Cell Culture

When researchers develop biomaterial they evaluate the biocompatibility property using standard biological protocols. The word biocompatibility means the ability of a material to perform appropriate host response without eliciting any local or systemic toxic effect. As a first step toward biocompatibility evaluation, the students and researchers perform cell culture experiments to assess cytotoxicity property. Now you look at the as sintered material and we do not know whether this material will be toxic to biological cells or not. If the material is toxic then the material substrate will not support cell adhesion or cell proliferation. In case a material is toxic or the material leaches toxic compound in culture medium then cell on the material surface will take the globular shape i.e. the sign of cell apoptosis or programmed cell death in molecular biology terminology.

Now we will follow the sequence of cell culture steps. 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. In the present case you see that a ceramic material is being sterilized in steam autoclave at 121 ^{o}C for 30-40 minutes. This autoclave looks more like the pressure cooker in mother's kitchen. After sterilization now we will place the sample in 8 well plate depending on number of samples or replicates 96 well plates can also be used. For any cell culture experiment one must use the control sample which should exhibit known behaviour in terms of non-cytotoxic behaviour. The control sample can be thin polymer disc. Now we will briefly show you media preparation, the media contains both nutrients and antibiotics to ensure cell growth and avoiding any contamination. As a part of media preparation Dulbecco's modified Eagles medium is mixed with fetal bovine serum and antibiotics. Then the cells are taken out from cryowells and dispersed in medium. Now you look at the micropipette which I will use to take cell along with media and release them in different wells. In order to ensure the cells will survive, the material with culture medium is kept inside the incubator, a constant flow of CO_2 is maintained so that air to CO_2 ratio constantly remains at 95 to 5. We will come after 1 day to take the samples out of the CO_2 incubator.

Samples will now be dried using ethanol series, in order to immobilise cell on substrate Glutraldehyde is used. The cells can now be stained using staining agents so that cytoskeleton, nucleus and mitochondria will appear in fluorescence contrast under fluorescence microscope. After following specific protocol now we will see the cells will appear on this material substrate. The cells as you see on the computer screen have expanded as well as proliferated from the material substrate as evident from clear morphological changes one can quantify the number of cells on material surface using image analysis software and compare the relative efficacy of different materials to support cell proliferation and growth when all are cultured together under the similar conditions.