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RESEARCH ARTICLE

Contraceptive Response of Beta Phase Titanium Copper Alloy for Intrauterine Gynaecological Application

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ABSTRACT

The Contraceptive response of the Titanium Copper alloy (TiCu) specimens was tested to quantify motility rate, glycolytic metabolism of oxygen and glucose uptake of cultured spermatozoa in the presence of biomaterial specimens; using Krebber Ringer Phosphate (KRP) simulated Solution. The relationship and correlative effects of the in vitro contraceptive response (motility rate, glycolytic metabolism) of Beta-phase TiCu alloy was investigated with a view to developing an improved alternative intrauterine contraceptive alloy for gynaecological application, which replaces the existing Cu IUD 380. The monoelement, Cu IUD380 is associated with fragmentation, expulsion, and infection and biocompatibility cytotoxicity problems.

This research innovated the design and production of Beta 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 ,Ti17.0% Cu, and the control reference material-Copper. The TiCu alloy specimens were produced by powder metallurgy technique in an inert environment.

Further invitro experimental investigations and Minitab Software Design analyses of contraceptive response integrity and the effect of surface treatment were all carried out. The phase constitution was observed using X-ray Diffractometry (XRD), and the microstructure examination adopted via the Scanning Electron Microscopy (SEM). The results indicated the effect of microstructure on mechanical compatibility of the specimens (TiCu) alloys). The microstructure of the Ti17%Cu showed very refined crystalline formation in beta-grade metallurgical phase which enhanced the mechanical strengthening. The invitro simulated test on contraceptive response of the Ti17%Cu alloy, with tests on glycolytic metabolism rate (oxygen and glucose uptake) and percentage motility test indicated that Ti17%Cu has effective response to antifertility in the endometrium.

***Keywords**: Contraceptive Response; Beta Phase Titanium Copper Alloy; Intrauterine Gynaecological Application; Beta phase TiCu*

Introduction

Currently, the advancement in the study of biomaterials and biomedical implants have yielded results, with knowledge being garnered in the field of immunology, histopathology, reconstructive surgery, gynaecology, ophthalmology, veterinary medicine, dentistry, orthopaedic, cardiovascular surgery, neurosurgery. The major design consideration is the biomaterial acceptability and affinity to the human body and this defines the biocompatibility. However, contraceptive biomaterials are highly needed for gynaecological application. The application of metals and alloys for biomedical contraceptive within the endometrium host environment is a research innovation that is yet to be fully explored. Williams (2013), a biomedical expert, reported that heavy metal ions possess spermicidal properties, and anchored his research finding on the effects of Cu^{2+}, Zn^{2+}, Fe^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+},Co^{2+}, Ag^{2+} on the spermatozoa motility of human, ram, bull, rabbit and dog.

A medical insert into the human body, known as Biomedical Implant, is created from metals, polymers and ceramics which are usually implanted through surgical methods (Ali, 2004). The biomedical parameters for their acceptability to perform this contraceptive-spermicidal function within the host biological environment are designed in conformity with standards of ASTME (American Standard for Testing of Materials (Amir, 2015). Intrauterine biomaterials function effectively by preventing Oval fertilization within the endometrium.

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Biomaterial research reports established that human tissue is mainly organised 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 883^0c it changes to body centred 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 gynaecological 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).

Many researchers have worked on mono-elements, copper(cu) and silver (Ag) materials to function as contraceptive device, but there is no record of research on the application of binary alloy, specifically beta(β) –phase (Bcc) Titanium copper (TiCu) alloy for gynaecological contraceptive application. Summarily, alloying of the elements (Ti and Cu) in an inert condition, within the temperature of 928^OC -1005^0C achieved a eutectic (BCC) beta phase.

Without experimental investigation of all these factors, the premature failure and infection of the biomaterial implants within the host environment would remain challenging research issues (Atkinson and Jobbins, 1981). Phase microstructure transformation effect on the development of intrauterine contraceptive binary alloy for gynaecological application (in vitro) is the basis of this research paper. The aim of this study is to develop a novel biomedical Beta Phase Titanium - Copper (Ti Cu) alloy for long term intrauterine implant application. The objectives of the study are to develop beta phase Ti – Cu alloy, assess the biocompatibility and the in vitro contraceptive response mechanisms of the newly developed alloy (Udeh,2021).

Materials and Method

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 1005^0C 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 1005^0C (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 983^0c -1005^0c 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 endometrin. Amir et al (2015) collaborated this result of Titanium Copper alloy fabrication and adopted the powder metallurgy approach.

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 centred cubic structure (Bcc) (Udeh, 2021).

Figure 1: 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.

