



# Effect of Surface Roughness of Ceria-Stabilized Zirconia/Alumina Nanocomposites on Adhesion of Mouse Gingival Junctional Epithelial Cells

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## Introduction

In implants, zirconium (Zr) abutments are potential substitutes to titanium (Ti) and offer better aesthetic result<sup>1</sup>. Furthermore, Zr can replace metals in both clinical medicine and dentistry owing to its high level of mechanical strength and excellent bioactivity<sup>2</sup>. In 1998, improvements in nanotechnology developed a strong ceria-stabilized tetragonal zirconia polycrystal / alumina (Ce-TZP/Al<sub>2</sub>O<sub>3</sub>) nanocomposite, which has an interpenetrated intragranular nanostructure. Compared with conventional 3Y-TZP, Ce-TZP/Al<sub>2</sub>O<sub>3</sub> exhibits greater flexural strength and fracture hardness, making it appropriate for dental implants use<sup>3</sup>. Epithelial tissue sealing on the dental implant surface is particularly important for the long-term stability of dental implants. Our previous studies on animals confirmed the improvement of the osseointegration strength of Ce-TZP/Al<sub>2</sub>O<sub>3</sub> treated with hydrofluoric acid on acid-etched Ti implants<sup>4</sup>. Furthermore, mirror-polished surfaces have been shown to enhance the biological affinity and adhesion strength of gingival fibroblasts<sup>5, 6</sup>.

## Objective of the study

To achieve the strong epithelial sealing of Ce-TZP/Al<sub>2</sub>O<sub>3</sub> implants, we investigated the effect of surface roughness on adhesion of mouse gingival junctional epithelial cells.

## Materials and Methods

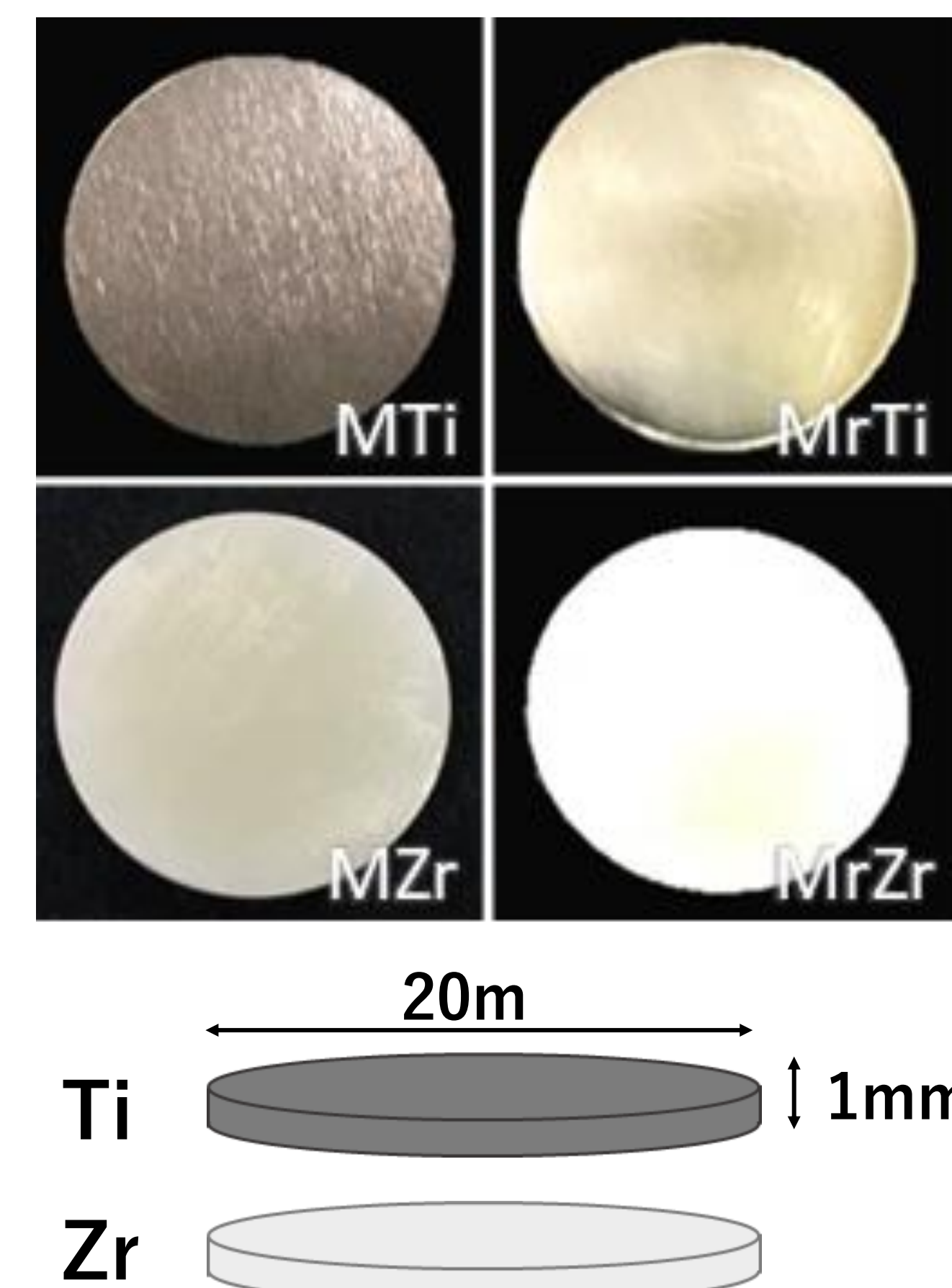
### 1. Sample

- Ce-TZP/Al<sub>2</sub>O<sub>3</sub> , Ti to produce the disks.
- Machined and mirror polishing of the manufactured disks.  
Mirror-polished surfaces : MrZr, MrTi  
Machine-polished surfaces : MZr, MTi

