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In vivo evaluation of a novel dexamethasone-heparin-double-coated stent for inhibition of artery restenosis and thrombosis

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Abstract To evaluate the efficacy and safety of dexamethasone-heparin-double-coated stent (DHDCS) on inhibition of artery lumen reduction and neointimal hyperplasia in porcine model we carried out this study. Bare mental stents (BMS, $n = 12$), protein-coated stents (PCS, $n = 12$), heparin microballoon-coated stents (HMCS, $n = 12$), and DHDCS ($n = 12$), prepared by the spray drying method, were implanted into the selected internal iliac artery, external iliac artery, sacrococcygeal artery, and femoral artery of each of the selected pigs ($n = 12$), which were randomly divided into four groups on average. Thirty days and ninety days after the implantation, aorta angiography was performed on all the 12 mini-pigs to evaluate the artery lumen reduction. Subsequently, in order to analyze their histological appearance, the pigs were killed, and their arteries with the stents inside were taken out, embedded in plastic for hard histological section and hematoxylin-eosin (H.E.) staining, and examined by light microscopy and scanning electron microscopy (SEM). The artery lumen reduction and average neointimal hyperplasia in the group of DHDCS were significantly lesser than those in the other three groups of BMS, PCS, and HMCS. This study shows that DHDCS is capable of inhibiting the proliferation of intima and lumen area reduction of the target artery within stents, and effectively and safely reducing the incidence of regional thrombosis and restenosis for a short term.

1 Introduction

Coronary artery disease (CAD) has become a common threat to human life, and different kinds of stents, including bare metal stents (BMS) and drug-eluting stents (DES), have been used to offer a minimally invasive treatment option through percutaneous coronary intervention (PCI). Moreover, PCI can cause injury to blood vessels, as well as neointimal hyperplasia and thrombosis that may result in vessel occlusion. In addition, in-stent restenosis has been reported to have an incidence of 10–40% [1, 2]. Thus, the problem of how to modify and optimize the stent properties and performance so as to reduce unexpected side effects is a task that must be resolved.

At first, BMS served as a scaffold to open and support the occluded arteries after balloon angioplasty in patients, and had relatively good efficacy in the short term. Recently, advances in coronary stent technology have produced different types of stents, such as DES made from BMS coated with different polymeric matrices containing drugs that have been introduced into clinical therapy for patients. These DES have been designed and made to release a controlled amount of anti- proliferation drugs that can reduce smooth muscle cell growth and prevent inflammatory response. Sirolimus-eluting stents (Cypher®, Cordis) and paclitaxel-eluting stents (Taxus®, Boston Scientific) have been the most extensively studied and widely

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used in patients with CAD, primarily because of their capability for restenosis reduction [3]. In addition, stents coated by hydrophilic polymers and phosphorylcholine-containing polymers carrying heparin have been used in order to decrease thrombosis. However, there are still a high percentage of patients using the DES mentioned above who experience artery narrowing, restenosis, thrombosis [4], and even sever acute heart stroke or myocardial infarction, which result in the need for revascularization by re-intervention or coronary artery bypass graft (CABG). In-stent restenosis and thrombosis are common and potentially life-threatening complications of PCI, so prevention of neointimal hyperplasia and resistance to thrombogenesis are of vital importance [5–7].

Previous publications have described the suitability of zein microspheres and tableted microspheres with sound blood material interactions, good tissue response and biocompatibility in vivo as a sustained-release forms of ivermectin (IVM), implying a potential application in tissue engineering for preparing scaffolds, which are composed of microspheres encapsulating bioactive components for stimulating cell differentiation and proliferation [8–16]. Dexamethasone is widely known for its pharmacological action of anti-adhesion, and antioxidant and anti-inflammatory properties and heparin for its anti-thrombotic efficacy which have beneficial effects on vessel response to injury, and neointimal hyperplasia and thrombosis, ensuring that the injured vascular endothelia remain smooth and maintain their integrity.

