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Synthesis of Poly(Vinyl Alcohol) and/or Poly(Vinyl Acetate) Particles with Spherical Morphology and Core-Shell Structure and its Use in Vascular Embolization

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Summary: Poly(vinyl alcohol), PVA, is the most frequently used material in embolization of tumors, aneurisms and arteriovenous malformations due to its low toxicity, good biocompatibility and desirable physical properties. It is well known that PVA particles cannot be prepared by direct polymerization of vinyl alcohol. Its synthesis is typically performed by the suspension polymerization of vinyl acetate to produce poly(vinyl acetate), PVAc, followed by the saponification of the PVAc particles. This work shows that, using the suspension polymerization technique, it is possible to obtain spherical particles with a core-shell structure of PVA/PVAc with regular morphology, instead of particles with irregular shapes and sizes, as usually found in many commercial embolization products. Therefore, this work presents the production of PVA/PVAc spherical particles that can be used to occlude blood vessels, eliminating the disadvantages of commercial PVA. *In vivo* clinical tests with white "New Zealand" rabbits undergoing kidney inflammation reaction have shown that these spherical particles are much more efficient for vascular embolization.

Keywords: biomaterials; core-shell polymers; embolization; poly(vinyl alcohol); suspension polymerization

Introduction

Vascular embolization is an important strategy to combat malignant tumors, aneurisms, arteriovenous malformations and uterine fibroid. This technique consists of injecting a fine material through catheter in the blood vessel next to a tumoral region in order to interrupt the nutrients supply to the lesioned area. Thus, the tumoral region tends to shrink, allowing for the tissue recuperation after some time.[1] Different types of materials have been mentioned in literature as useful for embolization, such as metallic coils, silicone rubber, carbon microspheres and poly(vinyl alcohol) – PVA – particles.[2] However, PVA particles have been the most used materials due to its advantageous properties such as good biocompatibility and elasticity, high compressibility, good chemical resistance to acids, bases and detergents, among other properties.[3,4] Nevertheless, typical PVA commercial particles show some undesirable characteristics related to irregular (flock-like) morphology and particle aggregation (responsible for the difficult flow of the particles through the catheter, potentially causing the catheter occlusion), fast biodegradability (responsible for the potential recanalization of the treated vascular vessel),[5] and its comparative high cost.

The first medical use of PVA particles is due to Grindlay and Clagget,[6] who applied

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PVA particles as a filling material after pneumonectomy (surgical intervention procedure to remove the entire lung). Many other PVA medical applications have been reported since then, e.g., as a skin substitute in burn patients,[7] in patients with rectal prolapse,[8] and for closure of cardiac defects.[9]

Tadavarthy *et al.*[4,10] presented the first successful use of PVA as an embolic agent in an application where complete vessel occlusion was achieved. According to Siskin *et al.*,[11] since the first embolization applications, PVA particles have been successfully used to embolize vessels in patients with a variety of diseases, including head and neck arteriovenous malformations and tumors,[2] lower gastrointestinal bleeding,[12] hepatic neoplasm,[13] bone metastases from renal cell carcinoma,[14] and hemoptysis caused by cystic fibrosis.[15]

Before commercially embolic PVA became available, particles were prepared by rasping or blending a block of PVA or by punching out PVA plugs.[11–16] Then, these particles were passed through sieves to be separated in more similar sizes since they showed large difference in their average particle diameters.[16]

Derdeyn *et al.*[16] showed that the large variation in size and the irregular morphology of PVA particles are the main characteristics responsible for catheter occlusion. As a consequence, other materials, such as gelatin, polysaccharides (dextran) and albumen, were tested combined with PVA particles as embolic agents but none of them was capable of reaching the stage of clinical application.[17] Bendszus *et al.*[18] showed that Trisacryl® gelatin microspheres present better efficiency in embolization procedure than commercial PVA with irregular morphology and different sizes.

