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Application of a mixture of glycol polyethylenes for the preparation of microcorrosion casts — an observation

Jerzy A. Walocha[1], Adam J. Miodoński[2], Maria Nowogrodzka-Zagórska[2], Radosława Kuciel[3], Janusz Gorczyca[1]

[1]*Chair of Anatomy, Medical College of Jagiellonian University, Kraków, Poland*

[2]*SEM Laboratory of Clinic of Otolaryngology, Medical College, Jagiellonian University, Kraków, Poland*

[3]*Institute of Medical Biochemistry, Medical College, Jagiellonian University, Kraków, Poland*

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Preparation of microcorrosion casts that can be used for observation in SEM is a laborious, time-consuming procedure. The authors paid particular attention to the process of dissection of the microcorrosion casts. This prompted the authors to reconstruct the plastic mass, produced by the firm Gurr (Great Britain) in the 1970s, which was used by them in previous research to immerse the cast in order to minimise the damage. By using easily obtainable polyethylene glycols, characterised by different physical and chemical features, in order to obtain smooth surface of the section, a low-toxic mixture was composed, which protected the microcorrosion casts sufficiently and did not interfere with the physical and chemical properties of the cast.

key words: microcorrosion casts, SEM, polyethylene glycol

INTRODUCTION

In recent years microcorrosion technique has undoubtedly played a major role in techniques used in anatomy. Studies of blood vessels were carried out with the aid of injection methods, using different types of filling masses. At the end of the 19th century scientists used very simple filling substances in respect to their chemical composition and mode of preparation, such as linseed varnish dyed with carmine or barium putty, which allowed them to obtain amazingly good results [3]. In Poland, the long-term head of the Chair of Anatomy, Professor Ludwik Karol Teichmann was a precursor, populariser and inventor of new injection techniques and injection masses used for injections. The tradition of such investigations has persisted in the Chair [7, 13].

In the 20th century, Indian ink with the addition of groundwork-binder — i.e. gelatine or animal serum, Berlin blue, Latex, Microfil and contrast medium, were used for injection of blood vessels. The substances were characterised by different size of molecules, and because of usually high viscosity (such as Latex) were not able to fill sufficiently the microcirculation. Besides they required two different colouring-stuffs to be employed to visualise independently the arterial and venous bed of the studied anatomical structures.

New injection materials, introduced during the second half of the 20th century, involved synthetic resins characterised by low viscosity and small dimension of molecules, i.e. Mercox or Batson's mass. They allowed filling of practically all blood vessels

Address for correspondence: Jerzy A. Walocha, Chair of Anatomy, Medical College of Jagiellonian University, ul. Kopernika 12, 31–034 Kraków, Poland, tel: +48 12 422 95 11, fax: +48 12 430 30 06; e-mail: mburbani@cyf-kr.edu.pl

and after their solidification, yielded microcorrosion specimens which represented casts (replicas) of the vascular system of the studied organ or tissue, including the capillary network.

However, a true turning point in angiologic methodology was the application (1970) of scanning electron miscroscope (SEM) in studies of microcorrosion vascular casts [1, 2, 4, 8–11, 14–16, 18, 20]. Thanks to high separating abilities and conspicuous sharpness, which characterises this type of microscope, new possibilities of the studies of the vascular bed were opened, specifically at the level of the microcirculation. Further progress was possible thanks to the presentation of morphological criteria [14], which allowed the differentiation of types of blood vessels and which were based on the impressions left by endothelial cells and their nuclei on surfaces of the vascular casts. The replica technique of vascular bed SEM analysis during angioarchitectonic studies of different organs or tissues yielded a lot of new information.

MATERIAL AND METHODS

The studies were carried out on microcorrosion casts of vascular structures of human uterine tumours, which were taken from cadavers during autopsies performed in the Chair of Forensic Medicine, Medical College of Jagiellonian University.

After an introductory rinsing with heparinised solution of 0.9% NaCl (3–5 minutes), the whole vascular bed was perfused with a solution consisting of: 0.66% paraformaldehyde and 0.08% glutaraldehyde prepared in 0.2 N cacodylate buffer (pH 7.4; 37°C) with Lignocaine (10 ml of 2% Lignocaine per 100 ml of buffer).

Subsequently, the vascular bed was filled with synthetic resin Mercox CR-2L (Japan, Vilene Comp., Ltd, Tokyo), mixed with the appropriate amount of the accelerator, suggested by the manufacturer. After the injection was carried out manually using a 20 ml syringe, the whole material was left in a water bath (55–60°C) to allow the injection mass to polymerise [15].

The prepared specimens were then washed several times in warm running and distilled water. Next, they were placed in 10–15% solutions of KOH for maceration. The maceration process took approximately 30 days. A solution of KOH was renewed every day after a delicate rinsing of the specimen in large amounts of distilled water. Then the microcorrosion casts were cleaned of any remnant of tissues in a water solution of 5% trichloric acid and they were rinsed in distilled water over several days. After freezing in the distilled water, they were freeze-dried in a lyophiliser (Liovag G2 Aqua Fina, Germany). Dried specimens were placed in exsiccators, in the presence of phosphorus pentoxide, to remove remnants of condensation water.

