Preparation and characterization of injectable fibrillar type I collagen and evaluation for pseudoaneurysm treatment in a pig model

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Objective: **Despite the efficacy of collagen in femoral artery pseudoaneurysm treatment, as reported in one patient study, its use has not yet gained wide acceptance in clinical practice. In this particular study, the collagen was not described in detail. To further investigate the potential of collagen preparations, we prepared and characterized highly purified injectable fibrillar type I collagen and evaluated its use for femoral artery pseudoaneurysm (PSA) treatment in vivo using a pig model.**

Methods: **Purified fibrillar type I collagen was characterized using electron microscopy. The effect of three different sterilization procedures, ie, hydrogen peroxide gas plasma (H_2O_2), ethylene oxide gas (EtO), and gamma irradiation, was studied on both SDS-PAGE and platelet aggregation. Different collagen injectables were prepared (3%, 4%, and 5%) and tested using an injection force test applying a 21-gauge needle. To evaluate the network characteristics of the injectable collagen, the collagen was suspended in phosphate buffered saline (PBS) at 37°C and studied both macroscopically and electron microscopically. To determine whether the collagen induced hemostasis in vivo, a pig PSA model was used applying a 4% EtO sterilized collagen injectable, and evaluation by angiography and routine histology.**

Results: **Electron microscopy of the purified type I collagen revealed intact fibrils with a distinct striated pattern and a length <300 μm. Both SDS-PAGE and platelet aggregation analysis of the sterilized collagen indicated no major differences between EtO and H_2O_2 sterilization, although gamma-irradiated collagen showed degradation products. Both 3% and 4% (w/v) collagen suspensions were acceptable with respect to the force used (<50 N). The 4% suspension was selected as the preferred injectable collagen, which formed a dense network under physiologic conditions. Testing the collagen in vivo (n = 5), the angiograms revealed that the PSA partly or completely coagulated. Histology confirmed the network formation, which was surrounded by thrombus.**

Conclusions: **Collagen injectables were prepared and EtO sterilized without major loss of structural integrity and platelet activity. In vivo, the injectable collagen formed a dense network and triggered (partial) local hemostasis. Although optimization is needed, an injectable collagen may be used as a therapeutic agent for femoral PSA treatment. (J Vasc Surg 2010;52:1330-8.)**

Clinical Relevance: **This study explores the use of collagen for femoral artery pseudoaneurysm (PSA) treatment. The efficacy of collagen has been reported in one patient study, but it has not gained acceptance for PSA treatment. The current study combines the characterization of a collagen preparation with its evaluation using a porcine model for PSA. First, injectable collagen was prepared and the optimal sterilization technique selected, addressing structural characteristics and platelet activity. Next, the collagen preparation was evaluation for PSA treatment using a porcine model. The results of this study may support the design and application of new collagen injectables.**

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A femoral artery pseudoaneurysm (PSA) is a complication that may occur after arterial puncture for invasive cardiovascular procedures. When an arterial puncture site fails to seal, arterial blood jets into the surrounding tissues and forms a pulsatile hematoma. In 0.2% to 7.7% of the femoral arterial interventions, a femoral PSA is formed that needs treatment.[1-4] When a PSA is left untreated, rupture or distal embolization may occur. PSA enlargement may give rise to pain, neuropathy, and venous obstruction. Since spontaneous repair of larger pseudoaneurysms is unlikely, a reliable and easy method to induce local hemostatic clotting is desirable. Various techniques (eg, ultrasound-guided compression repair, percutaneous thrombin injection, balloon occlusion, stent graft placement) have been used to stop arterial bleeding to replace standard compression treatment and shorten hospital stay.[5-10] Thrombin

treatment is highly effective. However, in some cases (especially complex pseudoaneurysms), it is not efficient and even contraindicated. Contraindications for the use of thrombin injection are the wide and/or short necked pseudoaneurysms.[11] Several cases have reported thrombin failure in these complex pseudoaneurysms.[12-14] Particularly in these cases, injectable collagen may be an attractive agent, since collagen is highly viscous and collagen fibrils aggregate and deposit at the site of injection. In one study, the potentials of injectable collagen for PSA treatment in patients was described, and a very high success rate was reported.[15] To follow up on this study, we prepared a well-characterized injectable collagen preparation and evaluated its use in a porcine model for PSA treatment.

Collagen is a well-known structural extracellular matrix protein. It has found ample usage as a biologic material for medical applications indicating its versatility as a biomaterial, including its use as a drug delivery device[16,17] and as a matrix for the repair and regeneration of tissues.[18-20] In addition, it is used as an adjunct for hemostasis.[21-23] Collagen-based plugs are widely used to manage the arterial puncture site and to prevent the extravasation of arterial blood.[24,25] When collagen comes in contact with blood, platelets become activated and their shape changes, resulting in the release of hemostasis-stimulating compounds, platelet adhesion, and aggregation to the vessel wall. This process is also known as primary hemostasis.[26,27] Collagen is biocompatible and biodegradable and allows remodelling in the host after implantation. In addition, collagen can be fabricated in various physical forms like gels, films, and sponges. These characteristics, in combination with its hemostatic activity, make collagen a good alternative for current treatments of femoral PSA.

