J Mater Sci: Mater Med (2008) 19:1265–1274
DOI 10.1007/s10856-007-3236-x

Poly(2-hydroxyethyl methacrylate) emboli with increased haemostatic effect for correction of haemorrhage of complex origin in endovascular surgery of children

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Received: 18 December 2006 / Accepted: 5 July 2007 / Published online: 21 August 2007

Abstract Poly(2-hydroxyethyl methacrylate) (PHEMA) embolization particles with enhanced haemostatic properties were prepared by bulk or suspension polymerisation of 2-hydroxyethyl methacrylate (HEMA) followed by particle soaking in ethamsylate solution. The particles accelerated thrombus formation as evidenced by blood analysis of rabbits with implanted emboli. Usefulness of both spherical and cylindrical PHEMA particles with enhanced haemostatic effect was demonstrated on the embolization of arterial anastomosis, fistulas of the lower extremity and abdominal cavity, haemangioma and arteriovenous malformation of the head of several children.

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1 Introduction

Interventional radiology employing endovascular embolization of blood vessels is widely used in vascular surgery with the aim to exclude the pathological blood supply to the tumour starving then its cells to death. It is also used as a measure reducing blood loss during surgery and as a treatment of bleeding of various origins. Literature data demonstrate extensive indications for endovascular occlusion of blood vessels in various forms of angiodysplasia, haemangiomas, especially of complex anatomic localization, which is necessary as a single-step treatment, as well as in combination with other effective methods [1]. The aim of proximal embolization is an occlusion of magistral blood circulation with preservation of anastomoses on segmental and subsegmental level. Combined occlusion consists of termination of both distal and proximal blood circulation of a single organ or blood vessel basin. Endovascular occlusion is classified as a single-step or a total multistep measure.

Therapeutic embolization is based on the introduction of an occluding embolic material into the blood vessel via a catheter. The efficiency of the embolization in haemorrhages primarily depends on selection of embolic materials [2]. Desirable requirements for an embolic material include non-toxicity, non-antigenicity, hydrophilicity, stability, radiopacity, and possibility to control localization of emboli in vascular bed. Even that up to now a universal embolization material has not been prepared, a variety of agents was used for the embolization. They include small pieces of autologous tissue (blood clot, fat, fibrin sealant, and albumin), dura mater and cartilage tissue; however, all of them show only a short-term effect. Other degradable materials are based on poly(2-hydroxyethyl acrylate) [3], poly(lactic acid/glycolic acid) [4], dextran crosslinked gelatine [5] or chitosan microspheres [6]. Alternative embolization by means of balloons,

e.g. detachable latex balloons suffers from other drawbacks, such as difficult manufacture and construction, high cost, and impossibility to use them for occlusion of magistral arteries larger than 5 mm. Embolization with Gianturco coils, which are not carried away by blood flow and remaining in the place of introduction when they revert to a three-dimensional shape that fills the vessel [7, 8] and embolization with metal coils [9] also suffer from some drawbacks. In some cases, inflammation occurs. A negative aspect of endovascular occlusion by electrocoagulation consists in deep burns of surrounding tissues. Liquid embolization agents include ethanol solutions [10], silicone, solidifying emulsion, estrogen/ethanol and poly(vinyl acetate) [11, 12]. Tissue glues (bucrylate, isobutyl 2-cyanoacrylate [13], polyurethane [14], Trombovar, sodium tetradecyl sulphate [15], acrylic glue) belong to this category of embolization materials. Also injectable temperature-responsive systems forming a gel in the body were proposed for vascular embolization, e.g. poly(*N*-isopropylacrylamide-*co*-*N*-propylacrylamide-*co*-vinylpyrrolidone) [16], poly(ethylene glycol-*co*-lactic acid-*co*-glycolic acid) emulsion with Lipiodol [17], or poly(*N*-isopropylacrylamide-*co*-2-hydroxyethyl methacrylate) in combination with a chemical crosslinker [18]. Cyanoacrylate derivatives and polymer solutions, however, can cause the catheter to adhere to the artery, and they require the use of organic solvents [13]. Embolization with solid materials includes Teflon particles of irregular shape, Ivalon (poly(vinyl alcohol) sponge [19]), microfilamentous collagen, gelatine sponge (Spongel) [20] inducing recanalization, metallic beads, pollen, poly(methyl methacrylate) particles. Recently, Embosphere^{TM} microspheres (trisacryl gelatine, Biosphere Medical, Rockland, MA, USA) were marketed [21, 22]. In general, biocompatible particles of regular, at best spherical shape are needed.

Previously, we reported the preparation and clinical application of poly(2-hydroxyethyl methacrylate) (PHEMA) emboli [23–25]. PHEMA emboli are inert; they do not induce injury and inflammatory reaction of the vascular wall. They provide a good immediate occlusion effect due to their ability to swell in the blood vessel lumen. PHEMA emboli almost do not degrade because of their three-dimensional crosslinked structure. Biostability of PHEMA implants (e.g. intraocular lenses) was proved during their 50 years of application in ophthalmology [26]. Yet, the emboli can be inadequate in urgent situations in massive arteriovenous haemorrhages, when the result depends on the speed of thrombus formation.

