Nickel-free stainless steel avoids neointima formation following coronary stent implantation

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Abstract
SUS316L stainless steel and cobalt–chromium and platinum–chromium alloys are widely used platforms for coronary stents. These alloys also contain nickel (Ni), which reportedly induces allergic reactions in some subjects and is known to have various cellular effects. The effects of Ni on neointima formation after stent implantation remain unknown, however. We developed coronary stents made of Ni-free high-nitrogen austenitic stainless steel prepared using a N2-gas pressurized electroslag remelting (P-ESR) process. Neointima formation and inflammatory responses following stent implantation in porcine coronary arteries were then compared between the Ni-free and SUS316L stainless steel stents. We found significantly less neointima formation and inflammation in arteries implanted with Ni-free stents, as compared to SUS316L stents. Notably, Ni2+ was eluted into the medium from SUS316L but not from Ni-free stainless steel. Mechanistically, Ni2+ increased levels of hypoxia inducible factor protein-1α (HIF-1α) and its target genes in cultured smooth muscle cells. HIF-1α and their target gene levels were also increased in the vascular wall at SUS316L stent sites but not at Ni-free stent sites. The Ni-free stainless steel coronary stent reduces neointima formation, in part by avoiding activation of inflammatory processes via the Ni-HIF pathway. The Ni-free-stainless steel stent is a promising new coronary stent platform.

Keywords: stent, nickel free, stainless steel, neointima, coronary

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1. Introduction
In-stent restenosis following coronary stent implantation remains an important problem following percutaneous coronary intervention (PCI) [1, 2]. Despite intensive efforts to improve the design and metal composition of stents, the rates of in-stent restenosis induced by bare metal stents range from 22 to 49% [2–5]. Drug-eluting stent technologies have markedly reduced in-stent restenosis rates to 0–31% [5–10], but drug eluting stents cause a new problem, namely late
thrombosis [11], though a recent meta-analysis reported that a cobalt–chromium (Co–Cr) everolimus-eluting stent had a lower rate of stent thrombosis than bare metal stents during the 2 years after implantation [12]. To suppress stent thrombosis, the current American Heart Association/American College of Cardiology guidelines recommend dual anti-platelet therapy (DAPT), entailing the use of clopidogrel plus aspirin, for at least 12 months after drug-eluting stent implantation and for a minimum of 1 month after implantation of a bare metal stent [13, 14]. However, DAPT increases the risk of major bleeding [15, 16] and poses a challenge if non-cardiac surgery is necessary: while cessation of anti-platelet therapy increases the risk for stent thrombosis, its continuation increases the risk of surgical bleeding [17]. Furthermore, a recent meta-analysis showed that while implantation of drug-eluting stents after ST-segment elevation myocardial infarction reduced the need for target-vessel revascularization, as compared to bare metal stents, the drug-eluting stents were associated with increased risks of very late stent thrombosis and reinfarction. Mechanisms proposed to cause this late stent thrombosis include delayed endothelialization and vascular healing, late acquired stent malapposition and hypersensitivity reactions [18, 19]. Therefore, it would be highly desirable to fabricate coronary stents that have better profiles with respect to the pathological processes involved in in-stent restenosis and vascular healing.

At present, SUS316L, Co–Cr and platinum–chromium (Pr–Cr) are the most widely used coronary stent platforms [9, 10]. Ni is added to these metal alloys to increase their flexibility [10, 20]. However, Ni is known to induce biological responses in animals. For instance, Ni allergy is a major cause of contact hypersensitivity and affects millions of people worldwide [21, 22]. Ni allergy is also reportedly associated with higher risks of restenosis after PCI [23, 24], though conflicting data have been reported [25]. Ni\(^{2+}\) solubilized in sweat and other body fluids serves as a sensitizing allergen in Ni-induced contact hypersensitivity, and Ni\(^{2+}\) has been shown to directly activate inflammatory processes. For instance, Ni\(^{2+}\) triggers rapid expression of surface adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) on endothelial cells, as well as chemokines such as MCP-1 [26, 27]. Mechanistically, it has been proposed that the hypersensitivity depends on activation of Toll-like receptor 4 and hypoxia inducible factor (HIF) signaling, and downregulation of the anti-apoptotic factor cellular Fas associated death domain-like interleukin 1 beta-converting enzyme-like inhibitory protein (cFLIP) [21].

