# IMPLANTATION OF STENT COATED WITH ANTIBODY AGAINST MESENCHYMAL STEM CELLS PREVENTS RESTENOSIS

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**ABSTRACT**

***Objectives**: To investigate the relationship of implantation of stents coated with mesenchymal stem cells (MSCs) and restenosis of coronary artery in a canine model.*

***Methods**: 1) The ethyl cellulose as a coating substrate and antibody against MSCs were used to coat the stents. 2) A canine coronary artery stenosis model was established via intravascular balloon dilatation. Stents (n=20) were implanted into the distal end of the injured coronary artery with a standard catheter-balloon technique. Then, the uncoated stents (n=20) were implanted to the proximal end of the damaged segment as controls. Animals were sacrificed 4 weeks later. The stents were collected and the vascular lumen and intima were evaluated by intravascular ultrasonography (IVUS) and vascular morphology.*

***Results**: Minimal lumen diameter (MLD) and intimal area (IA) significantly decreased in animals receiving implantation of coated stents, as compared to the control group (P<0. 05) while minimal lumen area (MLA) significantly increased (P <0. 05).*

***Conclusion**: MSCs coated stents can rapidly repair the damaged tunica intima and prevent the post-operative restenosis.*

***Key words**: Mesenchymal stem cells; stent; restenosis.*

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## Introduction

Coronary heart disease (CHD) has been one of the most common and serious diseases threatening human health. Since Gruentzig et al for the first time performed percutaneous tranluminal coronary angioplasty (PTCA) in 1977, interventional therapy has become an effective method to treat CHD. However, the fact is that even though percutaneous coronary intervention (PCI) has revolutionized the treatment of CHD, the restenosis still remains to be a great challenge for interventional cardiologists[1,2]. Thus, to prevent restenosis is crucial to improve the therapeutic efficacy.

The overgrowth of vascular smooth muscle cells (SMCs) due to the proliferation and migration of SMCs is a major pathophysiological feature of intrastent restenosis. Great effort has been applied to develop therapeutic strategies to inhibit the overgrowth of SMCs. Although the sirolimus and paclitaxel-eluting stents can inhibit the proliferation of SMCs, they may suppress the repair of endothelial cells simultaneously, which may induce subacute and tardive thrombosis[3,4]. Therefore, to early establish a functional endothelial layer after vascular injury has been shown to prevent neointimal proliferation and thrombosis[5,6].

Under certain conditions, bone marrow mesenchyme stem cells (MSCs) will migrate to the injured sites and differentiate into vascular endothelial cells for repair in order to prevent thrombosis. Since the MSCs can express specific antigens, the stents were coated with antibody against MSCs, and then circulating MSCs could bind to these stents and differentiate into vascular endothelial cell for repairing the impaired vascular intima.

This study was to investigate whether the stents coated with antibody against MSCs could prevent in vivo restenosis in a canine model.

## Materials and methods

### *Animals*

A total of 20 canines (Qinglongshan Experimental Animal Center, Nanjing) weighing 16-20 kg were used in the present study. These canines were fed regularly and allowed to accommodate to environment for 1 week before operation. One day before operation, the canines were treated with aspirin (100 mg) and clopidogrel (Plavix; 75 mg). Animals were fasted and then anesthetized with ketamine hydrochloride (i.m. 20 mg/kg body weight).

### *Main reagent and instruments*

Percoll solution (Institute of Biotechnology, Chinese Academy of Medical Sciences), newborn bovine serum (Hangzhou Sijiqing Biological Engineering Materials), Dulbecco's Modified Eagle Media (DMEM, Thermo Fisher Biochemical Products Co., Ltd.), hydrochloric acid, ketamine (Jiangsu Hengrui pharmaceutical companies), brominated deoxyuridine (Brdu, Sigma Company), inverted phase contrast microscope (Olympus), and Coronary Stent System (Abbott Vascular) were used in the present study.

### *Cell Culture*

The aspiration of iliac bone marrow was performed, and MSCs were isolated by using density gradient centrifugation. The MSCs were maintained in DMEM containing newborn bovine serum. Cells were observed under an inverted phase contrast microscope.

### *Preparation of antibody-coated stents*

In brief, 5 g of ethyl cellulose was dissolved in a solution containing 20 ml of ethanol and 30 ml of xylene. Stents were immersed in the cellulose solution and then taken out 10 min later for dry by airing. These steps repeated once. Then, above stents were immersed in a solution of antibody against MSCs at 4°C overnight. Finally, the antibody-coated stents were treated with sterilized penicillin solution overnight.

### *MSCs marker*

When the cell confluence reached about 70%, MSCs were collected and suspended in Brdu stock solution (10 μmol/L). Incubation was done for 24 h.

