INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

People suffering from degenerative joint diseases or injured in accident need surgery to replace the damaged joints or fix the fracture bones using suitable implants. Polymers, composites, ceramics, metals and alloys have been widely used to fabricate implants for various applications [1]. However, metals and alloys are the backbones of orthopedic surgical procedures. A significant increase in the use of implants is noticed in recent years, mainly due to the high demand driven by increasing population, accidents and aging of a large population. Biomaterials may be defined as "materials which are utilized to manufacture different structures for implantation, to repair the diseased or broken parts of biological structures for restoring their functions" [2]. The first and most important requirement of a biomaterial is that it should not cause any toxicity, allergy and inflammation during implantation, i.e., it should be biocompatible. The choice of metallic materials for biomedical applications is based on their acceptability in the human biological environment. Since, the biological environment is harsh and implants undergo static and dynamic loading, they should have sufficiently high mechanical properties like strength, hardness, endurance limit, and resistance against wear, corrosion and corrosion fatigue in human biological environment. Usually, the service life of biomaterials is about 15-20 years. The patients need to go for MRI, following implantation to observe the fixing of implant; therefore, implant materials should be non-magnetic.

1.2. Various Modes of Failure of Orthopedic Implants

1.2.1. Improper Loading of Implants

The service condition of implants is very complicated. Static and dynamic loads are applied on implants depending on the patients' activities. The load applied on different parts of the body varies; the load encountered by the hip, knee and femur is about four, three and two times the body weight, respectively [3]. Fresh bone grows by apposition, which involves forming a new matrix and degrading the older matrix. There are four different types of cells in bone: osteocytes, osteoclasts, osteoblasts and bone lining cells on the surface of bone. Osteoclasts digest away the old bones while osteoblasts lay down new bones. Bones have a unique property of self-healing, which is governed by the proper functioning of these cells [4]. Temporary implants are used for holding the broken ends in close proximity to promote healing and union of the fractured ends. Both, bones as well as implants, share the load. However, implants may fail due to localized plastic deformation caused by overloading and high stresses [5].

1.2.2. Fatigue of Implants

An active person generally walks about two steps, i.e., one cycle per second and people may take one to two million steps per year. It means that the walking frequency is 1 Hz [6]. Therefore, orthopedic implants, subjected to dynamic/cyclic loading during service, may fracture due to fatigue. Fatigue is highly sensitive to surface of implants. Typically, fatigue cracks initiate from the implant's surface, propagate to a critical depth and ultimately lead to catastrophic failure of implants. Implants have intricate shapes and there are many stress raisers like threads, holes, shoulders, etc., within the implants. These uneven structures act as crack initiating sites and orthopedic implants fail due to fatigue [7]. It has been observed that nearly 90% of the fracture surface of cementless hip prosthesis of Ti-6Al-4V alloy was mainly due to fatigue [8].

1.2.3. Corrosion and Corrosion Fatigue of Implants

Implants in human body are exposed to a harsh environment, including blood, water, sodium, chlorine, proteins, temperature, etc. The human biological environment is supposed to contain a saline solution of ~ 0.9 %. Its temperature remains at 37 ± 1 °C and its pH is ~7.4. The composition of human body fluids, like interstitial fluid and synovial fluid, is given in **Table 1.1**.

Table 1.1. Composition of selected components of human body fluids [9].

Component	Interstitial fluid (mgL^{-1})	Synovial fluid (mgL^{-1})
Na^+	3280	3127
K^+	156	156
Ca^+	100	60
Mg^{2+}	24	-
Cl^-	4042	3811
HCO_3^{3-}	1892	1880
HPO_4^{2-}	96	96
SO_4^{2-}	48	48
Organic acids	245	-
Protein	4,144	15,000

There are different simulated body fluid (SBF) solutions like Phosphate buffer saline (PBS), Hank's solution, Ringer's solution and Minimum essential medium (MEM), which are generally used. The chemical ingredients of these SBF solutions are listed in **Table 1.2**.

The pH in the vicinity of implant, may change from 3 to 9, due to biological changes caused by diseases and infections [1,11]. The change in pH significantly affects the corrosion of implants. Pitting, stress corrosion cracking, crevice corrosion and fretting corrosion are the common forms of corrosion that can occur to implants.

Table 1.2. Composition of simulated physiological fluids/solutions [9,10].

Component	PBS (gL^{-1})	Ringer's (gL^{-1})	Hank's (gL^{-1})	MEM (molL^{-1})
NaCl	8.0	8.6	8.0	1.16×10^{-1}
CaCl$_2$	-	0.33	0.14	1.80×10^{-3}
KCl	0.2	0.3	0.4	5.36×10^{-3}
MgCl$_2$.6H$_2$O	-	-	0.10	-
MgSO$_4$.7H$_2$O	-	-	0.10	-
Mg$_2$SO$_4$	-	-	-	8.11×10^{-4}
NaHCO$_3$	-	-	0.35	$2.38\text{-}2.62 \times 10^{-2}$
Na$_2$HPO$_4$	1.15	-	-	8.98×10^{-4}
Na$_2$HPO$_4$.12H$_2$O	-	-	0.12	-
KH$_2$PO$_4$	0.2	-	0.06	-
Phenol red	-	-	0.02	-
Glucose	-	-	1.0	-
Amino acid	-	-	-	5.5×10^{-3}

The disrupted oxide layer due to fatigue is unable to repassivate immediately in an aggressive environment and fatigue may be accelerated due to the corrosion of the exposed region [12]. Pits facilitate nucleation of fatigue cracks in metallic materials [13]. Aqueous environment accelerates the process of crack initiation and reduces the fatigue life of implants [14,15]. Hence, fatigue strength/life decreases drastically because of combined effect of corrosion and fatigue, known as corrosion fatigue [16]. Moreover, fretting between implant and bone makes it difficult to repassivate and further accelerates the fatigue. The chances of crevice corrosion are higher in temporary implants, such as at the interface of plates and screws. There is a very high possibility of crevice-corrosion assisted corrosion fatigue. Amel-Farzad et al. did failure analysis of an orthopedic stainless steel implant (barrel plate), which was implanted in the patient's thigh for two

years. Pits and cracks were visible on surface of the implant with unaided eyes and the reason for failure was corrosion fatigue, assisted by a crevice corrosion mechanism [17].

