J Artif Organs (2009) 12:61–66
DOI 10.1007/s10047-008-0446-z

BRIEF COMMUNICATION

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Development of novel covered stents using salmon collagen

Abstract In this study, we newly developed self-expandable or balloon-expandable covered stents with a biodegradable salmon collagen (SC) film. The SC-covered stents were fabricated by placing a bare stent in a mixture of acidic SC solution and a fibrillogenesis-inducing buffer (pH 6.8) including a cross-linking agent (water-soluble carbodiimide), and subsequent incubation at 4°C for 24 h and lyophilization. The stents obtained were completely covered with an SC film having a nanofibrous structure (fibril diameter, about 70 nm). On immersion in water, the film is converted to a gel with slight swelling. There was no rupture of the SC cover after mounting on a balloon catheter or after expansion. Preliminary implantation was conducted by placing the balloon-expandable covered stents in the common carotid arteries of beagles. One month after implantation, angiography showed that all stented arteries were patent with no significant neointimal thickening. In conclusion, SC is potentially useful as a cover material of endovascular stents to enhance patency.

Key words Covered stent · Salmon collagen · Balloon-expandable stent · Self-expandable stent

Received: April 1, 2008 / Accepted: November 27, 2008

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Introduction

Since conventional metallic stents are unable to inhibit smooth muscle overgrowth and extracellular matrix production leading to restenosis, much effort has been concentrated on finding new methods to prevent the outgrowth by covering the stent pores using cover films.[1–10] Synthetic polymers such as polytetrafluoroethylene (PTFE),[1] polyethylene terephthalate (PET),[3] or segmented polyurethane (SPU)[6–8] have been used as the cover films. However, these polymers have been shown to elicit severe inflammatory reactions that may induce occlusion of small-caliber arteries if the cover materials lack surface treatments such as drug coating.[6,8,9] Excessive accumulation of cellular and matrix components on the surface of the implanted stents can cause substantial luminal narrowing and possible occlusion in small-caliber vessels. Thus, the development of biocompatible covered stents with naturally occurring polymers[5,10] or autologous tissues[7] has been attempted to achieve successful stenting.

The use of biodegradable materials for the cover film has potential for maintaining patency while achieving total coverage of the struts of the stent with connective tissue and endothelium. In addition to biodegradability, mechanical properties such as tensile strength and flexibility are needed to withstand the extending force during deployment of the stents. In light of these points, collagen seems promising because it is able to self-assemble through in vitro fibril formation into a fibrillar matrix with extra strength and biological stability. Our previous studies demonstrated the development of bio-inspired salmon collagen (SC) fibrillar gel, which served as a scaffold for cell culture.[11,12] The use of such marine-derived collagen has been proposed as a potentially pathogen-free alternative to established collagens from livestock, which have the risk of transmitting infectious diseases including bovine spongiform encephalopathy. In addition, theoretical advantages favoring the use of collagen substrates include rapid endothelialization of the stents with subsequently decreased platelet adhesion. In this study, we developed novel

covered stents using SC. The prepared stents were implanted into the common carotid arteries of beagles as a preliminary experiment. The potential of the SC-covered stents are discussed.

Materials and methods

Preparation of SC-covered stents

SC was kindly supplied by Ihara and Company, Ltd. (Otaru, Japan). The SC-covered stents were prepared according to the procedure shown in Fig. 1a. An acidic SC solution (0.5% in diluted HCl, pH 3) was mixed with a fibrillogenesis-inducing buffer (pH 6.8) including 25 mM sodium phosphate, 58.3 mM NaCl, and 100 mM water-soluble carbodiimide (WSC, Dojindo, Tokyo, Japan) at a ratio of 2:3. Self-expandable Nitinol stents (Sendai stent: length, 18 mm; diameter, 5 mm; Piolax, Yokohama, Japan) or balloon-expandable stents (Palmaz–Schatz: length, 10 mm; diameter, 3 mm; Johnson and Johnson Medical Japan, Tokyo, Japan) were placed on a mandrel (diameter, 5 mm for self-expandable stent, 3 mm for balloon-expandable stent) and incubated in a cylinder mold (inner diameter, 8.6 mm for self-expandable stent, 6 mm for balloon-expandable stent) including the SC-buffer mixture at 4°C for 24 h. The stents covered with the cross-linked SC fibrillar gel (gel thickness, about 1.5 mm) were frozen at –80°C for 1 day and were then lyophilized, resulting in SC-covered stents.

Microscopic observation

The SC-covered stents were coated with Au by using an ion coater (E-1010; Hitachi, Tokyo, Japan) and subjected to observation under a high-resolution scanning electron microscope (SEM; JSM-6500F; JEOL, Tokyo, Japan). The SEM apparatus was operated at 5.0 kV.