In 2002, Hamraoui et al treated PSA patients with bovine fibrillar type I collagen aiming for a less invasive percutaneous treatment.[15] According to this study, the use of collagen is a safe and effective strategy. To further elaborate on the use of collagen for PSA treatment, we prepared well-characterized collagen fibrils, selected the optimal sterilization procedure, and evaluated its use for the treatment of PSA using a recently described porcine model.

METHODS

Materials. Unless otherwise stated, chemicals were obtained from Merck (Darmstadt, Germany) and were of the highest purity available.

Preparation of injectable fibrillar type I collagen. Fibrillar type I collagen was isolated from 6-month-old bovine Achilles tendon (deep flexor) applying extraction procedures using neutral salt solutions, chaotropic agents, dilute acid, and acetone.[28] After purification, type I collagen was lyophilized using a Sublimator 500II lyophilizer (Zirbus Technology, Bad Grund, Germany) and sterilized using either ethylene oxide gas (EtO; 24 hours at 35-45°C and at 50%-80% relative humidity; Sterigenics, Zoetermeer, The Netherlands), hydrogen peroxide gas plasma (H_2O_2; 6 mg/L at 35°C for 30 minutes), or gamma irradiation (Gamma; 20 kGy from a ^{60}Co source in an X-ray pallet irradiator (JS 9000; Isotron BV, 's-Hertogenbosch, The Netherlands)). Type I collagen suspensions were prepared by incubation of 3.0, 4.0, or 5.0 g type I collagen in 100 mL MilliQ water for 16 hours at 4°C. The suspensions were homogenized (20 times on ice) using a Potter-Elvehjem homogenizer with an intervening space of 0.35 mm.

Characterization of injectable fibrillar type I collagen. Scanning electron microscopy (SEM) was used to analyze the morphology and length of the purified collagen fibrils, and transmission electron microscopy (TEM) was applied to study the ultrastructure of the collagen fibrils. Collagen samples were frozen at −20°C and subsequently lyophilized. Poly-D-lysine coated glass inserts were incubated with collagen suspensions (~0.5 mg collagen/mL 0.25 M acetic acid), for 16 hours at 4°C, washed three times with MilliQ water, and air dried. Inserts were mounted on stubs and sputtered with an ultra thin layer of gold in a Polaron E5100 coating system (Quorum Technologies, Neuhaven, United Kingdom). The morphology of the collagen fibrils was studied in a Philips XL30 ESEM FEG apparatus (Philips, Eindhoven, The Netherlands) at an accelerating voltage of 10 kV, and length studied with a JEOL JSM-6310 SEM (JEOL Ltd, Tokyo, Japan) at 15 kV; 100 fibrils were randomly measured at ×500 magnification in 16 fields. The ultrastructure of the collagen fibrils was analyzed in the diluted collagen samples using TEM. The samples were incubated on formvar-coated grids for 1 hour at 21°C, washed with 0.1 M phosphate buffer (pH 7.4), washed two times with MilliQ water, stained with 0.1% (w/v) uranyl acetate, and examined in a JEOL 1010 TEM (Tokyo, Japan).

The purity and intactness of the type I collagen before and after sterilization was analyzed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Samples were incubated at 95°C for 10 minutes under reducing conditions (5% (v/v) 2-mercaptoethanol) and analyzed on an 8% (w/v) gel.[29] Only potential impurities and degradation products will penetrate the gel since collagen fibrils are insoluble in sample buffer. Gels were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 solution (ICN Pharmaceuticals, Calif), containing 50% (v/v) methanol and 10% (v/v) acetic acid.

To evaluate the network characteristics of the type I collagen under physiologic conditions, the injectable collagen suspension (4% (w/v) in MilliQ), was injected in phosphate buffered saline (PBS, pH 7.4) at 37°C. In addition, to study this on the electron microscopic level, a collagen suspension (0.5% (w/v) in MilliQ water) was 1:1 diluted with PBS at 37°C. Collagen suspension (0.5% (w/v) in MilliQ water) 1:1 diluted in MilliQ water was used as a control. The collagen was analyzed using the JEOL JSM-6310 SEM apparatus as previously described.

