

Biocompatibility of Titanium surface coated with ZrO₂-TiO₂ through Atomic Layer Deposition

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Abstract

Titania (TiO₂) and zirconia (ZrO₂) nanoceramics have been employed in biomaterials because they are biocompatible, chemically stable, hydrophilic, antimicrobial, and corrosion resistant. These nanoceramics can be deposited on substrate surfaces through Atomic Layer Deposition (ALD) at atomic level. The aim of this study was to understand biocompatibility of nanoceramics. Our result indicate that ZrO₂ and ZrO₂-T nanoceramic coating is biocompatible with epithelial cells and may improve their degree of cell attachment by examining live and dead cells analysis and quantifying various known genes of HAT7 cells that participates in cell adhesion and attachment. Scanning Electron Microscopy analysis shows attachment projections from ECM to titanium disc at both 24 and 48 hrs. Nanoceramic coating of ZrO₂ and ZrO₂-T is a promising method to improve the bio-functionality of titania alloy V as implant abutment material. Further research can be expanded *in vivo* experiments epithelial growth onto nano-ceramic coated custom abutments.

Objectives

- 1) Titanium (V) discs coated with ZrO₂, ZrO₂-TiO₂ nanofilm are biocompatible and have increased gene expression than those in control.
- 2) Titanium (V) discs coated with ZrO₂, ZrO₂-TiO₂ nanofilm have increased adherent epithelial cell density than control.

Method

Titanium grade V disks were polished using grit sandpaper to average Ra (roughness) within 3µm. Control, ZrO₂ and ZrO₂-TiO₂ nanoceramic thin film of 30-50nm thickness were deposited through ALD. Epithelial HAT7 cells were cultivated with disc in DMEM medium. Coding cRNA was separated from live cells, quantified, converted to DNA, and real-time quantitative PCR was completed for selected genes from protein databank with bioinformatics. Cellular attachments were analyzed with live/dead cells in SEM.

