

MGSZ AND ZNSZ NANOCOMPOSITE COATINGS ON MEDICAL GRADE 316L SS – A SUITABLE APPROACH FOR MECHANICAL, ANTIMICROBIAL AND BIOLOGICAL PERSPECTIVES

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology in B. Tech Biomedical Engineering

By

KRISHA H (39240022)

CHELSEA HANNAH A (39240012)



**DEPARTMENT OF BIOMEDICAL ENGINEERING
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

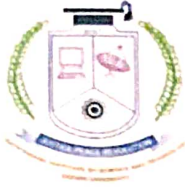
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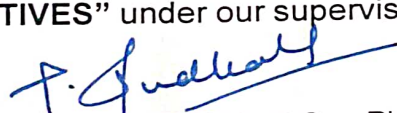
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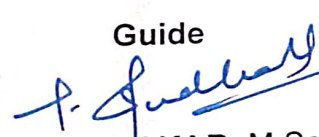
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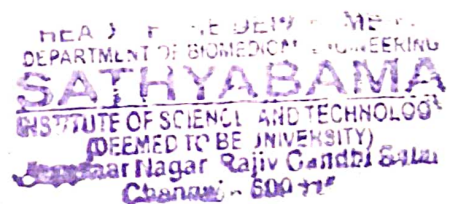
This is to certify that this Project Report is the bonafide work KRISHA H (39240022) and CHELSEA HANNAH A (39240012) who carried out the final year project entitled "MGSZ AND ZNSZ NANOCOMPOSITE COATINGS ON MEDICAL GRADE 316L SS – A SUITABLE APPROACH FOR MECHANICAL, ANTIMICROBIAL AND BIOLOGICAL PERSPECTIVES" under our supervision from October 2022 to April 2023.


Dr. T. SUDHAKAR, M.Sc., Ph.D.

Guide

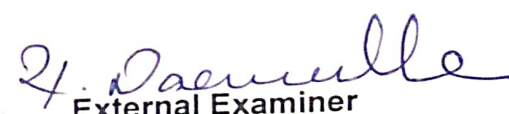

Dr. T. SUDHAKAR, M.Sc., Ph.D.

Head of the Department



Submitted for Viva voce Examination held on 18th April 2023


Internal Examiner


External Examiner

DECLARATION

We, and KRISHA H (39240022) and CHELSEA HANNAH A (39240012), hereby declare that the Project Report entitled "MGSZ AND ZNSZ NANOCOMPOSITE COATINGS ON MEDICAL GRADE 316L SS – A SUITABLE APPROACH FOR MECHANICAL, ANTIMICROBIAL AND BIOLOGICAL PERSPECTIVES" done by us under the guidance of Dr. T. SUDHAKAR is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in B. Tech Biomedical Engineering.

DATE: 18.04.2023

1. Chelsea hannah A

2. Krisha H

Chelsea Hannah A
H. Krishna

PLACE: CHENNAI

SIGNATURE OF THE CANDIDATES

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ABSTRACT

Surface tailoring of stainless steel is an effective approach to prevent the chances of bacterial contamination and improve mechanical and biological properties. In this view, we have developed MgSZ and ZnSZ nanocomposite coated stainless steel using thermal spray technique. Different techniques such as XRD, (X-ray diffraction), SEM (scanning electron microscope), Raman spectroscopy and XPS (X-ray photoelectron spectroscopy) were used to characterize the coated samples. Wear test analysis revealed that surface modified 316L SS samples exhibited better wear resistance property. Further, MgSZ and ZnSZ nanocomposite coated 316L SS samples performed well in the biological and electrochemical aspects rather bare 316L SS samples.

Keywords: ZnSZ, MgSZ, Nanocomposite coating, Surface modification, Bioimplants, Thermal spray, antimicrobial activity

TABLE OF CONTENTS

CHAPTER NO	TITLE	PAGE NO
	ABSTARCT	i
	FIGURES	v
1	INTRODUCTION	1
	1.1 Types of coating	2
	1.1.1 Polymer coating	2
	1.1.2 Nitrite Based Surface Coating	2
	1.1.3 Oxide based Surface coating	2
	1.1.4 Ceramic nanocomposites	3
	1.2 Type of Coating techniques	4
	1.2.1 RF magnetron sputtering	4
	1.2.2 DC magnetron Sputtering	4
	1.2.3 Thermal Evaporation	4
	1.2.4 Thin film anodes	5
	1.2.5 Pulsed Laser Deposition	5
	1.2.6 Pulse Length and Pulse Energy	
	1.2.7 Plasma Spray Method	5
	1.2.8 Electron Beam Evaporation	6
2	LITERATURE REVIEW	8
3	AIM AND SCOPE	13
	3.1 Aim	13
	3.2 Scope	13

4	MATERIALS AND METHODS	14
	4.1 Synthesis of nanocomposite coating	14
	4.1.1 Preparation of magnesium stabilized zirconia and zinc stabilized zirconia	14
	4.1.2 Substrate preparation	14
	4.1.3 Deposition of MgSZ and ZnSZ by plasma spray technique	15
	4.2 Invitro evaluation studies	16
	4.2.1 Hemolysis studies	16
	4.2.2. Protein adsorption studies	16
	4.2.3 Mineralisation	16
	4.2.4 Tribological property studies	17
	4.3 Characteristic testing	19
	4.3.1 XRD	19
	4.3.2 FESEM EDAX	19
	4.3.4 CONTACT ANGLE	20
	4.4 Bacterial Studies	
	4.4.1 Cytotoxic analysis	17
	4.4.2 Microbial Type Culture	17

5	RESULTS AND DISCUSSION	18
	5.1 Synthesis of nanocomposite coatings	18
	5.2 Characteristic evaluation results	18
	5.2.1 XRD analysis	18
	5.2.2 SEM analysis	19
	5.2.3 Contact angle	21
	5.3 Invitro studies	22
	5.3.1 Hemolysis test	22
	5.3.2 Protein adsorption studies	23
6	CONCLUSION	24
	REFERENCES	24

FIGURES

FIGURE	DESCRIPTION	PAGE.NO
4.1	(a) unpolished SS316L (b) polished SS316L (c) plasma spray coated SS316L	15
5.1	XRD analysis result	19
5.2	FESEM of ZnZrO ₂	20
5.3	FESEM image of MgZrO ₂	20
5.4	EDAX for MgZrO ₂ and ZnZrO ₂ graph	21
5.5	Contact angle of MgZrO ₂	22
5.6	Contact angle for ZnZrO ₂	22
5.7	Graphical representation of hemolysis	23
5.8	Standard graph for Protein adsorption	23
5.9	Microbial Type Culture	36

