

# Embolization with Cellulose Porous Beads, I: An Experimental Study

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**PURPOSE:** To study the possibility of using cellulose porous beads (CPBs) as a solid embolic material for the permanent occlusion of blood vessels. **METHODS:** Unilateral renal arteries of 12 adult mongrel dogs were embolized with CPBs. Selective renal angiograms were obtained immediately, 1 hour, 4 weeks, and 12 weeks after the embolization. The dogs were killed 1 hour (n = 4), 4 weeks (n = 4), or 12 weeks (n = 4) after the procedure. The kidneys were removed en bloc and examined histologically. **RESULTS:** The CPBs were easily injected through a microcatheter, were readily controlled with radiodensity, and did not adhere to the catheter. Renal angiograms obtained after embolization disclosed complete occlusion of the renal artery. Microscopically, the CPBs traveled to vessels with approximately the same diameter size. Larger vessels were occluded by aggregations of particles that left no open spaces. We found no disruption of the vessel wall, no evidence of perivascular hemorrhage, and no inflammatory changes of the vessel wall or the surrounding tissues. **CONCLUSION:** The CPBs were easy to use; they reached distal sites and produced a homogeneous and permanent occlusion without specific inflammatory changes. The good results of this experimental study led to a clinical trial of CPBs.

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

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Embolization is an accepted form of endovascular therapy, and nonabsorbable particulate materials have been used clinically. However, some of those materials involve tedious preparation, unequal suspension, high friction, difficulty in injecting through a microcatheter, incomplete occlusion, and recanalization. We have developed a particulate material, cellulose porous beads (CPBs), that is composed of cellulose only, is exceptionally uniform in size, is charged positively, and has a specific gravity similar to whole blood. We investigated the properties and characteristics of this material and studied the techniques of embolization with CPBs in an animal model. CPBs were found to

be suitable for distal, superselective, and permanent occlusion.

## Materials and Methods

CPBs (manufactured by Asahi-Kasei, Nobeoka, Japan) are microporous cellulose microspheres that can be used in cell culture. In the unique production process, a solution of cellulose is converted to very small droplets, which are then frozen and fixed. They are globoid in shape and have many micropores. They are of uniform size and accurately calibrated; the mean error of their diameter is within 10  $\mu\text{m}$ . The average diameter of pore openings is 30  $\mu\text{m}$ . They have an effective surface area of 180  $\text{m}^2/\text{g}$ , a volume of 54  $\text{mL}/\text{g}$ , and a specific gravity of 1.03 $\text{g}/\text{mL}$  in the dry state, and carry a net positive charge (1.2  $\text{mEq}/\text{g}$ ) (Fig 1). They are subjected to close quality inspection at Asahi Chemical Industry, including endotoxin testing, bacteriologic examination, testing for heavy metals and water content, and determinations of their degree of swelling and their capacity to hold an electric charge.

CPBs of two sizes (150 and 200  $\mu\text{m}$  diameter) were suspended in iodinated contrast material within stopped glass bottles at about 2000 pieces of CPB per millileter and sterilized for 20 minutes in a steam autoclave at 121°C at 11 Pa. The number of CPBs was obtained by counting the aliquots with a Coulter multisizer 2 (Coulter Electronics Limited, Beds, England). The quality of sterilization of each batch was bacteriologically confirmed.

