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RESEARCH ARTICLE

Biocompatibility of Improved Beta Phase Titanium Copper Alloy for Intrauterine Gynaecological Application

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ABSTRACT

The analytical study of the tissue biocompatibility status of intrauterine contraceptive beta phase Titanium Copper Alloy for gynecological application within the endometrium environment was investigated in comparative analysis with existing monoelement copper prototype Cu-T380(IUD).The biocompatibility assessment of the existing Cu-IUD380 evaluates problems of fragmentation micro-structural load deformation - failure mode which cause expulsion phenomenon , tissue bio-corrosion phenomenon, cell cytotoxicity phenomenon, microbial pathogenic skin rash reactions, Pelvic Inflammatory Disorders(PID) , with copper allergenic cramp resulting in infection phenomenon. These adverse phenomenal effects of expulsion, corrosion, cytotoxicity and subsequent infection associated with copper Intrauterine Device necessitated the development of an optimally improved beta phase biomaterial alloy, Titanium Copper alloy (TiCu alloy). This research innovated the design and production of Beta((β) phase TiCu alloy in the categories of specimens; 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 Ti-Cu alloy specimens were produced by powder metallurgy technique in an inert environment. The biocompatibility of the manufactured samples was investigated by Cellular Adhesion Test and Mitochondria (MTT) Activity Test methods. Ti17%Cu recorded a higher cell viability of 155% and 0.029mg/L copper ion release which establish it (Ti-Cu) as a possible replacement to the existing prototype biomaterial (Cu-T380(IUD).

***Keywords**: Beta Phase Titanium Copper Alloy; Intrauterine Gynaecological Application; Biocompatibility*

Introduction

Biocompatibility of a biomaterial refers to its ability to perform appropriately within the human or animal environment. The clinical affinity and interaction of the human body and biomaterial is referred to as biocompatibility, which raises the issue of cytotoxicity, cell viability and cell proliferation status of the biomaterial (Hiderbran & Hornez, 1998). Biodegradation is the analytical investigation of the degree and sequence of failure/failure rate of biomedical implants as a result of factors associated with corrosion and wear. The biocompatibility of a gynecological insert or biomedical plant measures the degree of affinity between the implant and the environment, with emphases on better interfacial bonding, reduction in pollution of adjoining cells and total abatement of risk of cell damages after implant. The biocompatibility and engineering application of implants are closely associated with the chemical and mechanical properties of the material used in the implant construction, and the degree of relationship between the chemical, mechanical properties and the human environment of the implants prior to their establishment in the biomedical utility. Also, it is necessary to clarify that since most medical implants are installed through surgical implantation,

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there is every need for familiarities of the repair and regeneration responses, before it is possible to define the biocompatibility of the implant (Singh, 2006).

The research interest in ensuring biocompatibility of a gynecological implant was weighed on the basis of achieving optimal functioning of the implant within the environment of installation, thus reducing the risk of incessant replacement due to failure, and probable high cost associated with any surgical operation involved in the introduction of a replacement implant. These risks of premature failure, shortened service life and non-functionality of implant were investigated on the bases of biocompatibility of the implant, and the subsequent assessment of the wear or degradation of the implant. Biocompatibility of implant halts the risks and complications that are commonly experienced with medical implants which are infections, surgical malfunctioning and implant failure. The prevalence of increasing population and high rate of infections are reasons for future development and improvement on functionality of implants. Above all, gynecological biomaterials must be biocompatible to avoid any allergic reactions to the patient, and ensure smooth functioning of the implant for a long run (Ganzi et al, 2017).

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 and George, 2015). The application of metals and alloys for biomedical contraceptive within the endometrium host environment is a research innovation, not yet explored fully. There is a phase transformation from alpha to beta phase at temperatures above 883°C. Below 882.5^{0}c, Titanuim exists as alpha-phase (α) material and the crystal structure is hexagonal close packed (Hcp), but above 883°c it changes to body centered cubic system (bcc) in beta (β) phase. It possesses high passivity and regenerative properties that is, ability to repair itself and form protective covering with dense oxide film (Coating, 2003), hence, it is usually considered for biomaterial. Furthermore, it also has low young modulus is very close to that of the bone (Williams, 1990). This 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 (α +β). 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 (Coating, 2003). 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 & Bonni, 2005).

