



TI6AL4V SURFACE MODIFICATION BY HYDROXYAPATITE POWDER MIXED ELECTRIC DISCHARGE MACHINING FOR MEDICAL APPLICATION

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ABSTRACT

Titanium surface modification by the Hydroxyapatite (HA) mixed Electric Discharge Machining (EDM) is an alternative and promising technique to enhance the biocompatibility and to promote the biological performance in bone, which is dependent on surface properties, such as surface roughness, chemistry, and wettability. HA powder is used for the first time with electrical discharge machining to improve osteoblastic cell activity on the developed surfaces for Ti6Al4V. Different HA concentrations in deionized water were tested as an experimental variable during EDM. Abrasive polishing and electrical discharge machined control surfaces without powder addition also analyzed to compare the results. The surface characteristics of analyzed samples were evaluated by Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS), X-Ray Diffractometry (XRD), white light interferometry, and contact angle measurements. The wettability tests suggest that the hydroxyapatite powder mixed EDM'ed surfaces shows highly hydrophilic characteristics compared the other surfaces, abrasive polished and EDM'ed without powder addition in the dielectric. The results from the MTT assay revealed that those surfaces modified using HA powder addition in distilled water dielectric liquid promoted the most significant cell attachment/growth. The results indicate that HA powder mixed EDM offers a promising method for the surface modification of biomaterials such as titanium alloys.



Keywords: Ti6Al4V, surface modification, Hydroxyapatite, Powder Mixed Electrical Discharge Machining, Osteoblast, Cell Attachment and Growth

1. INTRODUCTION

Recently, the importance of biomaterials has been increasing in parallel with the developments of medicine and engineering. These materials used for the repair of injuries, inflammations, tumors, gangrene, implants and could be metals, ceramics, polymers, and composites. The most commonly used metals for such applications are Ti and Ti alloys, as well as stainless steel. Metals for biomedical applications are usually coated to prevent corrosion and metallic ion release that have possible toxic effects to biological being. Working on various coating methods, facilitate surface coating or obtain functional surfaces to inhibit such side effects of metals have become the focus of researchers. Nowadays Electric Discharge Machining (EDM) is considered as a challenging alternative for obtaining functional surfaces for medical applications. Especially the properties of the surface and subsurface layers obtained in the EDM method are worth investigating in this regard.

In general, the topmost layer on the EDM'ed surface is the resolidified structure generated due to the thermal actions of the sequentially applied sparks. The heat affected structures in solid state lay underneath the resolidified layer that is followed by the unaffected base material. The creation of nanostructural resolidified layer is vital for the development of biocompatibility. Formation of γ phase and κ carbide in recast layer observed when Fe-Al-Mn work material EDM'ed in kerosene dielectric liquid. So, the formation of the nanostructure oxide layer in EDM could improve the work material biocompatibility [Chen et al., 2008]. Besides, nano ($\delta + \gamma$) hydrides are also essential for the formation of nanostructural oxide layers. $\alpha \rightarrow (\alpha + \delta) \rightarrow (\delta + \gamma) \rightarrow \gamma$ conversion occurs in resolidified layer throughout the EDM. Moreover, short duration pulses provide nanoporous TiO_2 on EDM'ed titanium surface [Peng et al., 2010]. Yang et al., [2013] and Lee et al., [2013] have observed nanoscale pores in anatase TiO_2 surface when EDM of Ti-6Al-4V work material when using water as dielectric liquid. Also, better biocompatible surfaces were obtained when using high pulse current and duration during EDM [Lee et al., 2016]. Fabrication of biocompatible microdevices is also possible by micro EDM with the desired surface roughness values recommended for biological applications [Murali and Yeo, 2004]. Corrosion resistance is also a key factor for dental implant applications. Comparison of EDM'ed and conventionally finished (grounded and polished) surfaces revealed a reduction in corrosion resistance of Co-Cr and grade II cpTi alloys [Ntasi et al., 2010].



Obtaining functional surfaces with the aid of powder additives in dielectric liquid also compelled researchers interest. In this way, TiC and nickel enriched resolidified layers formed on the surface with the proper selection of powder additives [Uno et al., 2001]. 150 μm thick TiC layer with a hardness of 1600 HV on carbon steel is possible when using Ti powder additives in EDM [Furutani et al., 2001]. Using reduced discharge energy improve the hardness of the resolidified layer up to 2000 HV [Furutani et al., 2009]. A titanium layer formed onto tungsten carbide work material when using Ti powder mixed EDM. The hardness of the layer reached up to 1750 HV with fewer occasions of cracking on the surface [Janmanee and Muttamara, 2012]. Similarly, tungsten powder mixed EDM produced a tungsten-rich and hard resolidified layer on steel [Kumar and Batra, 2012]. SiC powder mixed EDM'ed Ti-6Al-4V surfaces also indicate material migration from the suspended dielectric liquid. [Yasar and Ekmekci, 2013]. Al powder provides the least surfaces roughness, the best surface topography, and material removal rate, followed by SiC, Gr, Cr and Fe in sequence in powder mixed EDM [Jabbaripour et al., 2013]. The obtained recast layer can be considered as a kind of coating with excellent mechanical properties when tool steel is machined molybdenum powder mixed EDM [Amorim et al., 2017]. Similarly, a continuous strengthened TiC and TiSi₂ is formed on Ti-6Al-4V when using SiC abrasive-mixed EDM with magnetic stirring [Li et al., 2017].