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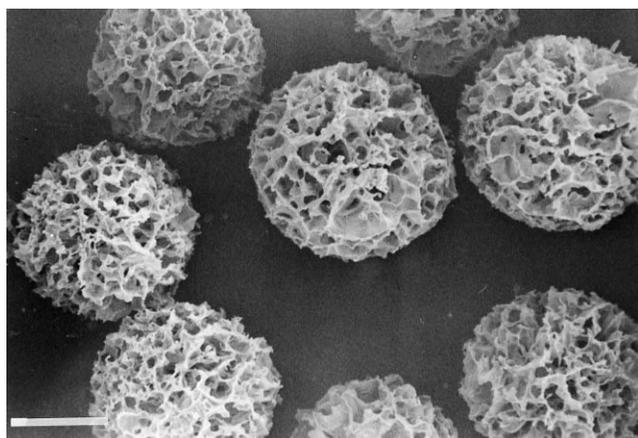


Fig 1. Scanning electron micrograph of 200- $\mu\text{m}$ -diameter CPB microspheres. They are spherical and have many pores, which are about 30  $\mu\text{m}$  in diameter. Bar represents 100  $\mu\text{m}$ .

CPBs were first passed through a Tracker-18 catheter (Target Therapeutics, Fremont, Calif) and a Balt Magic catheter (Balt, Montmorency, France) to examine their compatibility for delivery. For the *in vivo* experiments, 12 adult mongrel dogs weighing about 15 kg each were used. They were premedicated intramuscularly with 0.01 mg atropine sulfate and 10 mg ketamine chloride per kilogram. The dogs were anesthetized by an intravenous injection of 10 mg of thiopental sodium per kilogram for intratracheal intubation. Additional thiopental sodium (5 mg/kg) was administered intravenously as needed. Muscle relaxation was produced by suxamethonium chloride, and the experiment was performed under controlled mechanical ventilation. During the experiment, the arterial carbon dioxide pressure was maintained between 35 and 40 mm Hg and the partial pressure of arterial oxygen was kept at 100 mm Hg or higher. A 5F introducer sheath was positioned in the right femoral artery and 2000 units of heparin was injected. At the end of the procedure, protamine sulfate was injected to reverse the effect of heparin.

Renal preembolization angiograms were obtained by using a 4F polyethylene catheter with the tip of the catheter positioned in the orifice of the artery. Under fluoroscopic control, a unilateral renal artery was then embolized slowly until the flow was arrested (usually after 6 mL), using a small syringe (1 mL) to obtain better control and prevent overinjection of the particulate matter. The volumes used for embolization also contained 2000 pieces of CPB per millileter. Selective renal angiograms were obtained immediately, 1 hour, 4 weeks, and 12 weeks after embolization. All dogs, except those killed 1 hour after embolization ( $n = 4$ ) were allowed to recover from anesthesia. They were killed 4 weeks ( $n = 4$ ) or 12 weeks ( $n = 4$ ) after the procedure. Two animals in each group were embolized with CPBs measuring 150  $\mu\text{m}$  and 200  $\mu\text{m}$  in diameter, respectively. The kidneys were removed en bloc, fixed in 10% neutral buffered formalin, cut into 3- $\mu\text{m}$  sections and stained with hematoxylin-eosin, Giemsa, and elastica van Gieson.

All experiments were performed with the permission of the Animal Experimentation Ethical Committee and according to the Animal Care Guidelines of the Committee.

