Safety and efficacy of local periadventitial delivery of sirolimus for improving hemodialysis graft patency: first human experience with a sirolimus-eluting collagen membrane (Coll-R)

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Abstract

Background. Neointimal hyperplasia causes a high rate of hemodialysis synthetic graft failure. Thus, therapies that inhibit neointimal hyperplasia are urgently needed. The Coll-R is a sirolimus-eluting collagen matrix designed for intra-operative perivascular implantation around the graft-venous anastomosis. Sirolimus is an anti-proliferative drug that has proven clinical utility in suppressing neointimal tissue growth in coronary artery disease when delivered locally to the vascular wall by an endovascular drug eluting stent.

Methods. A cohort of 12 chronic hemodialysis patients underwent surgical placement of 13 polytetrafluoroethylene grafts + Coll-R and were followed for up to 24 months. The primary endpoint was safety (freedom from device related adverse events). Secondary endpoints were pharmacokinetics...
of sirolimus release, success of Coll-R implantation and primary unassisted graft patency.

**Results.** There were no technical failures, infections, vascular anastomotic or wound-healing problems. Whole blood sirolimus levels rose to a mean peak of 4.8 ng/mL at 6 h and fell to <1 ng/mL at 1 week (n = 5). Twelve and 24-month primary unassisted patencies were 76 and 38%, respectively, and the thrombosis rate was 0.37/patient-year.

**Conclusions.** Perivascular implantation of the Coll-R during graft surgery safely delivered sirolimus to the vascular wall. Systemic sirolimus levels were sub-therapeutic for muscle (neointimal) proliferation at the venous anastomosis. Safety and patency in this small study were sufficiently encouraging to justify randomized controlled trials to further test the efficacy of the Coll-R.

**Keywords:** hemodialysis access; neointimal hyperplasia; sirolimus; thrombosis; vascular access

**Introduction**

The most common cause of hemodialysis synthetic graft failure is neointimal hyperplasia at the venous anastomosis [1–3]. Consequently, 12-month primary unassisted patency has been poor, ranging from 23 to 43% in published studies [4–8]. Percutaneous transluminal angioplasty with or without stenting is widely used to treat stenosis, but recurrence is common [9–12]. The durability of angioplasty is impaired by the trauma of the procedure, which fuels further neointimal proliferation. Hence, multiple procedures are often needed to treat recurrent stenosis, but these inevitably lead to graft abandonment.

The native arteriovenous fistula is the preferred vascular access because mature fistulas survive longer than grafts. However, this advantage is offset by the fact that 20–60% of fistulas fail to mature sufficiently to support dialysis [13–16]. Thus, cumulative survival of fistulas and grafts from the time of surgery are similar at 2 years [17]. Moreover, patients who receive a fistula after starting dialysis remain catheter dependent longer than those who receive a graft [13]. It follows that grafts will continue to have an important role in hemodialysis [17, 18].

There is an urgent need for therapies that prolong graft patency by inhibiting development of neointimal hyperplasia. Local pharmacotherapy appears to be the most promising approach since systemic therapies have had little effect on graft failure [3, 8]. However, preliminary studies of locally delivered treatments have had mixed results. For example, perivascular placement of implants containing allogeneic aortic endothelial cells have improved patency in porcine models of grafts and fistulas [19]. However, a Phase I/II safety trial in humans did not show a significant patency benefit [20]. Similarly, paclitaxel has inhibited neointimal hyperplasia in porcine models when applied to grafts as a wrap or coating [21, 22]. However, a randomized controlled trial to assess the benefit of a paclitaxel-eluting mesh was terminated early because of a higher rate of infection in the paclitaxel group [23].

Locally eluted sirolimus (rapamycin) is a promising new approach to preventing neointimal hyperplasia in vascular accesses. Sirolimus is a cytostatic macrolide lactone with immunosuppressive, anti-inflammatory and anti-proliferative properties that acts on a variety of cell types, including smooth muscle [24, 25]. Sirolimus has proven clinical utility in suppressing neointimal tissue growth in coronary artery disease when delivered locally to the vascular wall by an endovascular stent [26–28].

