# Effect of Staphylococcus aureus embedded on titanium foam which uses as biomedical implant

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**ABSTRACT**
*The aim of this research is to investigate the impact of the Staphylococcus aureus, on the titanium foam. . It has been attempted to fabricate the foam with different pore size like 50%, 60%, 70% and 80% with different volume fraction of used space holder cenosphere through powder metallurgy route. According to many research papers porosity plays an important role as bio implant material. It has been prepared different size titanium pellets i.e.15mm and 12mm to see the effect of the surface area of the fabricated sample of implant material. Recently, In medical field, solid implant material is in use like Titanium, Steel, Ni-Titanium rod but due to the lack of porosity these implant material does not perform well osseointegration. It has been attempted to observe the roll of the bacteria Staphylococcus aureus in the above said fabricated titanium foam. It has been found that porosity in this foam played the key role as it worked as transporter. Foam has greater pores i.e.80% is more sensitive to develop the bacteria while 50% porous titanium foam is less sensitive meanwhile another sized porous titanium foam had been showed moderate capability of growing the bacteria. Through the all physical, mechanical, chemical and biological observation, titanium foam can be beneficial for the future use. It can be helpful in medical field as cheap and light weight implant material.*

***Keyword:*** - Biofilm, MTT Assay, Staphylococcus aureus ;Titanium foam

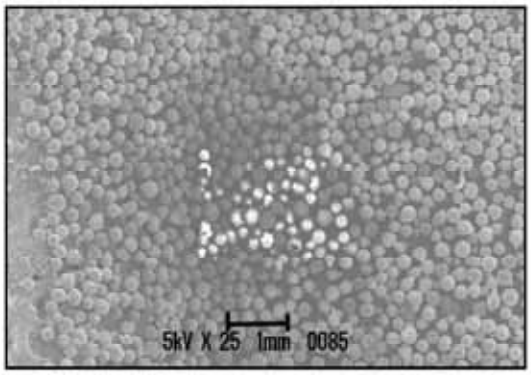
## I INTRODUCTION

Bio implant is not only an emerging subject in the field of medical science [1-3]. In fact, it has become more prominent now. Porous material is becoming more promising compare to solid material due to tailor-made young modulus and cell or tissue proficiency, It has become more significant now [4]. Recently many researchers attempted to synthesize open cell foam by using polyurethane [5]. Titanium attached with the matrix of polyurethane and debind with the further heat treatment to compose grille of titanium. This worked better instead of solid form of metals [6]. There are many methods to prepare metal foam like Gas Injection (Hydro/Alcan) [7], Blowing Agents (Alporas) [8],Solid-Gas Eutectic Solidification (Gasar) [9], foaming of Powder Compacts (Foaminal/Alulight) [10,11], Foaming of Ingots containing blowing agents (Form grip/Foam cast) [12] etc. The new inventions daily revealing in this regard. Although some researchers have trust on this statement that one day will come when injured or crumbled body parts will grow automatically with the help of either implant or stem cells [13]. Research has been conducted to show the cell viability into the titanium foam [14-15]. Another research revealed the effect of Staphylococcus epidermidis on the titanium foam [16].Some previous research pursued on the relation between the titanium foam and the bacteria Staphylococcus epidermidis [17]. It was found that Titanium foam didn't help to induce the biofilm itself. It has been proved in another previous experiment. This may be due to the fact that Titanium is corrosion free material [18] in biofluid and helps in cell growth along the cell boundary.

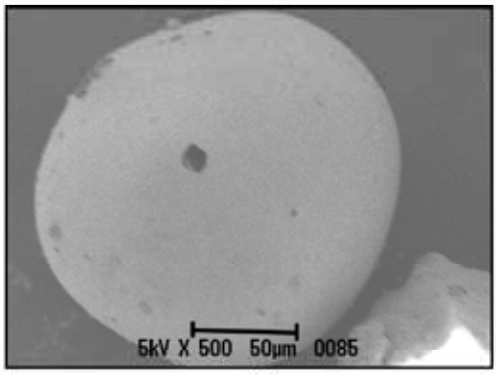
In the present study, titanium foam is prepared through spaceholder technique using cenosphere as spaceholder which has not been studied earlier. The cenosphere might give extra strength to titanium foam and provides porosity for cell adherences. But the cenospheres contain silicate particles and hence it is required to study the cell prolifications in such foams and the influence of Staphylococcus aureus on cell growth and biofilm formation.

