Development of Nickel Free Nitrogen Stabilized Austenitic Stainless Steel for Biomedical Applications

THESIS SUBMITTED IN PARTIAL FULFILLMENT
FOR THE AWARD OF DEGREE

Doctor of Philosophy

by

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I, ***"Chandra Shekhar Kumar"***, certify that the work embodied in this thesis is my own bona fide work and carried out by me under the supervision of ***"Dr. G. S. Mahobia"*** from ***"July 2016"*** to ***"October 2021"***, at the ***"Department of Metallurgical Engineering"***, Indian Institute of Technology (BHU), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma. I declare that I have faithfully acknowledged and given credits to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not willfully copied any other's work, paragraphs, text, data, results, *etc*., reported in journals, books, magazines, reports dissertations, theses, *etc*., or available at websites and have not included them in this thesis and have not cited as my own work.

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ACKNOWLEDGEMENTS

I take this opportunity to express my sincere thanks and gratitude beyond words to my supervisor, Dr. G. S. Mahobia for his consistent help, encouragement and valuable discussions during the entire duration of my Ph.D. work. I would like to express my deepest sense of gratitude to my revered Guru Prof. Vakil Singh, under whose able guidance the present research work was conceived. I would not have been able to complete this thesis work without their blessings, invaluable efforts and constant encouragement.

Besides my supervisor, I would like to thank my RPEC members: Dr. Amit Rastogi (IMS-BHU) and Dr. Kausik Chattopadhyay, Department of Metallurgical Engineering, for their support and insightful comments. I would like to convey my sincere thanks to Prof. Sunil Mohan (Head, Department of Metallurgical Engineering, IIT (BHU), Varanasi), Prof. N. K. Mukhopadhyay and Prof. R. K. Mandal (Former Heads, Department of Metallurgical Engineering, IIT (BHU)), for providing all necessary facilities to conduct my research work. My sincere thanks to Prof. O. P. Sinha, Prof. N. C. Santhi Srinivas, Dr. Joysurya Basu and other faculty members of the department for their cooperation and inspiration.

I am highly thankful to Dr. Sanjeev Kumar Mahto (School of Biomedical Engineering) for providing facility to carry out cell culture study and related valuable discussion. I am also thankful to Dr. Suruchi Poddar and Neelima Varshney from School of Biomedical Engineering, for their help during cell culture study. I am thankful to Dr. Gaurav Singh (IMS-BHU) for helping in *in vivo* animal study. I also acknowledge Prof. Rajiv Prakash (Prof.-in-charge, CIFC, IIT (BHU)) for extending necessary characterization facilities. I am thankful to Prof. I. Samajdar, IIT Bombay, for providing facility to carry out residual stress analysis. My sincere thanks are due to Mr. Girish Sahu (CIFC, IIT (BHU)) for his constant help in conducting SEM studies.

I am thankful to my friends, Pramod, Asnit, Shubham, Deepak Singh, Sharvan, Amit, Biswajit, Sandip, Jaydeep, Nitesh and seniors Dr. Sanjeev Rana, Dr. Vaibhav Pandey, Dr. Rupendra Singh Rajpurohit, Dr. Manish K Kishore Singh, Dr. Vivek Pandey, Dr. Vikash Shivam, Dr. Yagnesh Shadangi and Mr. Ch. V. Rao for their needful help and making joyful and memorable moments at IIT (BHU), Varanasi.

I am also thankful to all the laboratory, library and workshop staffs specially Shri Sushil Ji, Shri Balwant Ji and Shri Mangal Patel for helping in tensile and fatigue samples preparation, Shri Lalit Kumar Singh for helping in transmission electron microscopy and Shri Kamlesh, Shri Minz for helping in various mechanical testing. I am also thankful to all office staff of the Department particularly Shri R P Yadav, Shri Rishabh Tiwari and Shri J P Patel for providing necessary help as and when desired.

Last, but not the least, I would like to express my deepest gratitude to my family, my parents, for educating me, for their unconditional support and encouragement to pursue my interest. I would also like to thank my elder brother Mr. Shashi Shekhar Kumar for his constant encouragement and support.