In this paper, we report a novel type of double-coated stent, which was produced with a Firebird bare metal scaffold modified and coated with corn protein zein microsphere that carried dexamethasone and heparin through a layer-by-layer technique capable of sustained release drug delivery in an attempt to minimize restenosis and thrombosis. Firstly, we prepared the corn protein zein microsphere containing dexamethasone and heparin, respectively, which can be physically retained on the Firebird bare metal scaffold surface. Secondly, the stents were pre-coated with the dexamethasone zein microsphere by a spray method and dried at room temperature in a vacuum environment. As a third step, the monolayer stent surface was top-coated with the heparin zein microsphere and dried using the same method as described above; then the manufactured dexamethasone-heparin-double-coated stent (DHDCS) was sterilized by ultraviolet radiation. Finally, in order to evaluate its function of preventing artery vessel neointimal hyperplasia and thrombosis in vivo, the DHDCS, together with other types of stents, was implanted into the porcine arteries; 30 and 90 days later, imaging analysis was performed, and the artery vessel with stents was examined with a light microscope and a scanning electron microscope (SEM).

2 Materials and methods

2.1 Preparation and properties of DHDCS

All the Firebird bare metal stents, whose project number was 08XP0500700, were 3.5 mm in diameter and 18 mm in length, and were supplied by MicroPort Medical (Shanghai) Co., Ltd. The fabrication of the corn protein zein microspheres containing dexamethasone- and heparin-coated stents was accomplished by the Shanghai Institute of Organic Chemistry, Chinese Academy of Science.

2.2 Animals

The total of 12 healthy pigs used in the study,regardless of sex and with a body mass of approximately 15–25 kg, were form the animal laboratory center of the Shanghai Sixth People's Hospital, which is affiliated with the Shanghai Jiaotong University School of Medicine. All experiments were performed in accordance with the Chinese legislation on Regulations for Experimental Animals Administration (The People's Republic of China Science and Technology Commission Publication No. 2, Chapter IV, 1988), and approval was granted by the Shanghai Jiaotong University Ethics Review Board (SYXK-Shanghai, 2006-0010). In addition, the handling of the animals involved in the investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Altogether, 48 stents were implanted into the selected internal iliac artery, external iliac artery, sacrococcygeal artery, and femoral artery of each of the pigs ($n = 12$), which were randomly divided into four groups on average: group A, bare mental stents (BMS, $n = 12$); group B, protein-coated stents (PCS, $n = 12$); group C, heparin microballoon-coated stents (HMCS, $n = 12$); and group D, DHDCS ($n = 12$). The artery selection standard was that the blood vessels have similar shear stress and blood flow dynamics. Therefore, under the condition of distal abdominal aorta angiography, the arteries approximate 3 mm in diameter were selected to fit for the stent implantation ensuring that each group contains equivalent numbers of similar arteries.

2.3 Angiography and stent implantation

After the porcine had been fixed on the operating table with endotracheal intubation and ventilator-assisted breathing, intramuscular injection with ketamine (10–15 mg/kg) for infrastructure anesthesia and intravenous injection with 3% pentobarbital sodium (20–30 mg/kg) for intravenous anesthesia were administered. When the procedures of neck skin preparation, iodophor and alcohol disinfection,

and fleet covering of the surrounding field of operation were finished, longitudinal incision of the left paratracheal region was performed to expose the left common carotid artery. A F6 catheter for artery angiography was placed into the artery through which a dose of 200 μ/kg heparin was injected. Under sterile conditions, and the supervision of the guide wire in the catheter and the lower segment of the abdominal aorta angiography, a total of 48 scaffolds 18 mm × 3.5 mm in size were implanted into the selected internal iliac artery ($n = 12$), external iliac artery ($n = 12$), sacrococcygeal artery ($n = 12$), and femoral artery ($n = 12$) of the 12 mini-pigs. The diameter ratio of the stents and the target vessels was 1.1:1 to 1.2:1 [17], and the sustained vessel expansion pressure and time of the stent balloons were 6–10 atm and 20–40 s. Repeated angiography was administered to observe the patency of the target vessel, with or without filling defects, dissection, thrombosis, or distal vessel spasm, and to measure the values of the target vessel immediately after stent implantation; that is, the diameter of the balloon dilation, the baseline vessel diameter, and the minimal lumen diameter. All the mini-pigs were fed in the animal laboratory center. Signs of infection, body temperature, diet, action, and the condition of incision healing were monitored throughout the study, especially within the 3 days post-implantation; 250 μ/kg of heparin and 1.6 million units of penicillin were administered to all the animals twice a day through subcutaneous and intramuscular injection, respectively. In addition, tablets of aspirin 50 mg in dose were given orally to each mini-pig once a day until they were administered to euthanasia.