It is well known that PVA cannot be prepared by direct polymerization due to the tautomerism of vinyl alcohol monomer. However, PVA can be obtained by the saponification of a poly(vinyl ester), such as poly(vinyl pivalate) – PVPi and poly(vinyl acetate) – PVAc.[19] The final particle size distribution and shape of PVA particles play an important role on surgical intervention procedures, avoiding particles aggregation and catheter occlusion and allowing for the proper vessel occlusion. According to Lyoo *et al.*,[20] PVA particles with irregular morphology and size accounted for inflammatory reaction in the wall of the embolized vascular tissue. Different polymerization techniques can be used to obtain PVAc, but as the particle morphology is an important feature to the product application, the suspension polymerization technique is most often employed due to the possibility of obtaining spherical particles with regular morphology.

This work presents the development of a new experimental methodology capable of producing spherical PVA/PVAc microparticles with controlled morphology to be used in vascular embolization, eliminating the disadvantages of commercial PVA particles. The particles were clinically applied, *in vivo*, in kidneys of New Zealand white rabbits to cause tissue ischemia and verify the inflammatory reaction. It is shown that, when compared to commercial PVA particles, PVA/PVAc particles present better performance as far as vessel occlusion is concerned.

Experimental Methodology

Materials

Vinyl acetate (VAc) monomer was supplied by Spectrum Laboratories Inc. (Ft. Lauderdale, FL), with a minimum purity of 99.9%. The initiator (benzoyl peroxide, BPO) with a minimum purity of 99% was supplied by Fluka (Seelze, Germany). The suspending agent [poly(vinyl alcohol), PVA] with a weight-average molecular weight of 78000 Da and a degree of hydrolysis of 85% was supplied by Vetec Química Fina, Rio de Janeiro, Brazil. Nitrogen was supplied by AGA S/A, Rio de Janeiro, Brazil, with 99.9% purity. Tetrahydrofuran (THF) was supplied by Tedia Brasil, Rio de Janeiro, Brazil, with a minimum purity of 99.9%. Sodium hydroxide (NaOH) was supplied by

Spectrum Laboratories Inc. (Ft. Lauderdale, FL), with a minimum purity of 99%. Distilled water was used as the suspending medium. All chemicals, except water, were used without further purification.

PVA/PVAc particles were synthesized in a sequential two-stage process. In the first stage, suspension polymerizations of VAc were carried out in a batch reactor. In the second stage, PVA/PVAc particles with a core-shell structure were prepared by a partial saponification of PVAc beads. The scheme of the experimental unit used in both stages is presented in Figure 1.

Suspension Polymerization Procedure

Reactions were carried out in a 1-L jacketed glass reactor (FGG Equipamentos Científicos Ltda, São Paulo, Brazil) at 70 °C, with a total organic load of 30 wt%, under an inert nitrogen atmosphere in order to keep the reaction environment free of oxygen. Initially, the reactor was fed with distilled water, containing the specified amount of suspending agent (PVA). When the desired temperature was reached, the reagents and the initiator (BPO) were added. The system was kept under isothermal conditions with a constant agitation of 1,000 rpm. The reactions were interrupted after 2 hours of reaction and the PVAc particles were filtered, thoroughly washed with distilled water and then weighed for the saponification procedure. The basic polymerization recipe is presented in Table 1.

Saponification Procedure

PVA/PVAc beads were prepared by saponification of PVAc beads in an aqueous solution of NaOH solution at 30 °C for 5 h. The reactor vessel (see Figure 1) is fed with PVAc particles obtained through suspension polymerization, 190 mL of a 40%