I also wish to thank all my friends and the persons whose names have not been mentioned on this piece of paper for extending their cooperation directly or indirectly.

Chandra Shekhar Kumar

Dedicated to
My Beloved Parents

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Abbreviations

AISI	American Iron and Steel Institute
ALP	Alkaline Phosphatase
ASTM	American Society for Testing and Materials
BDT	Brittle to Ductile Transition
Co-Cr	Cobalt-Chromium
CSO	Cotton Seed Oil
DBTT	Ductile to Brittle Transition Temperature
DMG	Dimethyl-glyoxime
DMEM	Dulbecco’s Modified Eagle’s Medium
FBS	Fetal Bovine Serum
FCC	Face Centered Cubic
GPa	Giga Pascal
GPMT	Guinea Pig Maximization Test
HCF	High Cycle Fatigue
IARC	International Agency for Research on Cancer
IGC	Intergranular Corrosion
ISO	International Organization for Standardization
IREL	Increment Rate of Endurance Limit
LCF	Low Cycle Fatigue
MARC	Measure of Alloying for Resistance Against Corrosion
MEM	Minimum Essential Medium
MPa	Mega Pascal
MRI	Magnetic Resonance Imaging
MTT	3-[4,5-Dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide
OCP	Open Circuit Potential
PBS	Phosphate Buffer Saline
PREN	Pitting Resistance Equivalent Number
PS	Physiological Saline

RT	Room Temperature
SBF	Simulated Body Fluid
SCE	Saturated Calomel Electrode
SEM	Scanning Electron Microscope
SFE	Stacking Fault Energy
SMAT	Surface Mechanical Attrition Treatment
SMRT	Surface Mechanical Rolling Treatment
TEM	Transmission Electron Microscope
Ti	Titanium
USPed	Ultrasonic Shot Peened
USP	Ultrasonic Shot Peening
UHMWPE	Ultra-High Molecular Weight Polyethylene
UTS	Ultimate Tensile Strength
WHO	World Health Organization
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffraction
YS	Yield Strength

Symbols

Hz	Hertz
^{o}C	Degree Centigrade
mgL^{-1}	Milligram Per Liter
gL^{-1}	Gram Per Liter
$molL^{-1}$	Moles Per Liter
$\mu g/cm^2/week$	Microgram Per Square Centimeter Per Week
mm	Millimeter
H_v	Vickers Hardness
wt%	Weight Percent
k	Kilo
µm	Micrometer
V	Volt
θ	Theta
kN	Kilo Newton
h	Hour
Sec	Second
Min	Minute
E_{corr}	Corrosion Potential
E_{bd}	Breakdown Potential
i_{cr}	Critical Current Density
i_{corr}	Corrosion Current Density
$\Delta\varepsilon_t/2$	Total Strain Amplitude
$\Delta\varepsilon_e/2$	Elastic Strain Amplitude
$\Delta\varepsilon_p/2$	Plastic Strain Amplitude
$\Delta\sigma/2$	Stress Amplitude
N_f	Fatigue Life
$2N_f$	Number of Reversals to Failure
σ_t	Cyclic Tensile Stress

σ_c	Cyclic Compressive Stress
$\leq$	Less Than or Equals to
$\geq$	Greater Than or Equals to
$=$	Equals to
~	Approximately
R	Stress Ratio
N_i	Number of Cycles for Crack Initiation
N_p	Number of Cycles for Crack Propagation
mJ/m^2	Milli Joule per square meter