## Results

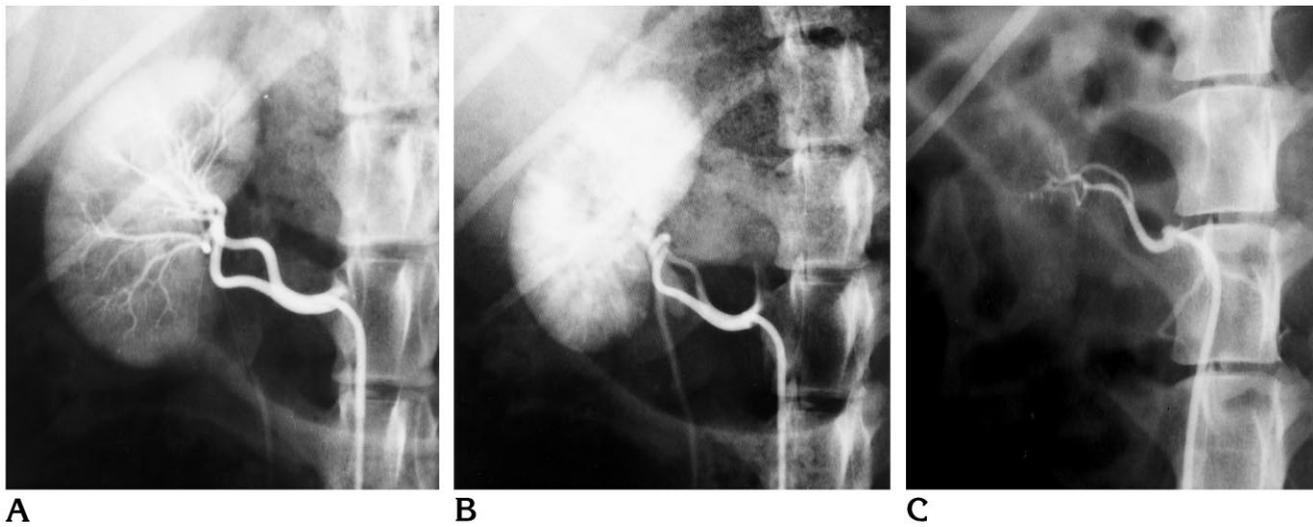
None of the dogs showed any outward signs of abnormality during the observation period. The wet volume of CPB was similar to the dry volume. When suspended in contrast material, the CPBs were isogravitational and remained in suspension for prolonged periods without clumping. They were easily injected through both microcatheters and readily controlled with radiodensity, tolerated long infusion times, and did not adhere to the catheter.

A renal angiogram taken before embolization showed the typical canine arterial vasculature. CPBs produced a smooth reduction in flow; however, severe stasis was seen in the immediate phases after embolization of the main renal artery. Renal angiograms obtained 1 hour, 4 weeks, and 12 weeks after embolization revealed complete occlusion of the renal artery (Fig 2).

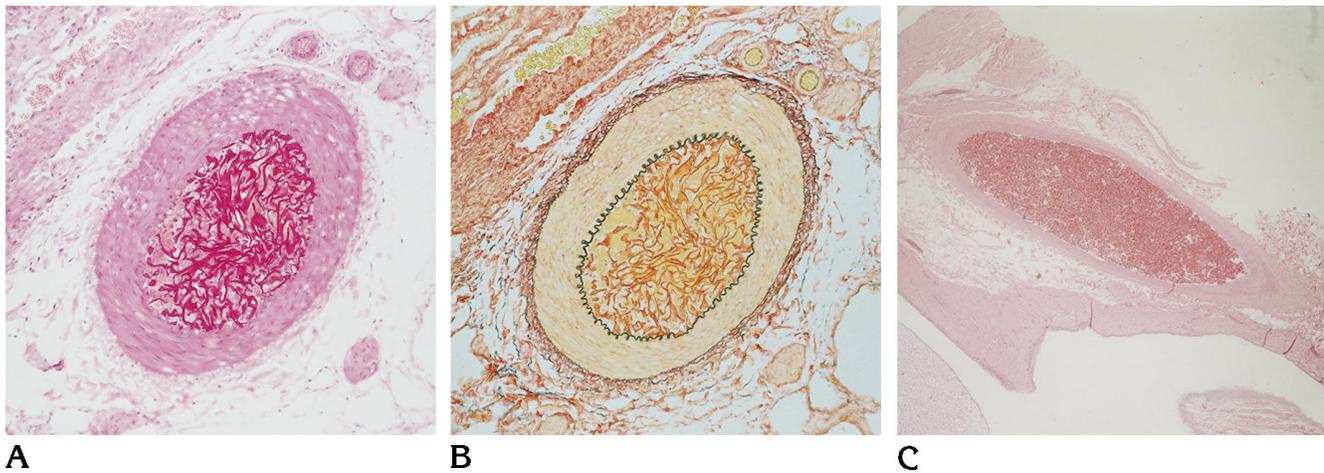
The gross kidney specimen revealed thrombosis of the main renal artery. During the 12 weeks after embolization, a progressive, marked decrease in renal size occurred. Microscopically, CPBs of 150  $\mu\text{m}$  and 200  $\mu\text{m}$  diameter traveled to vessels with approximately the same diameter size. Larger vessels were occluded by aggregates of many particles, which left no open spaces. There was no damage to the vessel wall and no evidence of perivascular hemorrhage. Sections obtained 1 hour after embolization showed fresh thrombus formation (Fig 3). Sections prepared 4 weeks and 12 weeks after embolization showed permanent occlusion of the vessels with infarction. Several old white and red blood cells were found within the CPB thrombus. No inflammatory changes of the vessel wall or the surrounding tissues were seen. The particles were not resorbed nor did their appearance change during the 12-week observation period (Fig 4).