1.3. Metallic Biomaterials

Several metallic materials are commonly used as implant materials. Titanium, cobalt-chromium-based alloys, stainless steel, tantalum, gold and niobium are widely used metallic biomaterials nowadays, in medical applications [18]. In actual practice, these materials were developed for industrial applications. However, they showed such an excellent mechanical and chemical response that they have been used for biomedical purposes. Metallic implants passivate by forming a passive protective film on the surface. Still, there is leakage current and the metallic implants corrode in the body environment. Eventually, they leach out metallic ions, which may have hazardous effects. Cobalt ions inhibit the osteoblast function [19]. Moreover, cobalt metal, present in Co-Cr alloys, is considered carcinogenic, mutagenic and toxic to reproduction [20]. The toxic biological response of vanadium and aluminium, present in titanium alloys, has been observed [21]. Vanadium and aluminium powders inhibit the growth of L929 and MC3T3-E1 cells [22]. Stainless steels are iron-based multicomponent solid solutions consisting of iron, chromium, nickel, manganese, molybdenum as substitutional elements, and carbon, nitrogen as interstitial elements. Stainless steels can be classified into five classes: austenitic, ferritic, martensitic, duplex and precipitation hardening. The crystal lattice structure of austenitic stainless steel is FCC. The austenite phase in the conventional stainless steel is stabilized by nickel. Austenitic stainless steels are non-magnetic and have a good combination of strength and ductility. Mechanical properties of the commonly used 316L are compared with those of the different widely used metallic alloys, as implant materials, in **Table 1.3**.

Table 1.3. Comparison of various mechanical properties and applications of metallic alloys [1,23].

Material	316L (annealed)	Cast Co-Cr alloy	Titanium	Ti-6Al-4V	Human bone
Young's modulus (GPa)	211	241	121	121	30
Vickers hardness (H_v)	190	300	-	-	26.3
Yield strength, YS, (MPa)	280	490	470	970	-
Ultimate Tensile strength, UTS, (MPa)	650	690	710	1000	137
Total elongation (%)	45	8	30	12	1.5
Fatigue limit (MPa)	280	300	300	-	-
Type of implant	Orthodontic wire, plates and screws, bone fixation	Artificial joints, bone fixation	Stent, plates and screws, artificial joints, orthodontic		-

The strength, hardness and corrosion resistance of 316L are poor in respect of titanium and Co-Cr alloys [24]. Therefore, there is a possibility of failure after a while; hence, the application of 316L stainless steel, as a permanent implant is restricted. However, 316L is popular among these metallic alloys due to its comparatively lower cost [25] and is used to manufacture temporary fixation devices such as nails, plates and screws.

1.4. 316L Austenitic Stainless Steel as a Biomaterial

Different classes of stainless steel differ in their magnetic behaviour, as martensitic and ferritic stainless steels are ferromagnetic, whereas fully austenitic stainless steels are non-

magnetic [26–28]. Therefore, austenitic stainless steels are the choice for biomedical applications due to the complete absence of ferromagnetism. The materials for implants should possess sufficient strength, good corrosion resistance in human body, high wear resistance, high fatigue and corrosion fatigue strength, and adequate cell response in the physiological environment [18,29]. Austenitic stainless steels possess several attractive properties like high formability because of FCC crystal structure, low yield to tensile strength ratio, which is increased by cold working, good corrosion resistance and non-magnetic behavior [25,30–35].

In medical applications, AISI 316L, ASTM F138 and ASTM F1314 stainless steels are mostly used. The 316L possesses acceptable load-bearing capability and biocompatibility [30,34]. However, it has a poor reputation in terms of corrosion resistance in the environment of body fluid. Intergranular, pitting, fretting, crevice corrosion are the most common forms of corrosion of stainless steels [1,24]. Colangelo et al. examined orthopedic appliances of 316 stainless steel after being removed from human body. Corrosion damage occurred on 91% of all multicomponent devices, whereas corrosion was observed for 37% of the individual components [36]. Walczak et al. [37] examined eleven surgically retrieved implants of austenitic stainless steel 316L. Out of these, nine of them had areas of corrosion, covering length of 1-5 cm of the stem, partially or over the entire surface. Corrosion was found in layers and the nickel content of the alloy, which was initially 13%, was persistently absent, indicating nickel's preferential release. In addition to that, detailed survey of failed stainless steel implants was conducted and corrosion was found as one of the major causes of failure [1,3,12]. Therefore, it is clear that during implantation, 316L stainless steel corrodes, though it has an inherent property of resisting corrosion and degradation, and it results in release of iron, chromium and nickel ions in the body during implantation. It should be noted that

the chance of corrosion increases with the duration of implantation. Corrosion, wear, fatigue, fretting, corrosion fatigue and their synergistic effects are responsible for the failure of implants [5,36,38]. The metallic debris released due to corrosion accumulates in the human body [39,40]. Nickel, present in 316L, is toxic to human health and may inhibit cell growth [41,42].

Many researchers have studied nickel sensitivity to human body and found nickel as a possible allergen [43–45]. Nickel (Ni) causes allergic reactions resulting in eczema, swelling, itching, reddening, and carcinogenic and teratogenic effects in human body [46–48]. The first allergic reaction to orthopedic implant was reported for stainless steel fracture plate and it was described as an eczematous rash [49]. After that, many similar incidents were reported when patients suffered from problems in the periphery of implants [50–52]. The allergy potential of nickel, released from metals and alloys, is generally determined by the DMG tests. However, it has been observed that the materials with negative DMG test can also release a considerable amount of nickel, which may cause sensitization [53]. The lung fibrosis, kidney diseases and cardiovascular problems associated with high nickel intake are also reported [41]. IARC of WHO estimates Ni-alloys as possibly carcinogenic to the human body [54].

Moreover, many researchers studied the role of the corrosion products released from 316L stainless steel on the cellular response. Bone marrow cells, exposed to 0.1% and 1% corrosion product of 316L, were impaired and resulted in cell death, respectively, after 18 days [55]. The corrosion products of 316L changed the cell morphology of smooth muscle cells and induced cell necrosis [42]. The ALP activity was reduced significantly in MC3T3-E1 cells cultured on 316L due to increased concentration of Ni in cells contacting 316L [56]. Nickel inhibited cell proliferation of bronchial epithelia cells [57]. The toxic effect of nickel nanoparticles was observed on the human lung

epithelial A549 cells [58]. Cell death of U937 cells caused by Ni was found dependent on dose and time [59]. Therefore, it is clear that corrosion product of 316L containing nickel may impair and damage contacting cells and hinder the growth of cells.