The Coll-R is a drug-eluting combination product of a collagen membrane (a biocompatible, biodegradable matrix) and sirolimus that has suppressed neointimal hyperplasia in animal models of isogenic and autologous vein grafts [29, 30]. The Coll-R is implanted as a sleeve around the adventitial surface of the venous anastomosis of the graft at the time of surgery (Figure 1). Sirolimus is then eluted from the Coll-R with a goal of inhibiting smooth muscle (neointimal) proliferation at the venous anastomosis. This is the first report of Coll-R applied to grafts in humans.

**Materials and methods**

We studied 12 stable patients who were scheduled to undergo polytetrafluoroethylene (PTFE) graft placement. At study entry, 11 of the 12 patients were new to dialysis. Ten patients were dialyzing with central venous catheters, and one had two previous failed native arteriovenous fistulas. One patient received a second graft after the first failed during the study. It was the vascular surgeon’s judgment that synthetic grafts rather than fistulas were the optimal accesses in these patients. Their veins were judged to be small on physical examination, and a ‘catheter last’ approach was preferred, which emphasizes placement of a graft rather than a fistula [18]. This avoids continued catheter exposure during the months that are often needed for successful fistula maturation. The study received ethics committee approval and all patients gave written informed consent. A total of 13 Coll-R graft surgeries were performed in the 12 patients at the Center of Angiology and Vascular Surgery, Tbilisi, Georgia from December 2005 through December 2006, and the study ended in December 2008.

The grafts were placed in the arm, with the anastomosis between the radial or brachial artery and the cephalic or basilic vein; PTFE (7.0 Gore-Tex) sutures were used. Heparin (5000 U) was given systemically during surgery to inhibit thrombosis. Each patient received two Coll-R sleeves around the venous anastomosis during surgery (Figure 1). The sleeves were stabilized in place with fine 6.0 prolene sutures. All patients received 325 mg aspirin and 75 mg clopidogrel daily for 4 weeks after surgery. These drugs were intended to decrease the probability of post-operative thrombosis; it is unlikely that that such short-term therapy significantly inhibited development of neointimal hyperplasia [16].

**Fig. 1.** Figure shows how Coll-R sleeves are wrapped around graft (Coll-R #1) and vein (Coll-R #2) at graft-vein anastomosis.
Successful local delivery of sirolimus depends upon elution of the drug from the collagen matrix and delivery to the local vascular tissue. The graft and vein sleeves delivered 200 and 400 μg of sirolimus, respectively, for a total dose of 600 μg. Whole blood sirolimus levels were measured by liquid chromatography/mass spectrometry [31].

The primary endpoint of the study was safety, evaluated at protocol-specified time points: pre-hospital discharge, 30-day post-surgery, and monthly thereafter for 24 months or until patients were no longer available for observation because of death, transfer or other reasons. Safety was evaluated as freedom from device related adverse events at 12 and 24 months. Safety parameters included freedom from death, infection, bleeding or peri-graft hematoma, early (<30-day post-surgery) graft occlusion, loss of suture integrity or interference with wound healing.

Secondary endpoints included technical feasibility of implantation of Coll-R at the anastomosis, pharmacokinetics of sirolimus release into the blood (evaluated by analysis of whole blood levels) and graft patency evaluated for up to 24 months. Primary-unassisted patency was defined as the time interval from graft placement until graft thrombosis or need for a procedure (percutaneous or surgical) to correct stenosis at the venous anastomosis or downstream vein when a clinical diagnosis of graft dysfunction was made. Secondary assisted patency was defined as the interval from graft placement until abandonment despite procedures designed to maintain graft patency or patency of the venous anastomosis or downstream vein. Grafts underwent routine clinical monitoring (physical examination, adequacy of dialysis, etc), but there was no formal surveillance program (e.g. graft blood flow or duplex ultrasound visualization of stenosis). Our approach to access maintenance agrees with numerous publications that indicate that surveillance does not improve graft outcomes [32–35].

