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# Application of Duracryl® Plus in Making of Corrosion Casts for Macro- and Microscopical Studies of the Renal Vasculature and Collecting System in Domestic Swine

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The lack of more concrete data about application of the acrylic resin Duracryl® Plus in making corrosion casts of renal blood vessels and collecting system motivated us to undergo undertake this study. We aimed to perform modified technique for making corrosion casts for 3-dimensional (3D) studies of the kidney blood vessels and collecting system and for replicating microvasculature using Duracryl. As a result, corrosion casts of the whole renal arterial network including glomeruli were obtained. The number of cortical glomeruli in kidney, which arteries were injected with casting media with dilution of 1:2, was significantly smaller than the dilution of 1:5. With regard to collecting system, the lower dilution of casting media allowed the filling of small calices while the media with a higher dilution – the collecting tubules system of the renal medulla and even of the cortex.

In conclusion, we found that the Duracryl is suitable for making three-dimensional casts of the renal arteries and collecting tubules and also microvascular corrosion casts for stereomicroscopy and scanning electron microscopy.

*Key words:* Duracryl® Plus, corrosion casts, kidney.

## Introduction

The anatomists have tried to improve the casting media, the method of injection, and the method of removing the surrounding tissues in order to produce more accurate replica of the biological structures for five centuries. The casting or injection media must have physicochemical properties making them appropriate for the scanning electron microscopy (SEM) study of microvessels, such as low viscosity, rapid polymerization, minimal shrinkage during hardening, resistance to corrosion, cleaning, dissection and drying procedures, allow replication of endothelial structures, electron conductivity, resistance to electron bombardment [1, 8].

Many other authors have used the microvascular corrosion casting/SEM method to study not only vascular but non-vascular structures of various organs and tissues under different conditions and during development and aging [3, 5].

The semi-polymerized methyl methacrylate resin has been considered the most appropriate casting medium for the SEM study of microvascular beds due to its low viscosity, which allows the infusion of the small vessels including the capillaries and its propriety of electron reflection [5, 9]. Duracryl as a representative of these resins is a self-polymerizable acrylic resin occurring as a bicomponent system: polymer and monomer. The polymer is a methyl polymethacrylate, in the form of low powder particles, while the monomer is liquid methyl methacrylate [13]. Duracryl is one of the most widely used dental materials in the stomatology [3, 5].

There are single data of applying Duracryl in the corrosion method. Mazensky and Danko [6] observed morphological variations in the origin and course of the rabbit arteria vertebralis using Duracryl Dental (Spofa-Dental, Czech Republic) for making corrosion casts.

The existence of many similarities in the pig and human intrarenal arteries define the swine as the best animal model for investigation of kidney function and pathology [10]. Anatomical knowledge about the macro- and microvascularisation and collecting system of kidney is important for performing intrarenal surgery with minimal blood loss and minimal injury to parenchyma [4].

In this regard, we aimed to perform modified technique for making 3-dimensional casts of the kidney renal vasculature and collecting system and for replicating microvasculature by using acrylic resin Duracryl® Plus.

## Materials and Methods

### 1. Animals and materials

The left kidneys were taken from 12 mixed breed Landras and Danube white, male pigs slaughtered at age of 6 months and weighing 97 to 105 kg. Two groups of kidneys (with almost equal weight – 183.30 ± 7.04 g for the first group, and 184.96 ± 6.47g for the second group) were chosen to be filled later with a corrosion cast medium in two different concentrations. Then the obtained kidneys were prepared for performing the renal vascular and collecting system corrosion casting method using Duracryl® Plus (Spofa/Dental Product, Czech Republic) as a medium.

### 2. Method

The corrosion casting method includes the following seven steps:

#### 2.1. Precasting treatment

Before injecting the casting media into the blood vessels, the complete removal of the blood was done in order to fill the entire arterial tree with the resin. For this reason, the kidneys are perfused by syringe with saline solution through a cannula (silicone tube 5 cm long for each kidney) placed and into the renal artery and ureter, as well.

#### 2.2. Injection of Duracryl® Plus

After the precasting treatment, two different concentrations of the injection medium were prepared. The first one is consisted of 1 part Duracryl's base added to 5 parts of catalyst to initiate the polymerization, but the second one – 1 part base to 2 parts catalyst. To obtain the endocasts a yellow resin (volume 15 ml) was injected manually by syringe into the ureter to fill the kidney collecting system and a red resin (volume 20 ml) was also injected into the main trunk of the renal artery to fill the arterial tree.

