

Efficacy and Biosafety of a New Bioresorbable Vascular Scaffold Covered with Biodegradable Film in Rabbits: An In Vivo Study

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Background: We developed a new fully bioresorbable vascular scaffold covered with biodegradable poly-L-lactic acid film (Firesorb-C) for coronary artery perforation. Our vitro tests have demonstrated that Firesorb-C was technically feasible but its biosafety and efficacy warranted further validation in vivo.

Objective: The aim of this study was to evaluate the biosafety and efficacy of Firesorb-C in rabbits.

Methods: Firesorb-C was deployed at the zone from the abdominal aorta to the right iliac artery in five rabbits. Angiography was conducted for evaluation of the immediate efficacy and 6-month biosafety and biodegradability of the Firesorb-C. Meanwhile, optical coherence tomography (OCT), histological light microscopy (HLM) and scan electron microscopy (SEM) were performed to evaluate the biosafety.

Results: All Firesorb-C applications were successfully implanted without procedure-related complications. In all treated rabbits, angiography showed that the Firesorb-C had completely sealed the opening of the left iliac artery without blood flow in its branches but with full patency of the right iliac artery immediately post-procedurally, while the covered membrane of Firesorb-C had been degraded and blood flow was restored in the left iliac artery and its branches at 6 months. OCT also found that the occluded left iliac artery had been reopened and the stented segment was almost fully endothelialized without in-stent restenosis at 6 months, meanwhile HLM and SEM confirmed comparable results.

Conclusions: Firesorb-C is associated with excellent efficacy, biosafety and biodegradability in rabbits. It shows promise as a replacement for conventional covered stents for treatment of coronary artery perforation or for use in other clinical situations.

Key Words: Biodegradable covered scaffold • Coronary artery perforation • Poly-L-lactic-acid • Rabbit

INTRODUCTION

Coronary artery perforation (CAP) during percutane-

ous coronary intervention (PCI) is a non-infrequent and potentially lethal complication, which accounts for 0.1–2.5% of the total number of PCI procedures.^{1,2} Although recent interventional skills and techniques, materials and equipment have improved considerably, CAP remains unavoidable due to more and more complex interventions in our daily practice.³ In addition, high-pressure post-stent dilatation to prevent stent malapposition was also regarded as a high risk factor to CAP.^{4,5}

For a small CAP in distal breaches, embolization with coils or autologous fat particles, and prolonged balloon inflating are usually sufficient for hemostasis,^{6–8} while for a big CAP in the proximal coronary artery, urgent sur-

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gical drainage or covered stent implantation is frequently required.⁹ In the clinical real world, covered stents have become a mainstay of treatment for CAP.¹⁰⁻¹² There are two major covered stents available: polytetrafluoroethylene-covered stents (Jostent, Abbott and Nuvasc, Cardiovasc; Symbiot, BostonScientific),¹³⁻¹⁹ and pericardial-covered stents (covered with heterologous pericardial tissue).²⁰⁻²² Additionally, if no commercial stents are available, there are several methods for making covered stents by hand for urgent use.

Even so, the currently used conventional covered stents for CAP have been associated with poor clinical outcomes, especially in-stent thrombosis and restenosis.²²⁻²⁶ As shown in the recent study by Pavani M et al., though the two major covered stents could successfully fix the ruptured vessel wall, there was an unacceptably high rate of clinical events and stent thrombosis at long-term follow up.²⁷ The major drawbacks of these covered stents are as follows: 1) limited flexibility, deliverability, luminal occupation and easy dislodgement due to their bulky profile; 2) strong local vascular inflammation response, poor endothelialization and prone thrombosis owing to the stent's components; 3) permanent occluding of side-branches, collateral circulation leading to ischemic events because of nonbiodegradable stent materials.

To overcome the aforementioned shortcomings of the conventional covered stents, together with MicroPort Medical Company (Shanghai, China), we have developed and manufactured a novel covered scaffold, which is a fully bioresorbable vascular scaffold covered with biodegradable polylactic-L-acid film (Firesorb-C). This study was conducted to determine the efficacy and biological properties of Firesorb-C in vivo.

