Shrinkage of Renal Tissue After Impregnation via the Cold Biodur Plastination Technique

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ABSTRACT

Thorough dehydration is a key for good plastination and invariably it leads to shrinkage. Shrinkage during plastination has been studied to lesser extent. Shrinkage was studied in 10 pig kidneys including regional shrinkage (cortex, medulla, sinus) and at which stages of the process (dehydration, impregnation, curing) shrinkage occurred. Kidneys were fixation by perfusion of 10% neutral buffered formalin solution via the renal artery. The vessels and ureter were filled with colored silicone (Dow Corning, Silastic E RTV Silicone Rubber) and the kidneys were cut into one centimeter transverse slices. Two slices of each kidney were plastinated via the classic von Hagens' method. Slices were photographed at the same focal length after preparation and at the end of each stage of plastination. Slice surface area was determined by a point-counting planimetry method. Post dehydration shrinkage of the kidney was 10.21% while post impregnation 10.11%. After completion of plastination, total area of kidney slice shrinkage was 19.72%. Cortical area shrunk 12.81% after dehydration and 13.16% after impregnation. After plastination, cortical area had shrunk 24.28%. No significant shrinkage occurred in the medulla and sinus. Results demonstrate that kidney shrinkage during impregnation is as intense as during dehydration. Significant shrinkage occurred in the renal cortex but not in the medulla and sinus. This demonstrates that different tissue types, even in the same specimen, have different rates of shrinkage during dehydration and impregnation. Therefore, plastinated specimens should be used carefully in research where obtaining measures is important. Anat Rec, 294:1418–1422, 2011.

Key words: kidney; plastination; shrinkage

INTRODUCTION

Dehydration, one of the central points for carrying out the plastination process (von Hagens, 1979), invariably leads to shrinkage (Tiedemann and Ivic-Matijas, 1988; Brown et al., 2002). Cold (−25°C) acetone has become the standard dehydrant for plastination. Ten percent shrinkage is generally considered acceptable for specimens produced by this process. Shrinkage during the plastination process has been studied briefly. However, studies designed to compartmentalize at which stage

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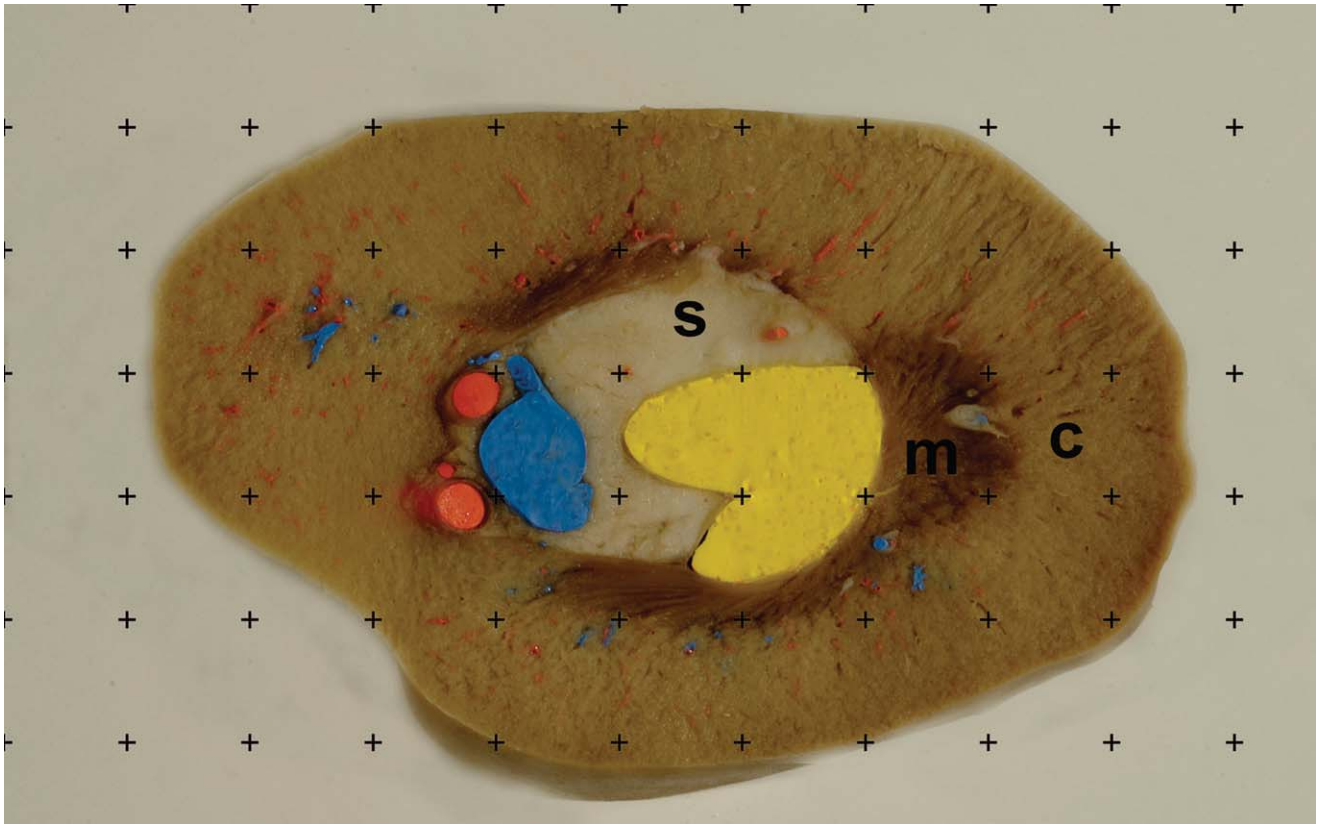