## Discussion

Many embolization materials of particulate type have been developed and tested in animal models or applied clinically. Both temporary (autologous clot, gelatin sponge, microfibrillar collagen) (1-7) and permanent (silicon spheres, lyophilized dura, polyvinyl alcohol, dextran mi-



**Fig 2.** *A*, Preembolization angiogram shows the typical canine renal arterial system. *B*, Angiogram obtained 1 hour after embolization shows complete occlusion of the main renal artery and the radiodensity of the CPB cast. *C*, Angiogram obtained 12 weeks after embolization shows no evidence of recanalization or collateralization of the embolized main renal artery.



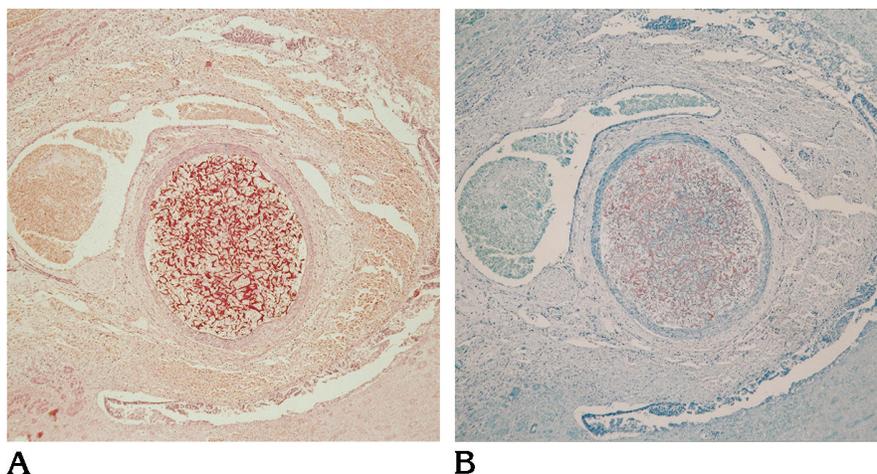
**Fig 3.** Sections obtained 1 hour after embolization with 200- $\mu$ m-diameter CPBs show fresh thrombus formation. The CPBs traveled to vessels approximately 200  $\mu$ m in diameter without stretching the vessel wall or rupturing the internal elastic lamina. CPBs were stained a strong red by hematoxylin-eosin. Large vessels were packed with aggregated CPBs. There was no disruption of the vessel wall and no evidence of perivascular hemorrhage (*A*, hematoxylin-eosin, original magnification  $\times 40$ ; *B*, elastica van Gieson, original magnification  $\times 40$ ; *C*, hematoxylin-eosin, original magnification  $\times 10$ ).

crosspheres) (8–21) agents are available. The oldest material for permanent occlusion is silastic spheres, introduced by Luessenhop and Velasquez (22). Problems common to these materials are their unequal size and unequal suspension, the difficulty in injecting them through a microcatheter, and an inflammatory response of the vessel wall and surrounding tissues. Recanalization is sometimes encountered even with permanent embolization agents.

