Collagen Microbeads: Experimental Evaluation of an Embolic Agent in the Rete Mirabile of the Swine

F. Turjman, T. F. Massoud, H. V. Vinters, C. Ji, M. Tardy, G. Guglielmi, and F. Viñuela

PURPOSE: To evaluate the histologic and angiographic effects of collagen microbeads as an embolic agent in the swine rete mirabile. **METHODS:** Human collagen particles (380 ± 100 μm) of spheroidal shape and smooth surface were used to embolize the rete mirabile in five swine. Control angiograms and pathologic examinations were obtained immediately and sequentially from 3 to 35 days after embolization. **RESULTS:** The collagen particles were easy to inject through microcatheters. Embolization was always angiographically complete and persistent for at least 5 weeks. Histologic studies showed occlusion of 25% to 50% of the rete vessels. After 3 and 5 weeks' follow-up, transmural and adventitial chronic inflammation was present. Inflammatory infiltrates included lymphohistiocytic cells and scattered eosinophils. The foreign-body giant-cell reaction was pronounced. No evidence of angionecrosis or focal hemorrhage was shown. **CONCLUSIONS:** Collagen microbeads are a promising experimental embolic agent, with potential future applications in humans.

Index terms: Interventional materials, particles and microspheres; Animal studies

AJNR Am J Neuroradiol 16:1031–1036, May 1995

Embolization is well recognized as a valuable tool in the preoperative treatment of a wide variety of lesions with vascular components, including vascular malformations and hypervascular tumors. Although a wide array of embolic agents is available, the need for new and more effective particulate agents has emerged. Particles with a spherical, smooth shape and an accurately calibrated size range are required. Collagen possesses interesting properties as a biomaterial. It has been used as an embolic material solely in its microfibrillar form (crashed sponge or powders). Collagen microbeads recently have become available. The present study was undertaken to evaluate the physicomorphologic characteristics and embolic properties of the collagen microbeads in an animal model.

Received July 13, 1994; accepted after revision November 28.

Dr F. Turjman's work is supported by a grant from the Ministère des Affaires Etrangères from France, and by the prize Innovalyon from the City of Lyon, France.

From the Departments of Radiological Sciences (Endovascular Therapy) (F.T., T.F.M., C.J., G.G., F.V.) and Pathology (Neuropathology) and Laboratory Medicine (H.V.V.), University of California at Los Angeles Medical Center, and Imedex (M.T.), Chaponost, France.

Address reprint requests to Dr F. Turjman, Department of Radiology, Hopital Neurologique et Neurochirurgical, 59, Blvd Pinel, BP Lyon-Montchat 69394, Lyon Cedex 3-France.

AJNR 16:1031–1036, May 1995 0195-6108/95/1605–1031

Materials and Methods

Preparation of the Collagen Microbeads (Imedex, Chaponost, France)

The extraction, purification, and characterization of the collagen obtained from fresh placentas were previously described (1, 2). An acid solution of human type I-III collagen is first prepared. The oxidization by periodic acid yields cross-linked collagen. The particles are obtained by applying a patented prilling process to the collagen solution (3). Final sterilization of the microbeads is achieved by 25 kGy gamma irradiation.

Morphology of the Microbeads and Particle Size Distribution

Microbeads were observed under a light microscope to examine their shape and to check their size distribution. One hundred particles were randomly verified in a sample. The sphericity of the particles was evaluated by calculating the ratio between the largest (d1) and the smallest (d2) diameters. When this ratio tends to one, the particle tends to be spherical. The prilling process allows the preparation of microbeads of different sizes. In this study, the chosen

mean diameter was 380 μm. It appears to be optimal in our animal model.