In Vitro Contraceptive Test

The contraceptive response was tested to quantify the motility rate and glycolytic metabolism of oxygen and glucose uptake of cultured spermatozoa in the presence of TiCu specimens using Krebber Ringer Phosphate (KRP) solution.

Preparation of Spermatozoa

The Semen was collected and brought to the laboratory within 60 minutes of collection. The semen is maintained at 37°c for 15 to 30minutes. The spermatozoa are prepared by washing it free of seminal plasma and diluted with 5 volumes of free calcium Kreber Ringer phosphate (KRP) buffer (0.12m NaCl, 0.005m KCl, 0.0124m $MgSo_4$, 0.016m Sodium Phosphate, PH 7.4 at 37°c. It was then centrifuged at 740xgl (r_{av}=17cm) for period of ten (10) minutes at 20°c. The aliquots was removed and the spermatozoal suspension collected for use, with each specimen placed into a container. The spermatozoa were mixed by adopting gentle agitation.

Estimation Test of Motility

Flasks containing 1.01 + 0.1 x 10^8 spermatozoa using the 300 Sq mm of the specimens to be tested and 3mm glucose in KRP buffer were incubated for 3hrs with constant shaking at 37°c in a water bath , with Spermatozoa added at intervals of 30,60,120 and 180 minutes.

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Evaluation Test of Metabolism

A special container having small flasks, and in which manometer was immersed in a thermostatically controlled bath assisted in measuring the oxygen consumption of the spermatozoa. Approximately, 1.01 + 0.1 x 10^8 spermatozoa were added to each flask, which contained 3Mm glucose (0.5x10^{-6}gm of glucose), and the 300Sqmm of the specimens under test.

Results and Data Analysis

Microstructure Examinations and Mechanical Strength

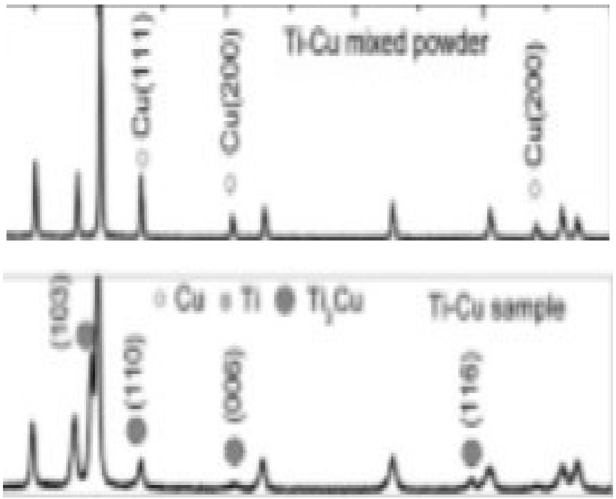
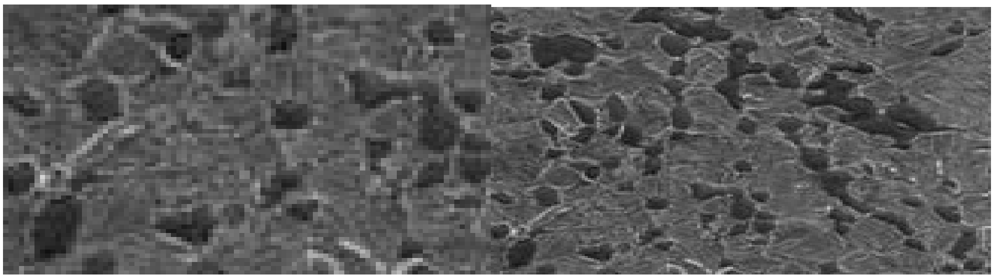
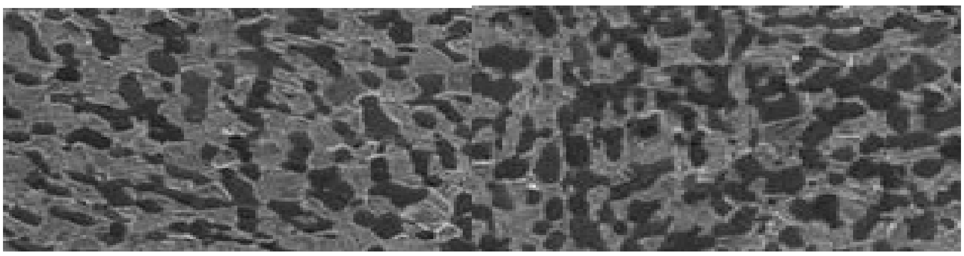


