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RESEARCH PAPER

Effects of Temperature on the Flexibility of Cow's Tendon in Silicone (S10) Plastination

Sadia Nure Afroze[1*], Nahid Farhana Amin[2], Mst. Zobaida Akter[3], Tahmina Akhter[4] Mohammad Monjurul Haque[5], Sabekun Nahar[6]

[1]Department of Anatomy, Dhaka Dental College, Dhaka, [2]Department. of Anatomy, Bangladesh Medical University, Dhaka, [3].Department of Anatomy, DG Health, Dhaka, [4]Department of Anatomy, Holy Family Red Crescent Medical College, Dhaka, [5]Department of Surgery, Dhaka Medical College Hospital, Dhaka, [6]Department of Anatomy, National Institute of Burn and Plastic Surgery, Dhaka.

Abstract

Background: Temperature is a major factor affecting the quality of plastination. The present study was conducted to explore a suitable method for plastination in Bangladesh by comparing the effect of cold and room temperatures on the tendon flexibility throughout the plastination.

Objective: The objective of the study is to compare the percentage of change in flexibility of cow's tendon in silicone (S10) plastination at cold temperature with those at room temperature.

Methods: An experimental study was performed at Bangabandhu Sheikh Mujib Medical University (BSMMU) currently known as Bangladesh Medical University, Dhaka, Bangladesh. Forty (40) pieces of tendon were obtained from approximately one-year-old cows (*Bos indicus*) slaughtered at a government-authorized slaughterhouse in Dhaka, Bangladesh, following international animal sacrificing protocols. Ethical clearance was obtained from the Institutional Review Board BSMMU. The specimens of tendon were divided equally into two groups: twenty (20) processed at cold temperature and the other twenty (20) at room temperature. Then the percentage of change in flexibility was measured at every stage of plastination: fixation, dehydration, forced impregnation and gas-curing and then compared between two temperature groups. The overall percentage of change in flexibility from fixation to gas-curing was also measured.

Results: The percentage of change in tendon flexibility was smaller (yielding greater flexibility) at cold temperature (29.56 ± 12.60) than at room temperature (30.47 ± 9.96) during fixation, although difference was not statistically significant (p = 0.787). However during dehydration, the change in flexibility of tendon was larger (yielding lesser flexibility) at cold temperature (49.46 ± 16.62) than at room temperature (45.43 ± 15.97), but the difference was also not significant (p = 0.433). The percentage of change in tendon flexibility was smaller (yielding greater flexibility) at cold temperature (42.43 ± 22.44) than at room temperature (45.18 ± 15.94) during forced impregnation, although difference was not statistically significant (p = 0.499). Moreover, the percentage of change in tendon flexibility was smaller (yielding greater flexibility) at cold temperature (29.81 ± 14.89) than at room temperature (39.14 ± 19.53) during gas-curing, which is reasonably close to the significant level (p = 0.062). The overall change in flexibility of pieces of tendon was smaller (yielding greater flexibility) at cold temperature (84.01 ± 8.22) than at room temperature (88.07 ± 5.27), with a reasonably close to the significant level (p = 0.074).

Conclusions: The cold temperature plastination showed consistent changes towards better preservation than room temperature across all stages of the process but was not statistically significant. The near significant results of gas-curing and overall flexibility indicates cold temperature palstination may show better outcome to preserve the properties of tendons..But, Further research with larger sample sizes is recommended to confirm these results.

Keywords: Plastination, tendonflexibility, Silicone (S10) method, temperature,preservation

Introduction

The study of cadavers and biological specimens remains indispensable in the field of anatomical education. When these specimens are plastinated, they become durable, dry, odorless, non-toxic, easy to handle, and virtually maintenance-free, compared to the specimens preserved by traditional methods such as embalming.[1] Use of plastinated specimens reduces the irritation and harmful effects caused by chemical preservatives like formalin, alcohol, etc.[2] Despite of the increasing number of medical colleges

***Correspondence:** Dr. Sadia Nure Afroze, Department of Anatomy, Dhaka Dental College, Dhaka.*

Email: sadialubna2386@gmail.com

ORCID ID: 0009-0007-8177-1032

in Bangladesh, there is a persistent shortage of cadavers, organs and prosected specimens; body donation is not commonly practiced due to prevailing religious beliefs and social stigmas.