#### Analysis of the surface morphology

Scanning electron microscopy SEM

Surface roughness - Ra



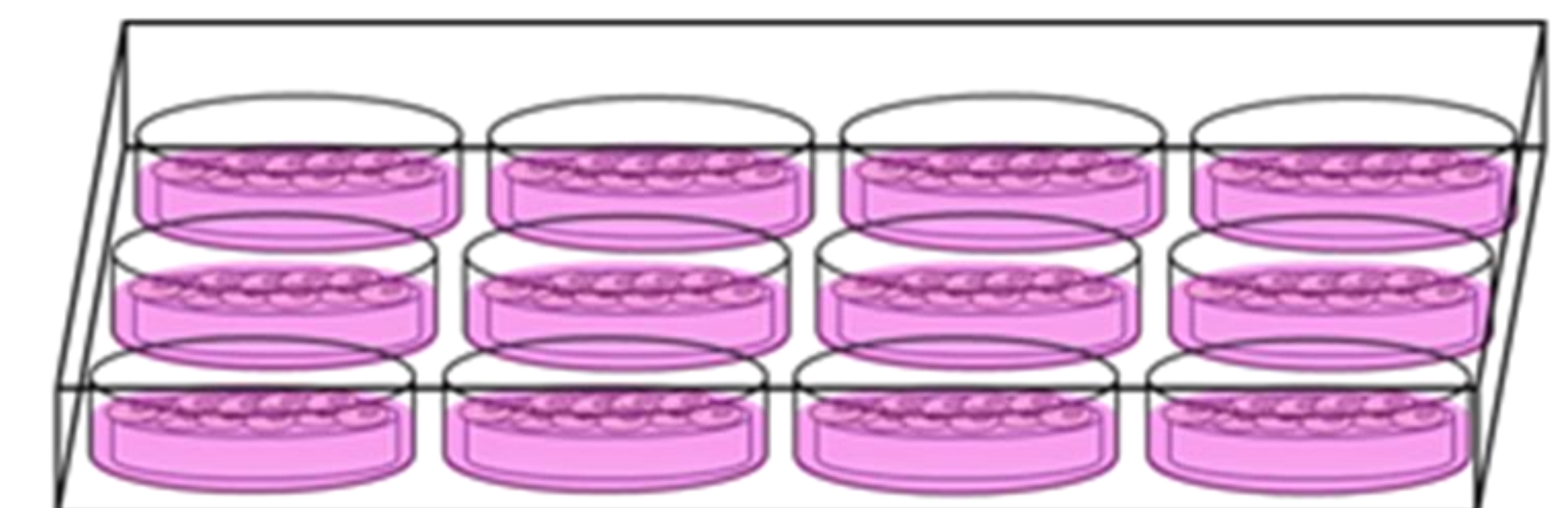
### 2. Cell culture

【Cell】 The adhesion of Mouse Gingival Junctional Epithelial Cells (JE-1)<sup>7</sup>

【Medium】 CnT-Prime Epithelial Proliferation Medium with 1% Penicillin Streptomycin

【Seed】 12-well plates at a density of  $1.0 \times 10^5$  cells/cm<sup>2</sup>

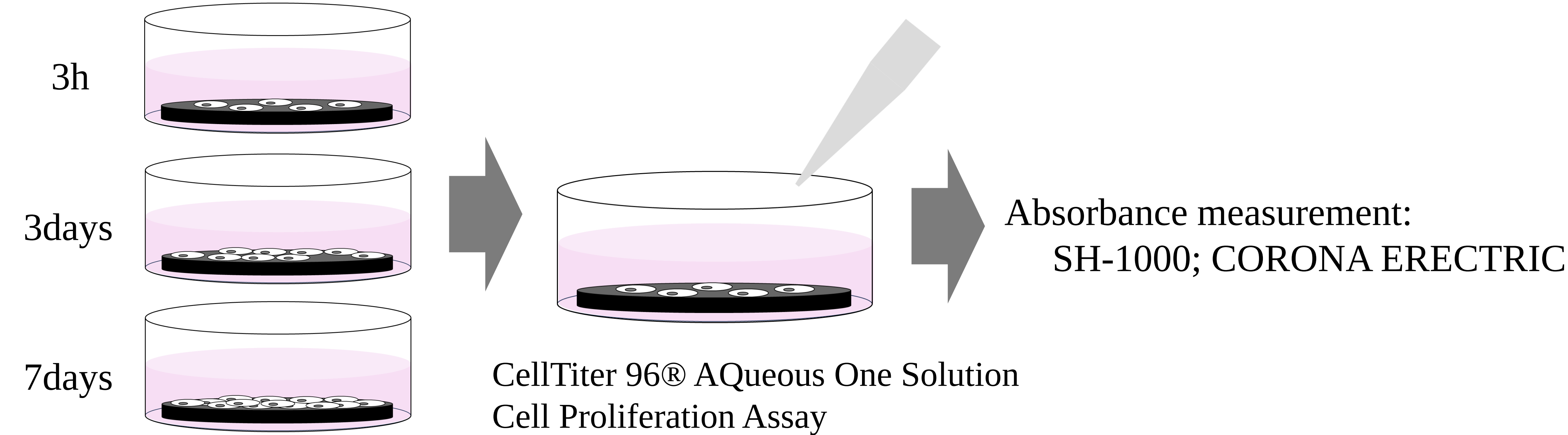
【Cultivation】 3 h, 3 days or 7 days.



