RESEARCH PAPER

Investigation of Antibacterial Properties of Biomaterials used for Orthopaedical Applications

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ABSTRACT
Biomaterials have gained significant importance in aerospace, marine and medical applications in recent years. The growing incidence of antibiotic-resistant bacteria represents a major obstacle in the implantation of medical devices, making it essential to develop new biomaterials that have built-in antibacterial properties. This paper aims at investigating the antibacterial properties of various biomaterials and also to study the effect of coating on the resistance to bacterial growth on the substrate materials. The investigation additionally intended to suggest suitable materials and surface modification techniques for enhancing antibacterial characteristics, making the material ideally suited for implants. In addition, the present study showed that the metal coating mediated surface modification showed a notable antibacterial activity against *Escherichia coli* by reducing the growth of the microbe.

Keywords: *Bio-materials, coating, Escherichia coli and Bacterial growth.*

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1. INTRODUCTION

Biocompatibility of a material inherently possessing host tissues became the main thrust research area. Superior biocompatibility materials are suitable for applications interrelated to tissue and bone transplantation (1). The alloys such as stainless steel, titanium and cobalt along with other alloys, have been extensively studied and used in clinical applications as implant materials. However, inflammation related to implants remains a considerable factor contributing to implantation failure. Bacterial infections pose a serious intimidation to human health and have become a serious concern globally. In recent times, antibacterial metals have gained considerable attention due to its resistivity against antibacterial, excellent mechanical properties and biocompatibility nature. Through successful integration of various antibacterial agents, antibacterial metal alloys have been produced. This comprise antibacterial titanium alloys, stainless steel, antibacterial magnesium and its alloys, antibacterial zinc and its alloys, antibacterial cobalt alloys and also other antibacterial metals and alloys (2). The physical vapour deposition method was used to deposit non-toxic and non-allergenic β-type TNTZ alloys with durable CrN, TiN and ZrN coatings, and their corrosion and surface properties were examined under suitable environmental conditions. Additionally, the hardness, resistance to adhesion and antibacterial properties of the coatings were analyzed and reported in view of their prospective biomaterial applications (3).

The development of Fe-Mn-Zn alloys which are biodegradable has been developed through alloying and Spark Plasma Sintering (SPS). Antibacterial testing revealed that Fe-30Mn-5Zn confirmed improved bactericidal efficacy against S. aureus and E. Coli. The testing results indicated that integration of Zn was critical in improving the corrosion rate, mechanical properties, and antibacterial activity. These finding indicates that Fe-Mn-Zn alloys be capable of providing increase mechanical strength, antibacterial properties and biodegradability (4). To promote wound healing, different antibacterial agents, which consist of cationic organic agents, antibiotics, antibacterial nanoparticles are infused into a range of dressing material formulations, electrospun scaffolds, including films, hydrogels, sponges, and foams. Additionally, the advantages and limitations of biomaterials with varying morphologies used as wound dressings discussed in detail. Hence, it provided an overall

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perspective on the refined design and enhancement of injury dressings (5). The processes involved in adhesion of bacteria and formation of biofilm following the insertion of implants into the body are examined, with a primary importance on the advancement of antibacterial surfaces that are presently gaining attention in this domain. Gegeneration of tissue plays a vital role once the implants are incorporated within the body. Since infection prevention and regeneration at the implant site occur simultaneously, the creation of materials capable of performing dual-functional that inhibit both bacterial infection and persuade tissue regeneration is crucial (6).

