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High-Nitrogen Nickel-Free Stainless Steel: An Attractive Material with Potential for Biomedical Application

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Metal materials such as stainless steel, cobalt-based alloy, and NiTi alloy used in clinic contain a certain amount of nickel (Ni) for the structure stability. Nickel ions can gradually dissolve into the human body even with much slow corrosion rate, causing allergic, inflammation, local tissue proliferation, and other adverse reactions to some people. To avoid these adverse effects of nickel, a new high-nitrogen nickel-free stainless steel (HNNFSS) using nitrogen and manganese instead of nickel to stabilize the austenite structure is developed, which exhibits much better mechanical properties, good corrosion resistance, and biocompatibility. Compared with the conventional orthopedic metal implants, the adhesion strength of HNNFSS implant with the surrounding tissue in animal is found to be stronger with new bone formation, promoted by both alkaline phosphatase (ALP) and osteocalcin (OCN) expressions. The vascular stent made of HNNFSS shows lower restenosis rate compared with the traditional 316L stainless steel stent, which is attributed to the promotion of endothelial cell and inhibition of smooth muscle cells, and superior blood compatibility. In conclusion, results from large number of studies show that high-nitrogen nickel-free stainless steel as a new kind of high-performance biomedical metallic material possesses great application potential.

1. Introduction

Biomedical materials refer to a class of high-tech materials used for diagnosis, treatment, repair. or replacement of diseased biological tissues or organs, to improve or restore their biological functionality. They mainly include biomedical metallic materials, biomedical organic materials (e.g., organic polymer materials), biomedical inorganic nonmetallic materials (e.g., bioceramics, bioglass, and carbon materials) and biomedical composite materials, etc. They are defined as inanimate materials used in medical devices that interact with biological systems,[1] which is an interdisciplinary subject covering medicine, material science, biology, and chemistry. According to the performance characteristics of various biomedical materials, they are applied to the suitable parts to achieve therapeutic effects. Among them, medical metallic materials have been the most widely used biomedical materials in clinical practice due to their excellent comprehensive properties such as high strength and toughness, fatigue resistance, easy machining, and formability. Biomedical metallic materials are the preferred implant materials for hard tissues such as bone and teeth that need to bear high loads, as well as stents for interventional therapy, and thus are widely used in various medical devices used in orthopedics, dentistry, interventional therapy, and other important medical applications. At present, the most widely used biomedical metallic materials include stainless steel, titanium and its alloys, cobalt-based alloys, and nickel–titanium shape memory alloys.[2–4] In quest of performance and biosafety enhancement, many biomedical metallic materials with better performance have been developed and applied lately. Among them, the high-nitrogen nickel-free stainless steel (HNNFSS) is a new biomedical metal material with high strength, high plasticity, high local corrosion resistance, and excellent biocompatibility. Different from conventional medical stainless steels, nitrogen (N) instead of nickel (Ni) has been employed to stabilize the austenite in HNNFSS to avoid the potential risks of the Ni, which may cause sensitization, teratogenicity, and other hazards to human body. Therefore, the application of HNNFSS in medical field has been widely focused in research and industry.[5–11]

In view of the significance of mechanical characteristics and different corrosion environment for biomedical devices, lots of studies performed on the mechanical properties and corrosion resistance of HNNFSS demonstrated its obvious advantages. Studies show that an increase in N content increases the strength of stainless steel linearly in a certain range.[12] Meanwhile a higher work hardening capacity can be obtained. The strengthening mechanism of stainless steel includes deformation strengthening, grain refinement strengthening, solid-solution strengthening and precipitation strengthening, etc.[13,14] In terms of fatigue performance, HNNFSS exhibits higher stress levels to the strain cycling than the conventional steel, and its fatigue resistance increases with nitrogen alloying but the effect

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becomes negligible after a limit.[15] Given that many medical devices go through mechanical loading, fretting fatigue behavior of HNNFSS studied in a simulated body fluid exhibited its higher fretting fatigue limit than that of 316L stainless steel (316L SS) both in air and in the phosphate-buffered saline (PBS (-)). A decrease in fatigue limit of HNNFSS by fretting was much smaller than that of 316L SS in these two environments.[16] Therefore, HNNFSS can be regarded as a biomedical metallic material with higher fretting fatigue strength.

The implanted metallic devices come into contact with human tissues from the beginning of service. Because high water content in tissues or liquid environment can induce corrosion of the implanted devices, the corrosion performance of HNNFSS as a biomedical metallic material is very important. A large number of studies show that the pitting corrosion resistance of the stainless steel increases linearly with the increase of N content.[17–19] The mechanism of influence of N on pitting corrosion resistance of the stainless steel has been explained from various aspects.[20–25] The influence of cold deformation on pitting corrosion resistance and corrosion fatigue performance of HNNFSS was studied during the cold deformation process and the periodic stress experienced by implanting medical device, respectively. The results showed that when the N content is low, the pitting corrosion resistance of HNNFSS is obviously reduced by cold deformation. However, when the N content is high enough, the cold deformation will not affect the pitting corrosion resistance of the steel.[26,27] Fatigue limit of HNNFSS was found to be obviously increased with the increase of prestraining. In a suitable range, the cold deformation had a beneficial effect on the corrosion fatigue strength of HNNFSS in Hank's solution.[28]

Furthermore, owing to no nickel addition, HNNFSS possess excellent biocompatibility. A large number of studies have shown that, compared with traditional metal materials (316L SS and L605 cobalt-based alloy), HNNFSS exhibits excellent compatibility for osteoblasts, endothelial cells, smooth muscle cells, and fibroblasts.[29–34] In terms of blood compatibility, HNNFSS also showed lower amount of platelet adhesion and longer clotting time.[35–37] Moreover, HNNFSS implantation in animal bone showed bonding performance with surrounding bone tissue, indicating that HNNFSS could promote the osteoblast infiltration and growth. The vascular stent made of HNNFSS also showed obvious advantages in vivo.[32,38] Compared with the 316L SS stent, HNNFSS stent had smaller intima hyperplasia area and lower restenosis rate. These results well affirmed the HNNFSS biocompatibility.