## 3. Biological test

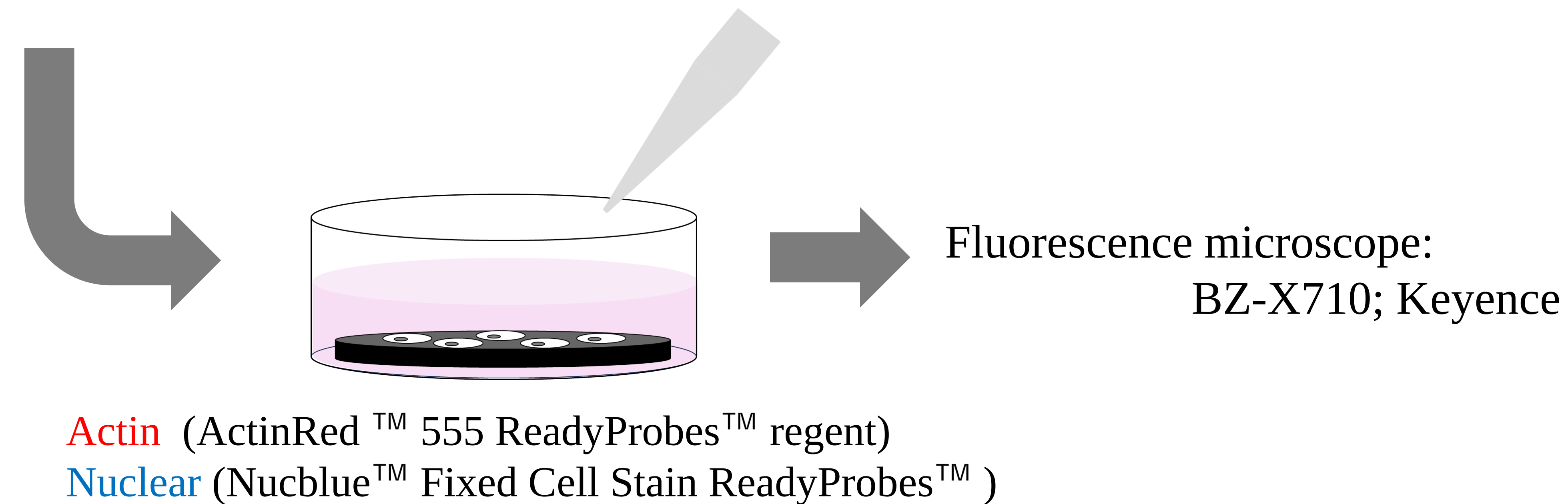
### ① Cell proliferation

Cultivation



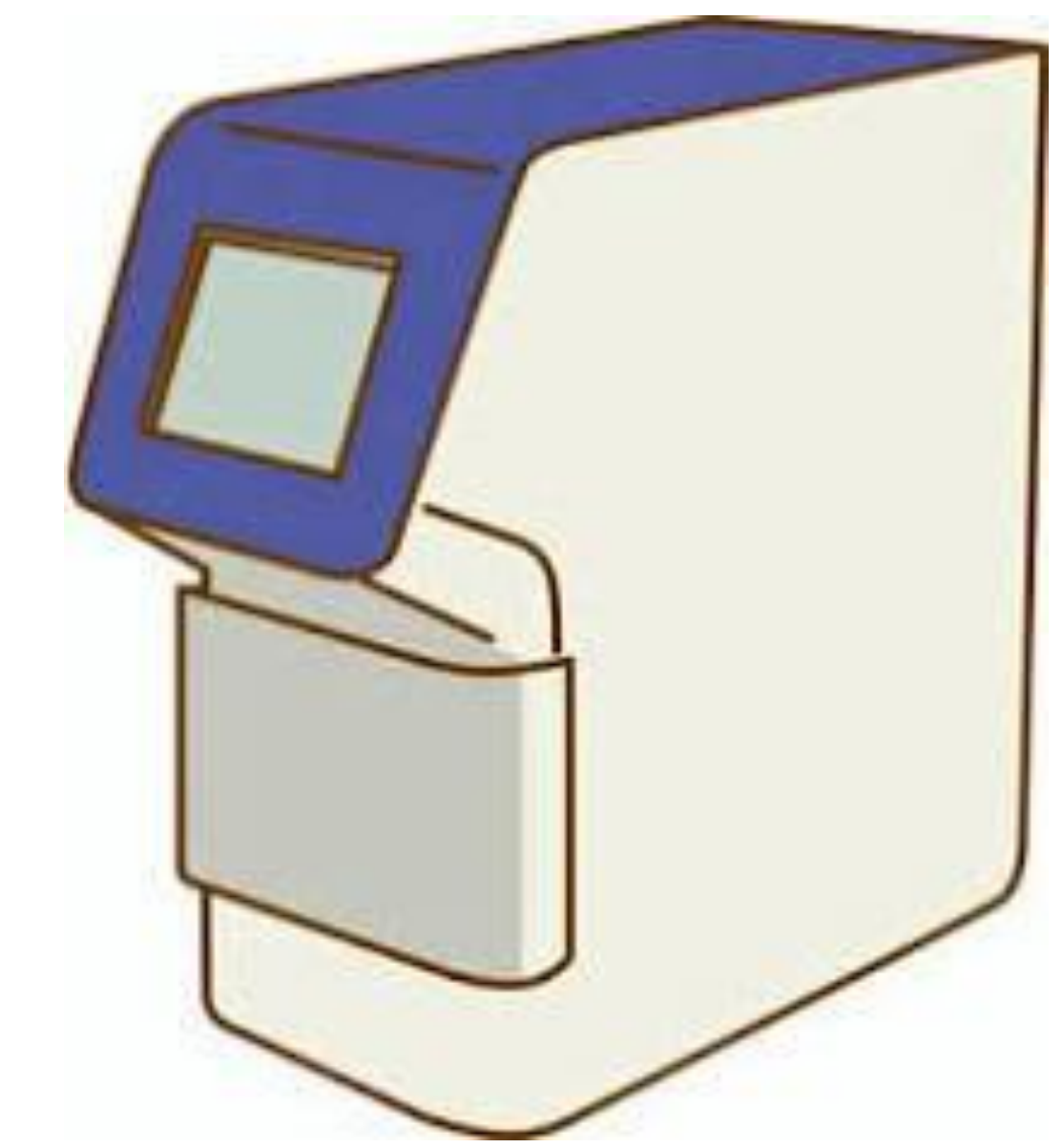
### ② Immunostaining of cultured JE-1

JE-1 were cultured on each disks for 3h, 3days and 7days

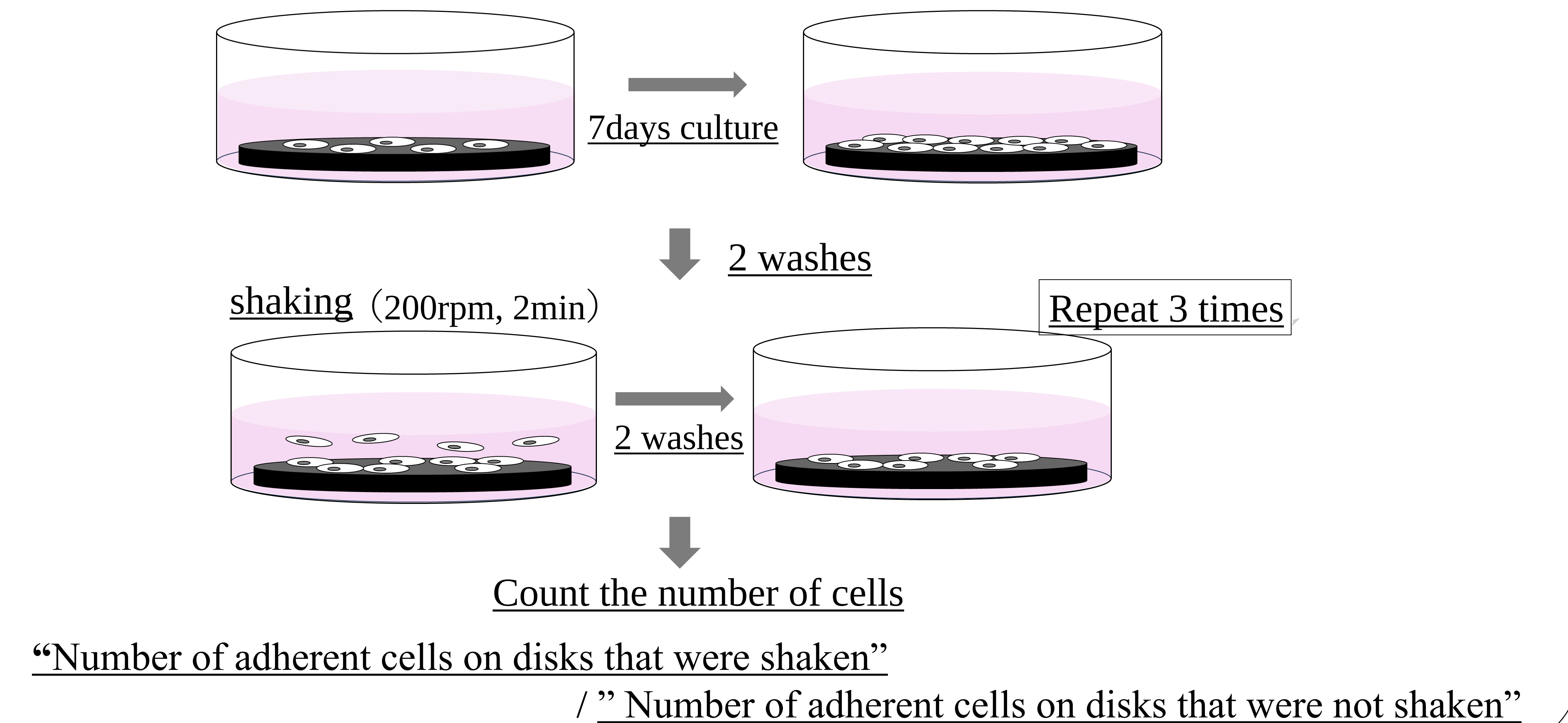


