Chapter 6

Result and Discussions

ANTIBACTERIAL AND CYTOCOMPATIBILITY BEHAVIOR OF SINTERED ALLOYS

6.1 Introduction

This chapter of the thesis discusses the results obtained after the antibacterial and cytocompatibility test of the sintered alloys. The cytocompatibility test of the samples is tested with MG-63 animal cells. The result obtained after the cell adhesion, proliferation, and viability of the cells on the surface of the alloys are discussed in this chapter. The antibacterial property of the alloys is tested in the presence of the S. aureus and E. coli bacteria. The presence of copper in the alloys made the alloys antibacterial. A detailed discussion of the results is presented in this chapter.

6.2 Antibacterial properties of the sintered alloys

The antibacterial activity of all four test samples was calculated by the plate count method and shown in Fig. 6.1 and 6.2, respectively. The typical *E. coli* and *S. aureus* colonies of bacteria were incubated for 24 hours on the surface of CP-Ti (control sample) and Ti-5Cu-(0, 5, 10, 15) % Nb prepared at 900 ºC. Bacterial colonies incubated directly on a negative sample (nutrient broth) are also compared. Many bacterial colonies of *E. coli* and *S. aureus* were found on the negative sample and control sample. This demonstrates that neither the negative nor the control sample has antibacterial properties. Also, here the negative sample has much more colonies than the control sample. This is because the bacteria got more nutrients, and in 24 hours of incubation, the population of bacteria increased; these colonies were uncountable. The control sample has a specified well-placed bacterial colony. On the contrary, the test samples had not found any colony for both *E. coli* and *S. aureus*, which strongly exemplifies that all four test samples exhibit antibacterial properties. Here it is observed that the addition of niobium does

not affect the antibacterial property of the test samples. The previous study says that Ti (1-5%Cu) has an excellent antibacterial property, and for an excellent stable antibacterial property, at least 5% copper should be present in the alloy (J. Liu et al., 2014; Shirai et al., 2009). The antibacterial properties of all four test samples in this study are showing better results than those of the previous reports (Li et al., 2017; J. Liu et al., 2014; Tao et al., 2020). Copper is a vital micronutrient for human health since it involves the absorption and utilization of iron; it also helps release protein and enzymes and reduces the cholesterol level in the blood. The copper also helps in the formation of red blood cells. However, excess copper intake is also hazardous for human metabolism. The antibacterial rate of all four test samples is approximately 100 %. This result is quite similar to the S. C. Tao et al. (Tao et al., 2020), which also reported that by increasing the incubation time and sintering temperature of the porous Ti-3Cu microwave sintered alloy, the antibacterial property also changes. The best result was obtained at 800 ºC sintering temperature and incubation period of 12 hrs and more. Compared to that result, this research also shows the same effect at 900 ºC sintering temperature and 25 hrs of the incubation period for both *E. coli*. and *S. aureus* bacteria. It can be noted here that the negative sample in both *E. coli*. and *S. aureus* bacteria have a vast (uncountable) inoculated bacterial colony because of the availability of nutrients. Also, in the negative sample, the bacterial colony in both *E. coli.* and *S. aureus* had accumulated in the centre of the petri dish, and this is because the bacteria concentration was gathered at a particular place after spreading. The antibacterial property in the test samples is because of the presence of Ti_2Cu. This compound is an inherently antibacterial property, and in the XRD pattern, the peak of Ti_2Cu can be observed easily. Table 6.1 reported the data obtained after the antibacterial test. Based on the results all the four sintered alloys had found nearly 100% antibacterial property.