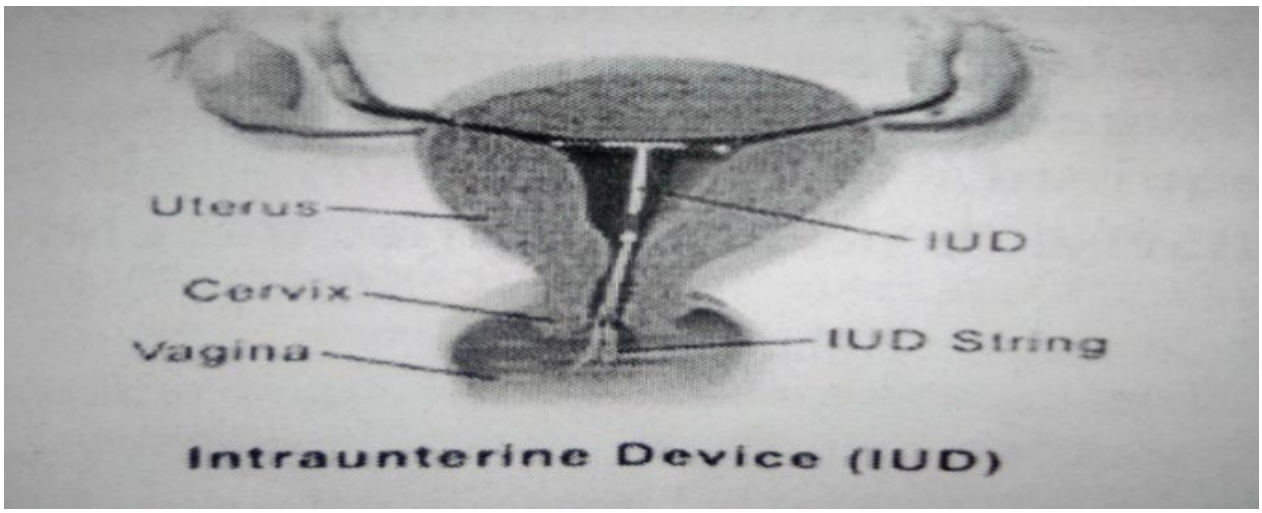


Figure 1: Insertion of IUD T380 in the Endometrium Ref; **Kalpana Gupta (2009)**

Biocompatibility of Titanium Based Alloys

Biocompatibility of a biomaterial refers to the ability of this artificial material used as an implant to perform appropriately within the host environment. It encompasses the clinical affinity and interaction of the human body and the biomaterial. There are new types of Ti β phase alloys which are composed of elements such as Ta, Zr, Nb, cu and Sn. These Ti β phase alloys possess very strong biocompatibility status, and very good mechanical properties, with high strength, low young's modulus and good cold workability properties, making them to be widely and effectively used in recent years. Research has established that high percentage of some elements with β- stabilizing

properties in Ti alloys such; as Cu and Mo are not so suitable for biomaterial applications because of possible release of their metallic ions into the surrounding tissues, which raises the issue of cytotoxicity (Hiderbrand & Hornez, 1998).

Materials and Method

The alloy samples were manufactured 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 1005^0C and compaction pressure of 500MPa. The copper element (control reference material) powder is also processed in the inert environment at the temperature of 1005^0C.

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

The Titanium powder and copper powder were each weighed out differently, and ball milled differently for 4-7hours, and then was pressure compacted up to 500MPa, to develop the specimens (TiCu Alloy), being 30mm in diameter, and under vacuum conditions of 983°c -1005°c for 135-190 minutes, and allowed to cool in furnace to room temperature of 30°c. The thermocouple inserted into the bottom punch was used to measure the temperature. Specimens of diameter 30mm and a thickness of 2.5cm were sliced-off from the Titanium-copper specimens for the various tests using dies and punches of graphite.

***Figure 2*:** Developed beta phase Titanium copper specimens and copper specimen

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.

Biocompatibility Test

The biocompatibility tests of the specimens were investigated by the process of Cellular attachment (adhesion) and mitochondrial activity (MTT) test of human osteoblast-derived cells compared to the bare substrate. Under normal

conditions, the human body fluids have about 0.9% saline solutions of mostly Na^+ Cl^- and other trace ions as well as amino acids and a range of soluble proteins (Rojas, 2009). The samples were placed in 8 cultured plates and cells each, were plated and left to adhere for 3.5hrs, and after this duration, 500 micro-liters of culture medium were added.