The aim of this study was thus to develop PHEMA hydrogel with enhanced haemostatic effect, to determine optimal doses for occlusion of blood vessels in arteriovenous malformations and fistulas in children in localizations difficult to access and to investigate its properties in the treatment of the above disorders. The hydrogel particles combine two effects: mechanical occlusion of blood supply by means of emboli and local potentiation of thrombus formation. Such a combination is ideal for discontinuation of massive haemorrhages in various forms of angiodysplasia of complex localization or in traumatic bleedings. Another goal was to examine the effect of such a material on blood clotting process.

2 Experimental

2.1 Materials

Monomers 2-hydroxyethyl methacrylate (HEMA; Röhm GmbH, Germany) and ethylene dimethacrylate (EDMA; Ugilor S.A., France) were distilled in vacuum before use. 2,2′-Azobisisobutyronitrile (AIBN; Fluka) was twice crystallized from ethanol. Dicynone (ethamsylate; OM Pharma, Geneva, Switzerland), solvents and other chemicals were used as received.

2.2 Preparation of PHEMA emboli

Preparation of spherical emboli and their classification according to size was described earlier [23]. For preparation of cylindrical emboli, a mixture of 40 wt.% of monomers (HEMA, EDMA, 2 wt.% and AIBN, 1 wt.%, relative to total monomers) and 60 wt.% water filled in glass capillaries ranging from 0.5 to 2 mm in diameter. Polymerisation started at 70 °C and continued for 8 h. After cooling, PHEMA emboli were pushed out from capillaries with a mandrel. Then, the hydrogel was thoroughly washed by boiling in water to remove all unreacted by-products. The degree of purity of hydrogel emboli was checked by UV spectrometry [23]. Absorbance of washing water at 190 nm (1 cm cell) has to fall below 0.3 AU to ensure that 1 g of emboli does not release more than 10^{-3} g of low-molecular-weight impurities [23]. Such an amount is known to be non-irritating. Resulting cylindrical emboli, on average 20–30 pieces depending on the diameter from 0.5 to 2 mm and length 10–15 mm, were put into bottles filled with 20 mL of distilled water and sterilized in an autoclave at 120 °C and 0.12 MPa for 30 min. Optical micrographs were taken with an Opton (Zeiss, Jena, Germany) microscope.

2.3 Preparation of emboli with enhanced haemostatic properties

PHEMA emboli (0.1 g of dry weight) were soaked in a water solution containing 0.1 mL of 12.5 wt.% ethamsylate for 24 h under sterile conditions. Ethamsylate was

adsorbed, not chemically bound to PHEMA particles, and its release from PHEMA emboli was described earlier [27]. The ethamsylate content in PHEMA emboli (0.05 g per g of PHEMA) was determined by weight difference before and after drying for 24 h at 100 °C.

2.4 Animal experiments

Experiments were carried out on 40 rabbits breed chinchilla of 2–3 kg weight according to EU guidelines for care and use of laboratory animals. The number of rabbits at the given time points is denoted in Table 1. After proper preparation of the skin of medial surface of the thigh (treatment with 5% iodine and 70% ethyl alcohol solutions), a 3 cm section of skin was made parallel to the vessel bundle under local anaesthesia with 10 mL of 0.25% Novocain solution, and femoral arteries were isolated. Transverse arteriotomy 2–3 mm long was performed in the upper third of femoral artery. Catheterisation of femoral artery was performed in the direction of blood flow with silicone catheter of 0.5 mm ID. The investigated modified emboli were introduced via catheter into the lumen of artery in the distal direction. After embolization (1 embolus 0.5 mm in diameter, 10–15 mm long), the catheter was removed. The proximal and distal artery sections were ligated in 30 cases, the arteriotomy site in the artery wall was sutured by knotty sutures and atraumatic threads Prolen 7/0, without lumen contraction in 10 cases. The wound was sutured.

The procedure used for histological preparations has been reported earlier [23].

2.5 Morphological analysis of blood elements and determination of blood coagulation properties

To investigate the effect of ethamsylate-modified cylindrical emboli, 0.5 mm in diameter and 10 ± 3 mm long, on the thrombus formation and on the blood vessel wall in experiments with 20 rabbits, they were implanted in their femoral artery. As a control, standard ethamsylate-free cylindrical PHEMA emboli of the same size were implanted in 20 other rabbits in analogous experiments. All tests were evaluated in 20 min–6 h in acute experiments and in 1–100 days in chronic experiments. As a criterion of the influence of PHEMA emboli with enhanced haemostatic properties on blood coagulation, the following indicators of starting blood were considered: changes in the number of thrombocytes (L^{-1}), fibrinolytic activity (h, min), fibrinogen (g/L), fibrinase (%), thrombin time (s), prothrombin index (%) and activity of antithrombin XIII (%). Based on the investigation of morphological changes in both blood and tissues surrounding the emboli at different time periods after endovascular occlusion, clinical characteristics of the investigated emboli and their suitability for endovascular occlusion were evaluated.

2.6 Diagnostic methods

Angiography was performed with a Philips Allura (Netherlands) X-ray system. Angiographic catheters from Cook (Denmark) and BALT (France), and also Y-connectors and guide wires were used in this report. Scanning was programmed with regard to the blood flow rate in vessel magisterial to the affected zone in order to fix not only the arterial, but also venous phase. At a high velocity of blood flow, the scanning speed was 6 frames/s during first 2 s, and then 1 frame/s during 8–9 s.