METHODS

Characteristics of Firesorb-C

Firesorb-C (Patent No. ZL201610628607.3) is a monorail-designed stent system composed of a bioresorbable vascular scaffold platform with a strut thickness of 100-120 μm (Firesorb, MicroPort Medical Co., Shanghai, China) and a highly expandable membrane mainly made of biodegradable poly-L-lactic acid (PLLA) copolymer with a thickness of 60-80 μm before dilatation, and 20-40 μm

after complete dilatation (co-developed by MicroPort Medical Co. and Fujian Medical University Union Hospital). A hot compression modeling technique was twice used to ensure the scaffold and the membrane compacted closely, effectively reducing the risk of dislodging during the procedure (as shown in Supplement Figure 1). Firesorb-C expanded to 3.5 mm \times 23 mm where the membrane came into contact with the stent platform closely and uniformly without rupture as shown in the digital photographs and microphotographs (Figure 1 and 2).

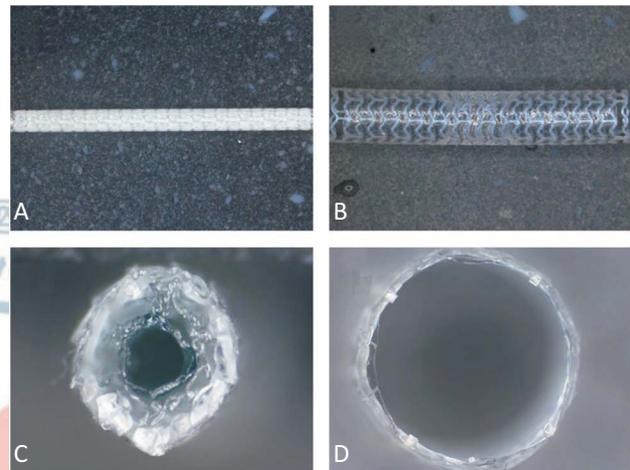


Figure 1. Digital photographs of Firesorb-C (20 \times). Before stent balloon inflation, the overall Firesorb-C shown in (A), the cross-sectional Firesorb-C shown in (C); after stent balloon inflation, the overall Firesorb-C shown in (B), the cross-sectional Firesorb-C shown in (D).

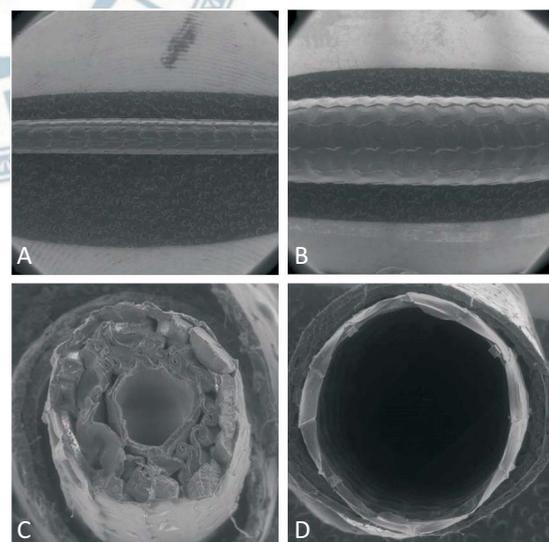


Figure 2. Microphotographs of Firesorb-C (20 \times). Before stent balloon inflation, the overall Firesorb-C shown in (A), the cross-sectional Firesorb-C shown in (C); after stent balloon inflation, the overall Firesorb-C shown in (B), the cross-sectional Firesorb-C shown in (D).

Our previous *in vitro* study had tested the scaffold's degradability. When coated in a 37 °C buffer solution, the weight of the membrane would be reduced to 20%-40% in 3-6 months, and the platform would gradually degrade within 2 years (as shown in Supplement Figure 2).

Animals

Five New Zealand white rabbits of both genders with an average weight of 2.8 ± 0.2 kg were all fed with a standard laboratory diet. All experimental procedures were performed in accordance with the National Institutes of Health guidelines for humane handling of animals and were approved by the animal research committee of Fujian Medical University.

