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QUALITY OF CORROSION SPECIMENS PREPARED FROM MATERIAL OBTAINED DURING AUTOPSIES — A PRELIMINARY STUDY

Abstract: Aim: Aim of this study was to assess the quality of the corrosion specimens obtained during autopsies of human body for scanning electron microscopy procedures.
Materials and Method: Ninety seven uteri were obtained upon autopsy of women aged 25–56 years, deceased due to causes not related to disorders of the reproductive system. Fourty three of them contained large subserosal uterine leiomyomata. Twenty uteri were injected with acrylic emulsion Liquitex R via the arteries or veins. Five of these uteri were next dissected and cut into slides on a microtom. The remaining uteri were injected with 60–80 ml of Mercox CL-2R resin, next macerated and studied under scanning electron microscope (JEOL SEM 35-CF scanning electron microscope at 20–25 kV)
Results: Best human specimens were obtained from the autopsies carried out possibly early after the decease, young aged (between 25 and 45) and died because of multitrauma not associated with the pelvic injury.
Conclusions: Specimens obtained from autopsies can be used for scanning electron microscopy however under several conditions, specially the time between death and undertaking the injection procedures and the age of the individual, because of the process of artherosclerosis.

Key words: corrosion casting, human uterus, SEM.

INTRODUCTION

Corrosion casting together with the scanning electron microscopy are the best currently available methods for studying the vascular angioarchitecture of different anatomical structures, among others of tumors [1]. The quality of corrosion specimens depends on many factors, i.e. time which passes between the moment of decease and injection of the vascular bed. This is why not so many studies have been worldwide undertaken, carried out on the material obtained from the cadavers during the autopsies [2, 3, 4, 5, 6].

In this study we tried to establish the conditions required for reception the best quality of the specimens. The study was carried out on 97 uteri obtained from the cadavers of females aged between 25–78. All uteri were obtained and

injected with synthetic resin Mercox CL-2R during first 72 hours after decease. After corrosion casting and coating with gold the quality of the specimens was examined using SEM.

Human uterus is supplied by two uterine arteries and two ovarian arteries. Usually following age of 50 uterine arteries are narrowed or even sometimes closed by atherosclerotic obliteration. The best specimens from analogous studies carried out on animals were obtained immediately postmortem, when the injection medium completely replaces the blood. This is why the best human specimens were obtained from the autopsies carried out possibly early after the decease, young aged and died because of multitrauma not associated with the pelvic injury.

MATERIAL AND METHODS

Ninety seven uteri were obtained upon autopsy of women aged 25–56 years, deceased due to causes not related to disorders of the reproductive system. Fourty three obtained uteri contained large subserosal uterine leiomyomata. The study was approved by the Ethics Committee of the Jagiellonian University Medical College (KBET/121/8/2007). The material was collected 6–24 h after death. Each uterus together with ovaries and cervical portion of the vagina was removed in such a way that relatively long fragments of uterine and ovarian vessels (arteries and veins) were retained.

Immediately after removal, the uteri were perfused via the afferent arteries with prewarmed (37°C), heparinized saline (12.5 IU/ml heparin; Polfa, Poland, containing 3% dextrane (70 kDa) and 0.025% lidocaine (Lignocaine; Polfa), until the fluid outflowing via the veins was completely transparent (~5 min). Twenty uteri were next injected with acrylic emulsion Liquitex R via the arteries or veins [17]. Five of these uteri were next dissected and cut into slides on a microtom. The slides were next examined using immunohistochemistry for von Willebrand factor. Next perfusion of the remaining uteri was continued using a solution of 0.66% paraformaldehyde/0.08% glutaraldehyde (Sigma) in 0.1 mol/l cacodylate buffer, pH 7.4 supplemented with 0.2% lidocaine. Finally, the vascular system o these uteri was injected with 60–80 ml of Mercox CL-2R resin (Vilene Comp. Ltd. Japan) containing 0.0625 mg/ml methyl acrylate polymerization initiator (Vilene Comp. Ltd.) and the uteri were left in a warm water bath (56°C) for several hours to allow polymerization and tempering of the resin.

