Clinical Anatomy 20:448–454 (2007)

ORIGINAL COMMUNICATION

Polyurethane Elastomer: A New Material for the Visualization of Cadaveric Blood Vessels

ERIC P. MEYER,[1] GERTRUDE M. BEER,[2*] AXEL LANG,[3] MIRJANA MANESTAR,[3] THOMAS KRUCKER,[4] SONJA MEIER,[5] DANIELA MIHIC-PROBST,[6] AND PETER GROSCURTH[3]

[1]*Institute of Zoology, University of Zurich, Switzerland*
[2]*Division for Plastic and Reconstructive Surgery, Bodenseeklinik, Lindau, Germany*
[3]*Division of Macroscopic Anatomy, Institute of Anatomy, University of Zurich, Switzerland*
[4]*Novartis Institutes for Bio Medical Research, Inc. (NIBRI), Discovery Technologies, Cambridge, Massachusetts*
[5]*Division for Plastic, Hand and Reconstructive Surgery, Department of Surgery, University Hospital (Academic Medical Center), Zurich, Switzerland*
[6]*Department of Pathology, University Hospital (Academic Medical Center), Zurich, Switzerland*

A multitude of various materials are available for the visualization of cadaveric vessels, ranging from natural materials like gelatin and latex to synthetic materials like silicone rubber or acrylates. To achieve a detailed overview of the vascular architecture in microvascular studies in experimental flap surgery, the injected material should have low viscosity to assure perfusion of even the smallest vessels. In addition, the material ideally should have either no or only minimal shrinkage, and should harden within a reasonable time, but retain sufficient elasticity and resistance to withstand tearing off the delicate vessels during subsequent dissection or casting. Because none of the available injection materials adequately combines these attributes, we evaluated the polyurethane elastomer ''PU4ii'' in latissimus dorsi muscles as a new material for the visualization of cadaveric vessels in comparison with the frequently used silicone rubber. The dissection of vessels injected with PU4ii proved easy largely because of its exceptional hardness. Even if not visible before dissection, the completely perfused vessels were easily palpated in the surrounding fat or muscle tissue of the microsurgical latissimus dorsi model. Despite the significantly higher hardness of PU4ii over silicone rubber (98 Sh-A vs. 12 Sh-A), PU4ii proved enough elasticity (20–25 N/mm^2 E modulus) and a high tear resistance (64–68 N/mm vs. 15 N/mm) preventing breakage during dissection even within the smallest vessels. In contrast to silicone rubber (and latex or gelatin), the high corrosion resistance and form stability of PU4ii also allowed building of casts for qualitative examination by scanning electron microscopy and quantitative analysis of the vessel density using micro-computed tomography with accurate 3D representation. In this study we show that PU4ii has physical characteristics that make it a multi-purpose material that allows at the same breath an excellent gross visualization of the architecture of cadaveric blood vessels as well as a detailed evaluation of casts by modern microscopic and or radiologic tools. Thus, the new polyurethane elastomer PU4ii is

*Correspondence to: Gertrude M. Beer, Gubelstrasse 19, 8050 Zurich, Switzerland.
E-mail: gertrude.beer@access.unizh.ch

Received 29 January 2006; Revised 29 April 2006; Accepted 9 July 2006

Published online 27 October 2006 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/ca.20403

in many respects superior to the widely used silicone rubber and can be strongly recommended as a visualization material for a comprehensive evaluation of cadaveric blood vessels in microsurgery. Clin. Anat. 20:448–454, 2007.

Key words: cadaver dissection; blood vessels; vascular injection; polyurethane elastomer; corrosion cast

---

INTRODUCTION

The visibility of exsanguinated cadaveric blood vessels has always been limited, making tracing of smaller vessels cumbersome. For this reason, as early as in the 19th century, methods were developed visualizing blood vessels within tissues and organs (Faller, 1948; Cole, 1979). Since then, a great variety of materials became available for that purpose. At the beginning, natural products such as ink or solidifying materials (e.g., gelatin, concentrated linseed oil, glue or starch) were used (Salmon, 1936; Piechocki, 1979; Steinmann, 1982; Taylor and Palmer, 1987). With the advent of modern chemistry, elastic materials like latex (Fisher, 1985) came into use, and more recently different synthetic materials like silicone rubber, polyester, or acrylic resins were introduced (Lametschwandtner et al., 1990). According to specific requirements, colored or radiopaque materials were added (Badran et al., 1984).