Tensile strength

Measurement of the tensile strength of the SC cover was performed using a tensile strength machine (RE-3305; Yamaden, Tokyo, Japan). The specimens (dumbbell shape; size of deformation part, 5 × 10 mm) were stress-loaded to rupture at a rate of 0.5 mm/s. The tensile strength denoted the amount of force required for rupture to occur.

Implantation

The experimental animals were beagles weighing about 10 kg ($n = 4$). All animal experiments were treated as acute experiments with clean conditions in compliance with the Principles of Laboratory Animal Care (formulated by the National Society for Medical Research) and the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, revised 1985). The research protocol (No. 07035) was approved by the ethics committee of the National Cardiovascular Center Research Institute. The balloon-expandable SC-covered stents mounted on a percutaneous transluminal angioplasty (PTA) balloon catheter (3.0 mm, 2 cm, SAVVY, Johnson and Johnson) were positioned into the common carotid arteries (approximately 5 mm) from the proximal side of the arteries. The balloon was inflated at a pressure of 8 atm for 30 s, deflated, and then slowly withdrawn, leaving the covered stent in place. Neither antiplatelet agents nor additional anticoagulants were administered during the 1-month follow-up period. After undergoing the above-mentioned procedure, the dogs were killed and the stents with the surrounding arteries were resected.

The arteries obtained were fixed in 5% glutaraldehyde in phosphate buffer (pH 7.4) for more than 48 h, dehydrated with an alcohol series, embedded in glycol methacrylate, and cut into cross sections in the circumferential direction (thickness 3–5 μm). The tissue sections were subjected to hematoxylin and eosin staining and were then observed under a light microscope.

Results

The balloon-expandable or self-expandable SC-covered stents were successfully fabricated in two steps; gelation of SC solution on the stents and subsequent lyophilization (Fig. 1a). Figure 1b shows the gross appearance of the resulting self-expandable SC-covered stents. The SC film completely covered the stent struts without any damage. On immersion in water, the cover film converted to a gel with slight swelling (Fig. 1c), indicating that the thickness of the film would be almost unchanged after implantation. The thickness of the film was approximately 100 μm, as calculated from the SEM image in Fig. 2b, while the original thickness of the gel was around 1.5 mm.

The balloon-expandable SC-covered stent could be mounted on a commercially available PTA balloon catheter using the hand crimping apparatus (Fig. 1d–f). The stent was dilated by expanding the balloon with pressurized water. After balloon deployment, the shape of the stent was adequately maintained without shrinkage. There was no rupture of the cover film after deployment of the stents.

The SC cover had a nanofibrous structure with 70-nm-diameter collagen fibrils as revealed by SEM observations (Fig. 2a). The outside of the strut was covered with the thin collagen membrane (Fig. 2b). The tensile strength and the maximum deformation of the SC cover were 20.1 ± 1.7 kPa and 53.9% ± 21.5%, respectively ($n = 5$).

The balloon-expandable SC-covered stents were implanted into the common carotid arteries of beagles. One month after implantation, angiographs showed patent stents with no significant stenosis or occlusions (Fig. 3a). There was no sign of distal embolization or movement of the stents during the implantation period.

Typical images of histological transverse sections of the stents without or with an SC cover are shown in Figs. 3b,c,

