

## **Comparative Study of Temperatures Used in Silicone Impregnation of Porcine Hearts Plastination**

**Damri Darawiroj\* Adisorn Adirekthaworn Sayamon Srisuwattanasakul  
Kongkiat Srisuwattanasakul**

### ***Abstract***

Forced impregnation process is normally performed under vacuum at freezing temperature which requires expensive plastination apparatus. To reduce the cost in the plastination process, forced impregnation of porcine hearts under two different environments, which are at the freezing temperature (-20°C) and at room temperature (22-25°C), was compared. Twelve porcine hearts were collected from a slaughter house, and divided into the groups, six in each, and subjected to be plastinated by S10 standard technique at the two temperatures. Although the forced impregnation at room temperature, also the curing process, was more time-consuming, all hearts from both groups produced satisfying plastinated specimens. The gross structures, rigidity and color of the plastinated hearts from both groups were examined. The surface and internal structures of the heart, after dissection, were kept in shape and no differences were observed between the two groups. The color of the external and internal structures was brown. Moreover, no significant color difference was detected between the specimens although the natural color of the heart before plastination is more grayish. All plastinated hearts were rigid and slightly flexible. In conclusion, although forced impregnation at room temperature required more processing time than forced impregnation at freezing temperature, both could still produce an equal quality at plastinated heart specimens.

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**Keywords:** Heart, pig, plastination, temperature

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*Department of Anatomy, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok, Thailand 10330*  
*Corresponding author E-mail: Damri.D@chula.ac.th*

## บทคัดย่อ

### การศึกษาเปรียบเทียบ อิทธิพลของอุณหภูมิต่อลักษณะของหัวใจสุกร ในกระบวนการกำซาบพลาสติก

ดำริ ดาราวีโรจน์ อติสร อติเรกถาวร ศยามน ศรีสุวรรณาสกุล ก้องเกียรติ ศรีสุวรรณาสกุล

ตามปกติ ขั้นตอนการกำซาบสารพลาสติกเข้าสู่อวัยวะ (Forced impregnation) ของกระบวนการ Plastination จะต้องทำในสภาวะสุญญากาศที่อุณหภูมิต่ำ ซึ่งต้องใช้อุปกรณ์ที่ราคาสูง ดังนั้นเพื่อที่จะลดต้นทุนในการผลิต จึงได้ศึกษาเปรียบเทียบผลของการกำซาบสารพลาสติกเข้าสู่อวัยวะ ที่อุณหภูมิต่ำ ( $-20^{\circ}\text{C}$ .) และ ที่อุณหภูมิห้อง ( $22-25^{\circ}\text{C}$ .) โดยใช้หัวใจสุกรทั้งหมดจำนวน 12 ดวง แบ่งเป็น 2 กลุ่ม และนำมาทำการกำซาบด้วยสารซิลิโคน ด้วยวิธีมาตรฐาน (S10 standard technique) โดยกลุ่มแรกทำการกำซาบสารพลาสติกเข้าสู่เนื้อเยื่อหัวใจที่อุณหภูมิต่ำ และ กลุ่มที่สองทำที่อุณหภูมิห้อง จากการศึกษาพบว่าที่อุณหภูมิห้อง ขั้นตอนการกำซาบสารพลาสติก และการทำให้พลาสติกแข็งตัว ใช้เวลานานกว่าที่ อุณหภูมิต่ำ ลักษณะของตัวอย่างที่ได้จากทั้งสองกลุ่มมีลักษณะเหมือนธรรมชาติ แท่ง ไม่มีกลิ่น และมีความแข็ง ความยืดหยุ่น ไม่แตกต่างกัน โครงสร้างของหัวใจทั้งภายในและภายนอกยังคงรูปร่างปกติ ไม่มีการเหี่ยวของเนื้อเยื่อ สีของหัวใจทั้งสองกลุ่มเปลี่ยนจากสีเทาชมพู เป็นสีน้ำตาลอ่อนเหมือนกัน อย่างไรก็ตามจากการศึกษานี้สรุปได้ว่า การกำซาบสารพลาสติกสามารถทำได้ทั้งที่อุณหภูมิต่ำ และที่อุณหภูมิห้อง โดยคุณภาพของตัวอย่างที่ได้ไม่แตกต่างกัน

**คำสำคัญ:** หัวใจ สุกร การกำซาบสารพลาสติก อุณหภูมิ

ภาควิชากายวิภาคศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330

\*ผู้รับผิดชอบบทความ E-mail: Damri.D@chula.ac.th

## Introduction

Traditional anatomy education using cadavers was replaced by a multiple range of special study tool such as 3-dimensional models generated by computer or plastination techniques introduced by Gunther von Hagens in 1978 (von Hagens, 1979). The plastinated model made from real tissue is a valuable procedure for avoiding the hazardous agents used in conventional specimen preparation. Several plastination protocols and techniques have been employed to obtain better-quality outcome for different organs (von Hagens et al., 1987; Holladay, 1988). Fixatives, dehydration agents and different kinds of polymers have been examined and chosen for particular tissues (Bickley et al., 1987). During the process of silicone impregnation, specimens are kept under vacuum at freezing temperatures for a long period. Therefore, expensive vacuum apparatus is required for this stage. This study aims at facilitating the procedure and reducing the cost of plastination by optimizing the temperature during the process of forced impregnation using porcine hearts as a model and comparing the quality of plastinated tissue at different temperatures.