The literature survey above reveals the possibility of powder mixed EDM as an alternative technique for obtaining functional implant surfaces. Surface treatment of Ti-6Al-4V alloy by EDM for orthopedics applications provided better substrates for the adhesion, growth, and viability of MG-63 cells than the alloy plasma-sprayed with TiO₂ [Harcuba et al., 2012]. High current, pulse duration, and powder concentration in Powder Mixed EDM increase the recast layer thickness and provide a Ti modified hydrophilic surface without surface cracks [Chen et al., 2014]. Bioceramic oxides and carbide phases (TiC, SiC, and NbC) on the Powder Mixed EDM'ed β -Ti surface provide excellent adhesion to the substrate with improved hardness. Besides this, an acceptable corrosion resistance is obtained for dental implants [Prakash et al., 2015]. The fatigue endurance limit of 280 MPa is possible which is very suitable for orthopedic applications [Prakash et al., 2016a]. Similarly, according to the multi-objective optimization results, parameter sequence consisting of 13A peak current, 5 μs pulse duration, 8% duty factor and 8 g/l powder concentration is the most suitable choice to enhance the biocompatibility of β -Ti alloy [Prakash et al., 2016b]. Surface crack density on the machined surface decrease considerably on EDM'ed Ti-35Nb-7Ta-5Zr β -titanium alloy when using 4 g/l Si powder concentration in the dielectric liquid. The pore size of 200–500 nm in the interconnected surface is obtainable in the parameter sequence consisting of 15A pulse current, longer pulse interval and 8 g / l Si powder concentration [Prakash et al., 2017a].



Therefore, powder mixed EDM provides higher bone/implant interface strengths, nanoporous surface, suitable adhesion and growth of osteoblast-like cell (MG-63) [Prakash et al., 2017b].

Hydroxyapatite (HA) is one of the hardest molecules, and its crystal structure is similar to that of the human skeletal system so that it can be used instead of bone. Similar to the structure of the bone, the ratio of calcium to phosphorus (Ca / P) is around 1.67. The studies on the use of HA for medical applications in powder mixed EDM were first carried out in 2013 [Ekmekci and Ekmekci, 2013]. In their study, they performed HA precipitation on Ti alloy when using HA additives in the dielectric liquid. They showed that, the most suitable parameters are high pulse current and low pulse on duration. In their continuing work, they have indicated that secondary sparks have made deposition more effectively [Ekmekci and Ekmekci, 2016]. Recent work on this subject has showed an improved corrosion resistance for β -phase Ti implant by HA mixed EDM. Higher corrosion resistance and improved hardness, as well as 18–20 μm , recast layer containing biocompatible phases with excellent adhesion to the base surface could be achieved [Prakash and Uddin, 2017]. Similarly, orthopedic-implant materials based on titanium and titanium–tantalum alloys are EDM'ed using HA powder additives [Shih-Fu Ou et al., 2017]. In another study, a biocompatible and nanoporous hard layer is formed on metallic glass surface when using HA as powder additive in hydrocarbon-based dielectric liquid [Aliyu et al., 2017].

The main attractive point of the process is the ability to provide extreme heat and pressure cycles in microscale that could not be achieved by other known techniques. Therefore, the idea of adding the powder in a dielectric liquid and modifying the machined surfaces for specific needs promising an innovative and practical alternative technique. Nowadays, among the specific needs, the possibility of generation of biocompatible surfaces with the use of the process became an attractive alternative to the known surface modification techniques such as plasma spray, shot peening, abrasive blasting, aqueous spray, and pulsed laser deposition. The studies are in the initial state, and preceding studies are needed to understand the bioactivity of HA powder mixed EDM. Therefore, the surface characteristics, and biocompatibility of Ti6Al4V surfaces modified HA powder mixed EDM are analyzed in the study.

2. EXPERIMENTAL SETUP AND PROCEDURE

Test Samples Preparation: Ti-6Al-4V ELI (Grade 5 ELI) materials were used in this study. The samples with 8 mm length were cut from 10 mm diameter titanium rod. Samples were machined by using AJANEDM CNC983 brand die sink type EDM (Figure 1). Pure titanium was used as the tool electrode during EDM. Deionised water was used as a dielectric fluid.



Hydroxyapatite (HA) powder was added to dielectric fluid with different concentration (0, 5, 10, and 15 g/L). Other EDM process parameters kept constant for all experiments. The machining condition was selected from the previous experimentation as 12 μ s pulse on duration and 22 A pulse current [Ekmekci and Ekmekci, 2016]. As a control, two Ti samples were wet-abraded with # 600 to #1200 SiC abrasive paper followed by polishing with 1 μ m polishing cloth. One of the control surface was polished by keeping the orientation of the sample constant during polishing (direct polish) as shown in Figure 2-(a), the other one was polished with random orientation (random polish) as shown in Figure 2-(b). After machining, the samples were cleaned in acetone for 5 min., ethanol for 5 min., and pure water for 15 min. using ultrasonic cleaner and the samples were dried in the air after each cleaning processes.

