การประเมินคุณภาพของตัวอย่างทางกายวิภาคศาสตร์ซึ่งผลิตจากการพลาสติเนชันด้วยซิลิโคน KE-108 ที่อุณหภูมิห้อง

Evaluation of The Quality of Anatomical Specimens Produced with Room Temperature Plastination Using KE-108 Silicone

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ABSTRACT

This study was aimed at evaluating the quality of porcine hearts and kidneys produced from a room-temperature plastination using industrial KE-108 liquid silicone. All plastination processes, including formalin fixation, acetone dehydration, and forced impregnation with KE-108 silicone liquid, were conducted at room temperatures between 28-33 ^{o}C. The specimen's volume, surface hardness, and color parameters (brightness, green-to-red and blue-to-yellow shades) were measured and compared between the control groups (the fresh and the formalin-fixed organs) and the organs derived from the plastination process using 1.5%, 2%, and 5% catalyst ratios. The results showed that all porcine hearts and kidneys produced from this technique maintained their anatomical shape and structures. All color parameter values were not affected by the plastination process. Although the plastination process caused significant shrinkage and increased their surface hardness in both hearts and kidneys, the specimens still had good quality. To determine the effects of different catalyst-to-polymer ratios in the force impregnation on the quality of products, the results showed that specimens produced from different catalyst-to-polymer ratios had similar quality. These proved that this technique is suitable for producing anatomical teaching media.

Keywords: Quality, Silicone, Plastination, Room temperature, Organs

บทคัดย่อ

การศึกษานี้มุ่งที่จะประเมินคุณสมบัติของหัวใจและไตสุกรซึ่งผลิตจากวิธีพลาสติเนชันที่อุณหภูมิห้อง ด้วยซิลิโคน KE-108 ซึ่งเป็นซิลิโคนเหลว กระบวนการพลาสติเนชันทั้งหมดประกอบด้วย การคงสภาพด้วยฟอร์มาลีน การขจัดน้ำด้วยอะซีโตน และการกำซาบด้วยซิลิโคน KE-108 วิธีทั้งหมดนี้กระทำที่อุณหภูมิห้องระหว่าง 28-33 ^{o}C. การศึกษานี้มีการวัดและเปรียบเทียบคุณภาพด้านต่าง ๆ ของตัวอย่าง อันได้แก่ ปริมาตรของตัวอย่าง ความแข็งที่พื้นผิวและค่ากำหนดสี (ค่าความสว่าง เฉดสีเขียวถึงแดงและเฉดสีน้ำเงินถึงเหลือง) ระหว่างกลุ่มควบคุม (อวัยวะสดและอวัยวะถูกคงสภาพด้วยฟอร์มาลีน) กับอวัยวะที่ได้จากการพลาสติเนชันซึ่งได้จากการใช้สัดส่วนสารเร่งปฏิกิริยาต่อโพลิเมอร์ที่ 1.5% 2% และ 5% ผลการทดลองแสดงว่า ตัวอย่างหัวใจและไตสุกรทั้งหมดซึ่งผลิตจากเทคนิคนี้ยังสามารถรักษารูปร่างและโครงสร้างทางกายวิภาคศาสตร์ไว้ได้ ค่ากำหนดสีทั้งหมดของตัวอย่างไม่ได้รับผลกระทบจาก

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กระบวนการพลาสติเนชัน ถึงแม้ว่ากระบวนการพลาสติเนชันทำให้ทั้งหัวใจและไตเกิดการหดตัวและมีพื้นผิวที่แข็งขึ้นอย่างมีนัยสำคัญ ตัวอย่างทั้งหมดยังคงมีคุณภาพดี เมื่อพิจารณาผลการใช้สัดส่วนสารเร่งปฏิกิริยาต่อโพลิเมอร์ที่แตกต่างกันในกระบวนการกำซาบที่มีต่อคุณภาพผลิตภัณฑ์ ผลการศึกษาแสดงให้เห็นว่าตัวอย่างซึ่งได้จากสัดส่วนสารเร่งปฏิกิริยาต่อโพลิเมอร์ที่แตกต่างมีคุณสมบัติคล้ายคลึงกัน สิ่งเหล่านี้พิสูจน์ได้ว่าเทคนิคนี้เหมาะสมกับการผลิตสื่อการสอนทางกายวิภาคศาสตร์ได้เป็นอย่างดี

