Bacteria Culture

Implant caused infections are considered to be serious and common complications in implant surgeries, and the problem usually requires removal of prosthesis. This is a common concern for cardiovascular and orthopaedic implants. Although success of orthopaedic and dental implants is mostly dependent on the bone-implant osseointegration, the success and long term survival of these implants are also dependent on the presence of the bacteria surrounding the implants. To overcome these problems of bacterial infections, it is vital that the materials which are to be implanted be analyzed for bactericidal properties. After the biocompatibility test materials are therefore required to be tested for bacterial response. In general (unless specified) a very good antimicrobial properties are required. Thus bacteria culture tests are performed on the samples to assess bactericidal properties.

Bacteria culture test is performed by following a protocol. Sintered material is initially polished and roughness of the surface is measured. Bacterial adhesion is enhanced by the roughness of the material surface. So it is critical to evaluate the roughness before doing bacteria culture. The roughness can be evaluated using laser surface profilometer. As a part of sample preparation a material is to be sterilized in a similar manner any orthopaedic implant or surgical instruments are sterilized prior to the medical surgery. The sterilization depends on the type of material, for metals or ceramics the sterilization is done using steam autoclave while many polymers are sterilized using ultraviolet exposure. After sterilization the sample are ultrasonicated for 20 minutes and placed in 4 well plate or 24 well plate depending on number of samples. For any bacteria culture experiment one must use the control sample which should exhibit known behaviour in terms of bactericidal behaviour. The control sample is the thin glass disc. Now the samples are rinsed in 1X PBS
and cleaned by ethanol. Then the samples are ready to be seeded by bacterial suspension.

Media which is used for the cultivation of bacteria is Luria Broth(LB). To prepare the agar plate, dissolve 0.65 grams of Luria Broth and 0.75 grams of agar in 50 ml of distilled water. Autoclave the media and petriplates. Pour the liquid media in sterilized petriplates under sterilized laminar air flow. Leave it to set for few minutes (before proceeding further). Vortex the media containing bacteria properly to make the uniformity. Now using sterilized "inoculation loop" make parallel streaks of the bacteria suspension on the agar plates. Incubate the plates overnight at 37°C. Bacterial Cultures are observed on the streaks and isolated areas. Now prepare 50 ml of LB media by dissolving 0.65 grams of LB in 50 ml distilled water and autoclave. Take 10 ml of this autoclaved LB media in a test tube and inoculate it with single colony from agar plate with the help of sterilized wire loop and incubate the test tube for 4 hours. Observe the test tube; if the clear solution turns turbid then the cells are growing. 1 ml of this suspension is taken in eppendorf tube and centrifuged at 2000rpm for 5 mins. Pellet is collected and supernatent is discarded. This pellet is resuspended in fresh LB media and vortexed. This bacterial solution is diluted to 0.1 OD. This culture is used for seeding bacteria on the samples.

These seeded samples are placed in an incubator with 37°C for 4 hours. After 4 hours the samples are removed and cleaned with 1XPBS. Now the bacterial cell will be fixed with 2% Glutaraldehyde in order to immobilize the samples to the material surface. The samples are then dried using ethanol series and HMDS. Now the samples are gold coated using sputtering unit. These are now observed under Scanning Electron Microscope. By using image analysis software quantification of the bacteria is done on each sample. Thus density of bacteria adhered on the samples can be compared and anti bacterial property is
evaluated. These samples can also be stained using specific staining agents and can be observed in a fluorescence microscope.