Inhibition of neointimal hyperplasia in a sheep model of dialysis access failure with the bioabsorbable Vascular Wrap* paclitaxel-eluting mesh

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Objective: **This study evaluated the effect of a bioabsorbable mesh containing paclitaxel on neointimal hyperplasia in a sheep model of dialysis access failure.**

Methods: **Forty neutered male sheep were randomized to one of five parallel groups: no mesh; or a 3-cm × 6-cm mesh with 0.0, 0.3, 0.7, or 1.2 μg/mm^2 of paclitaxel for a total dose of 0.0, 0.6, 1.3, or 2.2 mg, respectively. Commercially available 6-mm internal diameter expanded polytetrafluoroethylene grafts were surgically placed between the left common carotid artery and the right external jugular vein. For those animals randomized to one of the mesh groups, the mesh was placed around the distal end of the graft and venous anastomosis. Patency was assessed at weekly intervals throughout the study. Animals were euthanized 8 weeks after implantation, and grafts and veins were harvested. After histologic processing, six cross sections were cut at the venous end of the graft and vessel. The primary and secondary efficacy outcome measures, respectively, were the area and capillary density of the neointima at the graft-vein anastomosis. Histologic analyses were also performed to investigate the effects of the paclitaxel-eluting mesh on the anastomotic site.**

Results: **Grafts occluded before the scheduled sacrifice in five animals, and they were excluded from the study and not replaced. Control animals developed significant neointimal hyperplasia at the cross section taken perpendicular to the graft at its most distal end: the neointimal area measured 10.5 ± 6.8 mm^2 in the no mesh group and 6.4 ± 3.2 mm^2 in the zero-dose mesh group ($P = .28$). In contrast, neointimal area was significantly reduced in the paclitaxel mesh groups: 0.9 ± 1.4 mm^2 in the 0.3 μg/mm^2 group ($P = .008$ vs zero-dose mesh), 1.3 ± 1.5 mm^2 in the 0.7 μg/mm^2 group ($P = .004$ vs zero-dose mesh), and 1.2 ± 1.4 mm^2 in the 1.2 μg/mm^2 group ($P = .008$ vs zero-dose mesh). Capillary density in the neointima at the graft-vein anastomosis decreased with paclitaxel and was significantly reduced in the paclitaxel mesh groups with 0.3 and 1.2 μg/mm^2 compared with the zero-dose mesh control (3.6 ± 2.9 vs 8.9 ± 5.6 per mm^2 [$P = .022$] and 1.1 ± 1.7 vs 8.9 ± 5.6 per mm^2 [$P = .001$] respectively). The paclitaxel mesh had no significant effect on healing of the anastomosis or on the thickness of the adjacent vein.**

Conclusions: **In this model, the paclitaxel-eluting mesh significantly reduced neointimal hyperplasia and neointimal capillary density without apparent toxicity to the adjacent vein. (J Vasc Surg 2007;45:1029-38.)**

Clinical Relevance: **Although synthetic grafts (most commonly expanded polytetrafluoroethylene) are currently used in approximately 40% of hemodialysis patients who require a permanent vascular access, primary patency rates remain poor. Most graft failures are caused by venous neointimal hyperplasia, and there are no proven pharmacologic interventions that effectively prevent it. This study provides evidence of the safety and efficacy of a bioabsorbable paclitaxel-eluting mesh for inhibition of neointimal hyperplasia in a sheep model of dialysis access graft failure.**

Maintenance of arteriovenous (AV) access is one of the most intractable issues in the management of hemodialysis patients.[1] Native AV fistulas, used in 43.7% of long-term hemodialysis patients in the United States as of August 2006,[2] have repeatedly proven to be superior to all other forms of dialysis access, both clinically and in cost effectiveness.[3] Accordingly, there is a concerted and ongoing effort underway in the United States (US) to encourage the placement of native AV fistulas for dialysis access whenever possible.[4] In many cases, however, it is not feasible to create a primary AV fistula, particularly in older, diabetic patients and in patients whose veins have been used for multiple venipunctures and intravenous infusions over the course of their prolonged illness.

Synthetic grafts, most commonly made from expanded polytetrafluoroethylene (ePTFE), are required when no suitable vein is available for fistula creation. Synthetic grafts have worse patency rates of 50% at 1 year and 25% at 2 years

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than native fistulas at 85% at 1 year and 75% at 2 years.[5] Despite this, approximately 39% of US hemodialysis patients—or roughly 116,000 patients—had a synthetic graft placed for vascular access in 2004. Synthetic vascular grafts have significant advantages over native fistulas: they are easier to create and cannulate, and they require less time to mature (2 weeks vs 2 months). These devices are preferred over central catheters, which have a high rate of infection and may produce central vein stenosis.

Eighty percent of PTFE grafts fail because intimal hyperplasia causes stenosis at the venous anastomosis.[6] If this problem could be eliminated or significantly mitigated to provide patency rates similar to native AV fistulas, then the many advantages of PTFE would make this an attractive option in many more patients. To date, no therapies are available to effectively inhibit intimal hyperplasia at the venous anastomosis. Angioplasty is not a durable treatment,[7-9] and repeat multiple procedures are often necessary.[5,6] Many systemically delivered drugs have been found to reduce intimal hyperplasia in various animal models of restenosis after vascular injury, but none have proven to have clinical benefit (eg, heparin, angiotensin-converting enzyme inhibitors, calcium channel blockers, steroids). Similarly, no systemic pharmacologic interventions to date have proven effective in the prevention or treatment of venous intimal hyperplasia in ePTFE dialysis grafts, although dipyridamole has shown some promise.[3,10,11]

In contrast, certain pharmacologic agents combined with a local intravascular delivery device are effective in reducing neointimal formation in other vascular applications. One such combination, the paclitaxel-eluting stent used in coronary angioplasty, has proven highly successful in preventing restenosis and major adverse coronary events.[12-17] Even though the drug remains active for only a few weeks, the benefits of this therapy are durable, persisting for as long as 4 years.[18] A similar, local therapy approach would be very attractive for dialysis access grafts, particularly if it had no adverse effect on healing and could be applied easily at the time of surgery. The Vascular Wrap (Angiotech Pharmaceuticals, Inc, Vancouver, British Columbia, Canada) was developed for this purpose. It is a mesh containing paclitaxel and is composed of polyglycolic-polylactid acid, which fully degrades by hydrolysis in vivo between 60 to 90 days. We tested this local therapy in a well-characterized sheep model of dialysis access stenosis.[19]

METHODS

Experimental design and conduct. The in-life component of the study was conducted at the research facility of BioDevelopment Associates, LLC (Bellevue, Wash) and was approved for compliance with regulatory guidelines concerning the care and use of animals by its Institutional Animal Care and Use Committee. The facility is licensed by the US Department of Agriculture and approved by the Office for Protection From Research Risks. All animal housing and care complied with the *Guide for the Care and Use of Laboratory Animals* (US National Institutes of Health Publication No. 85-23, National Academy Press, Washington, DC, revised 1996).