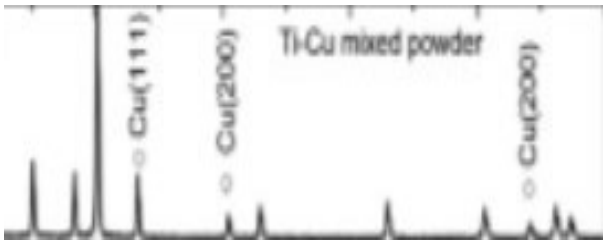
Cellular Adhesion Test

In the cell adhesion assay, the cells were incubated for a period of 24hrs in the culture medium at standard operating conditions. After this time, unattached cells were washed out three times with phosphate buffered saline (PBS) solution, and the remaining cells were fixed with 3.5% paraformaldehyde for 1 hour and then stained using 0.1% toluidine blue for 3 hours. The dye was extracted with 500 microliters of 0.1% sodiumdodecylsulfate (SDS). The number of attached cell was quantified by evaluating the optical density (or absorbance) at 595nm, which corresponds closely with cell number.

Mitochondria (MTT) Activity Test

The MTT test is used to evaluate the cell viability/proliferation of the Titanium alloy specimens and the copper reference material. The assay is used in the principle of the cleavage of tetrazolium salt (3-[4, 5 dimethyl thiazolyl-2-y]-2,5diphenyl tetrazolium bromide) to formazon by cellular mitochondrial dehydrogenases. The formazon dye is obtained from viable cells and quantified by measuring the absorbance of the dye solution at 570nm, which gives a reading to be directly proportional to the number of viable cells. For this, after the incubation period of (25, 80, and 96 hours) 50 micro-liters of MTT were added to this medium and for further incubation period of 3 hours. The supernatant and 500 microliters of dimethylsulfoxide (dmso) were added to each as well. After the period of 60 minutes (1 hour), of slow shaking, the absorbance at 570nm from the dye was measured.

Results and Discussion



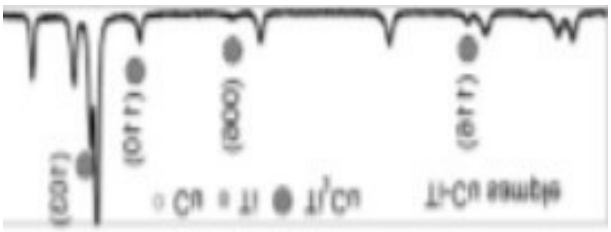
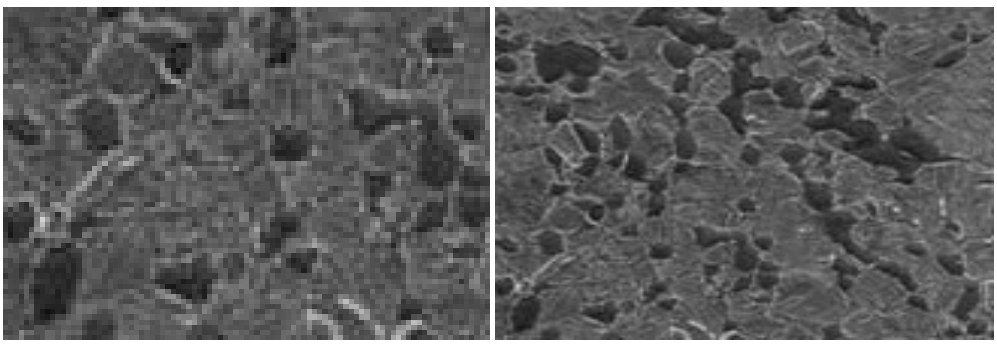
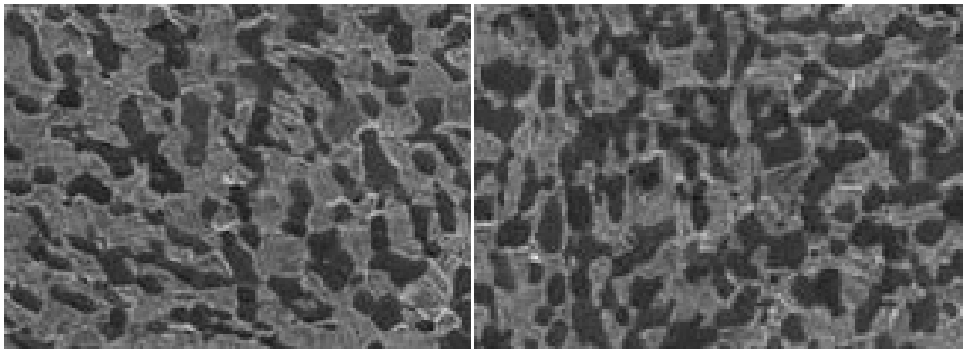