2.7 Catheterisation by femoral approach

Pulsation of the femoral artery was identified 1–2 cm below the inguinal ligament, the needle inserted to the pulsation and aimed towards the head and medially at an

Table 1 Changes of blood indicators 1, 14 and 60 days after occlusion of femoral rabbit artery with ethamsylate-modified emboli and unmodified emboli

Indicator	Starting blood	1 day		14 days		60 days	
		Unmodified	Modified	Unmodified	Modified	Unmodified	Modified
	$n = 6$	$n = 8$	$n = 6$	$n = 6$	$n = 7$	$n = 6$	$n = 7$
Fibrinolytic activity (min)	180 ± 17	260 ± 15	360 ± 13	200 ± 9	310 ± 21	210 ± 7.5	200 ± 10
Fibrinogen (g/L)	2 ± 0.2	3.7 ± 0.2	4 ± 0.3	2.5 ± 0.2	3.6 ± 0.5	2.6 ± 0.2	3 ± 0.2
Fibrinase (%)	50 ± 1	50 ± 8.7	400 ± 11	50 ± 6.7	300 ± 15	50 ± 6.7	100 ± 9.2
Thrombin time (s)	21 ± 1.5	23 ± 1.8	13 ± 0.9	20 ± 0.9	15 ± 1.2	19 ± 0.9	18 ± 0.8
Prothrombin index (%)	80 ± 9.5	85 ± 12	120 ± 12	90 ± 12	120 ± 11	85 ± 14	90 ± 11
Factor XIII activity (%)	95 ± 13.5	60 ± 8.9	60 ± 7.5	85 ± 7.5	68 ± 8.2	90 ± 8.5	70 ± 0.9
Thrombocytes no. ($\times 10^{-9}$ L^{-1})	250 ± 7.2	155 ± 11.4	185 ± 5.3	195 ± 15	175 ± 14	220 ± 11	210 ± 21

n, number of rabbits

angle of 20–30° to the skin. In adults, the artery is normally found 2–4 cm from the skin. In small children, the elevation of the needle is reduced to 10–15°, since the artery is more superficial.

2.8 Endovascular occlusion

Endovascular occlusion consists of the following steps: (1) superselective catheterisation of feeding vessel (by transfemoral or intraoperative approach), (2) diagnostic angiography, (3) endovascular occlusion and (4) control angiography.

To obtain reliable information about the nature of the affection, subsequent X-ray monitoring was taken. First, general arteriography of the investigated region was performed, and when alterations in the zone of haemangioma or angiodysplasia were exposed, complementary superselective arteriography was carried out.

If endovascular occlusion by transfemoral method was practiced, after superselective catheterisation of the required artery and diagnostic arteriography, occlusion with emboli with enhanced haemostatic properties was performed. In all operations, a suspension of spherical emboli was introduced first, followed by the cylindrical emboli. This kept the small-sized spherical particles from migration through the zone of arteriovenous fistula, which could induce major complications. Ethamsylate-modified cylindrical emboli 0.5 mm in diameter were placed in physiological saline (0.9% sodium chloride in water). An embolus was introduced one-by-one into a 2 mL syringe and pushed through a catheter into the embolized artery. The efficiency of the occlusion was ensured by keeping the sequence of emboli introduction, starting with the introduction of cylindrical emboli 0.5 mm in diameter and 3–5 mm in length, followed by longer emboli 1.5–1.8 cm. If necessary, the diameter of introduced emboli was increased to 0.6–0.7 mm.

After the introduction of each portion of the emboli, control arteriography was performed and, if necessary, emboli were added till complete vessel occlusion occurred. Total 20–60 emboli (i.e. 20–60 cm) were introduced in the 40–120 mL of physiological saline. Haemostasis occurred already during the introduction of the first emboli, which was observed in arteriography by the absence of leakage of the contrast agent outside the pathological formation. To obtain a more pronounced therapeutic effect, the emboli introduction was discontinued when the pressure during pushing emboli increased, which was associated with the absence of retrograde penetration of blood in the catheter.

If embolization was performed by intraoperative technique, an external carotid artery was isolated and ligated. A second free ligature was put more distally, arteriotomy was performed between them and a Teflon catheter with inner diameter no less than 0.5 mm was introduced and fixed to the distal ligature. Embolization was performed by the above method. The catheter was removed after embolization and control arteriography, the artery was ligated more distally to arteriotomy. The wound was sutured.

The amount of introduced emboli 0.5 mm in diameter, with an enhanced haemostatic effect, involved on average 67 ± 7 cm in diffuse arteriovenous malformations in poorly accessible localizations, and 50 ± 8 cm in local arteriovenous malformations. A smaller amount of emboli, 45 ± 5 cm, was required in intraoperative approach to endonvascular occlusion, which was associated with complementary artery ligature and development of the effect of proximal occlusion.

3 Results and discussion

In this report, PHEMA emboli were obtained by suspension or bulk polymerisation. While the former method produced porous particles of regular spheres with the size in the hundreds of micrometers (Fig. 1a), the latter method resulted in cylindrical emboli (Fig. 1b) with a diameter 0.5–2 mm depending on the diameter of selected capillary. The prepared PHEMA emboli were elastic, of spongy structure, and odourless, containing 40–90% of water. The hydrogel surface and porous structure with 1–10 μm pores supported adsorption of blood cell elements. After their introduction into blood vessel lumen, the emboli swelled within 2–3 min and then tightly adhered to the vessel forming a thrombus on the surface, which grew through the connective tissue.