When the polymerization was completed, the uterine tissues were macerated for 5–6 days by repeated baths in 10% potassium hydroxide at 37°C followed by washing with warm (50–55°C) running tap water. The obtained vascular casts were washed for the next 4–5 days in multiple changes of distilled water under mild vacuum conditions, cleaned in 5% trichloroacetic acid for 1–2 days, washed again in distilled water for 2–3 days and freeze-dried in a lyophilizer (Liovag G2; Aqua Fina, Germany).

The freeze-dried casts were examined macroscopically, gently dissected [7] to expose the vasculature of endometrium and stored in an exiccator containing phosphorus pentoxide until the microscopic examination. They were then mounted onto copper plates using colloidal silver and 'conductive bridges' [8] and coated with gold. The casts were examined using a JEOL SEM 35-CF scanning electron microscope at 20–25 kV.

RESULTS AND DISCUSSION

Corrosion casting is now regarded to be one of the best techniques useful for studying topography of microvessels. It offers high resolution of almost-3-D quality of SEM images [9, 10, 11]. The quality of corrosion specimens is dependent on many factors, i.e. time which passes between the moment of decease and injection of the vascular bed, temperature of the air, etc. Not so many studies have been worldwide undertaken, carried out on the material obtained from the cadavers during the autopsies [2, 12, 13, 14, 15, 16].

In this study we tried to establish the conditions required for reception the best quality of the specimens. Simple acrylic emulsion does not penetrate the smallest vessels [17, 18] (Fig. 1), what is also proved in immunohistochemical reaction (Fig. 2).

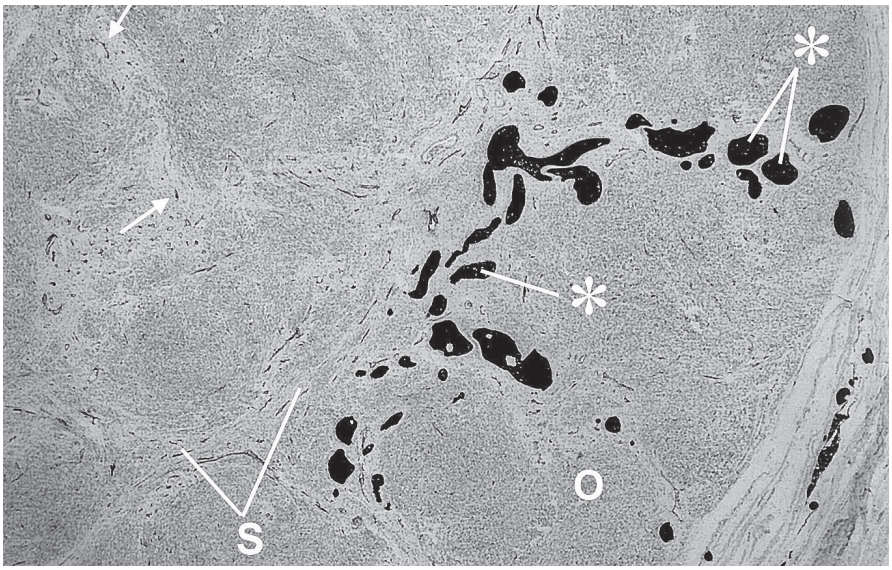

Fig. 1. Uterus of 46 year old female. Leiomyoma. Immunohistochemistry for von Willebrand factor. Specimen injected through the arteries with Acrylic emulsion Liquitex R (Binney and Smith, USA). Small not injected precapillaries and capillaries. O — outer leiomyomatous region; S — septa. Magn. 20 ×.