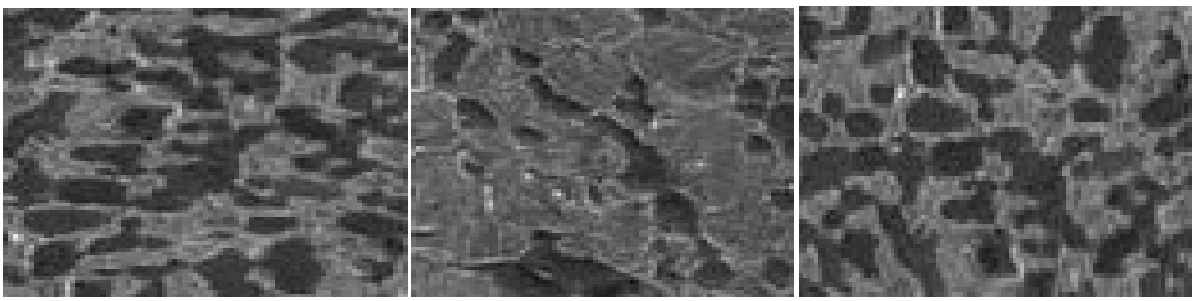
Figure 3**:** *XRD pattern of Ti 17% Cu and copper element*



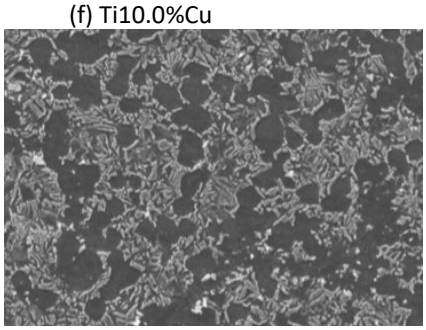
(a)Ti0.5%Cu (b) Ti1.0%Cu



(c)Ti2.0%cu (d) Ti5.0%Cu



(e)Ti15.0%Cu (f) Ti10.0%Cu (g) Ti17.0%Cu



(h)100%Cu

***Figure 4*:** SEM Microstructure Examination

Microstructure Examinations and Mechanical Strength

Figure 3 shows the XRD pattern of Ti 17% Cu and copper element, while Figures 4a-h display 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. The XRD indicated new peak identification at 40° and 70° for the Titanium Copper element. The microstructure of the Titanium Copper alloy, as shown in Figure 4a-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). According to Qizh & George, 2015, 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 (Mudda 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).

Biocompatibility

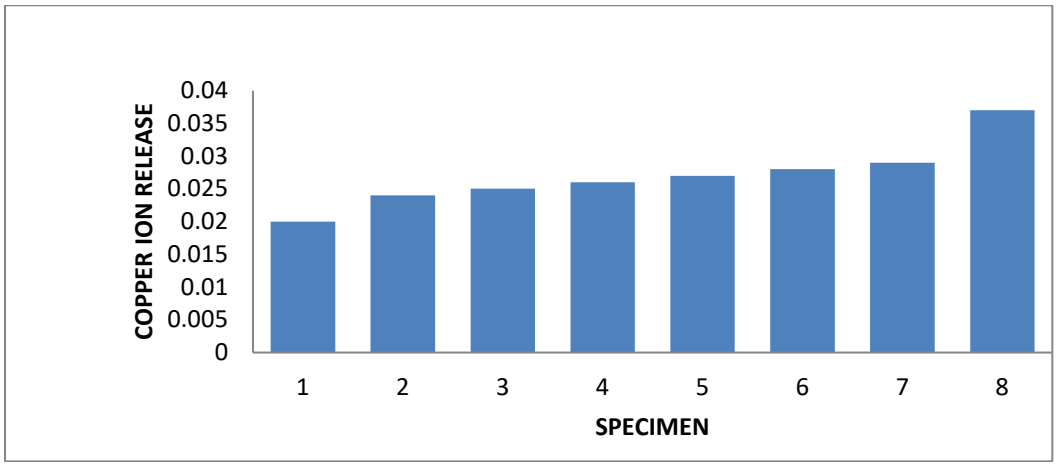


Figure 5: Copper ion Release (Cytotoxicity)