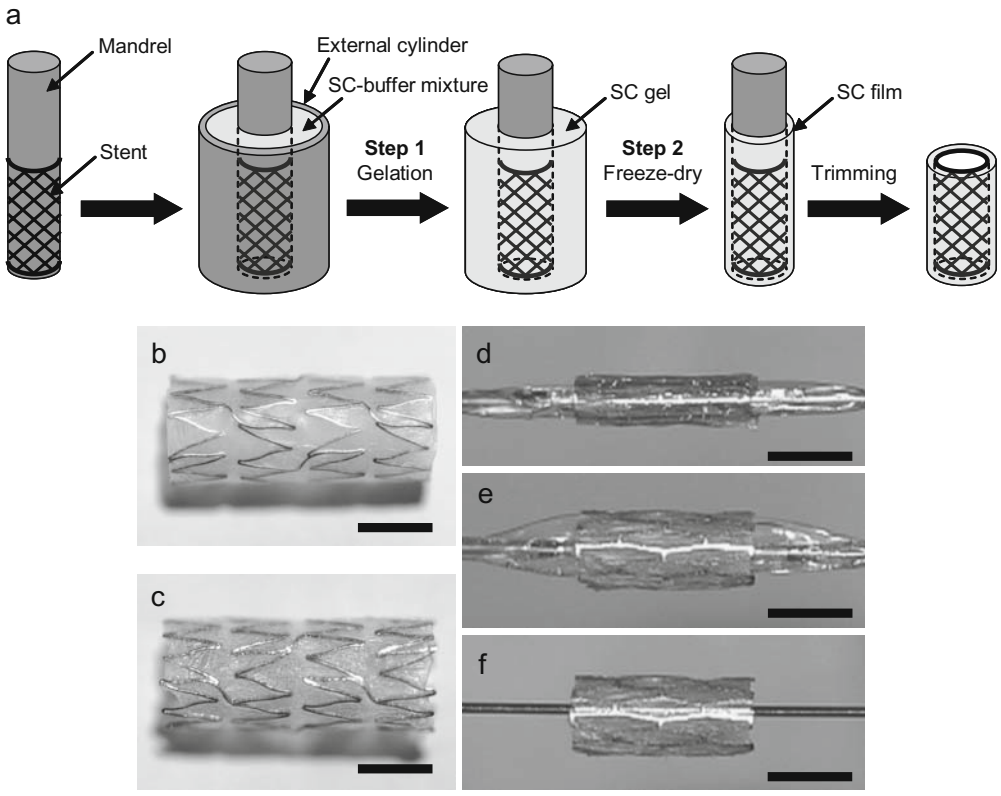

Fig. 1. Fabrication process of the salmon collagen (*SC*)-covered stents (**a**). The stent is placed on a mandrel and incubated in an SC–buffer mixture at 4°C for 24 h. Stents covered with the cross-linked SC gel were lyophilized, resulting in SC-covered stents. The gross appearance of the self-expandable SC-covered stent before (**b**) and after immersion in water (**c**). Gross appearance of the balloon-expandable SC-covered stent after mounting on a percutaneous transluminal angioplasty balloon catheter (**d**), after dilation by expanding the balloon (**e**), and after removal of the balloon catheter (**f**). *Bars* 5 mm

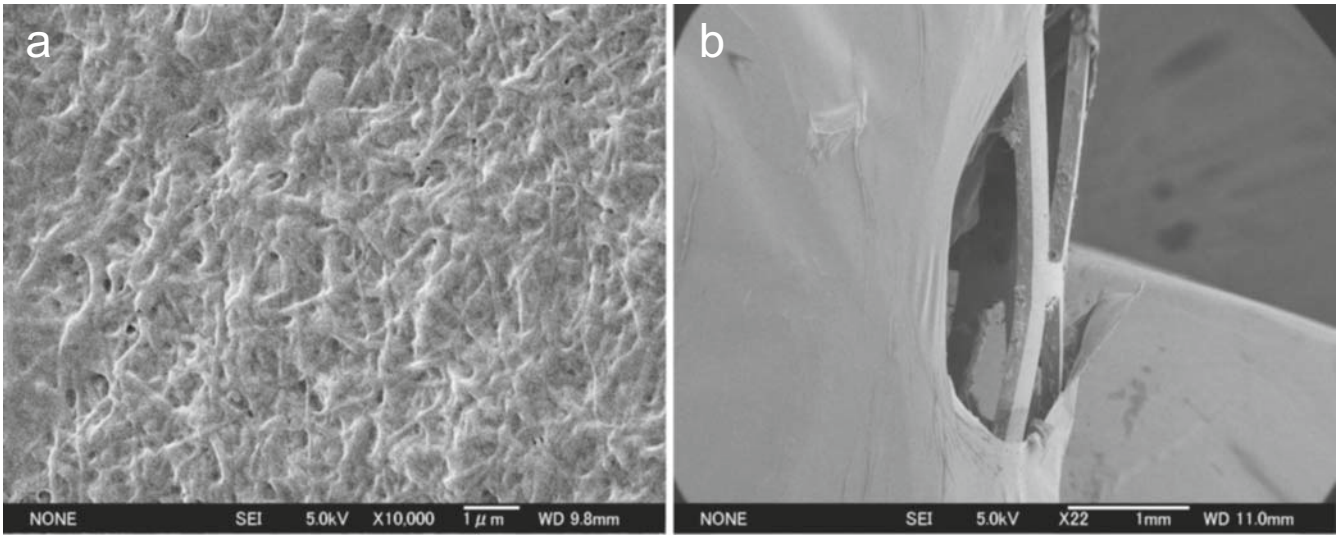

Fig. 2. Microscopic images showing the surface of the balloon-expandable SC-covered stent (**a**) and the surface of the SC cover with an intentionally made tear (**b**). Original magnifications: ×10 000 (**a**), ×22 (**b**)

respectively. In both types of stents, there was no thrombus or invasion of inflammatory cells. No significant intimal hyperplasia was observed and the luminal surface was smooth and flat. Endothelialization was complete (Fig. 3d). Cleavage of the SC cover was noticed in the neointimal layer without significant infiltration of inflammatory cells (Fig. 3e, arrowheads).