## II MATERIALS AND METHODS

**(a) Preparation of the Titanium Foam -** Titanium foam prepared through powder metallurgy route [19]. In this method Spherical shaped Titanium powder (99.9% pure & average size 22±3μm) supplied from Alfa Aesar used as a primary material while different sized Cenosphere i.e. 90.0 ± 8μm, 145.0 ± 11μm, 185.0 ± 15μm, 212.0 ± 18μm (supplied from M/s Cenosphere India) used as a space holder, to create porosity in the titanium foam. Micro structure of cenosphere of 90.0 ± 8 μm shown in Fig.No.1(a) while Fig.No.1 (b) presents its higher magnification micro graph




(a)




(b)

**Fig.No.1(a) Microstructure of cenosphere 90.0 ± 8 µm (b) Microstructure of cenosphere in large view**

The powder of pure titanium combined with spaceholder (cenosphere) by using 2 wt.% of diluted PVA (mixture of 95 wt% of water and 5 wt.% of poly vinyl alcohol). This mixture poured into the cylindrical die, pre wrapped with Zinc Stearate. The size of die was 15 mm in diameter and 80 mm in hight. The die was coated with zinc stearate so that flow ability can be increased at the time of cold compaction. In cold compaction method 75 MPa pressure applied at a cross head speed of 0.1mm/s in a cylindrical die by using 50 ton hydraulic press (M/s Columbia Engineering, Gaziabad,India) for 2-3 minutes. Green samples of 15 mm diameter and 10 mm height prepared through the cold compaction. The flow chart for foam preparation is shown in Fig.No.2. Initially these green samples incubated for 2 hrs after obtaining the 200$^0$C temperature in hot air oven so that moisture and organic binder is expelled. By using Vaccume sintering furnace (Vacuum Tech, Bangalore, India) these green samples are sintered under the condition of 1100$^0$C temperature and $10^{-4}$ mbar vacuum for 2 hrs. Green samples of titanium foam will kept in the closed stainless steel tube to protect the samples from the graphite fumes and the any kind of dust of generated from the green samples of furnace during sintering.

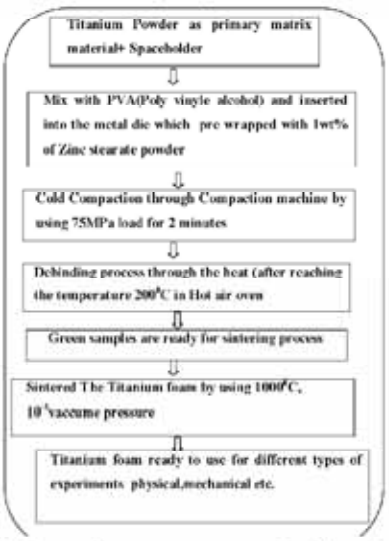



**Fig. No.2 Flow chart to prepare the Titanium Foam through Powder Metallurgy path**

Prepared titanium foam sample sectioned from 10 mm (hight) X15 mm (diameter) sized to 2mm (hight) X15 mm (diameter) size through diamond cutter (LECO VC 50) shown in Fig.No. 3.

**Fig. No. 3 Prepared titanium foam made by using +100 µm cenosphere mess size as spaceholder 50%,60%,70% and 80% volume fraction respectively**

Sintered samples polished through standard metallo graphic practice followed by cloth polish and finally etched with Keller's reagent (20 ml distilled $H_2O$, 20 ml $HNO_3$ (concentration of 70%), 20 ml HCl (concentration of 38%) and 20ml HF (concentration of 40%). Etched sample of Titanium foam examined through the physical (micro structure), mechanical (compression Test), chemical (corrosion Test, EDS) tests. Corrosion is the significant characteristics of metallic foam, because corrosion free character of this titanium foam will give the strength to approach for human being. Hank's solution used to test the

corrosion of the titanium foam. The composition of Hank's solution used for this study is shown in Table No.1.

