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RESEARCH ARTICLE

Antibacterial - Microbial Performance of Beta Phase Titanium Copper Alloy for Intrauterine Gynaecological Application

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ABSTRACT

The research study on the anti-bacterial property of beta phase TiCu alloy was handled by antibacterial test on Escherichia Coli (E.C) and Staphylococcus Aureus (S.A) colonies on the specimens. Research studies indicated that the Ti 17%Cu alloy specimen exhibited excellent antibacterial property, providing a great potential in clinical application for gynecological implants. The research of antimicrobial/antibiofilm activities of the beta phase contraceptive Titanium Copper alloy (TiCu) against these bacterial species showed an excellent antibacterial effect in vitro, thus inhibiting both Escherichia coli and Staphylococcus Aureus. This research innovated the design and production of Beta(β) phase TiCu alloy specimens in the categories of; Ti0.5%Cu, Ti1.0%Cu, Ti2.0%Cu, Ti5.0%Cu, Ti10.0.0%Cu, Ti15.0%Cu and Ti17.0% Cu, using Copper element as the experimental control reference biomaterial. The TiCu alloy specimens were produced by powder metallurgy technique in an inert environment. Experimental investigations and Minitab Software Design analyses on the specimens for combined antibacterial response in direct improvement of mechanical compatibility (tensile strength, hardness value, fracture toughness), biocompatibility–cell viability (cytocompatibility), was carried out in order to establish the suitability of Ti17%Cu alloy as a possible replacement to the existing prototype biomaterial (Cu-T380(IUD).

Keywords: *Antibacterial; Microbial Performance; Beta Phase Titanium Copper Alloy; Intrauterine Gynaecological Application*

Introduction

Most metals (Cu, Ag, Zn etc.) have been reported as being antibacterial agents in metallic matrix. Therefore, elements alloyed with these trace elements are used to develop antibacterial biomaterial metals for orthopedic, gynecological and dental implants. Copper element is a prominent material alloyed with other elements in order to establish antibacterial property. Recently, most metals having eutectic alloy composition are used as biomaterial with antibacterial response (Erlin, 2013). Titanium alloy exhibited excellent antibacterial property and corrosion resistance, providing a great potential in clinical application for gynecological implants.

Biomaterial research reports established that human tissue is mainly organized of self-assembled polymers (proteins) and ceramics (bone materials), having metal constituents as trace elements (Qizh & George, 2015). Titanium has a phase transformation from alpha to beta phase at temperatures above 883°c. Below 882.5°c, Titanium exists as alpha-phase (α*)* material and the crystal structure is hexagonal close packed (Hcp), but above 883^{0}c it changes to body centered cubic system (bcc) in beta *(β) phase,* because it possesses high passivity and regenerative properties that is, the ability to repair itself and form protective covering, with dense oxide film (Coating, 2003). It is usually considered for biomaterial, and the low young modulus is very close to that of the bone (Williams, 2013), which disallows the stress shielding effect associated with biomaterials of high modulus of elasticity, common to alpha (*α)* and alpha+beta *(*α +β) phases. Titanium alloys are differentiated into three metallurgical groups, which are; alpha (α), Beta (β) and alpha+beta. Research has shown that copper phase stabilizes Titanium alloys, and these are qualitatively used in gynecological biomaterial application, with very low modulus of Elasticity (which is below the α and α + β phase) , and very close to human femoral bone modulus of elasticity of between 38-40GPA (Sammons,2011). Titanium and its alloys in beta phase domain exhibit microstructure effects of Osseointegration, osteoconduction and osteoinduction properties of biomaterials .The Osteoinduction is an attribute of Titanium which guarantees bone healing process with formation of prosteoblasts, and the reduction of cracks and fractures initiated by corrosion process(Sutter and Bonni, 2005).

