washed in cooled acetone (-25°C) with the aid of a brush to remove ice, fragments and dirt from the slicing (Figure 4). Then they proceeded to the plastination process.

The plastination technique was basically performed according to the protocol proposed by von Hagens, Tiedemann and Kriz (1987), divided into 4 main stages: fixation, dehydration, forced impregnation and chemical cure/catalysis. Fixation had already been performed previously using a 10% formalin solution for tissue stabilization. Immediately after cleaning the cuts, as already described, the slices were placed in the vertical position in a plastic basket, separated from each other by perforated plastic screens to facilitate handling between the plastination steps, and then, the dehydration at low temperature (-25ºC), being carried out with 4 baths per week and immersed in acetone at concentrations 95, 95, 100 and 100% volume/volume (v/v), consecutively, inside a freezer.

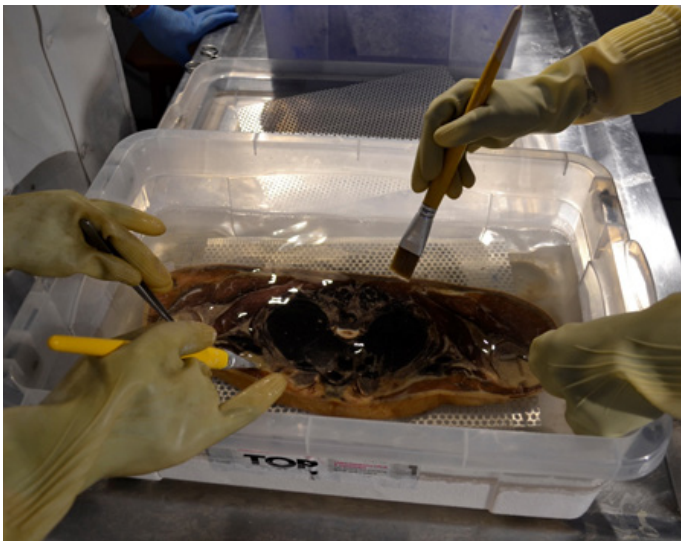

Figure 4. Washing process in cooled acetone (-25ºC) of the slices of the male human body destined for plastination.

The vertical accommodation of the slices separated by plastic screens aims to increase the contact surfaces of biological tissues in the dehydration and impregnation stages. Concomitantly with the first weekly dehydration bath, hydrogen peroxide was added to the cooled acetone to whitening the slices, with the final concentration of peroxide being 10 volumes (3% v/v). The bleaching was carried out at the same time as dehydration to avoid thawing of the cuts and possible loss or displacement of small structures. At the end of each bath, the purity of the acetone was checked by an acetonometer, considering that the dehydration step with acetone was concluded, having reached a purity greater than 99% v/v. Since then on, the cuts inside the baskets were immersed in the cold impregnation reactive mixture (-18°C), composed by a silicone PDMS (polydimethylsiloxanes) and the catalyst Dibutyltin dilaurate (DBTL), in the proportion of 100:1 mass/mass (m/m), respectively, already inside the vacuum chamber for 24 hours. After this period, the vacuum was applied slowly and progressively, having as a vacuum adjustment parameter the pattern of bubbles with one bubble/second at the same observation point (JONG; HENRY, 2007). Vacuum progression was measured with a digital and mercury manometer. When the appearance of bubbles on the silicone surface ceased and the maximum vacuum was reached by the pump, the step was considered complete. Thereby, the forced impregnation step lasted 26 days, reaching a minimum pressure (maximum vacuum) of 8 mmHg. Then, the slices were placed for drainage of excess silicone suspended in the chamber itself for 3 days (Figure 5)

The plastination of the sectioned body promoted an innovation not only for Brazil, but also for Latin America, in the context of anatomy teaching and in the area of museums. The pioneering spirit of the Museum of Life Sciences of the Federal University of Espirito Santo with the production of a specimen of high complexity and quality took place with a protocol that sought adaptations in order to make the processes less expensive and to make their reproduction more accessible for laboratories of smaller size, aiming at the diffusion of plastination technology and the improvement of teaching tools.

Conclusion

The plastination protocol used in this work show us that is possible to produce high-quality plastination of the slices with a modest cost and infrastructure.

The present work showed that the production of a sliced specimen can bring great benefits in teaching, research, and especially in biological sciences area as anatomy, pathology and health care degrees.

With plastination technology, the cuts of the body are more resistant, dry, odorless, easy to store and safer for the handlers, and, therefore, extremely advantageous for use in the activity of academic and museal education.

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