Original Article

A Novel High Nitrogen Nickel-free Coronary Stents System: Evaluation in a Porcine Model

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Abstract

Objective To study the safety of the novel high nitrogen nickel-free austenitic stainless steel bare metal stents (BMS) in a recognized porcine coronary model and to select a better grid structure of it.

Methods Three types of stents were randomly implanted in different coronary arteries of the same pig: 316L stainless steel BMS (316L-BMS) (n=12), novel high nitrogen nickel-free stents Grid A (NF-A-BMS) (n=12) and novel high nitrogen nickel-free stents Grid B (NF-B-BMS) (n=12). In total, eighteen animals underwent successful random placement of 36 oversized stents in the coronary arteries. Coronary angiography was performed after 36 d of stents implantation. Nine animals were respectively sacrificed after 14 d and 36 d for histomorphologic analysis.

Results Quantitative coronary angiography (QCA) showed similar luminal loss (LL) in the three groups: (0.21±0.17) mm for 316L-BMS, (0.16±0.12) mm for NF-A-BMS, (0.24±0.15) mm for NF-B-BMS (P>0.05). Histomorphomeric analysis after 15 d and 36 d revealed that there was also no significant difference among the three groups in neointimal area (NA) with similar injury scores respectively. High magnification histomorphologic examination showed similar inflammation scores in the three groups, but NF-A-BMS group had poorer endothelialization scores compared with NF-B-BMS group, 2.00±0.63 *vs.* 2.83±0.41 (P=0.015) at 15 d, which also could be proved by the scanning electron microscope. However, the difference could not been observed at 36 d.

Conclusion The novel NF-BMS showed similar safety as 316L-BMS during the short-term study. NF-B-BMS had better endothelialization than NF-A-BMS and this may owe to the specific strut units.

Key words: Austenitic steel; Bare metal stent; Endothelialization; Restenosis; Stent thrombosis

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INTRODUCTION

With the appearance of drug-eluting stents (DES), the restenosis rate of percutaneous coronary intervention (PCI) has dropped to below 10%[1] compared with 20%-30% of bare metal stents (BMS) generation[2]. However, with the increasing number of patients receiving intervention treatment, safety issue raised for long-term use of this technology. Stent restenosis (SR) and stent thrombosis (ST) are two of the most fatal problems unresolved. When the drug on the stent has been released completely, especially with the biodegradable coating on the new generation of DES also degraded gradually, there is only the metal stent platform retained in the blood vessel wall.

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Obviously, it is a major factor affecting the late event of blood vessels, which can’t be ignored. As a main component of traditional stainless steel, nickel is a potential allergenic factor for the blood vessel wall, which may cause cytotoxicity and inflammation[3-4] and eventually result in SR or ST. Improving the components of the stent platform may therefore reduce the late adverse events. Cobalt alloy stents[5-6], and platinum alloy stents[7] have been developed in western countries and they showed excellent outcomes in the late events. Fortunately, novel metal material, high nitrogen nickel-free austenitic stainless steel (Institution of Metal Research, Chinese Academy of Sciences) with independent intellectual property rights, has been used on the platform of BMS. This study aims to analyze the safety of the novel high nitrogen nickel-free austenitic stainless steel BMS in a recognized porcine coronary model and also to select a better grid structure of it.

METHODS

Materials

Stents All three types of stents were balloon-expandable BMS with the size of 3.0 mmx15 mm: commercial available 316L stainless steel BMS (316L-BMS) (SINO Medical Sciences and Technology INC, China), novel high nitrogen nickel-free stents Grid A (NF-A-BMS) and novel high nitrogen nickel-free stents Grid B (NF-B-BMS).

Animal The animal study was approved by the Institutional Animal Care and Use Committee of Peking University First Hospital and conformed to the Guide for the Care and Use of Laboratory Animals in Beijing. Eighteen Chinese Miniature Swine (30 kg to 55 kg and six months to eight months) purchased from China Agricultural University, China. The gender of the animals was ignored and they were fed a standard laboratory chow diet without lipid.