## Materials and Methods

Each of the twelve hearts obtained from slaughtered pigs was weighed and immersed in 10% formalin for 3 days. Specimens were then rinsed with

running water overnight. To expose the coronary arteries and their branches, fat and connective tissue around the coronary groove and the external surface of all the hearts were carefully removed. For the plastination procedure, the S10 standard technique was employed with some modifications (Henry and Nel, 1993). All specimens were then dehydrated by immersing them in a graded acetone at  $-20^{\circ}\text{C}$ . The degree of tissue dehydration was measured by acetometer on a daily basis. Specimens were initially placed in pure acetone for 48 hours and then transferred to two consecutive pure acetone baths. The dehydration process was ceased when the concentration of acetone was stable at 99%. Six specimens were then subjected to silicone impregnation in mixture of S10 and S3 (99:1 by volume) at room temperature ( $22-25^{\circ}\text{C}$ ), whereas the other six hearts were immersed in silicone and kept at  $-20^{\circ}\text{C}$ . Both groups were impregnated under vacuum until no acetone bubbles could be observed. After forced impregnation and the removal of excess silicone, all specimens were placed in a plastic-enclosed curing chamber containing the cross-linking curing agent (S6) and  $\text{KCl}_2$  to harden the infiltrated polymer. The curing of the specimens was completed following the absence of excess polymer from the specimens.

## Results and Discussion

All hearts from both freezing and room temperature groups produced satisfying specimens.



**Figure 1.** Plastinated porcine hearts produced by forced impregnation at freezing temperature ( $-20^{\circ}\text{C}$ ) (left) and at room temperature ( $22-25^{\circ}\text{C}$ ) (right); (A) aorta, (B) right atrium and (C) right ventricle.

They looked natural, were odorless, were durable and maintain their original shape. The average weights of the hearts were 458 grams and reduced approximately 47% after plastination (215 g). We concluded that the forced impregnation process of plastination performed at room temperature resulted in quality products comparable to the conventional protocol. The forced impregnation at room temperature was completed in 4 weeks, whereas the forced impregnation under  $-20^{\circ}\text{C}$  took only 3 weeks. The length of time for the forced impregnation at room temperature increased because the polymerization of the polymer mixture was slower than that at the freezing temperature (Henry and Nel, 1993). In addition, the curing process was extended from 3 weeks to 4 weeks for the plastinated hearts kept at room temperature. After the plastination, the gross morphologies, rigidity and color were examined. The surface structures of the two groups were not different. Of the plastinated hearts kept at room temperature, however, a very small amount of silicone still seeped out of the specimens and produced a degree of grease but no shrinkage was observed. Normally, tissue shrinkage is introduced by the different boiling temperatures of the intermediary solvent (acetone) and polymer (Henry and Nel, 1993). The incompatible temperature caused acetone to be extracted from the tissue too quickly and the polymer mixture was unable to be propelled into the tissue. Using ethanol as a dehydration agent also caused shrinkage at room temperature (Grondin, 1998). No shrinkage observed in some heart specimens points out that under room temperature, forced impregnation can be successful. The rigidity and flexibility of all specimens in both groups were not different. Nevertheless, compared to the original tissue, they were more rigid and less elastic. The external surface and the internal structures of the plastinated heart were kept in shape without any differences between the two groups. Although the

arteries and veins of all the plastinated porcine hearts were clearly seen and undamaged, the aorta was considerably harder and less flexible than other vessels. The natural color of the hearts before plastination was gray, whereas the color of the external and internal structures of the plastinated hearts was slightly changed to grayish brown. However, no significant color difference between each specimen was detected.

Generally, hollow organs, such as the stomach, the uterus and the heart, have to be dilated prior to fixation in order to assure a proper shape and a luminal size (Holladay, 1989; Henry et al., 1997). Many protocols of heart dilation have been reported (Baptista and Conran, 1989; Henry et al., 1997). In this experiment, however, none of the hearts were dilated because the specimens were damaged during initial specimen collection. Fixation of the heart should be done by introducing the fixative under pressure through one of the great vessels while the organ is suspended in a fixative bath (Bickley et al., 1987). Graded formalin (3 to 6 %) has also been used to preserve specimens before dehydration (Ripani et al, 1994). In our study, the hearts were immersed in 10% formalin for a few days without preparation of the features of the organs. The internal structures of the hearts were surgically demonstrated after the completion of the curing step to avoid morphological damages during plastination. Because a better chromatic preservation was obtained by fixation at a lower temperature than that room temperature (Ripani et al., 1994), the specimens in our study were preserved at  $-20^{\circ}\text{C}$ . The dehydration time should be as short as possible to prevent brittleness and low degree of flexibility. In this study, the dehydration process lasted 3 weeks. We used acetone as a dehydrating agent and volatile intermediary solvent because it boils readily under vacuum and is easily recycled by distillation (Bickley et al., 1987).

The temperature of the polymer also has an effect on acetone extraction. Because the extraction of the solvent from specimens was slower in cold polymer than in the room temperature polymer (Henry and Nel, 1993), an increased vacuum pressure was needed. In our study, the vacuum pressure started at 80 cm of Hg and was maintained at zero for 60-90 minutes. A discontinuous vacuum was set to prevent specimen shrinkage due to overrated acetone extraction. After complete impregnation, the excess polymer was removed from the specimens and a gas curing process was performed to harden the silicone. In our study, the specimens were hung in a curing container to maintain the shape of the plastinated organs and a slow cure procedure was performed.

Consequently, we concluded that porcine plastinated hearts could be produced in equal quality by forced impregnation either at freezing temperature or at room temperature.

Although the duration of the conventional procedure was shorter, the application of room temperature impregnation might be an alternative method for saving costs in heart plastination.

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