### 2.3. Polymerization of casting medium

Injected specimens were placed in a water bath (at room temperature) for 48 hours to complete the polymerization of the perfused casting medium.

### 2.4. Corrosive treatment

The surrounding tissues were macerated in order to obtain the renal vascular and collecting system casts. For this purpose, the injected specimens were immersed into a highly concentrated (20%) sodium hydroxide solution (at 60 °C for 24 hours).

### 2.5. Cleaning of the corrosion casts

Washing the cast in warm running water was performed to remove the white saponified materials resulted from the maceration of tissues rich of lipid.

### 2.6. Gross dissection and microdissection

For macroscopic study, cast samples were dissected longitudinally into two equal parts. Stereo microscopy (SM) which allows 3-dimensional microscopic viewing of the objects was used for microdissection.

### 2.7. Air-drying of the casts

### 3. Macrometric study

Macrometric parameters were determined by electronic digital caliper (with 0.02 mm precision), measuring the large and small diameters of major and small calices corrosion casts of porcine kidneys using two different concentrations of Duracryl® Plus.

### 4. SM observation of corrosion casts

Stereo microscope (МБС-10 USSR) was used for 3-dimensional microscopic viewing and for counting the glomeruli.

### 5. Statistical analysis

Five pieces (with area of about 2 $mm^3$ each) were obtained by microdissection from the lateral edge of each kidney opposite the transition of the ureter in the renal pelvis. The number of glomeruli was estimated by counting them on 10 microscopic fields (× 42) per each kidney. The data about glomeruli density (number/field × 42) are presented as mean ± standard deviation (SD). Large and small diameters of major and small calices corrosion casts per each kidney were measured and the data were presented as mean ± SD. Statistical data processing was done by using Student's *t*-test. The difference was considered as significant when $P < 0.05$.

## Results

The twelve corrosion casts made from normal porcine kidneys were subdivided into two groups (6 kidneys per each group) in order to establish the most suitable concentration of Duracryl® Plus for making the precision casts of the renal arterial tree and collecting system (**Table 1**). The results confirmed the existence of one more resin appropriate for corrosion casting method on kidneys. Duracryl® Plus used as a medium for corrosion casting method showed that its mechanical properties allow to make adequate casts of the entire arterial tree of the kidney to be made. *Arteria renalis* and all its branches were filled with resin in two concentrations, which made them clearly distinguished (**Fig. 1**). Besides, the glomeruli with afferent and efferent arterioles were also filled and well visible which proved that the resin due to its low viscosity and easy penetration can fill the smallest vessels of the microcirculatory bed (**Fig. 2**). However, comparing the results of the two concentration of Duracryl® Plus using stereo microscope, it was established that significantly (approximately 2 times) more glomeruli were filled and visible by applying the lower (1:5) concentrated resin (**Figs. 3 and 4**) than 1:2 one than the other one (1:2).

The degree of the collecting system filling with Duracryl® Plus was also detected. The macroscopic measurements revealed that the large diameter of major renal calices casts and the large and small diameter of small renal calices casts made from Duracryl® Plus used in concentration 1:5 were higher than those made from the higher concen-

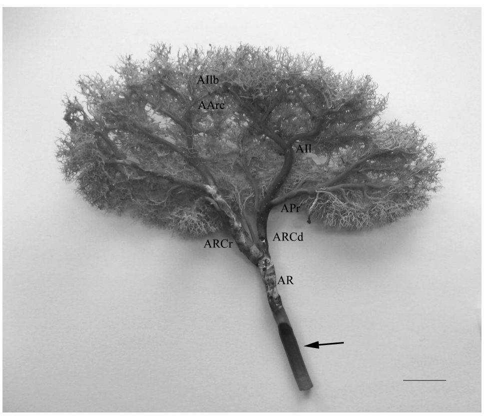


**Fig. 1.** Corrosion cast displaying arterial supply model of porcine kidney.
AR – renal arteria, ARCd – caudal renal artery, ARCr – cranial renal artery, Apr – prelobar artery, AIl – interlobal artery, AArc – arcual arteria, AIlb – interlobular arteria, Bar = 1cm

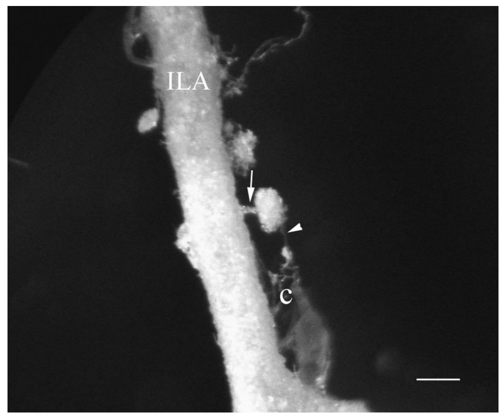


**Fig. 2.** Corrosion cast of interlobular arteria (ILA), afferent arteriole (arrow), cortical glomerulus and efferent arteriole (arrowhead) with capillary network (c). Concentration of the resin used (1:5). Bar = 140 µm