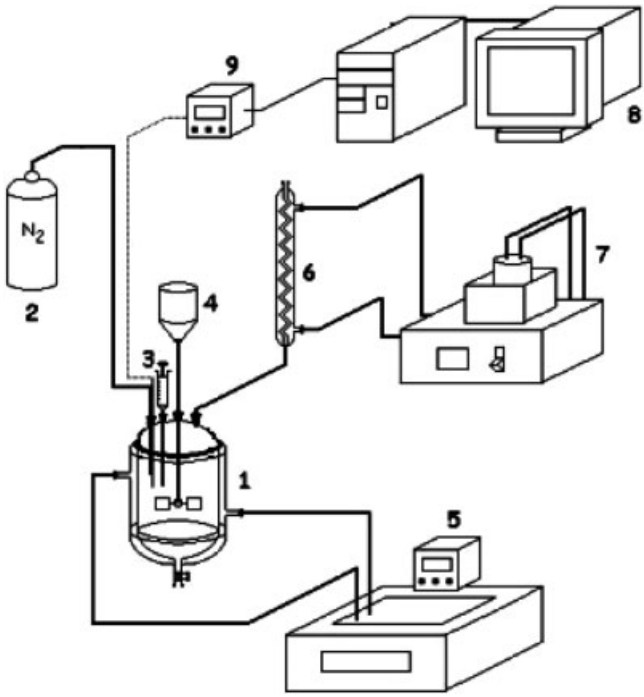

Figure 1.
Suspension polymerization and saponification experimental unit. (**1**) Glass reactor; (**2**) nitrogen cylinder; (**3**) sampling; (**4**) mechanical agitator; (**5**) heating bath; (**6**) reflux condenser; (**7**) refrigeration bath; (**8**) microcomputer for data acquisition (**9**) module of signal conditioning of thermocouple.

Table 1.
Basic Recipe for VAc homopolymerization and PVAc Saponification.

Homopolymerization Conditions	
Chemical	Weight (g)
H_2O	420
VAc	180
BPO	1.3
PVA	0.1
Stirring:	Temperature:
1000 rpm	70 °C
Saponification Conditions	
Chemical	Volume (mL)
H_2O	170
NaOH Solution	190
Stirring: 500 rpm	Temperature: 30 °C

aqueous solution of NaOH and 170 g of distilled water. After 5 h of operation, the reaction is interrupted and the particles are washed with distilled water, filtered and then dried in a vacuum oven at 30 °C. Saponification reaction takes place, predominantly, at the particles surface, producing particles presenting an outer PVA shell and an inner PVAc core. Table 1 presents the recipe used in the saponification stage.

PVA/PVAc Characterization

Polymer samples were characterized by X-ray diffraction (XRD), differential scanning calorimetry (DSC), gel permeation chromatography (GPC) and optical microscopy.

The melting temperature was determined by DSC measurements in a DSC7 calorimeter (Perkin Elmer, Torrance, California, USA) at heating rates of 10 °C/min, in accordance with the ASTM D3418-82. The crystallinity was determined in a Rigaku dmax 2200 X-ray diffractometer (Rigaku/MSC, The Woodlands, Texas, USA), using CuKα radiation. The weight-average molecular weight was measured through gel permeation chromatography (GPC). The system was composed of four columns (Phenomenex, Torrance, CA, USA) with gel porosities ranging from 10^3 to 10^6 Å. A refractometer (SFD RI-2000F, Schambeck, Germany) was used as the detector. The refractometer and the pumping system (Konic, Miami, FL, USA) were connected to a Pentium MMX 233 MHz personal computer for data acquisition and data handling. The calibration curve was built using samples of polystyrene with weights ranging from 10^4 to $2 \cdot 10^6$ and a polydispersity index lower than 1.05. THF was used as a mobile phase, and the analyses were carried out at 40 °C. The particle morphology of polymer particles was determined by optical microscopy with an Olympus SHZ10 stereomicroscope (Somerset, NJ, USA).

Results and Discussion

Polymer Particles Properties

Figure 2 shows the morphology of typical, commercially available PVA and PVA/PVAc polymer particles. According to Figures 2A–D the shape and size of commercial PVA are typically irregular. In industrial processes, PVA resins are frequently obtained by the alcoholysis of PVAc. Under usual reactions conditions, the base-catalyzed alcoholysis of PVAc is very rapid, leading to almost complete hydrolysis of PVAc. Due to very fast reaction rates, the partial hydrolysis of PVAc is quite difficult, when the saponification is performed in conventional processes. In addition, it is impossible to obtain particles with controlled morphology because the PVA resin is normally insoluble in the alcohol used (typically methanol), precipitating instantaneously as they are formed. For this reason, the particles illustrate in Figures 2A–D present irregular size and flock-like morphology.