คำสำคัญ: คุณสมบัติ ซิลิโคน พลาสติเนชัน อุณหภูมิห้อง อวัยวะ

Introduction

Plastination is a long-term preservative method based on polymer perfusion into the tissue under pressure (Von Hagens, 1979). Since several attempts have been made to develop techniques that can provide long-term preservation and reduce health risk associated with the use of formalin, which is a carcinogen and causes skin and mucous irritation (Balta *et al*., 2015), plastination was developed as an alternative for anatomical preservation from traditional fixation (Brenner, 2014). In modern anatomical education, specimens produced from the plastination have been proven to be effective learning tools for anatomical classes and self-study (Latorre *et al*., 2016; Klaus *et al*., 2017; Senos, 2024). In contrast to traditional formalin fixation, the plastination produced smooth and dry specimens, allowing direct handling and leading to the three-dimensional visual appreciation of anatomical features. The specimens are also valuable for studying anatomical aspects of clinical studies (Gómez *et al*., 2012; Suenaga, 2017).

Von Hogen's technique, based on the Biodur® silicone series, is generally considered the standard procedure for plastination. Since the procedure is conducted in freezing temperatures and requires expensive equipment, room-temperature plastination techniques were successfully developed for anatomical preservation (Henry, 2007; Raoof *et al*., 2007; Ottone *et al*., 2015). To reduce the cost and the time required for Biodur® silicone import and customs clearance, many industrial silicone polymers have been effectively employed for the plastination. These silicone polymers included Su-Yi Chinese silicone (Nanjing Su-Yi Plastination Factory, China; Zheng *et al*., 1998), PR-10 and PR-14 silicone polymer (Dow Corning, USA; Raoof *et al*., 2007), and NCS-10/11 silicone polymer (North Carolina Products, USA, Henry, 2007).

KE-108 silicone polymer is the room-temperature vulcanized type generally used in electronic and electrical applications and is commercially available in Thailand. The silicone mixture is diluted with thinner at 1:10 and blended with 5% (V/V). Catalyst-108 and the polymer mixture can be polymerized within 72 hours, at 23 ^{o}C (Shin-Etsu, 2025). Previous reports revealed that KE-108 silicone rubber was an eligible agent for plastination of various human organs, including brains, intestines, livers, and lungs (Sakamoto *et al*., 2006), and porcine hearts (Suenaga, 2017). In this study, we used porcine hearts and kidneys as anatomical samples because they are commonly used in many studies of the plastination technique, including the KE-108 silicone technique.

Thus, this study was aimed at analyzing the quality of porcine hearts, which represent solid organs, and porcine kidneys, which represent porous organs. The organs were preserved with the KE-108 silicone plastination at room temperature using different catalyst-to-polymer ratios. The findings

from this study will be applied for developing a novel plastination method.

Materials and Methods

The protocol regarding animal organ and tissue handling and experiments was approved by the Institutional Animal Care and Use Committee of Kasetsart University, Thailand, with I.D. number ACKU66-VET-059.

Organ preparation

Fresh porcine hearts and kidneys were purchased from a local vendor, transferred to the ice box, and transported to the laboratory within 2 hours. The heart and kidney samples were cleaned with tap water. The renal capsule and the perirenal fat pad were removed. All specimens were blotted dry with tissue paper. The heart was stuffed with cotton fiber to expand the chambers into their natural forms. For fixation, 5% (V/V) formalin solution was injected into the organs (cranial and caudal vena cava of the heart and renal artery of the kidney). All specimens were submerged in the fixative. The formalin solution was freshly replaced every 24 hours. The fixation was maintained at ambient temperatures for 7 days. The formalin-fixed specimens were rinsed with tap water overnight before submerging in a stainless-steel container filled with 100% acetone for dehydration and defatting for 1 month at ambient temperatures. The fresh acetone was replaced every 7 days.

Forced impregnation

The forced impregnation protocol in this study was modified from Sakamoto *et al*. (2006). The silicone is mixed with thinner at a ratio of 1:10 (V/V) to decrease viscosity and then added with Catalyst-108. In the present study, we modified the procedure by varying catalyst-to-polymer ratios in the silicone mixture. All specimens were randomly assigned for impregnation with three catalyst-to-polymer ratios (V/V): 1.5%, 2 % (Sakamoto *et al*., 2006), and 5% (the company's recommended ratio) and two control groups.