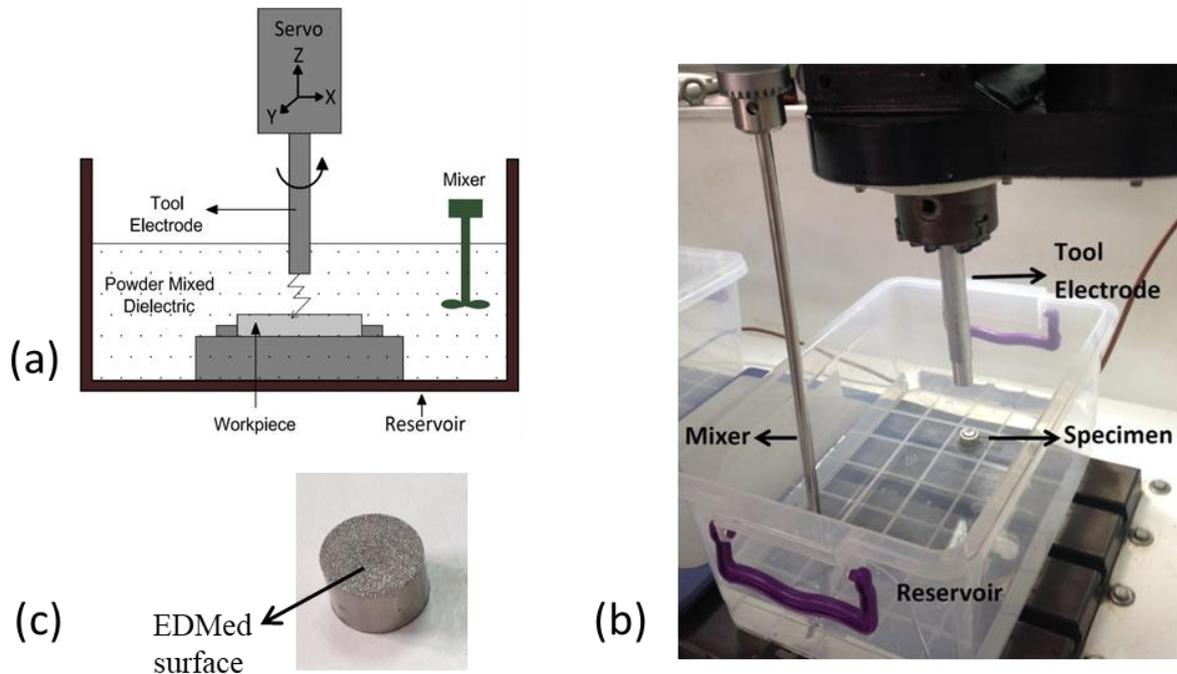


Figure 1. Experimental setup (a) schematic drawing, (b) reservoir view and (c) an EDMed sample.

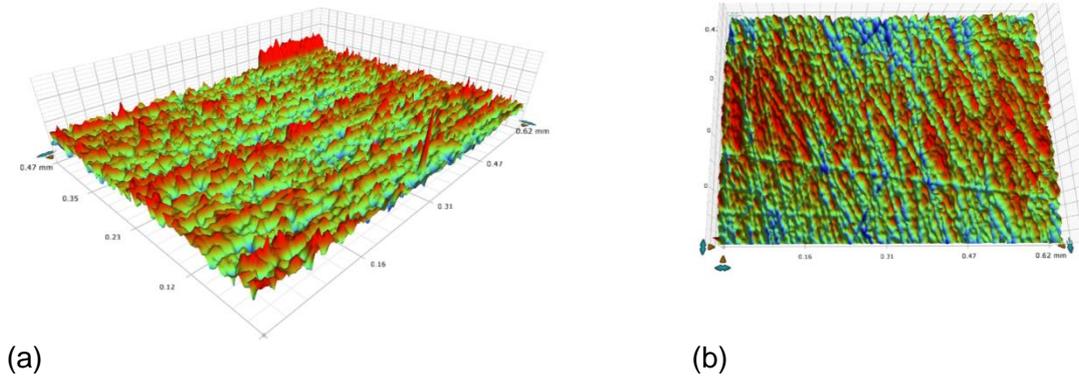


Figure 2. Control surfaces (a) directly polished ($S_a=61.5$ nm) and (b) randomly polished ($S_a=34.8$ nm). S_a is an areal surface roughness parameter measured using Bruker white light interferometer.

Surface Analysis: The surface morphologies and surface elemental composition of the EDMed samples were examined by using scanning electron microscopy (SEM, FEI Inspect S.) coupled with energy-dispersive x-ray spectroscopy (EDS). Phase composition of the EDMed surfaces were examined by using X-ray diffractometer (XRD, Rigaku MiniFlex) with settings (Cu, 30kV, 15mA). The samples were scanned at incident angle over a 2θ range of 30° to 90° . Bruker Contour GT-K 3D optical microscope (white light interferometer) with Vision 64 software was used for surface topography and surface roughness analysis.