In order to design a new type of emboli, ethamsylate (Dicynone) was chosen out of medications affecting haemostasis. The preparation stimulates blood clotting, preferentially by activation of tissue thromboplastin and increases adhesion of thrombocytes. Moreover, ethamsylate supports formation of high-molecular-weight mucopolysaccharides in capillary walls reinforcing thus blood vessel walls and decreasing their permeability. Because ethamsylate is assumed to be present in the emboli in low amounts, local haemostasis is accelerated, but not the general one.

3.1 Blood composition as a criterion for evaluation of the effect of modified emboli on haemostasis

Considering aims of the report, the effect of ethamsylate-modified emboli on general haemostasis was of primary interest. Indicators of the rabbit blood coagulation system were therefore investigated before and after introduction of

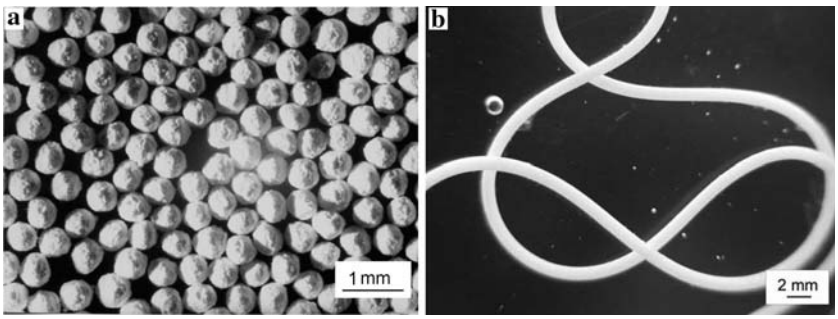

Fig. 1 Optical micrograph of (**a**) spherical PHEMA emboli 0.4–0.6 mm in diameter, (**b**) cylindrical PHEMA emboli 1.35 mm in diameter

ethamsylate-modified emboli: first minutes, 2 weeks and 2 months after embolization. Comparison of principal haemostasis indicators before and after embolization is summarized in Table 1.

Data of the coagulograms confirm the enhanced formation of thrombus in the acute experiment already within the first minutes after introduction of the ethamsylate-modified cylindrical emboli. Compared with the starting blood, fibrinase stimulating factor XIII (Hageman factor) increased eight times (from 50 ± 1% to 400 ± 10.9%); fibrinogen as a fibrin precursor twice (from 2 ± 0.2 g/L to 4 ± 0.3 g/L); thrombin time decreased twice (from 21 ± 1.5 s to 13 ± 0.9 s); fibrinolytic activity dropped twice (from 180 ± 17 min to 360 ± 13.2 min), factor XIII activity (antithrombin) decreased to 60 ± 7.5% and the number of thrombocytes fell to the lower limit of standard (from 250 ± 7.2 × 10^9 to 185 ± 5.3 × 10^9 in L). The remaining indicators of the coagulogram changed within the limits of the standard. The presented changes reflect the effect of ethamsylate: increase in fibrinolytic activity results from stimulation of tissue thromboplastin production, reduction in number of thrombocytes was due to their consumption during adhesion. When comparing blood indicators after embolization due to the ethamsylate-modified hydrogel emboli with changes in blood occurring after embolization with unmodified hydrogel, the conclusion can be drawn that ethamsylate-modified emboli induced the process of thrombus formation, as fibrinase increased eight times (from 50 ± 1% to 400 ± 10.9%), fibrinogen 1.2 times (from 3.7 ± 0.24 g/L to 4 ± 0.3 g/L); thrombin time was accelerated twice (from 23 ± 1.8 s to 13 ± 0.9 s); fibrinolytic activity dropped 1.5 times (from 260 ± 15 min to 360 ± 13.2 min) and antithrombin activity was reduced in the same way as with ethamsylate-modified emboli (to 60 ± 8.9%). The increase in blood coagulation was essentially caused by ethamsylate-stimulated plasmatic factors. Reduction in the number of thrombocytes after the introduction of unmodified emboli was lower than after the introduction of ethamsylate-containing emboli by 20% (155 ± 11.4 × 10^9 vs. 185 ± 5.3 × 10^9 thrombocytes in L) as a consequence of the embolization itself. The indicators demonstrated hypercoagulation of the third degree accompanied by the transition of intravascular coagulation to thrombosis. Comparative data of haemolysis indicators in acute experiment are shown in Table 1.

Average values of indicators for rabbit blood after 14- and 60-day occlusion with modified emboli compared with unmodified emboli are also presented in Table 1. Three days after the introduction of modified emboli (data not shown), a decrease in fibrinogen and in the number of thrombocytes was observed, which can be explained by their consumption. Fibrinolytic activity was reduced for a long time and normalized only within 2 months from the beginning of the experiment. If unmodified emboli were introduced, a moderate increase in the formation of thrombus was observed, which is associated with the release of low-molecular-weight compounds from the emboli, inducing aggregation of thrombocytes. Normalization of the indicators occurred within 14 days, when the process of thrombus formation was complete.