2.4 Re-examination of distal abdominal aorta angiography and quantitative analysis of artery stenosis within the stents

Thirty days and 90 days after the implantation, aorta angiography was performed on all the 12 mini-pigs to evaluate the artery lumen reduction. Distal abdominal aorta angiography was carried out, and quantitative artery image analysis software (GE Medical Equipment Co., Ltd., Germany) was applied to measure the reference value and minimum lumen vessel diameter of the artery within the stents, thereby reckoning the ratio of vessel dilation and the value of lumen loss.

2.5 Pathology analysis

The animals were killed by euthanasia with the hypertonic potassium intravenous injection, and explantation of stents was performed after re-examination by angiography. After the heparin saline rinsed, the narrowest 1/3 part of each artery was examined using a scanning electron microscope (S-450, Hitachi, Japan) to observe the morphologic appearance of the vessel intima. The remaining 2/3 part of the artery was fixed in a concentration of 4% methanal films for 24 h. After completing the processes of dehydration, methyl methacrylate (MMA) embedment, hard tissue transverse section (LEIKA SP1600, Germany) with a slice thickness of 100 μm, and H.E. staining, the vessel intima area was measured and converted into the average thickness of the vessel intima with MIQAS, a software for the quantitative analysis of medical images (Demand for Bio-technology Co., Ltd. Shanghai) equipped with an optical microscope (Olympus, BH2, Japan) and a camera (JVC TK-C1481 BEC, Japan). SEM was used to observe the surface and the inner microstructure of the scaffolds and the vessel neointima. The pathology analysis was carried out under blinded conditions by a pathologist.

The vascular injury standard was: 0 point inner elastic plate integrity; 1 point inner elastic plate fracture; 2 points, inner elastic plate and tunica media rupture; and 3 points, external elastic plate fracture [18]. The inflammatory response standard caused by stents implantation was: 0 point, no inflammatory cells; 1 point, scattered inflammatory cells; 2 points, half of the 25–50% circumference of the blood vessel with stents was surrounded by inflammatory cells; 3 points, the entire 25–50% circumference of the blood vessel with stents was surrounded by inflammatory cells [19]. The endothelialization classification was: 1 point, grade I, endothelial cells surrounding the lumen circumference approximately 25%; 2 points, grade II, endothelial cells surrounding the lumen circumference approximately 25–75%; and 3 points, grade III, endothelial cells surrounding the lumen circumference over 75% [20].

2.6 Statistics

SPSS 17.0 was used in the analysis. All the data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and analyzed by One Way ANOVA Analyses for group comparisons. Statistical significance was accepted at $P < 0.05$.

3 Results

3.1 Heparin-dexamethasone zein microsphere release in vitro, DHDCS pattern, and SEM

A test of the microsphere drugs' release in vitro proved that they undergo a surface degradation that allows for a stable release; the bursting release occurred within 4–8 h, followed by the stable release stage. Figure 1 shows the release procedure of coated heparin-dexamethasone zein microsphere (HDZM) in pepsin containing buffer.

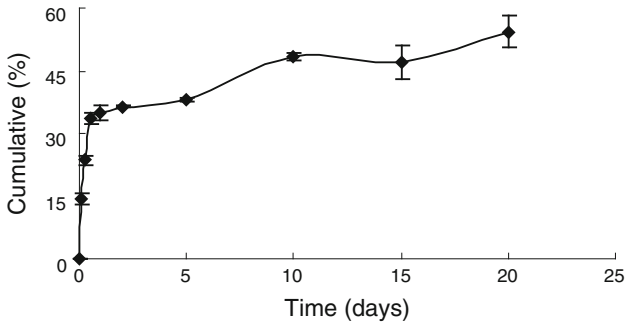

Fig. 1 The release procedure of coated heparin-dexamethasone zein microsphere (HDZM) in pepsin containing buffer

The coated drugs made up of zein microspheres, with encapsulation efficiency affected by the processing time, and the protein concentrations and amount of drug loading about of 20 and 2.5%, respectively, were sprayed and compressed onto the surface of the BMS and combined with it tightly. When expanded and rinsed in a simulated in vivo environment with a water flow velocity of 80 ml/s, much faster than the actual blood flow in the body for 12 h, the coating structure remained intact. Figure 2 shows the pattern of the DHDCS. Figure 3 shows the structure of BMS and SEM results of DHDCS pre-implantation.