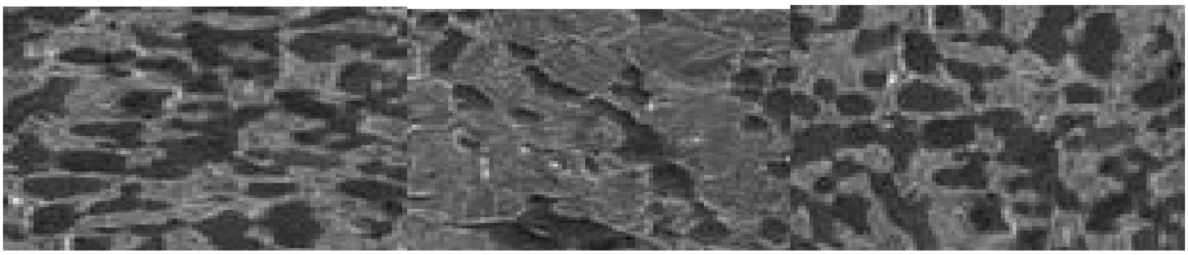
Figure 2: *XRD pattern of Ti 17% Cu and copper element*



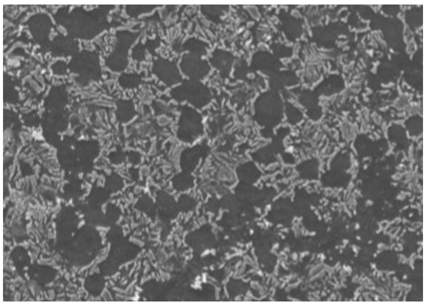
(a)Ti0.5%Cu (b) Ti1.0%Cu



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



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



(h)100%Cu

Figure 3: 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 3a-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 2 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 (Muddugaggadhar 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). 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, but when alloyed with a Beta phase stabilizer, copper, at temperature of 928^{o}C-1005^{o}C forms Titanium Copper alloy. There is a metallurgical phase transformation to Beta phase with Body centred cubic structure (Bcc) (Udeh, 2021)

Invitro Contraceptive Response

The in vitro test contraceptive result on the toxicity to human spermatozoa of the Titanium alloy species, and with reference to the control specimen copper element was evaluated and the result shown in Tables 2 and 3. A decline in the oxygen uptake, the quantity of glucose utilized and oxidized by spermatozoa was observed. Williams, 2013 opined that Trace metal elements such as Cu^{2+}, Zn^{2+}, Ag^{2+} et cetera, create contraceptive response resulting in immotility of the spermatozoa and inability of oval fertilization by the principle of actions of inhibition of both glycolytic oxygen and glucose uptake by the spermatozoa. This is as a result of high rate of oxidation of these metals, their action created an immotile environment that would create infertility in the cumulus oophorus of the endometrin.

Table 2: Percentage motility of spermatozoa

Specimen	Composition	0.1HR	0.5HR	1.0HR	2.0HR	3.0HR
1	Ti-0.5%Cu	60	55	40	20	8
2	Ti-1.0%Cu	54	50	30	10	5
3	Ti-2.0%Cu	49	40	25	8	4
4	Ti-5%Cu	43	30	10	6	3
5	Ti-10%Cu	40	20	0	0	2
6	Ti-15%Cu	35	10	0	0	0
7	Ti-17%Cu	5	0	0	0	0
8	100%Cu	3	0	0	0	0

Table 3: Glycolytic Metabolism Test

Specimen	Composition	Oxygen Uptake (10-6 Litre)		Glucose Uptake (10-6 Litre)	
		SP	SP + Sperm	SP	SP + Sperm
1	Ti-0.5%Cu	0.70	4.10	0.35	1.20
2	Ti-1.0%Cu	0.60	4.32	0.37	1.30
3	Ti-2.0%Cu	0.50	4.44	0.39	1.40
4	Ti-5%Cu	0.40	4.50	0.40	1.60
5	Ti-10%Cu	0.30	4.70	0.45	1.70
6	Ti-15%Cu	0.20	5.40	0.47	1.80
7	Ti-17%Cu	0.10	8.20	0.49	1.95
8	100%Cu	0.05	9.50	0.54	2.25

It was observed that Titanium-copper alloy specimens significantly reduced the percentage of motility and oxygen consumption by the spermatozoa with Ti-17% Cu reducing the motility to zero with half an hour. This is in accordance with the findings of Ahti (1978) that the copper ion release created immotile environment within the endometrin lining that inhibited the fertilization of the ovum by the immotile spermatozoa. This infertility and contraceptive action was created by the effect of copper ion release which inhibited spermatozoan movement in the endometrin (Zipper and Tatum, 1969).