All porcine heart samples were assigned as follows: the 1.5% catalyst ratio group (n=5), the 2% catalyst ratio group (n=3), and the 5% catalyst ratio group (n=3). The control group contained fresh hearts (n=5), 5% formalin-fixed hearts (n=3). All porcine kidneys were placed as follows: the 1.5% catalyst ratio group (n=5), the 2% catalyst ratio group (n=4), and the 5% catalyst ratio group (n=3). The control group contained fresh kidneys (n=5), and 5% formalin-fixed kidneys (n=3).

The polymer mixture volumes used were 1000 ml per heart and 300 ml per kidney. The dehydrated and defatted organs were placed and submerged in the polymer mixture-filled containers. The containers were subsequently placed into the vacuum chamber. The pressure in the vacuum chamber was gradually reduced as the pump was turned on. The initial pressure for the acetone bubbling was 0.2 bar. The pressure was then gradually adjusted to 0.03 bar and maintained for 6 hours. The impregnation ceased, as the acetone bubble was not observable. The post-processed products were removed from the containers and wiped with the catalyst.

Volume measurement

The volumes of porcine hearts and kidneys were measured in ml according to Archimedes' principle (Akgün *et al*., 2017).

Shore00 unit measurement for tissue hardness

The hardness of the specimens was measured on the surface of the left and right ventricles of each heart and two locations of each kidney using an HT-6510 type 00 shore durometer, Guangzhou Landtek Instruments, China (Yoon *et al*., 2017).

Color measurement

Color parameters based on the Commission International de l'éclariage (CIE) colorimetric system included L* for the brightness, a* for the green-to-red shade, and b* for the blue-to-yellow shade. The measurement was performed at two different surface sites per specimen using a WF-32 Precision colorimeter® (Shenzhen Wave Optoelectronic Technology, China).

Statistical analysis

The data acquired from each method were presented as mean ± standard deviation. The effects of the variables were analyzed with a non-parametric Kruskal-Wallis test. If the effects were significant, a post hoc pair-wise comparison between groups was performed using Dunn's test. All statistical analysis was performed with GraphPad Prism 10 for Windows version 10.4.1. A p-value of 0.05 was considered significantly different.

Results

The effects of plastination on organ quality were then described as follows.

The effects of KE-108 silicone plastination at room temperature on the appearance and color of porcine hearts and kidneys

After the plastination process, the porcine hearts and kidneys had smooth and dry surfaces without any odors. The anatomical details of the heart, including left and right auricles, the aorta, and the pulmonary trunk, were well-preserved and identical to the fresh and formalin-fixed organs (Figure 1A). All hearts had comparable brightness levels and green-to-red shade values (Figure 2A-B). However, the hearts impregnated with the 5% catalyst-to-polymer ratio had significantly higher blue-to-yellow shade values than the others (Figure 2C)

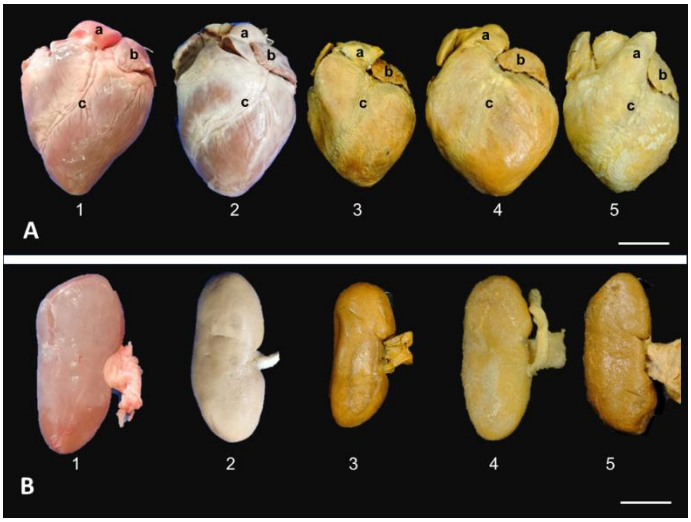

Figure 1 External appearance of (A) the porcine hearts and (B) kidneys, no.1 = fresh organs, no. 2 = formalin-fixed organs, no. 3= organs preserved with the plastination using the 1.5% catalyst-to-polymer ratio, no. 4 = organs preserved with the plastination using the 2% catalyst-to-polymer ratio, no. 5 = organs preserved with the plastination using the 5% catalyst-to-polymer ratio, a = pulmonary trunk, b = left auricle, c = paraconal interventricular groove, scale bar = 50 mm.