In general, the injection material should be easy to prepare and apply. It should be insoluble and resistant to the commonly used tissue preserving liquids. To precisely reproduce the entire vascular architecture, the injection material should feature low viscosity in order to perfuse the smallest vessels without leaking or noticeable shrinking. After curing, the material should retain sufficient elasticity and tear resistance to withstand rupture during vessel dissection. For the building of casts and the evaluation of replicated vessels with modern microscopic and radiologic visualizing tools, the injection material should have a high corrosion resistance and form stability.

None of the presently available injection materials combines these features adequately. After application some are either leaking (inks and dyes) (Kerrigan and Daniel, 1979) or are too soft (solidifying and elastic materials), or are rigid and brittle (acrylic resins) and have no corrosion resistance (natural products like gelatin and elastic products such as latex). Particularly, none of the available materials offers the desired hardness and elasticity along with an acceptable tear resistance. Yet, these features are crucial for the dissection of small vessels, such as in microvascular studies for the experimental development of new flaps in plastic surgery, including perforator flaps where the tiny vessels often have a long course within the source muscle (Heymans et al., 2004).

With regard to elasticity, silicone rubber has been the favorite injection material for dissection of vessels in microsurgery. However, it lacks sufficient tear resistance of small vessels that tend to rupture in the delicately embalmed material and has not been adopted for corrosion casts and related evaluations. To overcome these disadvantages we tested the polyurethane elastomer ''PU4ii'' (vasQtec, Zurich, Switzerland) as a new material for the visualization of cadaveric vessels in comparison with silicone rubber.

MATERIALS AND METHODS

Preparation of the Polyurethane Elastomer

The polyurethane elastomer PU4ii is an ambery, inherently fluorescent resin with a low prepolymerization, comprising the PU4ii resin and the hardener-L (slow), that can be stored and used at room temperature (Krucker et al., 2006). To lower the viscosity, the elastomer is mixed with the solvent ethylmethylketone (EMK, Merck, Germany). For the injection of blood vessels various dilutions of up to 30% (v/v) were tested without significant shrinkage or loss of replication quality (for the present application a 30% EMK solution was chosen). Then red, blue, or green pigment-containing paste (Farbpaste DW-0113-5[R], Astorit, Switzerland, 1–2% (v/v)) or radiopaque iodine (Lipiodol [R] Ultra-fluide, Guerbet, France, 20%) for X-ray or micro-CT (Wirkner and Richter, 2004) was adjoined. Shortly before injection, the hardener-L (ratio 100:18 (v/v), vasQtec, Switzerland, processing time 25 min) was added. The resulting mixture was thoroughly stirred, avoiding the formation of air inclusions. To remove occasional air inclusions, the mixture was evacuated in a vacuum system at a pressure of 30 mbar for 1–2 min. The resulting low viscosity elastomer was transferred into a syringe of suitable size and injected with dosed pressure. Low viscosity of PU4ii was retained for up to 20 min. The dissection of vessels could be started as early as 2 hr after injection. Final curing (for casting) was reached after 5 days at room temperature. At that time, the amount of shrinkage was maximally 5% (Krucker et al., 2006). Injected, embalmed specimens could be stored at room temperature over months without noticeable alterations of the injected PU4ii.

Ideally the preparation and injection of PU4ii should be performed under an actively vented hood or exhaust. Protective working clothes including butyl or vinyl rubber gloves and respiratory filters in case of insufficient ventilation (safety data sheet, ISO 11014-1) should be used. After curing, manipulation and disposal of the unused material is uncritical.