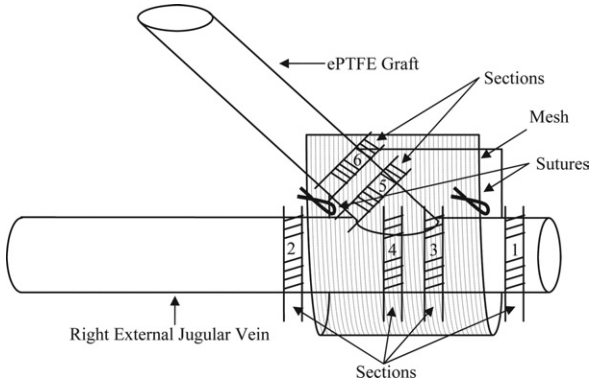

Fig 1. Schematic diagram of right external jugular vein and expanded polytetrafluoroethylene *(ePTFE)* graft anastomosis with applied mesh and histologic sections.

After the administration of heparin (150 U/kg intravenously [IV]), commercially available ePTFE grafts (Impra Flex ePTFE Vascular Grafts, 6 mm internal diameter, Bard Peripheral Vascular, Inc, Tempe, Ariz) approximately 8 cm in length were placed between the left common carotid artery and right external jugular vein in 40 sheep, one per animal. Animals were randomized (8 per group) to one of five treatment groups: no mesh; or mesh with 0.0, 0.3, 0.7, or 1.2 $\mu g/mm^2$ of paclitaxel, for a respective total dose of 0.0, 0.6, 1.3, or 2.2 mg. For those animals in the mesh groups, a sterilized 3-cm $\times$ 6-cm paclitaxel-eluting, bioabsorbable mesh was placed around the distal end of the graft-vein anastomosis. Surgeons applying the mesh were blinded to treatment group.

Graft patency was assessed immediately after implantation and at weekly intervals thereafter. Animals were euthanized 8 weeks after implantation and grafts and veins were harvested. After histologic processing, six cross sections were cut at the graft-vein anastomosis (Fig 1). Histopathologic and morphometric analyses of these samples were performed by a pathologist who was blinded to the treatment group of each animal.

Experimental procedures. In vitro release profiles for paclitaxel were obtained by placing samples of the mesh in a culture tube with phosphate buffer (pH 7.4) containing bovine albumin (0.8 g/L). The tubes were placed on a rotating disk (Glas-Co, Terre Haute, Ind) at 37°C. The amount of drug eluting into solution was assayed at various times up to 10 days. At these times, the buffer was removed from the sample and replaced with fresh buffer. The drug was extracted from the removed solution with 1 mL of dichloromethane, which was then separated from the samples by pipetting. After addition of a 1:1 acetonitrile and water solution, the paclitaxel content was analyzed by high-performance liquid chromatography. Results were calculated as the cumulative percentage of paclitaxel eluting over time.

Forty Columbia crossbred male lamb or yearling sheep (Nebeker Ranch, Los Angeles, Calif), neutered for ease of handling, were fasted for 12 hours before surgery and then sedated with intramuscular xylazine (0.2 to 1.0 mg/kg) and IV ketamine (3 to 10 mg/kg). After intubation, anesthesia was maintained with inhalation isoflurane. The neck was prepared for aseptic surgery.

Expanded PTFE grafts (6-mm internal diameter) were placed between the left common carotid artery and right external jugular vein, as reported previously.[19] After graft placement and restoration of blood flow, the vessels were allowed to equilibrate for several minutes. For those animals randomized to receive a mesh, the mesh was then applied to the distal (venous) anastomosis by pulling the long side (6 cm) of the mesh under the vein and up around either side of the distal end of the graft. One edge of the mesh was positioned as close to the heel of the anastomosis as possible. Sutures were placed at the proximal and distal ends of the mesh and sewn to nearby connective tissue to prevent slippage. The surgical site was then closed in layers.

Animals were administered flunixin meglumine (Banamine [Schering-Plough, Kenilworth, NJ], 1.0 mg/kg intramuscularly or subcutaneously as needed) as an analgesic to relieve postoperative pain. In addition, intramuscular ampicillin (5 mg/kg) was administered for 3 days beginning the day of the surgery.

Animals were fed daily PMI Rumilab Diet (PMI Nutrition International, LLC Brentwood, Mo). They were group-housed in wire-mesh enclosed pens with concrete floors for 4 to 10 days after surgery and were then moved to a fenced pasture with readily available shelter and supplemental feed, as described.

Immediately after implantation and at weekly intervals thereafter, graft patency was assessed by palpation or auscultation, or both, with a stethoscope for evidence of blood flow through the graft. Animals with graft failure occurring before the scheduled sacrifice were euthanized after confirmation of occlusion using Doppler ultrasonography and angiography. The remaining animals were euthanized between 56 and 58 days after graft implantation. Animals were sedated with intramuscular xylazine (0.2 to 1.0 mg/kg) and IV ketamine (3 to 10 mg/kg), intubated, and anesthetized with isoflurane. Contrast medium was injected through puncture of the midgraft, and angiograms were taken of the graft and vein at the distal anastomosis to verify patency. Animals received heparin (150 U/kg, IV) and were euthanized with an IV overdose of sodium pentobarbital.

The artery was immediately cannulated proximal to the arterial anastomosis and ligated beyond it. The recipient vein was opened to allow drainage, and the ePTFE graft was rinsed in situ with Ringer's lactate solution, followed by 10% neutral buffered formalin at physiologic pressure for several minutes. The specimens were excised and allowed to immersion-fix in formalin for a minimum of 24 hours before histologic processing. Six samples were then cut at the graft-vein anastomosis (Fig 1). Adjacent samples were cut at intervals of 2.5 to 4.5 mm. Specimens were paraffin-embedded, and cut as thin sections for histology. Slides were stained with hematoxylin and eosin. The study pathologist performed all qualitative and quantitative analyses in a blinded fashion. Assessment of the blinded histopathology observations relative to treatment group assignation was performed retrospectively.