Injection characteristics of type I collagen suspension. To determine the force that is needed to inject type I fibrillar collagen suspension, an injection force test was performed.[30] Different collagen suspensions (3%, 4%, and 5% [w/v] in MilliQ water) were extruded from a 1 mL

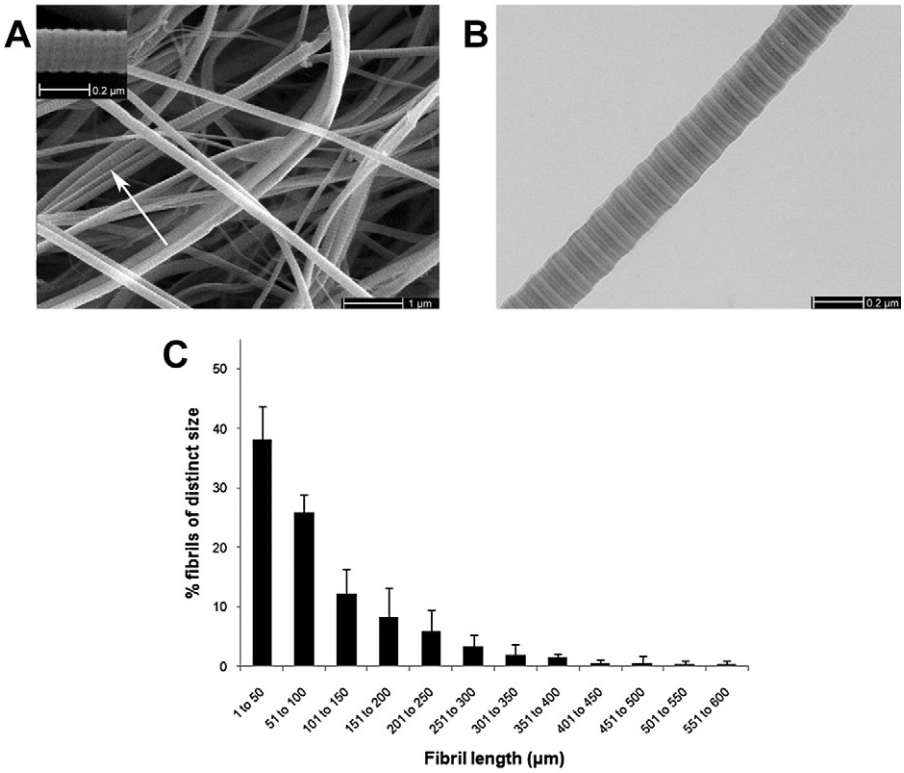

Fig 1. Morphology and length of purified type I collagen fibrils. Scanning and transmission electron micrograph of purified type I collagen fibrils (**A**) and (**B**), respectively, and the distribution of fibril length (µm) in the collagen preparation (**C**). Results are mean ± SD of three separate experiments. Bar in (**A**) is 1 µm and inset is 0.2 µm. Bar in (**B**) is 0.2 µm.

syringe with a 21-gauge needle (Ø 0.7 mm). The test was performed at a crosshead speed of 1.8 mm/s. The force was recorded by an MTS 810 testing machine combined with an MTS 458.20 microconsole (MTS Systems Corporation, Eden Prairie, Minn).

Platelet aggregation. Platelet aggregation and thrombus formation is dependent on the structure of the collagen preparation.[31] To assess the effect of sterilization of the injectable collagen on the platelet activity, platelet aggregation was measured. The test was performed in a four-channel whole-blood aggregometer model 590 (Chrono-Log Corp, Havertown, Penn) measuring the impedance (%), that is increased proportional with the amount of platelet aggregation.[31,32] Briefly, whole blood was collected by vena puncture from a healthy volunteer and collected in 3.2% (w/v) sodium citrate tubes (mean platelet count: $274{\cdot}10^3 \pm 30{\cdot}10^3/\mu L$). Whole blood was diluted with PBS in a 1:1 ratio and incubated for 15 minutes at 37°C. Next, 10 µL collagen suspension (0.05 mg/mL MilliQ water) was added to 1.0 mL diluted blood. All measurements were completed within 2 hours from the blood draw. The platelet aggregation of nonsterilized collagen was set on 1 arbitrary unit (AU). Single way ANOVA was used for statistical analyzes and $P < .05$ was considered statistically significant.

Evaluation of injectable collagen in a porcine model for femoral artery pseudoaneurysm. To study the effectiveness of injectable collagen fibrils for treatment of pseudoaneurysm a pig model was used as described.[33] This study was approved by the ethics committee (EC) of the Radboud University, Nijmegen, The Netherlands, and conducted under the supervision of veterinarians according to the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH publication 85-23 Rev. 1985). The pigs were housed at the Central Animal Facility, Nijmegen, The Netherlands. The animals were fed a normal laboratory diet and water ad libitum. Briefly, the femoral artery and femoral vein in the groin of an adult pig were exposed and temporarily clamped. A longitudinal arterio-venous anastomosis (side to side) with a 4 mm opening was prepared and about a 2 cm segment of the femoral vein was used as PSA model after closing the femoral vein both proximally and distally using nonabsorbable ligatures. The muscle layer and skin were closed. Amoxicillin (20 mg/kg/day) was injected intramuscularly for 4 days to protect the animal from bacterial infections. The animals received 2 mg/kg carbasalate calcium (Meda Pharma BV, Amstelveen, The Netherlands) and 0.75 mg/kg clopidogrel (Bristol-Myers Squibb/Sanofi Pharma-

ceuticals Partnership Bridgewater, NJ) every 48 hours for 4 days, as interim anticoagulant and pain medication.