Based on experimental evidences, it can be concluded that HNNFSS as a new biomedical metal material possess great clinical application potential, and it can endow medical devices with excellent performance combination of high strength, good ductility, durable stability, and biocompatibility.

2. Required Properties of Metallic Materials for Biomedical Devices

Among all biomedical materials, metallic materials hold an important place in the field of medical devices due to their excellent combination of mechanical properties. The main functions for metals in human body are fixation, connection, and support of the damaged tissues. However, the biomedical metallic materials are required not only to have excellent mechanical properties,[39] but also to have good biocorrosion resistance,[40] excellent biocompatibility,[41] visibility under X-ray,[42] and even some special biological functions according to the characteristics of clinical uses. The requirements of metal materials for vascular stents and orthopedic implants are summarized in the following context, in order to develop novel implant devices with better clinical effects, as shown in **Figure 1**.

2.1. Vessel Stents

Normal blood vessels are the tubular soft tissues that transport blood throughout the body to realize delivering oxygen and nutrients to the tissues and removing carbon dioxide and metabolic waste products from the tissues. When blood vessels are narrow, a supportive device needs to be inserted to restore the blood flow. Vessel stents, a special device, can restore the normal blood flow and avoid other critical consequences of vessel narrowing, which can be inserted into the affected vessel using X-ray digital subtraction angiography (DSA) and/or endoscopic guidance.[43–45] In view of the above clinical characteristics, vascular stents should have the following properties.

2.1.1. Support Capability

As a supportive medical device, vascular stent needs to support the pathological tissue to a certain extent. In order to make vascular stents more suitable for the diseased vessels, it is necessary to understand the mechanical properties of vascular tissues. Blood vessel walls are exposed to various mechanical stresses in vivo. In case of artery wall, its intraluminal surface is subjected to a mean pressure of ≈13.3 kPa (100 mmHg) and a pulse pressure of ≈5.3 kPa (40 mmHg). Wall shear stress of a few Pa is applied to the intraluminal surface due to the blood flow, and the wall shear stress oscillates during the cardiac cycle.[46] However, patients with vascular diseases usually show clinical hypertension and atherosclerotic plaques in the diseased vascular tissues. In order to ensure the effectiveness of vascular stents and reduce the risk of failure, it is necessary to appropriately expand the range of support strength during the stent design. Factors such as high blood pressure (a pressure of 23.9 kPa (180 mmHg)) and shear forces (a few Pa) exerted by blood flow on blood vessels, in addition to support for the hardened plaques, need to be considered. Then, what factors will affect the supporting performance of vascular stents? First, the mechanical properties of stent materials will determine the supporting performance of vascular stents. Second, the support performance of the stent can be further adjusted through the optimized design of the stent structure to finally meet the requirements of clinical use.

Metal Materials: Elasticity or plasticity for expansion and rigidity for the maintenance of dilatation and resistance to elastic recoil are required. The expandability and plasticity of a balloon-expandable stent and the elasticity of a self-expandable stent, as well as rigidity and resistance to the elastic recoil of blood vessels, are required for the stent materials. Conventional metals cover these properties when appropriate metals are selected.[47] The mechanical properties, including

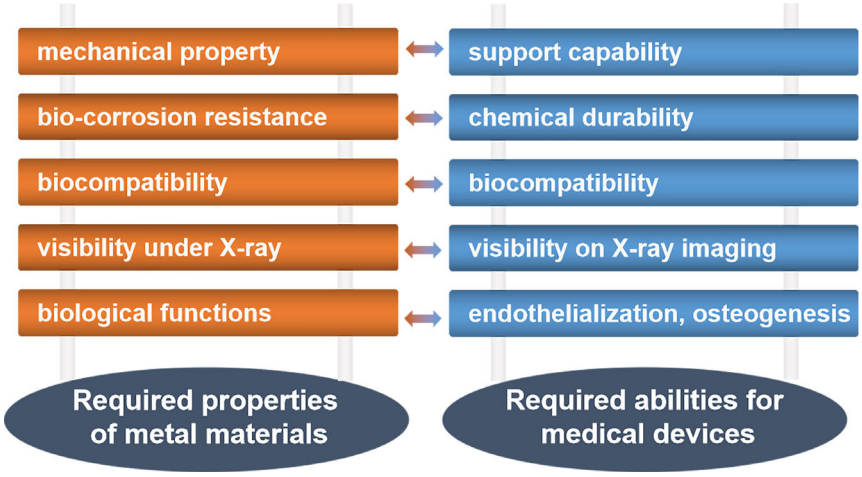

Figure 1. The relationship of required properties of metal materials and required performances of medical devices.

yield strength, tensile strength, and elongation at break of the traditional metal materials used for stents, are listed in **Table 1**. At present, cobalt alloy stents are most widely used in applications with excellent clinical effects. Therefore, if the mechanical properties of new metal materials are comparable with the cobalt-based alloy, or even higher than it, sufficient support performance of the new stent can be achieved with a smaller size.

Structural Design: The stent structure design is another key to determine the mechanical support performance of the stent. The radial support strength of the stent can be changed by adjusting the wall thickness, width, length of the stent mesh, and other parameters. The radial support strength of the stent will decrease with the reduction of width and thickness of the mesh. Therefore, the optimal design of the stent structure with appropriate support strength can simultaneously satisfy the effective support of the stent to the lesion site and minimize the pulling effect of the stent on the blood vessel to maximize the clinical benefits.