Figures 2E–F illustrate the polymer particles synthesized in a sequential two-stage process, proposed in this work. It can be observed that PVA/PVAc particles obtained by suspension polymerization are spherical, present smooth surface and regular morphology. It is very important to

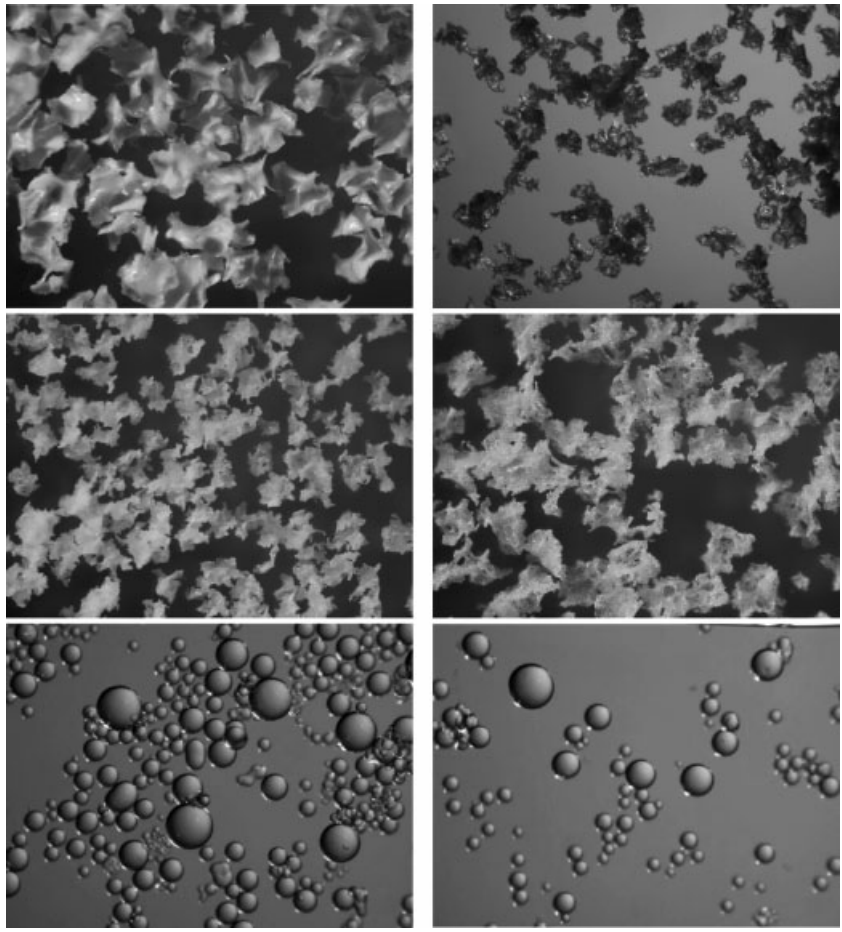

Figure 2.
Optical microscopy of polymer particles (Commercial PVA: A–D; PVA/PVAc: E–F) (**A**) Ivalon® – Nycomed; (**B**) TruFill® – Cordis; (**C**) PVA 200 – Cook; (**D**) Contour Emboli – Interventional Therapeutics Co.; (**E–F**) PVA/PVAc beads. (The size of the horizontal axis corresponds to 2 mm.)

emphasize that the particle size distribution and the spherical morphology of the polymer particles are very important features of embolic materials. In addition, polymer particles with good morphology allow for the proper vessel occlusion, providing suitable conditions for the surgical intervention procedures, avoiding the particles aggregation and the catheter occlusion.

Figure 3 illustrates typical DSC curves of the polymer resins. It can be seen that glass transition temperature (*Tg*) of the polymer is affected by the hydrolysis of PVAc, leading to increase of the *Tg* of the final material. The PVA/PVAc particles present higher glass transition temperature (41.5 °C) than PVAc particles (31.6 °C), which confirms that the final polymer material contains partially hydrolyzed PVAc chains. However, the core-shell structure of the polymer particles cannot be directly visualized from Figure 3 because of the relatively low saponification degree of the PVA/PVAc particles, which is much lower in the analyzed case than normally observed for commercial PVA grades. The glass transition temperature of typical