### ③ Real-time PCR analysis of adhesion protein expression

Adhesion protein: Laminin-5, Integrin $\beta$ 4  
Reagent: THUNDERBIRD™ Probe qPCR Mix  
Machine: Applied Biosystems  
StepOnePlus™ ; Thermo Fisher Scientific



### ④ Adhesion Assay to Evaluate the Strength

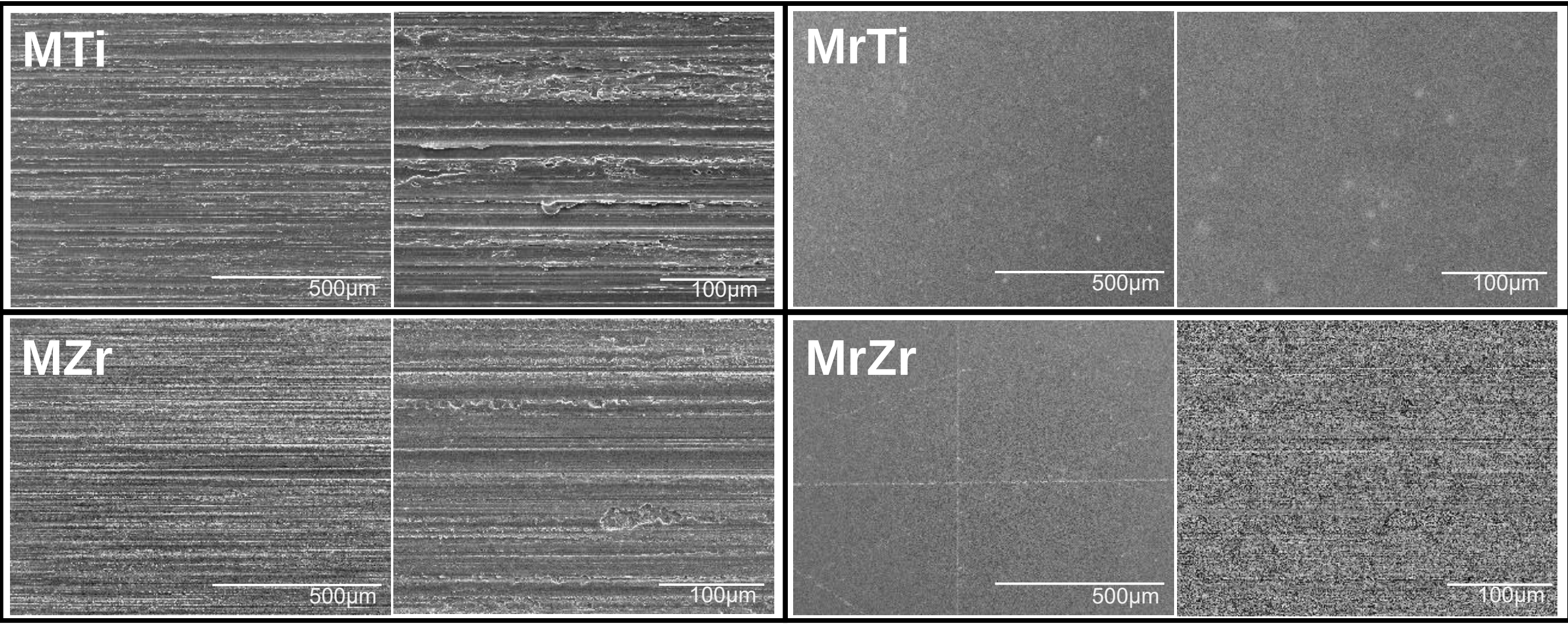


## 4. Statistical analysis

To evaluate the effects of cell proliferation, gene expression, and adhesion strength on substrates, we applied two-way analysis of variance. For multiple comparisons, we performed the Tukey test. Significance was considered for  $p < 0.05$ .