Outcome measures. Histopathologic assessment included characterization of cellular composition of vascular and perivascular tissue and graft in the vicinity of the venous anastomosis. Efficacy was measured by morphometric analysis of the cross-sectional samples. Quantitative analyses were made with computer-aided morphometry using Image-Pro Plus 4.5.1.22 (Media Cybernetics, Silver Spring, Md) for Windows XP (Microsoft, Redmond, Wash).

Morphometric measurements included the combined area of neointima and mural thrombus and area inside the graft (Appendix A, online only); the pathologist then used these measurements to calculate values for neointimal area and luminal area. Percentage of stenosis was calculated as the ratio of the luminal area to the area inside the graft. For these morphometric outcomes, section 5, cut from the graft at its distal (venous) end, was determined to be the cross-section of primary interest because it was the most proximate to the anastomosis. Data are also reported for the adjacent graft section (section 6), approximately 5 mm upstream from the venous anastomosis. Neointimal area was chosen as the primary efficacy end point because it is the most accurate measure of neointimal hyperplasia.

Because paclitaxel inhibits angiogenesis as well as cellular proliferation, capillary density was measured to determine if reduction in neointimal hyperplasia was associated with a reduction in neovascularization. Capillary density was measured in the neointima within the PTFE graft at the toe of the graft (section 4 or 3, whichever had the best arc of ePTFE graft with adjacent vein). The toe section was chosen because it is closest to the vein and has the most abundant capillary ingrowth. For this calculation, capillaries were identified and counted using sections stained with hematoxylin and eosin under light microscopy, using up to original magnification $\times 400$ as needed (Fig 2). The density was then calculated as the number of capillaries per mm^2 of neointima.

Statistical considerations. Animals whose grafts occluded before the scheduled sacrifice were excluded from the analysis. Histopathologic features were evaluated using a graded severity scale for semi-quantitative analysis. Results from the morphometric analyses were tabulated, and group means and standard deviations were calculated. A nonparametric approach was used rather than a parametric approach owing to the small sample size and the observation that the variance was inhomogeneous (see Results). Several transformations of the data were performed, but none were used because they still yielded large differences in standard deviations.

Nonparametric tests were used to compare groups. Specifically, pair-wise Fligner-Policello (FP) tests were used for the comparisons of interest. This test is similar to a Wilcoxon rank sum test but uses the Behrens-Fisher mod-

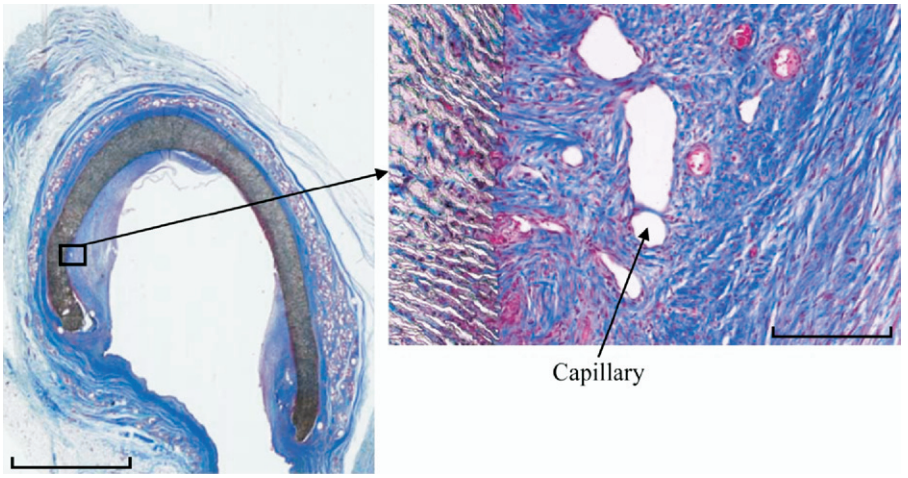

Fig 2. Example of a section through the toe of the graft (section 4) at low power showing where capillary density was measured in the neointima (*bar* represents 4 mm). The *inset* is a high power (*bar* represents 400 μm) from this region demonstrating multiple capillaries *(arrow)*.

ification, which allows for the variance in each group to be different.[20] Because standard tables for the FP test statistic only provide ranges of *P* values, exact two-sided Wilcoxon rank sum *P* values were also computed for all comparisons. Note that although the Wilcoxon *P* values are significant at the .05 level, standard FP tables only provide one-sided *P* values and thus a significant result is obtained only if the absolute value of the test statistic is greater than the critical value corresponding to $\alpha/2 = .025$. For simplicity of presentation, only the Wilcoxon *P* values are reported in the tables, figures, and text; the corresponding FP test statistics are provided in Appendices B and C (online only).

For each cross-section of interest, the zero-dose mesh group was compared with the control group with no mesh. All paclitaxel-loaded mesh groups were then compared with the zero-dose mesh group. The two graft cross sections, sections 5 and 6, cut from the graft at its distal (venous) end, were determined to be the cross sections of primary and secondary interest, respectively. The Fisher exact test was used to compare patency of control and drug-treated grafts. Linear regression analyses of the neointimal area and capillary density were also performed.

RESULTS

The in vitro drug elution profile of paclitaxel from the mesh at 0.3, 0.7, and 1.2 $\mu g/mm^2$ is shown in Fig 3. There was a rapid release of drug during the first 24 hours, followed by a more gradual release over the next 8 days. The rates of release increased with increasing drug concentration. At 1 week, 60% of the drug was released in the lower two concentrations (0.3 and 0.7 $\mu g/mm^2$), and 45% of the drug was released from the highest-dose mesh.

Animals appeared in good health and gained weight across all groups throughout the study. The average weight at the time of surgery was 35.5 kg. Surgical implantations of AV grafts, with and without mesh, were performed without complication or death.

Grafts occluded in five sheep before the scheduled sacrifice. Three of the five treatment groups were represented among the early occlusions: one graft in the zero-dose mesh group occluded after week 6; two grafts in the 0.3 $\mu g/mm^2$ paclitaxel mesh group occluded after week 3 or 4; and two grafts in the 1.2 $\mu g/mm^2$ paclitaxel mesh group occluded after week 3. In one animal in the 0.3 $\mu g/mm^2$ paclitaxel mesh group, the graft had eroded through the skin at its midsection, resulting in a kink that obstructed flow. The incisions in this animal were well healed, and there was no gross evidence of infection. The remaining four animals all had occlusive thrombi with no discernible cause.