1.5. Nickel Allergy: Government Policies and Directives and Need for the Development of Nickel-Free Austenitic Stainless Steel

It has been well established that nickel is toxic to the human body. Many countries have restricted nickel-containing metallic materials for biomedical applications, considering the harmful effects of nickel. Denmark has forbidden nickel releasing objects that are in contact with skin and the amount of nickel release has been more than 0.5 µg/cm^2/week since 1991. The European parliament has appealed to the member of states of the European community to forbid or restrict nickel-containing alloys for specific applications **(Directive 94127/EC from 30 June 1994)**. In a second directive (**2004/96/EC),** the limit of nickel release has been restricted to 0.2 µg/cm^2/week for the use in "piercing in human body". China has also restricted the release of nickel in body piercing to 0.2 µg/cm^2/week and in an article intended to come in direct and prolonged skin contact such as jewelry items to 0.5 µg/cm^2/week **(GB 28480-2012)**. The toxic effects of the materials containing nickel on the human body and many governments' policies have been a problem and have attracted global attention. Therefore, there is much scope and need for a technology to develop the next generation of a medical implant grade of austenitic stainless steel without nickel or negligible amount of nickel that overcomes the disadvantages of implant materials known in state of the art.

1.6. Role of Alloying Elements in High Nitrogen Austenitic Stainless Steels Without Nickel

The elements in the nickel-free stainless steel play a vital role in modifying different properties, such as phase formation, corrosion resistance, mechanical properties,

fabrication, weldability, etc. The primary alloying elements of the austenitic stainless steels stabilized by nitrogen are nitrogen, manganese, chromium and molybdenum.

1.6.1. Role of Carbon (C)

Carbon is a non-metallic element that is always there in iron-based alloys. It is an interstitial element and enhances the strength [60]. The influence of carbon on the corrosion behavior of alloys is decided by how does it exist. It may combine with chromium and form chromium carbide, due to which there is chromium depletion in the solid solution, which deteriorates the resistance against corrosion of alloys [61]. Chromium carbide forms when stainless steels are heated in a specific temperature range [62]. Therefore, carbon content is kept low in medical-grade of austenitic stainless steels to avoid precipitation.

1.6.2. Role of Chromium (Cr)

Chromium is like a wonder element for stainless steel, which is responsible for its high corrosion resistance. It improves both general as well as localized forms of corrosion resistance. A protective layer forms on the surface due to Cr, which is known as the passive layer or passive film. This film is of 1-3 nm thickness, responsible for the drastic reduction in corrosion rate [63]. Moreover, chromium helps in the addition of nitrogen to solid solution. However, it is a strong ferrite stabilizer and high amount of chromium increases the risk of formation of intermetallic precipitates such as sigma, chi, carbides and nitrides in the austenitic stainless steels [51].

1.6.3. Role of Molybdenum (Mo)

Molybdenum (Mo) is intentionally added in stainless steel to improve the resistance of stainless steel against localized types of corrosion such as pitting and crevice corrosion in harsh environments, like acidic and chloride-containing environments. Mo reduces the

intensity of oxidizing effect and decreases the tendency of breaking of the protective layer formed. Mo in the alloys in the presence of nitrogen causes significant increase in stainless steels' pitting resistance. Therefore, the role of Mo on the resistance against corrosion is higher when nitrogen is there [64]. Moreover, Mo increases solubility of nitrogen. However, Mo is a ferrite stabilizer. A high amount of Mo in stainless steel cannot be added, leading to an intermetallic σ-phase. The formation of intermetallic phases in stainless steel significantly decreases the toughness and corrosion resistance [51,52]. Further, Mo improves the strength of stainless steel by solid solution hardening.

1.6.4. Role of Manganese (Mn)

Manganese stabilizes austenite and increases the solubility of nitrogen in austenite. These two properties make it a suitable nickel substitute for austenitic stainless steel, without nickel [65]. Mn is added in AISI 200 grade of stainless steels where its content varies from 4 to 15.5 wt%. However, manganese has negative effect on resistance against corrosion. Wu et al. [66] reported that phases rich in Mn, in stainless steel, are more prone to release electrons quickly and act as an anode. The overall corrosion resistance of stainless steel decreases. The pitting potential was found lower in high manganese steels than in low manganese steels [67].

1.6.5. Role of Nitrogen (N)

Nitrogen is in gaseous form at room temperature. It is present in all the steels as an interstitial, quite similar to that of carbon. Its content varies from as little as 20 ppm to 1 wt%. The solubility of nitrogen is significantly less in ferritic steels than in austenitic steels. There are many advantages of adding the element nitrogen. The Ni_{eq} coefficient of nitrogen is 18, whereas that of nickel is 1 [51]. Therefore, nitrogen has strong austenite forming capability of 18 times more than that of nickel. It is considered the most suitable replacement of nickel in austenitic stainless steel. In addition to being a strong austenite

stabilizer, nitrogen is regarded to be relatively more effective as solid solution strengthening element in respect of carbon [60]. Interstitial nitrogen causes significant lattice distortion, which strengthens the steel. Moreover, pinning of dislocations by interstitial nitrogen, due to the electrostatic attraction, attributes to strengthening [68]. Further, nitrogen in stainless steel prefers to be in the neighborhood of chromium and there is Cr-N short-range ordering which may also contribute to the strengthening of steels [69,70]. Overall, there is significant strengthening of stainless steel due to nitrogen. Nitrogen significantly affects the dislocation structure of stainless steel, which attributes to fatigue behavior. A distinct role of nitrogen has been observed on stacking fault energy (SFE) in nickel and manganese-nitrogen containing austenitic stainless steels. There was increase in SFE with increase in nitrogen up to 0.40 wt%, after that, there was a decrease in SFE with a further increase in nitrogen content for nickel-containing stainless steel. However, an opposite trend was observed for high manganese-nitrogen containing stainless steel. Initially, it decreased with the nitrogen content and again was found to increase with increasing nitrogen content [71]. The stacking fault energy of 41 mJ/m^2 and 22.8 mJ/m^2 has been reported for Fe-15Cr-17Mn-0.8N and Fe-18Cr-10Mn-0.69N austenitic stainless steels, respectively [71,72]. The addition of nitrogen in austenitic stainless steel favors planar dislocation arrangement and reduces the tendency of cross slips, promoting cyclic softening. Cyclic softening of the austenitic stainless steel with high nitrogen has been attributed to the disordering of Cr-N short-range ordering [73].