Procedure

A total of twelve 316L-BMS, thirteen NF-A-BMS and thirteen NF-B-BMS were randomly assigned and placed in the left anterior descending (LAD), circumflex (LCX), or right coronary artery (RCA) (two types of stents per two arteries of one pig). In order to decrease the incidence of acute thrombosis, premedication with 300 mg aspirin (Bayer, Germany) and 75 mg clopidogrel (Xin Li Tai Pharmaceutical Co. Ltd, China) was administered before stent implantation for one day. Aspirin (100 mg/d) and clopidogrel (75 mg/d) were then given until sacrificed. Subcutaneous injection of 0.3 mg/kg ketamine (Double-Crane Pharmaceutical Company, China) was administered to all pigs prior operation for basic anesthesia, and then intravenous injection of 0.05 g/L sodium pentobarbital (Peking University First Hospital Animal Center, China) was given to keep the status of anesthesia and to extend the experimental time. Onwards, the groin of the pig was fixed and sterilized, and the femoral artery was punctured with Seldinger method. Then, 6F sheath was inserted with intravenous injection of heparin (100 U/kg). With 0.035 inch guide wire, 6F JL 3.5 and JR 3.5 guiding catheters were used for coronary angiography. According to the target vessel diameter, the stent size was selected (stent expanded diameter: vascular diameter, 1.1:1-1.2:1). Guide wire (0.014 inch) was sent to the distal coronary artery. Along the guide wire, the stent was pushed to the target vessel site and released by using 8 atm-12 atm single expansion for 5 s-10 s. The angiography was repeated to make sure that no serious dissection and thrombosis occurred. Then the sheath tube was withdrawn, and the puncture site was compressed to stop bleeding for 20 min-30 min, with intramuscular injection of 8×10^4 U/kg penicillin for preventing infection.

Quantitative Coronary Analysis (QCA) Evaluation

At 36 d of implantation, operation for repeat coronary angiography on 9 animals was performed. QCA was performed by CAAS 5.9 QCA Software (PIE MEDICAL IMAGINE, Netherlands). Coronary artery measurements including baseline vessel diameter, minimal lumen diameter immediately after implantation (I-MLD), reference vessel diameter (RVD), MLD of repeat angiography (R-MLD) and lumen loss (LL) were recorded.

Histopathological Evaluation

At 15 d and 36 d of implantation, 9 animals were sacrificed one after another. The heart was removed and perfused with heparin saline for 5 min at the pressure of 100 mmHg (1 mmHg=0.0133 kPa). The stent vessel segment was separated rapidly and fixed with 10% formaldehyde solution. The segments were embedded in methy methacrylate plastic for histological examination. The samples with stent were embedded by methyl methacrylate (Prepared by Peking University School of Stomatology), and sliced (50 μm) with a hard tissue microtome (LEICA, Germany). All sections were stained with hematoxylin-eosin (HE), and specimens were prepared for light microscopy examination. Optical microscope (LEICA, Germany) and Qwin PLUS V3.2.1 image analysis software (LEICA, Germany) were used to measure lumen area (LA), internal elastic lamina area (IELA), external elastic lamina area (EELA), neointimal area (NA) (internal elastic lamina area - lumen area), and percent area stenosis (PAS) [(internal elastic lamina area-lumen area) / lumen area × 100%]. Vascular injury score was determined by the method of Schwartz et al. (0=intact internal elastic lamina; 1=internal elastic lamina fracture; 2=internal elastic lamina and tunica media fracture; 3=external elastic plate fracture)[8]. Inflammation score was graded according to the inflammatory cells (0=no inflammatory cells; 1=scattered inflammatory cells; 2=inflammatory cells encompassing 50% of a strut in at least 25%-50% of the circumference of the artery; 3=inflammatory cells surrounding a strut in at least 25%-50% of the circumference of the artery)[9]. Stent endothelialization score was defined as the extent of the circumference of the arterial lumen covered by the endothelial cells and graded from 1 to 3 (1=0-25%, 2=25%-75%, 3=75%-100%)[10].

Statistical Analysis

Values in normal distribution were expressed as mean±standard deviation (SD). Group imaging, histological and integral data were analyzed with one-way ANOVA test. *n* representing the number of stents of different types. The value of $P<0.05$ was considered statistically significant. All statistical analysis were performed by commercially available SPSS 14.0 system software (IBM, USA).

RESULTS

A total of 36 stents of three types were successfully implanted in coronary arteries of eighteen pigs (Table 1). All of the animals (100%) survived in the intended study interval with on angiographic stent thrombosis or other clinical complications. The body weight and abnormal body temperature of the animals remained well throughout the study.

Quantitative image analysis of coronary angiography was showed in Figure 1 and Table 2. In the case of similar RVD and I-MLD among the three groups at baseline ($P>0.05$), there were no significant differences in R-MLD and LL statistically among the three groups ($P>0.05$).