The research investigations showed that Ti-Cu alloys can effectively serve as a contraceptive biomaterial and it is guaranteed to replace copper element as gynaecological implant, not withstanding that copper is good for contraceptive action.

The inhibition and immotile condition created by the tolerable level of copper ions in the Ti-17%Cu alloy, provided non- interference of the biomaterial with the adjoining tissue cells, thereby not causing cell proliferation or differentiation in its interaction with tissue cells.

Summarily, alloying of the elements (Ti and Cu) in an inert condition, with temperature above 828°c to obtain a eutectic (BCC) beta phase Ti 17%Cu alloy, affects the strengthening of the microstructure with the presence of Titanium copper alloy specimens and copper element releases copper ions which decrease the oxidative potential of the spermatozoa, thereby reducing the glycolytic metabolism due to absorption of oxygen by the specimens, resulting to the formation of free radicals (ROS).These radicals reacts with the glucose, breaking them down and causing weakness and immotility of the spermatozoa to fertilize the ovum in the endometrin. Ti 17%Cu specimen has the property to create immobility effect, and obviously quality contraceptive response as provided by the test results.

Experimental Tests Analysis

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 the characterization of Titanium based binary alloys.

Table 4: Experimental Results for Minitab Software Design Analysis

Samples	MOT (%)	GLY (%)
Ti-0.5%Cu	8	4.10
Ti-1.0%Cu	5	4.32
Ti-2.0%Cu	4	4.44
Ti-5%Cu	3	4.50
Ti-10%Cu	2	4.70

Ti-15%Cu	1	5.40
Ti-17%Cu	0.2	8.20
100%Cu	0.1	9.50

MOT – Spermatozoa Motility Rate
GLY – Glycolytic Metabolism Rate

Response Surface Regression: Motility Rate, versus Ti, Cu
Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.36955	85.34%	74.35%	*

Regression Equation ;

MOT = -84 + 0.53 Ti + 0.84 Cu + 0.00369 Ti^2

Response Surface Regression: Glycolytic Metabolism Rate; GLYM versus TI, Cu
Model Summary

S	**R-sq**	**R-sq(adj)**	**R-sq(pred)**
1.02616	**85.60%**	**74.80%**	*

Regression Equation;

GLY = 54 - 0.36 Ti - 0.45 Cu - 0.001428 Ti^2

The percentage motility for the samples indicated 8% for Ti 0.5% Cu, 5% for Ti 1.0%Cu, 4% for Ti 2.0%Cu , 3% for Ti 5.0%Cu ,2% for Ti 10% , 1%, for Ti 15% Cu, 0.2% for Ti17%Cu whilst copper has also 0.1%.

The Glycolytic Metabolism assessment indicated an oxygen uptake of 4.10x10^{-6} Litre for Ti 0.5% Cu, 4.32 x10^{-6} Litre for Ti 1.0%Cu, 4.44x10^{-6} Litre for Ti 2.0%Cu, 4.50x10^{-6} Litre for Ti 5.0%Cu ,4.70x10^{-6} Litre for Ti 10Cu% , 5.4x10^{-6} Litre for Ti 15% Cu , 8.2x10^{-6} Litre for Ti17%Cu with copper recording 9.5x10^{-6} Litre

The Glycolytic Metabolism assessment indicated a Glucose uptake of 1.20x10^{-6} Litre for Ti 0.5% Cu, 1.30 x10^{-6} Litre for Ti 1.0%Cu, 1.40 x10^{-6} Litre % for Ti 2.0%Cu , 1.60x10^{-6} Litre for Ti 5.0%Cu ,1.70x10^{-6} Litre for Ti 10% , 1.80x10^{-6} Litre for Ti 15% Cu , 1.95x10^{-6} Litre for Ti17%Cu. Whilst copper has 2.25x10^{-6} Litre

Generally, the various tests were subjected to the Minitab analysis, the variance of each specimen with Copper T-380 was established. The Ti-17% Cu specimen had factors and quality performance index over the other specimens as well as achieving contraceptive objective of the biomaterial.

Conclusion

Many researchers have worked on monoelements, copper(cu) and silver(Ag) materials to function as contraceptive device, but there is limited research on the application of binary alloy, specifically beta(β)–phase(Bcc) Titanium copper (Ti-Cu) alloy for gynaecological contraceptive application(Udeh,2021). Also, alloying of the elements (Ti and Cu) in an inert condition, within the temperature of 928˚C -1005˚C achieved a eutectic (BCC) beta phase. Finally, 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 Minitab software designed parametric model equations, thus proffering an improved alternative solution to intractable intrauterine contraceptive challenges of the existing mono-element copper IUDT380 biomaterial.

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