A high amount of nitrogen in austenitic stainless steel is problematic at cryogenic temperature as it drastically decreases the toughness. In general, austenitic stainless steels do not show brittle fracture due to sufficiently higher ductility. However, high nitrogen and manganese-containing austenitic stainless steels are exceptions, as they exhibit ductile to brittle transition [74–76]. Nitrogen in austenitic stainless steel provides stability

to the austenite phase. It suppresses thermal and deformation-induced martensite formation by lowering the M_s and M_{d30} temperature. M_{d30} temperature is an important parameter that is commonly used to describe the martensitic transformation induced by deformation in austenitic stainless steels of metastable nature. Many empirical relations have been proposed and have been summarised by Hahnenberger et al. [77]. The empirical relations proposed by Eichelmann and Sjoberg for M_s and M_{d30}, respectively, are given in **Eqs. 1.1 and 1.2** [78,79]**.** These equations signify the effect of different elements on M_s and M_{d30} temperatures.

$$M_s\ (°C) = 1350 - 28\ \%Si - 33\ \%Mn - 42\ \%Cr - 1665\ \%\ (C + N) - 61\%Ni \quad (1.1)$$

$$M_{d30}\ (°C) = 608 - 515\ \%C - 821\ \%N - 7.8\ \%Si - 12\ \%Mn - 13\ \%Cr -$$
$$34\ \%\ Ni - 6.5\ \%\ Mo \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad (1.2)$$

Where, M_S is martensitic start temperature and M_{d30} refers to temperature, at which, if the material is given 30% tensile strain, 50% α' martensite will form. Here, the concentration of elements is in wt%.

It is clear from these relationships that nitrogen has high potential for suppressing transformation to martensite significantly. However, austenitic stainless steel with high nitrogen showed martensitic transformation at cryogenic temperature (-196 °C) and consequently, a drastic reduction in the toughness was observed [80]. Tanaka et al. [81] studied the brittle to ductile transition (BDT) in high nitrogen austenitic steel without nickel. Samples tested at temperatures in the lower shelf region did not exhibit the sign of plastic deformation. Thus, it was speculated that difficulty in dislocation gliding was attributed to higher activation energy required for the BDT temperature. The very high value of activation energy was thought to be associated with an increase in Helmholtz free energy and internal stress for the dislocation gliding, caused by nitrogen [81]. Two

empirical relations have been reported to predict ductile to brittle transition temperature (DBTT) of nickel-free high nitrogen austenitic stainless steels. It depends predominantly on nitrogen content, as given in **Eqs. 1.3 and 1.4** [52,80]. Considering this negative effect of addition of nitrogen in stainless steel, limiting of nitrogen content within 0.9 wt% for biomedical applications was suggested [52].

$$DBTT = 300\ \%N - 303 \tag{1.3}$$

$$DBTT = 300\ \%N + 100\ \%C - 303 \tag{1.4}$$

In **Eqs. 1.3 and 1.4,** DBTT is in °C, and the concentration of nitrogen (N) and carbon (C) is in wt%.

Nitrogen improves the pitting and crevice corrosion resistance in the presence of chromium and molybdenum [65]. The theoretical pitting resistance of stainless steel in a chloride-containing environment may be estimated by pitting resistance equivalent number (PREN), which depends on stainless steel's Cr, Mo and N content. Uggowitzer et al. [52] have proposed an equation for the PREN number, as shown in **Eq. 1.5**. In another study [82], the formula for the PREN was proposed for manganese-containing stainless steels, as shown in **Eq. 1.6**. MARC is a new formula proposed for theoretical validation of corrosion resistance of materials as given by **Eq. 1.7** [83]. MARC is defined as the "measure of alloying for resistance against corrosion". It was correlated with temperature of critical pitting corrosion and critical crevice corrosion and both were observed to increase with MARC [84]. Therefore, beneficial effect of nitrogen on corrosion resistance can be understood as it significantly increases the PREN and MARC values.

$$PREN = \%Cr + 3.3\ \%Mo + 20\ \%\ N \tag{1.5}$$

$$PREN1 = \%Cr + 3.3\ \%Mo + 30\ \%\ N - 1\%\ Mn \tag{1.6}$$

$$MARC = \%Cr + 3.3\ \%Mo + 20\ \%\ N + 20\ \%\ C - 0.5\ \%\ Mn - 0.25\ \%\ Ni \tag{1.7}$$

In **Eqs. 1.5, 1.6 and 1.7,** the concentration of elements is in wt%. Nitrogen in stainless steel shows significantly high pitting resistance in chloride-containing environments due to its highly repassivation tendency [85]. It was found by Baba et al. [67] that almost 100 % of nitrogen dissolved into the solution transformed into NH_3 and suggested that NH_4^+ consumes the H^+ ions in the pit, which may suppress the growth of the pit and promote the repassivation. Enrichment of nitrogen at the interface of steel and the passive layer was observed, which may increase the stability of surface oxide film [86]. Wang et al. [87] observed CrN short-range ordering in the passive film and matrix for high nitrogen stainless steel. This short-range ordering is unstable on surface of the passive film. It dissolves in the solution to produce chromium oxide, which further strengthens the passive film. Therefore, enrichment of chromium and nitrogen in passive oxide film and at the interface of steel and passive layer, as well as inhibition in growth of pits due to formation of NH_4^+, enhance the pitting potential of the stainless steels containing nitrogen. Ahila et al. [88] observed the beneficial role of nitrogen on repassivation of Cr-Mn steel.

The presence of nitrogen in stainless steel promotes precipitation of Cr_2N when heated in the range of 500 – 1000 °C. The increase in nitrogen content significantly decreases the time of isothermal annealing required for nitride formation. However, austenitic microstructure without Cr_2N and σ-phase can be obtained by using a balanced range of nitrogen in Cr-Mn stainless steel [51].