Fourteen days after occlusion with ethamsylate-containing emboli, fibrinolytic activity remained reduced on average by 2 h (up to 310 ± 21 min) compared with the starting data, with unmodified emboli time of the fibrinolytic activity being reduced by 20–30 min (up to 200 ± 9 min). The fibrinogen level remained doubled (up to 3.6 ± 0.5 g/L) after introduction of the modified emboli and by 0.5 g/L (up to 2.5 ± 0.2 g/L) after introduction of the unmodified emboli. Fibrinase increased 6 times (up to 300 ± 15%) with ethamsylate-containing emboli and normal values were found after implantation of unmodified emboli (50 ± 6.7%). The thrombin time was accelerated by 5 s on average (up to 15 ± 1.2 s) after introduction of emboli with ethamsylate and was normalized after introduction of unmodified emboli (20 ± 0.9 s). The activity of antithrombin III (factor XIII) remained low (68 ± 8.2%) with ethamsylate-modified emboli, however, it was normalized with the unmodified emboli. The number of thrombocytes was moderately reduced after the introduction of modified emboli (down to 175 ± 14 × 10^9 L^{-1}). After analysis of results it could be concluded that the indicators remain changed for a long time after embolization with ethamsylate-containing emboli due to their stronger stimulating influence on the coagulation system of the organism.

It follows from Table 1 that all haemostasis parameters became almost normalized 2 months after embolization. A decrease in the fibrinogen level to the initial one was observed until for 90 days, which is obviously due to its consumption. Similarly, activity of antithrombin III recovered after 90 days. After implantation of unmodified emboli, a smooth decrease was observed until initial values were obtained 60 days after embolization. Comparison of the dynamics of changes in fibrinolytic activity after introduction of both types of the emboli showed that fibrinolysis sharply decreased and remained delayed for a long time after occlusion especially with ethamsylate-containing emboli. Undoubtedly, ethamsylate-containing emboli have an influence on the blood coagulation system, accelerating process of thrombus formation.

3.2 Histological changes in the blood vessel occluded with emboli with enhanced haemostatic properties

Ethamsylate-modified PHEMA emboli were implanted into the rabbit femoral artery. The sections of the artery and adjoining cellular tissue were histologically examined 1 day and 3 months after implantation (Fig. 2). One day after the embolization, the embolus bulk was homogeneous occupying partly the blood vessel lumen (Fig. 2a). The rest was filled with thrombotic matter, consisting primarily of a red-type thrombus and accompanied by small pieces of white thrombus. It is interesting to note that the elements of white thrombus were spontaneously exposed at the surface of the emboli. The presence of thrombotic matter indicated faster thrombus formation than with unmodified hydrogel documenting thus the effect of ethamsylate. As a result, the forming thrombus exerted backward pressure on the hydrogel embolus. The blood vessel wall at the site of emboli and thrombus localization was thick and stretched due to the swollen hydrogel and, in particular, due to a fast thrombus formation at the site of the embolus with absorbed ethamsylate. This was documented by stacking layers of interior elastic membrane. The artery wall was intact without any damage and inflammation (Fig. 2a). The artery segments more distal to the embolus site looked receded. Five days after embolization, histological preparations manifested an analogous picture as in one-day experiment. Later on (1–3 months), symptoms of thrombus organization were found, accompanied by fibroblasts penetrating into the emboli pores and well-developed network of collagen filaments (Fig. 2b). Resorption of the emboli was not observed (presence of giant cells) [27, 28].

It can be concluded from the above results that ethamsylate-containing PHEMA emboli accelerated the process of thrombus formation. This was confirmed by a large bulk of the thrombus, exceeding the volume of emboli occupying the vessel. Moreover, accumulation of thrombocytes and leucocytes at the surface of emboli indicated that the thrombus formation occurred first at the surface.

3.3 Clinical application of emboli with enhanced haemostatic properties in paediatric surgery—case report

After complex experimental investigation, ethamsylate-modified PHEMA emboli were used clinically. A striking example is the treatment of a 16-year girl T. She was hospitalised with complaints about pain in the pelvic region accompanied by long paramenia haemorrhage. As shown in Fig. 3a, the angiography clearly showed the arteriovenous anastomosis in the pelvic region and its many peripheral branches. The anastomosis was embolized with 1 g of ethamsylate-modified spherical PHEMA emboli, 0.4–0.6 mm in diameter, injected with a catheter, followed by injection of 170 pieces of ethamsylate-modified cylindrical emboli, 0.5, 0.6 and 0.85 mm in diameter. Angiography after embolization demonstrated the anastomosis receding due to its occlusion with the hydrogel and reduced blood supply into the zone of interest (Fig. 3b, c).

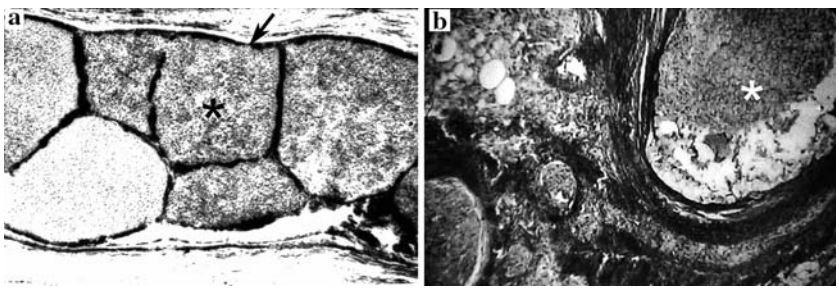

Fig. 2 Histomorphological section of the rabbit femoral artery containing ethamsylate-modified PHEMA emboli one day (**a**) and 3 months after the implantation (**b**). The artery lumen is filled with a mixed thrombus (asterisk), the vessel wall is stretched (arrow) indicating fast thrombus formation immediately after the application of emboli into the vessel. Stained with Reigert′s fuchselin (×90)