A two-tailed Fisher exact test found no statistically significant difference between the proportions of grafts with occlusion in the drug group (16.7%) vs the control group (6.3%, $P = 0.63$). Necropsies performed on the 35 animals with patent grafts at 8 weeks indicated that incisions were well healed, with no evidence of inflammation. Healing around the venous anastomosis was normal in all animals, with mild-to-moderate fibrotic encapsulation of the graft.

Histologic analysis. Compared with the no-mesh control group, the addition of mesh alone resulted in an increase in the thickness of the fibrous tissue encapsulating the graft (from 0.8 mm to 1.5 mm) and an increase in perigraft macrophages from minimal to mild. Macrophages were concentrated around disintegrating mesh debris. Among groups that received paclitaxel, there was more mesh debris at higher paclitaxel doses and fewer macrophages. In the no-mesh and mesh-alone control groups, the nodes of the graft wall were completely filled with spindle-shaped cells (presumably myofibroblasts). In contrast, almost no spindle-shaped cells were found in the nodes of the graft wall in the highest paclitaxel dose group; only degenerating blood and fibrin debris were present.

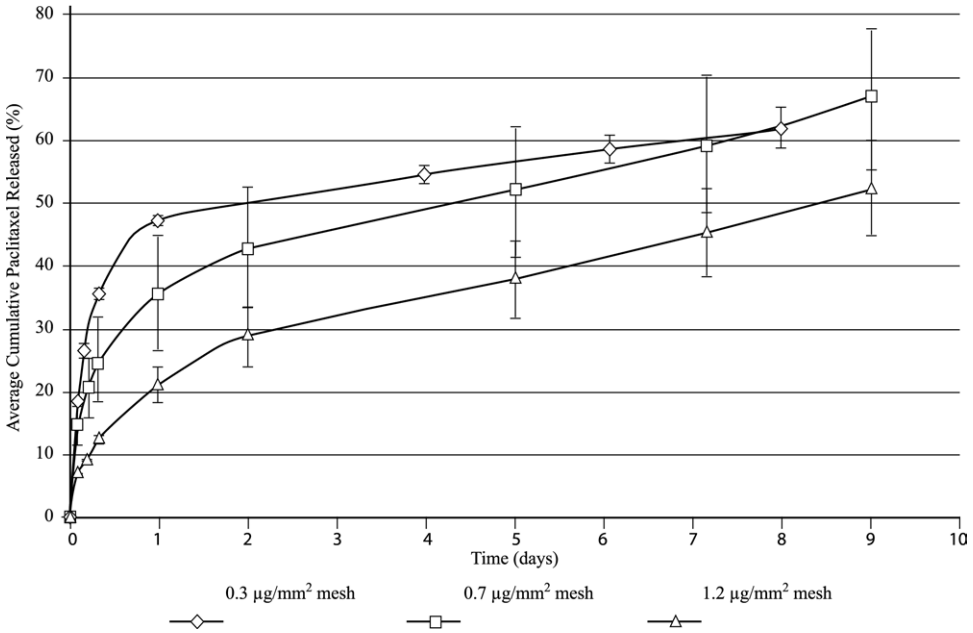

Fig 3. In vitro drug elution curves (presented with mean ± standard deviation) for paclitaxel from the polyglycolic-polylactid acid mesh at the three concentrations used in this experiment, 0.3 *(diamonds)*, 0.7 *(squares)*, and 1.2 *(triangles)* μg/mm^2.

There was no apparent dose effect of paclitaxel on perigraft inflammation or on the process of endothelialization (cells with the histologic appearance of endothelium covered the neointima in all groups). There was some enlargement of the vein at the anastomosis in most cases and some thickening of the vein; this was evident across all treatment groups. There was no evidence of loss of integrity of the vein wall at any dose. As we have found in our earlier work with this model, the vein adjacent to the graft did not develop hyperplasia.[19] Sections 1, 2, 3, and 4, taken through the vein, revealed no apparent atrophy or decrease in vein wall thickness, no surface thrombus, and an apparently intact endothelial lining (although immunohistochemistry was not performed for definitive cell typing).

Morphometric analysis. The nonparametric approach to data analysis was justified by the observation that the variance was very inhomogeneous among the groups as assessed visually using box plots. Although no official testing for unequal variances was performed, the standard deviation for the untransformed data for one group was often 10 times the standard deviation for another group in the same section.

At section 5, the first cross section through the graft above the anastomosis, neointimal hyperplasia was extensive in the group with no mesh and the group with zero-dose mesh (Table, Fig 4). Neointimal area was significantly lower in all paclitaxel mesh groups compared with the zero-dose mesh group ($P \leq .008$); sample photomicrographs illustrating this difference are presented in Fig 5. Section 6 had less neointima than section 5, but the effects of paclitaxel were similar (Fig 4, Appendix C. online only). Similar trends were observed for the outcomes of mural thrombus area and percentage of stenosis in both sections (Table; Appendices B and C, online only). Capillary density in the neointima at the graft-vein anastomosis (section 4 or 3) also decreased with paclitaxel (Table; Appendix B, online only). It was significantly lower in the mesh groups with 0.3 μg/mm^2 and 1.2 μg/mm^2 paclitaxel compared with the zero-dose group ($P = .022$ and $P = .001$, respectively). Linear regression analyses found an inverse relationship between neointimal area inside the graft and drug dose ($r^2 = 0.85$) and between capillary density in the neointima and drug dose ($r^2 = 0.77$). There was a strong, significant relationship between capillary density and intimal area ($r^2 = 0.99$, $P < .01$).