Sessile-Drop Contact Angle Measurement: The water contact angle of the samples was measured using a KSV CAM101 contact angle goniometer. Contact angle measurement conducted for 30 s time duration (20 f/s) for a single drop. Each drops volume during contact angle measurement was $1.5 \mu\text{L}$. Mean value of the contact angle was obtained at 10 s when the water droplet got mostly stable form.

Cell Culture: The cells described in this work are human bone Fibroblast cells, MG-63 (ECACC 86051601), which were purchased from Public Health of England. All cell culture work was carried out under aseptic conditions in a grade II laminar flow cabinet (EBSCO). Cells were maintained at 37°C in a humidified 5 % $\text{CO}_2/95$ % air atmosphere in Dulbecco's modified Eagle's medium (DMEM) (Sigma Aldrich, D6429) supplemented with 10 % foetal bovine serum (Sigma-Aldrich, 0804) and 1 % penicillin-streptomycin.

Cell Attachment and Growth: In order to determine if the cells (MG-63) preferred a particular machined surface they were seeded onto the surfaces at a density of 10,000 cells per well and left to incubate for 24hrs (37°C , 5% $\text{CO}_2/95\%$ humidity). After 24hrs, an MTT assay was carried out to determine relative cell growth on all surfaces through absorbance.



3. RESULTS

Analysis of Surface Topography and Roughness: Figure 3 shows the SEM and 3D white light interferometer images of EDM'ed and PMEDM'ed surfaces of the Ti6Al4V work material. As observed, HA PMEDM'ed surfaces characterized by distinct features and morphologies compared to the EDM'ed surface in deionized water without powder addition. The EDM'ed surfaces (Fig. 3(a) and Fig. 4(a)) display formation of smooth molten materials with very shallow craters, which resulted in lower average surface roughness (S_a) on the corresponding surface (Fig. 5). With the HA powder addition to the dielectric liquid, complex porous microstructures composed of overlapped micro pits and decomposed HA powders embedded into the molten surfaces, which could provide reliable anchorage for the particles and elements attached to the PMEDM'ed surfaces. More and deeper micro-craters were formed when HA concentration increased from 5 to 15 g/L, which led to high surface roughness, S_a (mean areal surface roughness) and S_z (maximum height from valley to peak) as shown in Fig.5 and Fig. 6, respectively, compared to polished and EDM'ed surfaces. The EDM'ed surfaces consisted of some microcracks (Fig. 4(a)) when treated with only deionized water. However, negligible or crack-free surfaces with porous structure were formed with HA PMEDM process (Fig.4(b-d)). Bruker 3D micrographs (Fig. (3)) also display micro roughness differentiation between different machining conditions. High concentration of HA led to increased micro roughness in micro-craters.

Analysis of Surface Composition by EDS/XRD: Element and phase composition of EDM'ed and PMEDM'ed surfaces were investigated using EDS and XRD analysis. Fig. 7 shows an SEM image of the PMEDM'ed surface with 10 g/L HA concentration. Associated EDS plot confirmed the presence of Ti, V, Al, and Ca elements. The Ca elements are existing due to the chemical decomposition of the HA powder under a high thermal source of energy during sparking. Fig. 8 shows the XRD patterns of the polished, EDM'ed and PMEDM'ed surfaces of Ti6Al4V samples. The phases are shown on the same graph to make a proper comparison. Polished Ti6Al4V sample contains more α -Ti phases where the β -Ti peak is observed before the maximum α -Ti at 40° . Samples which are machined in water dielectric and HA mixed dielectric contain TiO phase where O diffused from water dielectric liquid during machining that improves surface biocompatibility and bone cells osseointegration characteristics. It is known that, these bioceramics oxide layers have high corrosion resistance characteristics and commonly used as coating materials for biological application [Prakash et al., 20015]. HA penetrating the surface during PMEDM are observed approximately at 36° [Chen et. al., 2014]. So, the presence of HA-rich layer is confirmed that is crucial for medical applications.