Given the growing emphasis on the use of prosected specimens for teaching gross anatomy and the limited availability of cadavers and financial resources, plastination offers a valuable alternative for long-term specimen preservation. Although the technique itself is relatively straightforward, achieving consistently high-quality plastinates remains a challenge, as several factors influence the outcome; temperature is one of the most important factors.

As a tropical country, Bangladesh experiences temperature variations between 14°C to 30°C.[3] While it is generally accepted that cold temperature plastination (-15°C to -25°C) yields superior results, there is potential for effective plastination at room temperatures (15°C to 20°C) as well.[4-7] Success with the later would markedly reduce the associated costs of the process.

Flexibility is one of the key indicators of specimen quality. A plastinated specimen should retain its natural mechanical properties as much as possible while becoming dry, odorless and durable. Moreover, there is limited published information on how processing temperature affects the mechanical properties of silicone plastinated specimens and measuring flexibility contributes valuable evidence to improve plastination techniques.

Although a few studies in Bangladesh have examined the impact of temperature on plastination time and gross morphology of certain pig and goat organs using silicone (S10) plastination, the findings have been inconsistent. [8, 9, 10, 11] Furthermore, there had been no research conducted involving connective tissue like tendon Therefore, further research is needed to determine the optimal temperature conditions for achieving high-quality plastinates. The aim of this study is to compare the percentage of change in flexibility of cow's tendon in silicone (S10) plastination at cold temperature with those at room temperature.

Materials and Methods

The specimens were collected from the tendon of the back of the cow (*Bos indicus*). Fourteen (14) large pieces of tendons from the back of the cow were collected in normal saline and extra connective tissues from each specimen were removed. These fourteen (14) pieces measure about twenty-one centimeters in length and five centimeters in breadth (Figure 1A). Then tagging of all points of measurements was done (Figure 1B). Afterwards, the large pieces were cut into three small pieces measuring about seven centimeters in length and five centimeters in breadth (Figure 1C). Total (14 × 3) = 42 specimens of tendon were obtained. Finally, twenty (20) pieces of tendon were plastinated at cold temperature and designated as the 'Cold Temperature Group,' twenty (20) pieces of tendon were plastinated at room temperature and designated as the 'Room Temperature Group,' and the remaining two (02) pieces were discarded. Each of the pieces of tendon was considered as an individual sampling unit. After that, the flexibility of each tendon was measured at every stage of plastination and the change in flexibility was calculated by the difference between before and after every stage of plastination. Finally, the percentage of change in flexibility was calculated after every stage of plastination.

At first the specimens of 'Room Temperature Group' were plastinated. Then after completion of this procedure, the specimens of 'Cold Temperature Group' were plastinated in separate time due to the unavailability of forced impregnation and gas-curing equipment. Dehydration and forced impregnation stage were carried out at both cold (-19 °C to -23 °C) and room (20 °C to 28 °C) temperatures for both groups. But fixation and gas-curing stage were carried out only at room (20 °C to 28 °C) temperature for both groups.

Initially, fixation was carried out using the same method for both temperature groups. The pieces of tendon were immersed in 10% formalin within a plastic container for five days.[8-12] Following fixation, the specimens were thoroughly rinsed under running tap water overnight to eliminate residual formalin.

For dehydration, a plastic container was filled with acetone in a volume five times that of the specimens and the specimens were fully submerged.[13] For the '*Room Temperature Group,*' dehydration was performed using a series of 80%, 90% and 100% acetone at 20 °C to 28 °C in the laboratory outside the freezer. After the bath of 100% acetone, the concentration of acetone was found 98%. The dehydration is considered complete when concentration of water stabilizes at 1% or less.[2] As a