Surface texturing is the process of machining pre-defined geometrical pattern on a material surface by laser ablation process. It is the most effective technique of surface alteration process to enhance the material properties such as load carrying capacity, wear resistance and also to reduce the coefficient of friction (7). A surface texture on the implant material can be obtained through various techniques such as grit blasting, shot peening, etching and oxidation. The above methods are simple and quick, but the repeatability of these methods is lesser. As compared with the methods discussed above, surface texturing by laser beam provides the good repeatability, time reduction, clean and accurate method. It is well established and a potential technique for surface modification of implant (8,9). Titanium Dioxide (TiO_2) is a commonly used ceramic material recognized for its outstanding hardness, superior wear resistance, chemical stability, and excellent biocompatibility, making it as suitable for biomedical applications. TiO_2 operates as a protective top layer to minimize friction, resist corrosive bodily fluids, and improve surface integrity during motion. Its chemically inert characteristics also help to mitigate inflammatory responses when it comes into contact with human tissue. Additionally, TiO_2 showing a high dielectric constant and can act as a barrier to the progressin of electrons and ions, enhancing its corrosion protection properties on magnesium substrates (10).

The titanium aluminum nitride coating (Ti-Al-N) is accepted for its versatility and broad applicability. It is a chemical compound consisting of three elements: titanium, aluminium, and nitrogen. This characteristic stems from the TiN added with aluminium, which specifically facilitates the formation of aluminium oxide on the surface of the material and hence chance of increasing the operational temperature range of the coating. The structure achieves an optimal relationship between high hardness and residual compressive stress (11). By incorporating antibacterial characteristics improvement and cell-instructive with a controlled degradation mechanism, developed multifunctional hydrogel presents a strong foundation for implant based treatments, efficiently encouraging regeneration of tissue and restraining infection thereby tackling the ongoing issue of implant related disease in regenerative medication and medical applications (12).

2. MATERIALS AND METHODS

The following material combinations are used for the antibacterial test. Samples 1, 2, 4, 5 and 7 were machined from the bright bar stock. Samples 3 and 6 were prepared from the special surface treatment in terms of coating, TiO_2 and TiAlN were applied on the bare raw materials such as Magnesium Alloy (AZ91B) and Titanium Alloy (Ti6Al4V).

Sample 1: Magnesium Alloy (AZ91D)

Sample 2: Polyethylene (UHMWPE)

Sample 3: TiO_2 coated Magnesium Alloy (TiO_2 + AZ91B)

Sample 4: Magnesium Alloy (A231B)

Sample 5: Titanium Alloy (Ti6Al4V)

Sample 6: TiAlN Coated Titanium Alloy (TiAlN + Ti6Al4V)

Sample 7: Magnesium Alloy (AZ31)

Prior to the coating applied on samples 3 and 6, laser texturing being done on the substrate materials of magnesium and titanium alloys. Laser ablation process involves the heating, melting and evaporation processes. Fiber laser machine, 20 Watts capacity as shown in Figure. 1 (a) is used for creating circular pattern on the surface. Circular geometry created on metal alloy surface is shown in Figure. 1 (b). The deposition of coating on the textured surface having its own advantages such as improving bonding strength of the coating and acts as a reservoir and also entrap the wear debris while sliding (11).

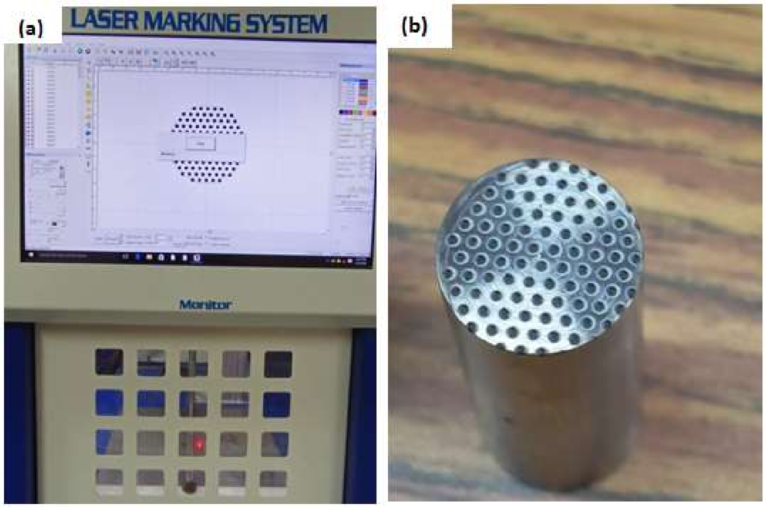

Figure 1. (a) Fiber laser machine used for texturing (b). Circular geometry dimple created with triangular pattern