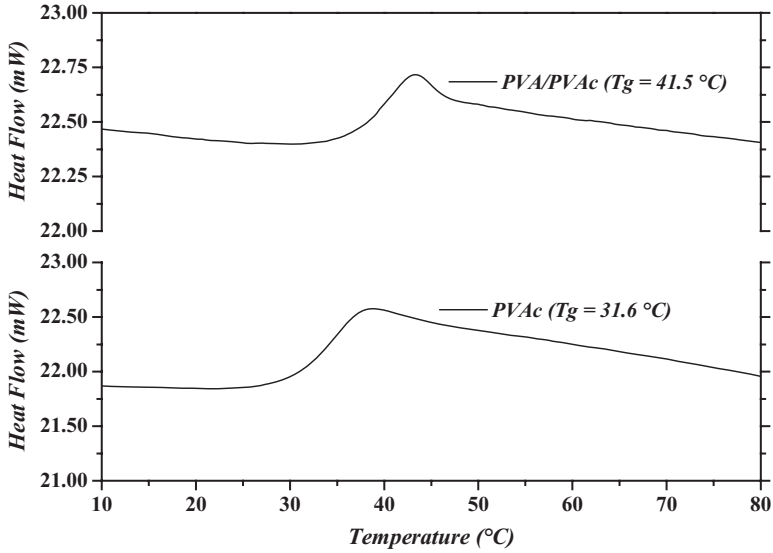

Figure 3.
Typical DSC curves of the polymer samples.

commercial PVA grades lies in the range of 75 to 85 °C, depending on the polymer stereo-regularity.[21]

The extent of saponification can be estimated with the help of the Fox equation.

$$\frac{1}{Tg_{1,2}} = \frac{1-\alpha}{Tg_1} + \frac{\alpha}{Tg_2} \quad (1)$$

where, $Tg_{1,2}$, Tg_1 and Tg_2 are the glass transition temperatures of the PVA/PVAc, PVAc and PVA polymer particles respectively. α is the weight fraction of PVA (actually, the overall extent of saponification) in the core-shell polymer particles. The degree of saponification for the sample described in Figure 3 was found to be equal to $\alpha = 0.380$. However, different extents of saponification can be obtained through proper manipulation of process variables, such as the NaOH concentration, the time of saponification and the size of the original PVAc polymer particles.

When dry PVA is put in contact with aqueous solutions, significant volume variation can take place in the polymer phase, as PVA particles interact with the aqueous phase and absorb water. In some cases, this can lead to catheter occlusion during the embolization procedures. It is important to emphasize that particle swelling is substantially reduced in the case of the partially saponified core-shell spherical particles. This may cause significant improvement of the embolization procedure, avoiding aggregation of polymer particles and consequently preventing the catheter occlusion. Another important characteristic of PVA particles is that PVA is partially soluble in the aqueous phase. Therefore, the dimension stability of the PVA particles can be improved quite significantly if it is anchored on an insoluble support, such as PVAc. This may also contribute with the increase of the efficiency of surgical intervention procedures, allowing for better and longer vessel occlusions. Therefore, there are many incentives for production of core-shell PVAc/PVA polymer beads.

In the conventional solution saponification processes, the control of the degree of saponification may be very difficult because of the very fast reaction rates. This leads to high degrees of hydrolysis and to formation of particles with irregular shapes. Generally, PVA commercial grades are available

with high degree of hydrolysis, in the range of 90–98%. In spite of that, the shell thickness and the shell/core ratio of core-shell PVA/PVAc particles can be controlled through proper manipulation of the NaOH concentration in the aqueous solution, of the saponification time and of the size of the precursor PVAc particles. This occurs because the reaction is controlled by diffusion, as water is not compatible with the PVAc polymer phase.

The X-ray diffraction analyses indicate the formation of PVA/PVAc particles with core-shell structure. Figure 4 shows the crystallographic patterns of the polymers determined using XRD measurements. It can be observed that the PVAc presents XRD patterns that are different from the patterns obtained for PVA/PVAc and commercial PVA particles. Figure 4 also shows that the PVA/PVAc patterns are similar to the commercial PVA patterns, indicating that the crystalline structure of the polymer surface is similar to the crystalline structure of the commercial PVA. In this case, PVA/PVAc beads present a core-shell structure, consisting of an outer PVA shell and an inner PVAc core.