Collagen Microbeads as Embolic Agents

Animal Model. The swine rete was chosen as the model for evaluating the new embolic agent. The ascending pharyngeal artery in swine has a special configuration. This artery breaks up into a multitude of fine channels (rete mirabile) situated at the base of the skull (Fig 1). The rete mirabile reconstitutes as the internal carotid artery with more distal visualization of the ipsilateral circle of Willis (4); the swine rete has been used previously to analyze acute and chronic angiographic and histologic changes after occlusion of the rete mirabile with microfibrillar collagens (5). The pig rete is easily accessible at autopsy, enabling pathologic correlation. Rete vessel size is 70 to 275 μm, with a mean of 154 μm (5).

Technique of Embolization. All animal experimentation was conducted in accordance with policies set by our university's Chancellor's Animal Research Committee and National Institutes of Health guidelines. Red Duroc swine maintained on standard laboratory diet, of mixed sex, and weighing 30 to 40 kg were used. After an overnight fast, each swine was premedicated with intramuscular 20 mg/kg ketamine and 2mg/kg xylazine. General anesthesia was maintained with mechanical ventilation and inhalation of 1% to 2% halothane after endotracheal intubation. The right femoral artery was punctured in the groin with introduction of a 6F sheath. Catheterization of the common carotid artery was performed using a 6 nontapered catheter. The ascending pharyngeal artery then was selectively coaxially cannulated with either a 2.7F (Target Therapeutics, Fremont, Calif) or a 1.8F (Balt, Montmorency, France) microcatheter. A superselective angiogram was performed before embolization of the rete mirabile. The microbeads were mixed with contrast medium to allow fluoroscopic control during the arterial injection. The mixture was slowly injected until total occlusion of the rete had occurred. Mixing ratio of spheres and contrast used in vivo was about 10%. Five animals were used and kept alive for 3, 7, and 9 days, and 3 and 5 weeks after embolization, respectively. At follow-up, a control angiogram was performed. In animals 1, 3, and 5, embolization of the contralateral rete was done during the same follow-up procedure.

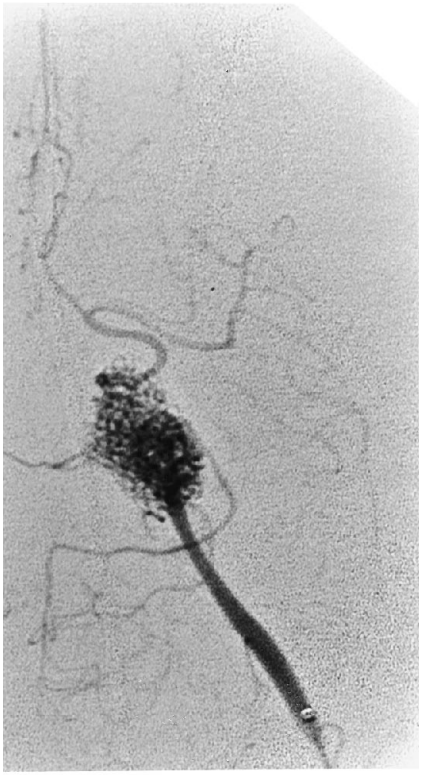


Fig 1. Selective left ascending pharyngeal angiogram demonstrating the normal rete mirabile and circle of Willis.

Histopathologic Verification. After standard euthanasia, all retia were removed at autopsy. After paraffin embedding and routine hematoxylin and eosin staining, 6- to 8-μm-thick sections from representative portions of the embolized or nonembolized retia were histopathologically examined.

Results

Morphology of the Microbeads

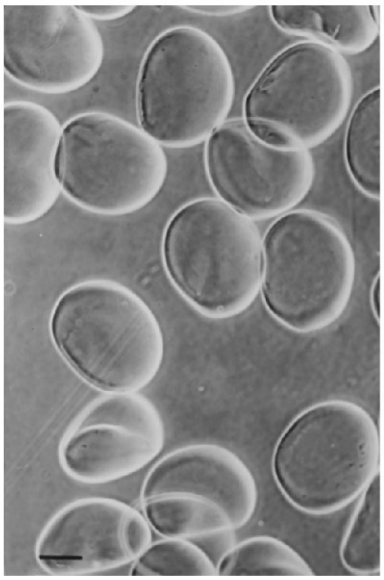
The ratio of the largest (d1) to the smallest (d2) diameter in our sample was found less than or equal to 1.2 in 36% of particles, between 1.2 and 1.5 in 55%, and greater than 1.5 in the remaining 9%. Therefore, the particles can be described as having a spheroidal shape (Fig 2). The surface contour of the particles was smooth under both light and electron microscopy (Fig 3).