Cadaver Embalming and Injection of PU4ii

From more than 100 specimens injected with PU4ii during the last 15 years, two cadavers were used for this study which were obtained by the donation program from the

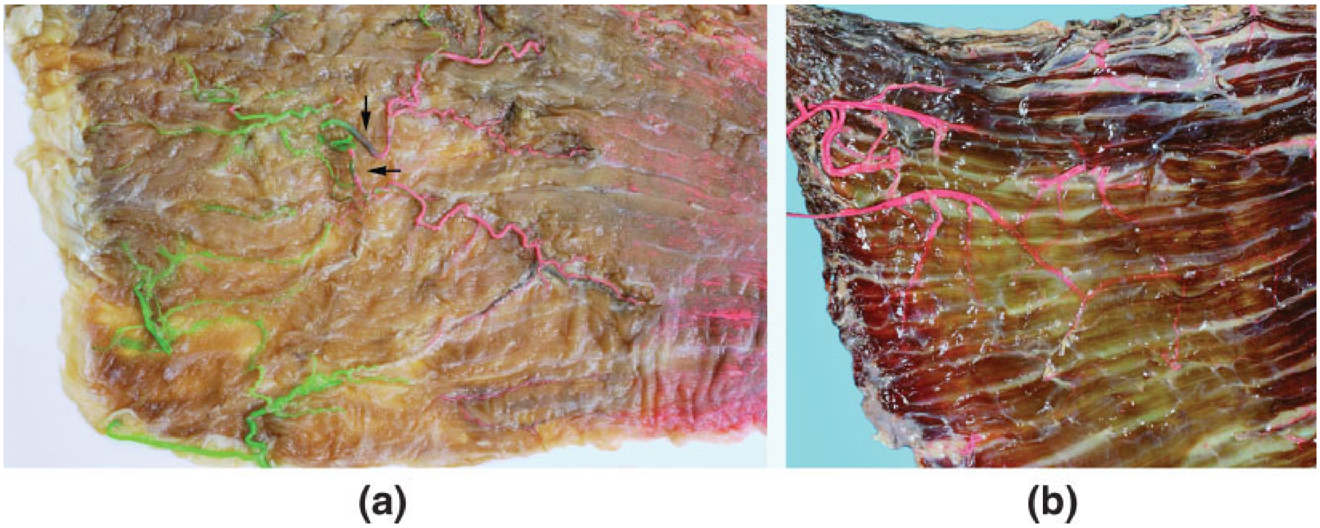

Fig. 1. Macroscopic anatomy of the latissimus dorsi muscle. **a:** Cadaver injected with PU4ii elastomer with branches of the thoracodorsal artery (red, arrow) and intercostals arteries (green, arrowhead). The vessels are tortuous, maintain their course, and can easily be palpated before dissection. **b:** Cadaver injected with red colored silicone rubber. The vessels are soft and more fragile, losing their original form and shape when dissected. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Institute of Anatomy, University of Zurich. At first, the vasculature was thoroughly rinsed and flushed mechanically at a hydraulic gradient of 1.5 m with Ringer's lactate (Pfizer, Switzerland) to prevent or remove thrombus formations. Then, the cadavers were perfused by Thiel solution (Thiel, 1992; Groscurth et al., 2001) via one femoral artery (fixation with formalin is possible as well). In the first cadaver from an 84-year old female, on both sides the thoracodorsal (red, blue) and (the three lowermost) intercostal arteries (green) were selectively injected with colored PU4ii and used as a model for testing. According to the dual blood supply of the latissimus dorsi muscle, the course of the vessels and their anastomoses were dissected. For comparison, in a 73-year old male cadaver, the thoracodorsal and intercostal arteries were filled with colored silicone rubber following standard protocol and dissected identically. Filling problems due to arteriosclerosis were circumvented by selectively injecting the target vessels, as arteriosclerosis is a common problem in our cadavers (mean age (80 ± 10) years, range 53–95, unpublished series of 88 cadavers). Generally, the thoracodorsal and the intercostal vessels are largely spared from major arteriosclerosis with the exception of constrictions at the outlet of the intercostal vessels at the aorta.

The dissected specimens injected with PU4ii were prepared for casting and various further microscopic and radiologic, qualitative and quantitative examinations.

Light Microscopy

Small fragments ($30 \times 10 \times 15$ mm^3) of the injected skin and subcutaneous tissue over both latissimus dorsi muscles were processed for routine light microscopy. The specimens were dehydrated in an alcohol series and embedded into paraffin wax. Sections of 8–10 μm were stained with Haemalaun-Eosin and van Gieson and evaluated for the presence of PU4ii in small vessels.