Analysis

Data are reported as median or mean ± SE. Kaplan–Meier survival analysis was used to analyze Coll-R graft outcomes.

Results

Twelve patients received 13 Coll-R grafts during the study (Table 1). Half of the patients were male, most had hypertension, and a small minority had diabetes mellitus. None had a diagnosis of peripheral vascular disease, coronary artery disease or congestive heart failure.

Upper arm grafts were the most common and the loop graft was the most common configuration. All surgeries were successful in that there were no failures to implant Coll-R around the venous anastomosis, tearing of Coll-R’s during implant procedures or ‘pull through’ tears related to sutures. There were no immediate post-operative thromboses or wound infections, and there was no limb swelling or other unusual occurrences. All incisions healed normally without any infections or suggestions of suture dehiscence.

Release of sirolimus from the Coll-R was tested in five patients by measuring whole blood levels after Coll-R graft surgery (Figure 2). Mean levels rose to a peak of 4.76 ± 1.04 ng/mL at 6 h and then rapidly fell to 1.70 ± 0.61 ng/mL at 48 h. Levels then fell more slowly to <1 ng/mL at 168 h. Thus, the mean peak level was less than recommended sirolimus trough levels in renal transplantation that generally vary from 5 to 15 ng/mL [36].

Patients were followed for as long as 731 days (24 months). Twelve- and 24-month primary unassisted patencies were 76 and 38%, respectively (Figure 3A). Twelve- and 24-month secondary assisted patencies were 84 and 52%, respectively (Figure 3B). The thrombosis rate was low at 0.37/patient-year.

Individual patient and graft outcomes are listed in Table 2. Graft #3 failed at 119-day post-surgery; the patient then received graft #7. Six of 13 grafts maintained primary unassisted patency during study participation, although Grafts 11 and 12 left the study early at 382 and 134 days, respectively, because of complications unrelated to the Coll-R or stenosis. Five grafts thrombosed, one had stenosis with prolonged bleeding, and another was dysfunctional because of a central vein occlusion. Graft #11 developed a pseudoaneurysm in the body of the graft, indicating that Coll-R was not a factor. The graft was treated with an interposition graft while retaining the original arterial and venous anastomoses. Two patients died for reasons that did not appear to be connected with the Coll-R.

Discussion

This first-in-human study demonstrates the technical success and safety of the Coll-R + graft combination in this

| Table 1. Patient and Coll-R graft characteristics and outcomes |
|-------------------|------------------|------------------|------------------|------------------|
|                  | Patients | No. of patients | 12               |
|                  |          | Median no. of months on dialysis | 1.5             |
|                  |          | No. with prior failed access (fistula) | 1/12            |
|                  |          | at study entry |                   |
|                  |          | No. of Coll-R graft procedures | 13              |
|                  |          | Median age at surgery (years) | 37              |
|                  |          | Age range (years) | 17–76            |
|                  |          | Male (%) | 50               |
|                  |          | Caucasian race (%) | 100             |
|                  |          | Body mass index (kg/m2) | 23.7 ± 1.6 |
|                  |          | Hypertension (%) | 83               |
|                  |          | Diabetes mellitus (%) | 8               |
|                  |          | Peripheral vascular disease (%) | 0               |
|                  |          | Coronary artery disease or congestive heart failure (%) | 0               |
|                  |          | Thrombosis rate | 0.37/patient-year |
|                  |          | 12-month primary unassisted patency | 76%             |
|                  |          | 24-month primary unassisted patency | 38%             |
|                  |          | 12-month secondary assisted patency | 84%             |
|                  |          | 24-month secondary assisted patency | 52%             |

Fig. 2. Whole blood sirolimus levels following implantation of Coll-R graft in five patients (mean ± SE).
cohort of 13 grafts in 12 patients. The surgical success rate was 100%, and the study met its primary endpoint (safety) in that there were no Coll-R related adverse events or safety concerns during the immediate post-surgical period or during 24 months of follow-up. Although sirolimus has been associated with surgical wound-healing problems [37–39], there were no such problems in this study. There were two deaths, but neither appeared to be related to the Coll-R.