At post-plastination, the kidneys maintained their shape, which resembled the fresh and the fixed-dehydrated organs (Figure 1B). No wrinkles or crevices were detected on their surfaces, and distorted contours in some kidneys produced from 2% catalyst ratio in the forced impregnation were observed.

Although the surfaces of the kidneys were turned brownish by the plastination, the average brightness, green to red, and blue to yellow shades of these kidney surfaces had no significant differences from the fresh and fixed organs (Figure 2D-F)

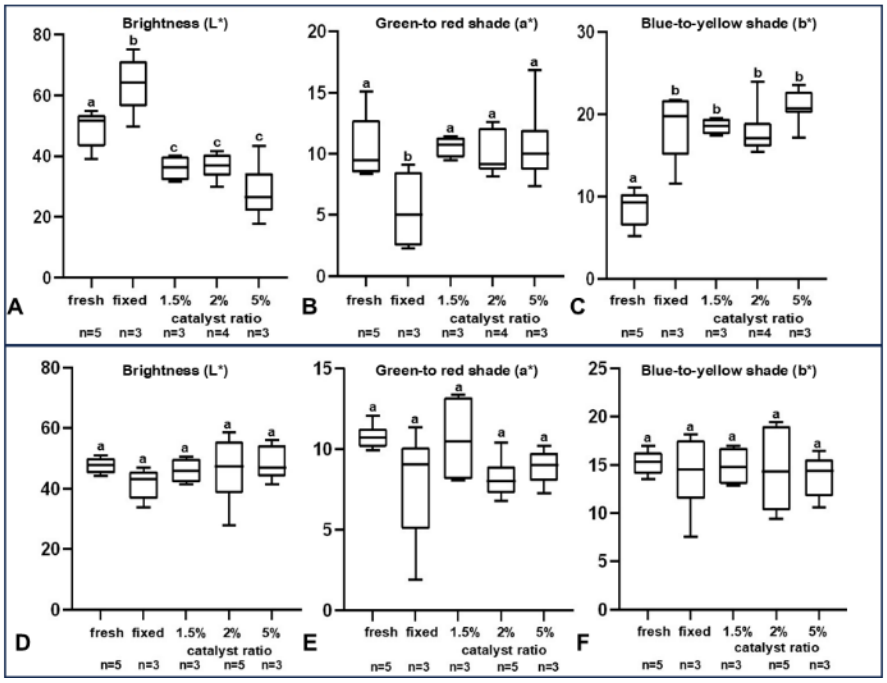

Figure 2 Boxplots of color values measured from (A-C) the post-processed hearts and (D-F). kidneys. a,b,c = different alphabets indicate significant differences. ($p < 0.05$)

The effects of KE-108 silicone plastination at room temperature on the volumes of the specimens

The hearts polymerized with the 1.5%, 2%, and 5% catalyst-to-polymer ratios had average volumes of 137.5 ± 22.35 ml, 200.00 ± 29.15, and 208.0 ± 25.53, respectively. Average plastinated heart sizes ranged from 40.6 to 60% of the fresh and fixed heart sizes (330 ± 85.44 ml, 320.66 ± 113.31 ml, respectively) and were significantly smaller than the control (Figure 3A). The average volumes of all hearts derived from the plastination were not significantly different from each other, despite the different catalyst ratios (Figure 3A)

The effects of the plastination on the kidney volumes were similar to those on the heart. The kidneys polymerized with the 1.5%, 2%, and 5% catalyst-to-polymer ratios had average volumes of 28.6 ± 10.69 ml, 36.0 ±13.44 ml, and 22.0 ± 9.84 ml, respectively. The average volumes of the plastinated kidney range from 16.6 to 27% of those of fresh organs. The plastination lessened the kidney volume significantly compared to that of the control. Different catalyst-to-polymer ratios provided similar results (Figure 3B)

The effects of KE-108 silicone plastination at room temperature on the surface Shore00 hardness unit

The plastination increased the surface Shore00 hardness units in both porcine hearts and kidneys, regardless of the catalyst-to-polymer ratio. However, the hardness units of the organs impregnated with any catalyst-to-polymer ratios were parallel to those of the formalin-fixed organs (Figure 4 A- B) It is worth noting that the thin-walled structures, such as the ureter and blood vessels, were more flexible than the whole organs.

