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# Investigating Biocompatibility of Laser Rapid Manufactured Porous structures of Titanium

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*Abstract*— **Titanium is one of the well-known materials in orthopedic and odontology due to chemical inertness, mechanical resistance, corrosion resistance and biocompatibility. In order to reduce the negative biologic response while maintaining adequate function of an implant in the body a suitable fabrication process is important. LRM is one of the advanced additive manufacturing processes that is capable of fabricating engineering components directly from a solid model. As a result, the fabricated structure is highly customized and expected to have biomechanical properties, which are comparable to those of autogenous tissues without any ill effects. This paper reports the cytocompatability studies of the laser manufactured porous Ti disc in cell culture of L929 cells in-vitro using Direct contact method.**

*Keywords*: **Laser rapid manufacturing, cytocompatability, titanium, in-vitro studies.**

## I. INTRODUCTION

Titanium processed via advanced powder manufacturing routes, such as additive manufacturing is receiving increased attention for mass-customization in product line. Medical implants are also one of the mass-customization products as the size and shape of these implants depends on a number of variables, including age, sex, origin, eating-habits and life-style. Recent advancement of sciences and technology propelled the demands of these implants due to increased expectancy index of an average human life and unwanted mishaps. LRM is one of the advanced additive manufacturing processes that is capable of fabricating engineering components directly from a solid model. As a result, the designed structure is highly customized and is prepared in less time. Appropriate selection of the implant biomaterial and its method of fabrication is a key factor for long term success of implants. The biological environment does not accept completely any material so to optimize biological performance; implants should be selected to reduce the negative biologic response while maintaining adequate function. In this era, synthetic polymers, ceramics, metals and metal alloys started replacing the naturally derived materials because they have better performance and more predictable results than the natural ones. Materials used for biomedical applications exist in different forms and they must possess specific properties to fulfil this role [1]. Materials such as metals are commonly used as implants and such metals have to possess properties, which will enable them to function inside the human or animal body [2]. Biomaterials, as they are also known, are expected to have biomechanical properties which are comparable to those of autogenous tissues without any side effects. The properties which determine whether a material is suitable for biomedical implant applications include biocompatibility, bio-adhesion, bio-functionality and corrosion resistance [3]. A systematic approach towards understanding the relationship between material properties and biological response is crucially dependent on knowledge about the surface properties of the investigated materials [4] and its compatibility in body environment in terms of cell growth and proliferation. In the present study the cytocompatibility of the laser manufactured porous Ti disc was determined in cell culture of L929 cells in-vitro using Direct contact method

### TITANIUM AS IMPLANT MATERIAL

Titanium and titanium alloys exhibit a high specific strength [5], which makes titanium an excellent choice for biomedical applications since 1970 [6]. Almost all commercially available per mucosal dental implants are made from CP-Ti as a result of the pioneering research of Brånemark and his co-workers [7]. CP-Ti and Ti-64 have an elastic modulus which is considered to be high when compared to the bone [2]. This factor therefore limit the application of CP-Ti and Ti-64 . A solution to the problems associated with the high titanium elastic modulus has been to use advanced manufacturing processes such as additive layer manufacturing (LRM) to

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make highly porous titanium structures of CP-Ti and Ti-64. These porous structures can be tailored to have excellent mechanical properties similar to those of human bone and are usually designed to facilitate bone in growth [8].

## MATERIALS AND METHOD

Ideally Implant materials should have biochemical properties same to those of natural tissues without any adverse effects, but there is large variation in these two [9]. This mismatch is responsible for implant failure. Laser Additive Manufacturing (LAM) is capable of fabricating porous structures with control on porosities and mechanical properties and hence, finds a vast potential in hard tissue replacement [10]. Thus, the optimum combination of above parameters can be used to fabricate various geometries. Figure1 Presents the processing parameters and their effects on the fabricated geometry.