Firesorb-C implantation

Surgical dissection was performed to obtain the right carotid artery under anesthesia with 3% pentobarbital (Sigma-Aldrich, US, 30 mg/kg, *im*) and xylazine hydrochloride (Jilinhua, China, 0.5 ml/kg, *im*). Then a 4 Fr vascular sheath (MicroPort, China) was inserted via the right carotid artery into the abdominal aorta. Angiography was performed to visualize the abdominal aorta and both iliac arteries; two PCI guide wires (Runthrough, Terumo, Japan) were introduced separately into the two iliac arteries. Subsequently, Firesorb-C was advanced and deployed at the target artery (abdominal aorta to the right iliac artery). After the stent implantation, post-procedural angiography was executed to evaluate the efficacy of Firesorb-C: whether there was sealing of the opening of the left iliac artery or not. When the procedure was complete, the right carotid artery was ligated and the rabbits were allowed to recover and resume feeding for subsequent study (see Figure 3).

A postoperative antibiotic (penicillin, NCPC, China, 400,000 U/d, *im*) was given to all the animals for 3 days. All the animals were also given aspirin (Bayer, Germany) and clopidogrel (Sanofi, China) 24 hours before catheterization and continued to the end of the experiment.

Follow-up

Six months after stent implantation, a small incision was made to expose the right femoral artery under anesthesia. Then a 4 Fr vascular sheath (MicroPort, China) was placed in the right femoral artery. Angiography was carried out to evaluate the biosafety and biodegrad-

ability of the Firesorb-C. Additionally, optical coherence tomography (OCT) was performed to evaluate the degradation of the covered membrane, endothelialization and thrombosis in the scaffold segment. After the procedure, and while still under anesthesia, the rabbits were sacrificed and the target arteries (including Firesorb-C) were excised and fixed by immersion in a buffered formalin solution and glutaraldehyde solution, respectively. Histological light microscopy (HLM) and scan electron microscopy (SEM) were carried out for comparison with the findings of OCT.

RESULTS

All Firesorb-C applications were successfully implanted without procedure-related complications, and all rabbits accomplished follow-up for 6 months without death.

Angiographic and OCT findings

In all treated rabbits, immediately after the procedure, angiography showed that the Firesorb-C had completely sealed the opening of the left iliac artery without

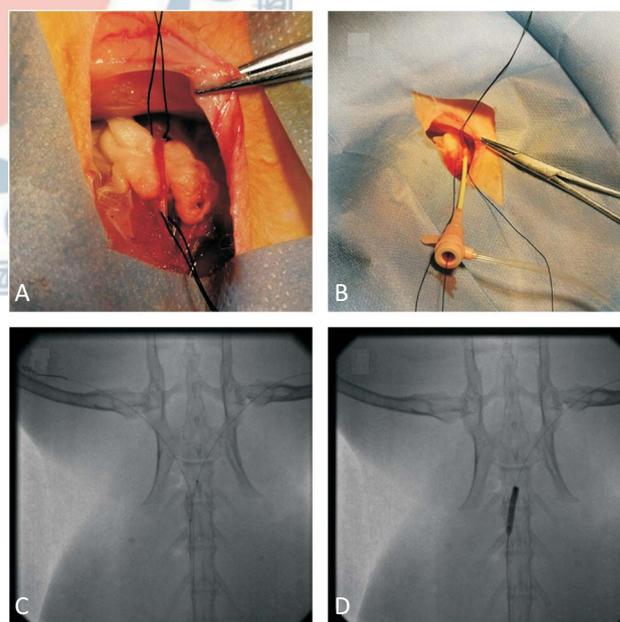


Figure 3. The procedure of poly(lactic-L-acid) film (Firesorb-C) implantation. Separate tissue to obtain the right carotid artery (A). Insert and fix 4 Fr vascular sheath and introduce percutaneous coronary intervention (PCI) guidewire (B). Locate the target artery by two PCI guidewires (C). Release Firesorb-C (D).

blood flow in its branches but with full patency of the right iliac artery; at 6 months angiography found that the covered membrane of Firesorb-C had been degraded and blood flow had been restored in the left iliac artery and its branches.