Four days after construction of the PSA model, the animals were anesthetised and the operation site was re-opened to evaluate if the PSA model was pulsatile and to directly inject the collagen preparation. Using a 21-gauge needle, 4% (w/v in MilliQ water) EtO sterilized 4% (w/v) collagen suspension (100-200 μL) was injected in the PSA compartment of six individual pigs, and the blood coagulation, host response, and network properties of the collagen evaluated. Intraoperative femoral artery angiography was performed to document the blood flow in the PSA model before and after collagen injection. A contrast agent (Xenetix 300; Codali Guerbet, Brussels, Belgium) was used as an intravascular flow tracer. The angiogram was performed by accessing the contralateral site with a 6F sheath (Cordis, Miami, Fla). A 5F angiographic catheter (Boston Scientific, Natick, Mass) was introduced and positioned across the aortic bifurcation in the ipsilateral proximal common femoral artery over a guidewire. All angiograms were performed with a Philips BV-25 C-arm image intensifier (Philips, Eindhoven, The Netherlands). After removal of the catheters, the wound was closed. Six days after the initial evaluation, a second angiogram was performed, and the animal was subsequently sacrificed by an overdose of intravenous barbiturate. The area of the pseudoaneurysm and the surrounding tissue was explanted and immediately fixed in 4% (v/v) formaldehyde in 10 mM phosphate buffer (pH 7.2) for 24 hours at 4°C and embedded in paraffin. Consecutive 5 μm sections were mounted onto glass slides, dewaxed in xylol, and rehydrated through a graded series of ethanol. Hematoxylin and eosin (H&E) staining was employed for histological evaluation.

RESULTS

Characterization of type I collagen. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to analyze the morphology and ultrastructure of the purified type I collagen fibrils. Both SEM and TEM showed intact fibrils with distinct striated pattern, and no impurities could be detected (Fig 1, *A* and *B*). The striated pattern reflects the three-dimensional organization of (triple helical) collagen molecules within a collagen fibril. The measured length of collagen fibrils (Fig 1, *C*) indicated that fibril length was <400 μm, 75% of the fibrils being <150 μm.

SDS-PAGE was used to biochemically characterize the collagen before and after sterilization (Fig 2). For native type I collagen, SDS-PAGE showed α_1 and α_2 bands that are typical for type I collagen (Fig 2; *lane 1*). No major differences were found before and after EtO sterilization or H_2O_2 sterilization (Fig 2; *lanes 2 and 3*). After gamma sterilization, however, a smear appeared on the gel, indicating collagen degradation products (Fig 2; *lane 4*).

Platelet aggregation. A whole-blood platelet aggregation test was used to analyze the effect of sterilization on platelet aggregation (in arbitrary units). The platelet aggregation of the native nonsterilized collagen was set to 1 arbitrary unit. The platelet aggregation of EtO, H_2O_2, and gamma was 1.05 ± 0.14, 0.63 ± 0.14, and 0.73 ± 0.09, respectively (Fig 3). Although EtO sterilized collagen was most comparable to the nonsterilized collagen, other ster-

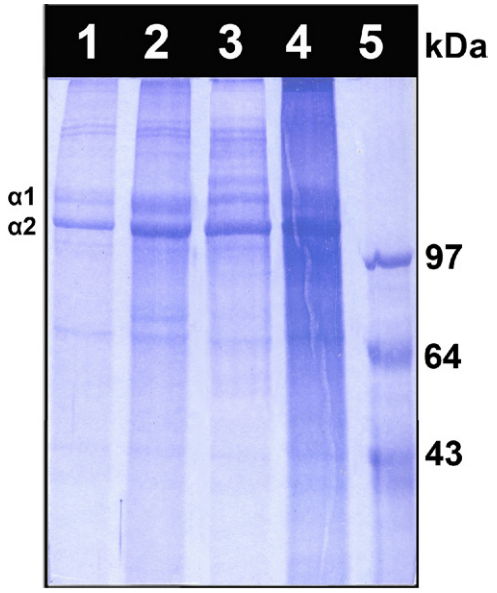

Fig 2. Effect of sterilization procedure on the integrity of collagen. SDS-PAGE under reducing conditions of fibrillar type I collagen before (*lane 1*) and after (*lanes 2-4*) sterilization, viz.: ethylene oxide gas sterilization (*lane 2*), hydrogen peroxide (H_2O_2) gas sterilization (*lane 3*), or gamma irradiation (*lane 4*). The low molecular weight marker is visualized in lane 5. This is a combined image taken from two separate gels (*lanes 1, 2, and 4 are from one gel*). Note that especially after gamma sterilization, collagen degradation products are observed.

