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Research Article

Alumina: As a Biocompatible Biomaterial Used in Dental Implants

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Abstract

Alumina is a ceramic bio inert biomaterial having purity 99.5% Aluminium oxide (Al_2O_3) and 0.5% Magnesium oxide (MgO), find its wide application in dental implant due to good bio-compatible property with adjacent tissue, better wear and aesthetic characteristics. This paper represents the in-vitro tests conducted to evaluation toxicity by cell culturing on Alumina biomaterial used in the dental implant by both direct contact and extraction method. In the present study in-vitro assessment of tissue bio compatibility was conducted on L929 cell line (mouse fibroblast). *In-vitro* test, the toxicity of Alumina specimen was done by computing percentage of viability in a cell cultured medium. An MTT system was used to measure the active cell activities with mitochondrial-dehydrogenases, which is an easy method which gives accurate and precision results. The results of biocompatibility *in-vitro* test by both Direct and Extraction methods confirmed that Alumina exhibits a highest cell growth of 93.05% and resulted with zero grade cytotoxicity. Alumina having good aesthetic characteristics i.e., colour of the implant matches with the tooth colour. Hence Alumina is a best candidate alternate implant material compared to other metal implants.

Keywords: Alumina (Al_2O_3); Bioinert material; Alternate dental implant; Cytotoxicity; MTT

Introduction

Alumina is traditionally referred as aluminium oxide, which is used as a bio-inert material. Aluminum oxide is the principal oxide of aluminium. Alumina forms the basis of a very wide and important range of ceramic articles and components. Its great usefulness as a material has resistance to hardness, withstand higher temperatures, less electric conductivity. Alumina is the major constituents have high density hence forms a high grade refractory material. As micrometer grain size alumina material, it provides good wear resistance and insulating property. Alumina do not induce direct bond with the host tissue, instead they are encapsulated by a characteristics thin layer of fibrous tissue after implantation.

Alumina having purity greater than 99.5% find its importance in implant application due to good bio-compatible property with adjacent tissue, better wear and friction property and Aesthetic characteristics. Alumina has less tensile strength due to its brittle nature of the material. Alumina with high density and higher purity has good corrosion resistant bio-compatible better compression strength and good wear resistant making it as a useful ceramic single piece implant [1].

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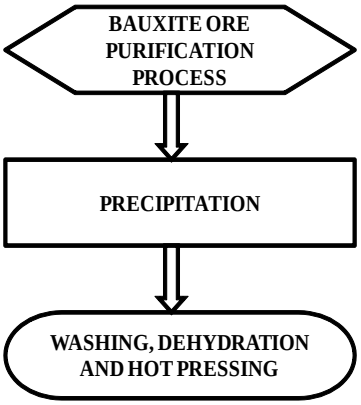

Figure 1: Flow chart of Alumina refining process.

Figure 1: Alumina specimen as per ASTM E384standard specification.

Alumina Refining Process

The oxide of alumina is the third most common element in the earth's crust, but it exists primarily in the form of bauxite ore. The most common mineral constituent of bauxite is gibbsite but the boehmite and diasporeoxy hydroxide phases are also found. Pure single crystal aluminum oxide is colorless and transmitting radiation over a wide spectral range. (Figure 1) shows the flow chart of alumina refining process [2].

Bauxite contains aluminum oxide and oxide impurities like Fe_2O_3, SiO_2, Ga_2O_3 and TiO_2. Bauxite ore is first crushed and purification is achieved through process of diluting bauxite in powder form with sodium hydroxide treated at 300°C under normal pressure. The above solution is later treated from normal temperature to super saturated temperature of sodium aluminate. Excess oxides are removed by seeding technique, where gibbsite is obtained by precipitating aluminum oxide. Once, precipitated material is washed and then dehydrated at temperature of 1000°C to 1200°C to convert low temperature forms alumina, a coarsely crystalline material within which large single crystals can be formed [3].

The bulk fusion and cooling of alumina powder yields fused alumina or aluminum oxide having hexagonal closed packed crystalline structure. This aluminum oxide powder is hot pressed at a pressure of 35 – 70 MPa at temperature of 1200°C – 1400°C using graphite dies to desired shape and size. The melting point of alumina is 2072°C and modulus of elasticity is 318 GPa [4]. Alumina is a single piece implant when compared to other two piece implant.

Application of Alumina

The following are the application of alumina

- Load bearing hip
- Knee prostheses
- Alveolar ridges
- Dental crown
- Dental screws
- Cardiovascular devices

Limitations of Alumina

The limitation of alumina is as follow

- Alumina is a brittle material hence; chances of fractures are more [5].