# Results

## Surface morphology



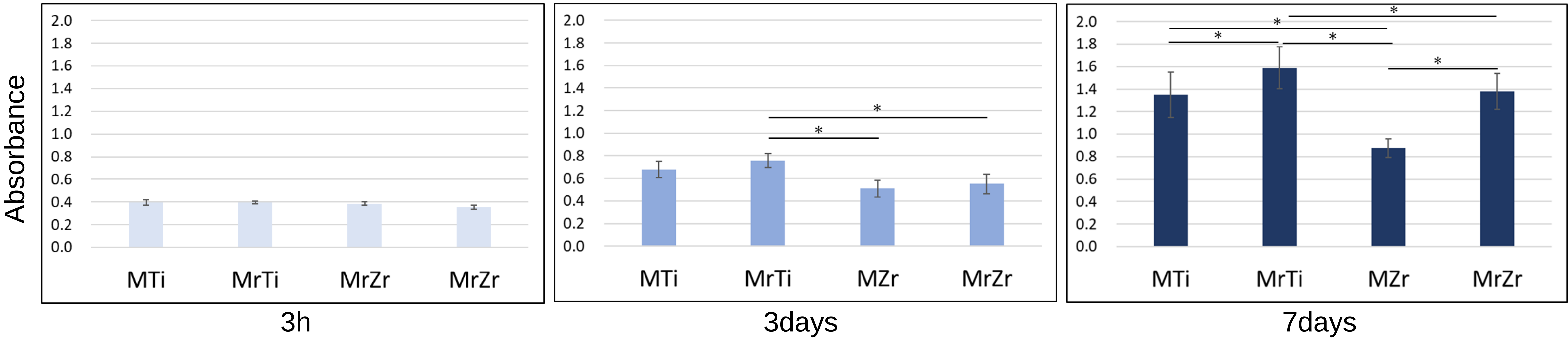
Different surface roughness profiles of Ti and Ce-TZP/Al<sub>2</sub>O<sub>3</sub> disks characterized by SEM

- The surface morphology of both the MTi and MZr disks was relatively smooth and flat, although the high magnification images showed a rough surface. In contrast, the SEM images of MrTi and MrZr disks showed very smooth and flat morphologies, without topographical or rough features.
- The Ra values of MTi and MZr were 0.91 and 0.92 µm, respectively. Also, the Ra values of MrTi and MrZr were 0.02 µm.

|         | MTi       | MrTi       | MZr       | MrZr       |
|---------|-----------|------------|-----------|------------|
| Ra (µm) | 0.91±0.03 | 0.02±0.004 | 0.92±0.05 | 0.02±0.004 |

Surface roughness measurement using Ra

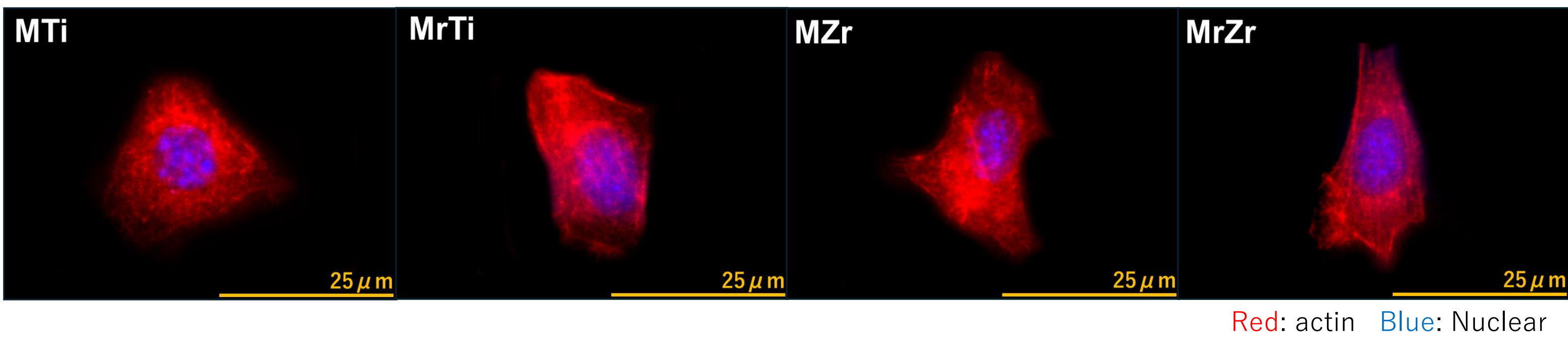
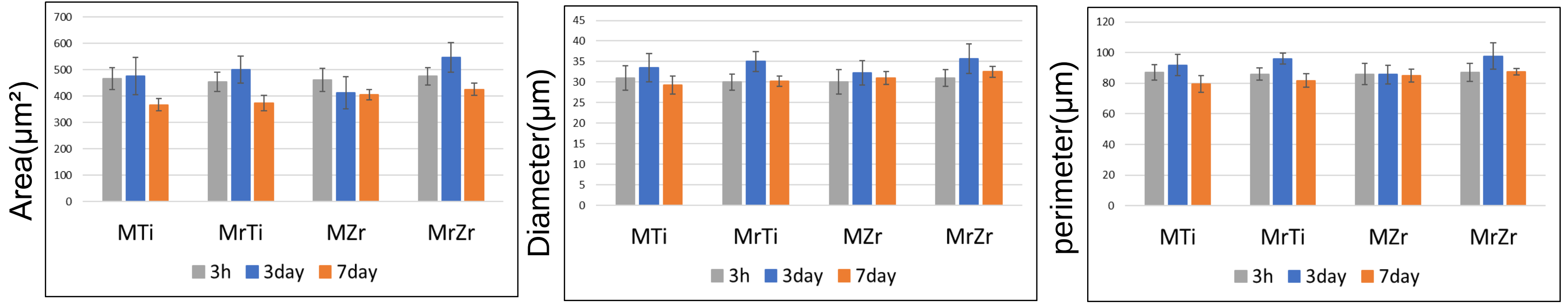
## Cell proliferation on Ce-TZP/Al<sub>2</sub>O<sub>3</sub>