Given the extensive medical application of stainless steel, its susceptibility to corrosion (metal ion release) and fatigue are important factors. Generally, elemental Ni is added to stainless steel to mitigate its negative characteristics. However, Ni\(^{2+}\) released from the amorphous surface layer of stainless steel reportedly causes allergic reactions and inflammation. For that reason, the idea of substituting N\(_2\) for the Ni has been considered. However, homogenous injection of N\(_2\) into stainless steel is not feasible using classical methods such as N\(_2\) adsorption [28]. We therefore developed Ni-free austenitic stainless steel with a high N\(_2\) content fabricated using an electroslag remelting (P-ESR) method under a pressurized N\(_2\) gas atmosphere [29]. This P-ESR method enables us to inject N\(_2\) homogenously into stainless steel, yielding Ni-free stainless steel that is highly resistant to corrosion [29, 30] and fatigue [31].

In this study, we produced a Ni-free stainless steel coronary artery stent and evaluated its utility in a porcine coronary model. This is one of the first reports examining the feasibility of using Ni-free stainless steel for treating heart disease.

The material of coronary stents must be strong to withstand the forces produced by the beating heart and also biocompatible, because local inflammation of the coronary artery wall can lead to in-stent restenosis [32]. Indeed, Ni allergy is thought to be one cause of in-stent restenosis [23]. We therefore speculate that a Ni-free stainless steel stent fabricated using our new method would have advantages over conventional Ni-containing stents.

In this study, we found that arteries implanted with the Ni-free stent showed significantly less neointima formation than arteries implanted with other bare metal stents. In addition, HIF activation by Ni\(^{2+}\) eluted from stent struts was a key contributor to neointima formation in arteries implanted with SUS316L stents, but was avoided in arteries implanted with Ni-free stents.

2. Materials and methods

2.1. Animals

All experimental studies were approved by the University of Tokyo Ethics Committee for Animal Experiments and strictly adhered to the guidelines for animal experiments of the University of Tokyo.

2.2. Ni-free high-nitrogen stainless steel (HNS) stent

Ni-free HNS ingot was first prepared using a N\(_2\)-gas P-ESR process [29–31]. Melting was conducted under 4 MPa of N\(_2\) gas pressure. FeCrN powder was used as the nitrogen source. Because nitrogen is an austenite-forming element, Ni-free austenitic stainless steel was successfully fabricated by adding soluble nitrogen up to more than 1 wt%. SUS316L stainless steel stents served as controls. The Ni contents of the Ni-free stainless steel and SUS316L were less than 0.005 and 12 wt%, respectively. To produce a seamless pipe 1.4 mm in diameter and 0.1 mm in thickness, a seamless pipe was machined first, followed by drawing with frequent annealing treatment. Stents were then formed through laser machining followed by surface smoothing using an electropolishing method.

2.3. Stent implantation into porcine coronary artery

Ten pigs were implanted with stents via femoral access according to the standard procedure, using balloon pressure set to expand the arterial diameter by a ratio of 1.2 : 1 [33]. The extent of the dilation of the stenting region was immediately assessed using intravascular ultrasound Eagles.
Eye Gold Catheter, (Volcano Corporation, California, USA), and expansion ratios were determined to be 1.20 ± 0.06 (SD). Each pig received one or two 3 × 12 mm stents in each of two out of three main coronary arteries (left anterior descending artery and left circumflex artery). A total of 20 coronary arteries received the control SUS316L stainless stents and 17 received Ni-free stainless stents. Coronary artery angiography and intravascular ultrasound imaging were performed 28 days after the stent implantation.

After coronary angiography, hearts were explanted from the thoracic cavity and flushed using pressure perfusion with saline. The arterial portions that received stents were removed and fixed in 10% buffered formalin, embedded in glycol methacrylate (Sakura Finetek Japan, Tokyo, Japan), and duplicate samples were cut into 5-μm-thick sections using published procedures [33]. The sections were cut at three positions: a proximal section, 2 mm distal from the proximal end of stent; a middle section, 6 mm distal from the proximal end; and a distal section, 2 mm proximal from the distal end. Of these, three from each group were subsequently examined histologically. Sections were stained with hematoxylin/eosin or Masson’s trichrome, or processed for immunohistochemistry.

2.4. Cell culture

Rat aortic smooth muscle cells (SMCs) were isolated as previously described [34] and cultured in Dulbecco’s modified Eagle medium/Nutrient F-12 (Ham’s, DMEM/F12, Life Technologies Corporation, California, USA) supplemented with 10% fetal bovine serum (FBS, Thermo Scientific; Hyclone Laboratory, Massachusetts, USA), except where otherwise indicated. Cells from two separate isolates were used between passages 8 and 12.