Preface

Metallic materials are backbone of orthopedic surgical procedures. Titanium, cobalt-chromium based alloys, stainless steel, tantalum, gold and niobium are widely used metallic biomaterials nowadays in medical applications. Implants are found to fail mainly due to fatigue, wear, corrosion, corrosion fatigue and their synergistic effect. Material debris resulting from corrosion and wear gets accumulated in the human body. The 316L austenitic stainless steel is a widely used steel for making medical devices, with many advantages such as easy processing, cost-effectiveness and favorable mechanical properties. However, appreciable amount of nickel ($\geq$ 10 wt%) in the 316L causes allergic reactions resulting in eczema, swelling, itching, reddening, carcinogenic and teratogenic effects in human body. These harmful effects of nickel triggered a need for austenitic stainless steel without nickel. Nitrogen and manganese are austenite stabilizers. Austenitic grades of stainless steel are non-magnetic, which is one of the most crucial requirements of implant materials. Replacement of nickel by nitrogen and manganese provides a stable microstructure and facilitates better biocompatibility in respect of the conventional 316L. Nitrogen in stainless steel significantly enhances corrosion resistance, strength and work-hardening rate. Numerous studies have been carried out on development of nickel free grade of austenitic stainless steel, however, very few of them have been developed and studied in detail from the point of view of biomedical application. It was found that very high amount of nitrogen increases brittleness of the material and restricts the use of stainless steel for a wide temperature range. A very high amount of chromium in stainless steel stabilizes the ferrite phase. Mo is added in stainless steels to improve the pitting resistance, however, very high amount of Mo promotes formation of ferrite and σ-phase. Therefore, there is a need for design of a stainless steel which is either free from nickel or has negligible amount of nickel with optimum amount of Cr, Mn, N and Mo.

The present investigation deals with systematic study of two grades of nickel free austenitic stainless steels. One is based on the Fe-Cr-Mn-N system (HNS) and the other is based on Fe-Cr-Mn-Mo-N system (HNS-Mo). Initially, HNS was studied for mechanical properties (tensile, hardness, low cycle fatigue and high cycle fatigue), corrosion behavior in simulated body fluid (SBF) environment and biocompatibility, and its behavior was compared with that of the conventionally used 316L austenitic stainless steel, containing nickel. Ultrasonic Shot Peening (USP) is a novel technique of surface modification in which grain size in the surface region of materials gets refined to nano scale and compressive residual stresses are induced in the surface region, and surface related properties of metallic materials are significantly enhanced. The objective of the present study was development of a nickel free grade of austenitic stainless steel for the replacement of 316L. The effect of USP on microstructure, corrosion, fatigue and biocompatibility behavior of 316L stainless steel was well documented. However, for the nickel free stainless steel, no study had been performed. Therefore, in the present investigation, study on the USP effect was not conducted for the 316L. Study on this aspect, was carried out only for the nickel free austenitic stainless steels HNS and HNS-Mo. In the present investigation, two different shot sizes of 2 mm and 3 mm were used for different durations of USP. HNS was characterized for the changes in microstructure, following USP, using the techniques like scanning electron microscopy, X-ray diffraction and transmission electron microscopy. It was also characterized for hardness, surface roughness and compressive residual stress.

Chapter-1 presents the brief introduction along with the literature review on requirement and development of nickel-free austenitic stainless steels. It also includes the effect of nitrogen on various properties. This chapter presents the description of USP and its effect on various properties of metallic materials. At the end of this chapter, the objectives of the present investigation are listed.

Chapter-2 presents the chemical compositions of three types of stainless steels used for the study and the details of sample preparation for various testing. It includes the experimental procedure for various mechanical, corrosion and biocompatibility testing along with the details of USP. This chapter also presents the process of sample preparation and operational details of X-Ray Diffraction, Scanning Electron Microscopy and Transmission Electron Microscopy, used for phase analysis and microstructural characterization.

Chapter-3 presents the comparison of various properties of HNS and 316L. HNS and 316L were tested for mechanical properties, corrosion resistance and biocompatibility. The HNS and 316L stainless steels are austenitic, free from carbide precipitates at grain boundaries and have negligible ferrite content. The inclusion rating, an important requirement of the implant material, was found within the limit of ISO 5832-1. The microstructure was found free from intergranular corrosion and grain dropping according to standard ASTM A 262 practice A and E. The breakdown potentials of 316 mV_{SCE} and 196 mV_{SCE} are found for the 316L and HNS steels, respectively, from potentiodynamic polarization tests in Ringer's solution. The yield strength of the 316L and HNS was found 279 MPa and 525 MPa, respectively in air. It should be noted that yield strength of the HNS is ~ 2 times that of the 316L. Also, the low cycle fatigue life of the HNS in air was nearly 2 times that of the 316L, irrespective of the strain amplitudes. However, the breakdown potential and endurance limit of the HNS were inferior to that of the 316L. The biocompatibility of HNS was studied in vitro and in vivo. The cell culture and proliferation study exhibited similar cell response for both HNS and 316L stainless steels.