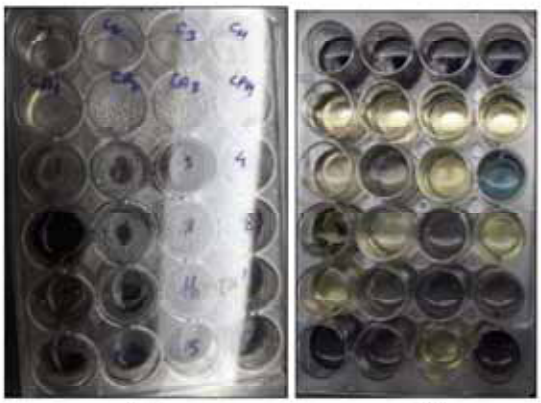
**Table No. 1**
**Composition of the Hank's solution**

| S.No. | Name of the component | Quantity |
|---|---|---|
| Q | $CaCl_2$ | 0.185g/l |
| 2. | KCl | 0.4g/l |
| 3. | $KH_2PO_4$ | 0.06g/l |
| 4. | $MgCl_26H_2O$ | 0.1g/l |
| 5. | $MgSO_4H_2O$ | 0.1g/l |
| 6. | $NaHCO_3$ | 0.35g/l |
| 7. | $Na_2HPO_4$ | 0.48g/l |
| 8. | D-glucose | 1.00g/l |
| 9. | Nacl | 8g/l |
| 10. | Distl Water | 1000ml |

Through experiment we obtained that the corrosion is very negligible in this foam. Ultra sonication test performed by using solvent ethanol (70%) at >20KHZ, so that all debris can ran off which may be present inside the titanium foam prior to the corrosion tests and after corrosion tests.

**(b) Bacteriological Test on Titanium foam**

Biological test was carried out on titanium foam with the help of cultured bacteria of Staphylococcus aureus (procured Staphylococcus aureus ATCC culture 9144 from Himedia India). In this process bacteria Staphylococcus aureus ($10^6$ bacterial cells /ml) is inoculated into each 24 well culture plate which is made up with sterile polystyrene and treated with trypsin. Then titanium foam is diped into the bacteria into culture solution.Titanium foams of various cenosphere contents (like 50%, 60%, 70% & 80%) were used in this investigation. Incubated the system is for 24 hrs., 48 hrs. and 72 hrs. in the bacterial incubator, where a temperature maintained at $37^0$C. The status of the live bacteria through colorimetric technique MTT Assay followed by spectrophotometer at 490nm web length is evaluated. A physical examination is performed through the naked eye and the required specific media was provided so that bacteria can be developed without any interruption. Staphylococcus aureus is a biofilm-producing gram positive coccus bacteria. One column of culture plate is occupied with the positive control of antibacterial gentamycin of 10mg/ml. used shown in Fig.No.4.



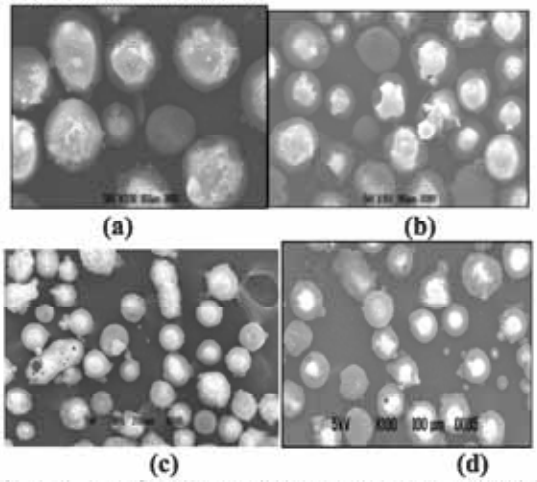
(a) (b)

(c)

**Fig. No. 4 In-vitro bacterial experiment (a) After 24 hrs (b) After 48 hrs (c) After 72 hrs**

## III RESULT

**(a) Micro structure-** Titanium foam is physically observed through SEM (Scanning Electron Microscope) and the matrix of titanium metal and porous structure of Titanium foam found. The micro structure of titanium foam exhibits the cell size of the foam (size of used space holder i.e. cenosphere) shown in Fig.No.5.



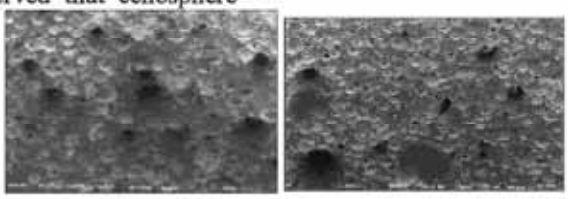
(a) (b)

(c) (d)

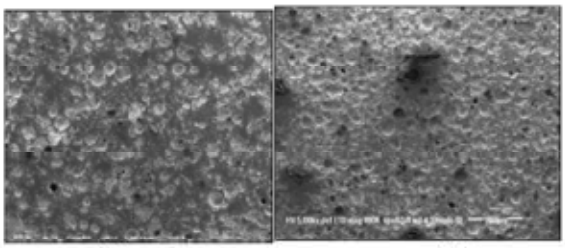
**Fig.No.5 Compilation of various types of used cenosphere as spaceholder (a) 212.0 ± 18 µm (b) 185.0 ± 15 µm**