2.5. Quantitative reverse transcriptase-PCR

Total RNA was purified from cultured cells using RNeasy (Qiagen, Hilden, Germany) and from in vivo samples using an Isogen PB kit (Nippon Gene, Tokyo, Japan) [35]. For quantitation of the transcripts, real-time polymerase chain reaction (PCR) was carried out using a LightCycler (Roche, Basel, Switzerland) and a QuantiTect SYBR green PCR kit (Qiagen, Hilden, Germany). The expression level of each gene was normalized to that of 18s rRNA, which served as an endogenous internal control. The sequences of the primers was previously described [36].

2.6. Immunohistochemistry

For immunohistochemistry, a rabbit polyclonal antibody against HIF-1α (Novus Biologicals, Colorado, USA) served as the primary antibody, and staining was accomplished using catalyzed signal amplification (Dako Cytomation Japan, Tokyo, Japan). The HIF1α signal intensity was enhanced using a heat-induced antigen retrieval method with citrate buffer (pH 6.0). To detect Smo-actin, we used a fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal antibody: clone 1A4 (Sigma Aldrich, Missouri, USA).

2.7. Measurement of Ni²⁺ ion release

Sample solutions were prepared by immersing five mirror-polished metal discs (10 mm in diameter, 1 mm in thickness) in 2.5 ml of saline for 7 days at 37 °C. Release of Ni²⁺ from metals was analyzed using a colorimetric method with dimethylglyoxime (DMG), as previously described [36] with minor modifications. To determine the Ni²⁺ concentration, 500 μl of saturated citric acid solution was added to 500 μl of sample solution, after which 500 μl of 0.03 mol l⁻¹ DMG solution was added. After incubation for 10 min, the absorbance at 540 nm was measured spectrophotometrically using a microplate reader.

2.8. Statistical analysis

Mean values were compared between two groups using Student’s t-test for unpaired values. To compare more than two groups, analysis of variance (ANOVA) was carried out, followed by Tukey–Kramer’s post hoc test for multiple comparisons to identify differences among specific groups. Values of P < 0.05 were considered significant.

3. Results

3.1. Visibility of Ni-free stainless stents on a clinical x-ray setup

Ni has little radiolucency, which enhances the visibility of SUS316L stainless stents on coronary angiography. Exclusion of Ni from stainless steel could therefore reduce the visibility of the stents. To test this possibility, we assessed the visibility of Ni-free stents within explanted porcine coronary arteries in a cardiac angiography system. Although the visibility of the Ni-free stents was slightly inferior to that of SUS316L stents, Ni-free stent struts were clearly visible (figure 1(a)). Moreover, the Ni-free stents were also clearly visible during their implantation in porcine coronary arteries. The maneuverability of the Ni-free stents did not differ from that of SUS316L stents of the same design.