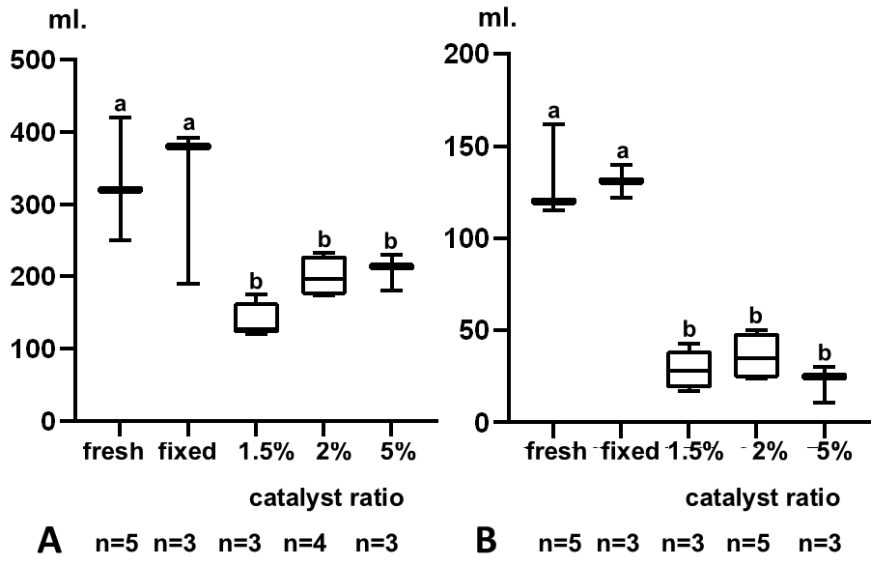

Figure 3 Boxplots of average volumes measured from (A) the post-processed hearts and (B) kidneys. a,b,c = different alphabets indicate the significant differences. ($p < 0.05$)

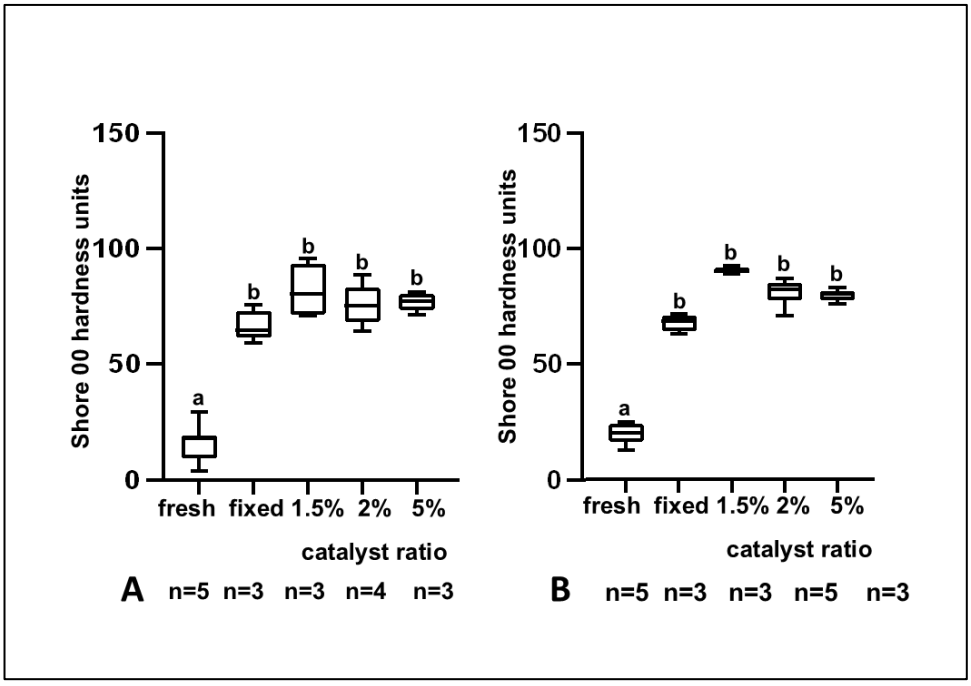

Figure 4 Boxplots of average surface Shore00 hardness units measured from (A) the post-processed hearts and (B) kidneys. a,b,c = different alphabets indicate the significant differences. ($p < 0.05$)