CHAPTER-1

INTRODUCTION

The term 'biomaterial' refers to a substance that has been designed specifically for the purpose of interacting with biological systems for medical use. This interaction can be therapeutic or diagnostic in nature (1). They are generally classified into three groups namely: metals, ceramics, and polymers. In recent times Stainless Steel (SS) is one of the widely used biomaterials for biomedical application because of their impeccable mechanical, physical and biocompatible properties. Stainless Steel (SS) has various day to day applications ranging from permanent dental implants, stents for physiological conditions and bone frames commonly used for supporting existing bones or destructed bones (1,2). In Addition to permanent implants, temporary implants have significant application details such as plates for construction of destructed bones and nails are screws required for the assembly of the implant. It has also been used for surgical tools like guiding templates and scalpels for surgery. One of the most denotable properties of Stainless Steel (SS) is its low cost, easy manufacturing techniques, good fatigue properties and corrosion resistance (3). Regardless, It is possible for SS to interact adversely with a harsh biological environment inside the human body, resulting in the formation of a biofilm and corrosion of the surface (2,3). As a result, SS surfaces do not possess the desired bio-functional properties such as hemocompatibility (cardiovascular implants) or osteoblast proliferation (bone implants, dental implants), nor do they prevent bacterial adhesion and the formation of biofilms. In order to solve this problem, it is essential to use a variety of surface finishing procedures (coatings, biofunctionalization methods, etc.) that have a direct effect on the surface characteristics of the material, while also maintaining its bulk properties (mechanical properties) (4). Even though stainless steel is a widely used biomaterial, there is still a limited number of reviews that critically evaluate various types of surface finishing strategies for preventing the adhesion of bacteria and the formation of biofilms on stainless steel (5,6). Due to ever alarming increase in bacterial infection and antibiotic resistant bacterial strains it possesses as a health care threat paving a way

for modified surface tailoring methods and strong expert knowledge on fields composed of surface modification techniques and interaction between microbial patterns and the substrate (6). This report briefly explains the different strategies for surface tailoring of Stainless Steel (SS) 316L using highly reactive radiofrequency plasma technology and initiatives to resist bacterial activities (6,7).

1.1 TYPES OF COATING:

1.1.1 Polymer coating

Polymer coatings interact as protective and decorative layers in the use of surface coatings for consumer goods. For additional applications, the polymer coatings have a durability which is limited often due to photodegradation (8). So super durable coatings and high performance are required for polymer coatings. Biodegradable polymer has been achieved to be beneficial for improving the degradation resistance of materials-based magnesium for applications in Biodegradable Mini implant. Especially the bulk polymer degradation is less effective than the degradation polymers. And the problem cannot be solved only by polymer coating. Through polymer coatings it can permeate body fluid mostly in bulk degradation polymers. However, the polymer alone coating has a very poor adhesive strength and the poor long-term performance has been reported (9,10). The mussel inspired Surface chemistry is one of the masses utilized strategies to surface functionalization, Practical industrial application does rather limited and small surfaces can only be treated in dip coating and coating vessel is dependent. The obtained via spray coating in Post functionalization with silver nanoparticles giving excellent embedded coating of a nanoparticle (11).

1.1.2 Nitrite Based Surface Coating

Nitrite Based Surface Coatings are a composition of nitrogen atoms (N) that is encompassed in a crystalline structure of varying metals that are Al, Cr, V, Ti etc. which causes internal stress within its compound (12,13). The metal undergoes physiochemical change and even difference in behavioral patterns. Attributes such as rigidity and resistance

1.1.3 Oxide based Surface coating

Induction-heat treatment is used for Oxide coatings and it is obtained by titanium Rutile that represents the growth of titania coating which is ensured by the Induction heat treatment (14). The surface morphology has the influence based on technological regimes can be studied in the experiment. The parameters of the oxide coating are obtained (15,16). Oxide coatings has its mechanical properties and in vitro biocompatibility prismatic crystals, needle like, the formation of dotted and the Plates these are the formation stages in Oxide coating. Hydroxyapatite colloidal nanoparticles are modified by Oxide coatings (17).

1.1.4 Ceramic nanocomposites

Due to remarkable properties Ceramic nanocomposites has been applied to a lot of industrial applications (18,19). The remarkable properties include good wear resistance in environment of high-temperature than the metals, resistance to corrosion and high temperature oxidation. The nano composite coatings are in the phase of metastable of a nano structure (20). The materials of new generation have the nanocomposite coatings minimally composes two phases those are two nanocrystalline or rather amorphous and phase of nanocrystalline. The materials of nanocomposite give improved properties Because of conventional materials and the sizes of grains are smaller comparatively (21,22). The hardness of the nanocomposite coating has increased in their own property (22). Compared to the harder component. The nanocomposite coatings can be obtained maximum two times or more than that of greater hardness in it. The increase in hardness is due to the nano structure of materials, the focus of cohesive is in between the neighboring grains of atoms and dislocation-induced plastic deformation (23,24). To achieve the better toughness and natural brittleness of ceramic films in oxide by just combining yttria stabilised zirconia (YSZ) And nano compost site films to produce gold. Nanocomposite recently develops in a biomaterial. In nanocomposite it has toughening mechanism which is a transformation phase of tetragonal to mono clinic in the phase of ceria stabilised zirconia (CSZ) (24). The ground specimens of surface in transformation can leads to increase in strength and surface compressive induced stress. By double torsion

method, Crack velocity curves come by cyclic and static fatigue. For reducing inflammation and by improving durability in surface tailoring of bioimplants is necessary. The stainless steel in medical grade is developed by calcia stabilised zirconia (CaSZ) and silver CaSZ (24,25). The performance of corrosion resistance, biological, mechanical and their microstructure.

1.2 TYPE OF COATING TECHNIQUES

1.2.1 RF sputtering

As with DC Sputtering, RF Sputtering runs an energetic wave through an inert gas in a vacuum chamber which becomes ionized (26). The target material or cathode which is to become the thin film coating is bombarded by these high energy ions sputtering off atoms as a fine spray covering the substrate to be coated. RF Magnetron sputtering uses magnets behind the negative cathode to trap electrons over the negatively charged target material so they are not free to bombard the substrate, allowing for faster deposition rates. Over time, positive ions are produced which accumulate on the surface of the target face giving it a positive charge (26,27). At a certain point this charge can build up and lead to a complete secession of sputtering atoms being discharged for coating. By alternating the electrical potential with RF Sputtering, the surface of the target material can be “cleaned” of a charge buildup with each cycle. On the positive cycle electrons are attracted to the target material or cathode giving it a negative bias. On the negative portion of the cycle – which is occurring at the radio frequency of 13.56 MHz used internationally for RF power supply equipment – ion bombardment of the target to be sputtered continues (28,29).

1.2.2 DC Sputtering

DC or Direct Current Sputtering is a Thin Film Physical Vapor Deposition (PVD) Coating technique where a target material to be used as the coating is bombarded with ionized gas molecules causing atoms to be “Sputtered” off into the plasma (30). These vaporized atoms are then deposited when they condense as a thin film on the substrate to be coated. DC Sputtering is the most basic and inexpensive type of sputtering for PVD metal deposition and electrically conductive target coating materials. Two major

advantages of DC as a power source for this process is that it is easy to control and is a low-cost option if you are doing metal deposition for coating (31,32).

1.2.3 Thermal Evaporation

Thermal evaporation methods include the simple case of emission at the heated source of the material which condenses on the cold substrate (33). The source can be a refractive wire or a crucible electrically heated and containing the material to be evaporate. The evaporation rate is determined by the vapor pressure of the source material at the evaporation temperature. The Joule heating of typical tungsten or molybdenum crucible allows reaching 2800 K, which provides appreciable vapor pressures for most metals. A deposition chamber under high vacuum ($<10^{-2}$ Pa) is necessary in order to avoid oxidation of the source. To prevent the harmful reaction between source and the crucible, the heating can be done rapidly and the process is known as flash evaporation (33,34). The properties of the deposited films are usually different if thermally or flash evaporated. Inductive heating of the crucible is also possible. The thermal evaporation of highly sensitive materials, in particular in ultrahigh vacuum (UHV) ($<10^{-6}$ Pa), is achieved by electron-beam (e-beam) heating. The main advantages of e-gun heating are to prevent contamination by the crucible and the high evaporation temperature reachable (35). Electron-beam sources are generated either by thermoionic emission or by field effect cathodes. Every evaporation process can be assisted by plasma, microwave, or electron resonance, in order to influence the deposition rate or the properties of the species condensing on the substrate.