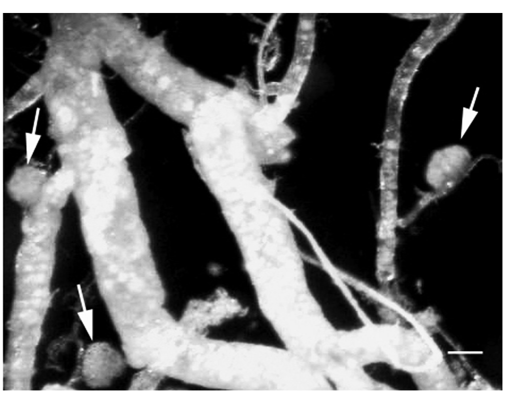

**Fig. 3.** Corrosion casts of cortical glomeruli (arrows) with higher concentrated resin. Bar = 100 µm

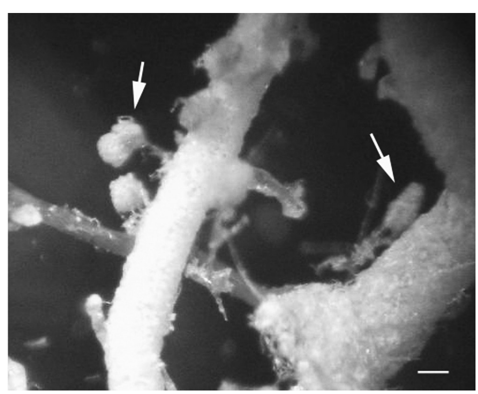

**Fig. 4.** Corrosion casts of cortical glomeruli (arrows) with lower concentrated resin. Bar = 100 µm

trated resin (**Table 1**). Our stereo microscope observations showed that the collecting tubules system of renal medulla and cortex of the entire kidney was more precisely filled with the lower concentrated resin (1:5) (**Fig. 5**). Higher concentrated resin filled the medullar and cortical parts of the collecting system in the cranial and caudal poles only (**Fig. 6**).

**Table 1.** Density (number/field ×42) of glomeruli, large and small diameters of major and small calices corrosion casts of porcine kidneys using two different concentrations of Duracryl® Plus as a medium. Data are presented as mean ± SD

| Parameters | Duracryl® Plus (concentration 1:5) | Duracryl® Plus (concentration 1:2) |
|---|---|---|
| Number of glomeruli | 4.28 ± 0.97 | 2.55 ± 1.04*** |
| Large diameter of major calices | 7.60 ± 1.47 | 9.63 ± 2.05 A |
| Small diameter of major calices | 6.30 ± 1.92 | 6.56 ± 2.23 |
| Large diameter of small calices | 11.99 ± 0.61 | 15.91 ± 1.58 B |
| Small diameter of small calices | 9.09 ± 0.62 | 11.42 ± 2.35 b |

*** $P < 0.001$ – statistical significant difference versus the number of glomeruli in lower concentration of Duracryl® Plus.
A – $P < 0.05$ – statistical significant difference versus the large diameter of major calices casts in lower concentration of Duracryl® Plus.
B – $P < 0.001$ – statistical significant difference versus the large diameter of small calices casts in lower concentration of Duracryl® Plus.
b – $P < 0.05$ – statistical significant difference versus the small diameter of small calices casts in lower concentration of Duracryl® Plus.

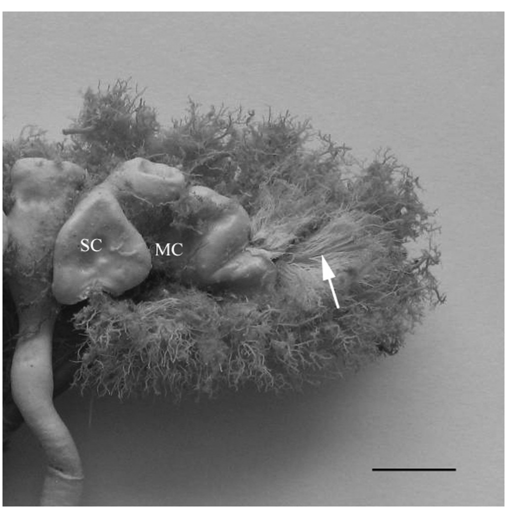


**Fig. 5.** Corrosion casts of major (MC) and small (SC) calices, collecting tubules (arrows) of renal cortex and medulla in lower concentration (1:5) of Duracryl. Bar = 12 mm