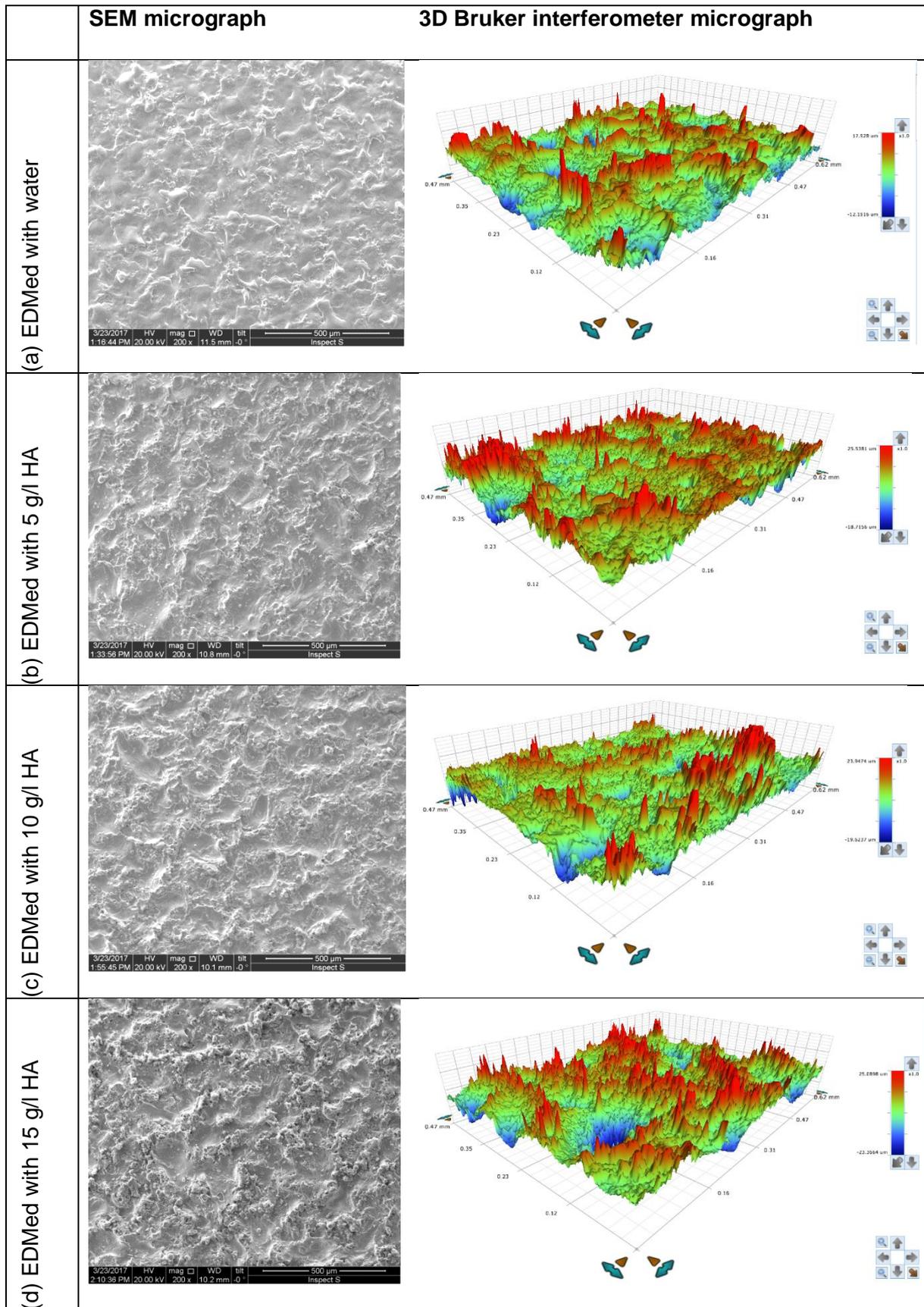
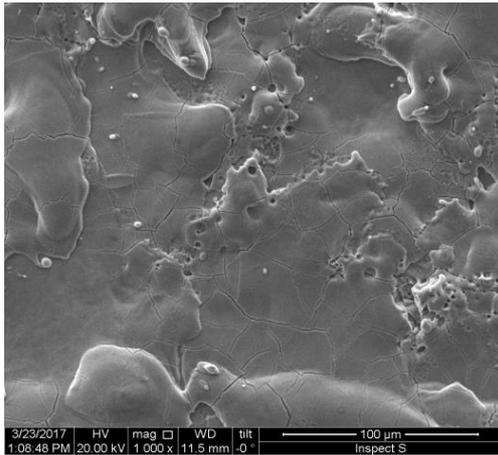
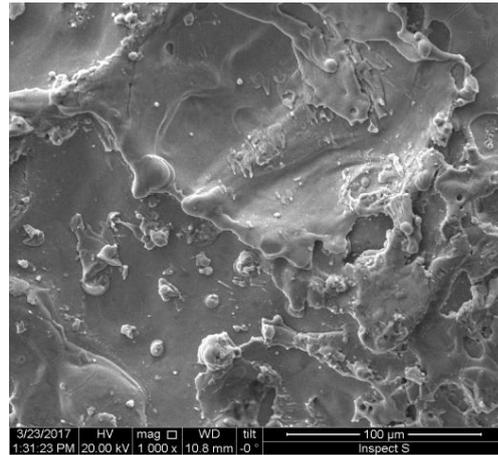


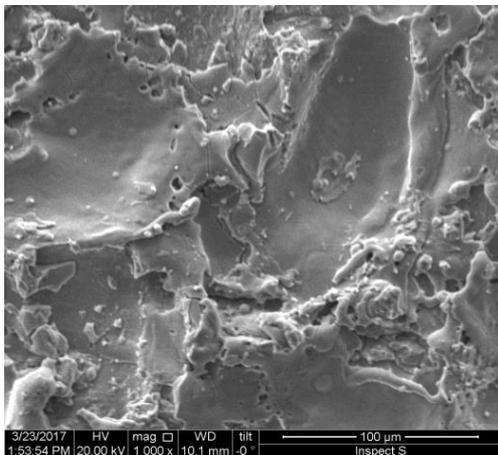
Figure 3. SEM and Bruker interferometer micrographs