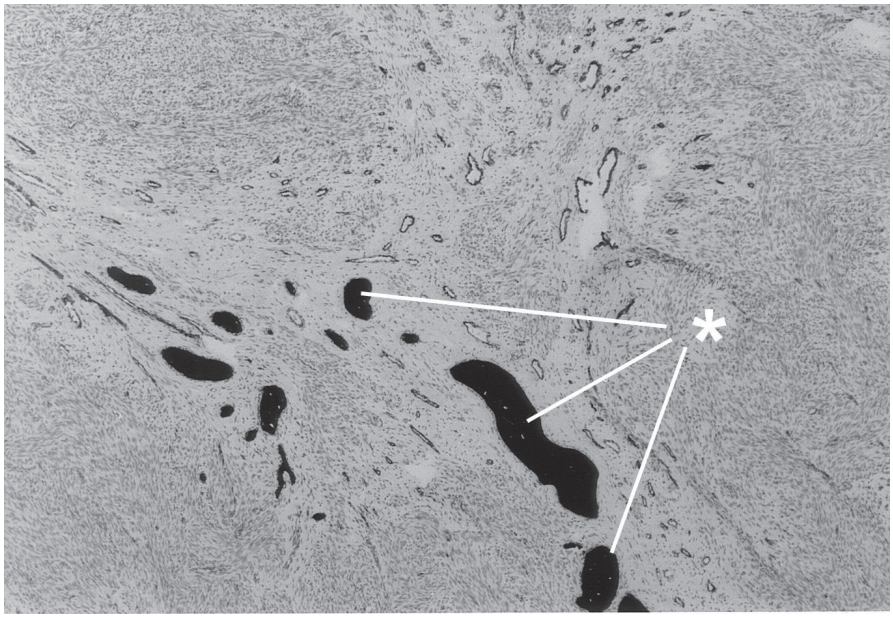

Fig. 2. Uterus of 51 year old female. Leiomyoma. Veins (*) injected with acrylic emulsion (Liquitex R, Binney and Smith, USA). Immunohistochemistry for von Willebrand factor. Magn. 40 ×.

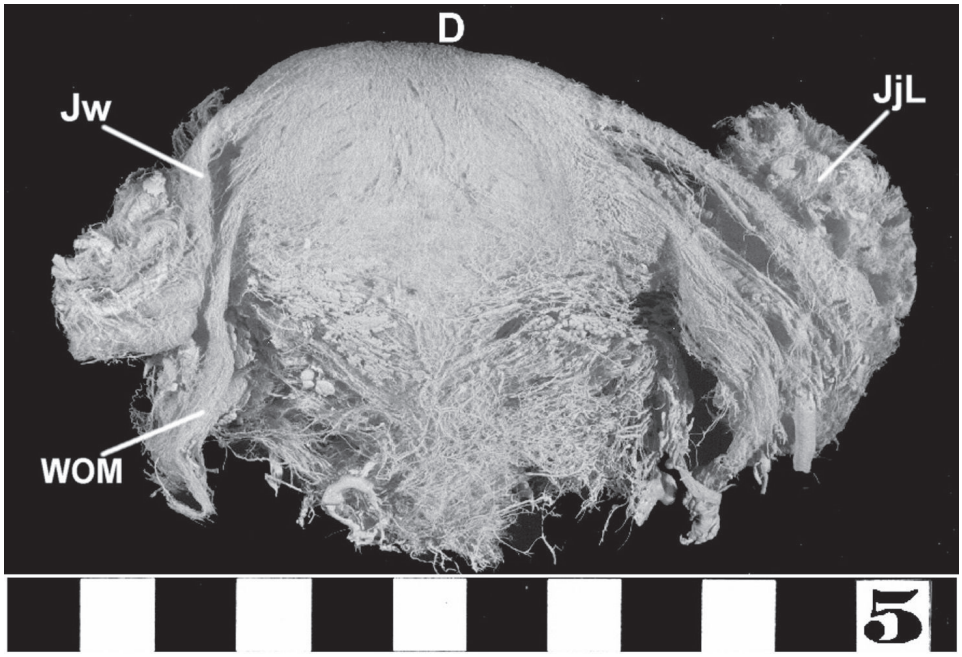

Fig. 3. Normal uterus of 25-year old female. A corrosion casts injected with Mercox. D — fundus; Jw — oviduct; JjL — left ovary; WOM — round ligament o uterus.