Discussion

In the development of covered stents, the choice of the cover material, which is critical to the successful development of these devices, is largely influenced by the material's biocompatibility and ease of fabrication. Further work on

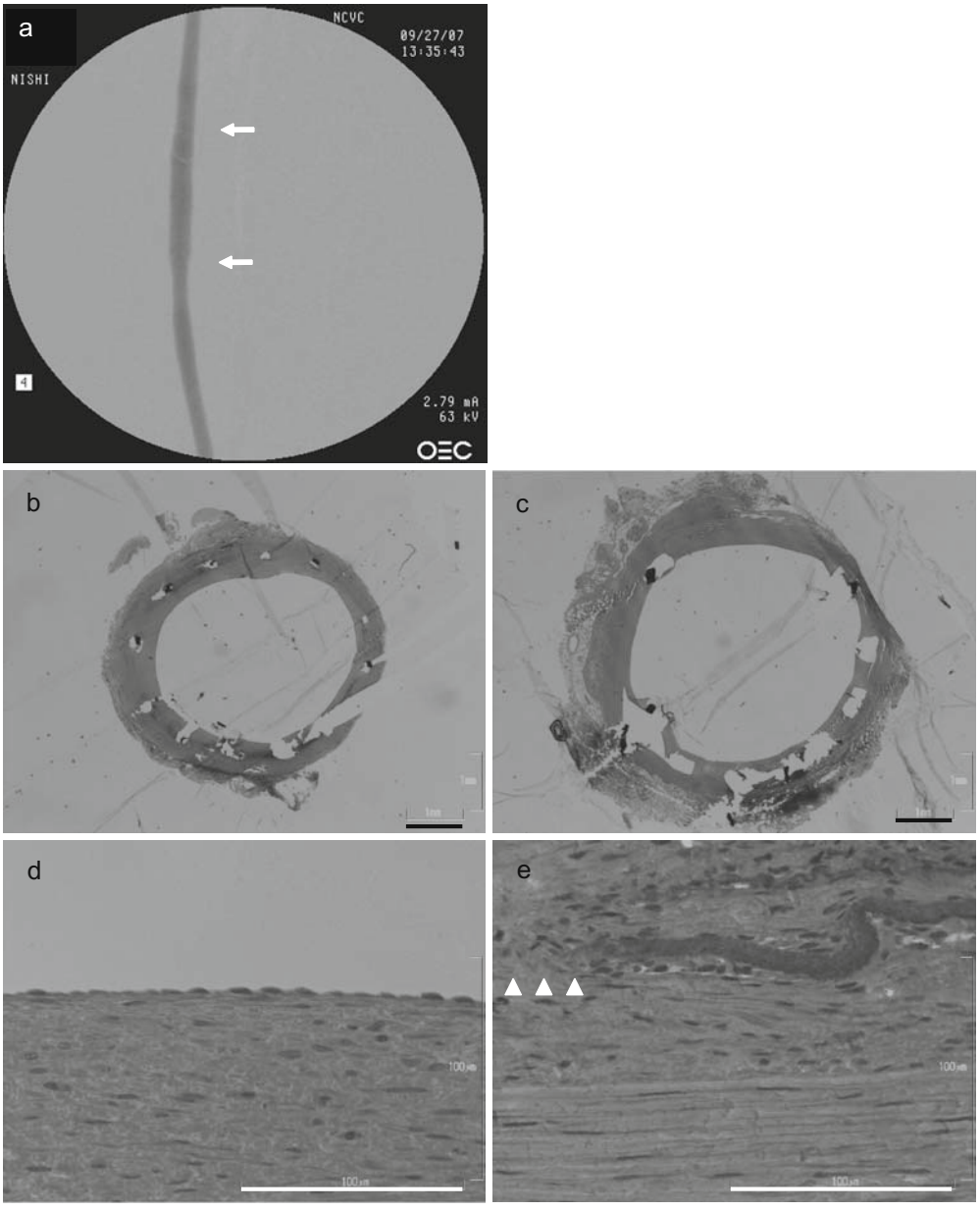

Fig. 3. Angiogram of the common carotid artery of a beagle 1 month after implantation of the balloon-expandable SC-covered stent (**a**). *Arrows* indicate the location of the implanted stent. Histological transverse sections of stents without (**b**) or with an SC cover (**c**). Luminal surface (**d**) and neointimal layer (**e**) of histological transverse sections of stents with an SC cover. *Arrowheads* indicate the cleavage site of the SC cover. *Bars* 1 mm (**b**,**c**) and 100 μm (**d**,**e**)

the development of covered stents using synthetic polymers has been performed to achieve long-term patency by coating a drug on the film to prevent early thrombus formation and intimal hyperplasia. Higher patency in the covered stents will be obtained by using a covering with appropriate biocompatibility.

