

# **Biocompatibility Improvement of High Entropy Alloy via Femtosecond Laser Treatment**

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## **Extended Abstract**

In recent years, metallic biomaterials have received considerable attention in the field of biomedical engineering due to their outstanding mechanical strength and acceptable biocompatibility. These materials are widely used in various clinical applications, especially in orthopedic and dental implants, which mechanical durability and load-bearing capacity are of critical importance [1], [2]. Orthopedic metallic biomaterials include 316L stainless-steel (SS), cobalt-chromium alloys, titanium alloys, and nickel-titanium alloys, which are preferred for their favourable mechanical properties [3], [4], [5]. However, several issues remain challenges with these materials, such as low corrosion-resistance, ion release, limited osseointegration, and mismatch in elastic modulus with surrounding tissues [6], [7]. Thus, novel research focuses on improving existing biomaterials and developing promising alternatives like high-entropy alloys (HEAs) for biomedical applications. For this purpose, present study focuses on evaluating the biocompatibility and osseointegration potential of the Titanium-Tantalum-Hafnium-Niobium-Zirconium (TiTaHfNbZr) high-entropy alloy. Additionally, femtosecond laser treatment was utilized to enhance the surface characteristics of the alloy. For comparative evaluation, 316L stainless-steel—widely preferred and commonly studied biomedical material—was selected as reference.

The surfaces of TiTaHfNbZr high-entropy alloy (HEA) and stainless-steel samples were firstly prepared to metallographic treatment through grinding and polishing, ensuring comparable surface properties between the two materials are consistent. Subsequently, average surface roughness ( $R_a$ ) parameters of the samples were measured via profilometer. Based on characterization results, HEA samples were treated with laser, so two conditions of HEAs were prepared for experimental comparison: untreated (HEA-control) and femtosecond laser-patterned (HEA-modified).

In order to assess the effects of material type and surface characteristics on cell proliferation, (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) MTT assay was performed following 3-day incubation period. Additionally, to evaluate the influence of material type and laser treatment on cell apoptosis, Annexin V/PI staining protocol was followed for same period. Finally, cell-material interactions were analysed with scanning electron microscopy (SEM).

Upon evaluation of the obtained results, the sample surfaces were first characterized using profilometry. According to the profilometer analysis, average surface roughness values of the control samples (SS and HEA-control) were found to be quite similar ( $\pm 5\%$ ). In contrast, HEA-modified sample exhibited a significant increase in roughness—approximately 25-fold—compared to controls.

Subsequently, MTT assay results, conducted to investigate the effects of material type and surface characteristics on cellular behaviour, revealed that HEA-control sample promoted approximately 250% more cell proliferation per  $\text{mm}^2$  than the stainless-steel sample. Furthermore, HEA-modified sample showed about 40% increase in cell proliferation compared to HEA-control.

In the apoptosis assay, the proportion of viable cells on HEA samples was approximately 20% higher than that observed on stainless-steel. Cell-material interactions examined via SEM showed that laser-treated HEA samples exhibited greater cell attachment and wider surface coverage compared to the untreated HEA-control. Additionally, when comparing control groups, the HEA surfaces demonstrated higher cell density and included more cellular extensions on the material surface.

Based on the findings, the investigated high-entropy alloy demonstrated significantly improved biocompatibility compared to stainless-steel, particularly in terms of cellular proliferation, adhesion, and viability. Furthermore, surface modification was also found to contribute positively to the material's biocompatibility. In this context, both the alloy and the applied surface treatment are considered to remarkably improve biocompatibility and promising strong potential as an innovative alternative for orthopedic implant applications.

## References

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