Particle Size Distribution

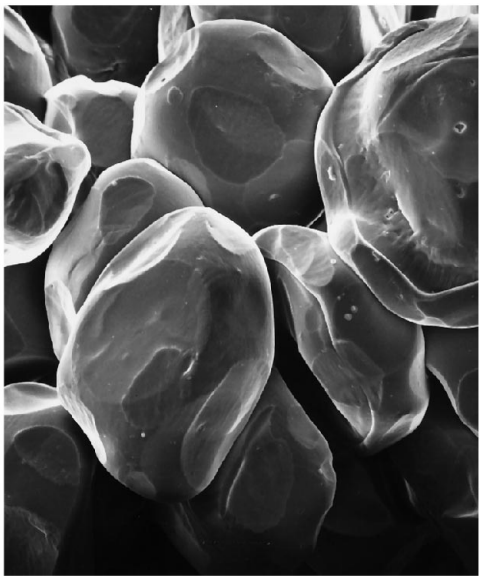
The mean diameters of the particles were d1 = 0.38 ± 0.05 mm and d2 = 0.3 ± 0.05 mm with a ratio d1/d2 = 1.29. Diameter d1 was never less than 0.28 mm or more than 0.48 mm for any of the particles.

Embolization Results

The microscopic appearance of the particles was found to be unchanged after in vitro injection through both types of microcatheters used; in particular, no fragmentation was demonstrated. Laboratory tests showed that the microbeads could be injected through catheters of size 1.8F. Various concentrations of microbeads (10%, 25%, 50%, and 66%) in contrast media were tested. Concentrations greater than 50% (ie, 1.2 mg/mL) often blocked the 2.5F cathe-



2



3

Fig 2. Light micrograph showing the spheroidal shape of collagen microbeads. (magnification, ×90).

Fig 3. Scanning electron micrograph showing the smooth contour of a particle. (magnification, ×250).

ters and, therefore, were not used in vivo. In the animal model, no clogging of microcatheters occurred during embolization.

All swine tolerated the surgical and endovascular procedures, with no general or specific neurologic ill effects.

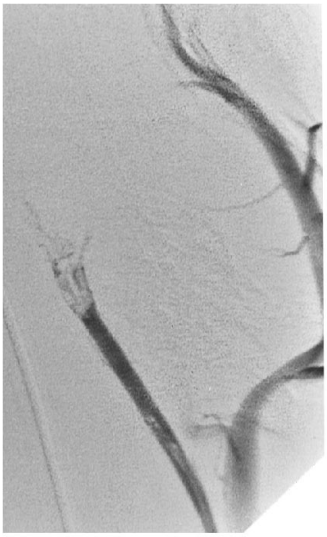
All retia were angiographically completely occluded after particulate embolization (Fig 4). The occlusion was persistent in all follow-up angiograms (Fig 5). In two cases, a partial recanalization of the superior segment of the rete by an arteria anastomotica and a ramus anastomoticus was demonstrated (Fig 6).

Histopathologic Results

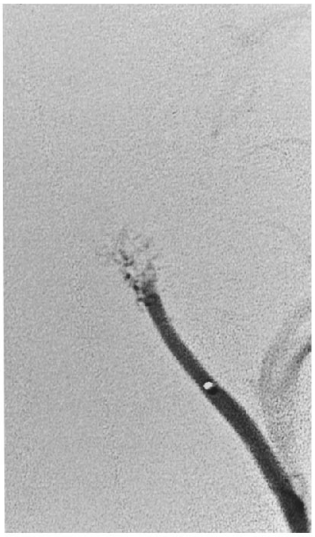
The microscopic appearance of the normal rete has been described previously as a vascular network similar in appearance to the nidus of a cerebral arteriovenous malformation.