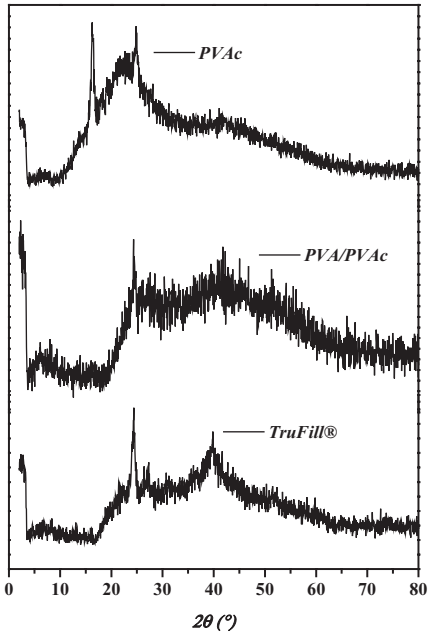

Figure 4.
XRD patterns of the polymers particles.

Figure 5 shows the molecular weight distributions (MWDs) of the polymer samples. It can be observed that the MWD of the PVA/PVAc polymer is different from the MWD obtained for the PVAc. The saponification step leads to modification of the shape of the initial MWD, significantly increasing the polydispersity index (PDI) of the polymer material, as shown in Figure 5. Despite the MWD modification, the final material presents unimodal distribution, indicating that mechanical properties of the precursor PVAc are only slightly modified, when the polymer is partially hydrolyzed.

It is well known that PVAc obtained from different processes is normally branched. Branching takes place at the secondary carbon of the main chain, due to the acidic character of the methylenic hydrogen. Branching frequency increases when PVAc is treated at mild temperatures for long times in the presence of small monomer concentrations. These are exactly the saponification conditions, which therefore explain the formation of larger polymer chains during saponification. Besides, saponification causes the cleavage of the acetate groups, which also explain the formation of polymer chains of lower molecular weights. Therefore, broadening of the molecular weight distributions during saponification could be already expected.

In vivo Tests

In-vivo clinical tests were executed for a comparative study between the effects of commercial PVA and PVA/PVAc spherical particles after intra-arterial embolization in white New Zealand rabbits' kidneys. Experimental results show that both particles are effective to cause tissue ischemia. However, the inflammatory reaction is more intense, as desirable, when PVA/PVAc particles are used.

The 48-hour embolized kidney (see Figure 6) presents acute ischemia, related to the obstruction of the inflow vessels. The 30-day embolized kidney (see Figure 6) presents a significant volume decrease,

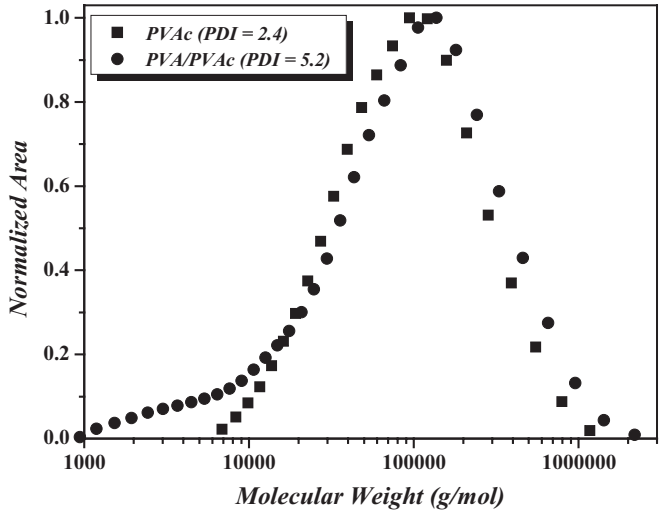

Figure 5.
Molecular weight distributions of the final polymer particles.

more conspicuous in animals tested with PVA/PVAc particles.

In Figures 7 and 8, a comparison between the performance of a typical commercial and the developed spherical embolization particles is presented. The comparison is carried out in terms of the vessel occlusion efficiency of each sort of particle.