Similarly, for all rabbits, OCT also visualized that the occluded left iliac artery had reopened, the membrane of Firesorb-C had degraded with almost full endothelialization and no thrombosis in the stented segment at 6 months (see Figure 4).

HLM and SEM findings

Histologically, vessel lumen was patent and new intima tissue had well-covered residual bioresorbable vascular scaffold (BVS) struts, and there was no inflammatory cell infiltration in all animals (see Figure 5).

Electron-microscopy found that there was complete endothelialization on the scaffold struts, which presented

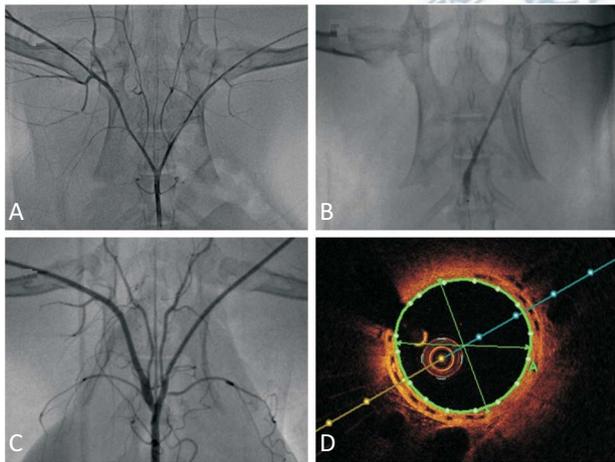


Figure 4. The results of angiography and optical coherence tomography (OCT) (17 \times). The angiography of the target artery before polylactic-L-acid film (Firesorb-C) deployment shown in (A), with blood flow in both iliac arteries and their branches. The post-procedural angiography showed in (B), without blood flow in the left iliac artery and its branches. The angiogram at 6 months showed in (C), left iliac artery and its branches had regained blood flow. The performance of OCT showed in (D), with pretty endothelialization and no thrombosis-event.

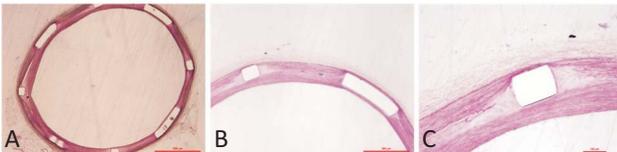


Figure 5. The histological evaluation of polylactic-L-acid film (Firesorb-C). At 6 months, Firesorb-C struts were fully covered with neointimal tissue, without inflammatory cell infiltration, shown in A (2 \times), B (4 \times) and C (10 \times).

as cobblestone in appearance with tight cell contact in the scaffold segment (see Figure 6).

DISCUSSION

This study was designed to examine the biosafety and efficacy of Firesorb-C in rabbits, as the report of a new, fully degradable, covered scaffold. Our main findings are as follows: (1) there was no death or thrombosis-related events among rabbits with Firesorb-C in the follow-up at 6 months; (2) Firesorb-C could seal the opening of the left iliac artery but would degrade to restore blood flow at 6 months, meanwhile with endothelialization; and (3) Firesorb-C had already degraded at 6 months in vivo, which was in accordance with a previous vitro study at nearly 3-6 months.

In this study, Firesorb-C had confirmed its technical success in rabbits, without death and procedure-related complications. From the results of post-procedural and six-month angiographies, the target artery with Firesorb-C kept patency, without any stent thrombosis or restenosis. The performances of OCT, HLM, and SEM all had shown almost completely endothelialization of Firesorb-C in rabbits. These results indicated that Firesorb-C is a biosafety option to replace conventional covered stents.

Firesorb-C was initially designed to provide a new fully degradable covered stent to substitute for the presently covered stents in the treatment of CAP. In this in vivo study, we focused on the abdominal aorta to the right iliac artery in New Zealand white rabbits. If the blood flow in the left iliac artery and its branches could vanish after Firesorb-C implantation, Firesorb-C would also have the potential to seal the breach of the coronary artery 6 months after implantation. The post-procedural angiography had confirmed the efficacy of Firesorb-C in the sealing of the vessel opening.