3.2. Neointima formation after coronary stent implantation was reduced with Ni-free stainless stents

Seventeen Ni-free and 20 SUS316L stents were deployed into the left anterior descending and left circumflex arteries.
Figure 1. Neointima formation following coronary stenting is reduced with Ni-free stainless steel stents. (a) Representative x-ray photographs of SUS316L stainless and Ni-free stainless stents within isolated porcine coronary arteries 28 days after implantation. (b)–(g) Coronary stents made of SUS316L or Ni-free stainless steel were implanted in the left anterior descending and circumflex arteries of pigs. The stented regions were then analyzed using radiography and intravascular ultrasound imaging, after which the stented arteries were harvested. In (b), representative angiographic views of the porcine left circumflex arteries 28 days after stent implantation are shown. All angulation was approximately right anterior oblique 50. Arrowheads indicate the ends of the stent-implanted sites in the left circumflex arteries. (c) Per cent in-stent restenosis evaluated using angiography; n = 20 and 17 in the SUS316L and Ni-free stent groups, respectively. *P < 0.05. (d) Representative sections of stented arteries stained with hematoxylin/eosin. L: lumen, N: neointima, M: media, bars: 500 µm. For morphometric analysis, luminal areas were defined as the blank areas within arteries. The smooth muscle layer, including the neointima and media, was defined as the inner side (high cell density) area of the vascular wall, while the adventitia was defined as the outer side (low cell density) area. The borders of the neointima (inner side of the smooth muscle layer) and media (outer side of the smooth muscle layer) were defined as the boundaries between two different cell alignments. In addition, in-stent neointima formation was evaluated at three positions within each stent (proximal, middle or distal), and the data from the position that showed the largest intimal area/medial area ratio were selected as representative for that stent. Results of histological analyses of the intimal area (e), intimal area/medial area ratio (f) and lumen area (g); n = 20 and 17 in the SUS316L and Ni-free stent groups, respectively. *P < 0.05.
coronary arteries. Ballooning pressures required to gain the aimed pre-post-artery diameter ratio (1 : 1.2) did not significantly differ between the two groups (data not shown), which indicates the compliance of the Ni-free stents was similar to that of the SUS316L stents. Angiography showed that all arteries were patent 28 days after stent implantation (figure 1(b)). Ni-free stents were associated with a significantly smaller per cent in-stent stenosis (figure 1(c)), smaller neointimal area (figures 1(d) and (e)), smaller intimal area/medial area ratio (figure 1(f)), and a significantly larger luminal area than SUS316L stents (figure 1(g)). We also performed morphometric analyses at three positions within the stents (proximal, middle and distal), but we found no specific tendency for positional effects on in-stent neointima formation (supplementary S1, available from stacks.iop.org/STAM/13/064218/mmedia). All stent struts were completely covered by the endothelium and vascular SMCs in both groups (figure 1(d)). Thus Ni-free stents avoided neointima formation with no evidence of unfavorable healing delay (e.g. uncovered stent struts).
Figure 3. Ni^{2+} activates HIF-1α in vascular SMCs. (a) Dissolution of Ni^{2+} from SUS316L and Ni-free stainless into saline solution, \(^* P < 0.05\). (b) Western blots of HIF-1α in cultured vascular SMCs supplemented with Ni^{2+} for 6 h under normoxic conditions. Ni^{2+} stabilized HIF-1α in cultured vascular SMCs under normoxic conditions. (c) Hypoxia modestly induced HIF-1α target genes (Vegfa, Adm2 and Pdgfb) in cultured SMCs; \( n = 3 \) in each group, \(^* P < 0.05\) versus control, \(^{**} P < 0.05\) versus 0.5 mM Ni under the same O\(_2\) conditions. \(^# P < 0.05\) versus the same Ni concentration group in 20% O\(_2\).

3.3. Vascular inflammation after stent implantation was reduced with Ni-free stainless stent

A number of basophilic mononuclear cells were observed, mainly surrounding stent struts, in the SUS316L stent-implanted arterial walls (figures 2(a) and (b)). By contrast, Ni-free stent-implanted arteries showed markedly fewer infiltrating cells (figures 2(a) and (b)). The SUS316L stent sites also showed extensive proliferation of neointimal cells, and much less neointimal cell proliferation was seen following Ni-free stent implantation (figure 2(a)). Moreover, the deposition of extracellular matrix, including collagen (figure 2(c), blue signal indicates collagen deposition), was greatly reduced in the walls of arteries implanted with Ni-free stents, as compared to SUS316L stents.
3.4. Ni$^{2+}$ is released from SUS316L but not from Ni-free stainless steel

Given the clear histological differences in the responses to Ni-free and SUS316L stents, we hypothesized that dissolved Ni$^{2+}$ ion might enhance neointima formation. To test this idea, we first determined whether Ni might be eluted from SUS316L stainless steel discs in saline solution. As expected, Ni$^{2+}$ was indeed eluted from the SUS316L stainless, but was undetectable in the saline containing Ni-free stainless discs (figure 3(a)).

3.5. Ni$^{2+}$ increases HIF-1$\alpha$ levels in arterial SMCs

SMCs are the major cell type forming neointimal lesions. For that reason, we next analyzed the effects of Ni$^{2+}$ on SMCs. As described previously in rat pleural mesothelial cells [37] and human lung epithelial cells [38], Ni$^{2+}$ increased the level of HIF-1$\alpha$ (HIF1A) protein, which is important for SMC proliferation and vascular remodeling [39] under the normoxic conditions (figure 3(b)). In addition, expression of downstream target genes of HIF-1$\alpha$, including Vegfa, Adm1, Adm2 and Pdgfb [40, 41], was markedly upregulated by Ni$^{2+}$ under normoxic conditions. As expected, hypoxia modestly increased levels of Vegfa, Adm2 and Pdgfb; however, hypoxia did not further increase the target gene expression induced by Ni$^{2+}$ (figure 3(c)). These results suggest that Ni$^{2+}$ eluted from SUS316L stainless may promote vascular remodeling by activating HIF-1 signaling in SMCs. A schematic summary of this study is shown in figure 4.