1.2.4 Thin Film Anodes

Most thin-film batteries use a lithium metal anode, which is easily deposited by thermal evaporation and gives the best battery performance (36). Such “Li-batteries” have the maximum battery voltage, discharge rate capability, and energy density. However, two drawbacks of the lithium anode, the low melting temperature (180 °C) and the high reactivity with air, has motivated development of other thin-film anode materials. As for a cathode, the thin-film anode must be a good mixed conductor which can accommodate a large density of Li^+ ions reversibly.

1.2.5 Pulsed Laser Deposition

Pulsed laser deposition is one of the multiple physical vapor deposition routes to

fabricate thin films, employing laser energy to eject material from a target in the form of a plasma (37). A substrate, commonly a single-crystal oxide, is placed in the path of the plume and acts as a template for the arriving species from the target to coalesce and self-assemble into a thin film (38,39). This technique is tremendously useful to produce crystalline films, due to the wide range of atmospheric conditions and the extent of possible chemical complexity of the target. However, this flexibility results in a high degree of complexity, oftentimes requiring rigorous optimization of the growth parameters to achieve high quality crystalline films with desired composition. In this tutorial review, we aim to reduce the complexity and the barrier to entry for the controlled growth of complex oxides by pulsed laser deposition. We present an overview of the fundamental and practical aspects of pulsed laser deposition, discuss the consequences of tailoring the growth parameters on the thin film properties, and describe *in situ* monitoring techniques that are useful in gaining a deeper understanding of the properties of the resultant films (40). Emphasis is placed on the general relationships between the growth parameters and the consequent structural, chemical, and functional properties of the thin films. In the final section, we discuss the open questions within the field and possible directions to further expand the utility of pulsed laser deposition (41,42).

1.2.6 Pulse Length and Pulse Energy

The initial stages of the interaction between laser light and the solid target are crucial (43). The energy density per pulse plays a strong role in this process, which is governed by the pulse length and the pulse energy (43,44). The typical timespan necessary for material ejection and plume formation is on the order of tens of picoseconds, although this time span is also dependent on the energy; however, further interactions are necessary between the laser light and the species in the plasma plume (45). It is also important to register if the removal of material takes place below or above the ablation threshold, which is the minimum laser intensity required to remove material. Below this threshold, most of the laser energy is converted into thermal energy and little material is ejected from the target (46).

1.2.7 Plasma Spray Method

Plasma spray coating is a flexible coating process that can easily produce a high-

performance surface coating material. In a plasma spray process, hot ionized gas is used to melt NiTi powder and drives the powder particle in such a way that it can easily adhere to and agglomerate on the coating surface. A schematic layout of the NiTi plasma spray coating process. In this process, two electrodes, anode and cathode, generate an electric arc with a temperature range of 15,000°C–20,000°C. This electric arc is used to heat inert gases such as argon and helium and form a plasma jet (Sharma, Jangra, & Raj, 2018). The source of water coolant is also used to reduce the overheating of electrodes. In a further process, a pressurized mixer of NiTi powder and carrier gas is deposited on the plasma gas stream. The powder mixer of NiTi melts when it interacts with the high jet stream of plasma gas and converts into the particle. This particle strikes on a substrate with high kinetic energy. The coating layer is formed by a particle-by-particle layer with a cooling rate of $106\text{--}108\text{K s}^{-1}$ due to variation between a thermal mass of NiTi particle and substrates. The degree of porosity can be changed by varying spraying parameters. The recombination between electrons and ions on a substrate's surface is due to powder heating in a plasma stream. It is to be considered that only a physical effect such as heating and acceleration occurs between the particles and plasma gas. The chemical reaction generally is assumed not to occur between plasma and particles due to argon and helium's inert behavior. During the experiment, a hot ionized gas of NiTi particles moves through the air toward the substrate. This oxygen is mixed with NiTi powder particles, and when it reaches the substrate, it forms an oxide layer such as TiO_2 . However, oxides are less fragile to the effect of oxidation than the metals.

1.2.8 Electron Beam Evaporation

In electron beam evaporation, the source material can be evaporated using high energy electrons in the form of an intense beam. A hot filament causes the thermionic emission of electrons, which can, after acceleration, provide sufficient energy for evaporating any material (47). In a typical case involving 1 A of emission accelerated through a 10 kV voltage drop, 10 kW is delivered upon impact [85]. To avoid melting the filament in the arriving evaporant, the filament is located out of sight of the evaporant as shown in Fig. 3.10 and the electron beam is pulled around to the surface by a magnetic field, B , the point shows the direction in this figure. The combined force, F ,

on an electron in electric (E) and magnetic field is known as Lorentz force. The electron beam (E-beam) evaporation process is a physical vapor deposition that yields a high deposition rate from 0.1 $\mu\text{m}/\text{min}$ to 100 $\mu\text{m}/\text{min}$ at relatively low substrate temperatures. The E-beam process offers extensive possibilities for controlling film structure and morphology, with desired properties such as dense coating, high thermal efficiency, low contamination, high reliability and high productivity (47,48). The deposition chamber is evacuated to a pressure of 10^{-5} Torr or lower. The material to be evaporated is in the form of ingots or a compressed solid. The E-beam can be generated from electron guns by thermionic emission, field electron emission or the anodic arc method. The electron beam is accelerated to a high kinetic energy and focused towards the starting material. The kinetic energy of the electrons is converted into thermal energy that will increase the surface temperature of the materials, leading to evaporation and deposition onto the substrate. The deposition rate depends on the starting material and E-beam power. The deposited film thickness can be measured in situ by a quartz crystal monitor. The evaporation of CNTs with MOXs (i.e., SnO_2 and WO_3) is a relatively new concept. A plausible mechanism for CNT/MOX co-evaporation can be drawn as follows:^{51,52} the E-beam is used to bombard the surface of the starting materials (i.e., CNT/ SnO_2 or CNT/ WO_3) (48). The MOXs (such as SnO_2 or WO_3) are evaporated at a temperature of $\sim 1500^\circ\text{C}$ in a high vacuum, while CNT fragments that are small and very light are carried into the vapor by surrounding SnO_2 or WO_3 molecules.

CHAPTER-2

LITERATURE REVIEW

Srinivasan Nagarajan *et al.*, 2012 Here, porous TiO(2)/ZrO(2) nanocomposite coated over 316 L stainless steels was studied for their enhanced performance in terms of its biocompatibility and corrosion resistance, following a sol-gel process via dip-coating technique. The immersion procedure resulted in the complete coverage of the TiO(2)/ZrO(2) nanocomposite (coated on the surface of 316 L stainless steel) with the growth of a one-dimensional (1D) rod-like carbonate-containing apatite. The TiO(2)/ZrO(2) nanocomposite coated specimens showed a higher corrosion resistance in the SBF solution with an enhanced biocompatibility, surpassing the performance of the pure oxide coatings. The cell viability of TiO(2)/ZrO(2) nanocomposite coated implant surface was examined under human dermal fibroblasts culture, and it was observed that the composite coating enhances the proliferation through effective cellular attachment compared to pristine 316 L SS surface.