The TiO_2 coating was utilized as the uppermost protective layer on Magnesium Alloy AZ31B pin specimens, using Plasma Spray Technology. The primary objective was to enhance the tribological performance of AZ31B, which, despite its mechanical compatibility with bone due to its low density and elastic modulus, suffers from poor corrosion and wear resistance. To address this challenge, a two-layered coating system was adopted. Before applying TiO_2, a Nickel-Chromium (Ni-Cr) alloy bonding coat was first deposited. The Ni-Cr serves a dual purpose: it provides a ductile, thermally compatible transition layer between the AZ31B substrate and the hard TiO_2 ceramic, while also ensuring excellent adhesion by creating a strong metallurgical bond. The presence of Ni-Cr further enhances oxidation resistance and prevents delamination of the coating during service. The TiO_2 coating, which was applied over the Ni-Cr bond coat, typically had a thickness ranging from 40 to 60 µm, while the bond coat thickness was approximately 10 to 20 µm,.

The TiAlN coating is applied using the physical vapour deposition method on a titanium alloy substrate, specifically Ti6Al4V. The coating thickness is maintained between 1 and 4 µm. This coating is characterized by its high resistance to heat and oxidation. The liberation of aluminium oxide (Al_2O_3) while TiAlN coating is heated which act as a guard from further oxidation. These results in improved hot hardness compared to other similar coatings. The colour of the TiAlN coating is influenced by the Ti: Al ratio, giving it a violet-bronze coloration. In our experiment, we coated titanium pin specimens with TiAlN to enhance their wear resistance.

3. ANTIBACTERIAL ACTIVITY OF COATING MATERIALS

UV-Visible spectrophotometer as in Figure 2 was used to assess the microbial growth in liquid broth medium by quantifying the light scattering on the cells at a particular wavelength (600 nm. With increasing time, the turbidity of the cells reduces the light transmission. As bacteria or cells divide, they increase turbidity, reducing light transmission. However, the quantification of cells typically uses the absorbance based on the Beer-Lambert Law by measuring the optical density of cells.

Figure 2. UV-Visible spectrophotometer was used for measuring the density of cells.

3.1 Culture and growth conditions of microbes used in this study

Escherichia coli was maintained on nutrient agar and broth media at 37 °C. In this study, 3.8×10^8 CFU mL^{-1} of *E. coli* ATCC 25922 from the nutrient agar was inoculated in the broth to perform the assays.

3.2 Effect of coating materials on the growth of *E. Coli*

The effect of coating materials such as Sample 1: Magnesium Alloy (AZ91D), Sample 2: Polyethylene (UHMWPE), Sample 3: TiO_2 coated Magnesium Alloy (TiO_2 + AZ91B), Sample 4: Magnesium Alloy (A231B), Sample 5: Titanium Alloy (Ti6Al4V), Sample 6: TiAlN Coated Titanium Alloy (TiAlN + Ti6Al4V), Sample 7: Magnesium Alloy (AZ31) was evaluated for their antibacterial activity against *E. coli* on nutrient agar medium by well diffusion assay. Briefly, 2.4×10^6 CFU mL^{-1} of *E. coli* was spread on the agar medium and well was created for fixing the sample materials on the centre of the laminate. The sterile coating materials was kept at the centre and incubate at 37 °C for 16 h. After incubation, the antimicrobial efficacy of the coating materials was observed by the zone of clearance around the materials.

3.3 Assessment of *E. coli* growth by spectroscopic method

In addition, the effect of coating materials as mentioned above was evaluated on the growth of *E. coli* in spectrophotometric analysis. The sterilized coating materials was kept in 24-well MTP in triplicate. After that, 1 mL of nutrient broth was added in each well followed by adding inoculum of 3.1×10^7 CFU mL^{-1} of *E. coli*. The laminate was incubated at 37 °C for 16 h and the growth was measured spectrophotometrically at 600 nm.