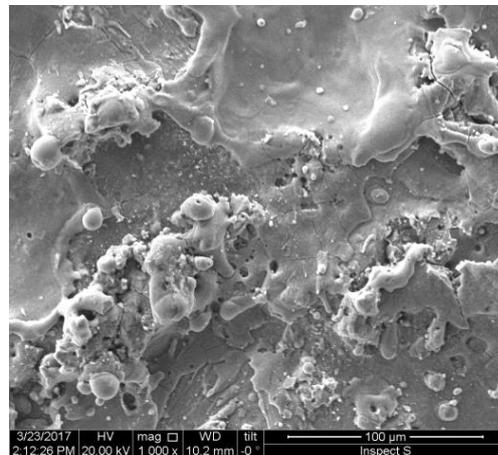
(a) EDMed with water



(b) EDMed with 5 g/l HA



(c) EDMed with 10 g/l HA



(d) EDMed with 15 g/l HA

Figure 4. SEM micrograph with 1000x magnification.

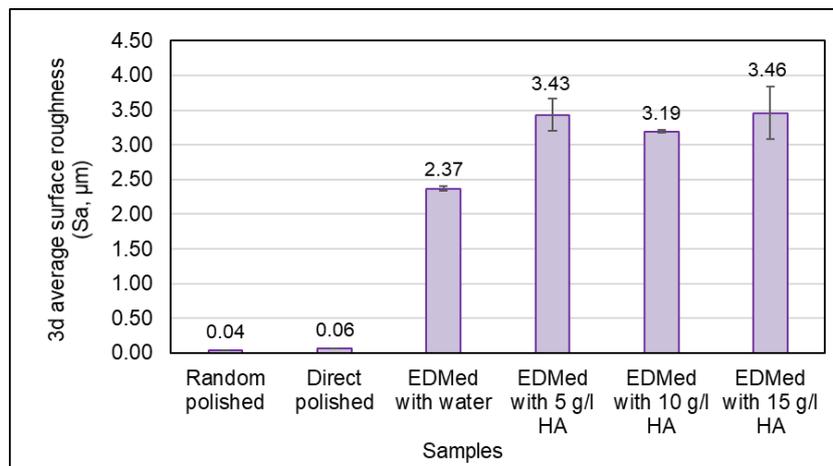


Figure 5. Average surface roughness (Sa) against samples treated by polishing and EDM and PMEDM.

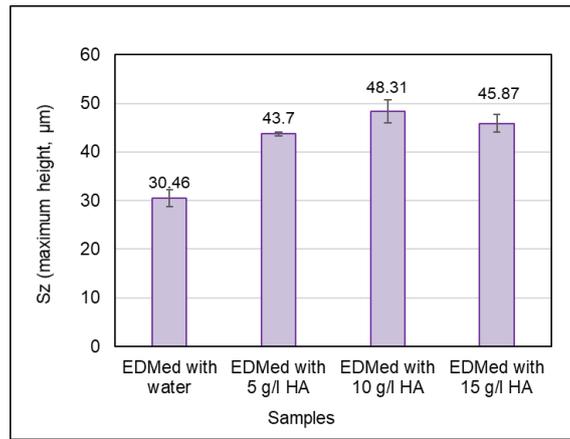
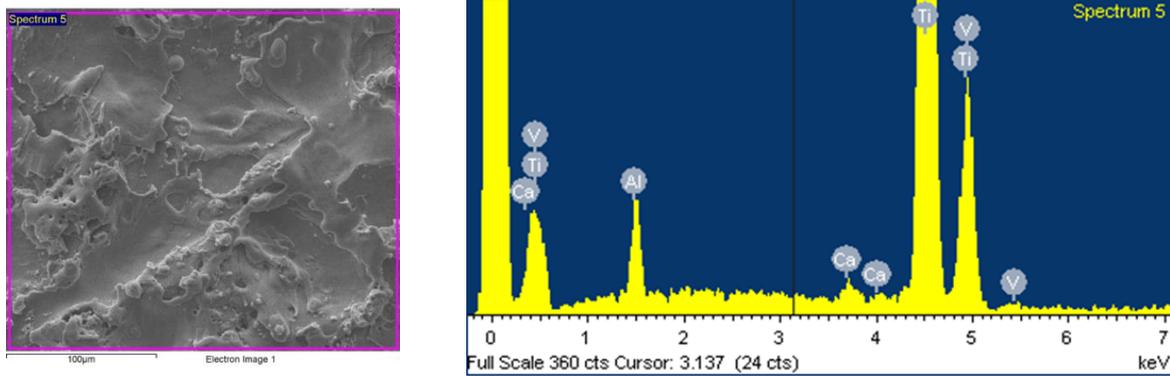


Figure 6. S_z measurement on EDM'ed and PMEDM'ed surfaces



(a)

(b)