Matic Resnik *et al.*, 2020 In this review, strategies for improving the antimicrobial properties of stainless steel (SS) are presented. The focus given is to present current strategies for surface modification of SS, which alter surface characteristics in terms of surface chemistry, topography, and wettability/surface charge, without influencing the bulk attributes of the material. As SS exhibits excellent mechanical properties and satisfactory biocompatibility, it is one of the most frequently used materials in medical applications. It is widely used as a material for fabricating orthopedic prosthesis, cardiovascular stents/valves and recently also for three-dimensional (3D) printing of custom-made implants. Despite its good mechanical properties, SS lacks desired bio functionality, which makes it prone to bacterial adhesion and biofilm formation. Due to increased resistance of bacteria to antibiotics, it is imperative to achieve antibacterial properties of implants. Thus, many different approaches were proposed and are discussed herein. Emphasis is given on novel approaches based on treatment with

highly reactive plasma, which may alter SS topography, chemistry, and wettability under appropriate treatment conditions. This review aims to present and critically discuss different approaches and propose novel possibilities for surface modification of SS by using highly reactive gaseous plasma in order to obtain a desired biological response.

Behzad Mojarad Shafiee *et al.*, 2020 In this study, various composites of bioglass/gelatin/polycaprolactone (BG/GE/PCL) were produced and coated on the surface of 316L stainless steel (SS) to improve its bioactivity. X-ray diffractometry (XRD), scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) were utilized to characterize the specimens. The results showed that bioglass particles were distributed uniformly in the coating. By increasing the wt.% of bioglass in the nanocomposite coatings, the surface roughness and adhesion strength increased. The corrosion behavior of GE/PCL (PCL-10 wt.% gelatin coated on 316L SS) and 3BG/GE/PCL (GE/PCL including 3 wt.% bioglass coated on 316L SS) samples were studied in PBS solution. The results demonstrated that 3BG/GE/PCL sample improved the corrosion resistance drastically compared to the GE/PCL specimen. In vitro bioactivity of samples was examined after soaking the specimens for 7, 14 and 28 days in simulated body fluid (SBF). The results showed a significant apatite formation on the surface of 3BG/GE/PCL samples. The cell viability evaluation was performed using 3-(4, 5-dimethylthiazol-2-yl)-2,5 diphenyltetrazoliumbromide (MTT) tests which confirmed the enhanced cell viability on the surface of 3BG/GE/PCL samples. The in vivo behavior of specimens illustrated no toxicity and inflammatory response and was in a good agreement with the results obtained from the in vitro test.

Pelin Erkoc *et al.*, 2021 Biocontamination of medical devices and implants is a growing issue that causes medical complications and increased expenses. In the fight against biocontamination, developing synthetic surfaces, which reduce the adhesion of microbes and provide biocidal activity or combinatory effects, has emerged as a major global strategy. Advances in nanotechnology and biological sciences have made it possible to design smart surfaces for decreasing infections. Nevertheless, the clinical performance of these surfaces is highly depending on the choice of material. This review focuses on

the antimicrobial surfaces with functional material coatings, such as cationic polymers, metal coatings and antifouling micro-/nanostructures. One of the highlights of the review is providing insights into the virus-inactivating surface development, which might particularly be useful for controlling the currently confronted pandemic coronavirus disease 2019 (COVID-19). The nanotechnology-based strategies presented here might be beneficial to produce materials that reduce or prevent the transmission of airborne viral droplets, once applied to biomedical devices and protective equipment of medical workers. Overall, this review compiles existing studies in this broad field by focusing on the recent related developments, draws attention to the possible activity mechanisms, discusses the key challenges and provides future recommendations for developing new, efficient antimicrobial and antiviral surface coatings.

Minho Lee et al., 2022 The ultrathin nanocomposite coatings made of zirconium oxide (ZrO₂), zinc oxide (ZnO), and titanium oxide (TiO₂) on stainless steel (SS) were prepared by the radio frequency sputtering method, and the effects of the nanocomposite coating on corrosion protection and antibacterial activities of nanocomposite coated SS were investigated. Scanning electron microscopy was conducted to observe surface morphology of nanocomposite coatings with distinct distribution of grains with the formation on SS substrate. From the electrochemical impedance spectroscopy results, ZrO₂/ZnO/TiO₂ nanocomposite coating showed excellent corrosion protection performance at 37 °C during immersion in simulated body fluid and saliva solution for 12 and 4 weeks, respectively. The impedance of ZrO₂/ZnO/TiO₂ (40/10/50) nanocomposite coated SS exhibited values about 5 orders of magnitude higher than that of uncoated SS with polarization at the low-frequency region. Cell viability of ZrO₂/ZnO/TiO₂ nanocomposite coated SS was examined under mouse fibroblasts culture (L929), and it was observed that the nanocomposite coating improves proliferation through effective cellular attachment compared to uncoated SS. From the antimicrobial activity results, ZrO₂/ZnO/TiO₂ nanocomposite-coated SS showed killing efficiency of 81.2% and 72.4% against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*, respectively.

Ahmed Abdel Nazeer et al., 2020 Resistance to stainless steel corrosion in marine-based industries requires more research into materials with an improved surface and enhanced protection by utilizing surface coatings. Herein, a thermally stable aramid–zirconia nanocomposite has been successfully prepared using the sol–gel method to produce a zirconia network-structure bonded to the polymer chain. Using thermal gravimetric analysis (TGA), the residue mass of zirconia retained after the thermal degradation of aramid-zirconia film was determined and found to be 10% by mass. The investigated nanocomposite (using 10% zirconia) was coated on the stainless-steel surface through a facile and effective spin coating method and its protection was examined in saline solution (3.5% NaCl). The aramid–zirconia nanocomposite coating (Ar-Zr10) was found to provide an outstanding corrosion resistance to steel surfaces which led to protecting it against the corrosive marine environment. The electrochemical impedance (EIS) measurements were carried out to evaluate steel resistance against dissolution in chloride solution in the absence and presence of the investigated coatings showed a corrosion protection efficiency of 99.3% using Ar-Zr10 compared to 92.1% using pure aramid. Moreover, the potentiodynamic polarization (PDP) plots showed a pronounced decrease in the corrosion current values which confirmed the formation of a passive layer which mitigated the corrosion reaction and ions diffusion. The water contact angle of stainless-steel coated with pure aramid and the aramid–zirconia was found to be 84.2° and 125°, respectively, confirming the hydrophobic nature of the hybrid coating Ar-Zr10. On the other hand, the results achieved through the electrochemical and surface techniques were used to clarify the protection mechanism. The aramid–zirconia nanocomposite coating showed a remarkable protection performance by controlling the charge transfer at the interface between the steel alloy and the electrolyte which prevented the alloy dissolution.

Rosiah Rohani et al., 2022 To produce green or eco-friendly products, the potential use of ZnO NPs biosynthesized from natural resources to replace conventional petroleum-derived polymers has been investigated. This review provides an insight into the growing trend of incorporating ZnO NPs into synthetic or semi-synthetic or bio-based polymeric materials via different synthesis methods as well as its characteristics and potential

applications in surface coatings and films. The antimicrobial potential of ZnO NPs to inhibit the growth of various types of microorganisms as well as its use in surface coatings or films to impart antimicrobial activities that prevent the spread of microorganisms, especially the COVID-19 virus, was also discussed.

Mehdi Shabani-Nooshabadi *et al.*, 2023 The synthesis of polyaniline montmorillonite (MMT) nanocomposite coatings on 316L stainless steel (316L SS) surface has been investigated by using the galvanostatic method. The synthesized coatings were characterized by Fourier Transform Infrared Spectroscopy (FT-IR), UV-visible absorption spectrometry and Scanning Electron Microscopy (SEM). The anticorrosion performances of polyaniline-MMT nanocomposite coatings were investigated in 0.5 M HCl medium by the potentiodynamic polarization technique and Electrochemical Impedance Spectroscopy (EIS). The corrosion rate of polyaniline-MMT nanocomposite coated 316L SS was found ~540 times lower than bare 316L SS and potential corrosion increased from -0.386 V versus Ag/AgCl for uncoated 316L SS to -0.040 V versus Ag/AgCl for polyaniline-MMT nanocomposite coated 316L SS electrodes. Electrochemical measurements indicate that polyaniline-MMT nanocomposite coated have good inhibiting properties with mean efficiency of ~99.8 % at 0.75 mA cm^{-2} current density applied on 316L SS corrosion in acid media. The results of this study clearly ascertain that the polyaniline-MMT nanocomposite has an outstanding potential to protect 316L SS against corrosion in an acidic environment.