3.6. HIF-1$\alpha$ activation was avoided with Ni-free stents

We next investigated whether HIF-1$\alpha$ signaling might be activated by SUS316L stent implantation in vivo. We detected expression of HIF-1$\alpha$ protein in SMCs within the neointima induced by SUS316L stent placement, whereas HIF-1$\alpha$ was not detected in the walls of arteries receiving Ni-free stents (figure 5(a)). Consistent with that finding, levels of mRNA expression of the HIF-1$\alpha$ target genes Vegfa and Pdgfb and the inflammatory cytokine Tnf were markedly increased by implantation of SUS316 stents, but not Ni-free stents (figure 5(b)). Among these factors, Tnf was induced not only in vascular walls of SUS316L stainless stent implantation.
group, but also those of Ni-free stainless stent implantation group, compared to unimplanted vascular wall (figure 5(b)). During the stent implantation, we dilated the vessel wall to increase the vessel diameter up to 1.2 times. Mechanical stress might also activate many stress response pathways. Ni-free stent might block Ni\(^{2+}\)-mediated injury including HIF1-\(\alpha\) pathway activation. However, mechanical stretch mediated injuries are of course not avoided and might result in weak induction of Tnf in the Ni-free stainless stent implanted group (figure 5(b)). These results suggest that Ni\(^{2+}\) eluted from SUS316L stents activates HIF-1\(\alpha\) within arterial walls, which in turn activates inflammatory processes, at least in part. Ni-free stents avoid this activation of inflammatory processes by Ni\(^{2+}\).
4. Discussion

In this study, we showed that Ni-free bare metal stents significantly reduce neointima formation, as compared to SUS316L stents, following implantation in porcine coronary arteries. We also showed that Ni$^{2+}$ is released from SUS316L and that it activates the HIF-1α pathway in cultured SMCs in vitro. Within the walls of arteries receiving Ni-free stents, this inflammation, SMC proliferation and HIF-1α activation were avoided, suggesting that Ni$^{2+}$ augments neointima formation by stimulating inflammatory processes, in part by activating HIF-1α signaling.

HIF-1α is a key transcription factor involved in mediating the hypoxic response and regulates cellular survival, metabolism and proliferation [42]. It is also known to activate angiogenesis, and is reportedly involved in the development of such cardiovascular diseases as myocardial infarction, atherosclerosis, abdominal aortic aneurysm, pulmonary hypertension, systemic hypertension and peripheral artery disease [43–45]. In addition to hypoxia, HIF-1α can be activated by various stimuli, including cytokines such as IL-1β, IL-4 and TNF-α [46, 47]. In SMCs, HIF-1α is activated by PDGF, FGFR2, EGFR and angiogenesis II and mediates cell proliferation and neointima formation [39, 48, 49]. In this study, we found less SMC proliferation and HIF-1α activation in coronary arteries receiving Ni-free stents, suggesting the lower level of HIF-1α signaling likely contributes to the diminished neointima formation seen with Ni-free stents.

Although HIF-1α is produced constitutively, under normoxic conditions it is hydroxylated by Fe$^{2+}$-containing prolyl hydroxylases (PHDs), which utilize O$_2$ as a cosubstrate for catalysis, and then undergo ubiquitination and proteosomal degradation [50, 51]. Under hypoxic conditions, by contrast, PHD activity is suppressed, leading to nuclear accumulation of HIF-1α and transcriptional induction of its target genes [50, 51]. In addition to hypoxia, Ni and Co ions also mediate HIF-1α activation, as these metal ions can substitute for Fe$^{2+}$ at the Fe$^{2+}$-binding site of PHDs, thereby inhibiting these enzymes [52, 53]. Consistent with that effect, we found that the presence of Ni$^{2+}$ led to HIF-1α activation and induction of HIF-1α target genes in cultured SMCs. Moreover, SUS316L stainless released Ni$^{2+}$ into saline solution, whereas Ni-free stainless did not (figure 3(a)), and SUS316L stents activated HIF-1α signaling within the arterial wall more prominently than Ni-free stents (figure 5). Collectively, these results strongly suggest that Ni$^{2+}$ released from SUS316L stainless stents promotes neointima formation via activation of HIF-1α signaling (figure 4).

The novel accomplishments summarized in this report are: (i) production of a coronary stent from Ni-free stainless steel, (ii) documentation of the favorable effects of the Ni-free stainless steel stent, as compared to the SUS316L stainless steel stent in vivo and (iii) provided evidence of a Ni$^{2+}$-dependent HIF1-α pathway leading to activation in smooth muscle cells in vivo and vitro. These results also identify Ni-free stainless steel as an attractive coronary stent platform.