MTS assay of JE-1 cultured on Ti and Ce-TZP/Al<sub>2</sub>O<sub>3</sub> for 3hours, 3days and 7days (n=4)

- It was found that cells were proliferating in each group of materials and Ra.
- No significant difference was observed at 3hours of incubation, but MrTi showed predominantly higher values than MrZr at 3days of incubation, and MTi showed predominantly higher values than MZr and MrTi showed predominantly higher values than MrZr at 7days of incubation.
- After 7days, Cell proliferation on Ti and Ce-TZP/Al<sub>2</sub>O<sub>3</sub> disks were enhanced on the Mirror-polished surfaces compared to Machine-polished, and Ce-TZP/Al<sub>2</sub>O<sub>3</sub> disks were consistently enhanced by 50%.

## Cell morphology

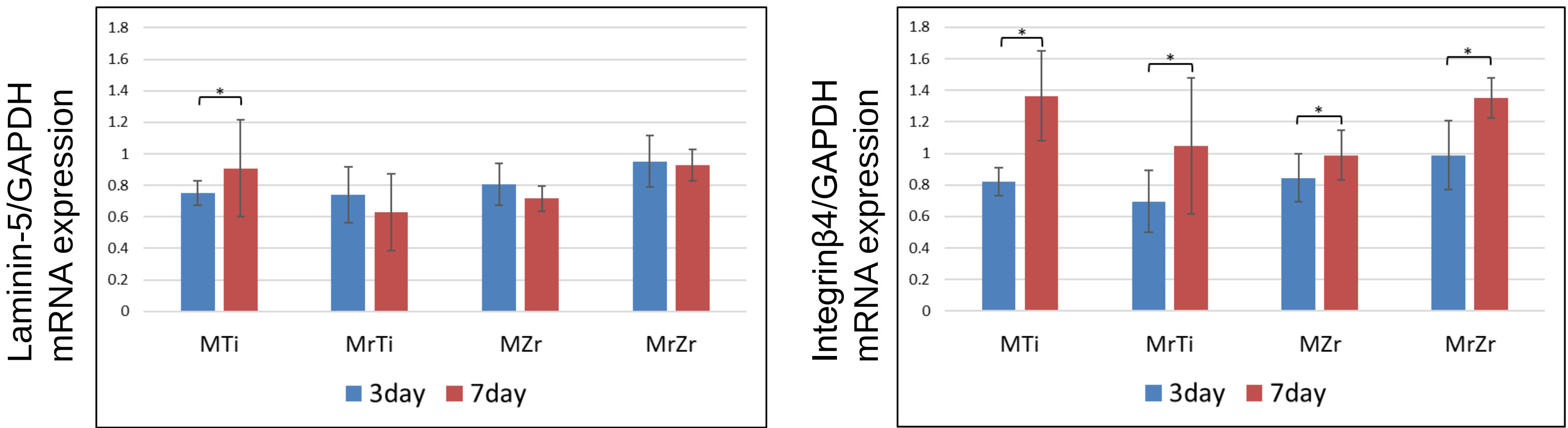


Fluorescence phase contrast microscopy of JE-1 cultured on Ti and Ce-TZP/Al<sub>2</sub>O<sub>3</sub> for 3hours, 3days and 7days (n=4)

• We observed the morphology and cytoskeletal organization of JE-1 on various Ce-TZP/Al<sub>2</sub>O<sub>3</sub> and Ti surfaces under a fluorescence phase contrast microscope.

• Fluorescent imaging with actin filament staining revealed that the area, diameter and perimeter of individual cells were independent of the material or Ra at 3 h, 3 days, and 7 days of culture.