Clear Specimen

The left latissimus dorsi muscle was dehydrated in pure alcohol and cleared with 99% methylsalicylate (Arcos Organics, NJ) according to the Spalteholtz technique (Piechocki, 1979) to obtain an overview of the density of vessels, the branching pattern of the dominant vascular pedicle and the overall architecture of the two different vessel sources, the thoracodorsal and the intercostal vessels. When the specimen was stored in water, the clearing process could be interrupted at any time and continued at a later date.

Vascular Corrosion Casting

To test the solvent resistance and the replica quality of PU4ii, portions of the right latissimus dorsi myocutaneous flap were processed for vascular corrosion casting by standard method (Lametschwandtner et al., 1990; Krucker et al., 2004). Briefly, the specimen was macerated in 7.5% KOH (temperature 55°C) and the remaining soft tissue was removed from the PU4ii with laundry detergent.

Scanning Electron Microscopy

Corrosion casts were mounted on aluminum stabs and sputtered with 10 nm gold. The specimens and their pattern of vasculature and the condition of the endothelial tissue were then studied with a Hitachi S4000 scanning electron microscope.

TABLE 1. Physical Properties of PU4ii and Silicone Rubber as Injection Materials for Cadaveric Vessels in Comparison

	p	
Physical properties[a]	PU4ii[b] (+ 30% EMK + hardener-L)	Silicone rubber
Color	amber	whitish
Viscosity (mPa)	4,000–5,000	20,000
Processing time (min)	5	60
Linear shrinkage (%)	0.1	6
Temperature resistance (°C)	100	200
Hardness (Sh)	98 A	12 A
E modulus (N/mm^2)	20–25	3
Elongation at break (%)	375[c]	500
Tear resistance (N/mm)	64–68	15

[a]Properties given for the standard mixtures without additional dilution or additional coloring, being available in the safety data sheet of all available materials. There is no hardness scale for solidifying materials like ink or gelatine. For all other indicated materials used to fill cadaveric vessels, the physical properties are too different to fit into a single scale being available for hardness. For comparison, natural materials like Latex have a hardness of 10 A, and acrylates like Mercox[R] have a hardness, that would surpasses the Shore scale of 0–100 by far.
[b]If even longer processing times are needed, the resin PU4ii can also be mixed with a slower hardener.
[c]For comparison, acrylates like Mercox[R] have an elongation at break of only 6%.

Micro-CT

For micro-CT, the digital visualization tool with morphometrical and architectural indices down to 6 μm, the casts were freeze-dried (Krucker et al., 2006) and scanned in a desktop cone-beam Scanner CT40 (Scanco Medical, Switzerland). Image analysis and quantification of vessels was carried out with the related software.

RESULTS

Dissection of PU4ii Filled Vessels

Because of its high hardness, the dissection of vessels injected with PU4ii elastomer proved easier than that with silicone rubber (12 Sh-A vs. 98 Sh-A). Even if not visible before dissection, the vessels could be well palpated in the surrounding fat or the latissimus dorsi muscle. Despite the considerable hardness, the vessels injected with PU4ii retained ample elasticity and tear resistance to avoid rupturing during dissection. For the bigger vessels, even substantial force was needed to tear them apart. Vessels injected with silicone rubber very easily ruptured or came apart (Fig. 1), which is a significant disadvantage for dissection of small vessels in microvascular flap preparation. The ideal combination of both, ample hardness and elasticity of the PU4ii, allowed a meticulous dissection even of the smallest vessels and the anastomoses between the differently colored thoracodorsal and intercostal vessels. During this dissection, even a number of hitherto unnoticed perforators of the intercostal vessels to the latissimus dorsi muscle and the overlying skin could be detected (Beer et al., in press).

Light Microscopy

The examination under the light microscope confirmed the superior perfusion qualities of PU4ii (Table 1). It was readily found in arteries of varying size filling the entire lumen (Fig. 2). Occasionally, also arterioles were found to be filled with PU4ii. Even venules were found completely filled with PU4ii, confirming the fact that PU4ii is well able to pass through the capillary system.

