Tissue Elimination of Large Vascular Corrosion Casting for Anatomy Education

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ABSTRACT

Objective: Vascular corrosion casting is crucial tool for three-dimensional study. Focusing on the casting for gross anatomy, large fatty tissue reacts with corrosive agents resulting in extensive saponification. Our study aimed to prevent saponification by a) finding the optimal corrosion temperature and concentration of corrosive agent and b) comparing the flow of the agent with conventional "non-flow" setting.

Materials and Methods: Phase I: pig fatty tissues, weighing 10 g each, were immersed in still (non-flowing) solution containing 0.5%, 1%, and 5% sodium hydroxide. Different temperatures were set to find the minimum soap-free temperature for each concentration. Phases II, III: 6 pig hearts were injected via the coronary arteries with polymethyl methacrylate. Three hearts were immersed in non-flowing 0.5%, 1%, and 5% NaOH solution, while another three were placed in a flowing solution. The flow was set in a vertical upward fashion in a specialized chamber while the outflow residue was collected from the system. The temperature was set at the minimum soap-free temperature. The durations of the corrosion were compared.

Results: The minimum soap-free temperatures for the 0.5%, 1%, and 5% concentrations were 55°C, 54°C, and 47°C, respectively. The corrosion times for the non-flowing 0.5%, 1%, and 5% concentrations were 216 h, 114 h, and 24 h, respectively. Flowing of the solution reduced the corrosion time by 25%-39% compared with the non-flowing.

Conclusion: The most efficient condition for soap-free coronary corrosion casting is 5% NaOH solution at a minimal temperature of 47°C.

Keywords: Coronary artery; Angiography; Saponification; Alkaline liquefaction; *Sus scrofa domesticus* (Siriraj Med J 2022; 74: 754-759)

INTRODUCTION

Cadaveric dissection is still the basis of anatomical education for medical students. Students' greatest challenge is understanding the complexity of the vascular system, which is hard to accomplish in a relatively limited time. To tackle this challenge, virtual 3D anatomy has been introduced to aid learning, but the lack of tactile feedback can leave kinesthetic learners behind.[1] On the other hand, the tangible model has shown better teaching outcomes than virtual dissection in the cardiovascular class.[2] This model can replicate or be derived from a real specimen (e.g., plastination or vascular corrosion casting).

Vascular corrosion casting has been utilized in vascular research for centuries.[3] However, since the 1960s, the research trend has shifted from the gross to the microscopic level in line with the rise of scanning electron microscopy (SEM). The casting technique has been developed accordingly to focus on the microscopic vessels of tiny specimens.[4-6] On the other hand, macroscopic cast production has been somewhat sidelined, with some

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http://dx.doi.org/10.33192/Smj.2022.89

technical difficulties remaining unresolved. Vascular corrosion casting consists of two steps: casting medium injection and the corrosion process. First, the medium is selected from certain polymers that have water-like fluidity in the monomeric form and rigidity in the polymeric form. Hence the medium is injected in the monomeric form and allowed to polymerize in the lumen of the blood vessel. The most popular medium is polymethyl methacrylate (pMMA). However, this polymer losses its strength at high temperature.[7] Therefore, unnecessary heat to the cast should be avoided during the whole process.