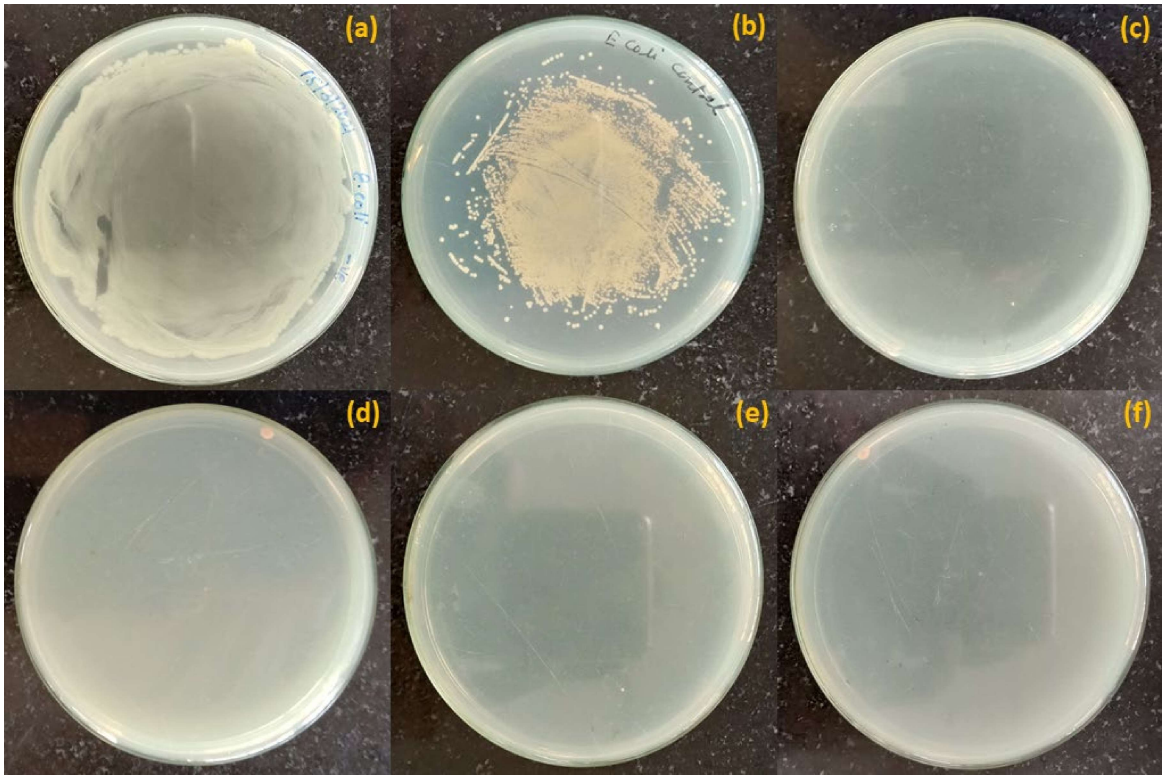

Fig. 6.1- Bacterial colony of E. coli. (a) negative sample, (b) control sample (CP-Ti), (c) S1, (d) S2, (e) S3, and (f) S4.

Table 6.1- Results obtained after the antibacterial test.

Samples	Number of bacterial colonies		Antibacterial rate (%)	
	E. coli.	***S. aureus***	***E. coli.***	***S. aureus***
Negative	countless	countless	-	-
Control	>10000	296	-	-
S1	1	1	≈100	99.66
S2	1	1	≈100	99.66
S3	1	0	≈100	100
S4	0	2	100	99.32