Biocompatibility

Biocompatible is measure of biomaterial ability to adhere to the biological condition to body. To determine biocompatible property, various biocompatibility tests are conducted and observations are made to evaluate the toxic effects of these materials when implanted in body. Typically material testing and analysis of a biomaterial component are conducted prior to any biological testing to check their cytotoxicity [6].

Cytotoxicity measures an ability of biomaterial to destroy living cell. Biocompatibility tests are categorized into two categories namely in vivo test and in vitro test. In invivo test experiments are conducted directly on the living cells or organisms usually humans and animals. In in-vitro test experiments are performed in test tube or outside living organisms. Once in vitro testing has been completed and satisfied, only then in-vivo testing will be performed to cross verify that the biomaterial will serve the purpose and that is to test biological response like irritation and other clinical testing.

The biomaterials undergo the following different kinds of tissue reactions:

1. **Toxic:** tissue cells death occurs
2. **Nontoxic and inactive biological nature:** fibrous growth between tissues.
3. **Nontoxic and biologically active:** bonding between tissues occur
4. **Nontoxic and dissolve:** replacement of adjacent tissue [7].

In-vitro test are conducted to evaluate whether tissue is biocompatible or not. These tests are done by cell culture method which is very effective method applied in medical field to evaluate diverse materials in medical industries [8].

Types of Cell Culturing Methods

The various types of cell culturing methods are:

1. Direct-contact method
2. Agar-diffusion method
3. Extraction method

For conducting the above mentioned test some of the variables of the experiment must be constantly monitored. Few of these constants are L929 cell line (mouse fibroblast), cell number, exposure time, sample size. The viability test was calculated comparing with control. The quantity of the cell death by viability test gives the measurement of cytotoxicity and in turn measures the biocompatible property of biomaterial tested.

Direct contact method: The test specimen is located directly on the cell line cultured medium. In this method blue haematoxylin is added to dilute the medium. Cytotoxicity is measured by the percentage of cell inhibition where death cells does not take up the stain and percentage of viability measures the stained cells which gives cell growth percentage.

Agar diffusion method: an alga made up of polymer is applied between the cells and sample. During this test the cytotoxicity is measured by comparing the active cells with death cells. Active cells take up stain which is red in colour [9].

Extraction process: Process of elution, in which the leachates from the contact bio-material is extracted and treated, assessment will be made directly on the human cells and medium by implanting it and check for any harmful effect in the potential behaviour and whether the bio-material is biocompatible with the adjacent tissue by surgical method [10]. The dimensions and geometry of implant plays a vital role after surgery, the material reaction with the corresponding adjacent tissue is monitored. So that any foreign particles present in implants may cause harmful toxic effect to the cell [11].

An ideal condition for implantation means implant must be easily and safely integrated to the surrounding adjacent tissues with very minimum time and the wound should heal as fast as possible and restore the damage one and replace it. But in real condition, now a day's the implantation done under normal condition has lead to the various harmful effect due to surrounding adjacent tissue problems like tissue over growth, improper bonding of implant with tissue, implant fracture inside and in certain serious problems implant may have to be removed from the body completely. The duration of time for the implantation should be as minimum time as possible [12].

To prevent implant failure, standard clinical procedure has to be followed. Only stable material should be chosen for implantation, if the unstable material is chosen more chances of implant failure occurs [13]. Stable material will not react with the adjacent tissue easily and will have minimum toxic effect on the body, enhances biological properties and are highly biocompatible. While designing implant Computer modelling and FEA should be carried out along with simulation for better understanding by advanced simulating tool to identify the peak stress and strain values that cause failure of implant. After fabrication of implant, both mechanical test and biological test should be performed to recommend it for clinical application or intended purpose [14].

In the present study in-vitro assessment of tissue bio compatibility was conducted on L929 cell line (mouse fibroblast) by direct contact test and agar diffusion test method. In-vitro test, the toxicity of specimen has been done by computing percentage of viability in a cell cultured medium. Alternative process is to calculate radio-isotope incorporated in DNA by counting the automated counters and other related activities of cell. An MTT system means measuring the active cell activities with mitochondrial-dehydrogenases. It is easy method which gives accurate and precision results [15].