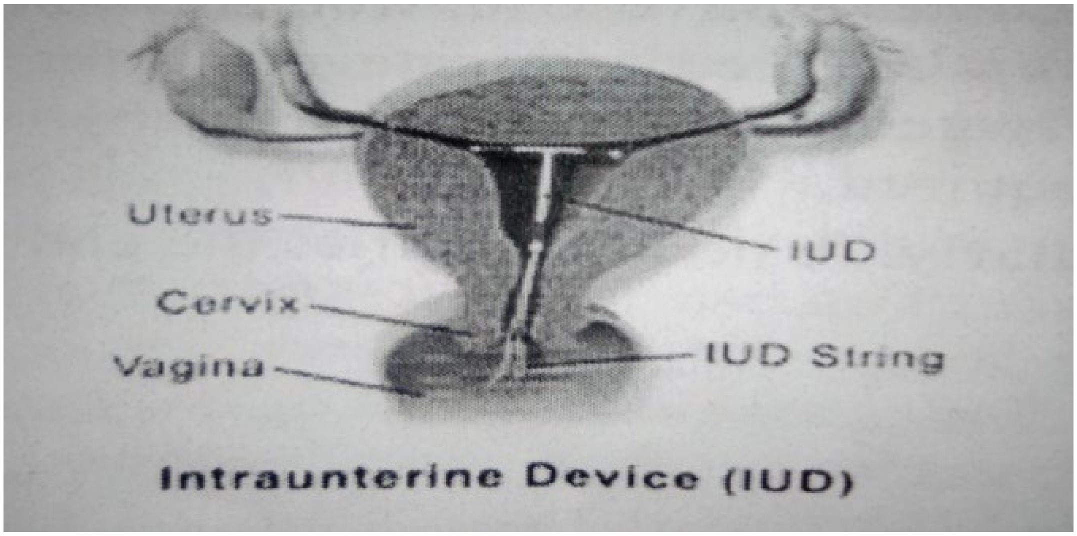


Figure 1: *Insertion of IUD T380 in the Endometrium Ref;* **Kalpana Gupta (2009)**

Materials and Method

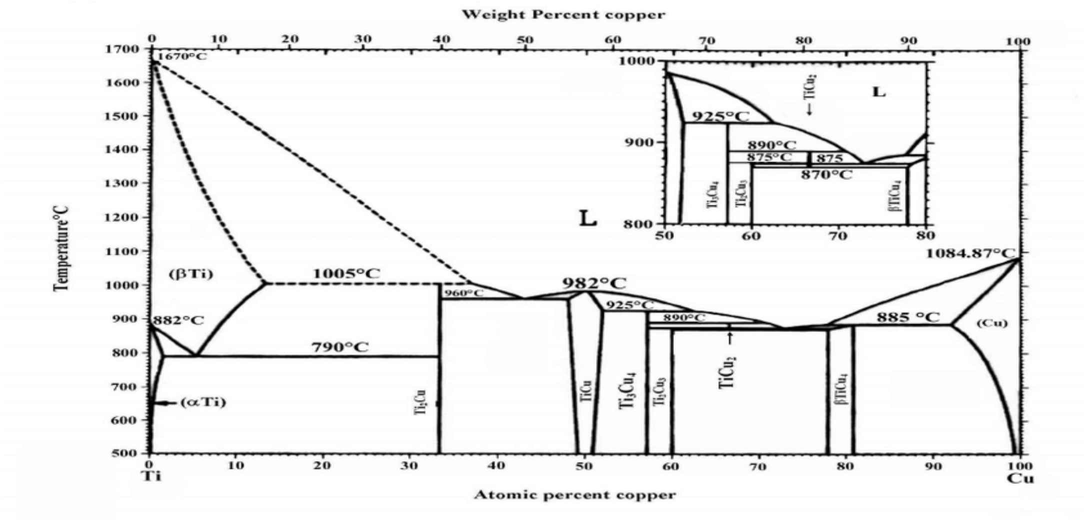


Figure 2: Phase Diagram of Ti-Cu Alloy (courtesy, Good fellow Inc, USA)