CHAPTER-3

AIM AND SCOPE

3.1 AIM

The aim of our project is we have developed MgSZ and ZnSZ nanocomposite coated stainless steel using thermal spray technique for biomedical application.

3.2 SCOPE

Stainless steel coatings have gained popularity in the medical industry for use in bone implants. Magnesium-stabilized with zirconium coatings applied using the plasma spray technique provide several benefits for bone implant applications.

The scope of using magnesium-stabilized with zirconium coatings for bone implants is broad, with several advantages over other materials. Some of the most significant benefits of this material include:

Biocompatibility: Magnesium-stabilized with zirconium coatings have excellent biocompatibility, making them an ideal choice for use in bone implants. The material is non-toxic and does not cause any adverse reactions in the body, making it safe for use in medical applications.

Corrosion Resistance: The material has excellent corrosion resistance, which is essential for bone implants. The coating protects the underlying metal from corrosion, ensuring the implant remains functional for an extended period.

High Strength: Magnesium-stabilized with zirconium coatings have high strength, making them durable and able to withstand the mechanical stresses associated with bone implants.

Osseointegration: The coating promotes osseointegration, which is the process by which the bone grows around the implant, integrating it into the body. This is critical for the success of bone implants as it ensures the implant remains stable and functional.

Reduced Inflammation: The material has been shown to reduce inflammation in the body, which is important for bone implant applications. Reduced inflammation promotes faster healing, reducing the recovery time for patients.

Overall, the scope of using magnesium-stabilized with zirconium coatings by plasma spray technique for bone implants is significant, and the material's benefits make it an ideal choice for medical applications. The coating's biocompatibility, corrosion resistance, high strength, osseointegration, and reduced inflammation make it an attractive option for bone implant applications.

CHAPTER- 4

MATERIALS AND METHODS

4.1 Synthesis of nanocomposite coating

4.1.1 Preparation of magnesium stabilized zirconia and zinc stabilized zirconia

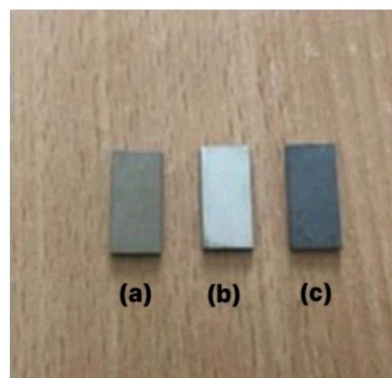
In simple terms ball milling simply involves the mechanical function of grinding powders finer particles. Usually, in ball milling the mechanical force breaks down reactants. The common advantages of ball milling make it preferred by all researchers due to its low cost, environmentally friendly, simplicity and its high yielding capacity. Typically, it is not only used for grinding powders but also for nanocomposite synthesis or oxide mixture. The mechanical activation by a ball milling to exponential increase the material reactivity and uniformity spatial distribution of elements. During this process the powders are put into a hollow vial along with the grinding balls (steel balls) and is set fixed in the machine the centrifugal force is formed while the vial is rotated. In this report chemical composition powders such as zinc oxide (10 %) infused zirconium (90%) and magnesium oxide (24%) and zirconium (76%) were taken and made into fine powders.

4.1.2 Substrate preparation

316 L SS stainless steel with a size of $20 \times 10 \times 1 \text{ mm}^3$ were prepared to deposit MgSZ and ZnSZ nanocomposite coatings (64). Before deposition, the substrates were mirror-polished using various grades of silicon carbide emery sheets (200, 600, 800, 1000, 1500, and 2000 pores per inches). Then, the samples were polished with $0.5 \mu\text{m}$ alumina suspension to obtain the micronized 316L SS samples (65). Finally, the samples were cleaned with soap solution and deionized water followed by ultrasonication for 15 min using acetone.

4.1.3 Deposition of MgSZ and ZnSZ by plasma spray technique

During plasma spraying, feedstock materials (in the form of powder, rod, or wire) are introduced into the plasma flame to be melted. The melted materials arrive on the surface of the target (SS 316L) after having been sufficiently heated and accelerated by the plasma jet. When the droplets impact the target, they are flattened and spread out on the substrate surface and form a coating through successive impingement. The velocity and temperature of powder particles or droplets are directly related to the plasma gas type. MgZrO₂ and ZnZrO₂ used for plasma spraying have particle sizes of 10–90 µm in diameter. Powders with this narrow size distribution are preferred in order to achieve uniform heating and acceleration of a single component powder. A constant powder feeding rate is a prime condition for achieving uniform coating thickness and good quality coatings.



***Fig.4.1 (a) unpolished SS316L
(b) polished SS316L
(c) plasma spray coated SS316L***

4.2 Invitro evaluation studies

4.2.1 Hemolysis studies

The hemolysis test was performed according to the standard procedure of ISO 10,993–4:2002. In this, test samples were initially placed into a 25 ml beaker

according to ISO 10,993–1 standard protocol. Then, 10 ml of saline was poured into a beaker containing test samples followed by an incubation at 37 °C for 30 min. Then we centrifuged at 1500 rpm for 10 min and the supernatant (saline solution) was collected for further analysis. 8 ml of whole blood containing anticoagulant agent was diluted with 10 ml of normal saline solution, from which, 0.2 ml was taken and poured into materials extracting saline solution. Normal saline and distilled water were used as a negative and positive control, respectively. Th solution was measured by UV-spectrophotometer at 545 nm. The rate of hemolysis (HR) was derived from the equation as follows,

$$HR (\%) = \frac{AS - AN}{AP - AN} \times 100 \quad (1)$$

Where, AS is the absorbance of test samples, AN and AP denote negative control and positive control, respectively. Finally, the red blood cells (RBC's) morphology was analyzed by a digital microscope (Olympus) after exposure to the samples. For all biological analyses, uncoated 316L SS samples were treated as control.

4.2.2. Protein adsorption studies

For the protein adsorption study, the coated and uncoated 316L SS surfaces were equilibrated in isotonic Tris buffer for about 20 h at 37 °C. Then, the samples were kept in a screw cap bottle containing 5 ml of plasma and incubated for 3 h. After incubation, the surface was gently washed by isotonic Tris-buffer followed by incubation with 2% sodium dodecyl sulfate (SDS) solution for 24 h in order to elute adsorbed proteins. Finally, the protein eluates were stored at – 70 °C and the concentration of adsorbed proteins were determined by Lowry's method. Triplicate experiments were done for data accuracy.

4.2.3 Mineralization

The test sample (MgSZ and ZnSZ) was soaked in 50ml of SBF solution and kept at 37°C, After the period of 72 hours the test sample was withdrawn from solution washed with deionized water and dried. The dried sample was subjected to EDX analysis; the results were compared to characterization of test sample.