Injection of Mercox allows to fill the whole vascular bed but due to the fact that following age of 50 uterine arteries are narrowed or even sometimes closed by atherosclerotic obliteration filling of the vascular bed may not be successful in all cases (Fig. 3, Fig. 4).

Fig. 4. Leiomyomatous uterus of 65 year old female — note separation of fibroid from the remaining tissue caused by poor injection of Mercox. M — myoma.

The best specimens from analogous studies carried out on animals were obtained immediately postmortem, when the injection medium completely replaces the blood [12]. This is why the best human specimens were obtained from the autopsies carried out possibly early after the decease, young aged (between 25 and 45) (Fig. 5) and died because of multitrauma not associated with the pelvic injury.

Capillaries are the first vessels destroyed in the process of putrification. High pressure of injecting medium may alter the physical resistance of its wall and result in extravasations (Fig. 6). Also the position of changes we wish to observe is important because more superficial structures may be destroyed in the process of corrosion (Fig. 7).

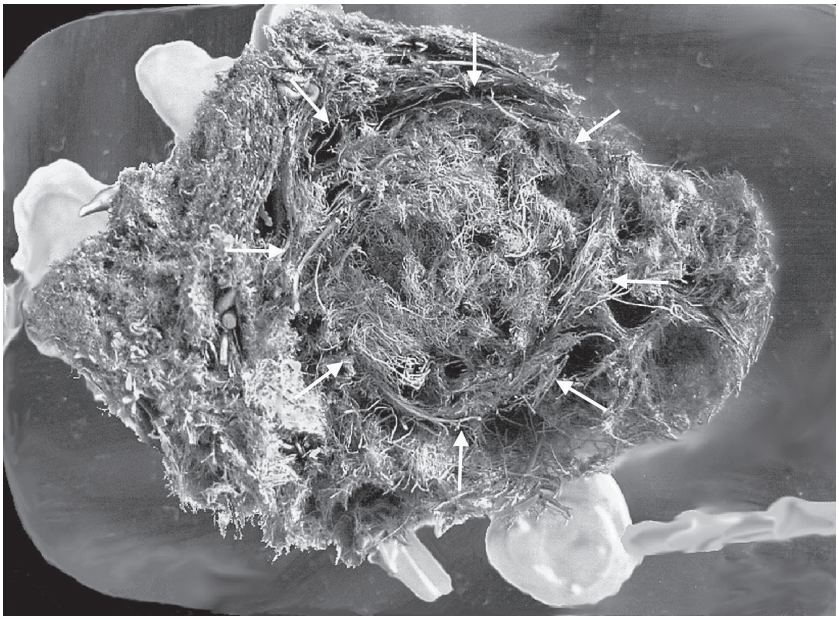

Fig. 5. Corrosion cast of 44-year old female, coated with gold, containing a single fibroid. Magn. 4 ×.