Clear Specimen

The transparent specimen revealed a high density of vessels with a rich vasculature throughout the whole latissimus dorsi muscle and gave a clear insight into a seldom occurring branching pattern of the thoracodorsal pedicle with four main vessels running in a vertical direction. Because the source arteries were filled with different colors (blue for the thoracodorsal and green for the intercostal vessels), the numerous big anastomoses between both vascular systems and the according territories of blood supply could also easily be identified (a common drawback, inherent to the method of clearing and not necessarily due to the injection material, was a certain amount of swelling of all vessels and thus the occurrence of oversized anastomoses (Fig. 3).

Vascular Corrosion Casting

The fluidity characteristics and the high corrosion resistance of PU4ii easily allowed the production of casts of the vessels supplying the latissimus dorsi muscle (Fig. 4a). The casts maintained perfect quality, including elasticity, tear-resistance, and form stability up to 100°C.

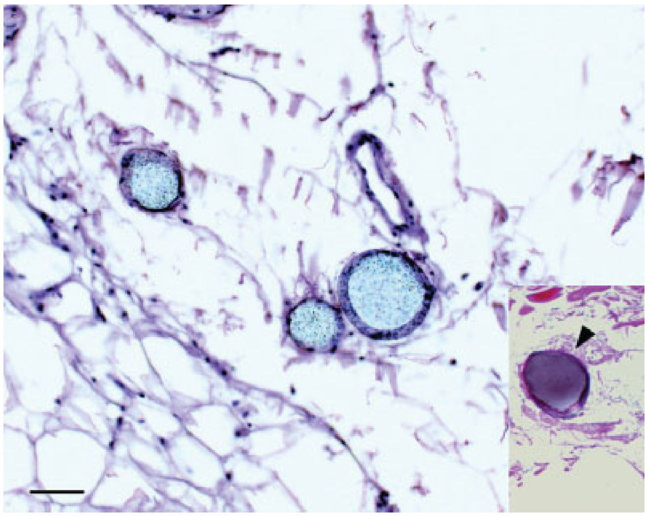

Fig. 2. Light microscopy of PU4iii injected latissimus dorsi myocutaneous unit. Three arteries of various sizes are completely filled with PU4ii; the accompanying vein appears empty (Haemalaun-Eosin x 125). Inset: Arrowhead points to the accumulation of PU4ii in a subdermal vein (Elastica van Gieson x 125). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The production of vascular corrosion casts was impossible with silicone rubber as it has no form stability.

Scanning Electron Microscopy

The qualitative examination of the casts in the SEM was possible and allowed good insight into the general arrangement of the microvasculature of the myocutaneous latissimus dorsi flap. Of equal importance, the SEM also allowed meticulous assessment of the surface condition of the endothelial tissue of both the arterial and venous system characterized by different cell imprints. For example, in the arteries, the surface of the cast showed oval shaped impressions resulting from the projections of the endothelial cell nuclei (Fig. 4b).

Micro-CT

Whereas SEM allows a detailed analysis of the vascularization mostly at the surface of the specimen, micro-CT scans offer the possibility to visualize the vessels also underneath the surface. In addition, reconstructions of micro-CT scans provide digital 3D data sets suitable for a thorough characterization and quantification of the entire vasculature (Fig. 4c). If vessels are color coded using a standard feature of the micro-CT software, easy recognition of vessel hierarchy is possible. In the conventional micro-CT, however, resolution of the smallest vessels is limited. In the present study, we set the nominal resolution at 6 μm.