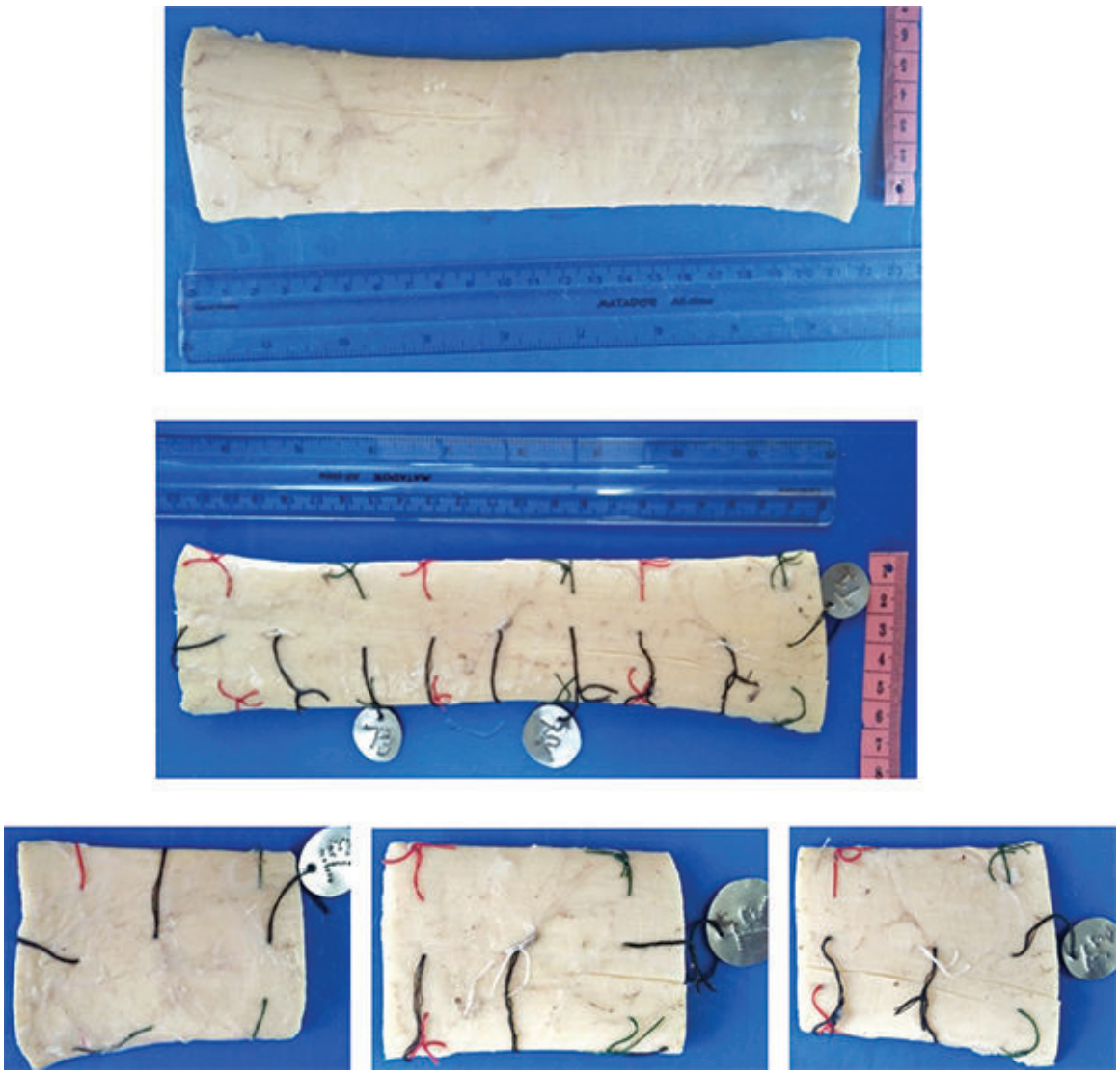

Figure 1: *Preparation of tendon from larger to smaller with tagging.*
A: Large piece of tendon.
B: Large piece of tendon tagged at all points of measurement.
C: Small pieces of tendons after cutting of large piece.