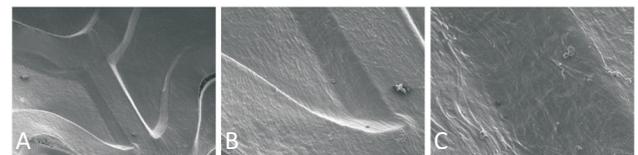


Figure 6. Electron-microscopies of polylactic-L-acid film (Firesorb-C). A (100 \times), B (300 \times) and C (1000 \times) shown that Firesorb-C was completely covered with endothelium at 6 months, without any interruption.

Compared to covered stents used presently, the prominent character of Firesorb-C is its biodegradability. The angiography at six months had shown that the blood flow in the left iliac artery and its branches had been restored, which revealed the membrane of Firesorb-C had already degraded. In fact, neither a bare-metal stent nor a drug-eluting stent for patients is an inevitable compromise for coronary lesions, severe pathological status converting to mild pathological status. To get milder pathological status or even natural status, a BVS was proposed. With its excellent biodegradability, Firesorb-C could gradually degrade with the lesions repaired, and ultimately restore the target vessel to normal vascular morphology without anything left in the vessel.

This biodegradability of Firesorb-C was attributed to the PLLA complex. The PLLA can improve their physical properties by developing copolymers or combining with other materials. In our previous *vitro* study, we had confirmed that Firesorb-C would gradually degrade with over time, finally into carbon dioxide and water. Multiple clinical studies have confirmed the biosafety and efficacy of biodegradable coronary stents or biodegradable polymer drug-eluting stents made by PLLA copolymer.^{28,29}

CAP tend to emerge in the complex intervention procedures, such as calcified and tortuous lesions.^{30,31} To improve the flexibility and passageability, Firesorb-C had reduced the thickness of the stent platform and film as much as possible, on the premise of ensuring sufficient radial support force. What's more, with the improvement of material processing technology, Firesorb-C is expected to become thinner still, with superior transportability and accessibility. Additionally, in the release process of conventional covered stents, owing to the resistance of the covered membrane, interventional cardiologists usually maintained higher pressure in the covered stent's balloon inflation to avoid the "dog-bone" effect, which would contribute to stent malaposition, and even to thrombosis and lipid plaque drift.^{32,33} For Firesorb-C, the biodegradable PLLA copolymer membrane could reduce resistance for excellent flexibility and intensity. And the compliance of the membrane was also evaluated in the *vitro* test we conducted previously. We found that even the burst pressure of the scaffold balloon still did not exceed the membrane burst pressure, let alone tear the film of Firesorb-C.

There are several limitations in our study: (1) we

had only quantitatively evaluated the biosafety and efficacy of Firesorb-C, but with high repeatability of the experiment and apparent results, we think it is enough to derive the conclusion that Firesorb-C has excellent biosafety and efficacy in rabbits; (2) we had roughly assessed the biodegradability *in vivo*, but did not determine the time that Firesorb-C started to degrade and when the sealed vessels regained blood flow (in addition, we did not assessed the biodegradability of the stent's platform of Firesorb-C); (3) just like other pre-clinical studies, the rabbit artery model perhaps did not fully represent human vascular conditions, including atherosclerosis and endothelial function, let alone simulate coronary perforation combined with severe calcification, tortuous lesions and chronic total occlusion lesions; and (4) the sample number of this study was limited. Further research is necessary to evaluate the details of the biodegradability of Firesorb-C.

CONCLUSION

Firesorb-C is associated with excellent efficacy, biosafety and biodegradability in rabbits and may be a promising replacement for conventional covered stents for treatment of coronary artery perforation or for use in other clinical situations.

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CONFLICT OF INTEREST

None of the authors have any kind of conflict of interest to declare.