Corrosion, the second step, eliminates the surrounding tissue, typically by alkaline solution (e.g., NaOH and KOH).[4-6,8,9] Alkalis, once dissociated in water, produce hydroxide ions that react with proteins in a process called solubilization[10], which is also known as alkaline liquefaction[11] and alkaline hydrolysis.[12] Meanwhile, hydroxide ions also saponify the fat, producing an undesirable by-product as a solid soap residue.[4-6,8,9] A constant heat at 40–60°C is applied to the solution for two purposes: (a) to increase the soap solubility in water and (b) to accelerate protein solubilization. Despite the heat input, this process could take up to 7–8 days[5] and some residue (e.g., soap) is usually still nestled in the cast (Fig 1), requiring additional washing by running water.[5,6,8,9] To reduce the corrosion time and cut the washing process, the circulating laminar outflow chamber (CLOC) system was developed.[13] The CLOC system (Fig 2) consists of three parts connected in one circuit: (1) the heater, (2) the pump, and (3) the chambers. The former two generate a circulating flow of hot corrosive solution to wash the tissue and fat away. The latter part, the chambers, are composed of a specimen chamber and outflow chamber. The solution flows to the specimen chamber, then to the outflow chamber, and returns to the heat and pump unit. The wash-out residue is contained in the outflow chamber and prevented from re-circulation. A recent study[13] demonstrated the feasibility of the system for the mass production of casts. However, two questions remain: (a) what is the optimal temperature for the corrosion process? and (b) How much time efficiency does the CLOC system have for corrosion process compared to conventional corrosion process? This study approached the first question by attempting to find the "minimum soap-free temperature", defined as the lowest temperature of the corrosive solution where solid soap is not formed during the corrosion process. This temperature, on the other hand, would minimize the heat damage on pMMA cast, hence presumably an optimal temperature for the corrosion process. The second question was tackled by comparing the duration of the corrosion process (corrosion time) between the conventional, non-flowing solution and flowing solution in CLOC system.

MATERIALS AND METHODS

Pig hearts and fatty tissues were purchased from a butcher shop in Bangkok Noi District, Bangkok. The pigs were slaughtered for commercial purposes at a registered slaughterhouse in Nakhon Pathom. The study is appoved by Siriraj Laboratory Animal Research and Care Center, Faculty of Medicine Siriraj Hospital, Mahidol University (Si 008/2022). Pig fat was cut from the subcutis into

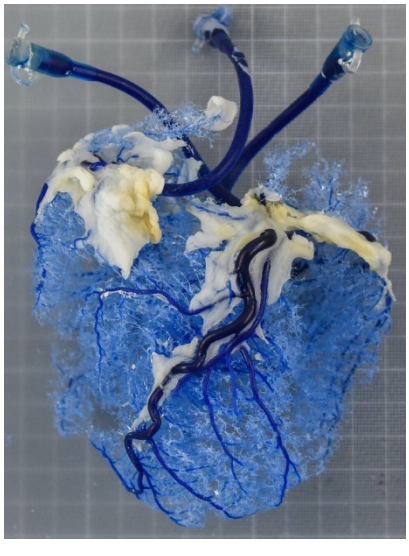

Fig 1. Photograph of a vascular corrosion cast that was processed via a corrosion step at too low a temperature. White solid soap residue is formed and can be seen attached to the cast.

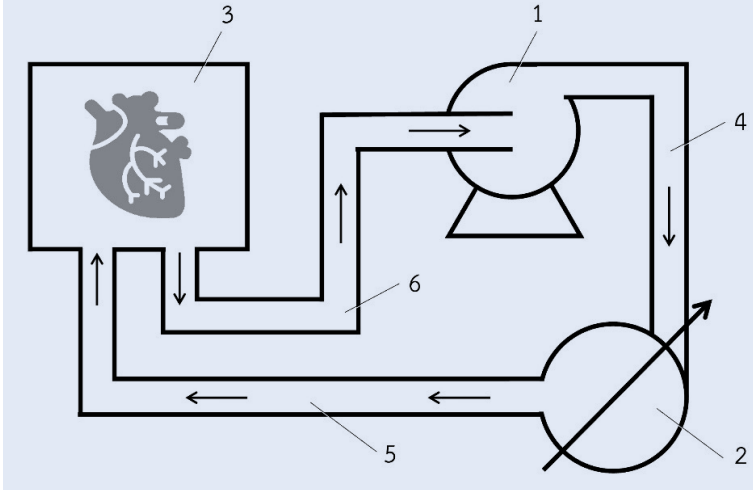

Fig 2. Diagram of the circulating laminar outflow chamber (CLOC) system. The system is composed of the pump (1), the heater (2) and the chamber (3) connected in a circuit (4, 5, 6). The arrows indicate the cyclic flow of the corrosive solution.