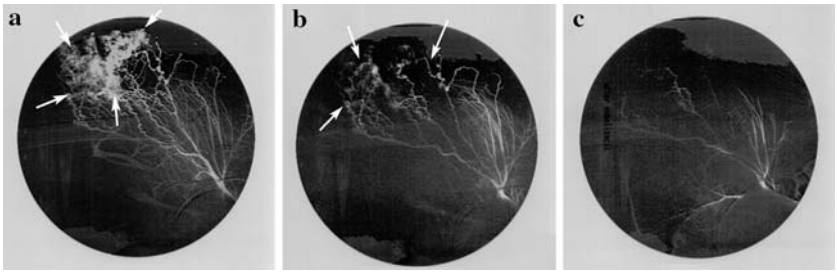

Fig. 3 Angiograms of arteriovenous anastomosis in the pelvic region of a 16-year girl T. (**a**) Arrows show arteriovenous malformation in glutealis artery in the pelvic region before embolization. (**b**) First stage during embolization with ethamsylate-modified PHEMA emboli. The arrows show partial obturation of arteriovenous malformation. (**c**) Arteriovenous malformation disappeared after finish of the second stage of embolization

As a result, haemorrhage and pain was stopped. Since the patient left the hospital, she has been in good condition.

A 12-year boy K., suffered from birth from arteriovenous fistulas of left lower extremity accompanied by muscle pain, swelling, local temperature increase to 41 °C and loss of motor activity. When he was hospitalised, his extremity was elongated by 3 cm. Figure 4 shows angiograms of anterior tibial artery before, immediately, 1 month and 1 year after embolization. Figure 4a shows the region with fistulas of the second and third toe. In the beginning, distal embolization was performed with 1 g of ethamsylate-modified spherical emboli 0.4–0.6 mm in diameter, which was followed by embolization with cylindrical PHEMA emboli 0.5, 0.7 and 0.9 mm in diameter. The absence of arteriovenous fistulas is documented in Fig. 4b. The same picture is observed in Fig. 4c–e. The patient was discharged from the hospital in good condition. One year after the embolization, no recanalization was observed (Fig. 4f), and the resulting absence of pain and increasing motor activity were noticeable.

In another case, a girl Z., 5 years old, was admitted to the hospital, suffering from severe pain in abdominal cavity, loss of body weight and decreased motor activity. Angiography of the patient demonstrated arteriovenous fistulas of abdominal cavity (Fig. 5a). Fistulas were occluded with 5 mL of ethamsylate-modified spherical emboli 0.4–0.6 mm in diameter, followed by 60 pieces of cylindrical emboli (1 mm in diameter, 1 cm length) to prevent emboli dislocation. The process of embolization is illustrated in Fig. 5b–d documenting disappearance of arteriovenous fistulas during embolization. This was accompanied by microcirculatory stasis. The absence of arteriovenous fistulas is documented also in Fig. 5e. The patient left the hospital in good condition without pain, with high motor activity and satisfactory body weight.

An example of poorly accessible inborn haemangioma of temporal region is the case history of a newborn baby girl L., 5 days old. The age of the child did not allow implementation of transcatheter embolization. Instead, intraoperative (open) embolization of temporal frontal

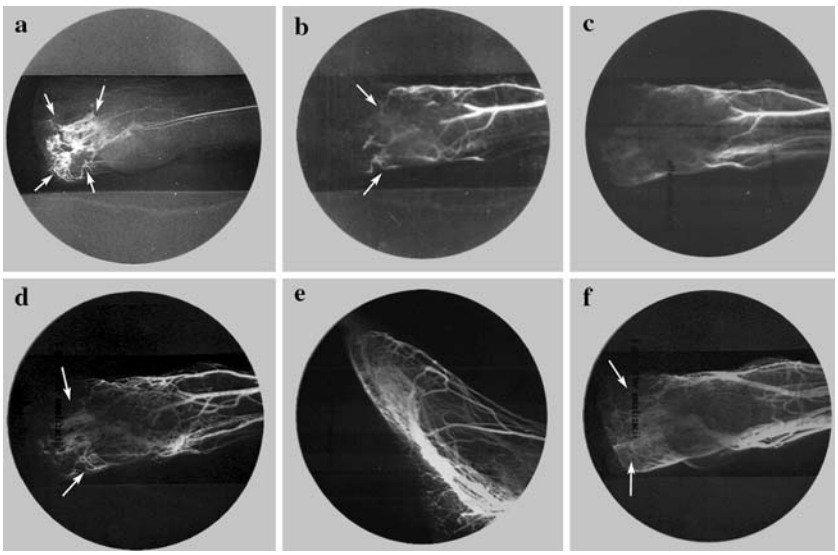

Fig. 4 Angiograms of arteriovenous fistulas of lower extremity of a 12-year boy K. (**a**) Before embolization (see arrows), (**b**) immediately after embolization, (**c**) 1 month after embolization, (**d**) arterial phase—front and (**e**) lateral view and (**f**) vein phase 1 year after embolization with ethamsylate-modified spherical and cylindrical PHEMA emboli. Pathological vessels disappeared after embolization—see the arrows in (**b**)–(**f**)

