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Enhanced Cell Adhesion on Severe Peened-Plasma Nitrided 316L Stainless Steel

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Abstract. Plasma nitriding is an effective technique to enhance the wear resistance of austenitic stainless steels. Recently, severe surface deformation techniques are extensively used prior to nitriding to enhance diffusion kinetics. In the present study, AISI 316L austenitic stainless steel is subjected to peening-nitriding duplex treatment and biocompatibility of treated surfaces is assessed through adhesion of the fibroblast cells. Three-fold increase in the surface microhardness is observed from the un-peened sample to the peened-nitrided sample; with severe peened sample showing intermediate hardness. Similar trend is observed in the number of the fibroblast cells attached to the sample surface. Spreading of some of the fibroblast cells is observed on the sample subjected to duplex treatment; while the other two samples showed only the spindle shaped fibroblasts. Combined influence of surface nanocrystallization and presence of nitride layer is responsible for the improved biocompatibility.

INTRODUCTION

Since several decades austenitic stainless steels are widely used biomedical materials owing to their superior corrosion resistance, biocompatibility and cost effectiveness. However, wear resistance of the austenitic stainless steels is relatively poor. There are several surface engineering techniques to produce uniform, hard layer on the surface of these alloys [1][2]. Out of them, plasma nitriding is widely used as the nitride layer on stainless steel surface improves the bio-compatibility along with improving wear resistance. In recent decades, severe deformation of the surface through different techniques, like- air blast shot peening, ultrasonic peening/surface mechanical attrition treatment, laser peening, surface rolling, high pressure torsion, etc are used as pre-treatment to plasma nitriding. Pre-treatment step results in surface nanocrystallization as well as increase the density of non-equilibrium defects on the surface. This, in turn, will improve nitriding kinetics[3].

Air blast shot peening is an effective, industrially viable and cost-effective technique to bring about surface nanocrystallization. In a recent study by the authors [4][5], significant improvement in the nitride layer thickness was observed when severe shot peening was employed as a pre-treatment step for low temperature plasma nitriding of AISI 316L grade stainless steel. Ultra fine grain structure evolved upon severe peening, lead to the development of significantly thick nitride layer during plasma nitriding treatment. In the present study, effect of this duplex treatment on the in-vitro biocompatibility is studied through the growth of fibroblast cells on the un-peened, severe peened and peened-nitrided samples. There are separate reports in the literature on the cell adhesion property of the severe peened 316L steel[6] as well as nitrided 316L stainless steel[7]. However, it would be interesting to study the synergetic effect of surface nanocrystallization and nitriding upon the duplex treatment.

EXPERIMENTAL DETAILS

Hot-rolled AISI 316L grade stainless steel sheets of 5 mm thickness were used in the study. Composition of the steel is given in Table.1. Sheets were cleaned ultrasonically in ethyl alcohol and distilled water. Test coupons of 20

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cm *25 cm were polished to surface roughness of about 0.2 µm and subjected to air blast shot peening treatment(M/s Curtiss Wright surface technologies, Bangalore). Peening intensity of 8A at Almen scale was maintained. In order to ensure the severe peening effect, coverage was set to 1000%. Samples were plasma nitrided (M/s Bhat Metals, Pune) at 400 °C for 4 hours. Details of the process parameters are given in the reference [5].

TABLE 1. Composition of the 316L grade stainless steel

Element	Cr	Ni	C	Mn	Mo	Si	S	Fe
Amount (wt %)	17.8	11.5	0.07	1.58	2.08	0.04	0.006	Balance

Microhardness on the surface of the samples was measured by using Schimadzu microhardness testing machine. Constant load of 200 g and dwell time of 15s were used for microhardness measurement.

Fibroblast (L929) cells were used to assess the cell adhesion property of the un-peened, severe peened and peened-nitrided samples. Top surface of the samples were slightly polished off to maintain uniform surface roughness for all the surfaces. 1 cm * 1 cm plates were autoclaved and kept inside 12-well plates used for cell seeding. 50 ml of fibroblast suspension was added each well, with cell density of 25000 cell per well. 2 ml of Modified Eagle's medium enriched with 10% fetal bovine serum was added to each well. Cells were incubated at 37 ° with 5% carbon dioxide atmosphere. Culture medium was changed on alternative day. After 4days, samples were removed from the well plates and washed with the phosphate buffer saline (PBS) and dried. In order to observe the cells under the microscope, they were fixed with 4% formaldehyde solution for 30 min and dried. Optical microscope (Carl Ziess make) and Scanning electron microscope (SEM, 6380LA, JEOL) operating at 10 kV were used to image the fibroblast cells on the steel surface.