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

Implant quality grade 316L (ASTM F138 and F139) has been used for orthopedic applications. However, ASTM F1314 and ASTM F1586, nitrogen strengthened austenitic stainless steels, were developed for biomedical applications [89,90]. The ASTM F1586 was developed in 1986 with higher fatigue strength and corrosion resistance than AISI 316L [90]. Due to the enhanced properties, the ASTM F1586 has been recommended for use as stem material in permanent hip replacement [91]. Nickel has not been removed entirely but partially by nitrogen, manganese, and carbon in these steels and these are put under the AISI 200 series [92].

With increasing attention towards nitrogen strengthened stainless steel and the toxic effects of nickel, further efforts have been made to replace nickel. The rationale for developing nitrogen stabilized austenitic stainless steel, free from nickel, is the harmful effect of nickel-containing alloys. Also, the high cost of nickel triggered a need to replace nickel in austenitic stainless steel with suitable substitutes [93]. The chemical composition of austenitic stainless steels developed for biomedical application is given in **Appendix A**. Developing austenitic stainless steel, free from nickel, for biomedical applications aims to find a composition that would lead to an austenite phase without delta ferrite at room temperature. Nickel (Ni) in austenitic stainless steel acts as an austenite stabilizer [51]. Therefore, using other austenite stabilizers like manganese (Mn) and nitrogen (N) is economical. A very high amount of nitrogen is needed to stabilize the austenite phase in nickel-free stainless steel. Uggowitzer et al. [52] introduced the philosophy for developing Fe-Cr-Mn-Mo-N based nickel-free austenitic stainless steel, which contained 15-18 % Cr, 10-12% Mn, 3-6% Mo and about 0.9% N, in a conference in 1995 (HNS 95). In the same conference, high nitrogen austenitic stainless steel, free

from nickel, was proposed for biomedical use by Menzel et al. [51]. After that, many studies related to the properties of high nitrogen and manganese stabilized stainless steels, without nickel, have been carried out. Some of the medical grades of nickel-free or negligible nickel austenitic stainless steels successfully developed are Biodur 108, P2000 (X13CrMNMoN18-14-3), PANACEA P558, BIOSS, 0Cr18Mn15Mo2N0.64, etc. [68]. Biodur 108 was listed in ASTM standard as ASTM F2229 in 2002 [94]. The ASTM F2581, high manganese and nitrogen grade of stainless steel with negligible nickel and considerably high carbon, is also developed for biomedical applications [95].

1.7.1. Philosophy of Alloy Design of Nickel-Free Nitrogen Stabilized Austenitic Stainless Steel

The microstructure of stainless steel is highly dependent on its chemical constituents. The role of alloying elements in stainless steels may be expressed in terms of nickel equivalent (Ni_{eq}) and chromium equivalent (Cr_{eq}), as shown in **Eqs. 1.8 and 1.9** [51].

$$Ni_{eq} = \%Ni + \% Co + 0.1\% Mn - 0.01(\%Mn)^2 + 18\%N + 30\%C \qquad (1.8)$$

$$Cr_{eq} = \%Cr + 1.5\% Mo + 1.5\% W + 0.48\% Si + 2.3\%V + 1.75\%Nb + 2.5\% Al$$

$$(1.9)$$

In **Eqs. 1.8 and 1.9**, the concentration of elements is in wt%. The elements contributing to Ni_{eq} stabilize the austenite phase, whereas the elements contributing to Cr_{eq} stabilize the ferrite phase. The Schaffler diagram is useful to theoretically estimate the phase based on the chemical constituents **(Fig. 1.1)**. It is clear from **Fig. 1.1** that if one wants to design stainless steel of a particular phase, then a proper combination of Ni_{eq} and Cr_{eq} needs to be chosen, which ultimately depends on the amount of various elements. Therefore, by varying the chemical constituents, the required phase or combination of phases can be found.
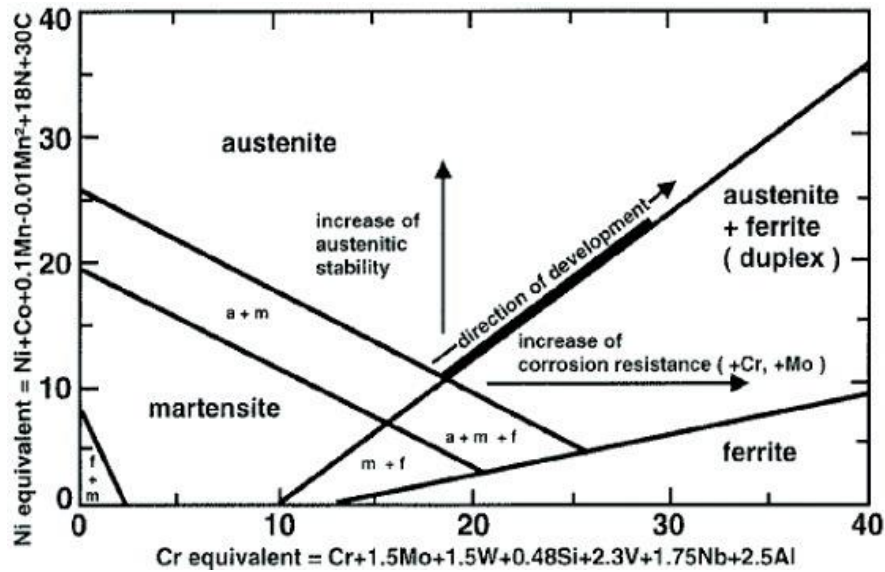
Fig. 1.1. Modified Schaeffler diagram for predicting stable phase of stainless steel from Cr and Ni equivalent [51].

Nickel in stainless steel stabilizes the austenite microstructure. Carbon (C), nitrogen (N), manganese (Mn) and cobalt (Co) also contribute to the austenite phase. Therefore, these elements may replace nickel to get a nickel-free austenitic stainless steel [65]. Carbon has a very high Ni_{eq}, but it cannot be used as a replacement, as it is harmful from a corrosion point of view. Cobalt is very expensive and its biocompatibility is also questionable [96]; therefore, it can be avoided. Nitrogen is the best choice for nickel replacement, as it has a very high coefficient of Ni_{eq}. However, a very high amount of nitrogen in austenite increases DBTT and decreases ductility [52,80]; therefore, it is suggested to limit the nitrogen content below 0.9 wt% to attain a homogeneous microstructure, free from precipitates, with adequate ductility.