The alloy samples were prepared using Powder metallurgy approach. Commercial pure copper powder named (cp-Cu) was used for the alloying, and also for the manufacture of control reference biomaterial (100%Cu). In the material design, the production of TixCu alloy specimens (x=0.5%,1.0%,2.0%,5.0%,10.0%,15.0% and 17.0%) is by powder metallurgy in an inert environment at eutectic maximum solubility of 17.0% copper in beta Titanium phase at 1005^{0}C and compaction pressure of 500MPa, as depicted above, in the phase diagram of Titanium Copper alloy. The copper element (control reference material) powder is also processed in the inert environment at the temperature of 1005^{0}C (Udeh, 2021). The Titanium powder and copper powder were each weighed out differently, and ball-milled differently for 4-7 hrs., and then were pressure compacted up to 500MPa, to develop the specimens (TiCu Alloy), being 30mm in diameter, and under vacuum conditions of 983^{0}c -1005^{0}c for 135-190 minutes, and allowed to cool in furnace to room temperature of 30 ^{O}C. The thermocouple inserted into the bottom punch was used to measure the temperature. Titanium-copper alloy (TiCu) specimen was prepared from Titanium powder and Copper powder (99.5% purity) at different percentage weight compositions as follows: (99.5% Ti 0.5%Cu), (99%Ti 1.0%Cu), (98.0% Ti 2,0%Cu), (95.0% Ti 5.0%Cu), (90.0% Ti 10.0%Cu), (85.0% Ti 15%Cu), (83% Ti 17%Cu). Specimens of diameter 30mm and a thickness of 2.5cm were sliced-off from the TiCu specimens for the various tests using dies and punches of graphite.

Ali (2004) confirmed that the manufacturing option of powder metallurgy approach of beta- phase Ti-Cu alloy specimen was very clinically acceptable due to its high degree of affinity with tissues in the endometrium. Amir et al (2015) collaborated the results of Titanium Copper alloy fabrication and adopted the powder metallurgy approach of this research.

Development of Samples (Ti-Cu) By Powder Metallurgy

The development and analysis of the beta phase Ti-Cu alloy (Bcc) specimens was innovated, using the highlighted characterized parameters which influenced the acceptability of the researched alloy, Titanium Copper alloy (Paul et al, 1988). Titanium as an element is allotropic, existing in more than one crystalline form, which at room temperature is Hexagonal close packed (HCP) and of Alpha phase (Amir et al, 2015), but when alloyed with a Beta phase stabilizer like copper, at temperature of 928°c-1005°c to form Titanium Copper alloy, there is a metallurgical phase transformation to Beta phase with Body centered cubic structure (Bcc) (Udeh, 2021).

***Figure 3*:** Developed beta phase Titanium copper specimens and copper specimen

Table 1: Composition of samples used

Specimen	Composition
1	Ti-0.5%Cu
2	Ti-1.0%Cu
3	Ti-2.0%Cu
4	Ti-5%Cu
5	Ti-10%Cu
6	Ti-15%Cu
7	Ti-17%Cu
8	100%Cu

Xrd Phase and Microstructure Examination

The X-ray diffraction (XRD) analysis and Scanning Electron Microscopy (SEM) Microstructure examination of the Titanium copper alloy specimens was conducted.

Antiseptic Microbial Test

The Antimicrobial Antiseptic properties are tested with cell bacterial colonies of staphylococcus Aureus (S.A) and Escherichia Coli (E.C). The antiseptic microbial tests on these samples, using colonies of staphylococcus Aureus and Escherichia Coli in plated and incubated media at 6-8 hours of incubation with the antibacterial rate, R, is calculated from the equation below (Meis Wang et al, 2014)

$$R = (N_{control} - N_{sample})/N_{conrol} \, \% \quad (1)$$

Where,

$N_{control}$ = average number of bacteria on the control dish

N_{sample} = average number of bacteria on the sample dish

R>99% denotes that sample has strong antibacterial property

R >90% denotes that sample has simple antibacterial property

Experimental Tests Analysis

The Data analysis adopted SPSS/ Excel flow sheet for graphical illustrations, and the determination of variance and the regressional relationship between the Ti Cu alloy test results and copper element are verified using the MINITAB approach in order to establish and validate the results.