4.2.4 Tribological property studies

The wear-resistant behavior of the coating was tested by a linear reciprocating nano tribometer (CSM Instruments, Switzerland). A steel ball (100Cr6) with a diameter of 2 mm was used as a sliding counter body. For analysis, applied load and sliding speed was constantly maintained at 1N and 3 cm/s, respectively. For each experiment, the test was carried out at three different locations at room temperature. The average friction coefficient value was determined. Error bars represent standard deviations.

4.3 Characteristic testing

4.3.1 XRD

The ability to visualize structures with the high resolution achieved by using electron microscopes provides the foundation for developing valid conclusions about functional relationships. Despite advances in other types of light (LM), atomic force microscopy (AFM), and electron microscopy (EM), scanning electron microscopy (SEM) remains distinct in its ability to examine dimensional topography and distribution of exposed features. In order to be imaged using SEM, the specimen needs a conductive surface and must be placed inside high vacuum. We analyzed our samples to determine the surface morphology.



Fig. 4.2 XRD

4.3.2 FESEM with EDAX

Powder X-ray diffraction (PXRD) is used to gain information about the crystallinity of

materials in the solid state. An amorphous (disordered, non-crystalline) material will not diffract, while a material with any ordering/crystallinity, even if mixed with amorphous material, will diffract. It is usually not possible to determine the structure of a material of unknown structure using PXRD, so if a structure determination is desired, single-crystal X-ray diffraction (SCXRD) is the preferred method. PXRD is a non-destructive technique, so the sample will be unchanged after data collection and can be saved and re-used for other purposes. The height of the sample has a significant effect on the 2θ angle of the peaks observed. This technique will identify the crystalline phases present in a material and thereby reveal chemical composition information.

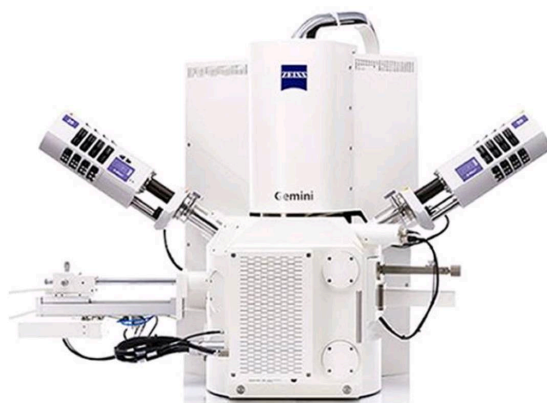


Fig.4.3 (ZIESIS) FESEM EDAX

4.3.4 Contact angle

The method measures the wetting contact angle using an air bubble at a solid–liquid interface. This method works best for hydrophilic substrates in which liquid spreads out yielding more difficulties to determine the contact angle with the sessile drop technique. E.g., for contact lenses and hydrogels.

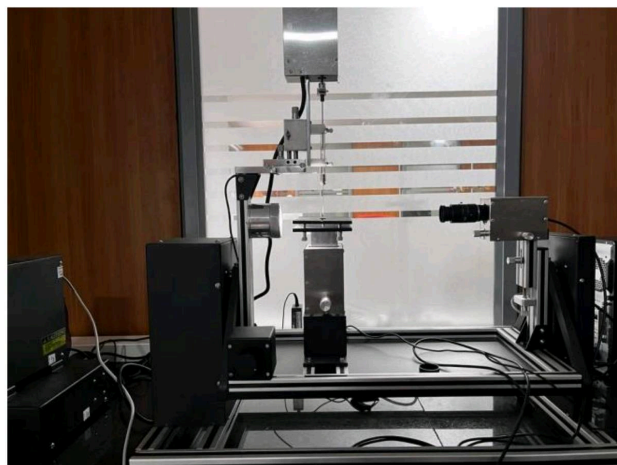


Fig. 4.3 (APEX) Contact angle

4.4 Bacterial Studies

4.4.1 Cytotoxic analysis

Human fibroblast (3T3) like cells were procured from National centre for Cell Sciences (NCCS), Pune, India. These cells were used to carry out cytotoxic behavior against test samples. Cells were cultured following the standard protocols suggested by the supplier. The cells were grown until confluency before the addition of mineralizing medium (α -MEM containing: 20% FCS (fetal calf serum), 100 μgml^{-1} streptomycins, 100 μgml^{-1} penicillin 50 mM L-ascorbate, 10^{-8} M dexamethasone, and 2 mM glutamine along with osteogenic supplements). All chemicals were procured from Sigma-Aldrich. Cells were incubated at 37 °C in presence of 5% CO₂ under standard humidified conditions. Test samples (MgSZ, ZnSZ coated, and uncoated 316 L SS) were kept in 6 well plates with human fibroblast with a density of 10⁴ cells/well. Incubation was done for 2 weeks at 37 °C under humidified conditions. Further, a quantitative analysis of cell cytotoxicity at different days was analyzed by a MTT assay.

4.4.2 Microbial Type Culture efficacy of the coatings

P. aeruginosa (MTCC-424) strain was obtained from Microbial Type Culture Collection and gene bank (MTCC), India. The lyophilized bacterial powder was cultured in nutrient broth at 37 °C for overnight incubation. After incubation, 0.1 ml of bacterial culture were added into a 200 ml conical flask containing nutrient broth. Finally, UV-sterilized test samples were immersed into the nutrient broth for 1, 2, 3 and 4 days at 37 °C in a shaking incubator. The incubated samples were gently washed with distilled water to remove the unbound cells on the samples. Then, acridine orange (AO) stain was added onto the samples and incubated for 10 min. After incubation, the samples were again gently washed and observed by epifluorescence microscopy (Nikon Eclipse E600, excitation filter BP 490; barrier filter 0515) to determine the proportion of viable bacteria.

CHAPTER-5

RESULT AND DISCUSSION

5.1 Synthesis of nanocomposite coatings

5.2 Characteristic evaluation results

5.2.1 XRD analysis

The crystallinity and its phase of as-deposited films were analyzed by XRD. Fig. 2 shows the XRD pattern of MgSZ and ZnSZ coating deposited onto 316L SS substrates. The acquired D-space values were well matched with the standard magnesium stabilized zirconia JCPDS card no. 80–1342. The obtained 2θ values are corresponding to (400), (422), (622), (800) and zinc stabilized zirconia, (111), (200), (220) and (311) planes respectively. Similarly, the θ values, 38.4 and 44.3 are corresponding to (111) and (200) planes and are in good agreement.

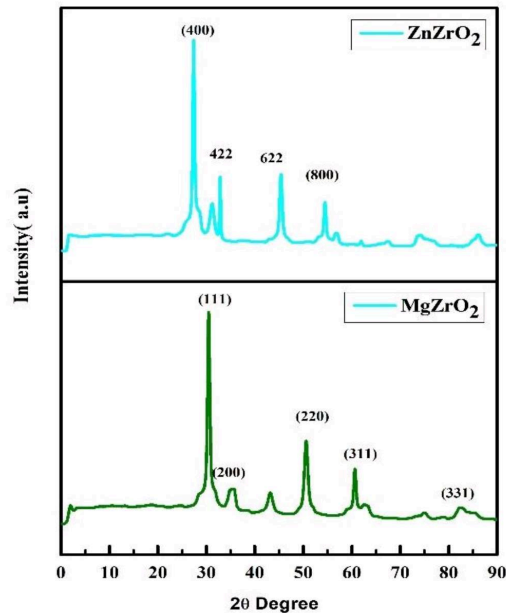


Fig 5.1 XRD analysis result

5.2.2 SEM analysis

The surface morphology of MgSZ and ZnSZ nanocomposite coatings on 316L SS substrates. The MgSZ coating (Fig. 5.2) was dense and the grains were observed. The SEM analysis of revealed hexagonal-shaped particles with clumped distributions, with an average size of 77nm. [24]. The results divulge that the growth of MgSZ grains was promoted. Besides, increases surface reactivity with MgSZ, ZnSZ and thus we obtained larger size grains [25].