Fig. 1. Kidney slice surface covered by Weibel grid, for measurement of total surface area and area of three renal compartments (cortex [c], medulla [m] and sinus [s]). Arteries (red), veins (blue) and collecting system (yellow) are filled with silicone.

shrinkage occurs were not found. Tiedemann and Iviv-Matijas (1988) were the first to quantitate water removal from gross anatomical specimens for plastination. They measured acetone percent change via the acetone in the reservoir and also measured penetration depth of acetone in tissue blocks versus time. Their findings indicate that only a 1:5 specimen to acetone ratio is needed. As well, they discovered that acetone percentages of 60–80% did not lead to shrinkage and freeze substitution lead to <10% shrinkage. Brown et al. (2002) and Reed and Henry (2002) used fluid displacement to quantify shrinkage of a variety of organs, which were dehydrated with either room temperature or cold acetone. Sora et al. (2002) used scanned images of slices and morphometrics to identify two-dimensional (area) shrinkage associated with cold (−25°C) acetone dehydration, which was 2.13%. As well, shrinkage associated with room temperature degreasing using acetone plus impregnation with epoxy resin plus curing was measured as 4.52%. No reports were found on quantitating shrinkage for each portion of the plastination sequence, that is, dehydration, impregnation, and curing. This study quantifies the shrinkage for each of these activities.

MATERIALS AND METHODS

Animals

Twenty kidneys, from adult Yorkshire pigs of both sexes, weighing 250 kilograms, were obtained from local slaughterhouses. The institutional animal review committee approved the research protocol.

Fixation

Cannulas were placed in the renal vessels and ureter. Fixation was by perfusion of 300 mL of 10% neutral buffered formalin solution via the cannulated renal artery. Post fixation, the vessels and ureter were filled with colored silicone (Dow Corning, Silastic E RTV Silicone Rubber) using a 60-mL syringe with digital pressure until excess resistance was felt. The kidneys were submerged in 10% neutral buffered formalin solution at room temperature for 4 days to allow the silicone to harden. The fixed, injected kidneys were flushed with cool tap water to remove the neutral buffered formalin. After flushing, 1-cm transverse slices were made using a rotary deli slicer. The slices were placed in a new 10% neutral buffered formalin solution and into the cold room (3°C) for 2 weeks.

Dehydration

Before dehydration by freeze substitution (Schwab and von Hagens, 1981), the caudal surface of two slices of each kidney were photographed at the same focal length using a digital camera. Slice area was determined by a point-counting planimetry method (Weibel, 1979) (Fig. 1). After photography, the slices were submerged in

cold (−25°C) 95% acetone with an acetone to specimen ratio of 10:1.