identical pieces weighing 10 g. On the other hand, the pig hearts were cannulated via the right and left coronary arteries. Then, 12 ml of polymethyl methacrylate (pMMA) (Ruthinium® group, Italy) was injected via the cannulae, 5 ml into right coronary artery and 7 ml into left coronary artery. The rate of injection was approximately 1 ml per second. The quality of the specimens, the technique, and the materials were all standardized. The specimens were allocated into three phases of experiments, as follow:

Phase I aimed to find the minimum soap-free temperatures; presumptive optimal temperature for corrosion process, at three concentrations of NaOH solution: 0.5%, 1.0%, and 5.0% (Fig 3). The volume of the solution was 3,000 ml, which, by calculation, contained an excessive amount of NaOH to complete the saponification reaction.[14] Each trial involved immersing three pieces of fat in three separate containers filled with the same NaOH concentration. The first trial started at an initial temperature of 45°C. The initial temperature was derived from the study on solubility of the tallow soap in aqueous solution.[15] After 24 h, the presence of solid soap was checked. If positive, the next trial would be repeated at a 1°C higher temperature and the presence of solid soap was checked for again. The cycle continued until the solid soap was completely absent and then the minimum soap-free temperature was declared.

Phase II aimed to measure the corrosion time (time efficiency) of the conventional corrosion process at the minimum soap-free temperature (Fig 4). The injected hearts were allocated to three concentrations of NaOH solution: 0.5% 1.0%, and 5.0%, one heart per one concentration. The temperature was set at the minimum soap-free temperature of each concentration. The corrosion process was checked regularly until tissue was eliminated completely from the cast.

Phase III aimed to compare the corrosion time (time efficiency) of the CLOC system with the conventional process (Fig 4). The CLOC system was assembled from three units: the chambers, the pump, and the heater (Fig 2).[13] The specimen chamber was fitted to contain one heart and was surrounded by an outflow chamber. The CLOC was connected to the pump and heater by a plastic tube in a circuit. The injected hearts were processed as described in phase II, but the corrosive solution was pumped at a flow rate of 600 ml/min. The temperature was set at the minimum soap-free temperature of each NaOH concentration.

RESULTS

In phase I, the minimum soap-free temperatures for the 0.5%, 1%, and 5% NaOH concentrations were 55 °C, 54 °C, and 47 °C, respectively (Table 1).

In phase II, the minimum soap-free temperatures successfully produced residue-free corrosion casts (Fig 5). In each case, heat-induced deformity was undetectable, and the anatomy of the vessel was preserved. The corrosion times of the non-flowing 0.5%, 1%, and 5% concentrations were 216, 114, and 24 h, respectively (Table 1).

In phase III, the circulating laminar outflow chamber (CLOC) system also produced residue-free and anatomically correct casts. The CLOC reduced the corrosion time for the 0.5%, 1%, and 5% concentrations to 132, 76, and 18 h, respectively (Table 1). Compared to the non-flowing group, the reductions were 39%, 33%, and 25% for the 0.5%, 1%, and 5% concentrations, respectively.