On histologic sections after embolization, the particles appeared as basophilic microbeads. They occluded most of the rete vessels with an homogeneous distribution (Fig 7). They still were easily identifiable 5 weeks after the embolization.

At 3 and 5 weeks after embolization, several vessels showed transmural and adventitial chronic inflammatory infiltrates in association with lumina that contain the microbeads. Inflammatory infiltrates included lymphohistiocytic cells and scattered eosinophils. A foreign-body giant-cell reaction was pronounced (Fig 8). No evidence of angionecrosis or focal hemorrhage was shown (Fig 9).



4



5

Fig 4. Immediately after embolization, the rete is occluded with collagen microbeads. External carotid arterial system fills by reflux.

Fig 5. Five weeks after embolization, there is continued occlusion of the rete; the trunk of the ascending pharyngeal artery remains open.

Discussion

An ideal embolic agent should have the following characteristics: (*a*) it must be effective in producing vascular occlusion; (*b*) it must be easily available; (*c*) it must be biocompatible;

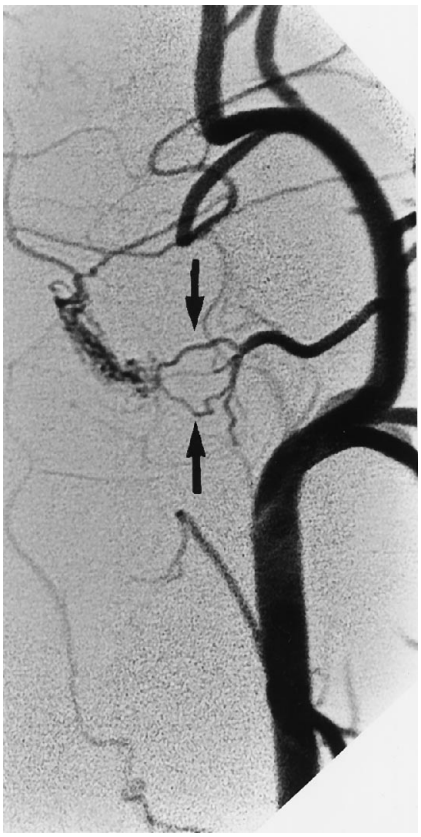


Fig 6. After embolization, left common carotid angiogram showing the ramus anastomoticus and arteria anastomotica artery (*arrows*). The proximal segment of rete embolized through the ascending pharyngeal artery remains occluded.

(*d*) it must easily pass through small, flow-directed catheters; (*e*) it must lead to a homogeneous embolization; (*f*) it must produce a relatively benign inflammatory response; and (*g*) it must not be carcinogenic.

Available Agents

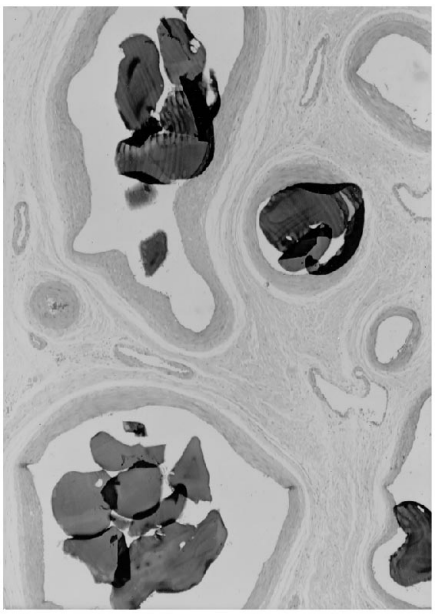
Available agents are classified as temporary or permanent, solid or liquid. Liquids may be embolized through catheters as small as 2F, whereas solids usually cannot. Thus, the circumstances in which vascular lesions are amenable to particulate embolization traditionally have been fewer than for liquids.