This first-in-human study was too limited in scope to draw reliable conclusions concerning patency, which was a secondary endpoint. Moreover, a control group would be needed to properly address patency but is typically not included in an early first-in-human study where the primary focus is safety. However, our results do provide a strong signal of patency that justifies further investigation. Primary unassisted patency was 76% at 12 months and 38% at 24 months. The 12 months primary patency exceeds that in previous studies, which have ranged from 23 to 43% (4–8). Also, the thrombosis rate of 0.37/patient-year was less than in recent studies, which have ranged from 0.41 to 1.03/patient-year [32].

This study reflects a growing interest in applying local treatments that prevent neointimal hyperplasia, rather than treating the sequelae (access dysfunction caused by stenosis) with angioplasty and stents or applying systemic pharmacologic agents that have provided modest benefits at best [3, 40]. Indeed, Lee and Roy-Chaudhury [3] have suggested that local therapies may be the only way to control the many factors involved in the pathogenesis of neointimal hyperplasia. The mechanisms of neointimal hyperplasia favor local treatments. In the traditional view of neointimal hyperplasia, vascular smooth muscle cells proliferate and migrate into the intima. However, recent evidence suggests that the adventitia may not be an innocent bystander—instead, it may be an important source of the cells that comprise neointimal tissue [1, 2]. It appears that fibroblasts contribute to the lesion by migrating from the adventitia into the intima, where they acquire the phenotype of myofibroblasts or smooth muscle cells. This process is accompanied by extracellular matrix deposition, adventitial angiogenesis and the presence of macrophages. Expansion of the intimal lesion leads to stenosis and access failure. Thus, local anti-proliferative treatments like Coll-R

![Fig. 3. Kaplan–Meier analysis of primary unassisted patency (A) and secondary assisted patency (B). Thirteen Coll-R grafts in 12 patients were followed for up to 24 months (731 days).](image)

### Table 2. Individual outcomes of 13 Coll-R grafts in 12 patients

<table>
<thead>
<tr>
<th>Graft no.</th>
<th>Patient age</th>
<th>Gender</th>
<th>Graft location</th>
<th>Graft configuration</th>
<th>Time to outcome (days)</th>
<th>Outcome</th>
<th>Primary unassisted patency preserved?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>Male</td>
<td>Forearm</td>
<td>Loop</td>
<td>730</td>
<td>Patent</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>76</td>
<td>Female</td>
<td>Forearm</td>
<td>Loop</td>
<td>730</td>
<td>Patent</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>Male</td>
<td>Upper arm</td>
<td>Loop</td>
<td>119</td>
<td>Stenosis with prolonged bleeding post-dialysis</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>Female</td>
<td>Upper arm</td>
<td>Loop</td>
<td>730</td>
<td>Thrombosis</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>37</td>
<td>Female</td>
<td>Upper arm</td>
<td>Straight</td>
<td>213</td>
<td>Thrombosis</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>Female</td>
<td>Upper arm</td>
<td>Straight</td>
<td>575</td>
<td>Thrombosis</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>Male</td>
<td>Upper arm</td>
<td>Loop</td>
<td>731</td>
<td>Patent</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>Male</td>
<td>Forearm</td>
<td>Loop</td>
<td>622</td>
<td>Central vein occlusion</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>70</td>
<td>Male</td>
<td>Upper arm</td>
<td>Straight</td>
<td>707</td>
<td>Thrombosis</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>57</td>
<td>Female</td>
<td>Upper arm</td>
<td>Straight</td>
<td>731</td>
<td>Patent</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>36</td>
<td>Male</td>
<td>Upper arm</td>
<td>Straight</td>
<td>382</td>
<td>Pseudoaneurysm at six months, died of sepsis at 15 months</td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>26</td>
<td>Male</td>
<td>Upper arm</td>
<td>Loop</td>
<td>134</td>
<td>Death from gastrointestinal bleed</td>
<td>Yes</td>
</tr>
<tr>
<td>13</td>
<td>17</td>
<td>Male</td>
<td>Forearm</td>
<td>Loop</td>
<td>298</td>
<td>Thrombosis</td>
<td>No</td>
</tr>
</tbody>
</table>

*aGraft #7 was placed in patient after graft #3 failed.*
that are applied to the adventitia may be particularly effective.