Corrosion resistance of stainless steel is also an essential factor for its use for the purpose of biomedical devices. Thus, a new grade of nickel-free austenitic stainless steel should be highly resistant to corrosion. Cr and Mo are the alloying elements, which significantly improve the corrosion resistance of stainless steel. They also increase the solubility of nitrogen in austenite. However, they increase the susceptibility of nitride (Cr_2N) and σ-

phase formation, ultimately affecting the corrosion and toughness of stainless steel. Mn has a great potential to suppress nitride formation in stainless steel and significantly increases the solubility of nitrogen in stainless steel. In stainless steel, the increase in solubility of nitrogen by Cr and Mo is insufficient because very high amount of nitrogen is needed. Therefore, it is essential to add Mn for increasing the solubility of nitrogen. In addition, Mn is an austenite stabilizer that will further promote the stability of austenite [51].

Based on the above philosophy, Uggowitzer et al. suggested using the composition of austenitic stainless steel, without nickel, with high nitrogen content in which (Cr + 1.5 Mo) ~ 23%, Mn ~ 11%, N ~ 0.9% [52]. Menzel et al. suggested composition for biomedical application as Cr ~18%, Mn ~ 18%, Mo ~ 2% and N ~ 0.9% [51].

1.7.2. Mechanical Behavior, Corrosion Resistance and Biocompatibility of Nickel-Free Nitrogen Stabilized Austenitic Stainless Steel

Nickel-free austenitic stainless steels have been developed earlier, based on Fe-Cr-Mn-N and Fe-Cr-Mn-Mo-N systems as given in **Appendix A** and studied for their mechanical behavior, resistance against electrochemical corrosion and biocompatibility, these are summarised in **Appendices B and C**. Fe-Cr-Mn-N austenitic stainless steel exhibited superior abrasion resistance in synthetic mine water and cavitation erosion resistance in distilled water than Hadfield steel [97]. The corrosion fatigue limit of Biodur 108 at 10^6 cycles was significantly higher in Ringer's solution [98]. Slip and twinning, both the mechanisms, were observed during the tensile deformation of Fe24Mn13Cr1Ni0.44N and Fe24Mn18Cr3Ni0.62N steels [99]. The high cycle fatigue cracks were preferentially initiated along annealing twin boundaries for Biodur 108 (ASTM F2229) [100]. The bend rotating fatigue strength of Fe24Mn13Cr0.44N at 10^7 cycles was 341 MPa at 40 Hz frequency and stress ratio (R) of -1 [101]. It was observed that there was no significant

difference in the fatigue strength of Fe-23Cr-1Mo-1N steel in air and PBS solution at 10^7 cycles, and it was found 320 MPa [102]. However, its fretting fatigue strength was reduced to 280 MPa and 240 MPa in air and PBS, respectively and was significantly higher than that of the 316L (180 MPa) [103]. The low cycle fatigue life of Fe-18Cr-18Mn-0.63N decreased with rise in strain amplitude. Significant cyclic softening was observed during the testing [104]. A substantial increase in tensile and high cycle fatigue strength, with an increase in content of nitrogen, was observed [105,106]. Fatigue limit of 425 MPa and 475 MPa was observed for 0.40 wt% and 0.60 wt% of nitrogen, at 10^7 cycles with 10 Hz frequency and R of 0.1. The influence of strain rate on strength in tensile loading and mechanism of deformation has been observed for austenitic stainless steels with high nitrogen. There was an increase in strength and decrease in ductility with increasing strain rate [107–109]. Cracks were found to nucleate along the grain boundaries or slip bands at a higher strain rate. However, they initiated preferably at inclusions during low straining (10^{-4}/sec) [107]. This steel exhibited two-stage strain hardening behavior [107,108].

The resistance of the high nitrogen-containing stainless steels against corrosion in an acidic salt solution is superior to that of conventional nickel stabilized austenitic stainless steel [66]. The protective film formed in NaCl salt solution was found stable. However, the stability decreased with the addition of H_2SO_4 [110]. The EIS study showed one inner passive layer, whose resistance increased with immersion time, related to uniform corrosion, and one outer porous layer [111]. The stainless steels containing high nitrogen showed higher resistance against pitting and crevice corrosion in 3.5 % NaCl at different pH of 1, 3, 6, 7 and 9. An increase in pitting potential and crevice pitting temperature (CPT) was found with an increase in the content of nitrogen [84]. Increase in nitrogen content in stainless steel stabilizes the passive film. The protecting ability and thickness of the passive film increase with increase in nitrogen content. The protective film formed

by passivation on high nitrogen steels showed n-type of semiconductor behavior and a decrease in donor density was found with an increase in nitrogen content [112]. It was suggested that the addition of nitrogen accelerates the dissolution process, which is attributed to the accumulation of passivation species and improvement in passivity [113]. Fe-Cr-Mn-Mo-N steels exhibited higher resistance against pitting as compared to 316L in Ringer's solution and artificial saliva solution under static and applied load [114]. P2000 steel showed good resistance against corrosion, cyclic loading and sliding wear in Ringer's solution, and its in vitro cell cytotoxicity was found adequate [115,116]. The P558 stainless steel was studied in MEM solution and its corrosion resistance was found comparable to that of ISO 5832-9 steel [117]. This steel demonstrated acceptable biocompatibility, both *in vitro* and *in vivo* [118]. Platelets adhered on the stainless steels stabilized by high nitrogen were less than that on 317L [119]. There was higher cell growth and enhanced osteoblast differentiation on steel with high nitrogen as compared to 317L [120]. MTT assay performed with HUVECs cells did not show a significant toxic effect. There was no significant effect on platelet adhesion and the hemolysis rate was under 5% [121]. The role of nitrogen on biocompatibility was studied extensively, there was increase in kinetic clotting time and decrease in adhered platelets, with increasing nitrogen content [119]. Increase in MG-63 cells cytocompatibility was observed with increase in content of nitrogen [120]. The cell cytotoxicity of MC3T3-E1 cells was assessed by MTT and alkaline phosphate assay (ALP). ALP mineralization showed good cell response with increase in nitrogen content. The observed hemolysis rate was according to the standard limit [122]. BIOSSN4 was extensively studied for tensile behavior, resistance against corrosion and biocompatibility. The YS and UTS of 00Cr18Mn15Mo2N0.62 steel were found 537 MPa and 884 MPa, respectively. It showed better resistance against corrosion in Hank's solution at 37 °C and blood compatibility than 316L [123]. The BIOSSN4 showed better blood compatibility than 316L [124].