DISCUSSION

In this study, application of a paclitaxel-eluting absorbable mesh to the distal end of ePTFE arteriovenous grafts nearly eliminated neointimal hyperplasia at 8 weeks in a sheep model of access failure. The mesh is a proprietary biodegradable copolymer that resorbs in approximately 2 to 3 months and elutes paclitaxel over 21 days (approximately half ≤7 days). This type of local therapy has the advantage of being easily applied at the time of surgery and having no systemic effects. In prior experiments using similar implantations of mesh with paclitaxel in an arterial position, we found no detectable systemic levels of paclitaxel even at doses as high as 1.2 mg (unpublished data). The dose required for local drug therapy (<2 mg, eluted over a period of weeks) is more than 140 times lower than

Table. Morphometric data at the graft-vein anastomosis*

Group	Neointimal area (mm^2)	*P*†	Mural thrombus area (mm^2)	*P*†	Luminal stenosis (%)	*P*†	Capillary density (per mm^2)	*P*†
No mesh (n = 8)	10.5 ± 6.8		2.6 ± 1.9		49.4 ± 22.4		11.9 ± 5.8	
Paclitaxel mesh (μg/mm^2)								
0.0 (n = 7)	6.4 ± 3.2	.28‡	3.8 ± 2.9	.40‡	40.9 ± 14.1	.87‡	8.9 ± 5.6	.28‡
0.3 (n = 6)	0.9 ± 1.4	.008§	0.2 ± 0.3	.002§	3.6 ± 4.3	.001§	3.6 ± 2.9	.02§
0.7 (n = 8)	1.3 ± 1.5	.004§	1.5 ± 2.3	.071§	9.9 ± 10.0	.001§	4.6 ± 6.0	.07§
1.2 (n = 6)	1.2 ± 1.4	.008§	0.6 ± 1.2	.008§	6.8 ± 5.6	.001§	1.1 ± 1.7	.001§

*Neointimal area, mural thrombus area, and luminal stenosis were measured in section 5, the cross section taken perpendicular to the graft at its most distal end; capillary density was measured in the neointima at the toe of the graft (sections 4 or 3, whichever had the best arc with the adjacent vein). Data are presented as means ± standard deviation

†All *P* values are based on pair-wise comparisons made using the Wilcoxon rank sum test; the ranges of *P* values based on the Fligner-Policello test statistic are reported in Appendix B.

‡*P* vs no mesh.

§*P* vs mesh with 0 mg paclitaxel.

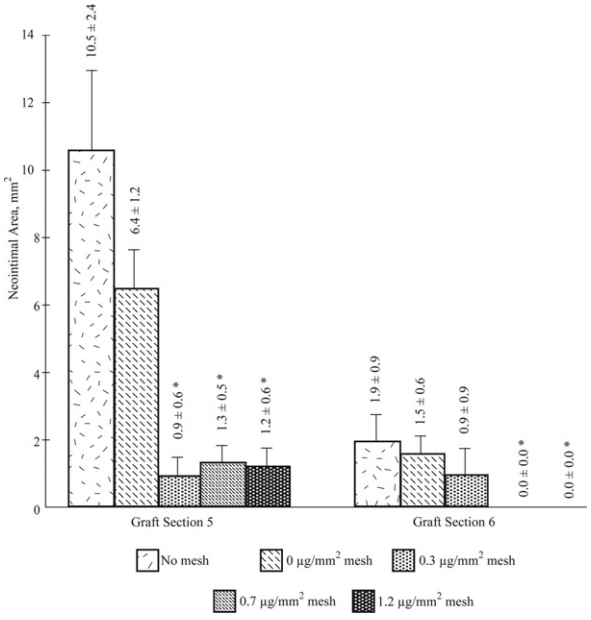

Fig 4. Dose effect of paclitaxel on neointimal area (presented with mean ± SEM). **P* < .05 vs 0 mg mesh group; there was no neointima formation with the two highest-dose mesh groups in graft section 6. All *P* values reported are based on pair-wise comparisons made using the Wilcoxon rank sum test; the ranges of *P* values based on the Fligner-Policello test statistic are reported in Appendices B and C, online only.

a single systemic dose that is used in a 50-kg oncology patient.

Paclitaxel, which acts by stabilizing microtubules, inhibits smooth muscle cell proliferation and angiogenesis.[21-24] This may explain the relative lack of myofibroblasts within the wall of drug-treated grafts. We also found that capillary density within the neointima was reduced with paclitaxel; thus, we have demonstrated an association between capillary density and neointimal mass.

Inhibition of angiogenesis is an effective approach to reducing the growth of tumors, which cannot grow beyond the limits of their blood supply. Although the results of the current study suggest a similar relationship for inhibition of intimal hyperplasia development in arterial venous grafts, additional studies will be required to demonstrate a causal relationship. It is also possible that neointima is reduced by inhibition of smooth muscle cell proliferation and that the reduced capillary density simply is a result of a decrease in neointimal mass. Because of the long time course of drug elution and the differential release rate of drug based on drug concentration, it is not possible to assess the association between drug dose and angiogenesis in these studies. Dose-effect relationships would be more appropriately studied in experiments using a series of systemic doses of drug.

Paclitaxel is known to inhibit neutrophil and lymphocyte activation.[25,26] Although the mesh was associated with an increase in the general inflammatory response in the surrounding tissues, there were fewer macrophages within the mesh debris in the paclitaxel groups. There was also more mesh debris in the paclitaxel groups, suggesting that the drug's anti-inflammatory effects affected the normal foreign body response to the mesh, thus retarding its degradation and absorption. There was no evidence of toxicity to the vein or disruption of the anastomoses by paclitaxel, which is nontoxic to cells that are not proliferating. If anything, the adjacent vein was slightly thickened in the drug-treated groups and in no case was the vein atrophied or aneurysmal. The absence of infections in this study suggests that the anti-inflammatory effects of paclitaxel did not predispose to sepsis.

There has been recent concern that drug-eluting stents in the coronary circulation may be associated with small increases in the rate of thrombosis. Yet, a recently presented meta-analysis of data from 3506 patients (Martin B. Leon, Transcatheter Cardiovascular Therapeutics, Eighteenth Annual Scientific Symposium, October 24, 2006) demonstrated freedom from stent thrombosis at 4 years of 99.1% for those who had received bare metal stents and 98.7% for those who had received paclitaxel-eluting stents (*P* = .29).