Results from pathology analysis were showed in Table 3. NA-one of the most crucial parameters on the stent efficiency evaluation was measured according to the histopathological photomicrographs (20x) of the stented coronary artery (Figure 2A-F). As shown in Figure 2G, the NA was not significantly different statistically among the three stent groups at 15 d and 36 d of implantation ($P>0.05$). Inflammation score and endothelialization score were

Table 1. Status of Stent Implantation

Type	Number	LAD	LCX	RCA
316L-BMS	12	6	6	0
NF-A-BMS	12	6	5	1
NF-B-BMS	12	6	5	1
Total	36	18	16	2

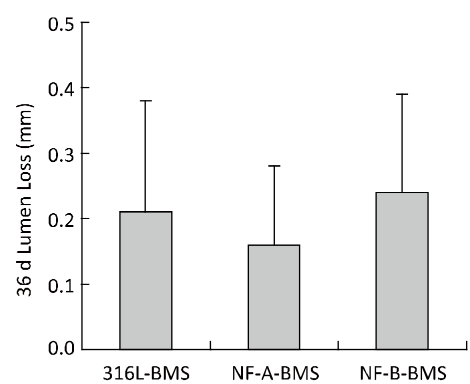

Figure 1. 36 d Lumen Loss in Different Groups.

Table 2. QCA Results at 36 d of Stent Implantation

QCA Results	316L-BMS (*n*=6)	NF-A-BMS (*n*=6)	NF-B-BMS (*n*=6)	*F*	*P*-value
RVD (mm)	2.59±0.27	2.56±0.35	2.42±0.07	0.720	0.503
I-MLD (mm)	2.58±0.45	2.70±0.29	2.65±0.16	0.189	0.830
R-MLD (mm)	2.37±0.48	2.54±0.23	2.41±0.14	0.467	0.636
LL (mm)	0.21±0.17	0.16±0.12	0.24±0.15	0.341	0.717

Note. RVD, reference vessel diameter; I-MLD, minimal lumen diameter immediately after implantation; R-MLD, minimal lumen diameter of repeat angiography; LL, lumen loss.

parameters used for the safety evaluation and were measured according to the histopathological photomicrographs (200x) of the stented coronary artery (Figure 3A-F). As shown in Figure 3H, NF-A-BMS group had poorer endothelialization scores compared with NF-B-BMS group (*P*=0.015) at 15 d by Post Hoc Tests (LSD), which also could be proved under the scanning electron microscope (Figure 4), but the difference was not observed at 36 d. There was no clear sign of thrombosis in all groups at 15 d and 36 d of stents implantation.

DISCUSSION

To our knowledge, the present study is the first to describe the novel high nitrogen nickel-free coronary stents system with independent intellectual property rights in the porcine coronary model. It is also a preliminary result of evaluation of biocompatibility and the grid structure initially. It was shown in this study that the novel coronary stent system demonstrates excellent safety as the commercially available 316L stainless steel stent system in QCA and pathology analysis.

As it is known, stent restenosis and stent thrombosis are two intricate and vital clinical phenomena after PCI in DES era. Three indispensable components of DES (metal stent platform, polymer and antiproliferative agents) have been improved stupendously. Considering that metal stent platform is the only part of stent being retained permanently in the vessel, it may be the chief culprit leading to the late adverse events.

316L stainless steel is the earliest and most widely used metallic material in coronary stent, containing about 14% nickel in mass percent[11]. However, allergic reactions and inflammation to nickel ions released from coronary stents inevitably may be one of the triggering mechanisms for SR[12] and ST. Nitrogen is a strong austenite formation element in the novel metal materials, which not only replaces nickel for austenitic structure stability but also significantly improves steel properties and corrosion resistance[13]. Furthermore, the nickel-free stainless steel shows better anti-platelet adhesion than that of 316L stainless steel *in vitro*[14], which could be a possible mechanism for the novel stent with less ST. In this study, no ST occurred in all three groups of animals and the inflammation scores among those groups were not different either, indicating the similar safety between the 316L BMS and the new type of BMS in the study duration.

The overexpansion porcine coronary model has been proved and widely applied for safety evaluation of coronary stent system. Although the vessel is lack of atherosclerosis, the model is still one of the best models that can imitate the SR mechanism well. In this study, LL, NA, and PAS were similar among three types in the two study endpoints in the terms of a similar expansion ratio and Injury scores. Expansion ratios and injury scores in different groups represented that the model was established successfully and different groups had similar baselines. The similar data implied that the new type of BMS had the equivalent effectiveness as the 316L BMS during the study.