Forced Impregnation

After dehydration, all slices were photographed, and the slice area was determined by using the point-counting planimetry method as before dehydration. After photographed, the specimens were impregnated via the classic von Hagens' method (von Hagens et al., 1987; Henry and Nel, 1993; Weiglein and Henry, 1993; deJong and Henry, 2007) using a cold silicone polymer/catalyst (NCS10/NCS3, Silicones, High Point, NC) reaction-mixture (Henry, 2007). The specimens were immersed into the cold reaction-mixture and allowed to equilibrate overnight. The next day, vacuum was applied and pressure was lowered 20 inches (50 cm) of mercury. Over a period of 35 days, the pressure was gradually reduced to 4 mmHg by observing bubble formation or lack there of. After 4 mmHg had been maintained for several days and bubble formation had nearly ceased, vacuum was released and pressure was allowed to return to ambience.

Post Impregnation

The next day (Day 1 after return to ambience), the basket of slices was raised from the polymer-mix, and the excess polymer was allowed to drain from the slices. Day 2, the slices were removed from the basket and allowed to finish draining of excess polymer at room temperature. Day 3 after impregnation, all slices were photographed to record and determine surface area.

After 1 week of draining at room temperature, the slices were placed in a closed environment containing a chain extender (NCS5, Silicones, High Point, NC). The chain extender was volatilized by an aquarium pump for 5 min daily for 1 week. The slices were manicured and turned twice a day. After exposure to the chain extender, a similar procedure was carried out using a crosslinker (NCS6, Silicones, High Point, NC) for 7 days after which time the surfaces were dry. The specimens remained in the closed environment for one more week to assure curing into the depths of the slices. The slices were now photographed for the third time after curing.

Point-counting Planimetry Method

Photographs of the 20 slices before dehydration, after dehydration, after impregnation, and after curing were analyzed. A Weibel B-100 translucent grid (Weibel, 1979) was placed over the photographs of the caudal surface of the slices, to estimate the area of the slices by using the "point-counting planimetry method" (Fig. 1). The total area of the slices, as well as the cortical, medullary and sinus areas, were determined. Thus, $A = a\,(p)\,\Sigma\,P\,u^2$, where: A = total area, a (p) = area associated with each point in the grid, Σ P = all points hitting an area in the slices and u^2 = the known bidimensional unit used in the test system (cm^2).

Statistical Analysis

The "*t*" student test was used to compare the area of kidney slices after each step of the plastination method and evaluate the significance of the shrinkage on each set of recorded values.

RESULTS

The total area of the kidney slice shrinkage was 10.21% (range: 2.84–22.70%) after dehydration and 10.11% (range: 3.15–28.44%) after impregnation. There was no significant shrinkage of the total area of the slices after curing. After all steps of the plastination technique, the total shrinkage of the kidney slice's surface area was 19.72% (range: 5.67–34.04%) (Table 1).

The cortical area of the kidney slice's surface shrunk 12.81% (range: 3.82–34.41%) after dehydration and 13.16% (range: 4.39–39.47%) after impregnation. After all steps of the plastination technique, the cortical area shrinkage of the kidney slice's surface was 24.28% (range: 11.47–45.89%). There was no significant shrinkage of the cortical area after curing (Table 2). As well, there was no significant shrinkage in the medulla (Table 3) and sinus (Table 4) compartments at any stage of the plastination process.

The visual contrast between renal cortex and medulla decreases after dehydration and almost disappears after impregnation and curing (Fig. 2). The color intensity of some of the pigments mixed with the silicone (Dow Corning, Silastic E RTV Silicone Rubber), which was injected into the vessels and ureter, was markedly decreased during the acetone baths. The yellow silicone became white and the red silicone became pink. However, the acetone did not modify the intense blue colored pigment (Fig. 2).

DISCUSSION

Although shrinkage has mainly been associated with the dehydration process during the plastination process (Schwab and von Hagens, 1981; Ripani et al., 1994; Brown et al., 2002; Reed and Henry, 2002), this report demonstrates that the kidney shrank as much during impregnation (10.11%) as during dehydration (10.21%). There was no significant difference in the shrinkage during these two stages. The most significant shrinkage of the kidney was the renal cortex ($P \leq 0.01$) during both dehydration and impregnation. There was no significant shrinkage in the medulla and sinus at any stage, demonstrating that different types of tissue, even in the same specimen, may have different rates of shrinkage during dehydration and impregnation. As the space of the renal sinus among the renal pedicle is filled by fat and acetone dehydration usually shrinks adipose tissue up to 10% (Reed and Henry, 2002) it would be expected an important shrinkage at this region. Nevertheless, because the main sinus structures (collecting system and vessels) were filled with silicone rubber (Dow Corning, Silastic E RTV), which cannot be dehydrated, the shrinkage observed was minimal.