We hypothesized that collagen represents a better cover material for several reasons. First, collagen is one of the extracellular matrix (ECM) components that are favorable to rapid endothelialization on the stent. The total coverage of the stent with connective tissue and endothelium would prevent restenosis. Second, cross-linking of the collagen with WSC might reduce thrombogenicity and increase mechanical strength of the collagen substrates. Several groups have already reported that platelet aggregation is reduced by glutaraldehyde cross-linking.[13] Furthermore, collagen is able to form fibrillar matrix under physiological conditions so that the fibrillar form provides extra strength and flexibility of the collagen substrate. Our group has demonstrated that simultaneous cross-linking during collagen fibril formation improves the mechanical properties and heat resistance of the SC gel.[12] Based on these points, we attempted the covering of stents using the collagen fibril formation of SC with simultaneous cross-linking.

The SC cover was able to sustain the mechanical deformation of the stents during deployment or constriction (Fig. 1b–f). The tensile strength (20.1 ± 1.7 kPa) of the SC film is considered to be sufficient for a cover material because researchers have reported a tensile strength of the order of 10 kPa for collagen-based vascular grafts.[14] Additionally, the thickness of the film (about 100 μm) did not revert to the original thickness of the gel (about 1.5 mm),

indicating that excess dilation of the vessel by gel swelling would not occur after implantation. Therefore, both self-expandable and balloon-expandable stents are expected to follow the shapes of the arteries while covering the lumen. The fibrillar mesh network may contribute to the flexibility and mechanical strength of the SC cover (Fig. 2). Preliminary short-term implantation showed that minimal intimal hyperplasia was observed on their implantation in the carotid arteries of beagles. This might be attributed to the chemical cross-linking effect.[13] We have reported that cross-linked salmon collagen grafts show a small amount of thrombus formation in vivo[15] and in vitro,[16] although native salmon collagen shows a large amount of thrombus formation (data not shown).

It can be speculated that the SC cover might be replaced with native vascular connective tissue after implantation because the partial cleavage of the SC cover was seen after 1 month of implantation, indicating biodegradation of the SC cover (Fig. 3e). Additionally, we have demonstrated that the original SC gel can completely be digested by collagenase in a short time,[17] indicating that the SC cover could be biodegraded by proteolytic enzymes. Furthermore, we are separately studying the culture of endothelial cells on the SC gel and the endothelial cells have been found to proliferate well.[18] Therefore, we believe that SC could maintain patency while achieving total coverage of the struts of the stent with connective tissue and endothelium. The implantation study showed that there was no significant difference in intimal thickening between the stents with and without the SC cover, indicating good biocompatibility of the SC cover. In contrast, synthetic cover materials are known to elicit severe inflammatory reactions, resulting in intimal narrowing and possible occlusion. Therefore, SC-covered stents may be able to cover coronary artery perforations, aneurysms, and fistulas without intimal narrowing.

To our knowledge, the present study represents the first attempt to develop a stent covered with collagen substrates, although we were unable to assess the long-term patency of the stents. The implantation period was fixed at 1 month based on our experience; in this period, the formation of the neointimal layer is usually maximal at around 1 month of stent deployment in normal animals, but a longer implantation period reduced the thickness of the neointimal layer because of the normal wound healing process, which was considerably different from the clinical course in human arteriosclerotic restenosis. When considered further, we need to evaluate these phenomena under conditions that bear a greater and direct resemblance to clinical applications; using hyperlipidemia animals with intimal hypertrophy produced by balloon injury should be considered.

In addition, some difficulties with stent implantation with the sheath were observed because the SC cover was sometimes peeled off from the surface of the stents by frictional forces when released from the delivery sheath in the self-expandable type. Therefore, we were unable to apply the self-expandable stent in the implantation study. We will refine the covering method so that the strut of the stent is fully covered with collagen substrate.

Conclusion

Novel SC-covered stents were developed. SC is potentially useful as an ideal cover material of endovascular stents. Therefore, it is anticipated that the SC-covered stents will acquire successful patency.

Acknowledgments This work was supported by the Special Coordination Funds for Promoting Science and Technology of the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT).

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