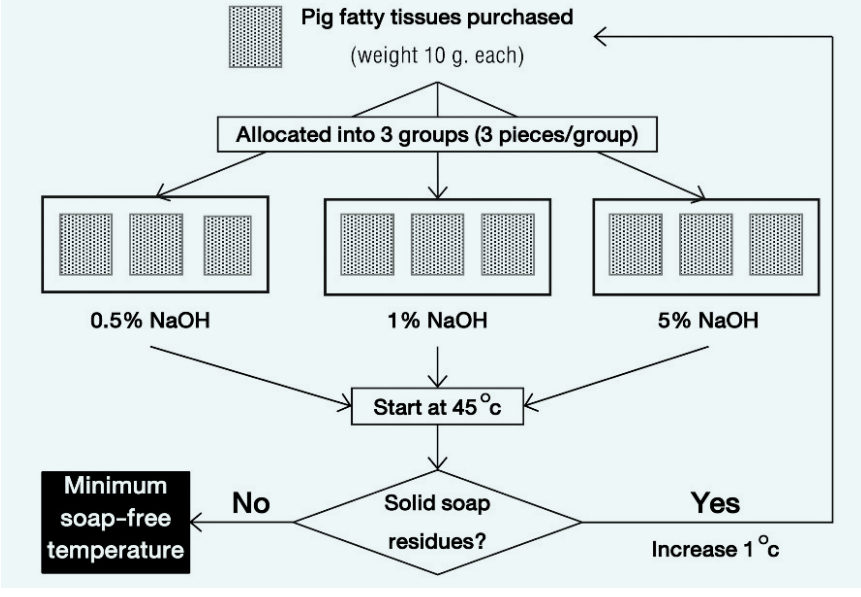

Fig 3. Flow chart depicting the phase I study aiming to find the minimum soap-free temperature at three concentrations of NaOH solution; 0.5%, 1%, and 5%.

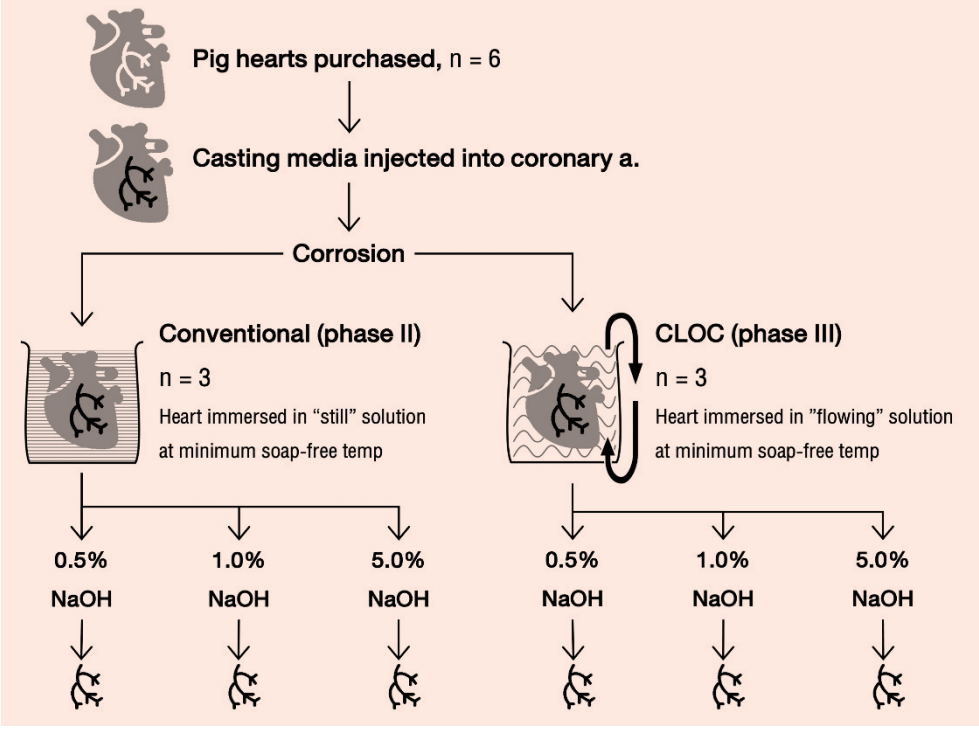

Fig 4. Flow chart depicting the phase II and phase III studies. Phase II aimed to measure the corrosion time of non-flowing NaOH solution at 0.5%, 1%, and 5% concentrations. The corrosion temperatures were set at the minimum soap-free temperatures. Whereas the phase III study aimed to measure the corrosion time in a flowing corrosive solution in the CLOC system.