3.2 Animals and stent explantation

Without severe complications, such as systemic infection, bleeding, lower extremity ischemia, stroke symptoms, and accidental pig death, surveillant prothrombin time (PT) and its international rates (INR) values of all the animals were in an acceptable range. All 48 stents including BMS Group, PCS Group, HMCS Group and DHDCS Group were taken out from the arteries with a few thrombosis in them except for within some BMS through gross observation and the stents maintained their original shape.

3.3 Quantitative distal aortic angiogram analysis

Distal aortic angiography 30 and 90 days after stent implantation showed that the lumen reduction in DHDCS group was less than that in the other three groups of BMS, PCS, and HMCS under a diameter ratio of stents and target vessels of 1.1:1 to 1.2:1; the differences had statistical significance (Tables 1, 2).

3.4 Analysis of histomorphology and pathology

The semiquantitative analysis of the target vascular histomorphology and pathology after 90 days of stent implantation indicated that, under similar vessel injury circumstances and compared with the BMS group, the DHDCS group had obvious suppression of intimal hyperplasia within the stents. Moreover, the average intima thickness of the artery in the DHDCS group was significantly smaller compared to those in other three groups. Although the inflammatory response was shown to be similar between the DHDCS group and the BMS group, it was better than that in HMCS group. The differences are statistically significant (Tables 3, 4; Figs. 3, 4).

3.5 H.E. staining and SE

The blinded outcomes of histomorphologic appearance of the H.E. staining and SEM 30 and 90 days after stent implantation is shown in Figs. 3 and 5 and Tables 5 and 6.

4 Discussion

Because they inhibit cell proliferation and reduce the incidence of in-stent restenosis at the sites of stents implantation, DES with rapamycin and paclitaxel have been widely used in the treatment of CAD. However, there are also limitations of these DES. Endothelium integrity is of utmost importance to prevent a thrombotic barrier, but the coated drugs are cytotoxic drugs, because they inhibit smooth muscle cell excessive proliferation and normal endothelial cell regeneration, thus delaying the process of vascular endothelialization after stents implantation and increasing the risk of delayed thrombosis. The longer the coated drugs take effect and the more persistently they

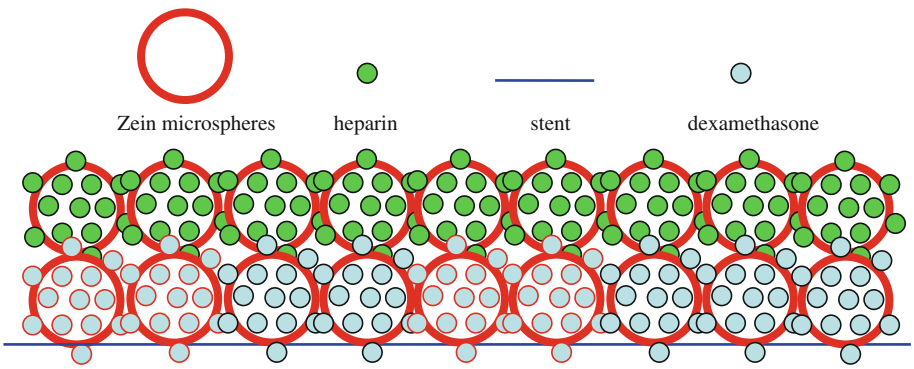

Fig. 2 Design and pattern of the DHDCS

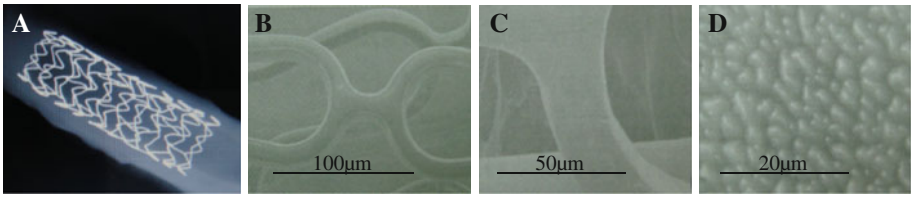

Fig. 3 The structure of BMS and SEM results of DHDCS pre-implantation. **a** BMS; **b** DHDCS ×120; **c** DHDCS ×240; **d** DHDCS ×880