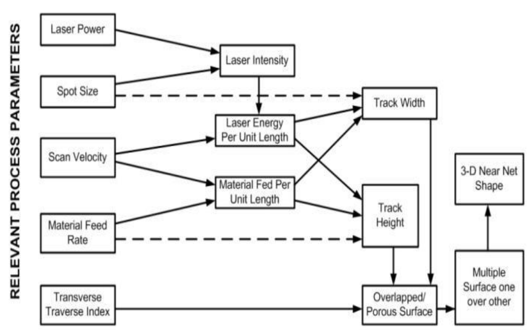


*Figure 1 : Relevant Processing Parameters and their effects*

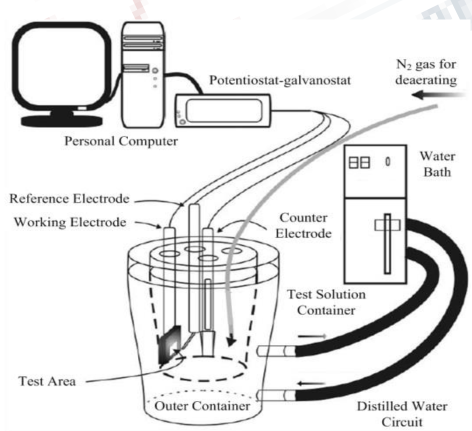


*Figure 2 : Schematic Diagram of Potentiostat*

After fabricating the sample was tested for biocorrosion as shown in fig.2. which is a potentiostat setup . The following setup is done to study the electrochemical behavior of LRMed Ti and pure Ti in SEM.[11]

Rapid Laser manufactured Titanium porous structures (discs and pins) with the following dimensions Pins: 85mg, 1mm x 5mm. , Disc: 2mm dia. disc were used as the investigating material. Both pins and discs were Cleaned with detergent, washed with water and PBS and sonicated . Materials were packed and sterilized by steam .

The cytocompatability of the laser manufactured Ti disc was determined using Direct contact method. L929 cells (ATCC) were sub cultured on to 35 mm dishes and up on 80% confluence the Ti discs in duplicate were placed on to the cells. The discs in direct contact with the cells were incubated for 24 hrs at 37$^o$C in a $CO_2$ incubator. High Molecular Weight Poly Ethylene was used as negative control and Copper was used as positive control. The cell monolayer was examined microscopically for the response around the test samples and imaged after 24h.

L929 cells were trypsinized, counted and 2500 cells were seeded on to the Ti disc and incubated for 24 hrs at 37$^o$C and at 95% relative humidity in a $CO_2$ incubator. After 24 hrs these cells were evaluated using Fluorescein di acetate (FDA) and Propidium iodide (PI) for assessing viability.

## RESULT AND DISCUSSION

Analysis of the ELECTROCHEMICAL behavior of LAM porous Ti in SEM On investigating electrochemical behavior of LRM Ti and compared that with the commercially pure Ti in SEM, The nature of stabilization of free corrosion potential (Ecorr) was similar for all the materials. The potential moved towards noble potential on immersion and stabilized in a relatively short period of time.

### *Analysis of the cytotoxicity and viability*

On testing cytocompatibility by direct contact method ,no vacuolization, detachment and membrane disintegration of cells was observed on contact with Ti discs and they maintained their morphology and shape. The Ti discs were shown to be non cytotoxic. Negative control (UHMWPE) was non cytotoxic while and positive control (Copper) showed severe cytotoxicity.**(Figure 3).**

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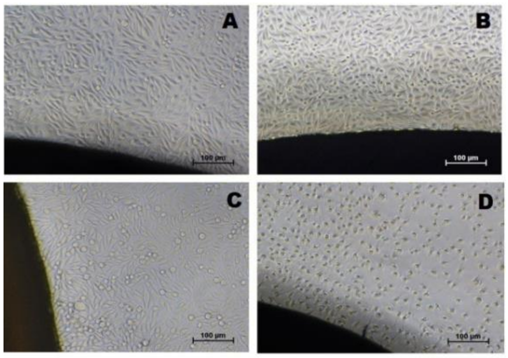