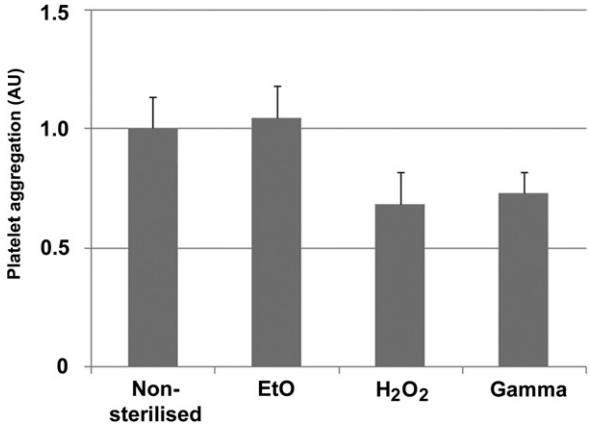

Fig 3. Effect of sterilization on platelet aggregation of fibrillar collagen. Collagen before sterilization was set on 1 arbitrary unit (*AU*). Results are mean ± SD for three separate experiments. Sterilized collagen showed no statistical significant differences in comparison to nonsterilized collagen ($P > .05$). *EtO*, Ethylene oxide gas sterilization; *Gamma*, gamma irradiation; H_2O_2, hydrogen peroxide sterilization.

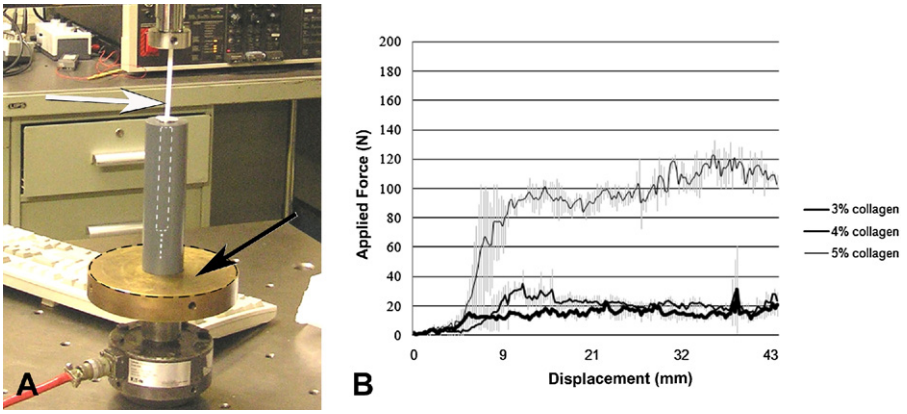

Fig 4. Evaluation of injectability. Macroscopic image of the displacement test set up with type I collagen suspension in a 1 mL syringe (**A**). Graph of displacement test (*1.8 mm/s with maximal distance 44 mm*) of 3%, 4%, and 5% (*w/v*) type I collagen suspension in a 1 mL syringe with a 2F (*0.7 mm*) needle (**B**). On the Y-axis, force is given in Newton, and on the X-axis displacement is shown in mm. Results are mean ± SD of three separate experiments. The *white arrow* and *white dashed line* indicate the 1 mL syringe in the displacement set up. The *black arrow* and *dashed circle* indicate the pressure plate.

ilization methods did also not significantly differ. EtO sterilized collagen was used for in vivo studies.

Characterization of type I collagen injectability. An injection force test was performed to analyze the effect of different concentrations of type I collagen on injectability (Fig 4, *A*). The force was measured while extruding a 3%, 4%, or 5% (w/v) suspension from a 1 mL syringe through a 2F (21 gauge, 0.7 mm) needle. The suspensions required a force of 18 N, 19 N, and 117 N, respectively (Fig 4, *B*). According to ISO regulations, both 3% and 4% (w/v) collagen suspensions are acceptable and can be used for manual injection (≤50 N according to ISO 7886-1:1997; Sterile hypodermic syringes for single use - Part 1: Syringes for manual use). The 4% (w/v) collagen suspension was selected as the preferred injectable collagen suspension.

To analyze the network properties of the injectable type I collagen under physiologic conditions, the 4% (w/v) collagen in MilliQ water (Fig 5, *A*) was applied to pre-heated (37°C) phosphate buffered saline (pH 7.4). Macroscopic evaluation indicated that the collagen formed a dense network immediately after extrusion in PBS (Fig 5, *B*).

SEM analyzes indicated a decrease in spacing between the collagen fibrils, which is in line with aggregation of fibrils into a network (Fig 5).