Table 1 Distal aortic angiography 30 days after stent implantation ($\bar{x} \pm s$, unit: mm)

Stent type	Balloon/vessel ratio	Reference vessel diameter	Instant implantation MLD	Re-examination MLD	Lumen reduction
BMS ($n = 12$)	1.15 ± 0.62	3.36 ± 0.28	3.48 ± 0.23	1.82 ± 0.32	1.42 ± 0.35
PCS ($n = 12$)	1.15 ± 0.56	3.38 ± 0.25	3.24 ± 0.33	1.95 ± 0.51	1.29 ± 0.68
HMCS ($n = 12$)	1.18 ± 0.61	3.27 ± 0.22	3.16 ± 0.25	2.12 ± 0.37	1.20 ± 0.43
DHDCS ($n = 12$)	1.18 ± 0.45	3.35 ± 0.19	3.41 ± 0.31	2.92 ± 0.45	0.61 ± 0.51[a]

MLD minimal luminal diameter

[a] Comparison with BMS GROUP under similar dilation ratio conditions, $P = 0.038$

Table 2 Distal aortic angiography 90 days after stent implantation ($\bar{x} \pm s$, unit: mm)

Stent type	Balloon/vessel ratio	Reference vessel diameter	Instant implantation MLD	Re-examination MLD	Lumen reduction
BMS ($n = 12$)	1.16 ± 0.53	3.29 ± 0.11	3.37 ± 0.31	1.88 ± 0.16	1.56 ± 0.29
PCS ($n = 12$)	1.15 ± 0.51	3.35 ± 0.19	3.26 ± 0.43	1.93 ± 0.15	1.44 ± 0.31
HMCS ($n = 12$)	1.18 ± 0.56	3.28 ± 0.24	3.21 ± 0.16	2.28 ± 0.25	1.87 ± 0.62
DHDCS ($n = 12$)	1.17 ± 0.54	3.25 ± 0.17	3.32 ± 0.22	2.67 ± 0.36	0.72 ± 0.39[a]

MLD minimal luminal diameter

[a] Comparison with BMS GROUP under similar dilation ratio conditions, $p = 0.042$

Table 3 Distal aortic angiography 90 days after stent implantation ($\bar{x} \pm s$)

Stent type	Vessel area (mm^2)	Inner elastic plate area (mm^2)	Neointima area (mm^2)	Vascular injury points	Inflammation points	Endothelialization points
BMS ($n = 12$)	0.98 ± 0.28	6.34 ± 1.38	5.27 ± 1.35	2.10 ± 0.58	0.95 ± 0.43	3.00 ± 0.00
PCS ($n = 12$)	0.85 ± 0.31	6.23 ± 1.34	5.14 ± 1.49	2.23 ± 0.52	1.17 ± 0.49	3.00 ± 0.00
HMCS ($n = 12$)	1.25 ± 0.86	4.12 ± 1.57	3.36 ± 1.25	2.33 ± 0.57	2.61 ± 0.56	2.84 ± 0.46
DHDCS ($n = 12$)	2.11 ± 1.29	5.36 ± 0.82	3.32 ± 1.13[a]	2.25 ± 0.64	1.36 ± 0.83[b]	3.00 ± 0.00

[a] Neointima in the DHDCS group was noticeably less than that in the BMS group, $P = 0.035$

[b] Inflammation points in DHDCS was noticeably less than that in the HMCS group, $P = 0.024$

Table 4 Average intima thickness of 90 days after stent implantation ($\bar{x} \pm s$, unit: μm)