4. RESULTS AND DISCUSSIONS

4.1 Antibacterial activity of coating materials against *E. coli.*

The coated specimen i.e. TiO_2 coated Magnesium Alloy (TiO_2 + AZ91B) (Sample 3) exhibited a potential antibacterial activity against *E. coli*, as evidenced by a distinct and well-defined zone of inhibition surrounding the material. In contrast, Sample 4 (Magnesium Alloy A231B) demonstrated only a limited zone of clearance, indicating comparatively weaker antibacterial efficacy. The remaining materials did not exhibit any inhibitory effect, as bacterial growth was comparable to that observed in the control. Overall, the findings confirm that coatings based on TiO_2 + AZ91B and, to a lesser extent, A231B possess measurable activity of antibacterial against *E. coli*. as shown in Figure 3.

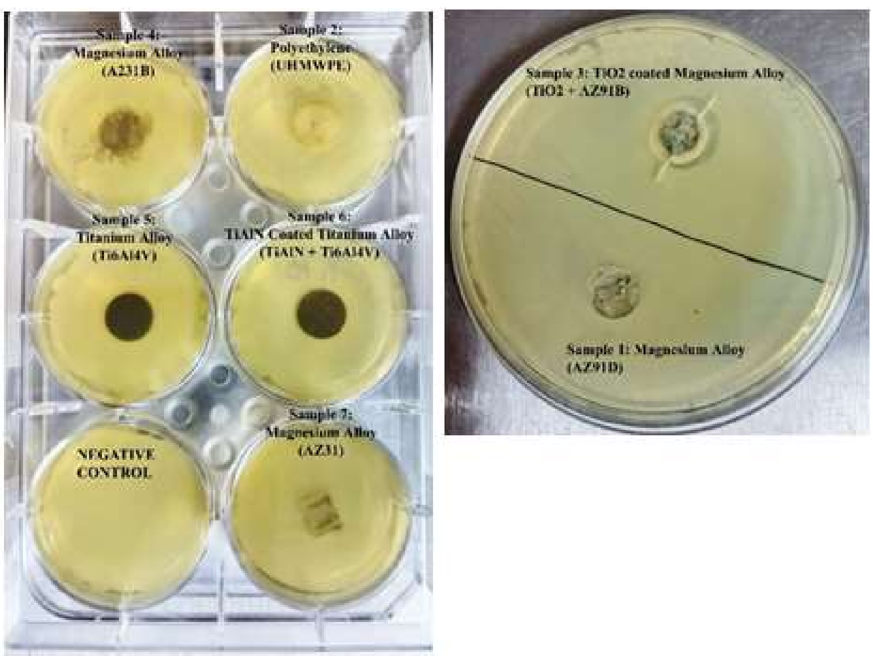

Figure 3 Antibacterial assement of coating materials by well diffusion assay

4.2 Spectrophotometric analysis of coating materials against *E. coli* growth.

The influence of various coating materials such as such as Sample 1: Magnesium Alloy (AZ91D), Sample 2: Polyethylene (UHMWPE), Sample 3: TiO_2 coated Magnesium Alloy (TiO_2 + AZ91B), Sample 4: Magnesium Alloy (A231B), Sample 5: Titanium Alloy (Ti6Al4V), Sample 6: TiAlN Coated Titanium Alloy (TiAlN + Ti6Al4V), Sample 7: Magnesium Alloy (AZ31) on the *E. coli* growth is illustrated in Figure. 4. Notably, an inhibitory effect on bacterial proliferation was observed in samples coated with TiO_2 coated Magnesium Alloy (TiO_2 + AZ91B). In addition, Magnesium Alloy (AZ91D) and TiAlN Coated Titanium Alloy (TiAlN + Ti6Al4V demonstrated a measurable reduction in absorbance relative to the control, indicating partial suppression of microbial growth. In contrast, the other coated samples exhibited no statistically significant impact on *E. coli* proliferation. Overall, spectrophotometric analysis corroborates that coatings comprising TiO_2 coated Magnesium Alloy (TiO_2 + AZ91B) possess appreciable antibacterial activity, as evidenced by the diminished growth of *E. coli*.