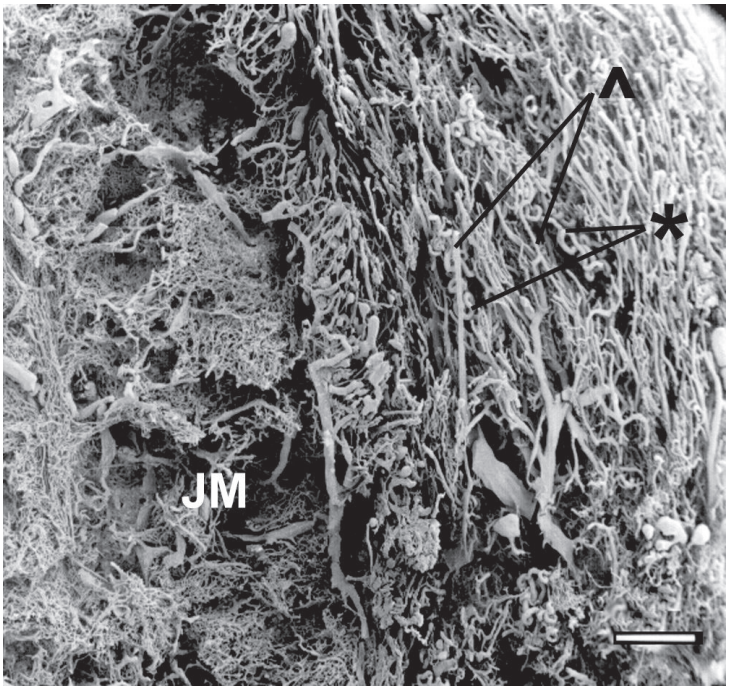

Fig. 6. Corrosion cast. SEM. Uterus of 65 year old female. Coronal section. Spiral arteries (*). JM — uterine cavity with visible extravasations.

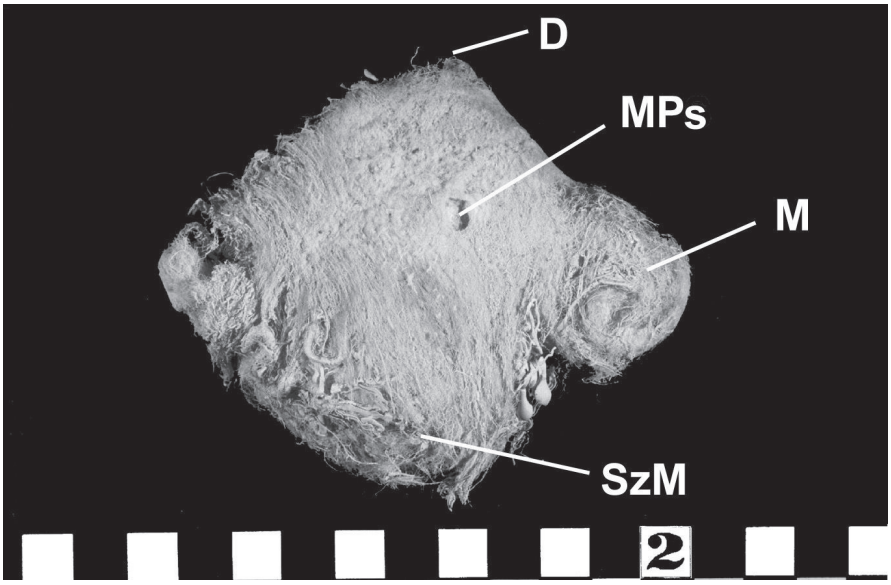

Fig. 7. Corrosion cast. Lyophilized specimen of uterus of 67 year-old female. M — myoma with visible tissue damages; MPs — damaged subserous leiomyoma; SzM — uterine cervix; D — uterine fundus.

CONCLUSIONS

Corrosion casting technique is widely applied in biological sciences. Its limitations are well known, although it does not need special equipment and may be carried out practically in all SEM laboratories. The vast majoraty of corrosion casting/SEM procedures has been performed on blood vessels. Large objects require casting after their removal from the body. This is why it is often difficult to obtain such cast of an acceptable quality. There is always a risk of intravascular coagulation, leading to a formation of an incomplete replica. This is why only several authors were able to obtain successful casts and analyze them in SEM. They were able to obtain them in organs collected upon autopsy performed within 24 hours after death. Also hormonal changes and age of the individual, as seen on the example of human uterus vasculature may play certain role which influents the quality of casting specimens.

CONFLICT OF INTEREST STATEMENT

None declared.

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