Collagen

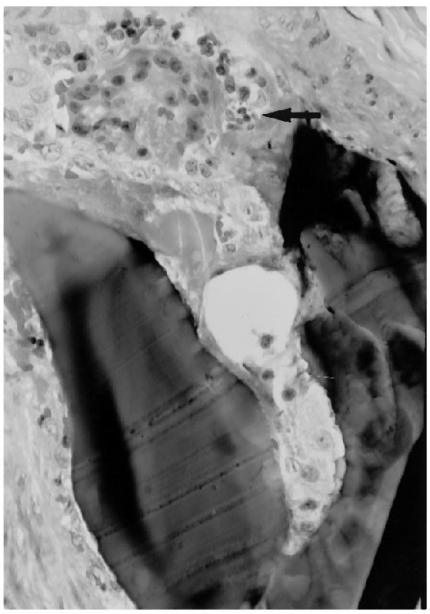
Collagen is a major structural constituent of the extracellular matrix of connective tissues. Collagen has numerous properties: it is resorbable, its stability dependent on the number of molecular cross linkages; it is hemostatic, being able to promote cellular growth, cell anchorage, and tissue repair; it has weak antigenicity; and it is able to aggregate platelets (6, 7). The elimination and/or inactivation of viruses and of nonconventional transmissible agents have been ensured during the industrial process of production, to avoid virus infections such as human immunodeficiency syndrome. The collagen should be highly purified, nonpyrogenic, and

Fig 7. Rete just embolized shows artery-type channels cut at various angles and levels. The particles are clearly visible inside the vascular channels (hematoxylin and eosin; magnification, ×16.1).

Fig 8. Rete embolized 7 days before. The particles occluded between 25% and 50% of the rete vessels. A foreign-body giant cell is identifiable close to the particles (*arrow*) (hematoxylin and eosin; magnification, ×104).



7



8

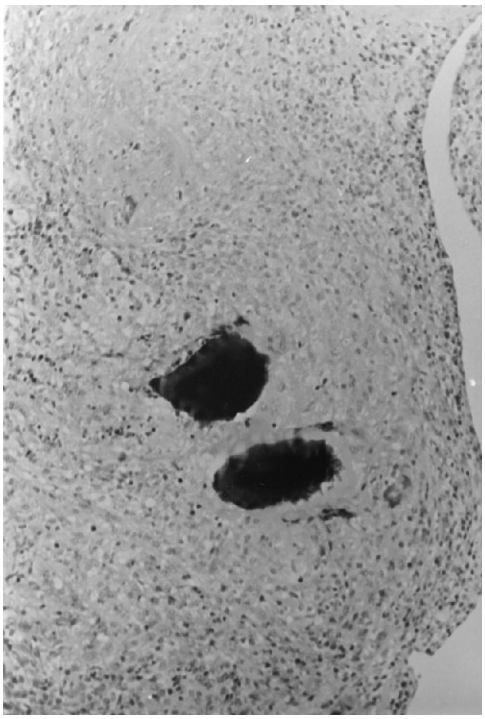


Fig 9. Five weeks after embolization, several vessels show transmural and adventitial chronic inflammatory infiltrates in association with lumina that contain the microbeads. Inflammatory infiltrates include lymphohistiocytic cells and scattered eosinophils. The foreign-body giant-cell reaction is pronounced. No evidence of angionecrosis or focal hemorrhage is shown (hematoxylin and eosin; magnification, ×40.6).

without telopeptides (8, 9) to avoid immunologic or severe inflammatory reaction.

Several collagen biomaterials have become available and have been shown to be useful for diverse medical applications; others are still in development (10–14). Various collagen embolic agents have been evaluated: glutaral cross-linked collagen (GAX) (15), microfibrillar collagen hemostat (MCH) (16), cross-linked collagen fibers (Angiostat) (17), and microfibrillar collagen (Avitene) (18). These products are different forms of microfibrillar collagen, but to our knowledge, collagen particles previously have not been available.