Table 3. Pathology Analysis at 15 d and 36 d of Stent Implantation

Pathology Analysis	15 d					36 d				
	316L-BMS (*n*=6)	NF-A-BMS (*n*=6)	NF-B-BMS (*n*=6)	*F*	*P* Value	316L-BMS (*n*=6)	NF-A-BMS (*n*=6)	NF-B-BMS (*n*=6)	*F*	*P* Value
LA (mm²)	5.28±1.09	4.39±1.30	5.59±0.74	2.060	0.162	3.86±1.45	4.15±1.77	3.91±2.37	0.040	0.961
IELA (mm²)	6.17±0.76	4.86±1.39	6.26±0.39	4.192	0.036	5.68±1.53	5.22±1.92	5.34±2.34	0.909	0.914
NA (mm²)	0.90±0.87	0.47±0.15	0.67±0.71	0.635	0.544	1.82±1.40	1.06±0.69	1.43±1.28	0.625	0.549
PAS (%)	14.56±13.20	9.97±3.05	10.59±9.72	0.364	0.701	30.50±19.88	21.49±10.11	27.73±23.57	0.364	0.701
Inflammation scores	1.00±1.27	0.67±0.52	0.67±0.82	0.263	0.772	1.67±1.03	1.67±0.82	1.67±1.21	0.000	1.000
Injury scores	0.50±0.55	0.17±0.41	0.33±0.52	0.682	0.521	0.33±0.82	0.33±0.82	0.50±0.84	0.082	0.922
Endothelialization scores	2.33±0.52	2.00±0.63	2.83±0.41	3.800	0.046	2.67±0.52	2.33±0.52	2.67±0.82	0.556	0.585

Note. LA, lumen area; IELA, internal elastic lamina area; EELA, external elastic lamina area; NA, neointimal area; PAS, percent area stenosis.

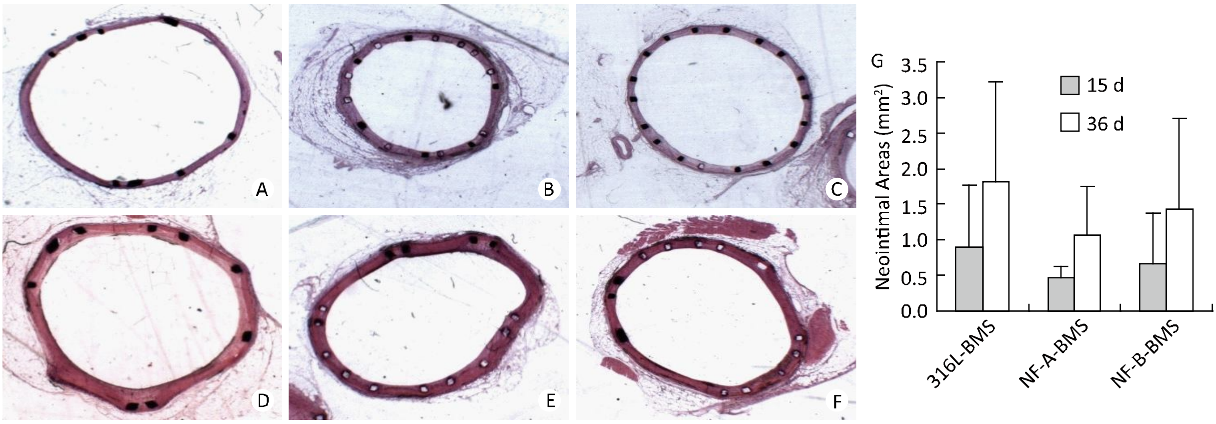

Figure 2. 15 d and 36 d histopathological photomicrographs (20x) of stented coronary artery and neointimal areas in different groups. (A), 316L-BMS group at 15 d (20X); (B), NF-A-BMS group at 15 d (20X); (C), NF-B-BMS group at 15 d (20X); (D), 316L-BMS group at 36 d (20X); (E), NF-A-BMS group at 36 d (20X); (F), NF-B-BMS group at 36 d (20X); (G), Efficiency of the stents is determined by neointimal areas. Each column represents the mean±SD in different groups and end points.