DISCUSSION

In this study, we show that the new polyurethane elastomer PU4ii has exceptional physical characteristics for the visualization of cadaveric blood vessels because it is fairly hard (therefore reduced breakage during dissection) and because it is fairly elastic at the same time, which enhances dissection of injected tissues. Combined with the ideal viscosity, and an otherwise unequalled tear and corrosion resistance, PU4ii is also the perfect material for the production of vascular casts. Beyond good visualization and easy dissection of vessels in microsurgery, the possibility of producing corrosion casts also allows a meticulous evaluation of the general vascular architecture, the endothelial condition of vessels, the branching pattern of vessels, the delineation of vascular territories, and an exact calculation of the vascular density. Thus, PU4ii has decisive advantages over the widely used silicone rubber in so far that PU4ii is the only injection material that can simultaneously be used with no or very minimal post treatment in modern imaging procedures, like the SEM, the micro-CT (resolution down to 6 μm), and the synchrotron radiation-based micro-CT (resolution down from 100 to 3 nm) (Castenholz, 1995; Minnich et al., 2001). Not even casts made with the commonly used acrylate resins are equally suitable for these imaging technologies, as the smallest vessels are insufficiently perfused and the cured materials are tremendously rigid and brittle. For acrylate resins (such as Mercox [R]), the elongation at break is below 10% in contrast to the PU4ii resin that can be stretched up to 375% until rupture (Krucker et al., 2006) (Table 1). These physical characteristics make PU4ii the preferred visualization and casting material for cadaveric vessels, especially in anatomical studies focusing on microvasculature in experimental flap surgery where a very targeted dissection of the vessels is necessary (Angrigiani et al., 1995a,b). The easy way to handle the vessels during preparation, dissection or further processing puts almost no limitations on the size of the tissue or organ of interest.

Recently it has been shown that PU4ii can also be used for injection of blood vessels in rodents. By this procedure, the vascularization of various organs including the brain can be studied in detail under experimental conditions (Krucker et al., 2006).

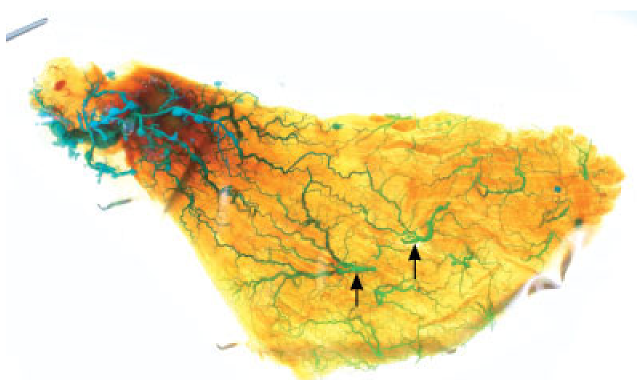

Fig. 3. Latissimus dorsi muscle cleared with methylsalicylate. The vessels filled with PU4iii are clearly visible as a very dense network. Thoracodorsal vessels are blue and the intercostal vessels are green. Arrows point to the anastomoses between the two source vessels. The caudal 1/3 of the latissimus dorsi muscle is exclusively supplied by intercostal vessels. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