Stent type	BMS	PCS	HMCS	DHDCS
Average intima thickness	36.0 ± 12.1	36.5 ± 5.8	29.9 ± 9.0	12.0 ± 4.4
BMS ($n = 12$)		$P = 0.91$	$P = 0.41$	$P = 0.02$
PCS ($n = 12$)	$P = 0.91$		$P = 0.13$	$P = 0.01$
HMCS ($n = 12$)	$P = 0.41$	$P = 0.13$		$P = 0.01$
DHDCS ($n = 12$)	$P = 0.02$	$P = 0.01$	$P = 0.01$	

Statistical significance was accepted at $P < 0.05$

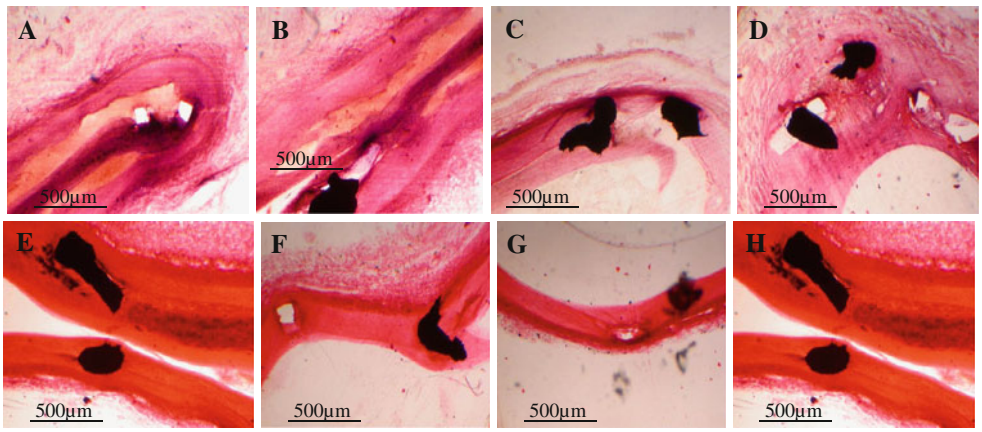

Fig. 4 H.E staining 30 and 90 days after stent implantation. **a** 30 days BMS ×10; **b** 30 days PCS ×10; **c** 30 days HMCS ×10; **d** 30 days DHDCS ×10; **e** 90 days BMS ×10; **f** 90 days PCS ×10; **g** 90 days HMCS ×10; **h** 90 days DHDCS ×10

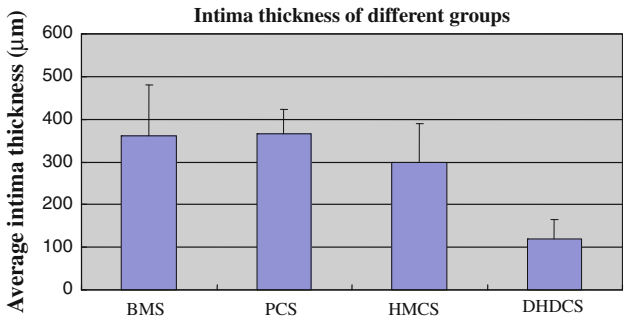

Fig. 5 Vessel intima thickness comparison of the four groups

infiltrate the tissue, the worse vascular endothelialization becomes. Presently, most drug-coated stents use only a single drug, while the incidence of in-stent restenosis is a multiple mechanism process. Since the drugs cannot affect all these mechanisms, their inhibitory effects are limited. As the most commonly used DES carrier cannot be degraded, it may increase vascular lesions of regional chronic inflammation to some extent and may be one of the factors that can cause delayed endothelialization [21–23]. Therefore, the prospects are not as optimistic as one might imagine, though statistically significant experimental data in real life had been obtained using DES [24]. In these cases, research on and the manufacture of a novel kind of coronary artery stent are imperative.

