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Effect of Ti, Nb, and Zr Additives on Biocompatibility of Injection Molded 316L Stainless Steel for Biomedical Applications

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Abstract : Background: Over the years, material research has led to the development of numerous metals and alloys for using in biomedical applications. One of the major tasks of biomaterial research is the functionalization of the material surface to improve the biocompatibility according to a specific application. 316L and 316L alloys are excellent for various bio-applications. This research was investigated the effect of titanium (Ti), niobium (Nb), and zirconium (Zr) additives on injection molded austenitic grade 316L stainless steels in vitro biocompatibility. For this purpose, cytotoxic tests were performed to evaluate the potential biocompatibility of the specimens. Materials and Methods: 3T3 fibroblast were cultivated in DMEM supplemented with 10% fetal bovine serum and %1 penicillin-streptomycin at 37°C with 5% CO2 and 95%humidity. Trypsin/EDTA solution was used to remove cells from the culture flask. Cells were reseeded at a density of 1×105cell in 25T flasks. The medium change took place every 3 days. The trypan blue assay was used to determine cell viability. Cell viability is calculated as the number of viable cells divided by the total number of cells within the grids on the cell counter machine counted the number of blue staining cells and the number of total cells. Cell viability should be at least 95% for healthy log-phase cultures. MTT assay was assessed for 96-hours. Cells were cultivated in 6-well flask within 5 ml DMEM and incubated as same conditions. 0,5mg/ml MTT was added for 4-hours and then acid-isoprohanol was added for solubilize to formazan crystals. Cell morphology after 96h was investigated by SEM. The medium was removed, samples were washed with 0.15 M PBS buffer and fixed for 12h at 4- 8°C with %2,5 gluteraldehyte. Samples were treated with 1% osmium tetroxide. Samples were then dehydrated and dried, mounted on appropriate stubs with colloidal silver and sputter-coated with gold. Images were collected using a scanning electron microscope. ROS assay is a cell viability test for in vitro studies. Cells were grown for 96h, ROS solution added on cells in 6 well plate flask and incubated for 1h. Fluorescence signal indicates ROS generation by cells. Results: Trypan Blue exclusion assay results were 96%, 92%, 95%, 90%, 91% for negative control group, 316L, 316L-Ti, 316L-Nb and 316L-Zr, respectively. Results were found nearly similar to each other when compared with control group. Cell viability from MTT analysis was found to be 100%, 108%, 103%, 107%, and 105% for the control group, 316L, 316L-Ti, 316L-Nb and 316L-Zr, respectively. Fluorescence microscopy analysis indicated that all test groups were same as the control group in ROS assay. SEM images demonstrated that the attachment of 3T3 cells on biomaterials. Conclusion: We, therefore, concluded that Ti, Nb and Zr additives improved physical properties of 316L stainless. In our in vitro experiments showed that these new additives did not modify the cytocompatibility of stainless steel and these additives on 316L might be useful for biomedical applications.

Keywords : 316L stainles steel, biocompatibility, cell culture, Ti, Nb, Zr
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