A MTT is [3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] an important substance made up of soluble water salt resulting in yellow liquid solution prepared in a media. MTT at this stage is converted to formazan soluble solution by enzyme (mitochondrial-dehydrogenases) of cell viability. This formazan solution is solubilised by DMSO solvent to convert from yellow to purple solution and it is measured spectrophotometrically depending on cytotoxic effect induced by the test specimen, the cell number may reduce or enhances. Hence, this leads to measure cytotoxicity [16,17].

There are certain criteria for selection of implant and implant should posses following:

- Biocompatible in nature Material must be bio-inert
- Good aesthetic property.
- Free from allergy
- Corrosion resistant and
- Wear resistant
- Economical
- Easily manufactured.

Materials and Methods

Material Preparation

Alumina block was sintered at a temperature of 1500°C for 1 Hr as per ASTM E384 standard specification and 20% of volumetric shrinkage was observed.

The Alumina specimen as per ASTM E384standard specification is shown in FIG 1.

TAB 1 shows the Dimensions of Alumina specimen prepared for biocompatible test.

Biocompatibility Testing Methods

The purpose of biocompatibility test or assay is to assess the effects of the leachates from the Aluminaon the L-929 mouse fibroblast cell line. For conducting biocompatibility test CO_2 incubator, P35 dishes, Autoclave, Test tube, Cell culture reagents such as DMEM, FBS, Pen strip and Trypsin were used. L929 mouse fibroblast cell line, treated in supplement of DMEM with Fetal Bovine Serum 10% in inactive condition, 100 IU/ml quantity of penicillin, 100 µg/ml

Table 1: Dimensions of Alumina specimen.

Dimensions	Before Sintering			After Sintering at 1500 °C			Volumetric Shrinkage
	Breadth (mm)	Depth (mm)	Length (mm)	Breadth (mm)	Depth (mm)	Length (mm)	Percentage (%)
	15	15	20	12	12	16	20

Table 2.1: Reactivity grades for Direct Contact Test [12].

Grades	Reaction level	Reaction Zone description
0	None	No cell death zone
1	Slight	Only Some cell death zone under specimen
2	Mild	Few cell death with limited zone to area below specimen
3	Moderate	Cell death zone extending specimen size up to 10mm
4	Severe	Cell death zone beyond the specimen more than 10mm

Table 2.2: Morphology grades indicating cytotoxicity reaction in extract [12].

Grades	Reaction level	All cell culture conditions and cytotoxic effect
0	None	No cell death or Cell growth unaffected
1	Slight	20% of Cell death occurs and slightly cell growth effected.
2	Mild	50% of Cell death in the medium and growth of cell is effected
3	Moderate	70 % of Cell death but completely not destroyed and growth of cell is effected
4	Severe	Cells are completely destroyed and cytotoxic.

streptomycin, 5 µg/ml of amphotericin with 5% CO_2 was humidified at 37°C temperature atmosphere till it reaches confluent stage. TPVG solution which contains 0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS was used to cell dissociation. Checking was done for cell viability. On other hand 10, 00, 000 cells and 50,000 cells of L-929 fibroblast cell line was seeded in a 96 well plated P35 dish respectively and incubation was carried out at temperature 37oC with CO_2 5% incubation for 24 hours as per ISO:10993-5 standard [12].

This research paper presents two methods of biocompatibility tests were carried out namely Direct Contact method and Extraction method.

Direct Contact Method: In Direct contact method sample specimen was cut and placed at the center of a cultured dish and incubated for 24 hours. Post incubation was carried out in which, the samples were removed carefully and cell morphology was observed under a microscope and scored according to the (Table 2.1).

Extraction Method: In Extraction Method, Leachates from the samples were suspended in a DMEM plain media and 100µl was added to the well containing L929 mouse fibroblast cells in a 96 well plate and incubated for 24hrs by adding 100 µl of MTT to all well. Separately, test solutions were discarded in each well after the process incubation [13,14]. Incubation was done for 4hrs in CO_2 content 5% atmosphere at 37°C. Removal of supernatant is done by adding DMSO of 100 µl quantity on plates and plate was solubilised by shaking gently to form Formosan. Micro plate reader having 590 nm wave lengths is used to measure absorbance by applying formula the viability and inhibition percentage was calculated. Later morphology grades indicating cytotoxicity in extract, reaction was given according to the (Table 2.2).

Percentage of Viability and Inhibition is computed by formula [12]:

% of Inhibition = 100 – (outer diameter of sample) x 100

% of Viability = 100 – % Inhibition.

