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Supplementary Materials

In Vitro Evaluation of the Biological Response to Clinically Relevant Alumina-Toughened Zirconia (ATZ) Surfaces

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Supplementary Files

Supplementary S1: Direct cytocompatibility

LDH is a cytosolic enzyme widely used as a marker of membrane integrity and cytotoxicity. When cell membranes are damaged, LDH is released into the culture medium, where it catalyzes the conversion of lactate to pyruvate, coupled with the reduction of NAD^+ to NADH. Diaphorase then utilizes NADH to reduce resazurin to resorufin, which can be detected fluorometrically (Ex/Em: 560/590 nm). The percentage of cell viability versus the cells cultivated on plastic, considered as the reference gold standard, was calculated using the following formula:

$$\text{Cytotoxicity (\%)} = \left(\frac{sample - spontaneous\ LDH}{MAX\ LDH - spontaneous\ LDH}\right) \times 100 \longrightarrow \text{Viability (\%)} = 100 - \text{Cytotoxicity}$$

where spontaneous LDH refers to the baseline LDH released under physiological conditions, and max LDH corresponds to the LDH released upon cell lysis induced by the provided lysis buffer.
Results are reported in **Figure S1**.

After 24 hours, the viability of cells cultured on the samples is comparable to that of cells cultured on plastic, considered the gold standard. After 72 hours, a decrease in cell viability was observed on the SLA-treated TiGr4. However, the viability remained above 70% (red line), the cytotoxicity threshold indicated by ISO 10993.

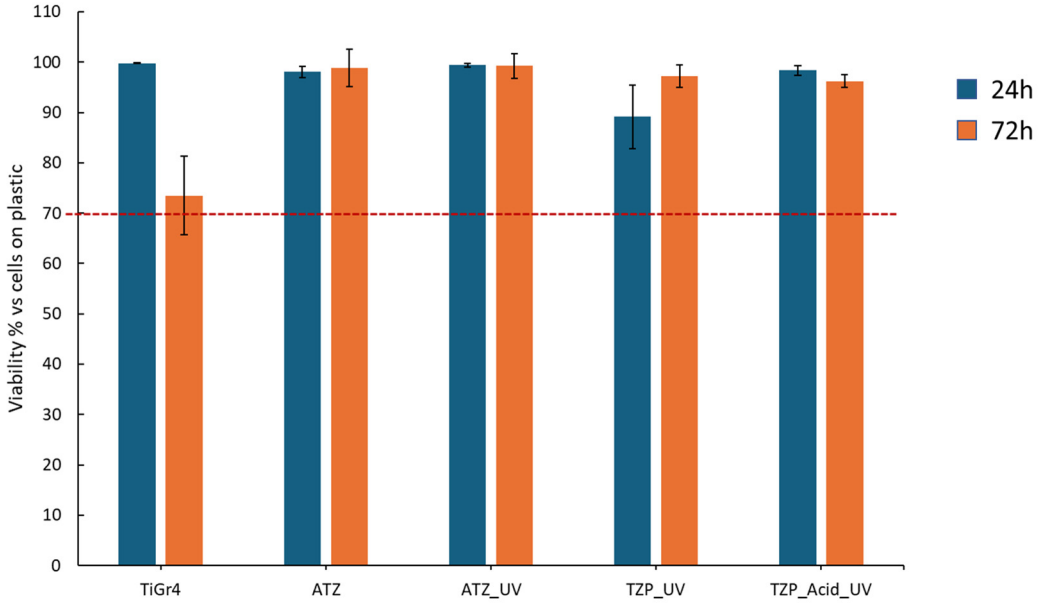

Figure S1. *Cell viability percentage calculated from LDH absolute values. The blue bars represent 24 hours, while the orange bars represent 72 hours. The red line indicates the cytotoxicity threshold defined by ISO 10993.*