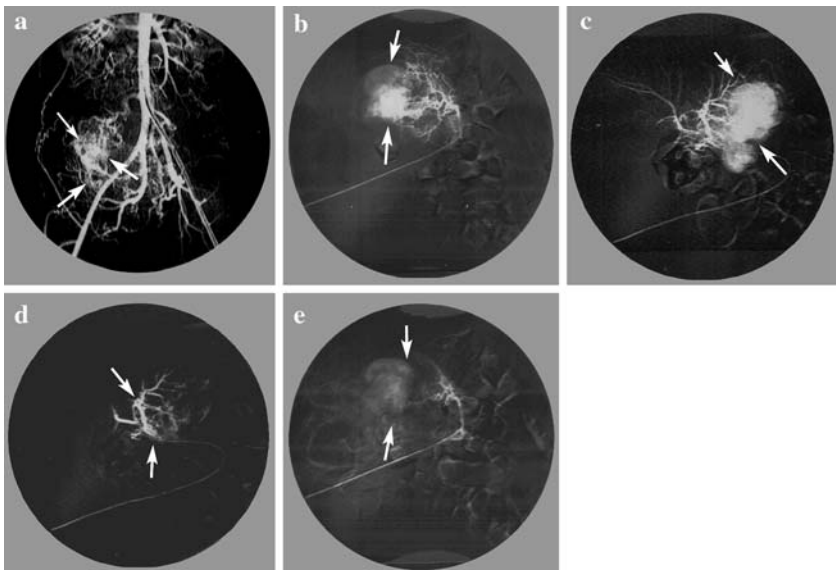

Fig. 5 Angiograms of arteriovenous fistulas of abdominal cavity of a 5-year girl Z. (**a**) Arrows show fistulas before embolization, (**b**–**d**) during embolization and (**e**) after embolization with 5 mL of ethamsylate-modified spherical PHEMA emboli and 60 pieces of cylindrical emboli. Fistulas disappeared in (**d**) and (**e**)

region was accomplished with 2 mL of ethamsylate-modified spherical PHEMA emboli 0.4–0.6 mm in diameter. Stages of intraoperative embolization are documented in Fig. 6a, b. Two years after embolization, the haemangioma diminished and the patient underwent plastic surgery to remove the residual haemangioma.

Finally, a boy patient G., 16 years old, suffering from arteriovenous dysplasia of cervical region was admitted to hospital. Neoplasm was indicated from the birth as a modest vascular nevus. Before admitting to a clinic, it increased to the size 8 × 8 cm during 6 months. When examined, a pulsating formation was diagnosed, in the centre of which scab as a sign of participating bleedings was found. With implementation of selective arteriography of external carotid arteries on both sides, participation of both cervical arteries supplying blood to the formation was revealed (Fig. 7a, c). With the aim to reduce the volume of blood loss during the resection, to decrease the size of the formation and to facilitate its removal and subsequent plastic surgery of the defect, both feeding arteries were occluded with 2 g (1 g for each artery) of ethamsylate-modified spherical PHEMA emboli 0.5 mm in diameter (Fig. 7b, d). The postembolization period was without complications. The patient was operated 2 days after embolization without complications, blood loss amounted to ca. 200 mL.

Figure 8 shows the wound both before and after the operation. Sutures were removed from skin and the patient was discharged from the clinic 10 and 14 days after the operation, respectively. Histological examination of the removed tissue confirmed arteriovenous dysplasia.

Complications during embolization and immediately after the operation were not observed in the above patients. Health of all children has improved, haemorrhage discontinued and the zone of arteriovenous malformation diminished. Involution of arteriovenous fistulas, formation of connective tissue at the place of arteriovenous fistulas and absence of recurrence of arteriovenous malformations were noted in the late postoperative periods from 3 months to 3 years. Haemorrhage did not recur, one child needed additional embolization. Results of clinical applications of ethamsylate-modified PHEMA emboli in urgent situations, such as in arterial haemorrhages of various intensity, confirm thus a new beneficial property of the emboli, namely their increased haemostatic effect.

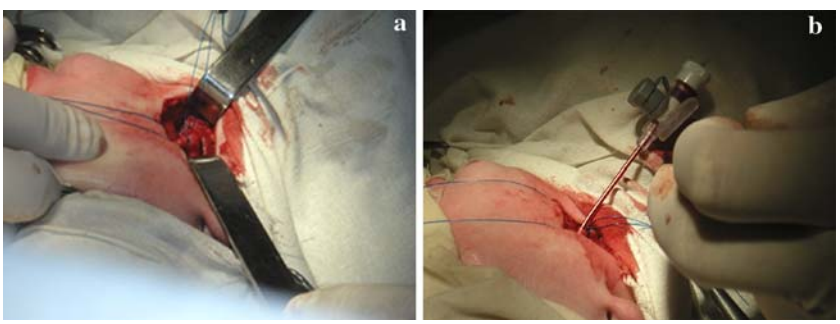

Fig. 6 5-day girl L. with inborn haemangioma of temporal region. Stages of intraoperative embolization: (**a**) isolation of exterior temporal artery, (**b**) catheterisation of exterior temporal artery for introduction of emboli