2.1.2. Chemical Durability

The service environment of vascular stents is relatively harsh, which is a complex environment with corrosive media (blood and/or body fluids), stress, and fatigue. This requires the chemical durability of the metal materials of the supportive platform, especially under the stress and fatigue environment. This chemical durability for metal materials is so-called corrosion resistance. For new type of metallic stent materials, it is particularly necessary to understand the corrosion resistance, stress corrosion resistance, and fatigue corrosion resistance of this new material to estimate the service life of the vascular stent.

Table 1. Mechanical properties of metal materials for stents.[47]

Materials	Yield strength [MPa]	Tensile strength [MPa]	Elongation at break [%]
Stainless steel (316L)	220–260	500–540	55–65
Co alloy (L605)[72]	450	1040	56

2.1.3. Biocompatibility

The stent implanted in the human body will be directly in contact with both vascular tissue and blood. An ideal stent can avoid activating the blood clotting factors in blood coagulation on the stent surface and promote fast adhesion, proliferation, and migration of endothelial cells on the stent surface. In particular, some metal materials will inevitably corrode and release some metal ions in the service environment. Some harmful ions (such as nickel, cobalt and chromium ions, etc.) may cause problems such as cytotoxicity or hemolysis/coagulation. Therefore, it is required that the stent materials should have good cytocompatibility and blood compatibility.

2.1.4. Visibility in X-Ray Imaging

The implantation and the follow-up process of the stent system are all performed under X-rays, which requires that the stent material should have good X-ray visibility to ensure accurate positioning during stent implantation.

2.2. Orthopedic Implants

Orthopedic implants are another major application field for biomedical metallic materials, which also requires materials with high mechanical strength and fatigue resistance, chemical stability, biological safety, and X-ray visibility. Orthopedic implants have the same performance requirements as vascular stents except for the special mechanics. In order to design an orthopedic implant that is well compatible with human bone tissue, it is necessary to understand the composition, structure, and physiological characteristics of bone tissues.

Bones are mainly composed of collagen fibers and apatite minerals at nanostructural level, crosslinked with type I

collagen, and the mineralized constituent BAp gives bone the strength and flexibility, respectively.[48] When a bone fracture occurs, small defects can be cured spontaneously. Subsequently, the bone fracture healing process is classified into three phases: inflammatory, reparative, and remodeling. But if the bone fracture is serious, orthopedic implants are often needed to promote bone tissue healing. The mechanical fit of orthopedic implants to human bone is an important factor. The elastic modulus of human bone is in the range of 10–30 GPa, while the elastic modulus of stainless steel is about 200 GPa, much higher than that of human bone, that can easily produce the so-called stress shielding effect. Therefore, it is necessary to reduce the risk of stress shielding by design of implant structure, and in this regard porous design can be an effective way.

3. Properties of High-Nitrogen Nickel-Free Stainless Steel

As a new kind of biomedical material, HNNFSS is mainly attributed to its unique feature of being nickel free, that is, zero nickel in the chemical composition design. In addition, it also shows better comprehensive mechanical properties, higher pitting corrosion resistance, and excellent biocompatibility. These excellent features of HNNFSS endow medical devices with excellent clinical effects; a summary of properties of HNNFSS is shown in **Figure 2**.

3.1. Mechanical Properties

The mechanical properties of HNNFSS are determined by the nitrogen content, heat treatment, and processing method. **Table 2** lists the chemical composition and mechanical properties of the HNNFSS developed in recent years.[41] Compared with traditional 316L SS, HNNFSS keeps the same elongation level, but its strength is obviously higher than 316L SS. Simmons et al.[12] found that both yield strength and tensile strength of HNNFSS showed a trend of continuous improvement with the increase of the nitrogen content in the steel. **Figure 3** shows the influence of nitrogen content on the tensile strength of austenitic stainless steel at room temperature. Although the strength of stainless steel increases with the increase of content, higher nitrogen content is not always better. Studies showed that the addition of nitrogen can significantly decrease the toughness–brittleness transition temperature of stainless steel.[49–53] However, when the nitrogen content is higher than 0.95%, the toughness–brittleness transition temperature of high-nitrogen stainless steel increases, thus increasing the failure risk of the material.

As most implanted metal medical devices need to support the human pathological changes or defected tissues in the clinical use and need to withstand the cyclic action of loading for a long time, the fatigue performance of medical metal materials is very important which will determine the service life of devices. Vogt et al. studied the fatigue property of HNNFSS and found that the fatigue resistance could be increased with nitrogen alloying, but there exists a limit at which the effect became negligible, as

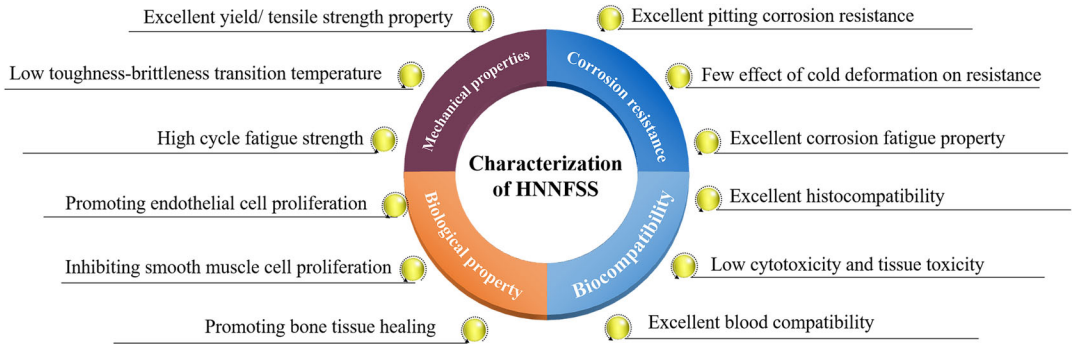

Figure 2. Summary diagram of properties of HNNFSS.