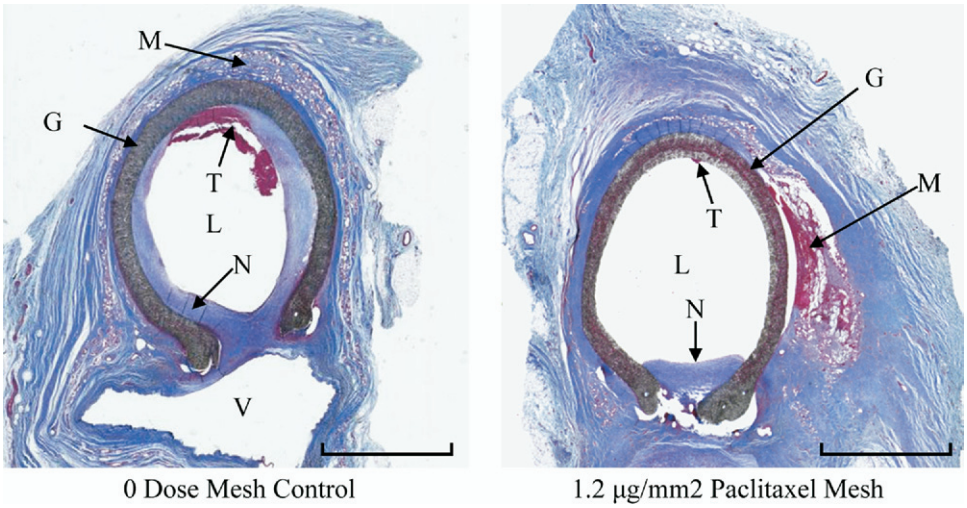

Fig 5. Left, Photomicrograph of a representative section 5 from the zero-dose mesh control and **(right)** 1.2 µg/mm^2 paclitaxel mesh group (*bar* represents 4 mm). *M,* Mesh, *G,* ePTFE graft; *N,* neointima; *T,* thrombus; *V,* vein; and *L,* lumen.

Furthermore, the reduction in luminal thrombosis in drug-treated grafts in our study was significant, suggesting that if anything, the drug reduces thrombosis in this setting. Four of the five graft occlusions that occurred were in the drug-treatment group, but this was not a statistically significant association. Our 12.5% graft occlusion rate (5/40) is similar to the 21% failure rate we observed in our initial description of this model.[19] At least one of the failures in the current study was technical due to kinking of the graft. The observed graft occlusions occurred at an early time (between 4 and 5 weeks) when intimal hyperplasia is very unlikely to have been the cause. This observation, plus the finding of reduction in thrombus in grafts at 8 weeks, suggests that most of the graft failures were due to technical causes, most likely kinking, which is difficult to avoid in the long and flexible neck of sheep.

Study limitations. No animal models exactly mimic the clinical problem of dialysis access failure, but the sheep model used in this study has been well characterized.[19] It develops a lesion of intimal hyperplasia that is very similar to that found in failed human access grafts. Both have the characteristics of neointimal hyperplasia that results from any vascular injury. Macrophages are common surrounding the ePTFE graft anastomosis, and smooth muscle cell proliferation, matrix deposition, and angiogenesis are prominent responses within the neointima.[27,28] The main differences between the sheep model and clinical access failure are the very rapid development of the stenotic lesion (although some patients form stenosing neointima very rapidly), the prominence of luminal thrombus, and the relatively large size of the vein. The sheep jugular vein is about twice the diameter of the usual, 3-mm to 4-mm antecubital vein used in patients, which may explain why there is less vein wall thickening than is seen in clinical specimens. Turbulence and shear resulting from high flow rates are more rapidly dissipated in the large vein than in smaller, antecubital veins in humans. In both instances, capillaries are prominent components of the lesion.

The beneficial effects of the Vascular Wrap paclitaxel-eluting mesh found in this study will need to be tested in clinical trials. It is encouraging to note that recently similar results with local paclitaxel delivery have been reported in other animal models of access failure: in dogs using local application of with an injectable copolymer, and in pigs using ethylene vinyl acetate wraps.[29,30] The dog study, although supportive, was limited (only five animals) and used histologic grading rather than quantitative analysis of intimal hyperplasia at the venous anastomosis. Full results of the pig study are not yet published, but paclitaxel caused a significant reduction in luminal narrowing (0.1% vs control of 37.9%) with minimal local side effects. This study used a nonabsorbable, paclitaxel-loaded ethylene vinyl acetate wrap, which is a solid film that could prevent incorporation of the device into surrounding tissue and could migrate out of position over time. Our method uses an absorbable mesh, which allows incorporation and will remain in place more reliably. Furthermore, application of the mesh is simple, and this material has met requirements for human use, allowing clinical trials to start in the near future.

Although the neointima found in our model closely mimics that of humans, there are differences, as outlined. Further, our study was only 8 weeks long, and it is not known if the benefit seen at this time will translate into the same long-lasting improvement in graft patency as it has for drug-eluting coronary stents.[18] The venous anastomosis of dialysis grafts is subjected to chronic injury due to high shear, turbulence, and contact with activated clotting factors released from the dialysis machine and from repeated upstream cannulation. This chronic injury is not mitigated

by application of the paclitaxel mesh, but it is possible that stabilization of the neointima during the early healing process will make it less susceptible to chronic injury. Long-term studies will be needed to determine if this approach can significantly improve graft patency. However, even an incremental improvement of patency would be clinically important in the case of ePTFE access grafts, which have an average patency of only 12 months.

Clinical implications. To date synthetic access grafts do not function nearly as well as native vein fistulas, although they have the clinical advantages of ease of placement, early use, and easy cannulation. Intimal hyperplasia at the venous end of ePTFE grafts is related to high flows, turbulence, compliance mismatch, endothelial injury, and platelet adhesion and activation at the site of injury. No effective pharmacology has yet been developed that can reduce intimal hyperplasia in the clinical setting.

Clinical trials with antiplatelet agents have failed to provide significant improvement in patency. Saratin, which inhibits platelet adhesion to the injured vascular wall, has shown promise in a canine model of dialysis access failure[31] but has not yet been tested in a clinical setting. Paclitaxel holds great promise because it is one of the two drugs—sirolimus[32] being the other—that have proven benefit in reduction of intimal hyperplasia after arterial injury in humans (ie, coronary angioplasty and stenting).

Improved patency of ePTFE access grafts would have a tremendous effect on dialysis access, which is the bane of long-term hemodialysis. The National Kidney Foundation Kidney Diseases Outcome Quality Initiative recommends that native arteriovenous fistulas be created when possible and synthetic bypass, such as PTFE, be used as a last resort owing to the inferior patency rate and increased risk of infection.[4] Any use of central venous catheters is discouraged because of their high complication rate. Unfortunately, many patients have unsuitable veins for fistula creation. This problem is likely to increase as the population develops more obesity and diabetes with resultant renal failure. Synthetic grafts would be preferable to native vein fistulas if their patency were as good because they are more easily implanted and cannulated and can be used after only a short period of maturation.