### *Implantation of stents*

After anesthesia, a 6F arterial sheath was inserted to the right femoral artery followed by insertion of a guiding catheter into the inlet of left coronary artery. Then the left anterior extension of the proximal and distal was selected and the diameter was measured by quantitative coronary angiography (QCA). According to the vascular diameter, a balloon (balloon/vessel: 1.1:1) with a stent was used. The balloon was inflated at a pressure of 2-4 absolute atmospheres for 15 sec of single excessive expansion of blood vessels, the coronary artery injury model was established. Thereafter, the antibody-coated stent (2.5 × 8 mm) was implanted into the distal left anterior descending artery according to the standard method in PCI while the uncoated stents were implanted in the proximal end. Finally, the over-the-wine balloon (3.0 × 8mm) was placed into the proximal to distal vessels.

Angiography showed that the anterior vessel was completely blocked. MSCs suspension was collected and injected into the over-the-wine balloon central hole slowly within 4 min. Then, the balloon was deflated and drawn out.

Routine coronary angiography was performed to check for vascular patency. After withdrawal of the arterial sheath, the wound was closed, and animals were housed in the Experimental Animal Center. After surgery, 3×106 U of penicillin was given intramuscularly for 3 consecutive days to prevent infection. The canines were also treated with oral clopidogrel (Plavix; 37.5 mg/day) and aspirin (100 mg/day). Four weeks later, animals were sacrificed.

### *Collection and preparation of samples*

At 4 weeks after stent placement, angiography was performed again under anesthesia to detect the stenosis. MLD and MLA were determined by QCA and IVUS. Thoracotomy was performed under deep anesthesia, and the canines were sacrificed followed by immediate collection of coronary artery. Coronary arteries were perfused and then fixed in 10% neutral formaldehyde for 24 h at a pressure of 100 mmHg (1 mmHg = 0.133 kPa). After fixation, sections of the coronary free segments which contain the stent were prepared. The intrastent neointimal area and medial area inside and outside the elastic panel around the area were compared between segments with placement of MSCs-coated stents and uncoated stents[7].

***Statistical analysis***

Data were expressed as mean ± standard deviation (SD). SPSS version 13.0 for Windows was used for statistical analysis. Student's t test was employed for comparisons of data between two groups. A value of P<0.05 was considered statistically significant.

## Results

Stents were successfully implanted in the proximal and distal end of anterior descending coronary artery of 20 canines, of which 4 died within 2h after stent implantation. The causes of death included ventricular fibrillation, bleeding and anesthesia accident. The remaining 16 canines survived until the end of study. The MLD measured by QCA before and after stent implantation is shown in Table 1. The MLA and pathological/histological findings were determined by intravascular ultrasonography (IVUS) and are shown in Table 2. MLD and IA significantly decreased in animals receiving implantation of coated stents, as compared to the control group (P<0. 05) while MLA significantly increased (P <0. 05). The images from the coronary angiography (Fig.1), IVUS (Fig.2), HE staining (Fig.3) and electron microscopy (Fig.4) were appended.

| Group | N | immediately after surgery | 4 weeks after surgery |
|---|---|---|---|
| MSCs-coated stents | 16 | 2.5531±0.09492 | 2.4913±0.10825* |
| MSCs uncoated stents | 16 | 2.5194±0.07767 | 2.4875±0.08112 |

**Table 1:** MLD after implantation of MSCs-coated stents and uncoated stents (mm, ±s).
*Note: *P < 0.05 vs MSCs uncoated stents*

| Group | N | MLA | intermnl elastic membrane area | external elastic membrane area | Intimal area | Medial area |
|---|---|---|---|---|---|---|
| MSCs-coated stents | 16 | 4.4494±0.10478* | 4.2031±0.81.34 | 5.3006±0.86101 | 2.2669±0.45808* | 3.1369±0.83091 |
| MSCs uncoated stents | 16 | 3.7369±0.34731 | 4.4769±0.95120 | 5.8113±0.72678 | 2.9681±0.69885 | 3.6425±0.89410 |

**Table 2**: MLA and morphological findings at 4 weeks after implantation of MSCs-coated stents and uncoated stents (mm2, ±s).
*Note: *P<0.05 vs MSCs uncoated stents*

## Discussion

In the present study, the bone marrow-derived MSCs were prepared. After multiple attempts, the cellulose was found to be very difficult to dissolve in a common organic solvent. However, it can dissolve in ethanol and xylene mixed solution. As MSCs express specific antigen, the stents were coated with specific antibody against MSCs. After implantation of these stents, the circulating MSCs can bind to above stents and then differentiate into endothelial cells to repair the injured artery and prevent restenosis. Our findings showed that, as compared to the uncoated stents group, animals had significantly reduced neointimal area and increased lumen diameter and area after implantation of MSCs coated stents.

Intrastent restenosis is a severe complication of stent implantation and may lead to recurrence of angina, and patients with intrastent restenosis usually require revascularization. The emergence of drug-eluting stents is a revolution in the interventional cardiology. Drug-eluting stents can significantly reduce the incidence of restenosis as compared to bare stents[8-11], which may be attributed to the inhibition of vascular intimal hyperplasia. However, the drug-eluting stents may also delay the repair of vascular endothelium, and increase the risk for acute and subacute thrombosis[12]. Ramin et al reported the acute stent thrombosis in 36 patients after implantation of drug-eluting stents. There is more number of cases of sudden death but phenomenon of thrombosis was not seen in the coronary artery of patients after drug-eluting stent implantation[13]. Therefore, to speed up the coronary balloon angioplasty and stent endothelialization of injured vessel segment is crucial to reduce the incidence of restenosis.