Table 2. Chemical compositions and mechanical properties of HNNFSS.[41]

HNNFSS	%Cr	%Mn	%Mo	%N	%Ni	Yield strength [MPa]	Tensile strength [MPa]	Elongation [%]
BIOSSN4[36,71,73]	17.9	15.3	2.02	0.45–1.0	0.2	559	938	54
PANACEA P558	17.4	10.18	3.09	0.48	0.08	600	923	54
24Cr–2Mo	24	–	2	1.0	–	–	1167	45
BioDur 108	21	23	0.7	0.97	0.3	586	931	52
24Cr–1N	24	–	–	1.0	–	–	1032	26
X13CrMnMoN18-14-3	18	14	3	0.75	0.05	590	1030	70
SS316L	16–18	2	2–3	0	10–14	220–260	500–540	55–65

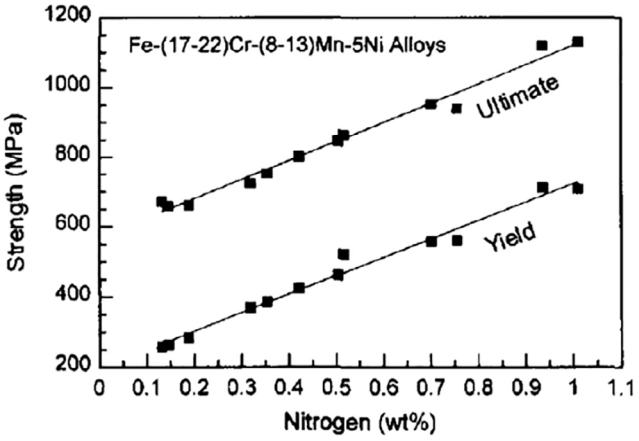

Figure 3. Effect of nitrogen content on tensile strength of austenitic stainless steel at room temperature. Reproduced with permission.[12] Copyright 1997, Elsevier.

shown in **Figure 4**.[54] Dai et al. studied the high-cycle fatigue strength of HNNFSS and found that the fatigue strength is higher than that of 316L SS; also, the effect of nitrogen on fatigue strength was studied which showed that nitrogen could increase the stacking fault energy and thus shorten the distance between interfatigue striations.[15]

3.2. Corrosion Resistance

Although HNNFSS is a kind of corrosion resistant metal material, it still faces the problems of biological corrosion, stress corrosion, and corrosion fatigue from the long-term service in the human body environment. Therefore, the corrosion performances of HNNFSS in the physiological environment need to be further understood, including the different liquid medium and the stress of surrounding tissue. Deformation even occurs in the processing and assembly of the devices. Compared with the traditional austenitic stainless steel, HNNFSS is developed by replacing Ni with N and Mn to stabilize the austenitic structure. Due to the difference in chemical composition, the corrosion behavior of the steel should be changed, so it is necessary to study the corrosion mechanism of HNNFSS.

Yang et al.[55] studied the anodic polarizations of HNNFSS, 316LSS, and Co28Cr6Mo alloy in Hank's solution, as shown in **Figure 5**. It can be found that HNNFSS had comparatively the best corrosion resistance with the highest pitting potential. Becerikli et al. compared the corrosion behaviors of different biomedical metallic materials in the cell culture medium (MEM) and found that P2000, a HNNFSS, showed significant improvement in corrosion resistance compared with 316L SS.[37] Yang et al.[56] also studied the corrosion behavior of HNNFSS in artificial saliva with *Streptococcus mutans*. The results showed that compared with 316L SS, the pitting depth of HNNFSS in the corrosive environment was lower, the corrosion current density was two orders of magnitude lower, and the pitting potential was 550 mV higher than 316L SS, indicating that HNNFSS is a more suitable metal material for dentistry. Then, why does HNNFSS have such excellent corrosion resistance? Chao et al. suggested that the better corrosion resistance of HNNFSS is mainly due to the formation of Cr_2N and enrichment of MoO_2 in the passivated film, which promote the stability of the passivated film.[57] In addition, some other studies proposed other mechanisms on the improved corrosion resistance of HNNFSS, including the mechanism of ammonium ion-regulating pH in pitting corrosion pits,[20,58] the mechanism of ammonium ion-consuming chloride ions,[21] the mechanism of N atoms improving quality of passivation film by filling the vacancies,[22] and the mechanism of N atoms tending to gather at active sites and reducing the active dissolution.[18]

Most biomedical materials need to undergo different degrees of cold deformation during processing and/or clinical use, and many studies have shown that the pitting corrosion resistance of austenitic stainless steel would be significantly reduced when the cold deformation reached a certain degree.[25,59–65] Wang et al. conducted a study on the influence of cold deformation on the corrosion resistance of HNNFSS, and the result showed that severe cold working was found detrimental to the pitting

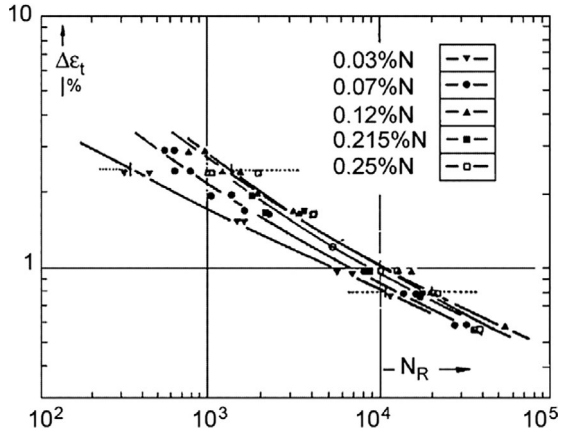

Figure 4. Coffin Manson curves of a set of 316L stainless steel alloyed with nitrogen (tests at room temperature). Reproduced with permission.[71] Copyright 2006, Elsevier.