`RESULTS AND DISCUSSION

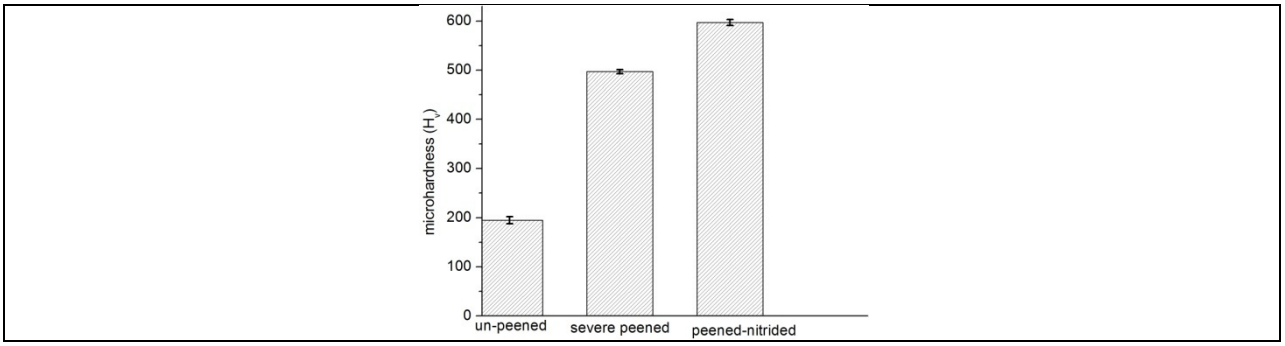

FIGURE 1. Microhardness of un-peened, severe peened and peened-nitrided samples

Figure 1 shows microhardness data of the un-peened, severe peened and peened-nitrided samples. It could be observed that there is a significant improvement in the hardness after severe peening treatment. This is primarily attributed to nano/ ultra fine scale grains on the surface. Apart from grain refinement, increase in the density of the non-equilibrium defects like dislocations, stacking faults, twins, etc also contributes to hardness [5][8]. Upon plasma nitriding, hardness further increases, owing to formation of the nitride layer on the surface. Overall, about 3-fold increase in hardness is observed after the duplex treatment.