Our Particles

In Vitro. Our particles fulfilled the requirement for possession of a smooth surface, which was demonstrated on scanning electron microscopy. Their injection was always easy, without clogging of the microcatheter. Furthermore, the microbeads were unchanged after injection through a microcatheter. The spheroidal shape of the microparticles allows easy determination of a good size distribution. Considering the mean diameter chosen (0.38 mm), we did not find any particles smaller than 0.28 mm. This size was considered optimal in the rete model, because it is just greater than the average size of the rete vessels. It could be used in human cerebral arteriovenous malformations, in which the average size of the nidus vessels is 156 μm (19).

In our study, the collagen microbeads were shown to be an effective embolic agent. In all instances, the injection of microparticles mixed with contrast medium allowed total occlusion of the rete. To achieve occlusion of the microvascular bed (rete), rather than the feeding artery, a low concentration of particles was used.

Pathology

In sections examined, the vascular and transmural inflammatory response was moderate, without signs of angionecrosis, focal hemorrhage, or severe vasogenic edema, but with a mixed inflammatory response, including foreign-body giant cells. This was considered a satisfactory result, because some degree of intraluminal inflammation actually may act in synergy with the embolization material to ensure effective thrombosis of the lesion.

Indications

In addition to the classic indications for particulate embolization such as preoperative embolization of tumors (to reduce intraoperative bleeding) and endovascular treatment of bleeding, the collagen microbeads could be used in the following two specific applications:

Arteriovenous Malformations. The ability to inject the collagen microbeads through flow-directed microcatheters (such as Magic, Balt) may represent a significant breakthrough in the endovascular treatment of cerebral arteriovenous malformations. The availability of an embolic agent that combines the safety features of using particles (when compared with glue, such as cyanoacrylates), and the ability to inject the particles close to the nidus, through a flow-directed microcatheter, is interesting.

Chemoembolization. A wide variety of drugs can be relatively easily bound to or mixed with the collagen, allowing the potential application of the collagen microbeads to chemoembolization. This therapeutic approach was proposed

by Kato in 1981 (21). It refers to the intraarterial administration of combined chemotherapeutic and vascular occlusive agents to treat malignant disease (22–25). The physicomorphologic characteristics of the collagen microbeads tested explain their suitability as a particulate embolic agent. Collagen microbeads appeared to be an efficient embolic material in experimental in vivo conditions. A variety of distinctive characteristics make it an attractive potential alternative in the treatment of cerebral arteriovenous malformations and in chemoembolization.

Acknowledgment

We thank Nadia Mehnana for manuscript preparation and Dr A. Fernandez and J. Tiollier for their help.