Direct Contact: (A & B) Ti porous discs showing fibroblast morphology of L929 (C) Negative Control (UHMPE) (D) Positive Control (Copper)

*Figure 3: Direct Contact*

For assessing viability live cells will take up FDA and fluoresce green and PI will be excluded, while dead cells takes up PI and fluoresce red. The cells were imaged with DMi6000, Leica inverted fluorescent microscope. **Figure 4.**

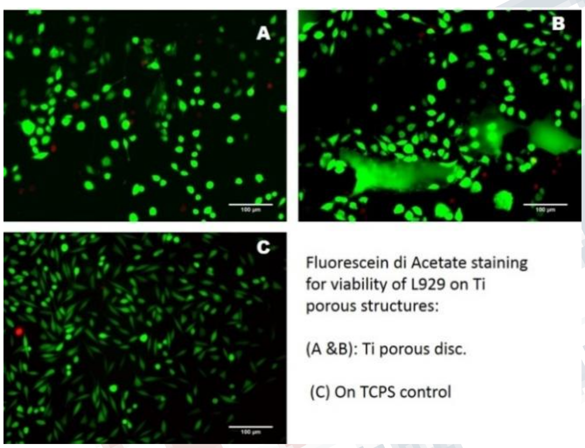



*Figure 4: Viability Staining FDA/PI*

**Analysis for cell morphology**

Morphology of L929 on the scaffold was evaluated using cytoskeletal staining of actin with Rhoda mine phalloidin (Invitrogen). The L929 cells on Ti porous discs were fixed with 4 % par formaldehyde, washed and stained with Actin phalloidin. Cells were observed through DMi6000, Leica inverted fluorescent microscope and showed typical actin cytoskeletal staining.

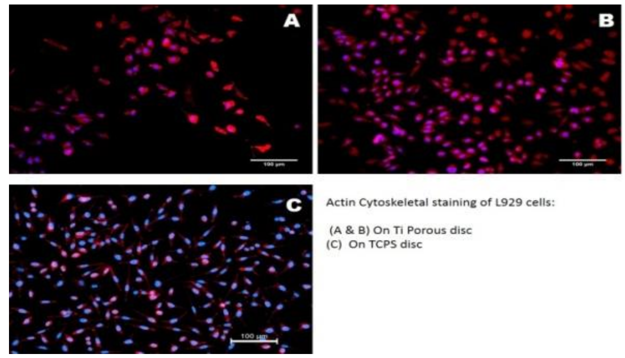
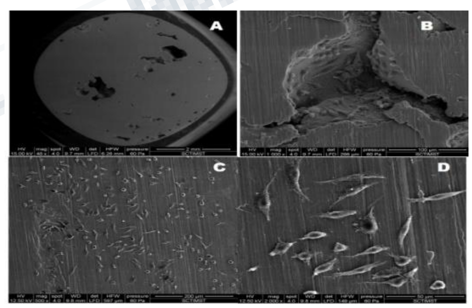



*Figure 5: Actin cytoskeletal staining*

**Analysis of Scanning Electron Microscopy for Cell Adhesion:**

L929 cells on Ti porous discs were fixed with gluteraldehyde, dehydrated using graded series of alcohol, treated with isoamyl acetate and then critical point dried. The disc with cells were then gold coated and viewed with EDAX scanning electron microscope. Surface morphology of the Ti discs were imaged, both with cells within the pores and on the surface of the disc. The cells were found to be well distributed and adhered on the Ti porous discs. SEM images also showed uneven pores on the surface **Figure 6.**




SEM: (A) Material alone (B) Cells in pores (C) Cells on surface (D) Evident cell morphology

*Figure 6: Scanning electron micrograph*

## CONCLUSION

From the available data we could confirm that the Laser rapid manufactured Ti porous structures are non cytotoxic and cytocompatible.

- Cells adhere well to the material.
- L929 cells were found viable and maintained their characteristic morphology.

Thus LRM can fabricate low-modulus implants, with tailored porosity and which are capable of achieving long-term *in vivo* stability.

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