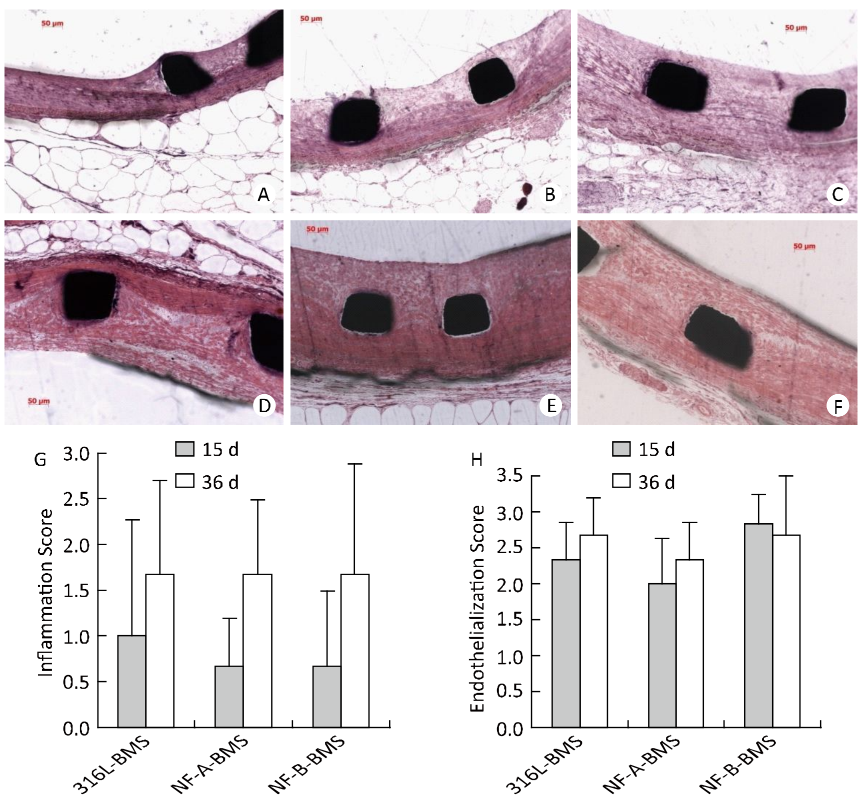

Figure 3. Histopathological photomicrographs (200x) of stented coronary artery, inflammation scores and endothelialization scores of 15 d and 36 d in different groups. (A), 316L-BMS group at 15 d (200X); (B), NF-A-BMS group at 15 d (200X); (C), NF-B-BMS group at 15 d (200X); (D), 316L-BMS group at 36 d (200X); (E), NF-A-BMS group at 36 d (200X); (F), NF-B-BMS group at 36 d (200X); (G-H), Safety of the stents is determined by inflammation score and endothelialization score. Each column represents the mean±SD in different groups and end points.

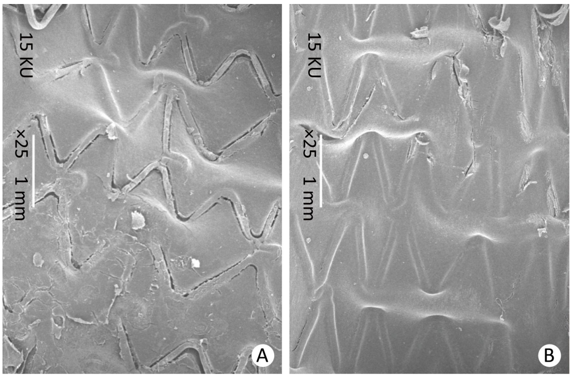

Figure 4. 15 d Scanning Electron Microscope. (A), NF-A-BMS group at 15 d; (B), NF-B-BMS group at 15 d.

Endothelialization is the key point of ST mechanism[15]. Although vascular repairing in pigs occurs more rapidly than humans, the endothelialization score and the results from scanning electron microscope are also two available indexes in the model. In our present study, NF-A-BMS with two different strut units showed lower endothelialization score and poorer endothelialization under scanning electron microscope compared with NF-B-BMS consisting of the single strut unit within 15 d of implantation, but this difference did not extend to 36 d of implantation. There were no significant differences between the two types of NF-BMS and 316L-BMS in terms of the two endpoints. It suggests that the single strut unit design of NF-B-BMS may be a better choice for endothelialization and ST prevention. However, the conclusion can't be made until longer endpoint observation and further study is therefore needed to be carried out. Owing to the SR, ST and neoatherosclerosis[16] may occur much later in patients with stent implantation, the duration of this study is too short to evaluate long term safety, and it is one of the major limitations of our present study. Further preclinical study including long term endpoints and clinical study should be conducted in order to evaluate the safety and effectiveness of the novel NF-BMS.

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