Experimental Design

Titanium Disc

10.00 mm x 10.00 mm x 1.00 mm
Material: Titanium Grade 5
Roughness: 0.2µm

Group 1
Control
Uncoated

Group 2
ZrO
Pure ZrO₂

Group 3
Mix
1:4 ZrO₂-TiO₂

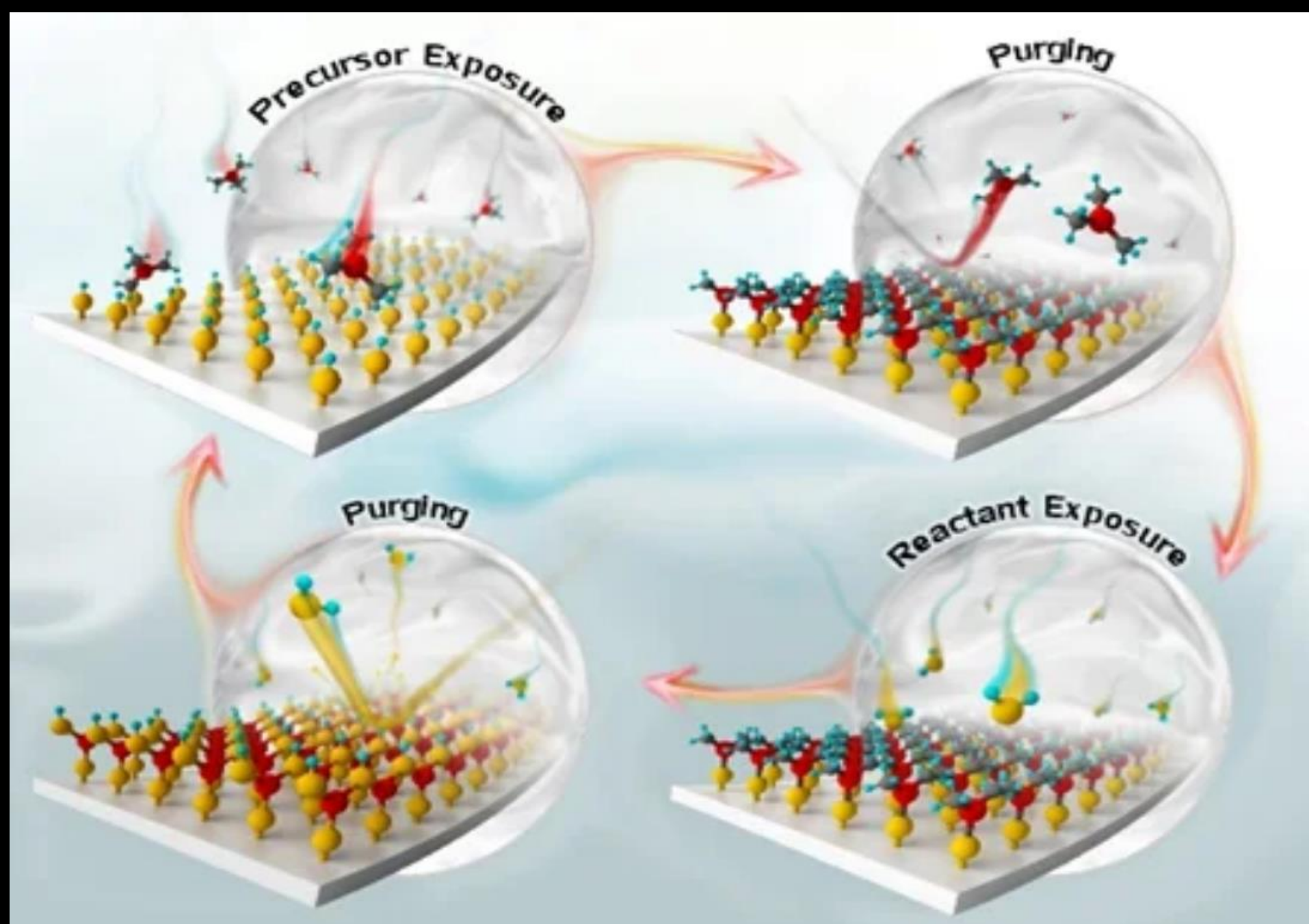
Cell Viability, Attachment, and SEM

HAT7 cells on Ti(V) discs:
Viability: **Live / Dead Assay**
Attachment interface visualization
- **Scanning Electron Microscopy**
- **Energy-Dispersive X-ray Spectrometry**

Gene Expression

Bioinformatics
cRNA Quantification
qRT-PCR
Laminin, Integrin,
Vinculin, Thrombospondin
Collagen type I α1
Collagen type IV α1
Versican, Periostin