Polyvinyl alcohol (PVA) foam has proved to

be a useful, biologically inert embolic agent for the treatment of lesions of the head and neck. It has been used for both surgical and primary therapeutic measures. This material was initially difficult to handle but it is now available in several convenient states for embolization. PVA has some features that make it an ideal agent for vascular occlusion. However, recent clinical and experimental studies have shown an increasingly high rate of recanalization after PVA embolization. A rat model has revealed that

Fig 4. Sections obtained 12 weeks after embolization show several old white and red blood cells within the CPB thrombus. No inflammatory changes of the vessel wall or the surrounding tissues were seen (A, hematoxylin-eosin, original magnification  $\times 20$ ). The particles stained pink were not resorbed nor did their appearance change (B, Giemsa, original magnification  $\times 20$ ).



PVA particles less than  $150\ \mu\text{m}$  in diameter tend to lodge within the vessel lumen even in vessels with a high flow rate (16). This factor is important because the incomplete filling of a vessel with PVA allows persistent blood flow and increases the probability of recanalization of such partially occluded vessels. Because of its irregular surface, PVA has a high friction coefficient (20), which permits particles to rest against the wall without completely occluding the vessel. Partial occlusion may cause the blood flow to stagnate, producing an embolus that consists of a combination of PVA and blood clot that will eventually recanalize with reendothelialization of the nonabsorbed PVA particles. Quisling et al (16) reported that complete vascular occlusion occurs only when the lumen is filled or packed with PVA particles. Furthermore, migration of PVA to adjacent nontarget vessels is likely to occur, particularly toward the end of the embolization process when flow-directed methods are used.

Dextran microspheres are efficient at reaching distal sites (10, 21). They are easily injected through microcatheters and do not incite a local inflammatory reaction. Flandroy et al (11) developed an embolic agent, (D, L) polylactide microspheres, which are spherical, smooth, and accurately calibrated. They are biodegradable and can be loaded with chemotherapeutic agents.

Our experimental study was undertaken to develop a better embolic agent for permanent occlusion in clinical settings. CPBs were easy to use because they stayed in suspension for a long time without clumping. This characteristic is probably related to their specific gravity

( $1.03\text{g}$ ), which is close to that of whole blood ( $1.05\text{g}$  to  $1.06\text{g}$ ), their positive charge ( $1.2\ \text{mEq/g}$ ), and their exceptionally uniform size (the mean error of a diameter is within  $10\ \mu\text{m}$ ). Consequently, CPBs could be injected smoothly without clogging the microcatheter, making it possible to perform superselective particulate embolization in territories traditionally limited by small catheter size to liquid embolic agents.

Inspection under a microscope showed that small vessels of approximately the same caliber as the CPB diameter were occluded without stretching the vessel wall or rupturing the internal elastic lamina. No inflammatory reactions of the vessel wall or the surrounding tissues were seen even 12 weeks after embolization, possibly because CPBs are composed of cellulose only and have a specific gravity, and their wet volume is similar to the dry state even after they are introduced into the bloodstream. Therefore, they passed farther into the vascular tree before lodging, and were distributed homogeneously. Positively charged CPBs attracted the negatively charged white blood cells, red blood cells, platelets, and fibrinogen. We think that this mechanism contributed to electrically induced thrombus formation. Consequently, mechanical blockage plus electrically promoted thrombus formation resulted in more permanent vascular occlusion. Larger vessels were packed tightly with many particles without leaving any open spaces. Therefore, the possibility of recanalization is significantly reduced because no recanalization could occur around the particles.

In conclusion, the CPBs were easy to use, they reached distal sites, and they produced a homogeneous, long-lasting, complete vascular

occlusion without specific inflammatory changes because of the advantageous characteristics of this materials. The good results obtained in this experimental study led us to undertake a clinical trial using CPBs (see the article that follows).

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