Brown et al. (2002) used small and large specimens to demonstrate that average of shrinkage in cold acetone (14.5%) is less than in room temperature acetone (20.2%) and methanol (22.6%). Specimen volume was obtained by fluid displacement pre and post dehydration. They used a variety of whole organs and demonstrated that different tissues shrink at different rates in cold acetone: 8.8% for dog testis, 10.7% for cat heart, 12.8% for

TABLE 1. Total shrinkage of kidney slice after each step of plastination and for the entire plastination process

Shrinkage	Pre dehydration	Post dehydration	Post impregnation	Post curing	Final
Average slice area (cm^2)	35.25	31.65	28.45	28.3	28.3
Standard deviation	8.97	8.29	6.92	7.37	7.37
Average shrinkage (%)	–	10.21	10.11	0.53	19.72
Standard deviation	–	2.58	2.89	1.69	2.98
Shrinkage *t*-test	–	4.95	6.24	0.4	10.43
Shrinkage significance	–	$P \leq 0.01$	$P \leq 0.01$	ns	$P \leq 0.01$

ns - not significant.

TABLE 2. Total cortical shrinkage of kidney slices after each step of plastination and for the entire plastination process

Cortical shrinkage	Pre dehydration	Post dehydration	Post impregnation	Post curing	Final
Average slice area (cm^2)	26.15	22.8	19.8	19.8	19.8
Standard deviation	5.82	5.27	3.65	4.75	4.75
Average shrinkage (%)	–	12.81	13.16	0	24.28
Standard deviation	–	2.5	2.87	1.95	2.41
Shrinkage *t*-test	–	5.99	4.68	0	11.78
Shrinkage significance	–	$P \leq 0.01$	$P \leq 0.01$	ns	$P \leq 0.01$

ns - not significant.

TABLE 3. Total medullary shrinkage of kidney slices after each step of plastination and for the entire plastination process

Medullary shrinkage	Pre dehydration	Post dehydration	Post impregnation	Post curing	Final
Average slice area (cm^2)	3.65	3.5	3.35	3.3	3.3
Standard deviation	1.66	1.47	1.46	1.42	1.42
Average shrinkage (%)	–	4.11	4.29	1.49	9.59
Standard deviation	–	1.04	0.99	1.15	1.31
Shrinkage *t*-test	–	0.65	0.68	0.19	1.2
Shrinkage significance	–	ns	ns	ns	ns

ns - not significant.

TABLE 4. Total sinusal shrinkage of kidney slices after each step of plastination and for the entire plastination process

Sinus Shrinkage	Pre dehydration	Post dehydration	Post impregnation	Post curing	Final
Average slice area (cm^2)	5.45	5.35	5.3	5.2	5.2
Standart deviation	3.97	3.69	3.83	3.83	3.83
Average shrinkage (%)	–	1.84	0.94	1.89	4.59
Standart deviation	–	1.12	0.94	1.02	1.21
Shrinkage *t*-test	–	0.4	0.24	0.44	0.92
Shrinkage significance	–	ns	ns	ns	ns

ns - not significant.

cat and dog kidney, and 24.6% for cat liver. The protocol in this study carried this one step farther by measuring shrinkage in various compartments of the kidney (cortex, medulla, and sinus) and demonstrated variability among tissue types, even in the same organ. This points out different propensities for and/or resistance to shrinkage during cold acetone dehydration, impregnation, and curing by different tissues. When measurements are taken before and after plastination to calculate shrinkage to be able to correct the final results (Sora et al., 2002; Sebe et al., 2005; Sora and Genser-Strobl, 2005), all tissue types present in the specimens should be considered. This is especially important when morphometry is made in specimens with two or more tissues with likely very different rates of shrinkage, that is, bone versus soft tissue. One must ponder and reckon these possible differences to obtain the best results. On the other hand, in morphometric studies, when only one kind of tissue is measured, and this tissue does not shrink during dehydration and impregnation, like bone, the correction is not necessary (Genser-Strobl and Sora, 2005).

Impregnation also decreased the contrast between the renal cortex and medulla, as demonstrated in Figure 1C. This demonstrates that a staining step used between dehydration and impregnation could be helpful to maintain the contrast among different tissues of the same specimen (deJong and Henry, 2007). As plastinated specimens are used for morphometric studies, researchers must be concerned with possible changes in boundaries between tissues if this is the focus of the measurement.