In Figure 7, it is shown that some red blood cells are mixed with PVA commercial particles. This is due to the irregular particle morphology which allows for the formation of vacant spaces which may be filled with blood. After the vessel occlusion, the blood clots and become a thrombus which is reabsorbed during the inflammatory reaction stage. Moreover, the commercial PVA particles degradation may create even more space and benefit the vessel recanalization.[5]

In Figure 8, it can be seen that PVA/PVAc particles are very close to the blood vessel wall causing formation of smaller spaces to be filled with blood. Therefore, the amount of blood within the interstices is

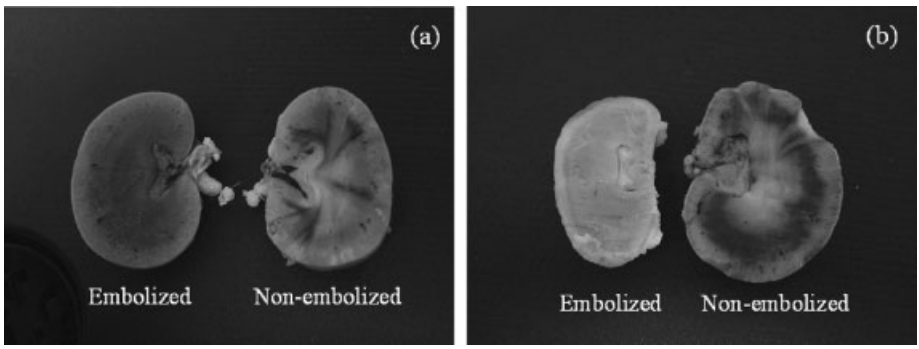

Figure 6.
Embolized kidneys after (a) 48 hours and (b) 30 days.

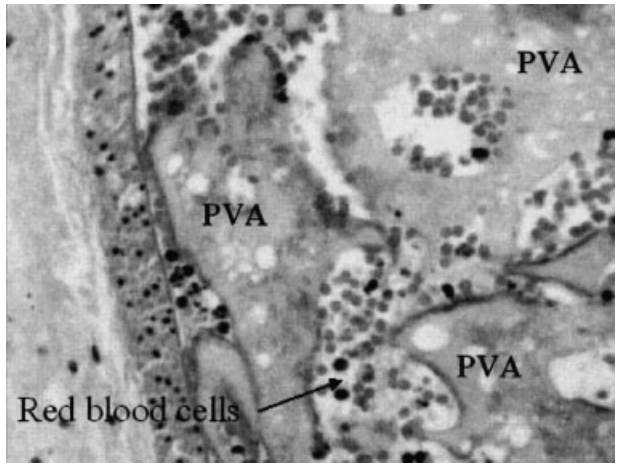

Figure 7.
Vessel microscopy with commercial PVA particles. (The size of the horizontal axis corresponds to 500 μm.)

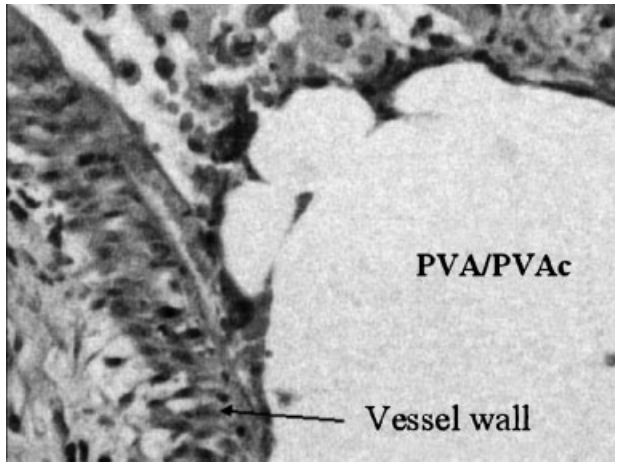

Figure 8.
Vessel microscopy with PVA/PVAc particles. (The size of the horizontal axis corresponds to 300 μm.)

smaller and not sufficient to allow the vessel recanalization[5], showing better results regarding vessel occlusion.

Conclusion

The results show that it is possible to obtain PVA/PVAc spherical particles with controlled morphology which are efficient for vascular embolization, easy to pass through the catheter and does not agglomerate in blood vessels, reaching more distant arterial segments when compared to commercial PVA particles. Besides, PVA/PVAc particles present better behavior in the vessel occlusion as tissue fibrosis is more intense when these particles are used.

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