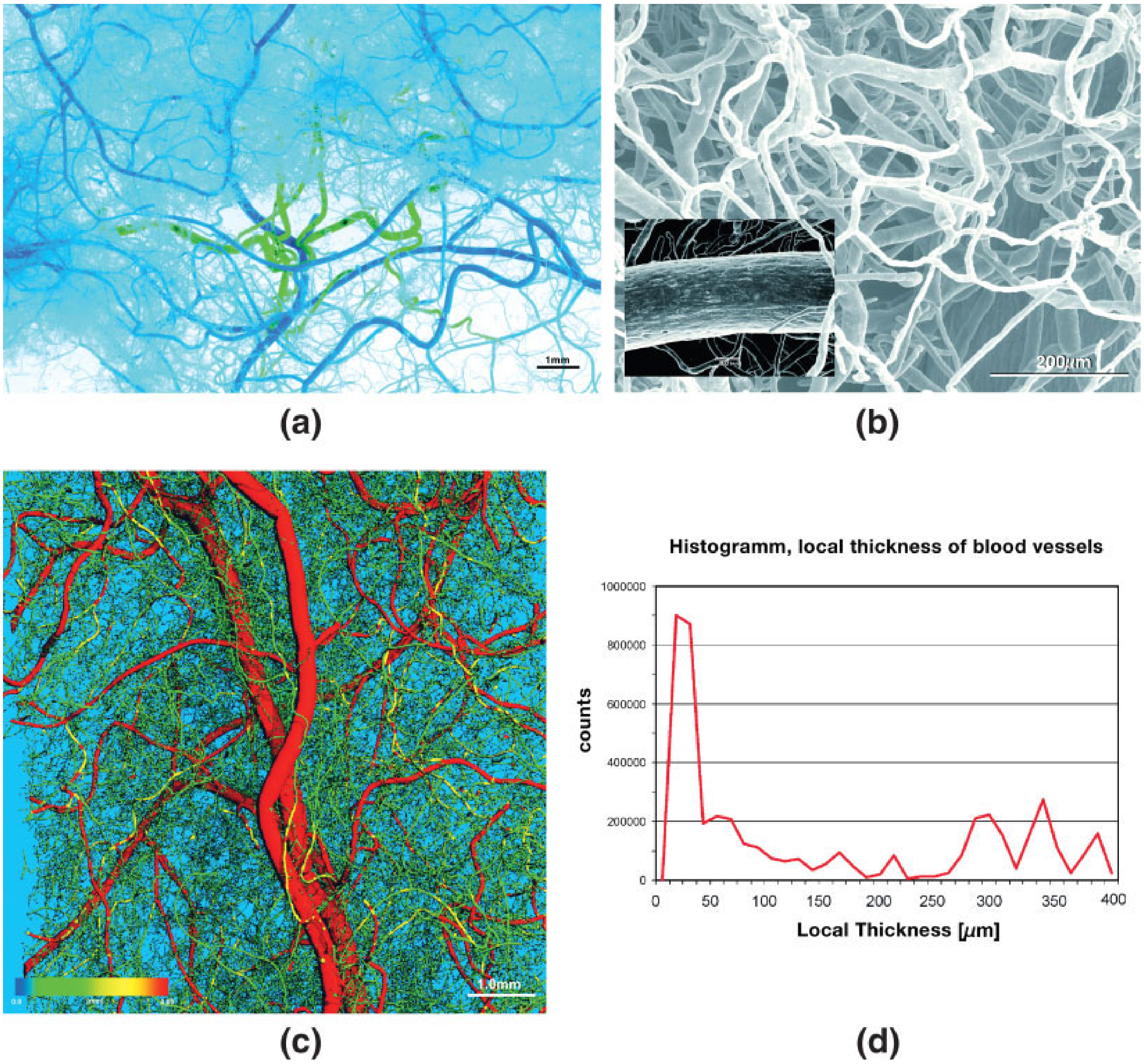

Fig. 4. Vascular corrosion cast of the thoracodorsal/intercostal vessels injected with PU4ii elastomer. **a:** Macroscopic view of a macerated area of the soft tissue overlying the latissimus dorsi muscle. Branches of the thoracodorsal artery are blue and those of the intercostal arteries appear green. The vast number of small and smallest vessels imparts the impression of a cloudy appearance within the cast, yet the dense meshwork of vessels gives additional rigidity and form stability to the cast. **b:** SEM micrograph from the cast shown in (a). Note the dense capillary network surrounding mostly fat cells (arrow). The arrowhead shows one of the blind ending vessels, indicating incomplete filling because of residual thrombus formation (this may be the case when cadavers arrive with delay at the institute and are not completely fresh any more). The inset shows the surface of an artery with the typical oval shaped impressions of nuclei from endothelial cells (overview at a magnification of 50 µm). **c:** Partial 3D reconstruction from micro-CT scan showing thoracodorsal vessels with color coded representation of the vasculature of the subcutaneous tissue overlying the latissimus dorsi. Finest capillaries are not shown. The distribution of vessel thickness is calculated quantitatively and plotted in the adjacent histogram (**d**) (a quantitative analysis of vessel density is also possible). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Concerning the disadvantages, the toxicity of PU4ii is comparable to that of the acrylates, and when freshly prepared may temporarily be more toxic than diluted silicone rubber.

Weighing the advantages and disadvantages of the new PU4ii elastomer, it is a new multi-purpose material, which is in many respects superior to silicone rubber and can be strongly recommended as visualization material for cadaveric blood vessels in microsurgery.