Figure 7. (a) SEM micrograph, (b) EDS analysis for samples EDM'ed with 10 g/l HA

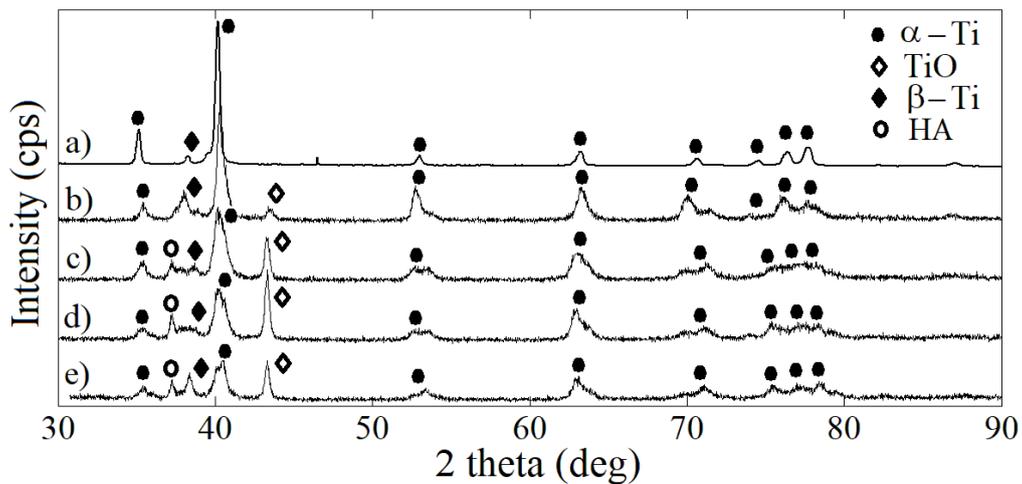
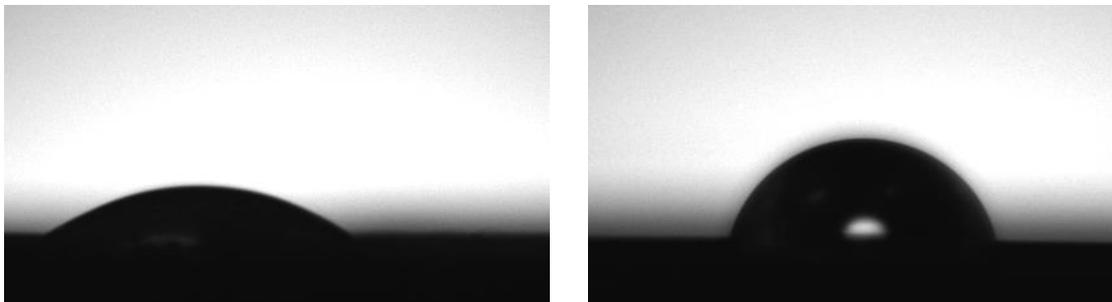


Figure 8. XRD analysis of the sample surfaces. **a)** Polished Ti6Al4V sample. **b)** EDM'ed sample in water dielectric. **c)** PMEDMed sample in 5 g/l HA mixed dielectric **d)** PMEDMed sample in 10 g/l HA mixed dielectric **e)** PMEDMed sample in 5 g/l HA mixed dielectric

Surface Wettability: The PMEDM was found to promote surface hydrophilicity due to high surface energy. Surface wettability was measured by contact angle measurement. Fig. 9 shows water drops on (a) the PMEDM'ed with 5 g/L HA and (b) polished surface, in which the contact angles measured from the drop shapes. The PMEDM'ed surfaces resulted in lower contact angles compared to EDM'ed and polished surfaces as shown in Fig. 10. However, optimal HA concentration must be sought to give the most beneficial surfaces with super hydrophilicity characteristic, which requires further research on it. Thus, the lower the contact angle, the higher surface hydrophilicity. Surface hydrophilicity increases the adsorption of cell adhesion, which in turn contribute to promote enhanced cell response, such as osteoblastic cell attachment and proliferation compared with hydrophobic Ti surfaces [Otsuka et al., 2012].



(a) EDMed with 5 g/l HA (Contact angle=33.7°) (b) Random polished (Contact angle=77.3°)

Figure 9. Contact angle measurement for wettability test.

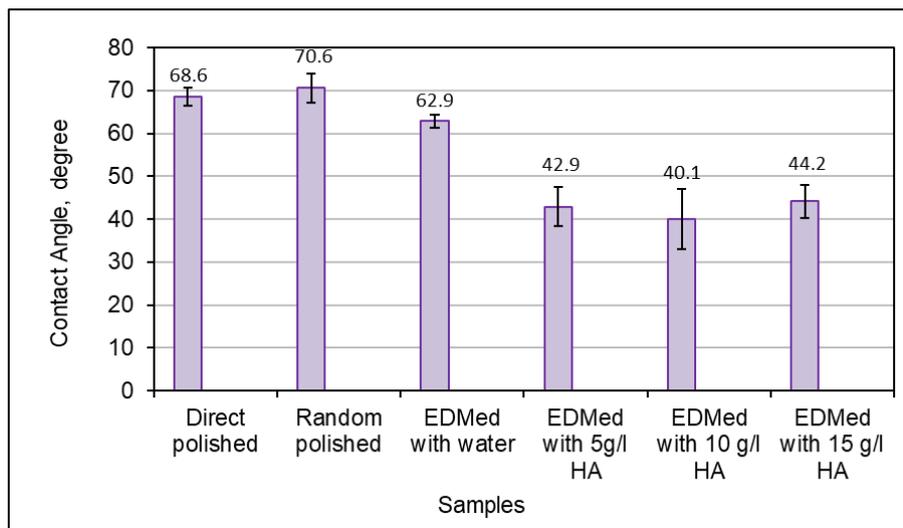


Figure 10. Contact angle measurement for different surfaces.