result, another 100% acetone bath was used. After completion of this bath, the acetone concentration was found to be 99%, thus the dehydration was considered as complete in room temperature. Room temperature dehydration allowed simultaneous 'defatting' of the specimen and duration of defatting at room temperature was considered as 3 days.[6,14] For the 'Cold Temperature Group', the dehydration procedure was same as the 'Room Temperature Group', except it is preceded by precooling which was done at 5 °C in a usual laboratory refrigerator (not in the deep freezer). Dehydration was made at deep freezer where temperature was in between -19 °C to -23 °C with the same graded of acetone series. After the first bath of 100% pure acetone, the purity was found 98%. So, for proper dehydration and defatting the specimens were placed in to 100% pure acetone at room temperature (20 °C to 28 °C) for 3 days, then the concentration of acetone became 99% and considered complete.[14]

Subsequently, impregnation was carried out. Specimens in the 'Cold Temperature Group' were treated at –19°C to –23°C in a deep freezer, while those in the 'Room Temperature Group' were treated

at 20°C to 28°C. The specimens were immersed in a plastination kettle containing a reaction mixture of BIODUR® S10/S3 (100:1 ratio) and kept overnight for acetone evaporation and equilibration. Vacuum was then gradually applied: pressure was reduced to 10 cm Hg on the second day, when the first bubbles appeared. From the third to fifth day, the pressure was lowered at a rate of 7 cm Hg, producing small bubbles. Between the sixth and ninth day, the pressure decreased at a rate of 8 cm Hg, with medium-sized bubbles forming. Finally, the pressure was reduced by 10 cm Hg, resulting in the formation and bursting of large bubbles. The rate of pressure decrease was maintained by observing the pressure gauge and also by observing the bubble formation on the reaction-mixture of the silicon surface. Then set the parameters daily using a manual vacuum controller. The vacuum controller semi-automatically decreased pressure once it had been programmed for that incremental decrease. Then the controller automatically decreased pressure to the set level to maintain bubble formation. The pressure parameters were set daily in conjunction with observation of bubble production. Impregnation was considered complete when bubble formation on the silicone surface ceased for 12 hours.

During gas curing, specimens were arranged on absorbent papers inside a curing chamber at 20 °C to 28 °C for both temperature groups separately. A cross-linker BIODUR® S6 (20 cc) and a desiccant ($CaCl_2$) were placed in the chamber. The S6 was vaporized twice daily using a small fan for 20 minutes each time. The specimens' surfaces were regularly checked, manicured and exposed to S6 vapor until completely dry.

For measuring the change in flexibility of a piece of tendon from the fresh stage and after every stage of plastination, a locally made flexibility machine was used (Figure 2). An older light microscope was modified by attaching a digital slide caliper, which has a fixed and a movable part, with it to form the flexibility machine. The objective lenses of the microscope were removed and a circular, metallic plate was attached at that place. The fixed part of the digital slide caliper was attached to the stage of the microscope and the movable part was moved with the circular metallic plate. The specimen was placed on the stage of the machine and was touched by the circular metallic plate by moving the adjustment knob. When the circular metallic plate just touched the upper surface of the specimen, the value of the digital measuring display was made zero (0). Then the specimen was compressed by moving the stage upward with the movement of the adjustment knob. The value of the change in flexibility of the specimen would be yielded directly on the display in millimeters (Figure 2).

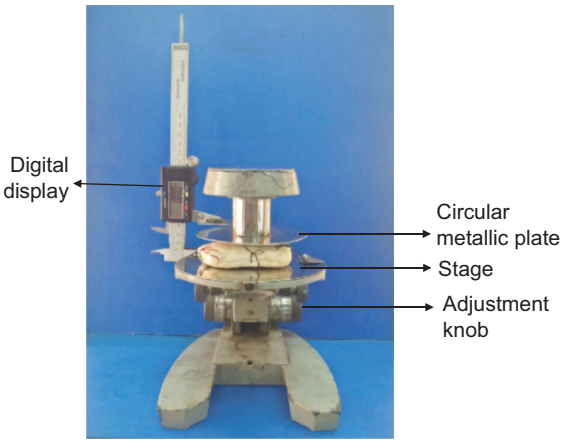

Figure 2: Procedure for measuring the flexibility of the specimen.