After the intervention treatment, the regional inflammatory response in the vessel kept the atheromatous plaque in a stable state, which ultimately led to acute myocardial infarction [25, 26]. Platelet and white blood cell adhesion occurred at the site of the stent implantation, resulting in endothelial cell injury, and cytokines and growth factors with the ability of promoting proliferation were produced [27, 28]. In addition, the systemic inflammatory response will be triggered, thus promoting the level of C-reactive protein (CRP) in blood, which is intimately related to the vessel restenosis within the stents [29]. Aside from performing an anti-inflammatory and anti-immune function,

Table 5 H.E. staining appearance within and surrounding the stents

Stent type	Stent implantation at 30 days	Stent implantation at 90 days
BMS	A few inflammatory cells infiltrating;	No significant inflammatory cells infiltrating;
	Regional thrombosis;	Regional thrombosis;
	Regional vessel wall thickening.	Regional vessel wall thickening.
PCS	A few inflammatory cells infiltrating;	Minimal inflammatory cells infiltrating;
	No significant thrombosis;	No significant thrombosis;
	Regional vessel wall thickening.	Regional vessel wall thickening.
HMCS	Minimal inflammatory cells infiltrating;	A few inflammatory cells infiltrating;
	A few thrombosis;	No significant thrombosis;
	No significant change in vessel wall.	No significant change in vessel wall.
DHDCS	No significant inflammatory cells infiltrating;	No significant inflammatory cells infiltrating;
	No significant thrombosis;	No significant thrombosis;
	No significant change in vessel wall.	No significant change in vessel wall.

Table 6 SEM appearance within and surrounding the stent

Stent type	Stent implantation for 30 days	Stent implantation for 90 days
BMS	Partial endothelial cell comparative well arrangement	Partial endothelial cell comparative confusion arrangement
	Some endothelial cell injury	Predominant endothelial cell injury
	Visible fibrous joint	Visible fibrous joint
	A few lipids and red cell conglutination	A few lipids and red cell conglutination
	Visible thrombosis	Significant thrombosis
PCS	Comparatively disorderly arrangement of endothelial cells	Comparatively disorderly arrangement of endothelial cells
	Visible endothelial cell injury	Severe endothelial cell injury
	A few fibrous joint	Visible fibrous joint
	Visible thrombosis	Visible thrombosis
HMCS	Partial endothelial cell comparative well arrangement	Partial endothelial cell comparative well arrangement
	Visible endothelial cell injury	Some endothelial cell injury
	Few fibrous joints	Visible fibrous joint
	Few lipids and red cell conglutination	Few lipids and red cell conglutination
	Few thromboses	Few thromboses
DHDCS	Significant endothelial cell well arrangement	Predominant endothelial cell comparative well arrangement
	Few endothelial cell injuries	No visible endothelial cell injury
	Few fibrous joints	No visible fibrous joints
	Few lipids and red cell conglutination	No lipids and red cell conglutination
	No visible thrombosis	No thrombosis

and inhibiting white blood cell adhesion and migration, dexamethasone can also adjust the functions of endothelial cells, platelets, and fibroblasts [30, 31]. Therefore, it is possible that dexamethasone has the ability to suppress restenosis within stents in theory. Some clinical studies have shown that dexamethasone-eluting stents are capable of preventing narrowing in the medium and long term [32, 33]. Although, another study found that dexamethasone eluting-stents did not relieve restenosis within stents, the said study had no control group and no convincing results [34].

Recently, a report of Italian REgistry study [35] on steroid-eluting stents in patients with acute coronary syndromes (ACS) indicates that dexamethasone-eluting stents (D-DES) implantation do not support any effective anti-proliferation action in patients with CAS, with the results of 6-month angiographic follow-up. But we think 50% of patients they included was intermediate-to-high TIMI risk stratification. The incidences of complications such as target vessel revascularization (TVR), non-Q-wave myocardial infraction (MI), restenosis and thrombosis were still unknown within similar patients without using the D-DES, so their conclusions should be inquired in further.

Due to the high incidence of mortality or myocardial infarction [36–39], secondary thrombosis after implantation within stents is another important problem to be tackled, and there is an urgent need to come up with prevention measures. Though anticoagulant drugs were administered after stents implantation, secondary thrombosis and simultaneous hemorrhage can still occur [40]. Heparin-coated stents could increase inhibition of platelet adhesion, and thrombosis; based on clinical application, by using heparin-eluting stents, the doses of systemic anticoagulant drugs, as well as the incidence of complications of hemorrhage and thrombosis can be reduced [41–43].