0Cr18Mn15Mo2N0.64 possessed a better combination of mechanical properties, resistance against corrosion in Hank's solution and blood compatibility than 316L [125]. It should be noted here that BIOSSN4, 00Cr18Mn15Mo2N0.62 and 0Cr18Mn15Mo2N0.64 steels are different in terms of nitrogen content which varied from 0.45 to 0.70.

Therefore, it is obvious from the extensive literature review that manganese and nitrogen stabilized austenitic stainless steels possess superior mechanical properties such as strength, hardness, fatigue strength, corrosion fatigue resistance, etc. They are biocompatible and have good corrosion resistance. They did not show any significant toxic or adverse effect on cell adhesion, proliferation and growth. There was a decrease in susceptibility to stress corrosion of Fe18Cr10Mn austenitic stainless steel with increased nitrogen content [126].

1.8. Surface Modification of Metallic Materials

Corrosion, fatigue, and functioning of cells are surface properties and these are susceptible to surface of the components. The surface roughness, size of grains and associated stress exert appreciable influence on fatigue life, corrosion resistance, cell adhesion and proliferation [127–134]. Enhancement of these properties without change in the chemical composition at the surface of biomedical material is a great challenge. There are many techniques such as laser shock peening [135–137], sandblasting [138,139], conventional shot peening [140], surface mechanical rolling treatment [141] and ultrasonic shot peening (USP) [142–145], which have been used to refine surface grains of different metallic alloys. These techniques have been found effective in grain refinement of micron size surface grains to nanometer size and induce residual compressive stresses up to a certain depth. Among these, USP is considered a more versatile, effective and fast processing technique. A thicker layer of refined grains is

produced by this technique, also it causes less roughness on the surface and higher compressive stress is induced as compared to the conventional shot peening [146].

1.8.1. Ultrasonic Shot Peening

The ultrasonic shot peening (USP) principle is to impinge surface by vibrating shots of hard steel through high-power ultrasonic waves. It is different from the conventional shot peening process in terms of the transfer of energy to the shots. The vibrating body triggers shots in USP, whereas pneumatic, centrifugal and blast machines are used in other peening processes. The USP set-up is shown schematically in **Fig. 1.2**.
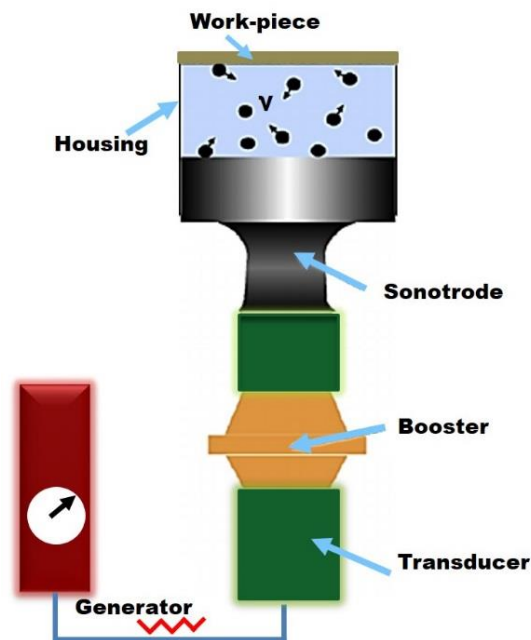

Fig. 1.2. Schematic representation of USP set-up.

It has an acoustic assembly that generates mechanical vibration with a very high frequency in the ultrasonic range. The generator produces ultrasonic signals translated into mechanical vibration by a transducer and amplified by an acoustic booster (**Fig. 1.2**). Finally, sonotrode emits mechanical vibration at high frequency in the ultrasonic range of 20 kHz. In this process, the vibrational energy of sonotrode is transferred to hard shots of steel with 1-8 mm diameter, kept in a closed housing, which repeatedly impacts the

surface of the workpiece in random directions with a high velocity (3-20 m/sec). With a very high kinetic energy, the impacts of shots plastically deform the surface of the material at a high strain rate of 10^3/sec. Eventually, severe plastic deformation of material occurs due to the repeated multidirectional random impacts of shots resulting in refinement of grains to nanolevel in the surface region [142,147,148].

Due to USP, a gradient microstructure is developed with very fine grains near the surface, which gradually increases with increasing depth from the surface to interior of the material, without any sharp interface. A significant improvement occurs in surface hardness and inducement of compressive residual stress, following USP [134]. The material's surface becomes rough without change in chemical composition due to the indentations made by the impinging shots with high kinetic energy. The depth of gradient microstructure, induced compressive stress and hardness of the USPed samples depend mainly on the process parameters. These include duration of USP, shot diameter, vibrational frequency and amplitude of oscillation [143,149–152]. This technique of surface nanostructuring of metallic materials was found to enhance the properties such as tensile strength, fatigue strength, wear resistance, cell adhesion, and resistance to electrochemical corrosion and corrosion fatigue [129,130,133,144,145,149,153,154].

A significantly large number of grain boundaries on the nanostructured surface, caused by USP, provide high density of nucleation sites for oxide formation [155]. It quickly forms a highly protective oxide film, rich in chromium, on the surface [156,157]. The delay in crack initiation and slow crack propagation caused by nanostructured surface and imparted compressive residual stress enhances the fatigue life of the metallic materials, following USP [134,158]. A very high number of grain boundaries at the USPed surface inhibits the slip band formation [158]. There is further delay in crack initiation and the rate of crack propagation in the presence of compressive stress induced by USP at the surface and fatigue life is significantly enhanced. USP changes surface

topography of the metallic material and improves the hydrophilicity/wettability [150]. The physicochemical nature of the surface greatly influences adhesion and proliferation of cells on the surface of metallic materials. The significant increase in the grain boundary of materials, following USP, provides many preferential sites for the adsorption of proteins, which increases the cells' biological function at the interface [129,131,159].