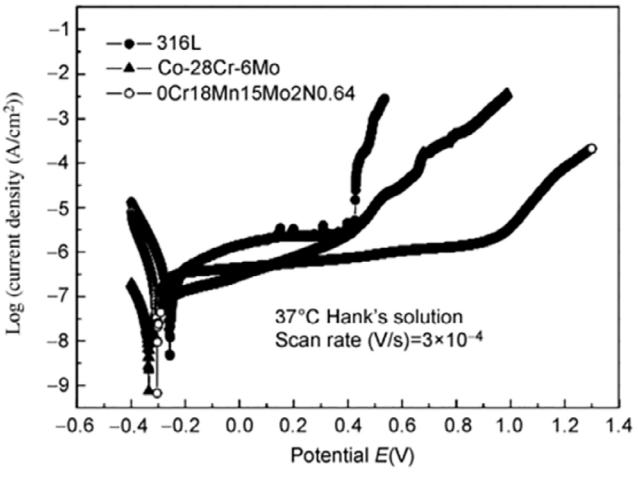

Figure 5. Anodic polarization curves of 316L SS, Co–Cr–Mo alloy, and HNNFSS in Hank's solution at 37 °C. Reproduced with permission.[55] Copyright 2009, Elsevier.

corrosion resistance of HNNFSS, but when the nitrogen content was further increased, this detrimental effect was completely eliminated (**Figure 6**).[26] In addition, the mechanism of resistance to the effect of cold deformation on pitting corrosion resistance of HNNFSS was further studied. It was found that nitrogen was significantly enriched as a form of short-range order [CrN] in the passive film and highly doped in the chromium oxide layer, which gave a self-healing ability to the coating.[27] Furthermore, what is the effect of cold deformation on the corrosion fatigue property of HNNFSS? It was found that the fatigue limit of HNNFSS in air and in the corrosive solution increased with the increase of cold deformation degree in a certain range (**Table 3**), indicating that prestraining had a beneficial effect on corrosion fatigue strength of HNNFSS.[28] The corrosion performance of metallic materials not only determines their service life, but also affects the biosafety of the materials after implantation.

3.3. Biocompatibility

Biocompatibility is the significantly important performance requirement for biomedical materials after implantation, including blood compatibility, cell compatibility, and tissue compatibility. HNNFSS have no nickel addition, which can avoid the cytotoxicity and tissue toxicity induced by nickel-ion leaching. Therefore, a lot of research has been carried out to study the biocompatibility of HNNFSS. Inoue et al. established a relationship among the dissolution of Ni ion, the promotion of endothelialization, and the inflammation to clarify the reason for the excellent biocompatibility of HNNFSS.[31] Biodur 108 alloy is a typical HNNFSS, which was the first to be used in clinic. Taking Biodur 108 alloy for example, which has been listed in ASTM standard in 2002 (ASTM F2229), the alloy has passed the standard biocompatibility assessments including cytotoxicity, irritation, acute systematic toxicity, pyrogenicity, mutagenicity, implantation with histopathology, and hemocompatibility test by Toxikon Corporation of USA.[66,67] Moreover, Li et al.[33] found that the cell toxicity test of HNNFSS showed no significant toxic side effects on MC3T3-E1 cells compared to the nitinol alloy (a NiTi alloy). Cell adhesion test showed that the number of MC3T3-E1 cells on HNNFSS surface was more than that on the nitinol alloy and they grew in good condition. Besides, HNNFSS showed obvious advantages in blood compatibility compared with traditional metal materials. Wan et al.[38] studied the effect of nitrogen content on surface and interfacial energies, platelet adhesion, and dynamic clotting time for HNNFSS; the results showed that with the increase of the nitrogen content, the quantity of platelet adhesion to the surface of HNNFSS significantly reduced while the dynamic clotting time gradually extended. Above all, HNNFSS exhibited excellent biocompatibility.[38,39]

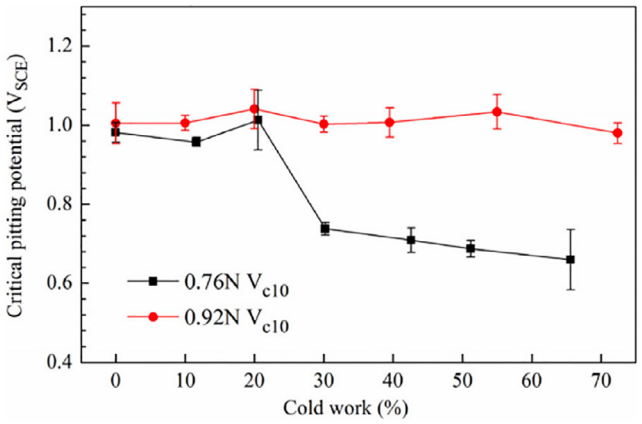

Figure 6. Variation of the critical pitting potential as a function of cold working level for 0.76 and 0.92 N in 3.5 wt% NaCl solution at 25 °C. Reproduced with permission.[26] Copyright 2017, Elsevier.

Table 3. Mechanical properties of different prestrained HNNFSS (MPa).

Sample	Fatigue limit in air	Fatigue limit in Hank's solution
0%	550	475
20%	850	750
35%	900	750

3.4. Biological Properties

In addition to excellent biocompatibility, HNNFSS also showed certain biological properties, such as promoting endothelial cell proliferation, inhibiting smooth muscle proliferation, reducing platelet adhesion, promoting bone tissue healing, etc., which are rarely reflected in the inert metal materials.