Evaluation of injectable collagen in a porcine model for femoral artery pseudoaneurysm. No complications due to the surgery were observed. In one of six cases, the femoral PSA model clotted directly after the operation and this model was excluded from the experiment. No bleeding complications occurred as a result of surgery.

Four days after surgery, the PSA was evaluated with angiography before and after collagen injection. When the PSA model was open (Fig 6, *A*), 100 to 200 μL 4% (w/v) type I collagen in MilliQ water was locally injected. In four of the five cases, the PSA model partly or completely coagulated within approximately 5 minutes after type I collagen injection, which was confirmed with angiography (Fig 6, *B*). In one case, both femoral artery and PSA coagulated. In this case, both femoral arteries were found open 6 days after injection leaving the PSA nicely closed (data not shown). After cross sectioning the injected PSA, both the collagen and the thrombus were macroscopically visible and could be localized (Fig 7).

Histologic evaluation. Six days after injection, a minor infiltration of inflammatory cells was observed (Fig 8, *A*). Specimens demonstrated a prominent thrombus and partial leukocyte infiltration toward the center of the thrombus (Fig 8, *B*). The PSA wall was clearly visible and the injected collagen was observed in a network that appeared to be interconnected with the thrombus.

DISCUSSION

Collagen is well known for its hemostatic activity and has been widely used as a biodegradable plug to prevent hemorrhagic complications after arterial puncture, ie, catheterization or coronary angioplasty.[34,35] To our knowledge, only one study describes the use of collagen treatment after femoral PSA occurrence.[15] Despite the reported efficacy of this study (97%), its use has not currently gained wide acceptance.[21] However, the promising results of this study warrant further investigations to the use of collagen for PSA treatment.

Compared with thrombin there may be several additional values for use of collagen for (complex) PSA treatment. The first advantage may lay in its physico-chemical properties. With fibrillar type I collagen as an injectable, the consistency of the material is important. Collagen swells at low pH,[36] making it possible to prepare a suspension or paste which can be injected. When the pH of the collagen suspension was increased to a physiological range (pH 7.4), the collagen fibrils formed a dense network. After applying the injectable collagen in the PSA model, fibrils aggregated

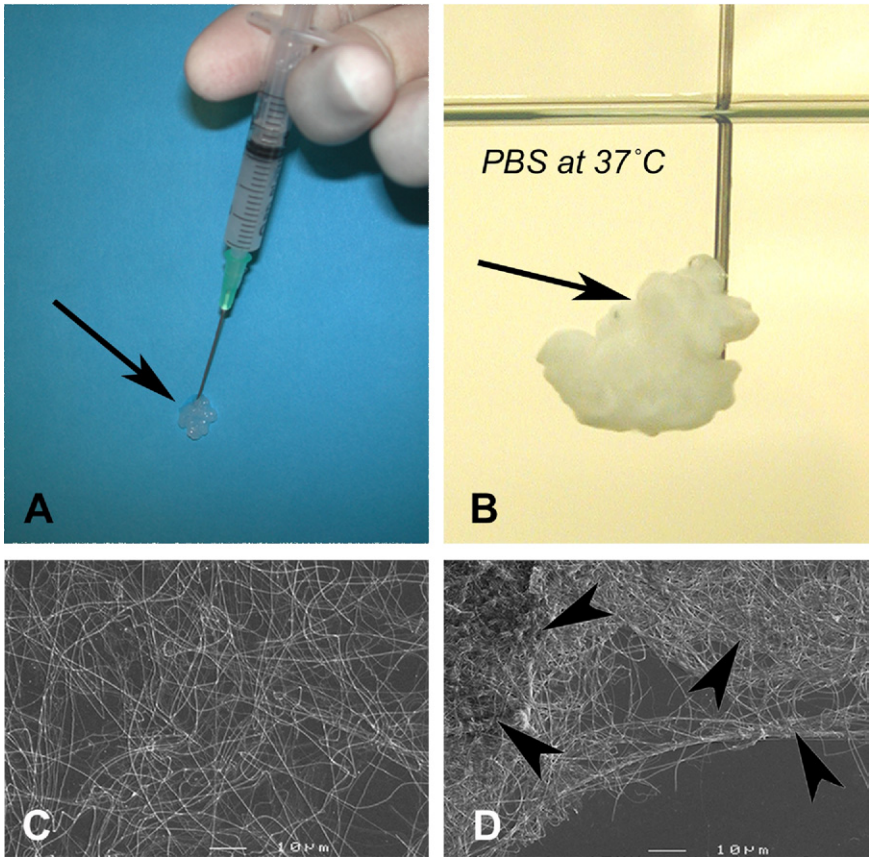

Fig 5. Aggregation of injectable collagen under physiologic conditions. Macroscopic images of 4% (*w/v*) type I collagen suspension in MilliQ water (**A**) and the network that was formed directly after injection of the type I collagen in pre-heated (*37°C*) phosphate buffered saline (*PBS, pH 7.4*) (**B**). Scanning electron microscopic images after applying a 0.5% (*w/v*) collagen suspension in MilliQ water or in PBS, resulting in an open fibril network (**C**) and an aggregated fibril network (**D**), respectively. Under physiologic conditions (*PBS, 37°C*), the collagen fibrils formed a dense network. *Arrows* in (**A**) and (**B**) indicate the aggregated type I collagen suspension. *Arrowheads* for (**D**) indicate the aggregated collagen fibrils. Bar is 10 μm in (**C**) and (**D**).