1.8.2. Role of Surface Nanostructuring on Various Properties of Metallic Materials

Several studies have been carried out, related to the influence of nanostructured surface on the mechanical behavior, cellular response and resistance against corrosion. The corrosion resistance of AISI 4140 low-alloy steel increased with an increase in the shot peening intensity [160]. The tribological behavior of metallic materials has been extensively studied following severe plastic deformation. There was increase in wear resistance due to the improvement in surface hardness and nanostructuring in the surface region [161–164]. The effect of ultrasonic shot peening (USP) and surface mechanical attrition treatment (SMAT) on tensile properties, corrosion resistance in various environments, wear rate, low cycle and high cycle fatigue, corrosion-fatigue are summarised in **Appendix D**. It is clear from this summary that parameters like duration of peening and shot size significantly influence the properties. There is a drastic reduction in the size of grains to nano level following SMAT and USP. The bigger shots have higher associated energy, deform the surface to a greater depth, and substantially affect the hardness and strength. The life of the component under high cycle fatigue depends on the strength of the material. Therefore, the specimens shot-peened with bigger shots have higher fatigue strength than those with smaller ones [158]. Fatigue life significantly increases in the high cycle fatigue regime; whereas there is little/no effect in the low cycle fatigue regime or higher stress levels. It may be due to relaxation of the compressive stress at higher stress levels during early cycling [134]. The LCF life of the 316L was unaffected at lower strain amplitudes whereas it decreased at higher strain amplitudes,

following SMAT [165]. Enhancement in corrosion resistance was observed due to the formation of intact and highly protective film in USPed samples. Still, defects density increased at the surface from longer duration of SMAT/USP and corrosion resistance decreased. There was increase in cell attachment, spreading and proliferation rate due to the significant increase in grain boundary, caused by SMAT and USP [129].

1.9. Surface Modification and its Impact on Properties of Nitrogen Stabilized Austenitic Stainless Steel

Cold rolling and USP were used to modify the surface condition of materials [105,166–168]. The various work related to cold working and USP of high nitrogen-containing stainless steels, free from nickel, have been summarised in **Appendix E**. Detailed investigations were made to observe effect of cold working on microstructure, hardness, tensile properties, fatigue, corrosion, corrosion-fatigue and biocompatibility behavior of the nitrogen and manganese stabilized stainless steels. A significant improvement in fatigue, corrosion fatigue and cell adhesion and proliferation was observed following cold working. However, both negative and positive effects have been observed for corrosion resistance after cold rolling. There is only one study related to the effect of USP on microstructure, hardness and low cycle fatigue behavior of nickel-free high nitrogen stainless steel [167]. LCF life of this steel, at total strain amplitudes of $\pm 0.60\%$, $\pm 0.80\%$ and $\pm 1.0\%$, was found to decrease following USP.

Deformation-induced martensitic phase transformation has been observed in 316L and 304L conventional austenitic stainless steels, following severe plastic deformation [158,169]. However, no phase transformation was observed in the high nitrogen-containing stainless steel following USP [167]. There was a deleterious effect of martensitic transformation on the corrosion resistance of austenitic stainless steel in SBF [170]. Martensite should not be present in biomaterials used for implantation, as it is magnetic.

1.10. Scope of the Present Investigation

Based on literature, it is concluded that nickel-free high manganese and nitrogen stabilized austenitic stainless steels are superior to 316L in terms of corrosion resistance, tensile strength, hardness and they exhibit acceptable biocompatibility both *in vitro* and *in vivo*. Austenitic stainless steels free from nickel and stabilized by manganese and nitrogen with different chemical composition ranges have been developed, as given in **Appendix A**. However, only a few of them have been systematically studied from a biomedical application point of view. The ASTM F2581, P2000 and P558 have comparatively high carbon content. Since carbon is deleterious from a corrosion point of view, these steels may not be a suitable substitute for 316. However, F2229 (Biodur 108) and BIOSSN4 have been developed with lower carbon content.

BIOSSN4/0Cr18Mn15Mo2N0.64 is based on the Fe-18Cr-15Mn-2Mo system with nitrogen content varied from 0.45 to 0.70. BIOSSN4 exhibited better tensile strength, corrosion resistance, hardness, cell cytotoxicity and blood compatibility than 316L. It was found suitable as a stent material. The corrosion fatigue of this steel has not been reported as per the ASTM F1801 standard, which is one of the essential criteria of metallic implant materials, especially for orthopedic implant applications. Biodur 108 has been extensively studied for corrosion resistance, tensile properties and biocompatibility and was found better than 316L. However, Biodur 108 was checked for corrosion fatigue only up to 10^6 cycles [98]. The corrosion fatigue results should be reported for 10^7 cycles according to ASTM F1801 standards.

Therefore, there is scope of development of a new grade of nickel-free high nitrogen-containing stainless steel for biomedical applications, with an optimized chemical composition without compromising with its chemical and mechanical properties with

adequate biocompatibility, which may be a potent replacement of 316L. It also needs extensive study related to corrosion, fatigue, corrosion fatigue and biocompatibility.

There is no detailed study on the influence of USP on corrosion resistance, biocompatibility and high cycle fatigue behavior of nickel-free nitrogen stabilized austenitic stainless steel. Also, the effect of duration of USP on LCF of high nitrogen-containing stainless steels has not been carried out. Thus, there is much scope for the investigation on the role of duration of USP on corrosion resistance in simulated body fluid environment, *in vitro* cell attachment, spreading and proliferation, and LCF behavior of nickel-free high nitrogen stabilized austenitic stainless steel. Further, there is no study related to high cycle fatigue and corrosion fatigue of nickel-free high nitrogen stabilized steels, following USP.

1.11. Objectives of the Present Investigation

➢ Development of austenitic stainless steel without nickel, stabilized by manganese and nitrogen, for orthopedic implant application.

➢ Characterization of the above steels for

(a) Mechanical properties

(b) Corrosion resistance in simulated body fluid environment

(c) Corrosion fatigue

(d) Biocompatibility

➢ Comparison of the above aspects with those of the conventional nickel-containing 316L austenitic stainless steel used presently.

➢ Examination of the effect of USP on microstructure, corrosion resistance, fatigue, corrosion fatigue and cell growth of the nitrogen stabilized austenitic stainless steel free from nickel.