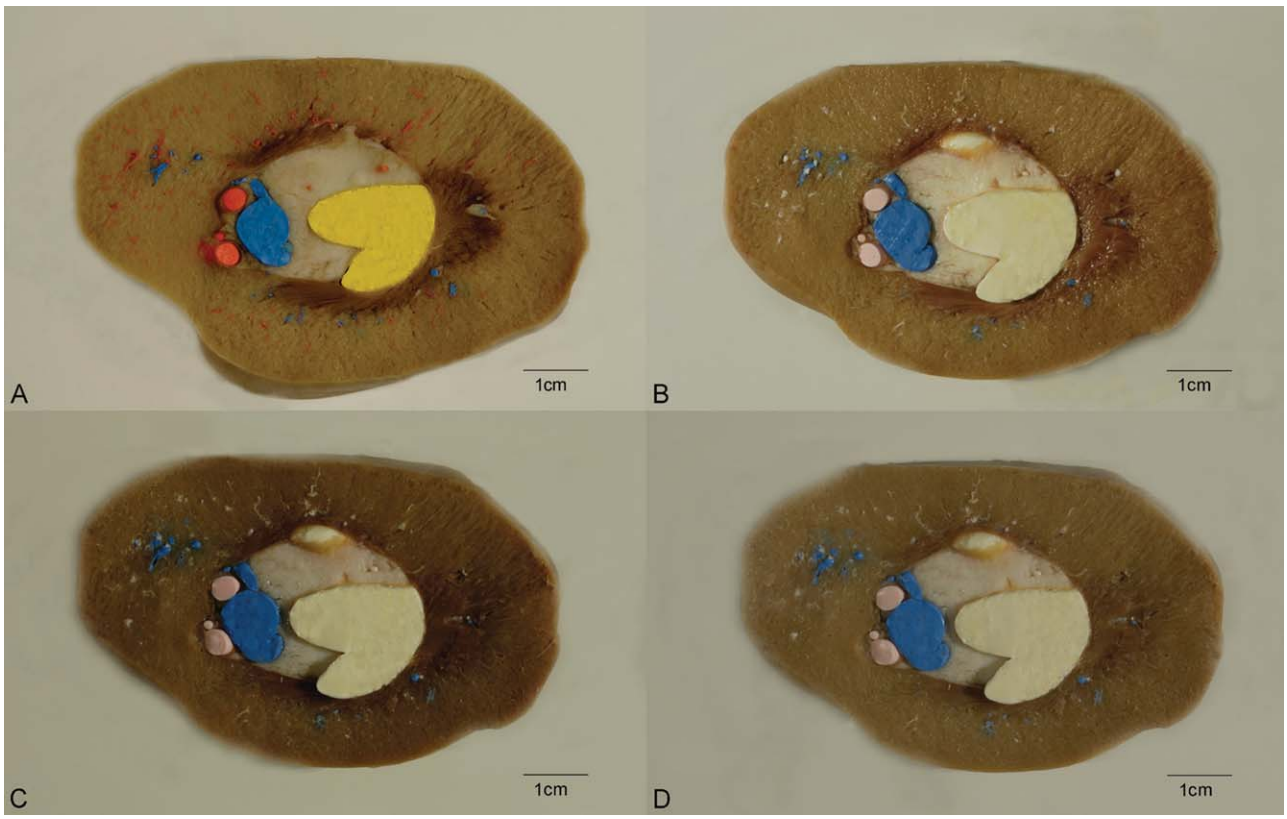

Fig. 2. Pig kidney: Transverse slices, silicone filled arteries (red), veins (blue) and collecting system (yellow). **A:** Before dehydration in acetone. **B:** After dehydration in acetone. **C:** After impregnation with polymer/catalyst reaction-mixture. **D:** After curing with chain extender (NCS5) and crosslinker (NCS6).

Some of the pigments mixed with the silicone (Dow Corning, Silastic E RTV Silicone Rubber), used for vascular and ureteral injection, lost their intense color during the acetone baths. The yellow silicone became white, and the red silicone became pink. However, the acetone did not modify the blue pigment, which suggests that the blue pigment was more stable in acetone. It would be reasonable to test the reaction between the pigments and the dehydrant solvents before their use in plastination, otherwise the pigment could be diminished during the dehydration stage.

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