Nano-Ceramic Thin Film Coating with ALD



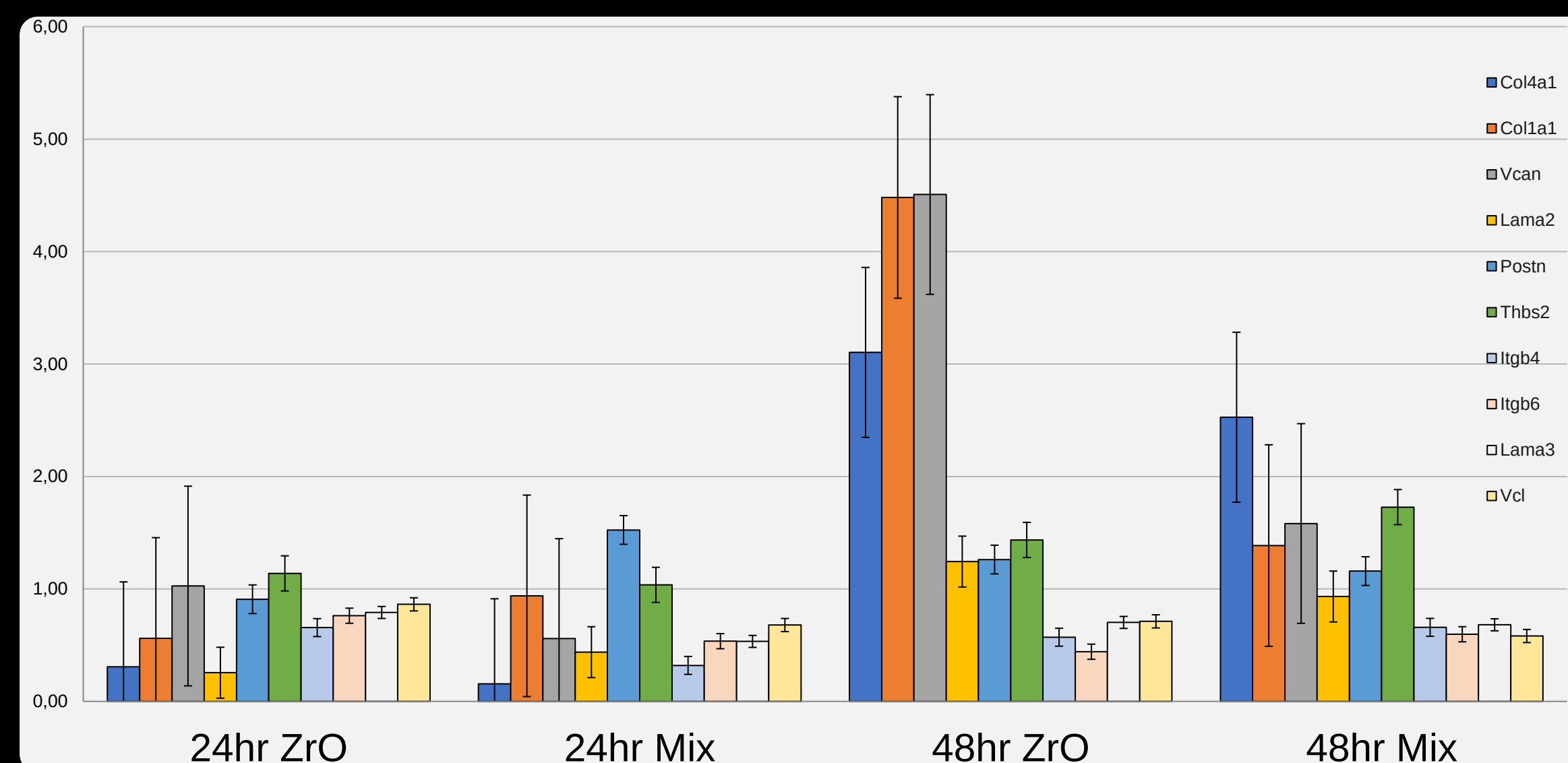
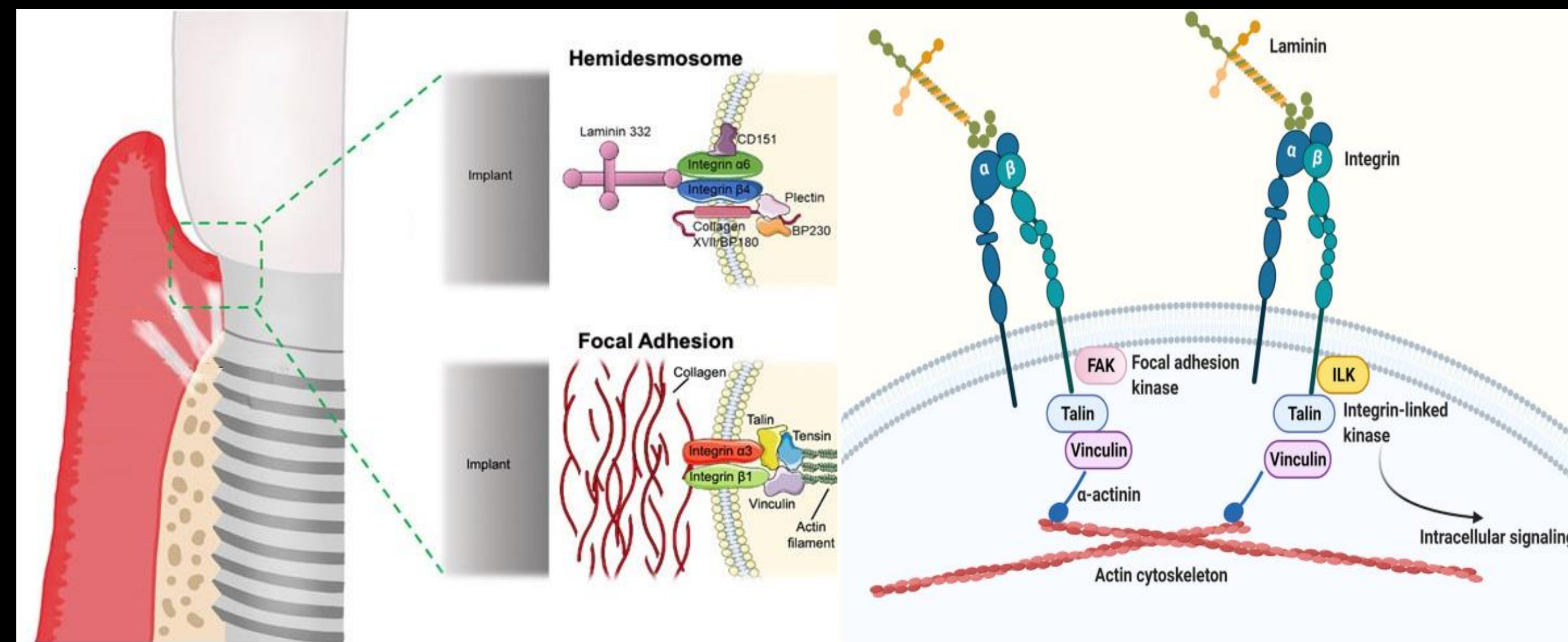
Uniformity