The attempts to prepare mixtures of polyethylene glycols for covering and dissection of the microcorrosion casts were dictated by the estimated application of such material, named "Aquax — water soluble wax" which was introduced in the 1970s by the Gurr company (Great Britain). Currently, no such material is commercially available. Based on the information received from the workers of BDH (which incorporated the Gurr company) it was suggested that "Aquax" was a mixture of several polyethylene glycols, characterised by different molecular masses and melting points. It was not possible to receive precise data about the composition of the material.

Because of the delicate walls of the vessels, which penetrate the mass of uterine tumours, the whole microcorrosion casts were completely immersed in the mixture of polyethylene glycols. The glycols manifested different molecular weights and different melting points and were water-soluble (Merck-Schuchardt; PEG 600: PEG 2000 at 1:20 ratio of components).

The specimens were immersed in a warm mixture of PEG (55–60°C), which was liquid, and then placed in an aluminium dish in an ice bath.

After condensation of the mixture in the form of a block, the immersed microcorrosion specimen was cut in established planes using a precise handsaw. The obtained sections (0.8–1 cm in width) were placed into a plexiglass dish (15 × 15 × 3 cm), with holes in the bottom and walls (0.5–1 cm). The dish, together with the specimen, was placed in the glass container filled with the distilled water. The bottom of the dish was placed above the level of the bottom of container filled with the distilled water. A magnet placed under the bottom of the dish was set in a rotatory motion after placing the water container on the plate of a magnetic mixer. The forced circulation rinsed the solidified mixture of PEG, soluble in water, off the specimen. As a result, the surfaces of the obtained dissections could preserve the network of the casts of blood vessels without malformation.

RESULTS

With the help of readily available chemical substances we reconstructed a mixture which did not interfere with the physical and chemical properties of the microcorrosion casts. This allowed the preservation of a quasi-three-dimensional character of the vascular structures of the studied organ, which also allowed us to cut the specimen safely, therefore lowering the risk of injury to the cast.

DISCUSSION

Microcorrosion casts can be dissected in different conditions:

— immediately after polymerisation of the casting medium is finished;
— during rinsing of the cast in distilled or tap water;
— embedded in frozen distilled water [5, 17, 21];
— while embedded in gelatine [6, 12] or after having been frozen in liquid nitrogen [6];
— after they are freeze-dried in a lyophilisator and mounted;
— repeatedly after examination of the superficial layer and its removal to examine the deeper layer;
— within the scanning electron microscope [19, 22].

The liquid mixture of PEG is transparent, which enables proper orientation of microcorrosion casts and demarcation of future planes of dissection, before it loses that ability after solidification. The edge of the plane of the dissection can be marked with the help of a thin surgical thread, which contacts the surface of the liquid PEG mass, a modification which allows the identification of the proper position after hardening of the mass. Polyethylene glycol [synonyms: alpha-hydro-omega-hydroxypoly(oxy--1,2-ethanediyl); polyglycol; polyethylene oxide; polyoxyethylene; PEG; $HO(C_2H_4O)_nH$] is soluble in water, alcohol and also in various organic solvents. Because of its low toxicity (lethal dose LD > 15 000 mg/kg) it is extensively used in the cosmetic and toiletry industry. It is a polymer of ethylene. Molecular formula:

$$HO\{-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-O\}_n-H$$

Depending on the molecular weight PEG is a transparent, colourless, odourless ropy liquid or a waxy solid substance. The molecular weight determines its properties. An increase in the molecular weight of PEG results in an increase in its viscosity and freezing temperature. PEG 600 is a substance widely used in experimental research, in which a need arises for immersing a structure in the liquid. Later, the glycol polyethylene "cover" is removed. The freezing point (19.5°C — near room temperature) is an important parameter. Although PEG is a water-soluble compound, its solubility is greatly reduced at the temperature approaching 0°C, in the majority of cases allowing experiments to run before the dissolution of PEG becomes pronounced (about 15–20 minutes). At higher temperatures (above 10°C) the dissolution of PEG mass is much shorter.

In order to prepare the described mass, PEG 600 and PEG 2000 were mixed in 1:20 ratio (Table 1).

CONCLUSIONS

1. The performed studies did not show physical or chemical interference of PEG mixture with microcorrosion casts.
2. Low toxicity of the mixture and ease of preparation cause it to be a useful and helpful material in the process of preparation of microcorrosion casts, for SEM studies.