3.4.1. Promoting Endothelial Cell Proliferation

Inoue et al.[31] studied the effect of HNNFSS with 1.03% N on endothelial cells, in comparison with traditional 316L SS. As shown in **Figure 7**, the proliferation number of endothelial cells on HNS (i.e., HNNFSS) after coculture was significantly higher than that on 316L SS, and the endothelial cells spread more easily on the surface of HNS. With prolongation of the coculture time, the difference between two groups became more obvious. In addition, Li et al. found that HNNFSS with 0.64% N content showed no advantage in promoting endothelial cell proliferation compared with traditional 316L SS, but the apoptosis rate of endothelial cells cocultured with HNNFSS extract was lower than that of 316L SS extract, which could be attributed to the nickel-ion dissolution.[68] These results indicate that HNNFSS has a biological property of promoting endothelial cells.

3.4.2. Inhibiting Smooth Muscle Cell Proliferation

Excessive proliferation of smooth muscle cells is the main factor leading to the in-stent restenosis (ISR) after stent implantation. Therefore, studying the action rule and mechanism of HNNFSS on smooth muscle cells is an effective way to reduce the risk of ISR for the stents made of HNNFSS. Related studies showed that, compared with 316L SS, HNNFSS can inhibit the proliferation of smooth muscle cells and then promote the apoptosis of smooth muscle cells. The mechanism was analyzed by

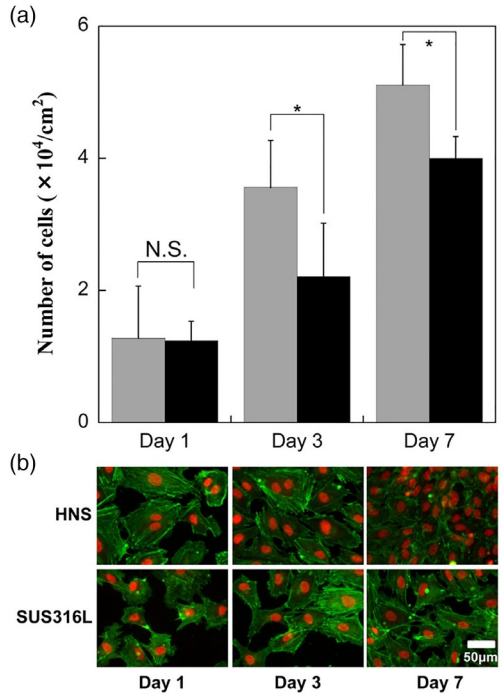

Figure 7. a) Proliferation number and b) spreading morphology of endothelial cells on 316L SS and HNS samples cocultured for 1, 3, and 7 days. Reproduced with permission.[31] Copyright 2014, John Wiley and Sons.

upregulating the expressions of Caspase-3, Caspase-8, Fas, ATG5, and ATG7 genes and downregulating the expression of Cyclin A and Cyclin E gene.[34] In addition to the in vitro cell-level studies, Fujiu et al. implanted HNNFSS stents into the coronary arteries of pigs to evaluate the intimal area, lumen area of the vascular with stent, inflammatory response, and related gene expressions, using 316L SS stents as the control.[69] The results showed that the new intima area of the HNNFSS stent group was lower than that of the 316L SS stent group, the lumen area was higher than that of the 316L SS stent group, and the inflammatory response was significantly lower than that of the control group, as shown in **Figure 8**. This was attributed to upregulating the expression levels of the HIF-1α target genes Vegfa and Pdgfa, and the inflammatory cytokine Tnf in the smooth muscle cells was downregulated on walls of stented arteries. In conclusion, HNNFSS can inhibit the proliferation of smooth muscle cells.

3.4.3. Reducing Platelet Adhesion

Platelet adhesion test is an effective method to characterize the antithrombotic properties of materials. Many studies have proved that HNNFSS has good characteristics of reducing platelet adhesion. Ren et al. evaluated the platelet adhesion by comparing the number and activation of platelets on surfaces of HNNFSS and 316L SS.[36] The results showed that the number of platelets on the surface of HNNFSS was lower, the number of activated platelets was also lower, thus the risk of thrombosis should be lower for HNNFSS. Li et al.[30] also compared the numbers of platelets adhered to surfaces of HNNFSS with different nitrogen contents and nitinol alloy and found that the adhered platelet numbers were significantly different between HNNFSS and nitinol alloy. However, different nitrogen contents of HNNFSS had no significant difference ($p > 0.05$).