Results and Discussion

Microstructure Examinations and Mechanical Strength

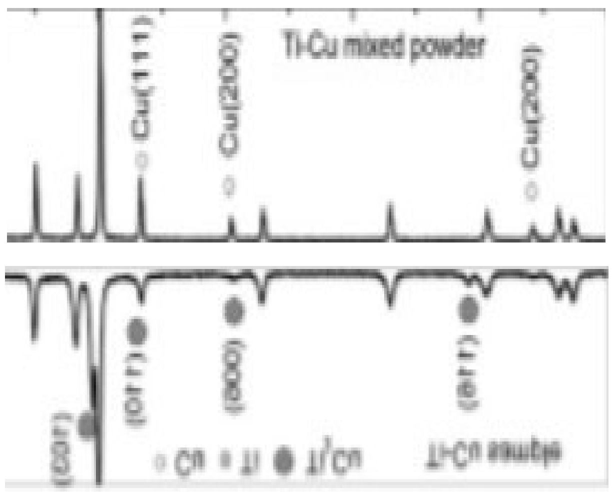
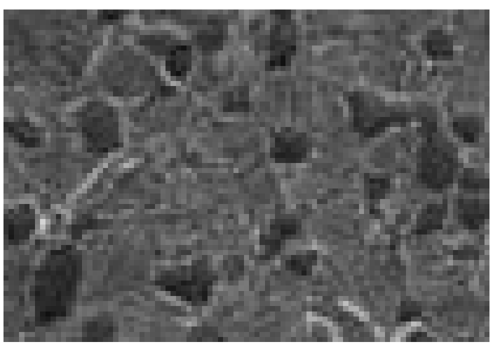
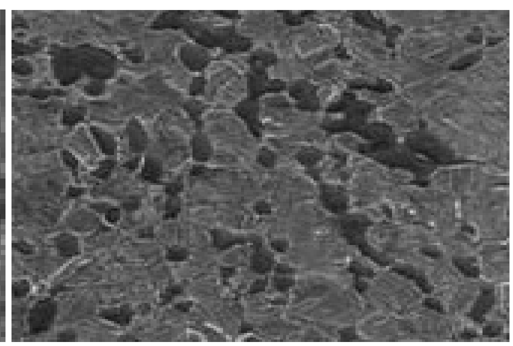


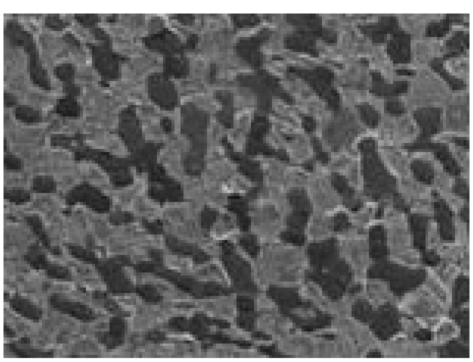
Figure 4*: XRD pattern of Ti 17% Cu and copper element*