Discussion

The present study showed that formalin fixation, a traditional method, could preserve specimens well. However, its harmful effect on health makes it unsuitable for anatomical preparation (Brenner, 2014). Plastination has been viewed as the best alternative (Von Hagen *et al*., 1987). We assessed porcine heart and kidney quality after plastination with KE-108 silicone at room temperature. The results showed that both hearts and kidneys maintained their anatomical features similarly to those derived from the formalin-fixed method.

Plastination elicited the surface color alternation in the hearts and kidneys. After plastination, the heart's brightness decreased, and the blue to yellow values increased. In contrast, the surface color values of the plastinated kidneys were not different from those of the fresh and formalin-fixed kidneys. This finding agreed with previous reports showing that the surface color of the specimens could change during each step of the plastination process (Bakici *et al*., 2019; Insal & Haziroğul, 2021; Baygeldi *et al*., 2022). In addition, variances in color changes were found between different types of tissues (Bakici *et al*., 2019)

Shrinkage has been constantly present in either whole organs or organ slices after plastination. The crucial factors include temperature, polymer viscosity (Monteiro *et al*., 2022), and tissue types (Okoye e*t al*., 2019).

During the forced impregnation, the amounts of acetone released from the tissues and the entry of the polymers must be balanced to maintain an organ's shape and size. Our plastination procedure at room temperature reduced the heart volumes to a lesser degree than the kidneys. This finding agrees with the work by Starchik and Henry (2015), which showed that the heart shrank less than the kidney. Since the heart is mainly composed of muscular tissue, and the kidney contains blood vessels and renal tubules, tissue porosity may be attributed to differences in shrinkage levels caused by plastination in various organ types (Okoye *et al.,* 2019)

The hardness of plastinated organs depends on the polymer types used to replace tissue fluid. For its flexibility, silicone is the polymer of choice (Von Hagen *et al*., 1987). KE-108 liquid silicone could render specimens' flexibility, as mentioned in the reports by Sakamoto *et al*. (2006) and Suenaga (2017). However, their hardness values were not accounted for. The current study showed that KE-108 silicone impregnation significantly increased porcine heart and kidney hardness, compared to the fresh organs. Nevertheless, the result revealed that these organs were in the formalin-fixed group were also hardened, and the hardness of both hearts and kidneys was slightly higher post-plastination. Since the formalin fixation was involved in the pre-silicone impregnation process, it suggests that the organ hardness was mediated mainly by formalin's protein denaturation effect (Brenner, 2014) and, to a lesser extent, by KE-108 silicone.

The catalyst ratio is related to the polymerization time (Sakamoto *et al*., 2006), which must be matched to the tissue entry of the polymer during the forced impregnation, thereby determining the specimen's quality. The present study demonstrated that the porcine hearts and kidneys processed with a 1.5% catalyst-to-polymer ratio, a 2% catalyst-to-polymer ratio used for plastination at freezing temperature (Sakamoto *et al*., 2006), and

a 5% recommended ratio had comparable qualities. This result was supported by Ottone *et al.* (2015), who suggested that the catalyst ratios for the silicone plastination at room temperature can be equal to or less than those used at cold temperature. In addition, the KE-108 silicone appeared to accommodate various catalyst ratios for its polymerization.

Conclusion

1. The KE-108 silicone plastination can be conducted at room temperature and produces specimens as the KE-108 plastination processed under freezing temperatures does.

2. The KE-108 silicone plastination at room temperature retained the specimen's quality similarly to the formalin fixation. However, the products from this plastination technique had no odor and were dry, making them safer for handling and easier for storage than those preserved with formalin technique.

3. The KE-108 plastination caused the color changes in porcine hearts but not in porcine kidneys. This suggests that different organ types responded to the plastination process differently

4. Different catalyst-to-polymer ratios used in this study had no significant effects on the quality of porcine heart and kidney

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