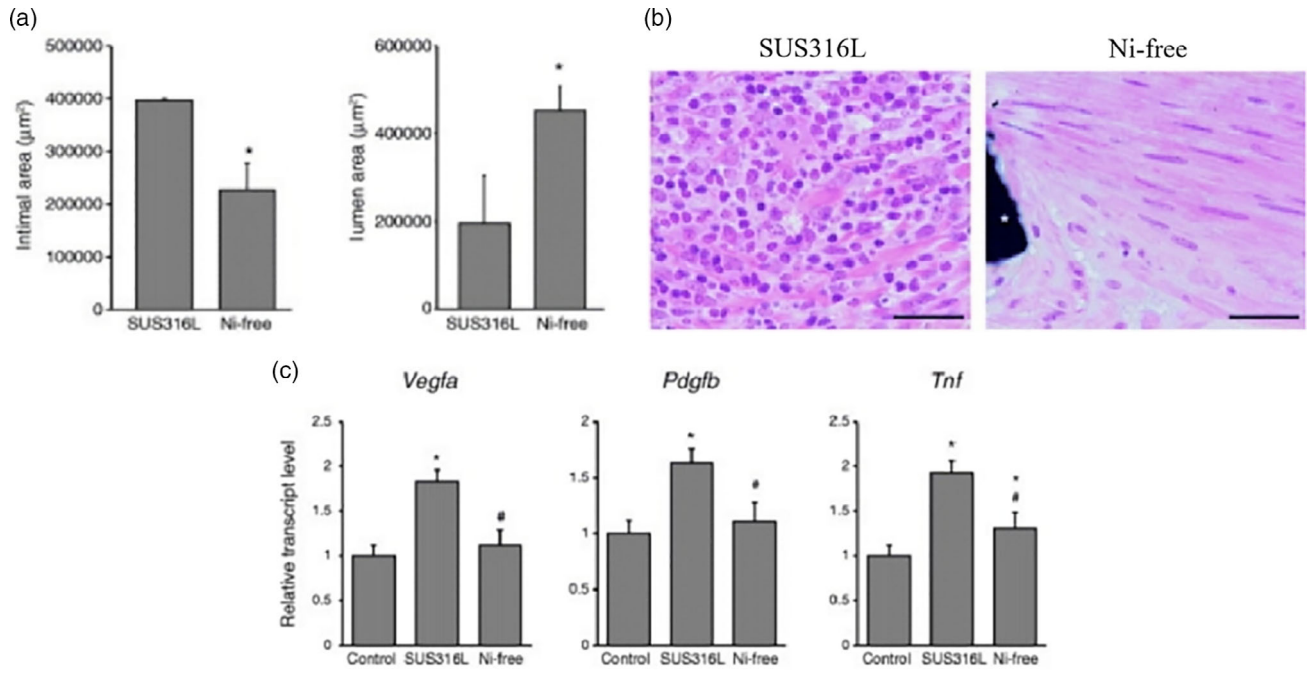

Figure 8. Tissue response of vascular after stent implantation: a) intimal area and lumen area; b) inflammatory cell infiltration; and c) related gene expressions. Reproduced with permission.[69] Copyright 2012, Taylor & Francis Informa UK Ltd-Journals.

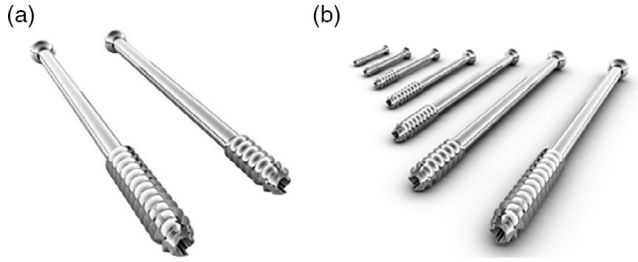

Figure 9. Commercially available medical device made of BioDur 108 alloy (a HNNFSS): a) cannulated bone screw developed by Zimmer; b) cannulated bone screw developed by Renovis.

3.4.4. Promoting Bone Tissue Healing

Bone tissue healing mainly depends on the osteogenic activity, and an important index to evaluate osteogenic activity is the expression of alkaline phosphatase (ALP). P2000 without nickel addition as a representative of HNNFSS was incubated with osteoblast cell and the traditional orthopedic implant materials (titanium alloy, 316L SS, CoCrMo alloy and plastic) were taken as controlled groups. The results showed that P2000 promoted excellent bone activity, obviously increasing the expression of ALP level and the related factors thus promoting the osteoblast proliferation.[56] Yu et al. implanted HNNFSS and 316L SS in the rabbit tibia to analyze the formation of new bone and the bonding of implant bone tissue around the implants. HNNFSS implants showed a significant promotion of the transformation of bone marrow mesenchymal stem cells to the osteoblast and had excellent bonding performance with surrounding bone tissue. In addition, its mechanism was mainly the hydrophilicity of material surface that was beneficial to cell adhesion and migration.[32]

Based on the above results, studies on the mechanism of biological functions mainly concentrated on cells' response in the implant surrounding tissue and characterization on the level of gene expression can establish clear material characteristics (e.g., surface charge, surface energy and dissolution ion, etc.) and response relationship in cell response and gene expression.