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References

[1] Dussaillant G R et al 1995 J. Am. Coll. Cardiol. 26 720
[2] Fischman D L et al 1994 New Engl. J. Med. 331 496
[3] Lau K W, Johan A, Sigwart U and Hung J S 2004 Singapore Med. J. 45 (5) 305
[4] Serruys P W et al 1999 New Engl. J. Med. 331 489
[5] Stone G W et al 2005 J. Am. Med. Assoc. 294 1215
[6] Morice M-C et al 2002 New Engl. J. Med. 346 1773
[7] De Luca G et al 2012 Arch. Intern. Med. 172 611
[8] Lemos P A et al 2004 Circulation 109 190
[9] Meredith I T et al 2012 J. Am. Coll. Cardiol. 59 1362
[10] Stone G W et al 2011 J. Am. Coll. Cardiol. 57 1700
[11] McFadden E P et al Lancet 364 1519
[12] Palmerini T et al 2012 Lancet 379 1393
[13] Farb A and Boam A B 2007 New Engl. J. Med. 356 984
[14] Levine G N et al 2011 J. Am. Coll. Cardiol. 58 e44
[15] Berger P B et al 2010 Circulation 121 2575
[16] Brott B C and Hillegass W B 2012 Ann. Intern. Med. 157 JC2
[17] Sharma A B et al 2012 Cardiac Interv. Today 26
[18] Nakazawa G 2011 J. Cardiol. 58 84
[19] Inoue K 2012 Thorosmsis 2012 219389
[20] Poncin P and Proft J 2003 Stent tubing: understanding the desired attributes Conf. on Materials and Processes for Medical Devices
[21] Schmidt M and Goebeler M 2011 J. Mol. Med. 89 961
[22] Costa M et al 2003 J. Environ. Monit. 5 222
[23] Koster R et al 2000 Lancet 356 1895
[24] Saito T et al 2009 Cardiovac. Revasc. Med. 10 17
[25] Romero-Braufel S et al 2012 Circ. Cardiovasc. Interv. 5 220
[26] Goebeler M et al 1993 J. Invest. Dermatol. 100 759
[27] Goebeler M et al 2001 Blood 97 46
[28] Kuroda D et al 2003 Mater. Trans. JIM 44 1363
[29] Sagara M et al 2002 Tetsu To Hagane-J. Iron Steel Inst. Japan 88 672
[30] Katada Y et al 2004 Mater. Manuf. Process. 19 19
[31] Maruyama N et al 2009 Mater. Trans. 50 2615
[32] Virmani R and Farb A 1999 Curr. Opin. Lipidol. 10 499
[33] Perkins I L et al 2009 J. Interv. Cardiol. 22 528
[34] Shimizu R T et al 1995 J. Biol. Chem. 270 7631
[35] Fujiu K et al 2005 Circ. Res. 97 1132
[36] Watanabe K et al 2011 Bunseki Kagaku 60 557
[37] Glista-Baker E E et al 2012 Am. J. Respir. Cell Mol. Biol. 47 552
[38] Pietruska J R et al 2011 Toxicol. Sci. 124 138
[39] Lambert C M et al 2010 Cardiovasc. Res. 88 196
[40] Semenza G L et al 2006 Novartis Found. Symp. 272 2 (discussion 8–14, 33–16)
[41] Knowles J et al 2004 Circ. Res. 95 162
[42] Semenza G L 2009 *Blood* **114** 2015
[43] Semenza G L 2001 *Trends Mol. Med.* **7** 345
[44] Bonnet S et al 2006 *Circulation* **113** 2630
[45] Tuder R M et al 2001 *J. Pathol.* **195** 367
[46] Hellwig-Bürgel T et al 1999 *Blood* **94** 1561
[47] Jiang H et al 2010 *Am. J. Physiol., Lung Cell. Mol. Physiol.* **298** L660
[48] Richard D E et al 2000 *J. Biol. Chem.* **275** 26765
[49] Schultz K et al 2006 *Am. J. Physiol. Heart Circ. Physiol.* **290** H2528
[50] Hayaishi O and Nozaki M 1969 *Science* **164** 389
[51] Jaakkola P et al 2001 *Science* **292** 468
[52] Maxwell P and Salnikow K 2004 *Cancer Biol. Ther.* **3** 29
[53] Batie C J et al 1987 *J. Biol. Chem.* **262** 1510