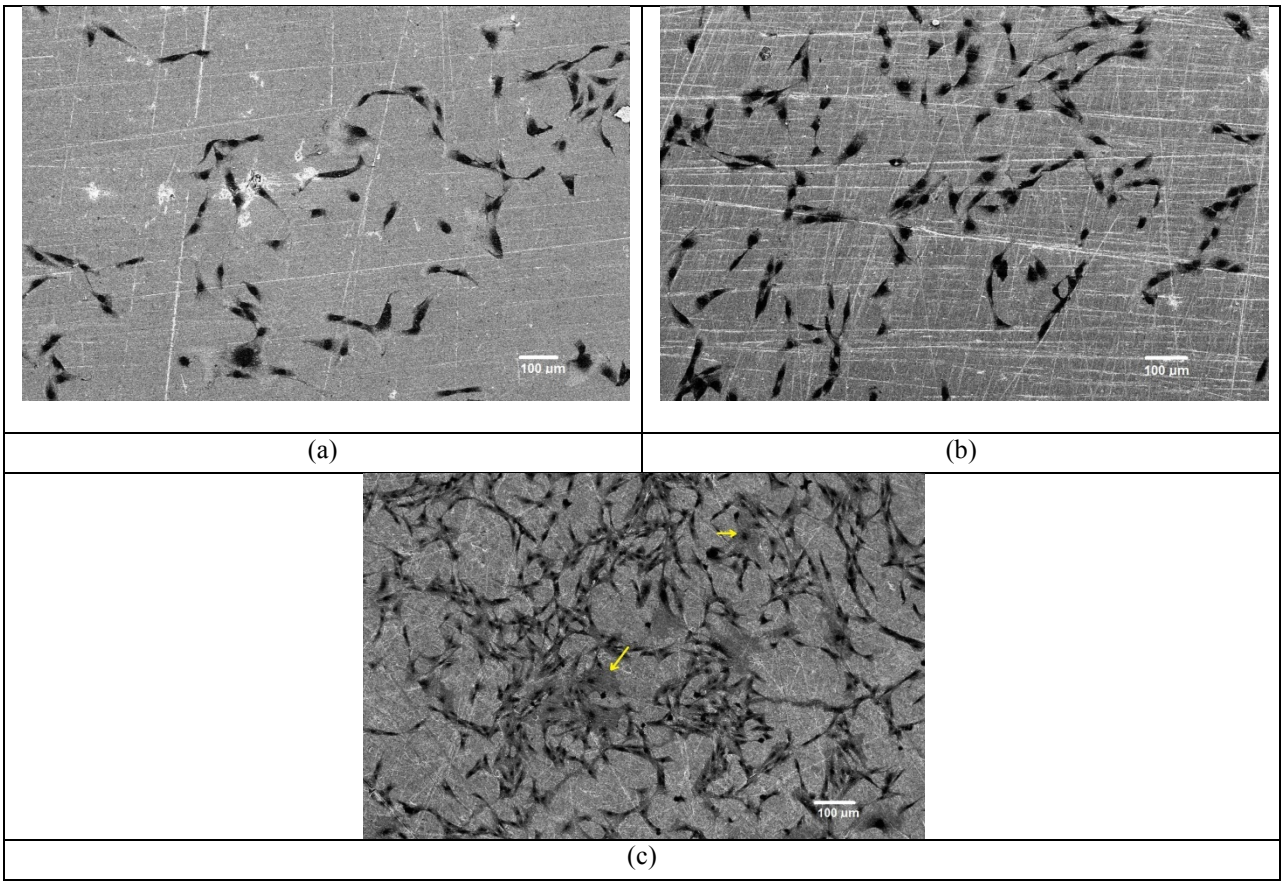

FIGURE 2. SEM micrographs of the fibroblast cells on the surface of (a) un-peened and (b) severe peened and (c) peened-nitrided samples

Figure 2 depicts the fibroblast adhesion on the surface of the un-peened, severe peened and peened-nitrided samples after 4days. Spindle shaped fibroblast cells are attached to the un-peened surface as seen in Fig. 2(a). It could be clearly observed from Fig. 2(b) that the number of the fibroblasts adhered to the sample surface increase upon severe peening. Along with nanocrystallization, peening process also increases the roughness of the surface. Surface roughness increases the cell adhesion through anchoring the cells to the substrate surface. However, in the present study influence of roughness is nullified by polishing off the rough surface. Hence, increased cell adhesion is largely attributed to refined grain size upon severe peening.

Number of fibroblast cells proliferated on the severe peened-nitrided sample is highest amongst three samples considered in the present study. It is known that nitrogen enhances the bio-compatibility of the steels [9]. Nanostructuring of the steel during severe peening stage results in nanocrystalline nitride layer after plasma nitriding treatment. Combined effect of fine grained surface and presence of nitrogen improves the adhesion of the fibroblast cells on the surface. It interesting to observe that, some of the fibroblast cells have spread on the surface as indicated by yellow arrows in Fig. 2(c). This indicates the higher degree of attachment of the cells on the peened-nitrided sample compared to the un-peened and severe peened samples for the same incubation period. This observation affirms the enhanced biocompatibility after the duplex treatment; indicating the faster osseointegration on the implant surface when used in service. Typical optical micrographs of the spindle shaped and spread fibroblast cells are given in Fig.3. Spreading of cytoplasm in Fig. 3(b) indicates better adhesion to the surface.

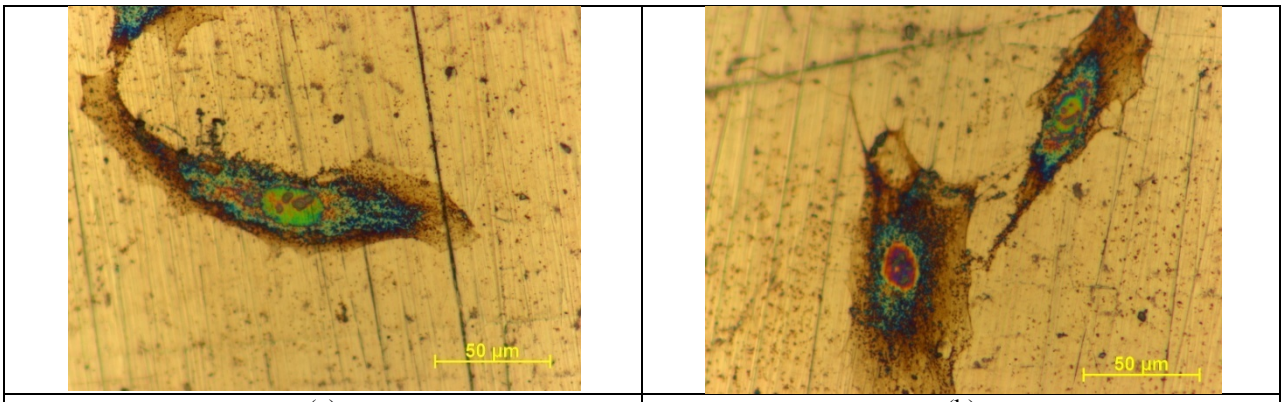

FIGURE 3. Typical optical micrographs of the (a) spindle shaped and (b) well-spread fibroblast cell on the surface of peened-nitrided sample

CONCLUSION

In the study, biocompatibility of the surface produced by the peening-nitriding duplex treatment is assessed through attachment of the fibroblast cells. Microhardness data showed that the surface layer obtained after the duplex treatment is harder than the un-peened and severe peened sample. Adhesion of the fibroblast is the severe peened sample compared to the un-peened sample. It is attributed to the surface nanocrystallization and increased defect density. Highest fibroblast density is obtained on the peened-nitrided sample owing to the presence of the nitride layer. Spreading of some of the fibroblast cells are also observed on the sample; affirming better biocompatibility after the duplex treatment.

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