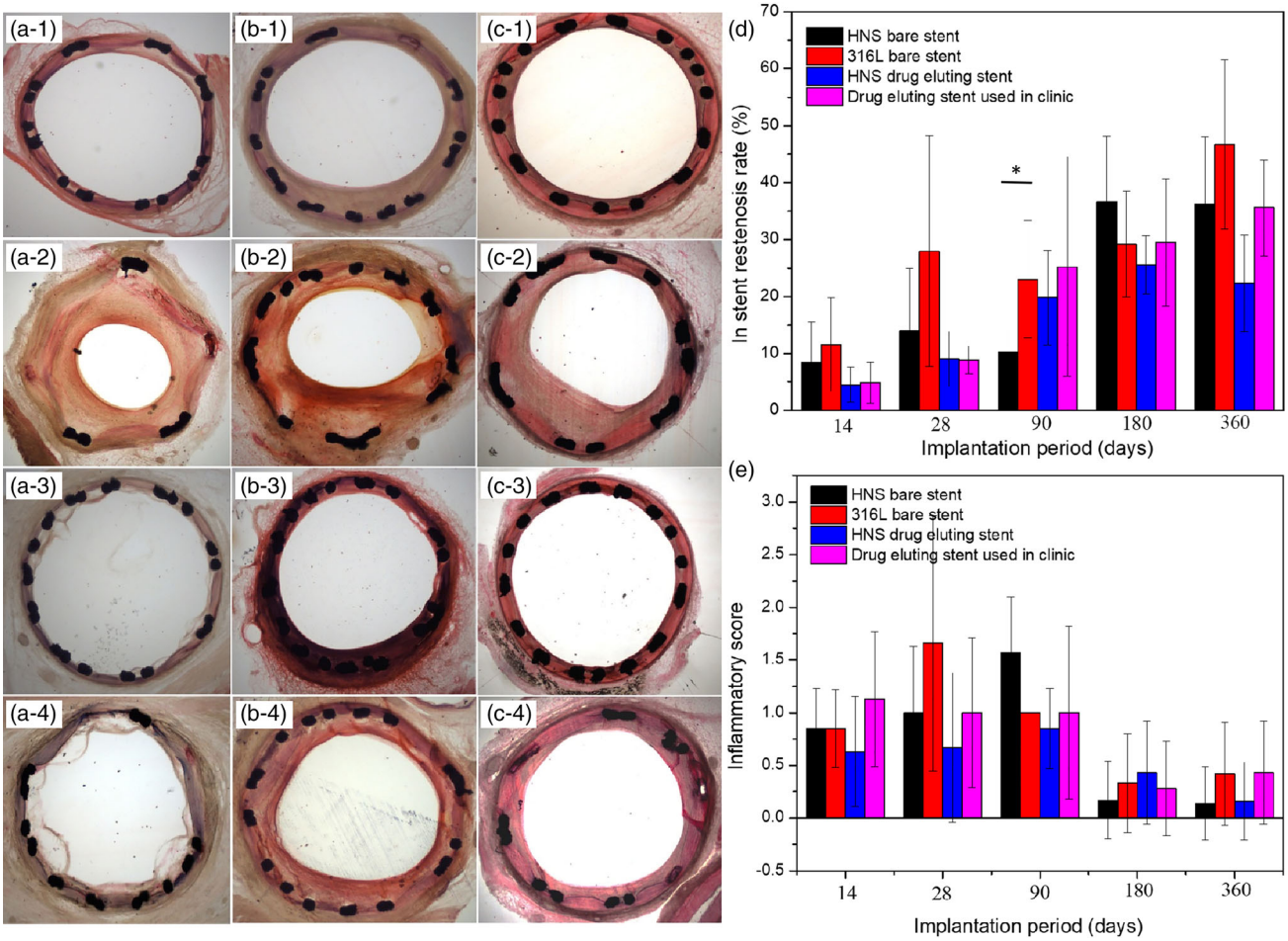

Figure 10. a–c) HE-staining morphologies, d) restenosis rate, and e) inflammatory of each stent group after different implantation times, *indicating a significant difference. Thereinto, a-1–a-4) after 14 days implantation, b-1–b-4) after 30 days implantation, and c-1–c-4) after 90 days implantation. Reproduced with permission.[70] Copyright 2020, Elsevier.

4. Application Status of HNNFSS in Biomedical Devices

Research and development of HNNFSS is taking place worldwide, including BioDur 108 alloy in the United States, P2000 alloy in Germany, P558 alloy in Switzerland, P548 alloy in Austria, and BIOSSN steel in China. Among them, BioDur 108 alloy developed by Carpenter Corp. has been listed in ASTM F2229-12 standard in the United States and has been applied in clinical applications for bone surgical implants and surgical instruments, including metal locking bone plate system, cannulated bone screw, etc., as shown in **Figure 9**.

HNNFSS has been applied to manufacture the drug-eluting stent (DES), which was implanted in the coronary artery of porcine to evaluate the biosafety as a coronary stent platform. **Figure 10** shows the comparison results of HE-staining morphologies, restenosis rate, and inflammatory score of different groups. Figure 10a–c shows the HE-staining morphologies of each stent group, where Figure 10a-1–a-4 shows the HE-staining morphologies of four stent groups after 14 days implantation. It can be observed that the Lumen stenosis occurred in the coronary arteries implanted with 316L stent. Two groups of DES were all exposed to the blood but only HNS stent was covered partly by neointima. Figure 10b-1–b-4 shows the HE-staining morphologies of four stent groups after 30 days implantation. It can be found that the lumen stenosis phenomenon on the 316L stent implanted in coronary arteries continued up to 90 days (Figure 10c-1–c-4). The two groups of DES inhibited the intimal hypertrophy; in addition, neointima thickness was moderate after implantation of HNS stent for 30 and 90 days. The results confirmed that the HNNFSS bare stents showed certain advantages compared with the traditional 316L SS bare stent in inhibiting restenosis; moreover, the DES further reduced the ISR rate to a certain extent. Compared with the commercially available L605 cobalt-based alloy DES, HNNFSS DES showed a trend of inhibition of restenosis, but the difference was not significant, as shown in Figure 10d. The inflammatory score of the vascular tissues with the stent segment was further performed as shown Figure 10e; the comparison results showed that the average inflammatory score of the HNNFSS stent group was lower than that of the 316L SS stent group, but no significant difference was observed.[70] No acute thromboembolic event occurred in the HNNFSS stent group (both bare stents and DES), while one in-stent thrombotic event occurred in the DES control group. Based on the evidence of nickel-ion dissolution-inducing stent restenosis and the fact of inhibiting platelet adhesion, HNNFSS as stent platform material can theoretically reduce the incidence of ISR and the risk of thrombosis. So far, more than 1000 cases of clinical trials of HNNFSS DES made by Zhongke Yian Medical Technology (Beijing) Co. Ltd., China, have been completed, and good clinical results are expected after a period of clinical follow-up.

5. Conclusion

HNNFSS is a new biomedical metallic material with high strength, high fatigue resistance, high pitting corrosion resistance, and excellent biocompatibility. In addition, HNNFSS would be suitable for those patients with nickel allergy. At present, it has been applied in orthopedic implants, surgical instruments, and coronary stents, showing significant clinical advantages compared with traditional medical metal devices. With the increasing awareness and attention regarding nickel allergy globally, nickel-free biomedical metallic devices could have great potential to gradually replace the currently used nickel containing medical devices to avoid the potential risks caused by nickel dissolution from the devices into human body.

Moreover, the addition of nitrogen element can endow materials more excellent biological functions. However, the studies about the transformation of nitrogen in the new biomedical metallic material during service has not been reported. Nitrogen is the building block of proteins, so the relationship between its transformation products and biological functions should be the critical point and the focus of future research, which may further expand the application of high-nitrogen nickel-free stainless steel.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biocompatibility, bone implants, coronary stents, corrosion resistances, high-nitrogen nickel-free stainless steels, mechanical properties

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