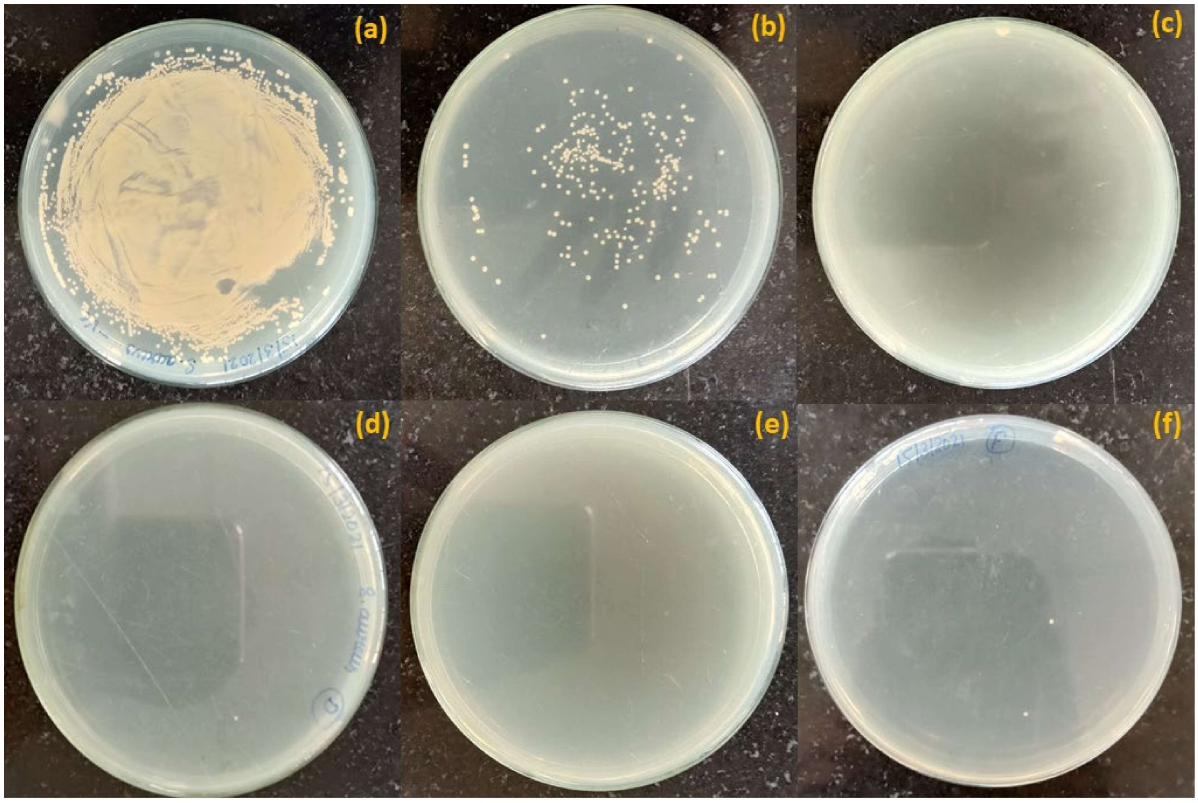

Fig. 6.2- Bacterial colony of S. aureus. (a) negative sample, (b) control sample (CP-Ti), (c) S1, (d) S2, (e) S3, and (f) S4.

6.3 Cytocompatibility assessment of sintered alloys

6.3.1 Cell culture and morphological analysis

The MG-63 animal cells were used for the bioactivity or cytocompatibility test of the samples S1 to S4. A glass cover slit is used as the control sample. The cell attachment to the developed samples is seen after 3, 5, and 7 days. The MTT assay technique is used for the proliferation and viability study of the samples. After three days of incubation, the fluorescent microscopic images of the human bone osteosarcoma MG-63 cells that were cultured on the different samples can be seen in Fig. 6.3. There is an appreciable increase in the cell coverage with an increase in the culture time, on all the samples, showing a gradual cellular growth with time. The presence of Nb in the sample S2, S3, and S4 with increasing percentage increases the

nuclei formation. The dark blue color in the image is showing the spread of nuclei, while the green colour actin cytoskeleton filaments on the samples. Figure 6.3 (a-d) shows the spread of the cell on samples S1, S2, S3, and S4, while Fig. 6.3 (e) of control glass strip. The cell growth on sample S4 is maximum compared to the other three samples. This is because of the presence of maximum percentage of Nb. The literature survey shows that niobium has excellent bioactivity or biocompatibility property. The cells have accumulated on the corner of the samples because of uneven surfaces.