Table 1. Physical and chemical properties of selected PEG

Physical-chemical properties of PEG 600	Physical-chemical properties of PEG 2000
1. Vapour pressure (20°C) < 0.1 hPa	1. Vapour pressure < 1 mbar
2. Specific density (20°C) 1.13 g/cm^3	2. Specific density (20°C) 1.21 g/cm^3
3. Flash point 270°C	3. Flash point 250°C
4. Solubility in water (20°C) miscible	4. Solubility in water (20°C) 600 g/l
5. M = 600 g/mol	5. M = 1800–2200 g/mol
6. pH value (100 g/l, H_2O, 20°C) 4–7	6. pH value (100 g/l, H_2O, 20°C) 4–7
7. Melting point 17–22°C	7. Melting point 49–52°C
8. Ignition temperature 380°C	8. Ignition temperature 420°C
9. Number of hydroxyl groups: 178–197	9. Number of hydroxyl groups: 51–63
	10. Bulk density 400–500 kg/m^3

REFERENCES

1. Aharinejad SH, Lametschwandtner A (1992) Microvascular Corrosion Casting in Scanning Electron Microscopy. Springer Verlag, Wien, New York.
2. Burger PC, Chandler DB, Klintworth GK (1984) Scanning electron microscopy of vascular casts. J Electr Microsc Techn, 1: 341–348.
3. Cole FJ (1921) The history of anatomical injections. In: Singer C (ed.). Studies in the history and methods of science. Oxford University Press. London, pp. 285–343.
4. Hodde KC, Nowell JA (1980) SEM of micro-corrosion casts. Scanning Electron Microscopy SEM Inc, Chicago, II: pp. 88–106.
5. Kikuta A, Ohtsukia A, Ohtani O, Murakami T (1980) Microvascularization of endocrine glands as studied by injection-replica SEM method. In: Motta PM (ed.). Ultrastructure of Endocrine Cells and Tissues. Martinus Nijhoff Boston/Hague/Dordrecht/Lancaster, pp. 313–320.
6. Kohler T (1984) Funktionelle Morphologie der Chorioidea bei Rind und Zwergziege. Habilitationsschrift zur Erlangung der Venia legendi an der Veterinär-mediz--inischen Fakultät, Universität Bern, pp. 1–141.
7. Kuś J (1969) Historia metod iniekcyjnych w naukach morfologicznych. Folia Morphol (Warsz), 28: 147–160.
8. Lametschwandtner A, Simonsberger P, Adam H (1976) Scanning electron microscopical studies of corrosion casts. The vascularization of the paraventricular organ of the toad. Mikroskopie Bd, 32: 195–203.
9. Lametschwandtner A, Miodoński AJ, Simonsberger P (1980) On the prevention of specimen charging in scanning electron microscopy of vascular corrosion casts by attaching conductive bridges. Microscopie (Wien), 36: 270–273.
10. Lametschwandtner A, Lametschwandtner U, Weiger T (1984) Scanning electron microscopy of vascular corrosion casts — technique and application. Scanning Electron Microscopy SEM Inc Chicago, II: pp. 663–695.
11. Lametschwandtner A, Lametschwandtner U, Weiger T (1990) Scanning electron microscopy of vascular corrosion casts — technique and applications: updated review. Scanning Microsc, 4: 889–941.
12. Leiser R, Luckhardt M, Kaufmann P, Winterhager E, Bruns U (1985) The fetal vascularisation of term human placental villi. Anat Embryol, 173: 71–80.
13. Miodoński AJ, Kuś J (1978) Metody iniekcyjne w badaniach układu krwionośnego. Wszechświat 7–8: 183––186.
14. Miodoński AJ, Hodde KC, Backer C (1976) Rasterelektronenmikroskopien von Plastik-Korrosion-Präparaten: Morphologische Unterschiede zwischen Arterien und Venen. BEDO Münster, 9: 435–442.
15. Miodoński AJ, Kuś J, Tyrankiewicz R (1981) SEM blood-vessel cast analysis. In: Allen DJ, Motta PM, DiDio LJA (eds). Three-dimensional microanatomy of cells and tissue surfaces. Elsevier/North Holland, New York, Amsterdam, Oxford, pp. 71–87.
16. Murakami T (1971) Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. Arch Histol Jap, 32: 445–454.
17. Murakami T, Itoshima T, Hitomi K, Ohtsuka A, Jones AL (1984) A monomeric methyl and hydroxypropyl methacrylate injection medium and its utility in casting blood capillaries and liver bile canaliculi for scanning electron microscopy. Arch Histol Jap, 47: 223–237.
18. Nowell JA, Pangborn J, Tyler W S (1970) SEM of the avian lung. Scan Electron Microsc IITRI Chicago I: pp. 249–256.
19. Reiss G, Reale E (1989) Osmium impregnation and micromanipulation. Their association in studies using secondary or backscattered electrons. In: Motta PM (ed.). Cells and Tissues. A three-dimensional approach by modern techniques in microscopy. Prog in Clinical and Biol Res, 295: pp. 563–570.
20. Walocha JA (2001) Unaczynienie mięśniaków macicy. Praca doktorska, Kraków.
21. Weerasooriya TR, Yamamoto T (1985) Three-dimensional organisation of the vasculature of the rat spermatic cord and testis. Cell Tiss Res, 241: 317–323.
22. Yoshida S, Kobayashi S, Domon T, Wakita M (1986) Microdissection of methylmethacrylate vascular casts in the scanning electron microscope. J Electron Microsc, 35: 276–279.