**(c) 145.0 ± 11 µm (d) 90±8 µm**

Micro structure of titanium foam also observed through FESEM (Field Emmision Scanning Electron Microscope)(Nova Nano Sem 430 Model) which had accommodated with an EDX machine facility(Model No.IE Synergy 250,with a detector of 50 $mm^2$ ability of detecting beryllium and above). Elemental analysis of titanium foam pellets observed that cenosphere micro balloons present in the titanium matrix. This characteristic play significant role in this investigation as it showed the porous structure of titanium foam in Fig. No.6 (a),6 (b) & 6 (c). It is noted from Fig.No.6 that the cenospheres are uniformly distributed in the matrix and a fraction of cenospheres get crushed during compaction and sintering.



(a) (b)



(c) (d)

**Fig. No. 6 Compilation of FESEM images of Titanium foam (a) 212.0 ± 18 µm size,50% (b)185.0 ± 15 µm size,50% (c) 145.0 ± 11 µm size,50% (d) 90±8µm size,60%**

**(b) EDS Analysis**

EDS analysis of Titanium foam sample EDS analysis is presented in Fig.No.7.The presence of titanium and titanium oxide is observed. Fig.No.7 (a) showed the EDX analysis of the holes which showed the presence of Al,Si,Ti and O indicating that these holes are nothing but cenospheres. Fig.No.7 (b) showed the EDX at the matrix (between neighbouring cenospheres).It depicts the presence of Ti O.It indicates that the matrix is consist of Ti primarily and a fraction of Ti get oxidized. Oxidation of Titanium takes place due to its high reactivity with oxygen.

Even at a vacuum of $10^{-4}$ mbar, fractions of Titanium get oxydized. It is represented that around 5 to 8 % Titanium

Thus get oxydized.

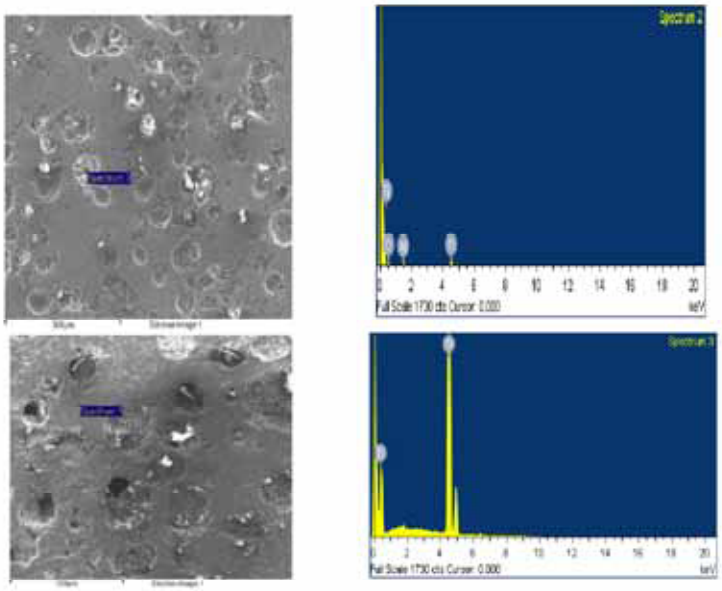


**Fig. No. 7 EDX analysis (a) at the holes and (b) in the matrix**

**(c) Mechanical Experiment**

**(i) Compression test**

Strength of Titanium foam inspected through compressive deformation characteristic by using Universal Testing Machine (Instron Model:8801) at strain rate of 0.01/s at room temperature. The deformative behaviour of the titanium foam depend on the used spaceholder (cenosphere) size 90.0 ± 8, 145.0 ± 11, 185.0 ± 15, 212.0 ± 18 and volume fraction i.e. 50%,60%,70% and 80%. The compressive strength as a function of cenosphere size and cenosphere content is shown in Table No. 2.It is noted that the foam with 60% cenosphere having its size of 145.0 ± 11 exhibited the best mechanical strength.