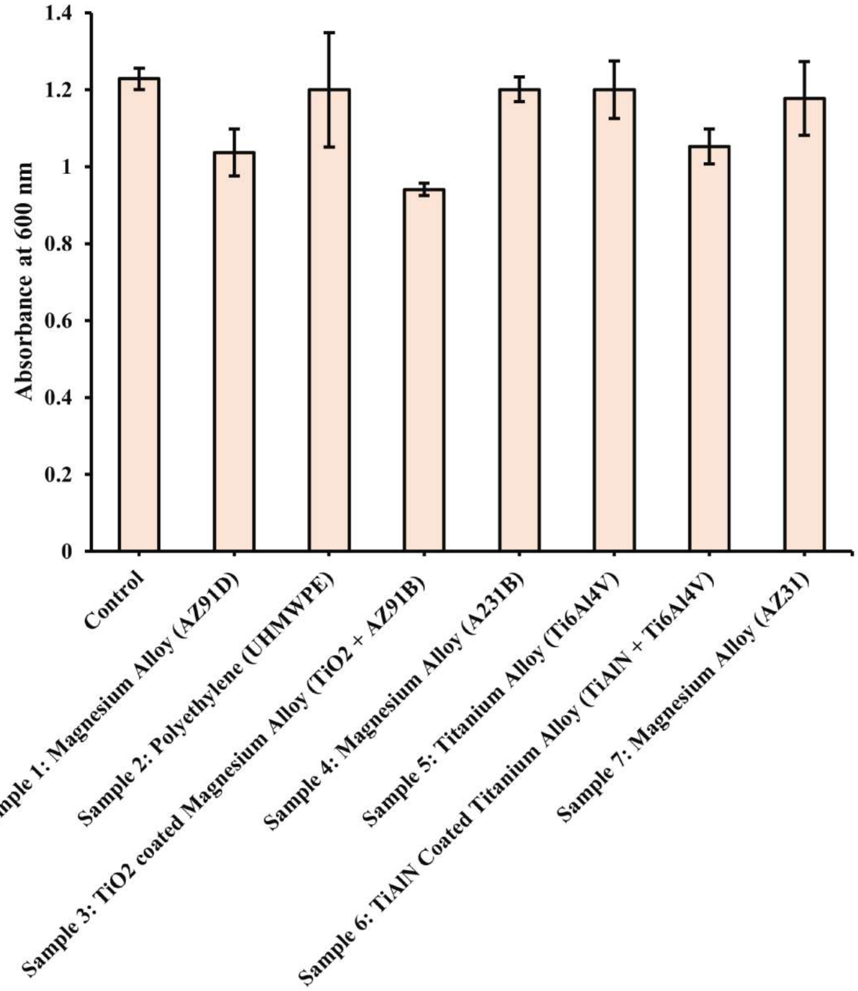

Figure 4 The impact of coating materials on *E.coli* by Spectrophotometric analysis

5. CONCLUSION

The antibacterial activity test carried successfully on coated and non-coated specimens using E-coli proliferation. Though all the samples inherently posses the anti-corrosion properties, its anti-bacterial properties are different. Among the samples tested, TiO_2 coated Magnesium Alloy (TiO_2 + AZ91B) possess appreciable antibacterial activity, as evidenced by the diminished growth of *E. coli*. which is well suitable combination for artificial joint replacement. Magnesium Alloy (AZ91D) and TiAlN coated Titanium Alloy (Ti6Al4V) outperforms other remaining materials considered for the testing.

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