CONCLUSION

Application of the paclitaxel-eluting mesh to the venous anastomosis was highly effective in inhibiting the neointimal growth that leads to arteriovenous graft stenosis in this sheep model of dialysis access failure. This effect was achieved without apparent toxicity to the adjacent vein. These short-term, promising results in this animal model will serve as a basis for longer-term, clinical studies to determine if the Vascular Wrap paclitaxel eluting mesh can durably improve the patency of ePTFE dialysis access grafts.

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AUTHOR CONTRIBUTIONS

Conception and design: TK, PT, DG, RA
Analysis and interpretation: TK, PT, DG, RA
Data collection: TK, PT, DG
Writing the article: TK, PT, DG, RA
Critical revision of the article: TK, PT, DG, RA
Final approval of the article: TK, PT, DG, RA
Statistical analysis: TK, PT, DG, RA
Obtained funding: PT, DG, RA
Overall responsibility: TK

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DISCUSSION

Dr Hugh A. Gelabert (Los Angeles, Calif). I wish to thank the authors for kindly remitting a copy of their manuscript in a timely manner. It is well written and clearly illustrated and for this I commend them.

The success of drug eluting stents has provided the impetus for this project, where drug-eluting mesh is used to modulate the hyperplasia response in a sheep model of AV graft dialysis access. An absorbable mesh was impregnated with paclitaxel (an agent which inhibits angiogenesis and smooth muscle proliferation) and this was wrapped around the venous anastomosis of a PTFE AV graft. The animals were followed for about 2 months, then euthanized.

The anastomoses were studied to assess diameter reduction and histological characteristics. The findings indicate a statistically significant reduction in the intimal hyperplasia at the venous anastomosis in the treated subjects. Concerns include the small number of subjects, the limited extent of the study period, and the limited parameters studied in this experiment. Still the findings are encouraging and merit further investigation.

Question one, the authors indicate that paclitaxel has been shown to inhibit neutrophil and lymphocyte activation, yet they claim there was no dose effect on perigraft inflammation. Please comment.

Question two, in the course of the experiment, five subjects were lost, as their AV grafts clotted. Were any of the clotted grafts subject to histological or morphometric analysis to assess intimal hyperplasia in these failures?

Question three, given the described effects of paclitaxel—the inhibition of neutrophils, lymphocytes, macrophages, smooth muscle cells, and capillary growth—what is the chance of increased prosthetic graft infections?

Dr Ted Kohler. Thank you Hugh, for a very thoughtful review and for your excellent questions. First, regarding the effects of paclitaxel on inflammation. We did note that there were fewer macrophages associated with the wrap in the drug-treated groups and that resorption of the wrap was delayed. We did not note a dose-effect escalation of these effects as the dose increased. This either may be because our lowest dose was already producing the maximum anti-inflammatory effect, or it could be related to the rather crude qualitative measures we had for this effect, which was not a primary focus of this study.

Next, with regard to the failed grafts, gross inspection determined a kink in one graft but no obvious cause of the failure of the other devices. We have found that it is not possible to determine the cause of failure using microscopic histology of clotted grafts: the histology is too distorted by the thrombus. The neck of the sheep is long and flexible, which makes these grafts prone to kinking. Early on, we removed the external ring support of the devices too far back from the venous anastomosis, and this may have contributed to kinking. The relatively early timing of these failures makes intimal hyperplasia an unlikely cause, and our finding of reduced mural thrombus in the drug-treatment groups at 8 weeks makes primary thrombosis seem unlikely. The failure rate the current experimental group was very similar to that we observed in our initial description of this model. Further, there was not a statistically significant difference between drug and control groups, although the numbers are too small for valid statistical comparison.

Finally, as far as I know, there have been no observations of increased infections in any of the many applications of paclitaxel. The slower resorption of the mesh along with inhibition of inflammation could theoretically increase the risk of infection. One nice aspect of this local approach is that the drug affects only a short segment of the graft at the venous anastomosis, where infection is rarely a problem. It is comforting to note that we did not observe infections in our model, but the numbers are relatively small. The drug is gone by a few weeks, so normal inflammatory processes should resume by that time (hopefully not contributing to intimal hyperplasia!). This question and the efficacy of this novel treatment in patients will be addressed in a large clinical trial that is currently being designed.

Dr James Watson (Seattle, Wash). That is a nice study, Ted. I just have a question. I have to apologize for my ignorance, but after the paclitaxel runs out, does it still confer protection against development of neointimal hyperplasia? In other words, will both groups start at the same rate after the paclitaxel runs out, and if so, do you need to consider a better way to provide long-term protection with paclitaxel?

Dr Kohler. That is a great question. As I mentioned in the coronary stent trials, patients maintain the benefit as long as 4 years but that is a very different situation. In the coronary stent where the neointima once it matures and perhaps endothelializes, then the patient is no longer at risk, whereas in dialysis access grafts, as we all know, there is an ongoing injury at that venous end. The hope is that if the neointima can form, mature and become more stable that it will be less prone to continue to stenose but that may not be the case and that is why we need the longer-term studies.

Also, PTFE has such a poor patency rate that even if we could get 6 or 8 months more out of this treatment, it would be a significant difference and I think a clinically meaningful difference. We may very well wish to go to a PTFE forearm loop before having to go to a basilica transposition which is a much bigger deal for our patients. With dialysis access, it is all about buying time.

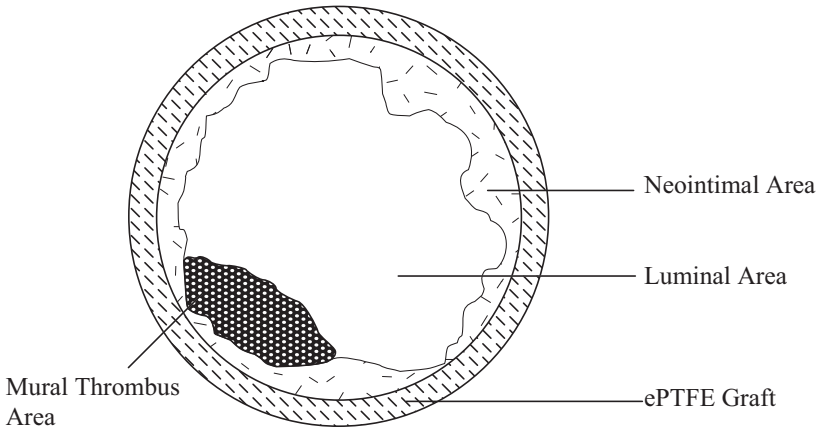
Dr Watson. Do you know if there is a potential for longer time paclitaxel elution? In other words, can you put something in that will give the drug for a long period of time or does that have its own. . .