TABLE 1. Minimum soap-free temperature at three concentrations of NaOH solution (phase I). Corrosion time in a non-flowing NaOH solution (phase II) and flowing NaOH solution in the CLOC system (phase III) at the minimum soap-free temperature.

	Concentration of NaOH solution (%w/v)		
	0.5	1.0	5.0
Phase I Minimum soap-free temperature (ºC)	55	54	47
Phase II Corrosion time in still solution (hours)	216	114	24
Phase III Corrosion time in flowing solution (hours)	132	76	18
Phase II vs III Reduction of the corrosion time	39%	33%	25%

DISCUSSION

The solubility of soap in water depends on the composition of fatty acid in the soap[16], the temperature of the water[16], and the co-existing electrolytes.[17] To explore these issues, we introduced a new term: the "minimum soap-free temperature", despite existing terms being available, such as the clearing temperature[17] and solubility curve.[15] Our rationale was based on the instrumental limitations and practicality. First, we could not measure the concentration of all the phases that were collectively dissolved in the soap solution; micelles, hydrated crystals, and monomers.[18] Second, we needed

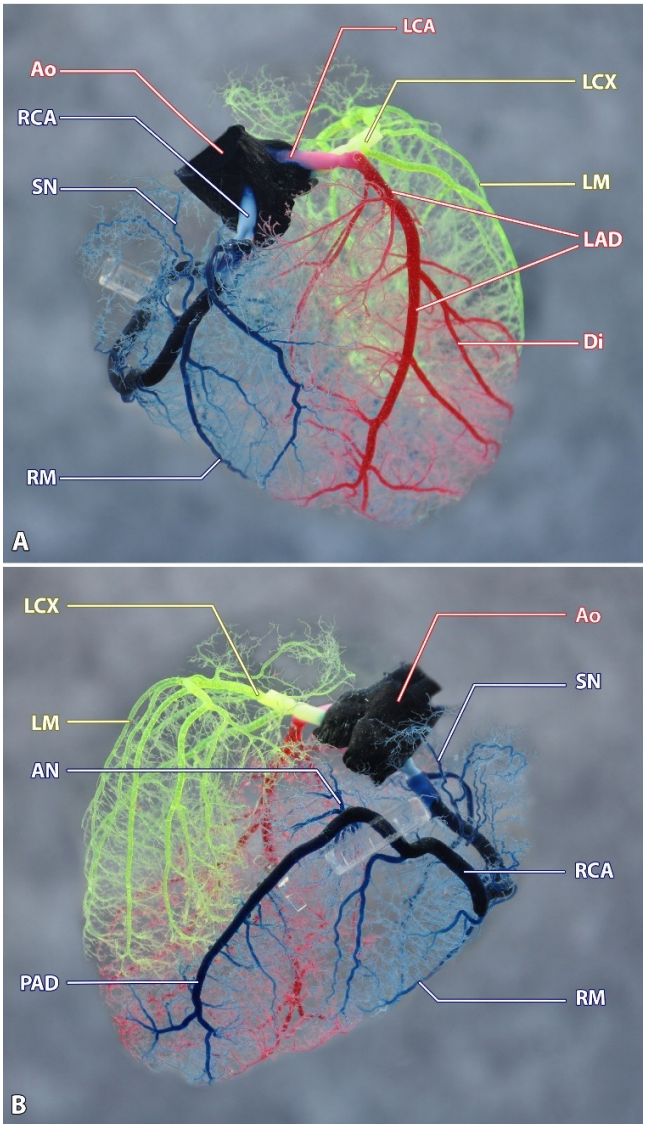

Fig 5. Photographs of a vascular corrosion cast produced at the minimum soap-free temperature. Three colors of pMMA were injected into three branches of coronary artery: red represents the left anterior descending artery (LAD); yellow represents the left circumflex artery (LCX); and blue represents the right coronary artery (RCA). The cast shows no heat deformity. No soap residue remained on the cast. Aorta (Ao), left coronary artery (LCA), left anterior descending artery (LAD), diagonal branch of left anterior descending artery (Di), left marginal branch of left circumflex artery (LM), sinoatrial nodal branch (SN), right marginal branch of right coronary artery (RM) and posterior descending artery (PAD)