Additionally, *in vivo* animal study of HNS did not show any adverse effect and was found biocompatible.

Chapter-4 presents the characterization of a new grade of austenitic stainless steel, free from nickel, and stabilized by nitrogen and manganese, which was designed with small addition of Mo and developed with the help of M/s Mishra Dhatu Nigam Limited. This steel was characterized for microstructure, corrosion behavior, mechanical properties and biocompatibility. Its characterization showed austenite microstructure with some annealing twins, absence of grain boundary precipitates and negligible delta ferrite. The breakdown potential of the HNS-Mo was found 310 mV_{SCE}, comparable to that of the 316L (316 mV_{SCE}). Its yield strength was found 540 MPa which is higher than that of HNS and ~ 2 times that of 316L. The endurance limit (maximum stress) corresponding to 10^7 cycles of the HNS-Mo decreased from 513 MPa in air to 475 MPa in simulated body fluid environment. HNS-Mo showed stress amplitude of ~ 213 MPa in SBF environment for 10^7 cycles at stress ratio of 0.1, higher than that of the reported value of 200 MPa of 316L. The HNS-Mo showed better mechanical properties and exhibited cell adhesion and proliferation, similar to those of the 316L and HNS. It was further studied extensively for biocompatibility, both *in vitro* and *in vivo*, and showed acceptable *in vitro* cytotoxicity according to ISO 10993-5. The irritation and skin sensitization, acute systematic toxicity and implantation study showed acceptable biocompatibility. Overall, the performance of HNS-Mo was found better in comparison with those of HNS and 316L, and was found comparatively more suitable for biomedical applications.

Chapter-5 presents the effect of USP on microstructure, corrosion resistance, biocompatibility and low cycle fatigue of HNS. There was no deformation induced martensitic transformation of the HNS after USP. The surface grains of the HNS were refined to 15 nm, following USP for 2 minutes with 3 mm shots. Surface hardness was increased by 42%, following USP for 2 minutes with 3 mm shots and there was a gradual decrease in hardness from surface to interior. Also, compressive residual stress was induced after USP and it increased with increase in the duration of USP. Corrosion resistance of HNS was increased, following USP for shorter duration. However, it decreased for 2 minutes of USP due to excessive surface damage. A peculiar effect of USP on LCF life of the HNS was observed. LCF life increased at lower strain amplitude but decreased at higher strain amplitude, following USP. LCF life of the HNS was increased to ~ 18 times by the USP with 3 mm shots for 18 minutes, it was mainly due to delay in crack initiation caused by nanostructured surface and associated compressive residual stress.

Chapter-6 presents the effect of USP on electrochemical corrosion in SBF, biocompatibility (cell culture and proliferation), high cycle fatigue and corrosion fatigue of HNS-Mo. A significant improvement in corrosion resistance, biocompatibility, fatigue and corrosion fatigue was observed, following USP. The breakdown potential of the HNS-Mo was increased from 310 mV_{SCE} to 370 mV_{SCE} and 395 mV_{SCE}, following USP for 30 seconds with 3 mm and 2 mm shots, respectively. Since, there was continuous increase in LCF life of the HNS at lower strain amplitude, with increase in the duration of USP, it shows higher positive effect of USP in the regime of high cycle fatigue. As, high cycle stress controlled fatigue life is more important for implant material, the effect of USP on high cycle fatigue life of the HNS-Mo was studied both in air and in SBF. High cycle fatigue life of the HNS-Mo improved significantly, following USP with 3 mm shots for 3 minutes; the endurance limit (maximum stress) increased from 513 MPa to 572 MPa in air and from 475 MPa to 572 MPa in simulated body fluid environment.

Chapter-7 presents the major conclusions drawn from the present investigation along with suggestions for future work.