In order to take a good use of the merits and synergistic effect of the two inexpensive and easily accessible drugs, the DHDCS was fabricated with the dexamethasone layer on the surface of the scaffold and then top-coated with the heparin layer loaded with the zein microsphere. In the DHDCS zein microsphere plays a role as the coated layer on the bare metal stent keeping its surface smooth and integrated and the carrier of drugs, which can reduce the vascular wall injury and blood flow resistance where the stents implanted. Dexamethasone plays its effect of anti-inflammatory, anti-immune, anti-adhesion, and heparin anti-coagulation, anti-thrombosis, respectively. Consequently, the target vascular endothelium will become intact and lumen unobstructed. Figure 1 shows that DHZM release is a course of surface release, and after a short time of bursting release it entered a relative longer time of stable release phase, which ensuring that the eluting drugs have an effective and sustainable concentration at the site of the stents in the culprit vessels. At the same time the additional effects of dexamethasone and heparin to the whole body can be reduced to the least.

After stents implantation, all the mini-pigs was administered with prophylactic antibiotics and routine

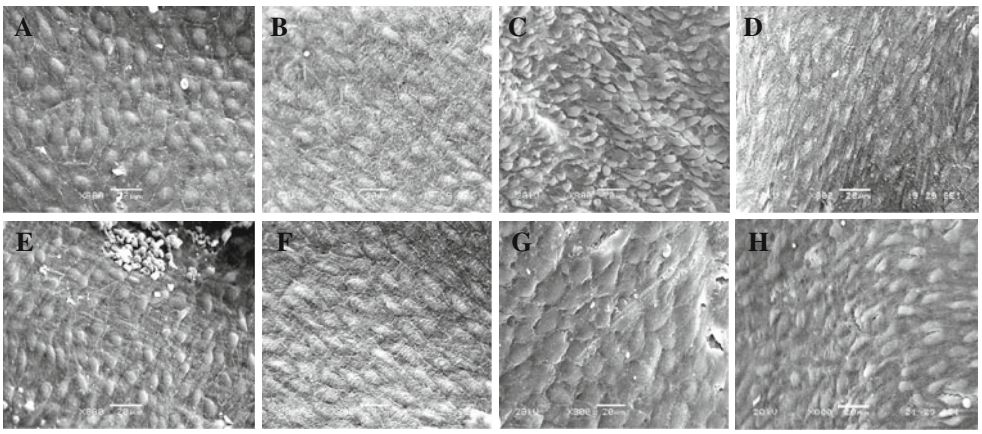

Fig. 6 SEM results 30 and 90 days after stent implantation. **a** 30 days BMS ×880; **b** 30 days PCS ×880; **c** 30 days HMCS ×880; **d** 30 days DHDCS ×880; **e** 90 days BMS ×880; **f** 90 days PCS ×880; **g** 90 days HMCS ×880; **h** 90 days DHDCS ×880

anti-coagulative medicines for 3 days, without clinical complications, such as systemic infection, bleeding, lower extremity ischemia, stroke symptoms, and accidental death, which demonstrated that the stents implantation in animals is safe. Through porcine experiments, we studied the target vessel appearance by imaging and pathology 30 and 90 days after stent implantation and found that the size of the arterial lumen loss and the intimal hyperplasia thickness within the stents of the DHDCS group were significantly lower than those in the BMS group (Tables 1, 2, 3, 4; Figs. 4, 5). Besides, there was no thrombosis within the stents of the former group and the endothelia cells were arranged well, while in the latter had thrombosis and the endothelia cells were arranged so well (Fig. 6; Tables 5, 6). This study shows that DHDCS is capable of inhibiting the proliferation of intima and reducing the lumen area in the target artery within stents, thus effectively and safely decreasing the incidence of regional thrombosis and restenosis in the short term.

The limitation of this study lies in the fact that it is a shorter-term research and not a longer-term observation, and also, the stents influences to the animals and human body may present some discrimination between them; thus, further experiments of the novel stents must be conducted to evaluate their sound functioning. In the future, we will carry out more research on this new type of coated stents with a view of providing safe and effective stents for interventional therapy on CAD.

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Conflict of interest No conflict of interest exists.

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