Table 2: Result of Cell Viability Test

Specimen	Composition	Cell Viability (%)
1	Ti-0.5%Cu	80
2	Ti-1.0%Cu	90
3	Ti-2.0%Cu	125
4	Ti-5%Cu	140
5	Ti-10%Cu	147
6	Ti-15%Cu	148
7	Ti-17%Cu	155
8	100%Cu	54

The Ti-Cu alloys developed have better cell viability than the reference copper with Ti17%Cu having the higher cell viability of 155% and Ti0.5%Cu recorded the least value of 80% among the developed Ti-Cu alloys. However, the reference copper could only record viability of 54%. Also, developed samples have copper ion release ranging from 0.020-0.029mg/L for samples 0.5-17% as against the reference copper element which records COIR of 0.037mg/L. This is because the samples are designed to monitor and evaluate the release of metal ions which is an index for cell viability status. The above finding also confirms the suitability the Ti-Cu alloys developed of the over the control reference copper material. The copper ion release of the samples is below the recommended tolerable value of copper ions for human systems. Ions release from metal or alloy results in very unnecessary toxicity reactions, discoloration of adjacent tissues and allergic reactions (Seni, 2015). Chen et al (2018) in their research on biomaterial applications confirmed that Ti-Cu alloys, as in the Ti-20%Cu alloy, have very tolerable copper ion release which gives acceptable cell compatibility and viability to these biomaterials. Also, the biocompatibility of the Ti-Cu alloy, being evaluated by the principles of adhesion viability / proliferation assays, showed that it agreed with the findings of Rojas et al (2009) which recorded high cell affinity of Titanium alloys, just like in Ti-17Cu%, with the interacting biological human tissues. Long et al (2005) opined that the cell compatibility is the degree of comfortability in the application of biomaterial within the human system without clinical issues developing. A preliminary study by Frederick & Silver, (1998) indicated that Ti-Cu alloys have no cytotoxicity effect on cells, thus giving acceptability to the research result of good cell compatibility of Ti 17%Cu alloy. There is complete integrity in terms of cell viability and zero tolerance for cell cytotoxicity.

Table 3: Experimental Results for Minitab Software Design Analysis

Samples	*COIR* (mg/L)	*VIA* (%)
Ti-0.5%Cu	0.02	80
Ti-1.0%Cu	0.024	90
Ti-2.0%Cu	0.025	110
Ti-5%Cu	0.026	120
Ti-10%Cu	0.027	130
Ti-15%Cu	0.028	140
Ti-17%Cu	0.029	155
100%Cu	0.037	54

COIR – COPPER ION RELEASE RATE

VIA - BIOCOMPATIBILITY VIABILITY PERCENT

Response Surface Regression: Copper Ion Release Rate versus Ti, Cu

Model Summary

S	***R-sq***	***R-sq(adj)***	***R-sq(pred)***
0.0017480	**92.72%**	**87.27%**	*

Regression Equation;

$$COIR = 0.168 - 0.00114\,Ti - 0.00131\,Cu - 0.000003\,Ti^2$$

Response Surface Regression: Viability-Biocompatibility, versus TI, Cu

Model Summary

S	***R-sq***	***R-sq(adj)***	***R-sq(pred)***
9.95772	**94.95%**	**91.17%**	*

Regression Equation;

$$VIA = -581 + 11.5\,Ti + 6.3\,Cu - 0.04769\,Ti^2$$

Conclusion

From the result above it was confirmed that copper powder uniformly distributed within the Titanium matrix, and this is an index for good mechanical strength. It was also established that the developed β-phase (BCC) Titanium Copper alloys specimens have enhanced biocompatibility than copper element. The adverse effect of released metal ions that pose health challenges like pelvic inflammatory disorders (PID) or copper allergies inherent in the existing copper device (Copper T380 IUD) has been dully checkmated in Ti-Cu alloy with an optimal value obtain in Ti-17% Cu sample. Hence, Ti-17% Cu is suitable gynecological biomaterials for endometrium application

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