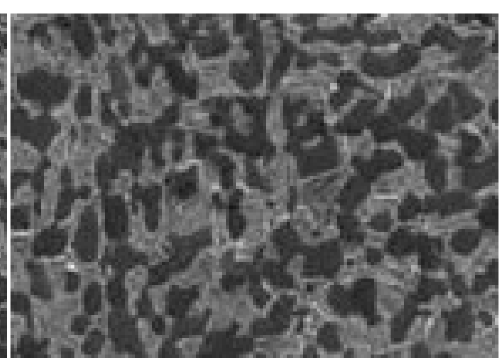
(a)Ti0.5%Cu



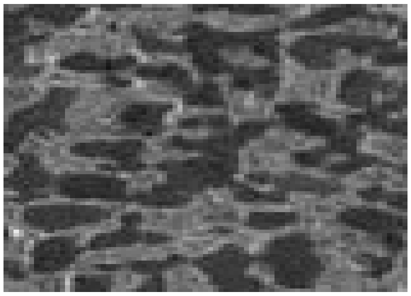
(b) Ti1.0%Cu



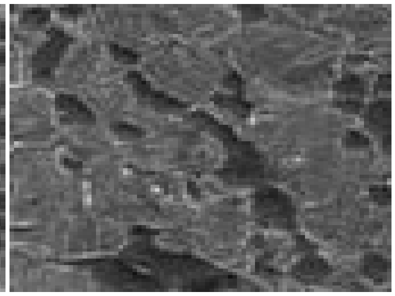
(c)Ti2.0%cu



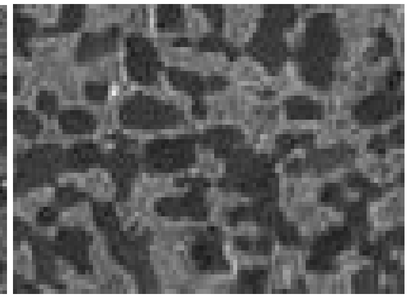
(d) Ti5.0%Cu



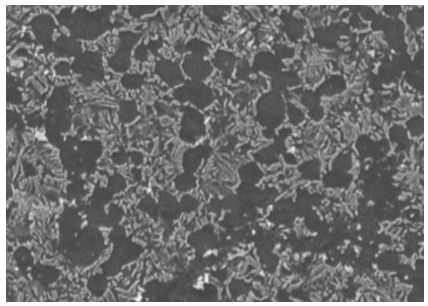
(e)Ti15.0%Cu



(f) Ti10.0%Cu



(g) Ti17.0%Cu



(h)100%Cu

Figure 5*: SEM Microstructure* Examination

The SEM microstructure analyses of the samples at the various compositions of copper in the Titanium matrix at Ti-Cu (x= 0.5, 1.0, 2.0, 5.0, 10.0, 15.0 and 17.0) together with the reference copper as shown in Figure 5a-h, indicates that the Copper powder is uniformly distributed within the Titanium matrix, and is an index for good mechanical strength. The scanning electron microscopy (SEM) showed the presence of inter-metallic Titanium copper (Ti_2Cu) which provided the interface for mechanical strength and good biocompatibility properties (Hugson, 2012; Sykaras, 2000). *Figure 4 shows the XRD pattern of Ti 17% Cu and copper element, it* indicated new peak identification at 40° and 70° for the Titanium Copper element. The result is in accordance with the findings of Qizh & George (2015) that the XRD and SEM microstructure analyses indicated the formation of Ti_2Cu inter-metallic beta phase, with Bcc structure for all the beta phase Titanium copper alloy specimens.

This research has proven that alterations in the surface roughness of Ti 17%Cu alloy influences the response of cells and tissues by increasing the surface area of the implant, and as such, improves the overall affinity of the biomaterial with the adjoining cells (Muddu*gaggadhar* et al 2011). The improvement of the surface texture improves the wettability of the implant (Ti 17Cu %) by the wetting fluid (blood), and ensures the cleanliness of the Ti 17%Cu alloy surface, thereby improving the cell adhesion and cell viability of the biomaterial (Sykaras et al 2000).

Antimicrobial - Antiseptic Test Results

The antiseptic microbial test on the specimens, in the cultured environment of each of Staphylococcus Aureus (S.A) and Escherichia Coli (E.C) colonies, indicated progressive antibacterial action with increase in the percentage composition of copper.