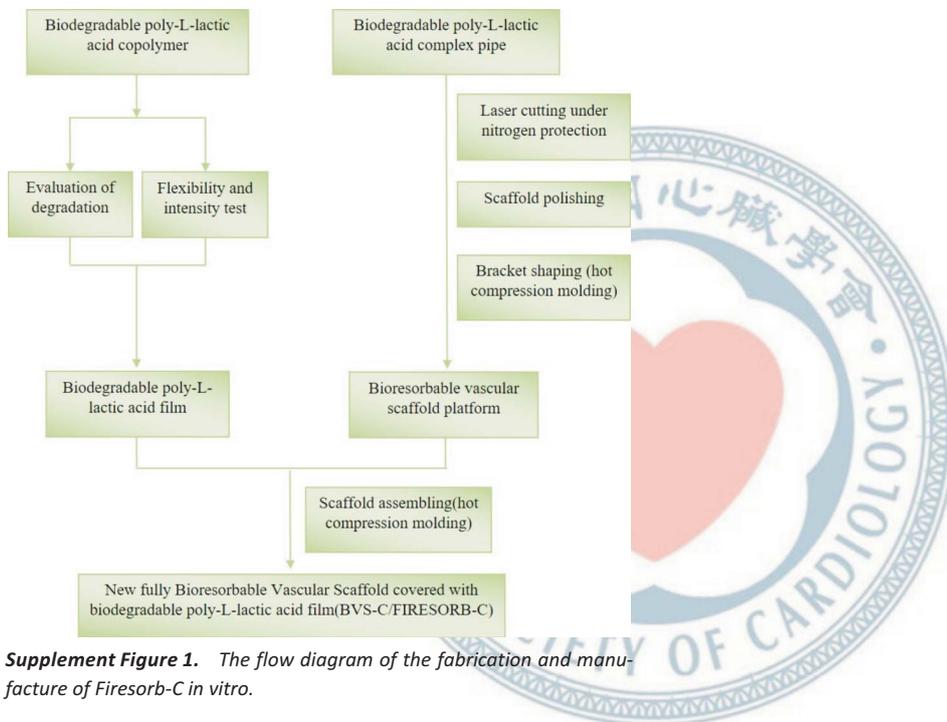
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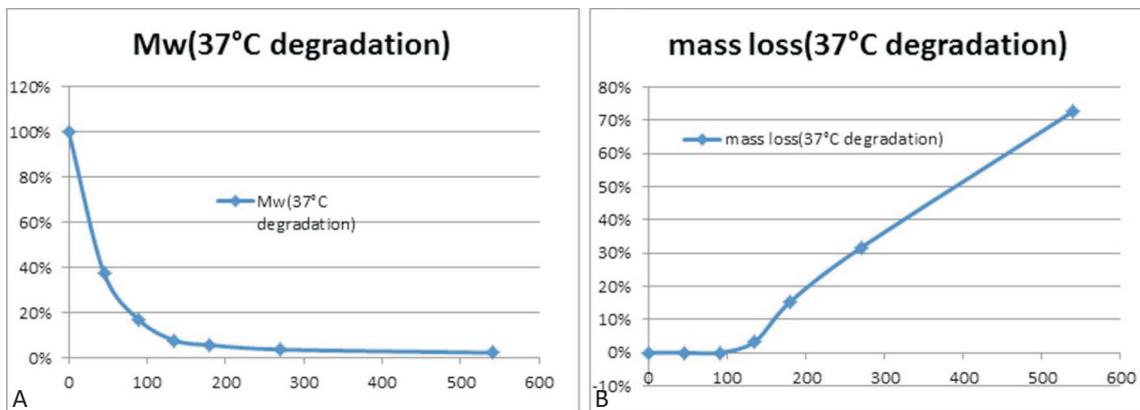
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SUPPLEMENT



Supplement Figure 1. The flow diagram of the fabrication and manufacture of Firesorb-C in vitro.



Supplement Figure 2. The degradability of Firesorb-C in vitro. (A) The weight of film from Firesorb-C, coating in 37 °C buffer solution, was reduced to 20%-40% in 3-6 months; (B) The platform of Firesorb-C would gradually degrade within 2 years.