Cell Attachment and Growth: In order to determine how the EDM'ed and HA PMEDM'ed surfaces affected cell adhesion and growth, a MTT assay was performed 24hrs after the cells were seeded on to the surfaces. Fig. 11 shows adhesion/growth of the cells to PMEDM'ed with 10 and 15 g/L HA concentration give the best cell absorbance compared to that of the EDM'ed and polished surfaces. Higher the value of absorbance reflects that the given surfaces promoted cells attachment and growth. However, the polished surfaces give better cells absorbance compared to the EDM'ed and PMEDM'ed with 5 g/L HA surfaces. These findings suggest that the HA PMEDM will be promising alternative technique to modify the Ti alloys to promote the cells bioactivity and surface biocompatibility. Higher absorbance values with higher HA concentration could be attributed to surface porosity, rough micro structure, Ca elements and oxide phases (TiO) constituents created during the PMEDM.

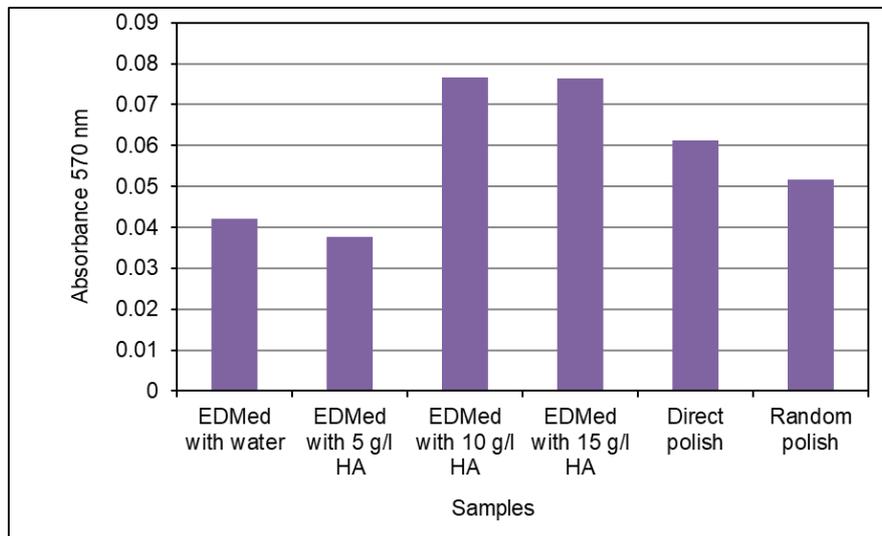


Figure 11. Surfaces were seeded with cells and left for 24hrs. An MTT assay was carried out to determine relative attachment/growth across all surfaces

4. DISCUSSION AND CONCLUSION

The Ti6Al4V work material, often employed as an implant material in dental and orthopedic applications, was treated by the HA mixed EDM to investigate its surface formation characteristics and *in vitro* osteoblast-like MG-63 cells response to the generated surfaces. The HA mixed EDM'ed surfaces exhibit higher surface roughness compared to the control surfaces treated by abrasive polishing and EDM with deionized water. HA concentration in the dielectric fluid is one of the influential factors to obtain surfaces which can promote cellular activity and biocompatibility. Surface wettability, indicated by the water contact angle measurement, is also affected by addition of HA powder. Highly hydrophilic surfaces with



contact angles around 40° were created by the HA mixed EDM compared to the polished surface with contact angle around 70° and EDM'ed surface with contact angle around 62°. The hydrophilic surfaces promotes cellular activity, which was observed in this work as well. The MTT assays demonstrate that better cell attachment and growth are observed on the surfaces treated by the HA (10g/L and 15 g/L concentration) mixed EDM compared to all other surfaces. It is crucial to mention that the alteration on the surfaces with powder mixed EDM is partially due to alteration of discharge mechanism, which resulted in several secondary discharges leading to particle migration and deposition. This discharge alteration phenomenon was detailed in authors' previous paper [Ekmekci and Ekmekci, 2016]. It can be concluded that the HA mixed EDM with high HA concentration provides very efficient and rich surfaces for enhanced biocompatibility and improved cellular response. Factors affecting these improvements could be attributed to the formation of HA-enriched layer composed of HA crystals and other elements (Ca), oxide phases (such as TiO and TiO₂), higher micro surface roughness, and hydrophilic surface characteristics. Further work could be needed to find optimal conditions including HA concentration to obtain better biocompatible surfaces, and further, *in vitro*, biological tests could be performed to investigate the cell adhesion, proliferation and differentiation on the HA mixed EDM'ed surfaces.

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