This kind of visualizing vessels in microsurgery with the application of modern evaluation tools does not only lead to an enhanced evaluation of the architecture of blood vessels but may lead to an enhanced understanding of the mechanisms of flap failure due to changes in the arrangement of vessels or inherent or oxygen-depleted changes of the endothelial tissue.

ACKNOWLEDGMENTS

The authors thank Mr. H. Sonderegger for the provision of the light micrographs, Alexandra Schuler and Urs Jauch for their excellent technical help, and Markus Burkhart (Scanco Medical) for his support and help with the micro-CT.

REFERENCES

Angrigiani C, Grilli D, Siebert J. 1995a. Latissimus dorsi musculocutaneous flap without muscle. Plast Reconstr Surg 96:1608–1614.

Angrigiani C, Grilli D, Siebert J, Thorne C. 1995b. A new musculocutaneous island flap from the distal thigh for recurrent ischial and perineal pressure sores. Plast Reconstr Surg 96:935–940.

Badran HA, El-Helaly MS, Safe I. 1984. The lateral intercostal neurovascular free flap. Plast Reconstr Surg 73:17–26.

Beer GM, Manestar M, Lang A, Kompatscher P. The bipedicled latissimus dorsi free and perforator flap: An anatomic study. Plast Reconstr Surg, in press.

Castenholz A. 1995. Examination of injected specimens by confocal laser scanning microscopy and scanning electron microscopy. Scanning Microsc 9:1245–1253.

Cole FJ. 1979. The history of anatomical injections. Studies in the history and method of science. In: Piechocki, R. *Makroskopische Präparationstechnik*. Stuttgart: Gustav Fischer Verlag.

Faller A. 1948. Die Entwicklung der makroskopischen Präparierkunst von Galen bis zur Neuzeit. *Acta Anatomica*, Suppl. 7.

Fisher J. 1985. External oblique fasciocutaneous flap for elbow coverage. Plast Reconstr Surg 75:51–61.

Groscurth P, Eggli P, Kapfhammer J, Rager G, Hornung JP, Fasel JDH. 2001. Gross anatomy in the surgical curriculum in Switzerland: improved cadaver preservation, anatomical models and course development. Anat Rec 265:254–256.

Heymans OY, Nelissen XP, Peters S, Lemaire V, Carlier A. 2004. New approach to vascular injection in fresh cadaver dissection. J Reconstr Microsurg 20:311–315.

Kerrigan CL, Daniel RK. 1979. The intercostal flap: an anatomical and hemodynamic approach. Ann Plast Surg 2:411–421.

Krucker T, Schuler A, Meyer EP, Staufenbiel M, Beckmann N. 2004. Magnetic resonance angiography and vascular corrosion casting as tools in biomedical research: application to transgenic mice modeling Alzheimer's disease. Neurol Res 26(5):507–516.

Krucker T, Lang A, Meyer EP. 2006. New polyurethane based material for vascular corrosion casting with improved physical and imaging characteristics. Res Tech 69:138–147.

Lametschwandtner A, Lametschwandtner U, Weiger T. 1990. Scanning electron microscopy of vascular corrosion casts-technique and applications: updated review. Scanning Microsc 4:889–940.

Minnich B, Bartel H, Lametschwandtner A. 2001. Quantitative microvascular corrosion casting by 2D- and 3D-morphometry. Ital J Anat Embryol 106:213–220.

Piechocki R. 1979. *Makroskopische Präparationstechnik*. Stuttgart: Gustav Fischer Verlag.

Salmon M. 1936. *Arteres de la Peau. Etude Anatomique et Chirurgicale*. Paris: Masson.

Steinmann W. 1982. *Makroskopische Präpariermethoden in der Medizin*. Stuttgart: Georg Thieme Verlag.

Taylor GI, Palmer JH. 1987. The vascular territories (angiosomes) of the body: experimental study and clinical applications. Br J Plast Surg 40:113–141.

Thiel W. 1992. The preservation of the whole corpse with natural color. Anat Anz 174:185–195.

Wirkner CS, Richter S. 2004. Improvement of microanatomical research by combining corrosion casts with microCT and 3D reconstruction, exemplified in the circulatory organs of the woodlouse. Microsc Res Tech 64:250–254.