and concentrated at the site of injection. Leukocytes infiltrated this collagen network, which is commonly found after applying collagenous biomaterials in vivo.[37] The consistency and the ability to form a network may be an advantage to reduce the risk of arterial influx of collagen fibrils. In addition, complex pseudoaneurysms have been described as contraindications for thrombin treatment.[11] Several cases reported thrombin failure in these complex pseudoaneurysms.[12-14] The soluble thrombin may quickly diffuse through the wide neck of the pseudoaneurysm toward the lumen of the artery. In most cases, these complex pseudoaneurysms need to be revised surgically. Especially in such cases, injectable collagen can be an attractive agent, since collagen is highly viscous and collagen fibrils aggregate and deposit at the site of injection, as shown in this study. The pseudoaneurysm model used in this study involves the preparation of an arteriovenous shunt between the femoral artery and femoral vein, where ~2 cm of the vein is segmented by proximal and distal closure using ligatures. This model presents a complex pseudoaneurysm (with a relative wide opening [~4 mm][33]) and makes the experimental model highly challenging with respect to pseudoaneurysm treatment.

A second issue may be the difference in immunogenicity between thrombin and collagen. There are several reported cases of immune complex reactions to bovine thrombin resulting in antibody formation. Although less for recombinant human thrombin,[38] the antibodies may cross-react with the coagulation cascade, resulting in factor inhibition and clinical coagulopathy. Forty percent to 66% of patients who underwent cardiac surgery and 20% of patients who underwent neurosurgery developed bovine thrombin-associated factor V antibodies.[39] This complication may be overcome by the use of collagen, since fibrillar bovine collagen is almost nonimmunogenic.

Other advantages may relate to technical issues. The injectability of collagen is critical for its clinical performance and for patient comfort, eg, in case of the use of collagen as

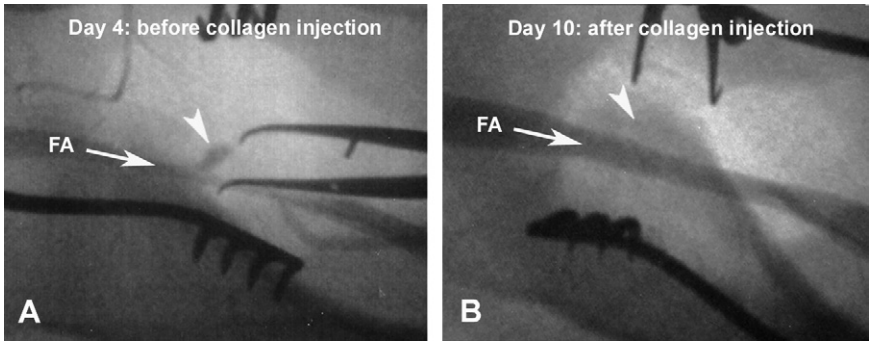

Fig 6. Angiograms of the porcine femoral pseudoaneurysm (PSA) (*animal no. 6*) at day 4 before collagen injection (**A**) and at day 10 (*6 days after collagen injection*) (**B**). Tweezers were positioned on the PSA segment (*white arrowheads*). In (**A**), the PSA was open. In (**B**), it was closed after collagen injection. *White arrows* indicate the direction of arterial blood flow in the femoral artery (*FA*).

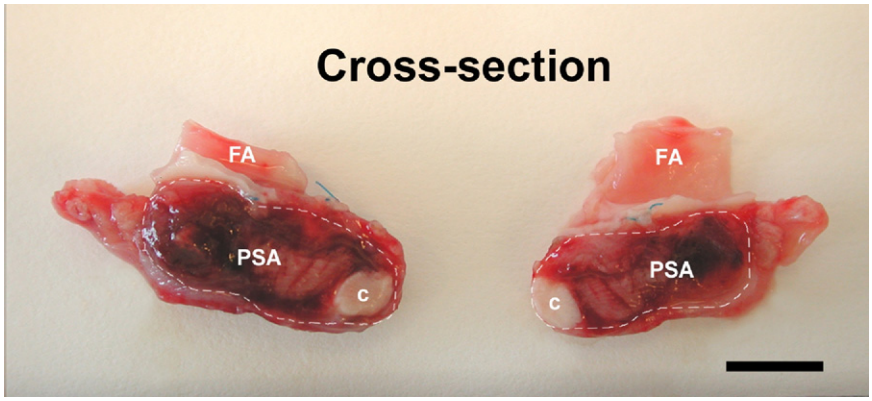

Fig 7. Macroscopic image of cross-sectioned pseudoaneurysm (*PSA*) model, 6 days after collagen injection (*animal no. 1, day 10*). *FA,* Femoral artery; *PSA,* pseudoaneurysm model with thrombus; *c,* injected type I collagen suspension (*4% [w/v] in MilliQ water*). The *white dashed lines* indicate the boundary of the PSA wall. Bar indicates 5 mm.