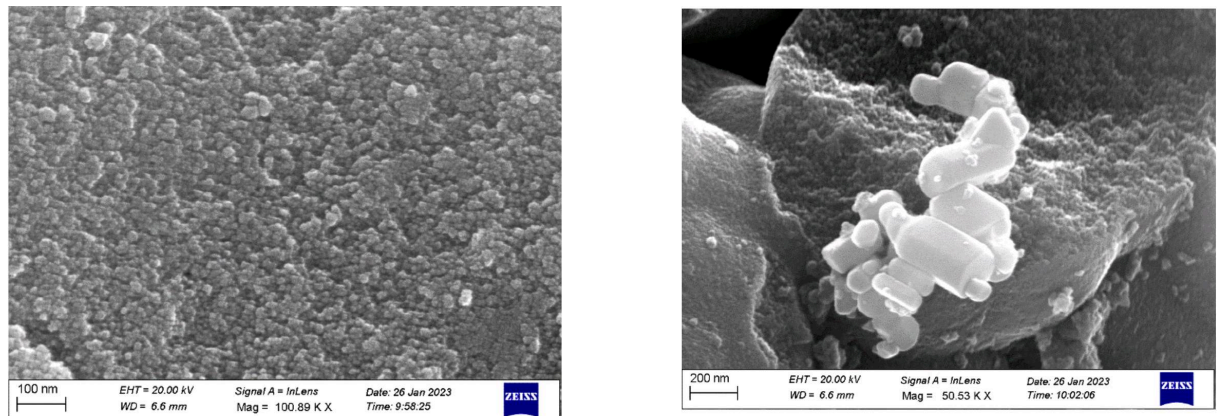


Fig 5.2 FESEM of ZnZrO2

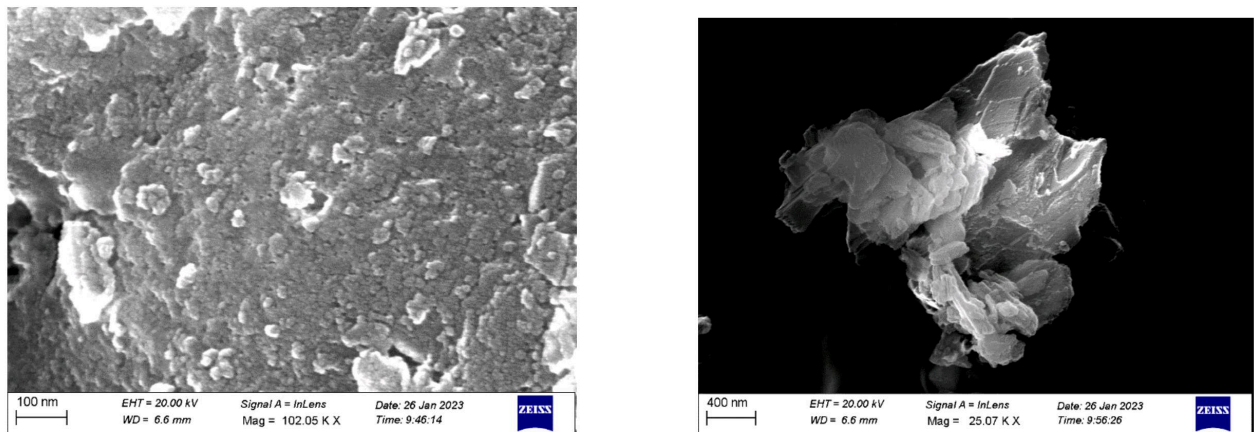


Fig 5.3 FESEM image of MgZrO2

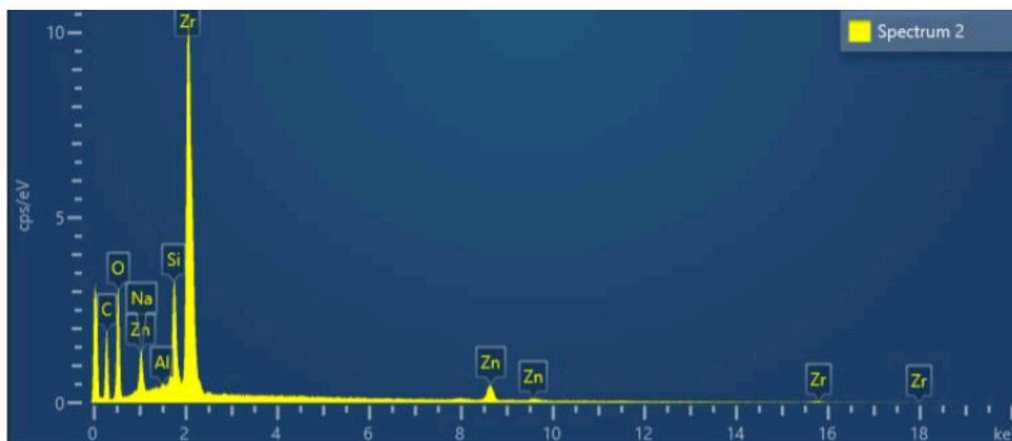


Fig 5.4 EDAX for $MgZrO_2$ and $ZnZrO_2$ graph

Spectrum 2 Element	Line Type	Weight %	Weight Sigma	% Atomic %
C	K series	38.38	0.57	61.58
O	K series	23.34	0.37	28.12
Na	K series	0.68	0.09	0.57
Al	K series	0.14	0.03	0.10
Si	K series	2.69	0.06	1.84
Zn	K series	5.28	0.18	1.56
Zr	L series	29.50	0.35	6.23

5.2.3 Contact angle

The studies stated that $MgSZ$ and $ZnSZ$ coatings exhibit the contact angle of 130° and 140° respectively and divulged its superhydrophobic nature. The hydrophobic nature of coated samples is due to the reduction of metal ions present in the surface of the coating creates oxygen vacancy thereby producing a more hydrophobic nature than on uncoated 316L SS substrates (70).