At first the measurement of flexibility of each piece was taken at the fresh stage which was considered as the flexibility before fixation. Then the flexibility of each piece was measured again after fixation. Then the change in flexibility of tendon after fixation was calculated by their difference ($f_B - f_E$). After that, percentage of change in flexibility of a tendon after fixation was also calculated by

$$\frac{(f_B - f_E) \times 100}{f_B}$$

In the same method percentage of change in flexibility of a piece of tendon in dehydration, forced impregnation and gas-curing were calculated. Here the measurement of flexibility before dehydration is same as the measurement of flexibility after fixation ($d_B=f_E$), the measurement of flexibility before forced impregnation is same as the measurement of flexibility after dehydration ($i_B=d_E$) and the measurement of flexibility before gas-curing is same as the measurement of flexibility after forced impregnation ($c_B=i_E$). Overall percentage of change in flexibility was also measured by $\frac{(f_B - c_E) \times 100}{f_B}$

For the comparison of percentage of change in flexibility, the values of the individual sample unit were expressed as means and medians (as there were non-normal distributions as well) for the two groups. Hypothesis testing was done for the differences between the two groups using the Mann-Whitney U

test with the help of software Statistical Package for Social Sciences.

Results

A smaller percentage of change in flexibility in any specific temperature indicates that the tendon underwent less alteration during plastination in that temperature. Therefore, it retained more of its original flexibility, yielding greater flexibility after plastination at that temperature.

After fixations stage, the percentage of change in flexibility of tendon was smaller at cold temperature (29.56 ± 12.60) than at room temperature (30.47 ± 9.96) (yielding greater flexibility). But the difference was not statistically significant (p = 0.787) (Table I). After dehydration stage, the percentage of change in flexibility was larger at cold temperature (49.46 ± 16.62) than at room temperature (45.43 ± 15.97) (yielding lesser flexibility). But the difference was not statistically significant (p = 0.433) (Table I). After the forced impregnation stage, the percentage of changes in flexibility was smaller at cold temperature (42.43 ± 22.44) than at room temperature (45.18 ± 15.94) (yielding greater flexibility). But the difference was not statistically significant (p = 0.499) (Table I). After gas-curing stage, the percentage of change in flexibility of tendon was smaller at cold temperature (29.81 ± 14.89) than at room temperature (39.14 ± 19.53) (yielding greater flexibility), and the difference was reasonably close to significance level (p = 0.062) (Table I).

In the 'Cold Temperature Group', the mean percentage of change in flexibility of tendon was the highest (49.46 ± 16.62) in the dehydration stage and the lowest (29.56 ± 12.60) in the fixation stage (Table I).

In the 'Room Temperature Group', the mean percentage of change in flexibility of tendon was the highest (45.43 ± 15.97) in the dehydration stage and the lowest (30.47 ± 9.96) in the fixation stage (Table I).

The overall percentage of change in flexibility was smaller at cold temperature (84.01 ± 8.22) than at room temperature (88.07 ± 5.27) (yielding greater flexibility) and the difference was reasonably close to significance (p = 0.074) (Table I). In this study, there is a trend in changes in flexibility that were smaller (yielding greater flexibility) in the 'Cold Temperature Group' than in the 'Room Temperature Group.' That means the plastinates of the 'Cold Temperature Group' were better than that of 'Room Temperature Group.'

Table I: *Comparison between the two temperature groups regarding the percentage of changes in flexibility of tendon in different stages of plastination*

Stage of plastination	Percentage of change in flexibility Mean ± SD Median±(25th and 75th percentile)		Probability (p) and significance	
	Room temperature	Cold temperature		
Fixation	30.47 ± 9.96 31.53(21.64, 38.30)	29.56 ± 12.60 28.36 (20.58, 38.74)	0.787	NS
Dehydration	45.43 ± 15.97 46.67 (33.99, 59.19)	49.46 ± 16.62 50.71 (36.41, 62.44)	0.433	NS
Forced impregnation	45.18 ± 15.94 44.95 (36.11, 59.36)	42.43 ± 22.44 43.77 (28.20, 58.01)	0.499	NS
Gas-curing	39.14 ± 19.53 39.84 (22.36, 49.62)	29.81 ± 14.89 30.14 (19.72, 36.07)	0.062	NS
Overall (from fresh stage to the end of plastination)	88.07 ± 5.27 88.89 (83.82, 91.12)	84.01 ± 8.22 4.37 (80.58, 88.08)	0.074	NS

n (Number of specimens in each group): 20; S:Significant (p≤0.05); NS: Non-significant
P-values were calculated using the Mann-Whitney U test.