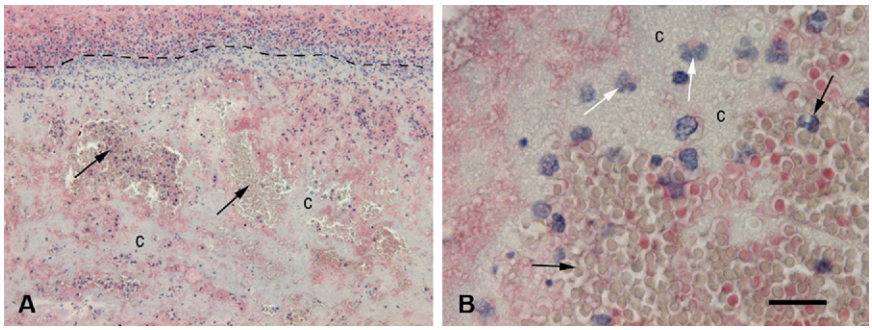

Fig 8. Microscopic images of coagulated pseudoaneurysm (PSA) model (*luminal site*) 6 days after type I collagen injection. Hematoxylin-eosin stained sections showed a clotted PSA filled with type I collagen (*c*) and coagulated blood cells (*black arrows*) (**A**) and (**B**). The wall of the PSA is indicated by *dashed line* (**A**). Leukocytes were found within the collagen network (*white arrows*) (**B**). *c,* Collagen network. Bar indicates 500 μm in (**A**) and 50 μm in (**B**).

a dermal filler.[40] Especially for percutaneous PSA treatment, it is important that the agent can be injected through a needle with a limited diameter. In the clinical study of Hamraoui et al, a 1% (w/v) collagen suspension was injected through a relatively large 9F (Ø 3 mm) needle, which may come with discomfort for the patient. In our suspension, the collagen content was higher (4% [w/v]) and could be injected through a 2F (Ø 0.7 mm) needle, which is comparable with the procedure using thrombin injections (Ø 0.6 mm).[21] In addition, one well-known technical

difficulty in the use of thrombin is blood clotting inside the needle tip resulting in injection problems.[41] With injectable collagen we did not observe this problem. The high consistency of the collagen may prevent the entrance of blood into the needle and subsequent clot formation.

Proper sterilization is an important issue when using injectable collagen for medical applications. The injectable collagen must be supplied as a sterile product, but the efficacy of the collagen must not be compromised by the sterilization technique. It has been described that techniques like gamma irradiation affect the structural properties of the collagen fibrils.[42,43] This may adversely affect the platelet aggregation and thrombus formation. For this reason, we tested different sterilization techniques and evaluated both biochemical and primary hemostatic characteristics. Both EtO and H_2O_2 sterilized collagen did not compromise the integrity of the collagen. We selected EtO for its tendency to have less influence on collagen-induced platelet aggregation. In vivo, the injectable collagen suspension did induce local hemostasis within an approximate 5-minute clotting time, but this was often partial. However, in one case we found that both the femoral artery and the PSA coagulated after collagen injection. Although the artery was open after 6 days, it demonstrates the existing risk of injecting hemostatic agents in femoral PSA. It has been described that a percutaneous thrombin injection in a femoral PSA may be associated with an increased risk of thrombus extension.[44] For thrombin injection, various methods are known to decrease this risk, eg, directing the needle away from the PSA neck[9] or placing a balloon in the PSA neck.[45] In case of high risk (complex) PSAs, these methods may also prevent the risk on distal embolization when using injectable collagen. Particularly for the complex femoral PSA treatment, it might be attractive to combine collagen fibrils with thrombin to not only initiate the primary hemostasis, but also induce the secondary hemostasis (clotting factor mediated). Although not tested in this study, such an approach has been successful in giant splenic artery pseudoaneurysm treatment.[46]

In view of our results, we conclude that an EtO sterilized, injectable 4% collagen could be prepared with defined structural characteristics and platelet activity. In vivo, the injectable collagen formed a dense network, which was (partially) hemostatic active. Injectable collagen may be an effective therapeutic alternative for current femoral PSA treatment, but additional studies are needed to prove its efficacy.

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AUTHOR CONTRIBUTIONS

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