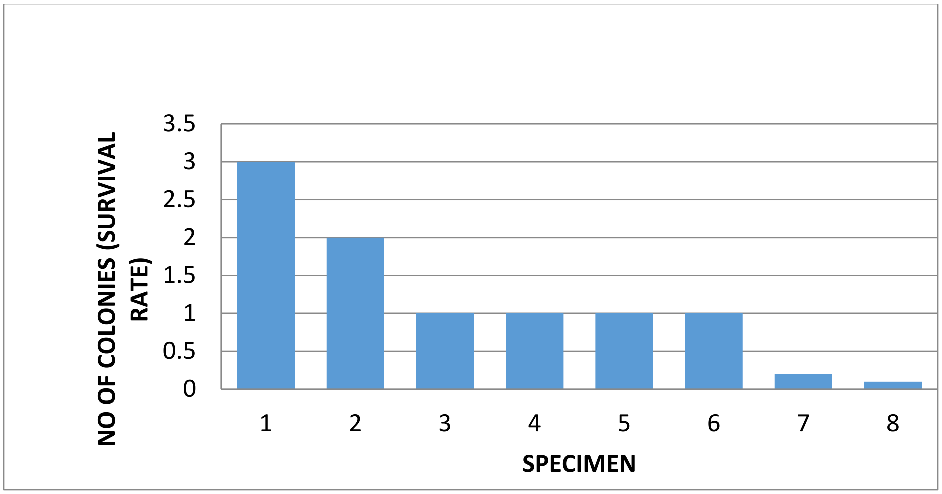


Figure 6: *Staphylococcus Aureus (S.A)*

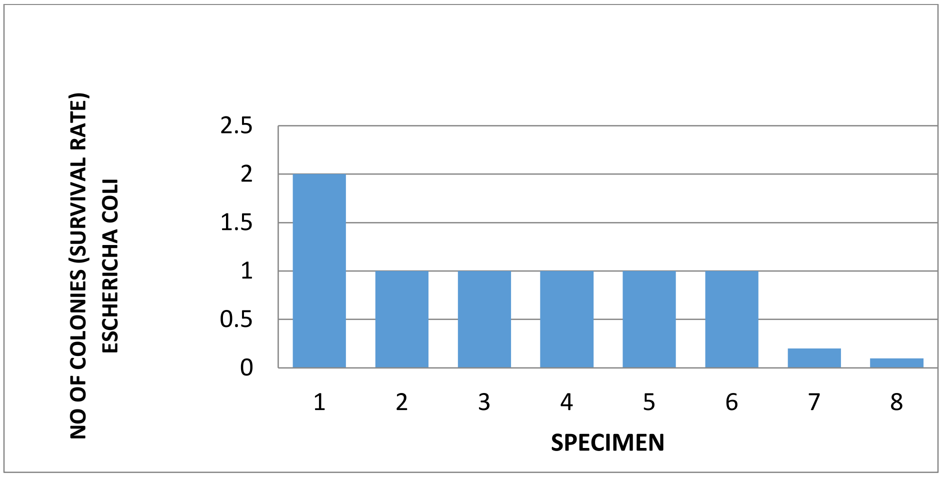


Figure 7: *Escherichia Coli (E.C)*

The antibacterial rates were calculated and plotted as shown in Figures 6 and 7. The antibacterial rate of Ti17%Cu alloy sample against E.Coli and S.Aureus is 99%, which confers it with the status of very strong antibacterial biomaterial. The surface treatment of Ti 17%Cu alloy, with deposition of Ti_2O oxide film, provided up to 99.6% antibacterial rating which is an improvement for biomaterial tissue interaction

Ali (2004) opined that Titanium alloys, exhibited excellent antibacterial microbial quality performance against Escherichia Coli (E.Coli) and Staphylococcus Aureus (S.A), with an approximate 99.9% antibacterial rate. The normal vaginal system is reported to host urinogenital bacteria and cause subsequent infections. The vaginal flora is disrupted by the presence of antibacterial biomaterial which has microbial and antiseptic properties (Kalpana, 2009). The Ti17%Cu response to the most common genital infections in the endometrium of staphylococcus aureus (S.A) and Escherichia coli (E.C) showed inhibition of the colony formation of these microbes, thereby providing good antiseptic qualities.

Validation of Results

The Minitab software analyses confirmed the statistical Deterministic correlation of the variables (R^2) for the parametric experimental analyses as $0.85 < R^2 < 0.95$. This regressional relationship further confirms the high acceptability of the research results in conformity with the research works of Jin et al (2015) and Erlin et al (2013) on characterization of Titanium based binary alloys.

Conclusion

The experimental result indicated that the biomaterial (Ti-Cu) which satisfied the in vitro biofunctional effects is also optimized to perform antibacterial- microbial problems associated with implant–tissue interaction. This experimental research has provided a knowledge interface in the development of a beta (β) phase biomaterial alloy (Ti17%Cu) with Bcc microstructure, that are anchored on software designed parametric model equations, thus proffering an improved alternative solution to intractable intrauterine challenges of the existing mono-element, copper IUDT380 biomaterial.

Summarily, alloying of the elements (Ti and Cu) in an inert condition, with temperature above 828oc, to obtain a eutectic (BCC) beta phase Ti 17%Cu alloy, affects the strengthening of the microstructure with;

1) The copper ion released by Ti-17%Cu alloy in the biological environment of the endometrin leads to high antibacterial properties without cell toxicity or proliferation
2) By alloying Ti and Cu in Ti 17%Cu alloy, it is very essential to get the percentage (cu-17%) that will effectively obtain good antiseptic microbial features.
3) The inter-metallic Ti_2Cu phase in the eutectic Ti-17%Cu alloy microstructure played key role in influencing the good antibacterial property

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