**Table No. 2**
**Compressive Stress (MPa) of different volume fraction and different size of cenosphere**

| volume fraction | Cenosphere size | Compressive Stress(MPa) |
|---|---|---|
| 50% | 215±18µm | 79±6.3 |
| | 185±15µm | 71±5.9 |
| | 145±11µm | 115±8.5 |
| | 90±8µm | 63±5.3 |
| 60% | 215±18µm | 79±8.2 |
| | 185±15µm | 50±4.0 |
| | 145±11µm | 135±9.6 |
| | 90±8µm | 79±7.2 |
| 70% | 185±15µm | 45±3.9 |
| | 145±11µm | 31±3.2 |
| | 90±8µm | 48±4.3 |
| 80% | 215±18µm | 65±6.0 |
| | 185±15µm | 158±13.2 |
| | 90±8µm | 43±3.6 |

**Table No. 3**
**Corrosion rate as per the volume fraction of the titanium foam after 30 days, 60days, 90days and 120 days**

| Cenosphere size | Volume Fraction | Corrosion Rate (mm/Y) | | | |
|---|---|---|---|---|---|
| | | | 60days | 90days | 120days |
| +212±18µm | 50% | -0.021±0.02 | 0.310±0.013 | -0.08±0.007 | -0.015±0.002 |
| | 60% | 0.051±0.005 | 0.120±0.014 | 0.025±0.002 | 0.003±0.002 |
| | 70% | -0.011±0.002 | 0.290±0.015 | 0.003±0.001 | -0.08±0.002 |
| | 80% | -0.061±0.005 | 0.440±0.034 | -0.015±0.002 | -0.0344±0.001 |

**(d) Chemical Experiment** - Immersion test of a set of foam samples are examined through the balanced salt solution i.e. Hank's solution. Cenosphere size of 215±18µm were conducted using balanced salt solution i.e. Hank's solution. The results are shown in Table No. 3. It is noted from this tests that the foam with 60% cenosphere exhibits least corrosion. This may be because of uniform distribution and strong bonding with matrix.

**(c) Biological Analysis**

**(i) Bacteriological Analysis** - Bio film production found in Titanium foam with 80% porosity due to the wide size of the foam. Bacteria flourished very well. A thin layer of biofilm occurred below the lower surface of foam and the surface of the culture plate. While in 70% porous titanium foam biofilm production was poor. We found small surface area of titanium foam; having 12mm size was not prone to enhancement of bacteria while wider size surface area of titanium foam was more prone to growth of the bacteria.

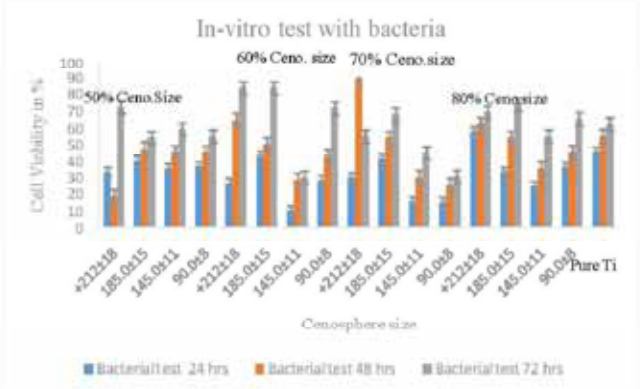
Observation took placed in 50% volume fraction porous titanium foam in which bacterial culture not promoted after 24 hrs , after 48 hrs observation; it was constant after 72 hrs. The minimal growth found in this type of foam. It may be possible due to the succinct pore. Thus we can say 50% of porous titanium foam can be helpful in medical field. Spaceholder also played important role. Cenosphere present in less quality in the foam not helpful to the growth of bacteria. The cenosphere size which used were 90.0 ± 8, 145.0 ± 11, 185.0 ± 15, 212.0 ± 18. The size of cenosphere 145.0 ± 11 and 185.0 ± 15, showed intermediate performance means it was not helpful to develop the bacteria (Staphylococcos aureus) shown in Table No.4 and bar chart 1. But foam with 90.0 ± 8 cenosphere size with 60 to 70% porosity exhibits higher degree of resistance against bacterial growth. It is further noted that the resistance to bacterial growth of these foam is even better than that of pure dense titanium.While the foam is coated with HAP its resistance is also improved against bacterial growth.