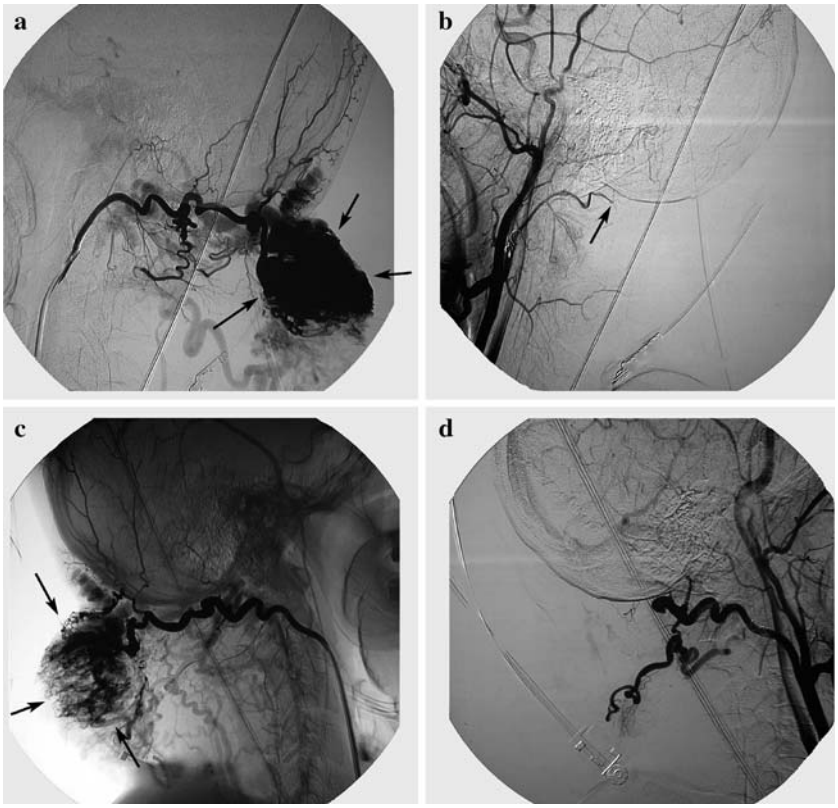

Fig. 7 Angiograms of a 16-year boy G., with arteriovenous malformation on the back side of the neck: (**a**, **b**) right and (**c**, **d**) left occipital artery; (**a**, **c**) arrows show arteriovenous malformation before embolization and (**b**, **d**) closed arteries with disappeared malformation after embolization with ethamsylate-modified PHEMA emboli

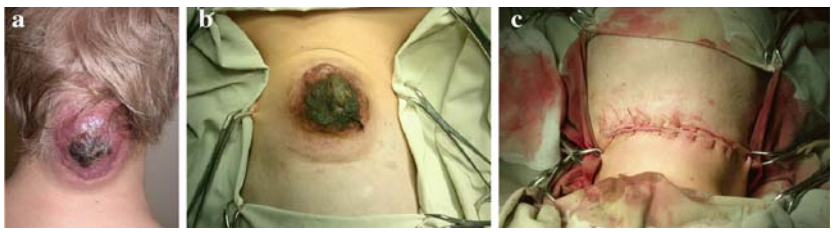

Fig. 8 Photographs of a patient G., 16 years, with demarcated form of arteriovenous malformation of the head: (**a**) before, (**b**) after embolization and (**c**) after surgical removal

4 Conclusions

PHEMA embolic material was obtained either by suspension or bulk polymerisation yielding spherical or cylindrical particles of defined size. They possess optimum characteristics including very good biocompatibility and non-adhesiveness and are accurately fixed in the blood vessel lumen. Analysis of changes of haemostasis parameters after embolization of femoral artery of a rabbit both with ethamsylate-modified and unmodified cylindrical emboli proved that modified emboli support faster formation of steady thrombus (within the first minutes after introduction). This indicated that stimulation of the thrombus formation occurred only locally. The process of local thrombus formation was so intensive that the changes in the coagulograms preserved for another 1–1.5 months, which was in contrast to the results obtained with unmodified emboli. Compared with unmodified emboli, a smaller amount of modified emboli was sufficient to achieve the necessary haemostatic effect, which is their best benefit. Indications of inflammation and damages of artery walls were absent. The absence of the effect of modified emboli on general haemostasis allows using them widely for clinical occlusion in various diseases. Owing to their small size, the emboli primarily shut off the microcirculation level of haemorrhage, where a large amount of blood vessels feed collateral circulation. Due to the blockage of microcirculatory vascular bed and obturation of feeding of tumour or regions of arteriovenous communications, new conditions are established to perform subsequent surgery (e.g. cryogenic, microwave destruction). If good results cannot be achieved with transcatheter (shut) embolization, e.g. due to the danger of emboli dislocation in other vessels, intraoperative (open) embolization is indicated.

The main feature of embolization with ethamsylate-modified PHEMA emboli is prophylaxis of haemorrhages.

Ethamsylate-modified emboli are clinically indicated in urgent arterial haemorrhages difficult to access, such as in lungs, haemorrhages in giant combined haemangiomas of head, face and neck, and various forms of angiodysplasia (arteriovenous internal, soft tissue and combined anastomoses particularly in the face and neck region). At the same time, they make it possible to reduce lethality from blood loss and the time spent in hospital.

Acknowledgement Financial support of the Center for Cell Therapy and Tissue Repair No. 1M0021620803 and Grant Agency of AS CR (grant KAN201110651) is gratefully acknowledged.

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