a more specific term to describe the absence of soap residue in the corrosion casting. There was no solubility curve for lard (pig fat) soap available to be compared with our data. Fortunately, the composition of fatty acid in lard, tard (cow fat)[18], and humans[19] is similar; namely about one-half oleic acid and one-quarter palmitic acid. Therefore, the solubility curve of tallow soap should be analogous to lard and human fat soap. Our minimum soap-free temperatures corresponded to the solubility curve of tallow soap.[15] This implies the validity of the experiment and applicability of the corrosion casting for human specimens.

Focusing on the saponification reaction, the results of phase I seemed counterintuitive in suggesting that a higher NaOH concentration required a lower minimum soap-free temperature. One might think that the more NaOH is added, the more sodium soap is produced, and thus a higher temperature is required to dissolve the soap. Nevertheless, all the NaOH concentrations in phase I, i.e., 0.5%, 1%, and 5%, contained a copious amount of NaOH that would overwhelm fat in the saponification reaction. The amount of sodium soap was therefore equal in every NaOH concentration. Next, the question is why an equal amount of soap produced at different NaOH concentrations dissolved differently. The quantities of the electrolytes Na^+ and OH^- were not actually measured, but the quantity and pH were certainly increased in the 0.5%, 1.0%, to 5% groups. Surprisingly, we could find no study in the literature describing the effect of the basicity of water on the solubility of sodium soap. On the other hand, we found one study[17] that demonstrated clearly that the more Na^+ is added to a solution, the lower the soap solubility is, and the higher temperature is required to completely dissolve it. If the latter concept is applied here, the highest concentration group, i.e., 5% NaOH, would be supposed to show the highest minimum soap-free temperature. However, the actual result contradicted this assumption. In practice, the data could be explained better by the protein solubilization reactions.

According to previous studies[11,12], 5% NaOH would show the highest rate of protein solubilization. By this assumption, the reaction might be so rapid that the structural proteins (e.g., collagen fiber) holding the adipocyte together would be dissolved before saponification could take place *in situ*. Therefore, the subsequent sodium soap would be produced from the free, colloidal phase of fatty tissue. In contrast to the lowest concentration group, i.e., 0.5% NaOH, the slowest solubilization might allow saponification to take place *in situ* while the structural proteins are still intact. Thus, the higher minimum soap-free temperature would be required to break the saponified tissue and dissolve it.

The success in phases II and III confirmed the validity of our minimum soap-free temperature. From these findings, we could set an appropriate temperature for the corrosion process for each NaOH concentration. Moreover, we could cut the corrosion time and subsequent washing process through the double actions of corrosion and washing in the CLOC system.

CONCLUSION

The most efficient corrosion process to produce a coronary vascular corrosion cast was 5% NaOH via the CLOC system at 47°C, which involved the shortest corrosion time at 18 h.

ACKNOWLEDGEMENTS

The authors are grateful to Phattarapong Predapramote of Department of Radiology, Faculty of Medicine Siriraj Hospital, Mahidol University for assistance with angiography.

Financial disclosure: None of the authors has a financial interest in any of the products or devices mentioned in this manuscript.

Abbreviations:
pMMA, polymethyl methacrylate
NaOH, sodium hydroxide
KOH, potassium hydroxide
CLOC, Circulating laminar outflow chamber
LAD, left anterior descending artery
LCX, left circumflex artery
RCA, right coronary artery

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