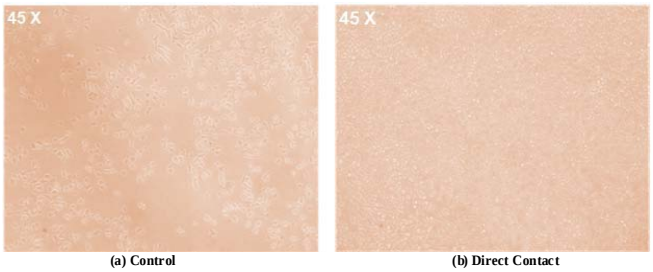

Figure 3.1: Microscopic image of Alumina (a) Control and (b) Direct contact.

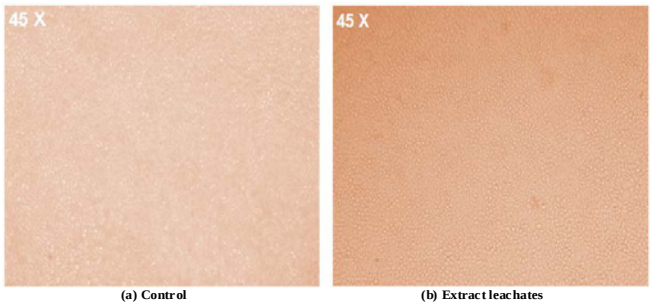

Figure 3.2: Microscopic image of Alumina (a) Control and (b) Extraction leachates.

Table 3.1: Reactivity level grade for Direct Contact test.

Sample	Grade	Reaction level	Figure No.	Result
Alumina	1	Slight	3.1(b)	Only some cell death zone under specimen

Table 3.2: Evaluation of effect of leachates on cell viability in L-929 cells.

Effect of leachates on cell viability in L-929 mouse fibroblast cells					
Compound Name	**Dilutions in %**	**OD at 590nm**	**% Inhibition**	**% Viability**	**Result**
Control	0	0.7471	0.00	100.00	Cell growth is **93.05 %** and Cell death is **6.95%**
Alumina	**50**	**0.5392**	**27.83**	**72.17**	
	25	**0.5597**	**25.08**	**74.92**	
	12.5	**0.5808**	**22.26**	**77.74**	
	6.25	**0.6614**	**11.47**	**88.53**	
	3.125	**0.6795**	**9.05**	**90.95**	
	1.562	**0.74**	**6.95**	**93.05**	

Table 3.3: Morphology grades indicating cytotoxicity reaction in extract.

Biomaterial	Grade	Figure No.	Result
Alumina	0	3.2(b)	No cell death or Cell growth unaffected

Results and Discussion

Biocompatibility In-Vitro Test Results of different methods are discussed below:

Figure 3.1 shows the microscopic image of a control and direct contact with Alumina bio material. In Figure 3.1(a) control which is an L-929 mouse fibroblast cell cultured in medium microscopic image and Figure 3.2 (b) microscopic image of Alumina biomaterial when place directly on the cultured L-929 mouse fibroblast cell medium. From the above microscopic observation reactivity grade is 1, which

has only some cell death zone under Alumina treated specimen.

Table 3.1 shows the reactivity grade for direct contact test method for Alumina. Direct contact test method result showed that Alumina biomaterial have grade 1 and slight reaction level and only some cell death zone under specimen, which is within recommended limit. Hence Aluminais a best candidate alternate material for metal free dental implant.

Figure 3.2 shows the microscopic image of a control and Extract leachates Alumina biomaterial is treated. In Figure 3.2 (a) control which is an L-929 mouse fibroblast cell cultured in medium microscopic image and Figure 3.2 (b) microscopic image of Alumina biomaterial leachates were suspended in a cultured L-929 mouse fibroblast cell medium.

Table 3.2 shows the evaluation of effect of leachates on cell viability in L-929 cells where the Alumina cell growth is higher i.e., 93.05 %compared to metal other implants.

Table 3.3 shows the Morphology grades indicating cytotoxicity reaction in extract, where Alumina exhibits 0 grade which results in No cell death or Cell growth unaffected. Hence Alumina can be considered as an alternate material to metal dental implant.

Conclusions

Biocompatibility in-vitro test conducted by both Direct and Extraction methods, confirmed that Alumina exhibits a highest cell growth of 93.05% and resulted with zero grade cytotoxicity. Alumina having good aesthetic characteristics i.e., colour of the implant matches with the tooth colour. Hence, Alumina having high cell growth percentage can be used as alternate biomaterial dental implant and other medical applications.

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