Discussion

As the tendon, kidney, hyaline cartilage and elastic cartilage are firm specimen, the morphological features of goat kidney, goat hyaline cartilage and goat elastic cartilage are closely similar with the morphological feature of cow tendon.

After the fixation stage, the change in the flexibility of tendon was smaller (yielding greater flexibility) at cold temperature than at room temperature, which is not statistically significant. Moreover, Akhter found the similar result for kidney but could not prove it.[15] According to Henry, Janick, and Henry more flexible specimens can be yielded by shortening the fixation time using a low concentration of formalin.[16] Although they used 10% formalin, as in the present study, their fixation period was reduced to 1-2 days, whereas fixation period of this study was 5 days. So, the mean percentage of change in flexibility of tendon did not show any consistent result regarding temperature.

After the dehydration stage, the mean percentage of change in flexibility of tendon was larger (yielding lesser flexibility) at cold temperature than at room temperature, but was not statistically significant. Paul proved similar results for hyaline cartilage, respectively, but Paul and Akhter proved the opposite result for elastic cartilage and kidney.[9, 15] Thus, it may be said that, the mean percentage of change in flexibility of tendon during dehydration showed inconsistent results regarding temperature.

After the forced impregnation stage, the mean percentage of change in flexibility of tendon was smaller (yielding greater flexibility) at cold temperature than at room temperature but was not statistically significant. Akhter and Paul proved similar results for kidney and elastic cartilage, respectively.[9, 15] However, Paul proved the opposite result for hyaline cartilage.[9] So, it may be said that, the mean percentage of change in flexibility of tendon during the forced impregnation stage showed an inconsistent result regarding temperature.

After the gas-curing stage, the mean percentage of change in flexibility of tendon was smaller (yielding greater flexibility) at cold temperature than at room temperature, which was reasonably close to the significance level ($p = 0.062$). Akhter proved a similar result for kidney.[15] Moreover, Paul also found a similar result for elastic cartilage but an opposite result for hyaline cartilage, but could not prove her result.[9] According to Tianzhong, Jingren, and Kerming, use of a low percentage of hardener produces more flexible specimens.[6] They used silicon mixed with varying ratio of hardener (1% to 5%) by covering on the surface of the specimens. Although a cross linker BIODUR® S6 (20 cc) and a desicant ($CaCl_2$) were used for gas-curing in the present study. Moreover, Mendez et al. stated that longer exposure to gas-curing agent produces less flexible specimens.[17] Their specimens achieved complete curing within one to four weeks. In contrast, in the present study, gas-curing required 12 days in both temperature groups. Thus, it may be said that, the mean percentage of change in flexibility of tendon during gas-curing showed inconsistent results regarding temperature.

The overall mean percentage of change in flexibility of tendon was smaller (yielding greater flexibility) at cold temperature than at room temperature, which was reasonably close to the significance level ($p = 0.074$). Furthermore, Paul proved a similar result for elastic cartilage, but proved the opposite result for hyaline cartilage.[9] Thus, it may be said that the overall mean percentage of change in flexibility of tendon showed inconsistent results regarding temperature. Thus, it may be concluded that there was a trend of obtaining more flexible specimens which means a better outcome in the 'Cold Temperature Group' than in the 'Room Temperature Group.

Conclusion

The percentage of change in flexibility of tendon showed a trend of more flexible specimen indicating a better outcome at cold temperature than at room temperature in the present study. But the differences were not significant. The sample size of the study was small and this was due to limitation of the size of the impregnation chamber. Moreover no formal sample size determination was done. If a formal sample size calculation would be done it would raise the strength of the study. Randomization of group selection was not done due to unavailability of logistic supports. Two groups could not be run in parallel way. So, further research with larger sample and randomize group selection is recommended to reach a definitive conclusion.

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Conflict of Interest: There are no conflicts of interest.

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Ethical Clearance: Permission was taken for carrying out the research from the Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU), .

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