The goal of local sirolimus delivery is to promote high local drug concentrations while avoiding the side effects of systemic administration. The blood pharmacokinetic profile of sirolimus in this study supports release of the drug from the Coll-R matrix with local delivery. The mean peak sirolimus blood level of 4.76 ng/mL was less than trough levels recommended for immunosuppression in renal transplantation when combined with calcineurin inhibitors (5–15 ng/mL) [36]. Moreover, blood levels fell to 1.70 ng/mL at 48 h and then fell to <1 ng/mL 1 week after Coll-R implantation. This result suggests that the risk of systemic immunosuppression is negligible. Our unpublished animal data indicate that the collagen membrane degrades in about 8–12 weeks and sirolimus is detected in local (venous) tissue for at least 8 weeks. Thus, sirolimus can be detected in local venous tissue long after systemic levels have fallen below the lower limit of detection (<1.0 ng/mL).

Nevertheless, when compared with the desired life of an access, Coll-R provides relatively short-term exposure of vascular tissue to sirolimus. This raises the issue of whether it can provide the needed long-term inhibition of neointimal hyperplasia. The following considerations, however, explain why this may indeed be the case. Many grafts develop stenosis followed by thrombosis during the first 90 days after implantation, suggesting that neointimal hyperplasia begins in the immediate post-operative period [32]. Li et al. [41] recently defined the temporal profile of cellular proliferation in a porcine graft model. They found that adventitial cells begin proliferating soon after the graft anastomosis is placed, peaking at approximately Day 7 with cellular proliferation essentially complete by Day 49. Neointimal tissue proliferation starts by Day 14 and peaks by Day 28. Hence, the key to suppressing neointimal hyperplasia may be to protect the venous anastomosis with local exposure to sirolimus during the period of greatest vulnerability to tissue proliferation.

Moreover, sirolimus has provided sustained suppression of neointimal tissue growth in coronary artery disease when delivered locally to the vascular wall. Sirolimus-eluting stents applied after balloon angioplasty have shown improved outcomes when compared with bare metal stents. For example, 5-year follow-up of the SIRIUS and RAVEL trials showed a sustained reduction in target lesion or target vessel revascularization procedures with no evidence of a late catch-up phenomenon [26–28].

The Coll-R matrix is applied directly to the freshly sutured vascular anastomotic site. Hence, an important safety concern was potential compromise of the integrity of the anastomosis, which could result in suture detachment or formation of an aneurysm or pseudoaneurysm. Furthermore, since Coll-R is implanted in the subcutaneous space, locally eluted sirolimus acts not only on the vascular wall but also on the surrounding tissue, potentially interfering with healing of the vascular incision and surgical wound [37–39]. Compromise of the vascular suture line and wound healing were primary safety end points, and none of the patients had such problems. One graft developed a pseudoaneurysm in the body of the graft, indicating that Coll-R was not a factor.

We should point out that although graft patency in this study was encouraging, patency may vary across patient populations and may depend upon a number of factors, such as patient selection, comorbidities and surgical technique. The patient population in this study was Caucasian, had a wide age range, was predominantly hypertensive but generally lacked comorbidities, such as diabetes mellitus, coronary artery disease or peripheral vascular disease, that could promote graft failure. In contrast, in the USA, such comorbidities and other races such as African American are common. Thus, large randomized studies that include other patient populations are needed to properly test the benefit of Coll-R on graft patency.

In conclusion, this small first-in-human study supports the concept that the periadventitiously implanted Coll-R can safely deliver sirolimus to the venous anastomosis of grafts without significant systemic immunosuppression. Randomized controlled trials are planned to further test the safety and efficacy of the Coll-R.

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