Conformality

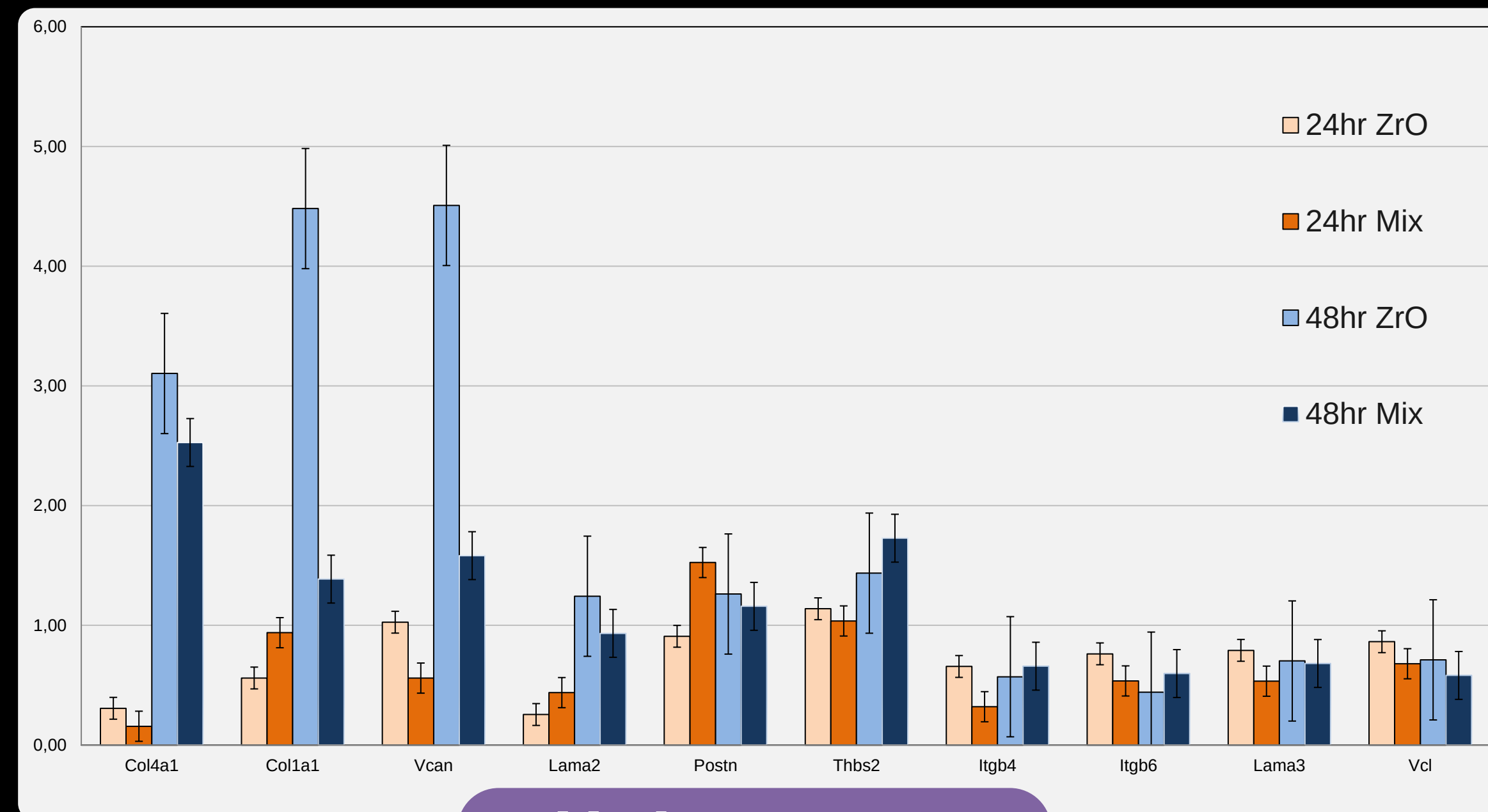
Surface Smoothness

Research

Genes Expression

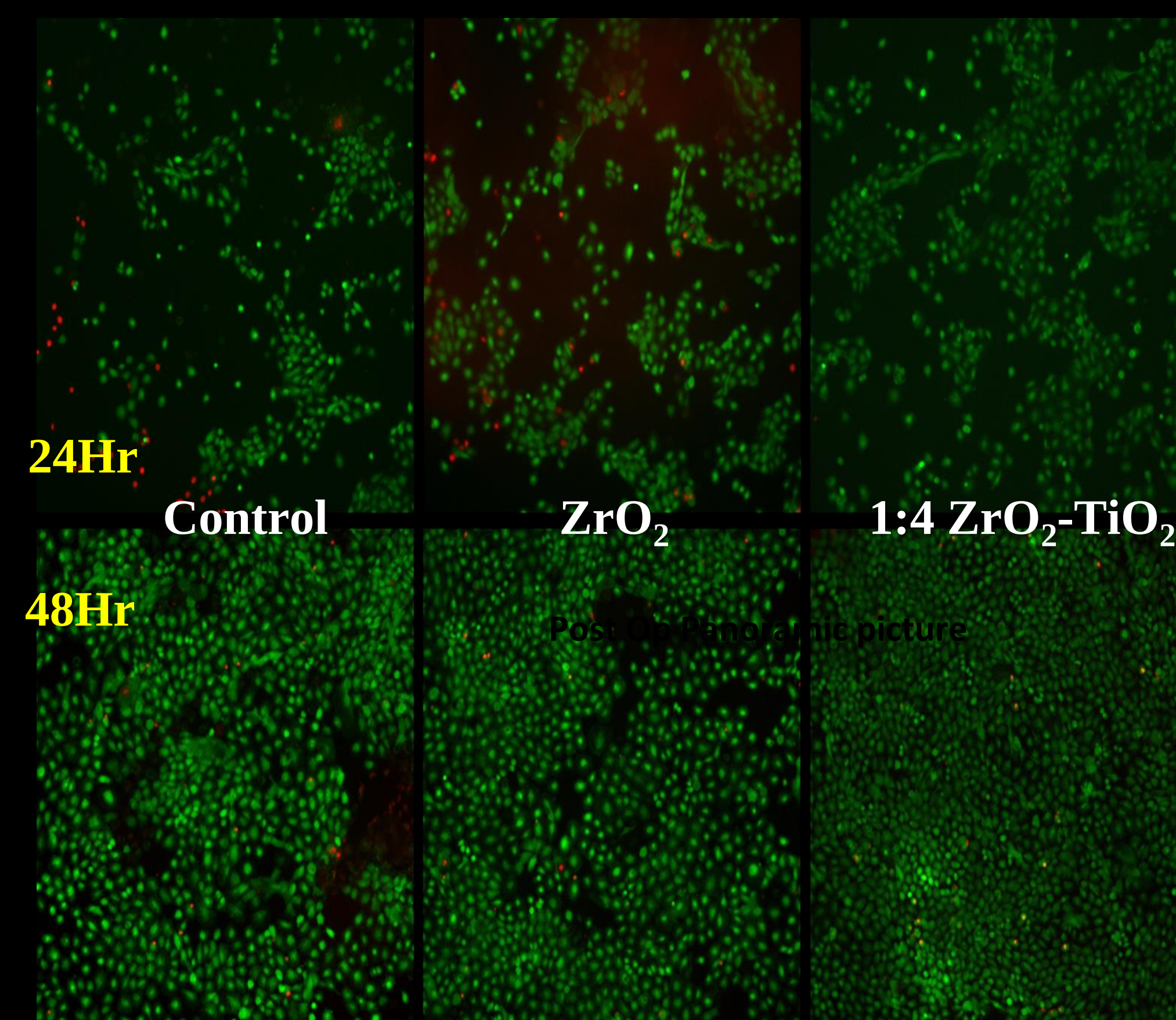


Fold Change per Time Point

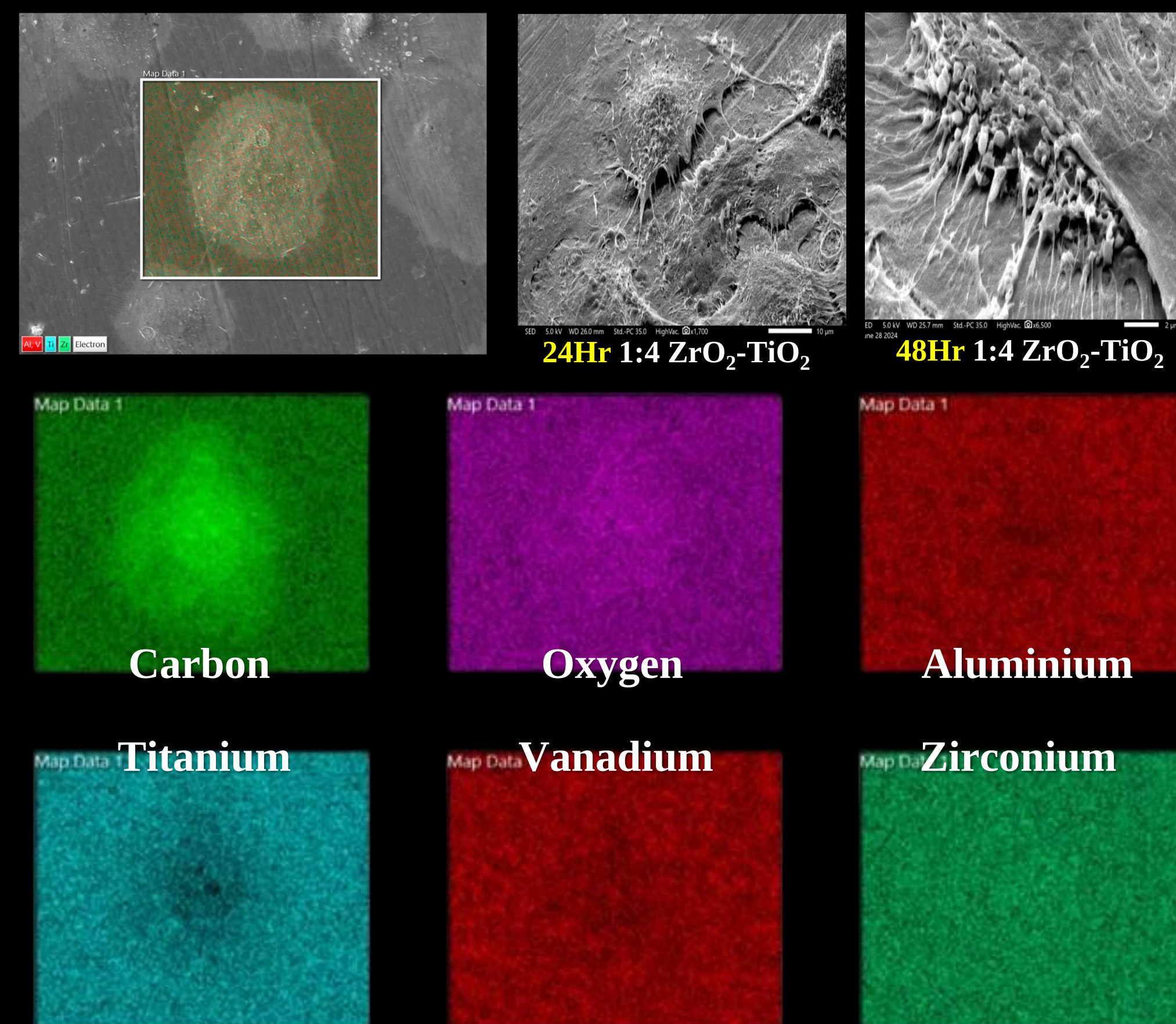


Fold Change per Gene

Live Dead Assay



ZrO₂ Coated Surface: Energy-Dispersive X-ray Spectrometry



Result

Dental epithelial cells were viable on in all groups, with the 1:4 ZrO₂-TiO₂ group demonstrating increased number of attached cells with attachment processes at the cell-disk interface at both 24 and 48 hours. Laminin and Integrin expression levels were not different amongst groups and were trending towards increased expression in the 1:4 ZrO₂-TiO₂ group across all time points. Initial cell adhesion as measured by total amount of dsDNA at 1, 3, 6, and 24 hour were not different amongst groups. Genes known to participate in cellular attachment such as Collagen IV, Collagen VI, Versican, Laminin, Periostin, Thrombospondin, integrin, Vinculin were examined. For all examined genes, 48 hours showed more mRNA expression compared to 24 hrs for both mixed and ZrO₂ coated groups.

Conclusion

Epithelial cells are biocompatible and viable in tested Atomic Layer Deposition deposited nanoceramic conditions of ZrO₂-TiO₂ and actively forming attachment to titanium *in vitro*. Nanoceramic coating of ZrO₂ and ZrO₂-T is a promising method to improve the bio-functionality of titania alloy V as implant abutment material.

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