**Table No.4**

**The table show bacterial growth kinetics profile with respect to time period i.e.24 hrs,48 hrs and 72 hrs**

| Volume Fraction | Cenosphere Size | Cell Viability in % | | |
|---|---|---|---|---|
| | | 24hrs | 48hrs | 72hrs |
| 50% | +212±18 | 33.33 | 18.57 | 72.46 |
| | 185.0±15 | 40.11 | 47.35 | 54.35 |
| | 145.0±11 | 35.6 | 45.3 | 59.42 |
| | 90.0±8 | 36.74 | 45.2 | 55.075 |
| 60% | +212±18 | 26.44 | 64.76 | 84.06 |
| | 185.0±15 | 43.15 | 50.51 | 84.06 |
| | 145.0±11 | 10.5 | 28.48 | 30.2 |
| | 90.0±8 | 28.32 | 43.08 | 71.74 |
| 70% | +212±18 | 29.89 | 89.52 | 55.07 |
| | 185.0±15 | 41.38 | 54.3 | 68.12 |
| | 145.0±11 | 15.6 | 30.2 | 44.93 |
| | 90.0±8 | 15.2 | 25.3 | 30.5 |
| 80% | +212±18 | 58.16 | 62.3 | 69.6 |
| | 185.0±15 | 33.42 | 54.38 | 73.92 |
| | 145.0±11 | 24.6 | 35.6 | 55.1 |

| | | | | |
|---|---|---|---|---|
| | 90.0±8 | 36..6 | 45.6 | 65.6 |
| Pure Titanium | | 45.56 | 55.3 | 62.4 |
| HAP coated sample | | 37.93 | 41.9 | 51.2 |
| | | 33.33 | 42.7 | 52.3 |




**Bar chart 1 : Bacterial cell viabiality (%) in different titanium foam**

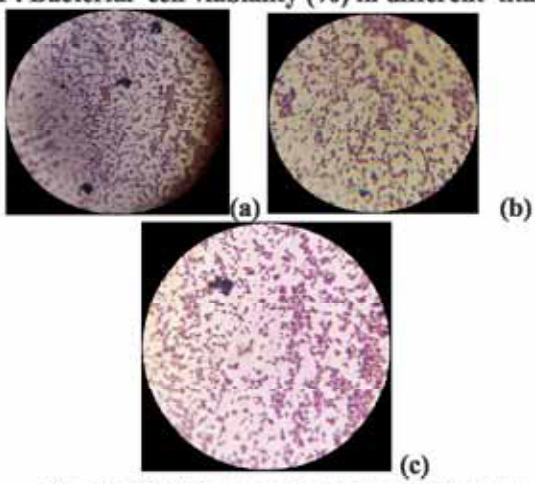



**Fig. No.(8) Microscopic images of S.aureus**

## V DISCUSSION

We concluded that Titanium foam with finer cenosphere and having 50 to 60% cenosphere can be helpful as implant. Another point which should be mention here is that antibacterial dose should also apply during the experiment. The only titanium foam could not helpful substance as antibacterial. The HAP coated titanium foam also showed significant bacterial growth. Wide surface area of titanium foam is more prone to growth of bacteria and bacterial production while finer size of cenosphere containing titanium foam is less susceptible for the bacterial growth. Another factor can be the cenosphere size of which we used for making titanium foam. It is recommended that titanium foam with cenosphere size 90.0±8 to 185.0±15 may be useful as bioimplant. Before going to apply into the human, antibacterial dose are required for further experiment. For better understanding further experimentation using different level of antibacterial doses may be concluded. The growth of bacterial film on titanium foams with various cenosphere size and cenosphere volume is shown in Fig.No. 8. It may be noted that from these figures that highly dense bacteria are grown in the film generated over titanium foam with coarser cenosphere size and higher cenosphere volume fraction Fig.No.8 (a). But the biofilm grow over the foam with fewer cenosphere size contain less bacterial density indicating slower growth of bacteria in such kind of foam Fig.No. 8 (b). In case of finar cenosphere size, liquid or bacterial proliferation in the foam may be less Fig.No. 8 (c). But in case of coarser cenosphere, bacterial proliferation is easier and it inoculated and grows easily.

## VI CONCLUSION

The following conclusion may be drawn from the present study:

(a) Cenospheres of different size ranges can be used for making titanium foam with different density and pore sizes.
(b) The bacterial growth depends on cenosphere size and cenosphere volume fraction.It is linear in case of finar cenosphere size and lower cenosphere content.
(c) Even HAP coated titanium foam also led to significant bacterial growth.
(d) It may be higher as compared to that in titanium foam with 90.0±8 size cenosphere having its volume fraction of 60 to 70%.
(e) For bioimplant application to prevent bacterial growth doses of antibacteria are required.

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