References

1. Tinois E, Tiollier J, Gaucherand M, et al. In vitro and posttransplantation differentiation of human keratino-cytes grown on the human type IV collagen film of a bilayered dermal substitute. *Exp Cell Res* 1991;193:310–319
2. Tiollier J, Dumas H, Tardy M, et al. Fibroblast behavior on gels of type I, III and IV human placental collagens. *Exp Cell Res* 1990; 191:95–104
3. Dumas H, Tardy M, Rochat MH, Tayot JL. Prilling process applied to collagen solutions: drug development and industrial pharmacy. 1992;18:1395–1409
4. Duboulay G. Comparative neuroradiologic anatomy of experimental animals. In: Newton TH, Potts DG, eds. *Radiology of the Skull and Brain.* St Louis: Mosby, 1974;2782–2783
5. Lee DH, Wriedt CH, Kaufmann JCE, Pelz DM, Fox AJ, Vinuela F. Evaluation of three embolic agents in pig rete. *AJNR Am J Neuroradiol* 1989;10:773–776
6. Kuhn K. The classical collagens types I, II and III. In: Kuhn K. *Structure and Function of Collagen Types.* Eds: Mayne R. and Burgeson R.E. - London: Academic Press Inc, 1987;1–42
7. De Lustro F, Condell RA, Nguyem MA, McPherson JM. A comparative study of the biological and immunological response to medical devices derived from dermal collagen. *J Biomed Mater Res* 1986;20:93–107
8. De Lustro F, Dasch J, Keefe J, et al. Immune responses to allogeneic and xenogeneic implants of collagen and collagen derivatives. *Clin Orthop* 1990;260:263–279
9. Dupont D, Gravagna P, Albinet P, et al. Biocompatibility of human collagen type IV in intracorneal implants. *Cornea* 1989;8:251–258
10. Laquerriere A, Yun J, Tiollier J, Hemet J, Tadie M. Experimental evaluation of bilayered human collagen as a dural substitute. *J Neurosurg* 1993;78:487–491
11. Morgon A, Disant F, Truy E. Experimental study of collagen as eardrum graft support in dogs. *Acta Otolaryngol* 1989;107: 450–455
12. Sarmah BD, Holl-Allen RTJ. Porcine dermal collagen repair of incisional herniae. *Br J Surg* 1984;71:524–525
13. Aprahamian M, Damgé C, Kerr-Conte J, Mutter D, Evrard S, Marescaux J. In vitro resistance of artificial connective tissues to human bile and pancreatic juice. *Biomaterials* 1992;13:697–703
14. Thompson KP, Hanna KD, Gipson IK, Gravagna P, Waring GO III, Johnson-Wint B. Synthetic epikeratoplasty in rhesus monkeys with human type IV collagen. *Cornea* 1993;12(1):35–45
15. Strother CM, Laravuso R, Rappe A, Ling Su S, Northern K. Glutaraldehyde cross-linked collagen (GAX): a new material for therapeutic embolization. *AJNR Am J Neuroradiol* 1987;8:509–515
16. Diamond NG, Casarella WJ, Bachman DM, Wolff M. Microfibrillar collagen hemostat: a new transcatheter embolization agent. *Radiology* 1979;133:775–779
17. Daniels J, Kerlan R, Dodds L, et al. Peripheral hepatic arterial embolization with crosslinked collagen fibers. *Invest Radiol* 1987; 22:126–31
18. Kaufman SL, Strandberg JD, Barth KH, White RI. Transcatheter embolization with microfibrillar collagen in swine. *Invest Radiol* 1978;13:200–204
19. Isoda K, Fukuda H, Takamura N, Hamamoto Y. Arteriovenous malformation of the brain: histological study and micrometric measurement of abnormal vessels. *Acta Pathol Jpn* 1981;31: 883–893
20. Lylyk P, Vinuela F, Vinters HV, et al. Use of a new mixture for embolization of intracranial vascular malformations: preliminary experimental experience. *Neuroradiology* 1990;32:304–310
21. Kato T, Nemoto R, Mori H, Takahashi M, Tamakawa Y, Harada M. Arterial chemoembolization with microencapsulated anticancer drug. *JAMA* 1981;245:1123–1127
22. Lindell B, Aronsen KF, Nosslin B, Rothman U. Studies in pharmacokinetics and tolerance of substances temporarily retained in the liver by microsphere embolization. *Ann Surg* 1978;187:95–99
23. Karakousis CP, Kanter PM, Lopez R, Morre R, Holyoke ED. Modes of regional chemotherapy. *J Surg Res* 1979;26:134–141
24. Lorelius LE, Benedetto AR, Blumhardt R, Gaskill HV, Lancaster JL, Stridbeck H. Enhanced drug retention in VX2 tumors by use of degradable starch microspheres. *Invest Radiol* 1984;19:212–215
25. Daniels JR, Sternlicht M, Daniels AM. Collagen chemoembolization: pharmacokinetics and tissue tolerance of cis-diamminedichloroplatinum (II) in porcine liver and rabbit kidney. *Cancer Res* 1988;48:2446–2450