Dr Kohler. There are local delivery devices that Steve (Hansen) has developed that could be used. The elution rate can be adjusted with this bioresorbable but not beyond a couple of months, so unless Rui Avelar has some trick up his sleeve that he has not told me yet, I think we are going to be limited in that respect.

Audience. That is a nice paper, Ted. I was going to ask you about the elution kinetics as well. Can the PTFE actually elute the paclitaxel? Is that an option rather than having the mesh be placed externally and also bioabsorbable stents possibly or some variant of that. How do you move from the model to a clinically practical application?

Dr Kohler. Those are great thoughts as well. We have talked about putting the drug directly into the PTFE. We are interested in doing that in 60-micron PTFE grafts, which have a lot more space within the graft material. The commercially available 20- to 30-micron grafts do not have as much space within the intranodal space to have drug, but it may be that having drug there is all we need. I like the idea of the wrap around the vein because that is the source for this neointima that is growing in. Actually the wrap is extremely simple to use so in terms of clinical application I think this is a extremely simple procedure to simply place the wrap and that is what the next clinical trial is going to be, but we are always thinking of other ways to deliver the drug.

APPENDIX A (online only).



$$\% \text{ Stenosis} = 100 \times \left(1 - \frac{\text{Luminal Area}}{\text{Graft Area}} \right)$$

Where Luminal Area = Graft Area - (Neointimal Area + Mural Thrombus Area)

Schematic diagram of morphometric measurements. (*ePTFE*, Expanded polytetrafluoroethylene.)

APPENDIX B (online only).

Morphometric data for section 5, the first cross section of the graft distal to the venous anastomosis

Group	Comparison	Statistic	Neointimal area (mm^2)	Mural thrombus area (mm^2)	Luminal stenosis (%)	Capillary density (per mm^2)
No mesh (n=8)		Mean ± SD	10.5 ± 6.8	2.6 ± 1.9	49.4 ± 22.4	11.9 ± 5.8
Paclitaxel mesh						
0.0 μg/mm^2 (n = 7)	Vs no mesh	Mean ± SD	6.4 ± 3.2	3.8 ± 2.9	40.9 ± 14.1	8.9 ± 5.6
		FP test	1.151	0.907	0.21	1.082
		P >	.1	.1	.1	.1
		P <	N/A*	N/A*	N/A*	N/A*
		Wilcoxon *P* =	.281	.3969	.8665	.281
0.3 μg/mm^2 (n = 6)	Vs mesh, 0.0 mg	Mean ± SD	0.9 ± 1.4	0.2 ± 0.3	3.6 ± 4.3	3.6 ± 2.9
		FP test	5.692	12.247	∞†	3.098
		P >	N/A*	N/A*	N/A*	.01
		P <	.01	.01	.01	.024
		Wilcoxon *P* =	.0076	.0023	.0012	.0221
0.7 μg/mm^2 (n = 8)	Vs Mesh, 0.0 mg	Mean ± SD	1.3 ± 1.5	1.5 ± 2.3	9.9 ± 10.0	4.6 ± 6.0
		FP test	6.11	2.16	14.318	2.16
		P >	N/A*	.025	N/A*	.025
		P <	.01	.05	.01	.05
		Wilcoxon *P* =	.0037	.0709	.0012	.0693
1.2 μg/mm^2 (n = 6)	Vs Mesh, 0.0 mg	Mean ± SD	1.2 ± 1.4	0.6 ± 1.2	6.8 ± 5.6	1.1 ± 1.7
		FP test	5.692	5.196	∞†	∞†
		P >	N/A*	N/A*	N/A*	N/A*
		P <	.01	.01	.01	.01
		Wilcoxon *P* =	.0082	.0082	.0012	.0012

FP, Fligner-Policello; *SD,* standard deviation; *N/A,* not applicable.

Note that capillary density was measured in the neointima at the toe of the graft in sections 4 or 3, whichever had the best arc with the adjacent vein.

*Standard FP tables do not provide upper/lower bounds for these *P* values.

†When all data points in one sample are less than all data points in the other sample, the Fligner-Policello test results a statistic that tends to infinity (∞), and therefore the *P* value tends to zero. Those differences are considered highly significant.

APPENDIX C (online only).

Morphometric data for section 6, the second cross section of the graft distal to the venous anastomosis

Group	Comparison	Statistic	Neointimal area (mm^2)	Mural thrombus area (mm^2)	Luminal stenosis (%)
No mesh (n = 8)		Mean ± SD	1.9 ± 2.4	6.3 ± 6.4	35.7 ± 28.5
Paclitaxel mesh					
0.0 μg/mm^2 (n = 7)		Mean ± SD	1.5 ± 1.6	5.7 ± 6.4	31.5 ± 29.1
	Vs no mesh	FP test	.161	.21	.323
		P >	.1	.1	.1
		P <	N/A*	N/A*	N/A*
		Wilcoxon *P* =	.8951	.8665	.7789
0.3 μg/mm^2 (n = 6)		Mean ± SD	0.9 ± 2.1	4.6 ± 7.0	23.8 ± 36.9
	Vs mesh, 0.0 mg	FP test	1.561	.585	1.162
		P >	.05	.1	.1
		P <	.1	N/A*	N/A*
		Wilcoxon *P* =	.1247	.5618	.2343
0.7 μg/mm^2 (n = 8)		Mean ± SD	.0 ± .0	.7 ± .7	3.3 ± 3.5
	Vs mesh, 0.0 mg	FP test	5.749	1.964	9.929
		P >	N/A*	.025	n/a
		P <	.01	.05	.01
		Wilcoxon *P* =	.0014	.0709	.0012
1.2 μg/mm^2 (n = 6)		Mean ± SD	0.0 ± 0.0	0.5 ± 0.7	1.9 ± 3.0
	Vs mesh, 0.0 mg	FP test	5.555	2.821	7.359
		P >	N/A*	.01	N/A*
		P <	.01	.024	.01
		Wilcoxon *P* =	.0047	.0367	.0047

FP, Fligner-Policello; *SD*, standard deviation; *N/A*, not applicable.
*Standard FP tables do not provide upper/lower bounds for these *P* values.