In recent years, with in depth studies on bone marrow stem cells, cellular biological interventions have been used in the treatment of myocardial remodeling after myocardial infarction. A prospective single-center study in Italy indicated that the implantation of stents coated with antibody against endothelial progenitor cells in high-risk population had a success rate of as high as 98%, and acute and subacute intrastent restenosis was not observed in these patients[14]. Amalia et al also reported that the bone marrow-derived MSCs could effectively reduce the incidence of carotid artery stenosis in a rat model[15].

As compared to other seed cells used in tissue engineering, bone marrow-derived MSCs have more potent differentiation capacity into car-

diomyocytes and vascular endothelial cells. Moreover, MSCs have the characteristic of adherent growth. Thus, partial-cell therapy for the treatment of local vascular lesions can be achieved on the basis of biological characteristics of MSCs.

Findings in the present study indicate that the implantation of stents coated with antibody against MSCs followed by injection of bone marrow-derived MSCs can prevent post-injury restenosis. However, feasibility and safety of this strategy in clinical practice are required to be further studied in future investigations.

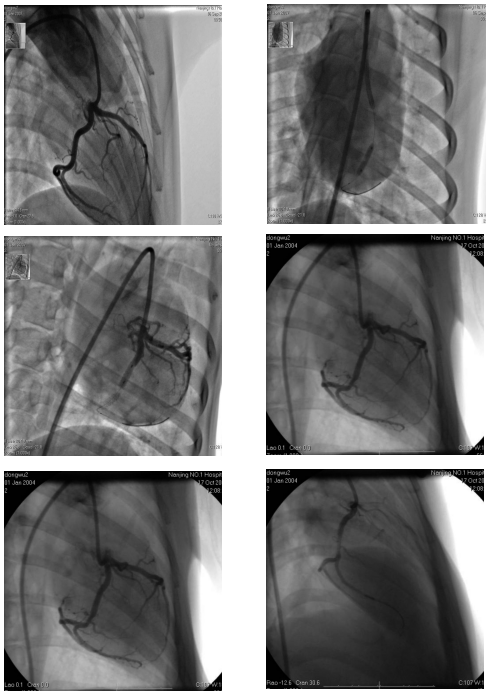

**Fig. 1:** Results of coronary angiography. (A) Coronary artery in coronary angiography before stent implantation. (B) Implantation of first stent. (C) Implantation of second stent. (D) Coronary artery in Coronary angiography after operation. (E) Coronary angiography at 4 weeks after surgery. (F) Intravascular ultrasonography at 4 weeks after surgery.

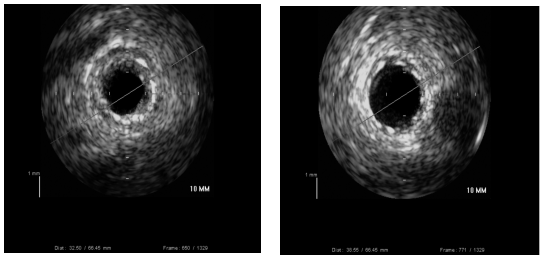


**Fig. 2:** Results of IVUS. (A) MSCs uncoated stents group presented with obvious accrementition. (B) MSCs coated stents group showed smooth intima.

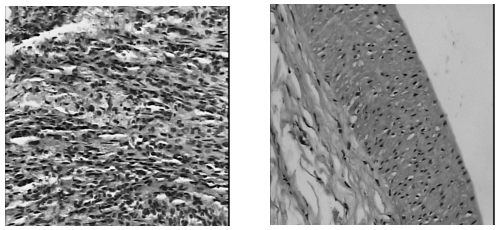

**Fig. 3:** Results of HE staining. (A) MSCs uncoated stents group (×200) presented with hemosiderin deposition accompanied by obvious inflammatory reaction. (B) MSCs coated stents group (×200) had no evident inflammatory reaction.

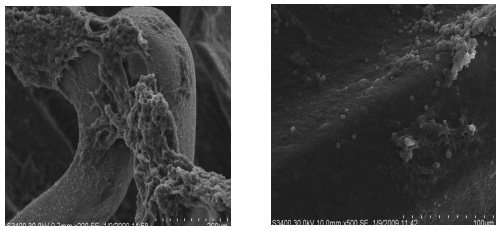

**Fig. 4:** Results of Scanning electron microscopy. (A) MSCs uncoated stents had rough surface. (B) MSCs coated stents had smooth surface.

## Conclusion

Mesenchymal stem cells via combining with their specific antibody on the surface of bare metal stents have potential of repairing the endothelial injury, with resultant decreased neointimal hyperplasia. Our study has some limitations.

This study was a small single-center study, so a large sample, multi-center study is needed for further confirmation. In this study, only four weeks were reviewed, and this effect can not continue to maintain, require longer follow-up study.

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