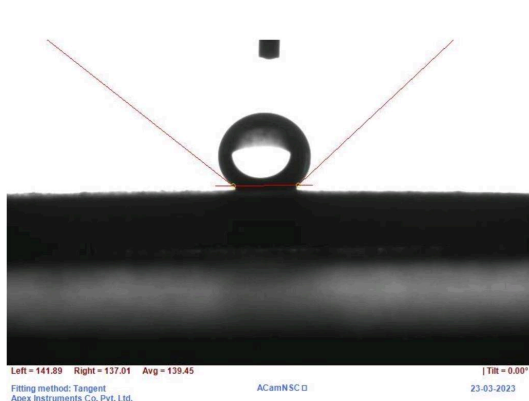


Fig.5.5 Contact angle of MgZrO₂

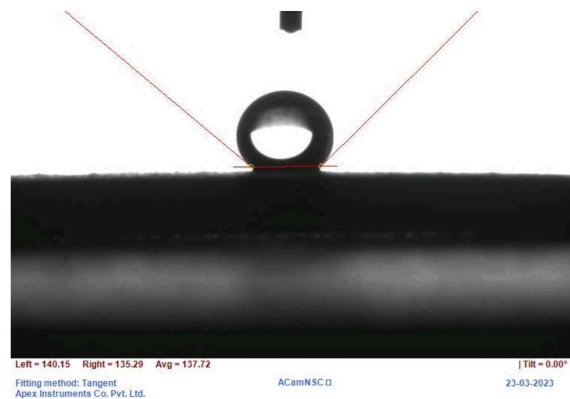


Fig 5.6 Contact angle for ZnZrO₂

5.3 Invitro studies

5.3.1 Hemolysis test

Hemolysis is the damage of red blood cells causing, release of hemoglobin into the bloodstream leading to inflammation. According to the ASTM protocol (ASTM F 756–00), the hemolytic ratio should be below 2.0% for the non-hemolytic condition. Fig. 12 shows the hemolysis behavior of MgSZ, ZnSZ coated, and uncoated 316 L SS samples. The inactivation behavior of platelets on the sample surface was inferred by higher platelet circularity. The hydrophilic surface enhances the formation of –OH groups, which may increase the binding of platelets activation reducing protein adhesion that hinders platelet adhesion activation (86). Further, 316L SS surfaces also exhibited non-hemolytic nature due to the adhesion of albumin protein, which strongly hinders the platelet activation (87). Coated and uncoated samples demonstrated hemocompatibility with a low degree of hemolytic activity (1.16, 1.81, 1.89 for MgSZ, ZnSZ coated, and uncoated 316L SS, respectively) (88). The morphology of human erythrocytes onto MgSZ, ZnSZ (Fig.5.7) and uncoated 316L SS samples remains normal as well. However, a few ruptured erythrocytes were observed in uncoated 316L SS samples. This might be due to the presence of nickel or chromium in the sample. Also, small, elongated erythrocytes were noticed in MgSZ coating.

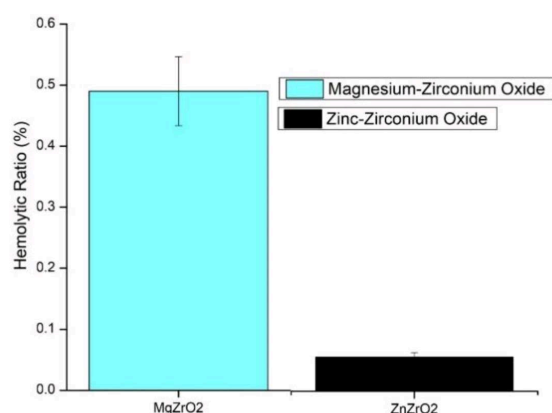


Fig.5.7 Graphical representation of hemolysis

5.3.2 Protein adsorption studies

Protein adsorption onto uncoated 316L SS, MgSZ, and ZnSZ and coatings (Fig. 13) was quantified by Lowry's method (66). MgSZ adsorbed 3.6 mg/ml, ZnSZ coatings evolved 3.52 mg/ml while uncoated 316 L SS adsorbed 3.05 mg/ml. There was no significant difference after 8 h of incubation. The improved protein adsorption on coated samples (MgSZ and ZnSZ) might be due to its hydrophilic nature, which facilitates the unfolding of adhered proteins paves the way to evolve interior functional groups, and enhance additional proteins binding sites (70,90). Further, uncoated 316 L SS showed considerable protein adhesion was due to the physisorption as well as the irreversible chemisorption mechanism (91,92,93). However, further studies need to be done to analyze specific proteins for better understanding.

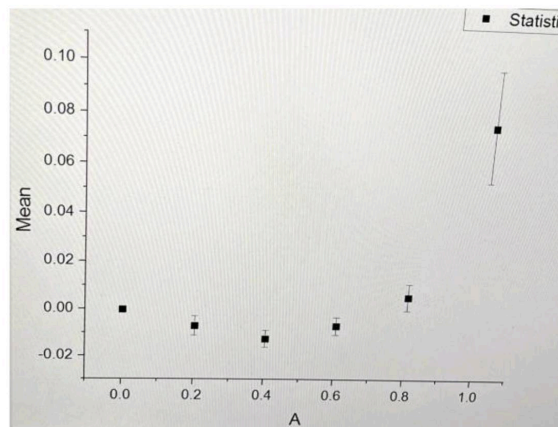


Fig 5.8 Standard graph for Protein adsorption

5.3.3. Microbial Type Culture

From the bacterial adhesion study, it is observed that coated samples exhibited very less bacterial property rather bare 316L SS samples. It could be due to the unfavorable environment of bacteria to attach onto coated samples. Hence, the study reveals that coated samples are better candidature for protecting bacterial adhesion than uncoated 316L SS sample.

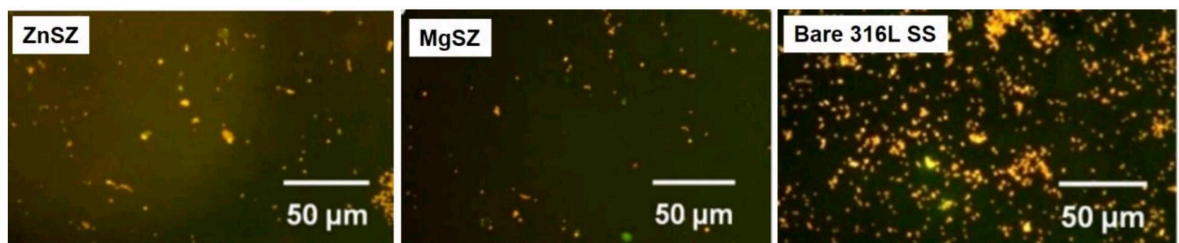


Fig.5.9 Microbial Type Culture

CHAPTER- 6

CONCUSION

Based on the findings presented, it can be concluded that the use of magnesium stabilized and zinc stabilized zirconium nanocomposites for biomedical implants is a promising approach. The in vitro studies evaluating hemolysis activity, protein adsorption, antibacterial properties, and cytotoxicity all demonstrated satisfactory results. Additionally, the XRD analysis revealed a satisfactory level of crystallinity, while the SEM analysis provided insight into the surface morphology. The contact angle analysis also demonstrated that the implant coating is superhydrophobic. Overall, these findings suggest that magnesium stabilized zirconium and zinc stabilized zirconium coatings on SS316 are suitable candidates for permanent implant fixations. This research provides important contributions to the field of biomedical engineering and serves as a foundation for future studies in this area.

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MgSZ and ZnSZ nanocomposite coatings on medical grade 316L SS – A suitable approach for mechanical, antimicrobial and biological perspectives

A. Chelsea Hannah^{#1}, H. Krishna^{#1}, T. Sudhakar^{#1}, Gobi Saravanan Kaliaraj^{#2}

¹School of Bio and Chemical Engineering, Department of Biomedical Engineering, Sathyabama Institute of Science and Technology, Chennai – 600119, India.

²Centre for Nanoscience and Nanotechnology, Sathyabama Institute of Science and Technology, Chennai – 600119, India.

[#]Authors are equally contributed

Corresponding Author: gsbiotech5@gmail.com, drsudhakar@gmail.com

ABSTRACT

Surface tailoring of stainless steel is an effective approach to prevent the chances of bacterial contamination and improve mechanical and biological properties. In this view, we have developed MgSZ and ZnSZ nanocomposite coated stainless steel using thermal spray technique. Different techniques such as XRD, (X-ray diffraction), SEM (scanning electron microscope), Raman spectroscopy and XPS (X-ray photoelectron spectroscopy) were used to characterize the coated samples. Wear test analysis revealed that surface modified 316L SS samples exhibited better wear resistance property. Further, MgSZ and ZnSZ nanocomposite coated 316L SS samples performed well in the biological and electrochemical aspects rather bare 316L SS samples.

Keywords: ZnSZ, MgSZ, Nanocomposite coating, Surface modification, Bioimplants, Thermal spray, Antimicrobial activity