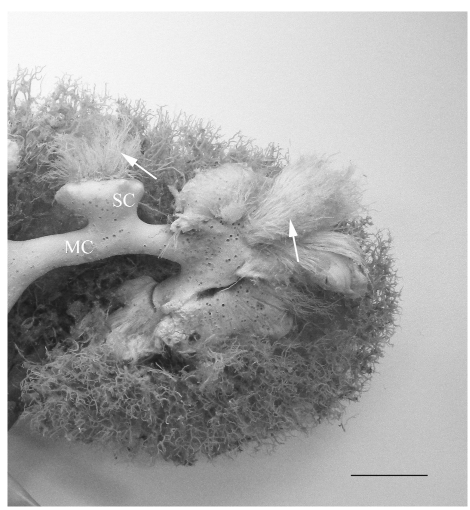


**Fig. 6.** Corrosion casts of major (MC) and small (SC) calices, collecting tubules (arrow) of renal cortex and medulla in higher concentration (1:2) of Duracryl. Bar = 15 mm

## Discussion

In this study, two concentrations of Duracryl® Plus (1 to 2 and 1 to 5 of base and catalyst, respectively) were compared in order to make more adequate corrosion casts of the renal arterial tree and collecting system. The application of Duracryl® Plus as a medium of corrosion casting technic on kidneys was established for the first time. This comparatively cheap resin allows to study entire arterial tree including glomeruli together with afferent and efferent arterioly arterioles similar to other certain, but more expensive resins such as Technovit 7100, Batson's # 17 and Mercox CL-2B [14]. The main advantage of Duracryl is that, among the resins, it is the cheapest. Many authors, who have studied the mechanical requirements of the dental materials, refer to their resistance to various types of forces and stresses, to surface hardness, to abrasion and compression forces for long time [2, 11, 15]. Our results showed that the mechanical properties of Duracryl® Plus also make this resin suitable for performing corrosion casting method in order to study in detail the features of the arterial tree and collecting system of the kidneys**.** Its low viscosity, rapid polymerization, minimal shrinkage during hardening, resistance to corrosion, cleaning, dissection and drying procedures, allow adequate replication of micro- and macrovessels, and collecting system, as well as in porcine kidney. This fact correlates with the results of Mazensky and Danko [6] that successfully used Duracryl in studying origin and course of the arteria vertebralis in rabbit. The authors conducted maceration in 2-4% KOH solution for a period of 2 days at 60-70 ºC.  However, in our study the best results about this step of the corrosion technic technique was received using 20% NaOH solution for 24 h at 60 ºC. Based on these facts, it can be concluded that species and tissue dependence exist.

So far, the anatomical relationships between the intrarenal arteries and the kidney collecting system in swine have been detected by Pereira-Sampaio et al. (2004) using polyester resin and methyl ethyl ketone peroxide as a catalyst.

The authors described the arterial tree without glomeruli and the collecting system consisted of pelvis, major and minor calices without medullar and cortical collecting tubules. It should be mentioned that, unlike them, we found that the number of cortical glomeruli in kidney, which arteries were injected with casting media with higher dilution, was significantly higher than the lower dilution. With regard to collecting system, the higher concentrated resin with higher viscosity allowed the filling predominantly of major and small calices which were bigger in diameter than those from the lower concentrated resin. Furthermore, media with a higher dilution filled the collecting system in the medulla and even in the cortex on the entire renal surface. The described differences of the resin can be explained by the better penetrating ability of the lower concentrated Duracryl® Plus. In this regard, we recommend lower concentrated Duracryl® Plus (1:5) in making corrosion casts. On the one hand, the differences between our corrosion technic with Duracryl® Plus and that used by Pereira-Sampaio et al. [10] can be explained by distinct penetration which is specific for each resin type. On the other hand, the concentration of the resin is also important.

## Conclusion

The results of the present study proved that the Duracryl® Plus is one of the most suitable resin for making three-dimensional casts of the renal arteries and collecting tubules and also microvascular corrosion casts for stereomicroscopy and scanning electron microscopy. The concentration of the resin is very important for preparing accurate casts.

Further investigations are needed to prove the suitability of this resin as a medium for corrosion casting method conducting on different organs of animals and humans.

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