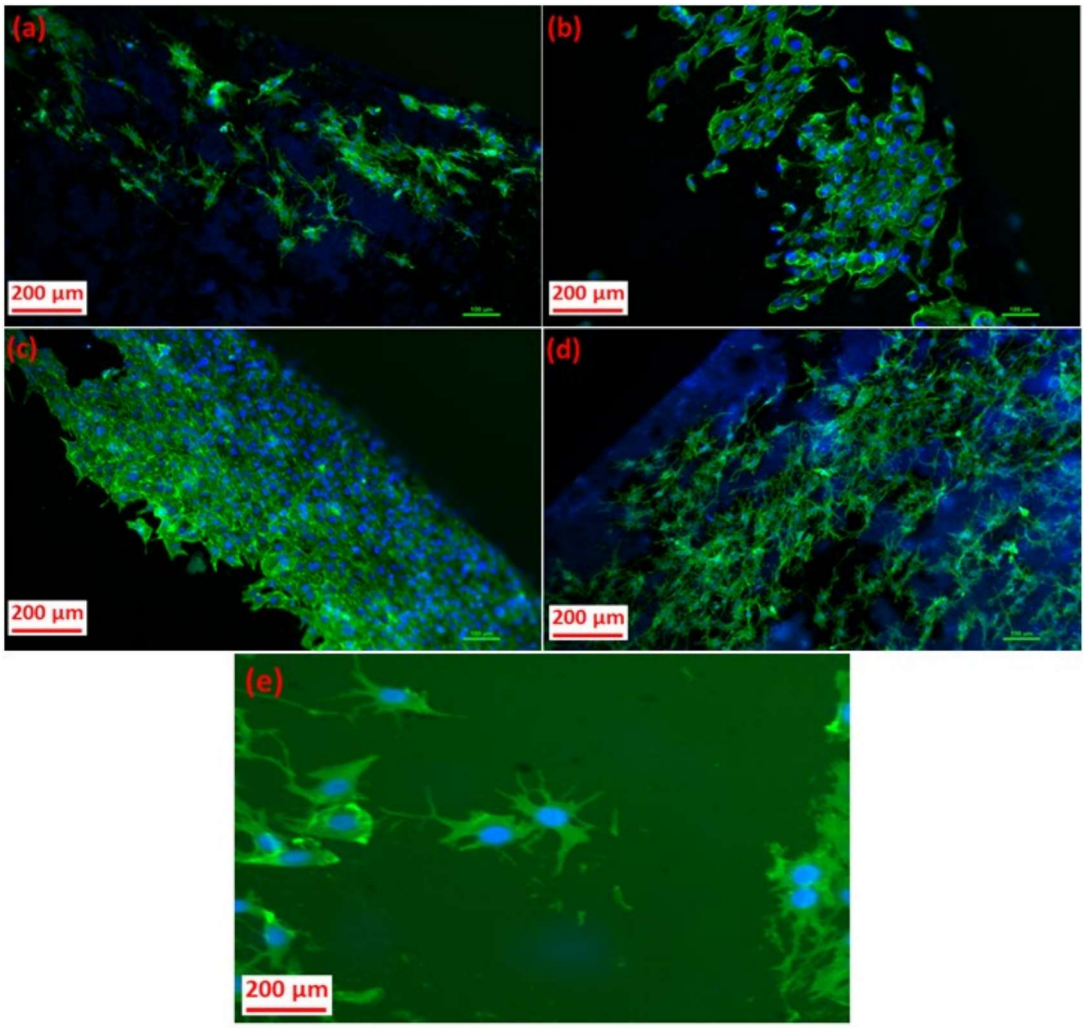

Fig.6.3- The cell culture images of MG-63 human bone osteosarcoma cells on the (a) S1, (b) S2, (c) S3, (d) S4, and (e) control samples after 3 days of incubation. Dark blue colour: nuclei staining; green colour: actin cytoskeleton filaments staining.

6.3.2 MTT Assay

The cell viability/proliferation behavior of MG-63 osteoblast-like cells was analyzed on samples S1, S2, S3, S4, and control samples using MTT assay. Figure 6.4 shows the histogram of the cell viability/proliferation for the MG-63 cells in different samples after incubation of 3, 5, and 7 days. It is clear from the histogram (Fig. 6.4) that there was a gradual increase in cell proliferation with the duration of incubation for all the samples. The mean percentage increment of cell proliferation of the MG-63 cells on sample S1 is ≈17% to ≈116%, on sample S2 ≈10.9% to ≈151%, on sample S3 ≈-14% to ≈101.5%, on sample S4 ≈8% to ≈111% compared to control sample with increasing incubation duration from 3 to 7 days, respectively. The cell proliferation on the samples from S2 to S4 increases with increasing Nb percentage in S1 (Kumar et al., 2021). The cell proliferation on the samples is found to be satisfactorily compared to the control sample with increasing the incubation duration.

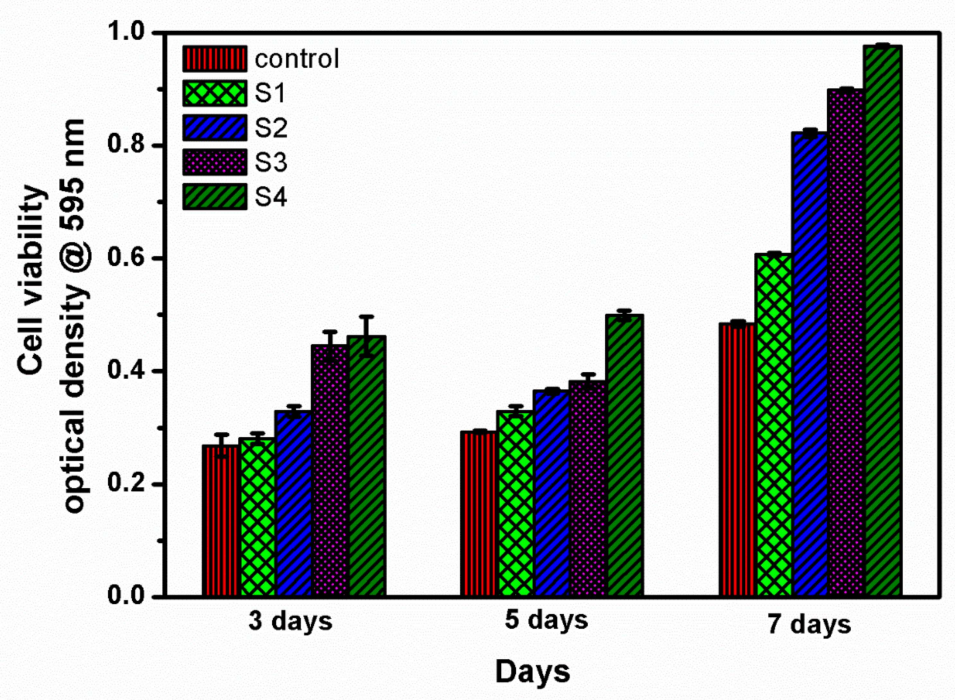

Fig. 6.4- MTT assay of MG-63 animal cells on S1, S2, S3, S4, and control samples on surfaces, the following culture for 3, 5, and 7 days.