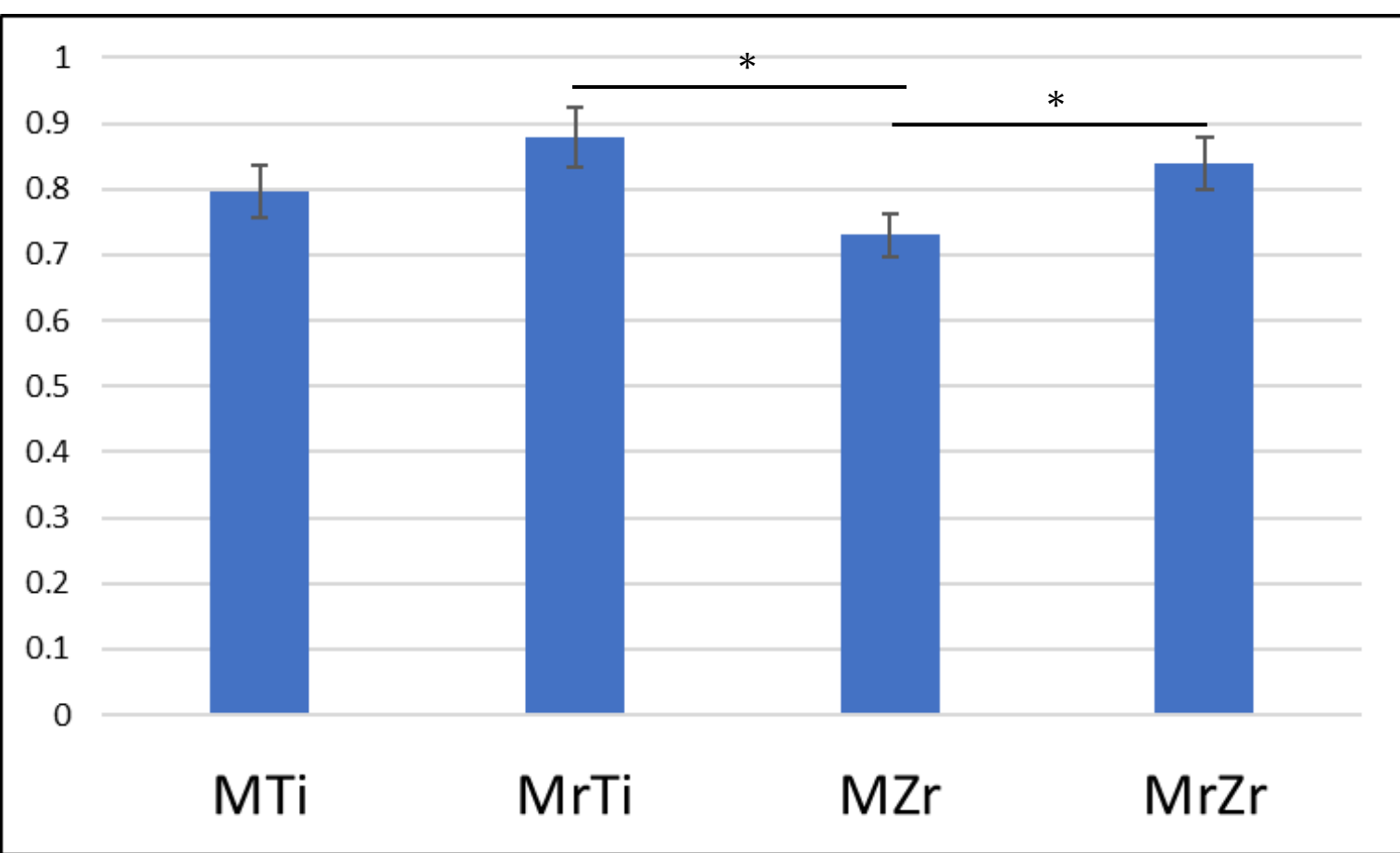
## mRNA expression of adhesive protein



Expression levels of mRNA for Laminin-5 and Integrinβ4 in JE-1 cultured on each disk for 3days and 7days (n=6)

- The epithelial cell adhesion proteins Laminin-5 and Integrinβ4 were found to be expressed in each group.
- On Ce-TZP/Al<sub>2</sub>O<sub>3</sub> disks, the expression of laminin 5 and integrin β4 in JE-1 tended to be higher with Mirror-polished surfaces than with Machine-polished.

## Adhesion assay



Adhesion assay of JE-1 cultured on Ti and Ce-TZP/Al<sub>2</sub>O<sub>3</sub> for 7 days. (n=4)

- Cell detachment due to shaking was observed in all disks cultured with JE-1.
- There was no significant difference between Ti and Ce-TZP/Al<sub>2</sub>O<sub>3</sub> disks with the same Ra.
- The value was higher in MrZr than in MZr, and the same tendency was observed in Ti.

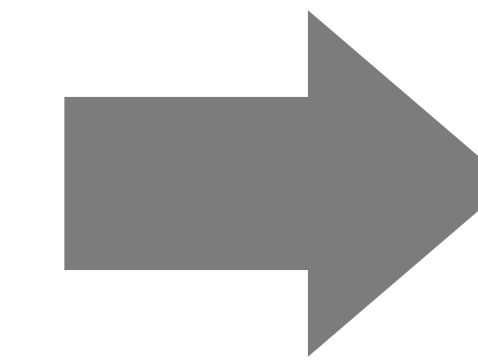
## Discussion

- Mirror-polished treatment modified adhesion strength and the cellular adhesive properties of individual epithelial cells. For example, the percentage of cells not detached by shaking stimulation on Ce-TZP/Al<sub>2</sub>O<sub>3</sub> were about 1.2 times higher on mirror-polish surfaces than on roughened surfaces at 7-day after culture. These results suggest that mirror-polished surfaces may retain cells better and thus have greater resistance to forces that cause cell detachment, even during the final stages of cell adhesion.
- Epithelial adhesion to the tooth surface is mediated by hemidesmosomes and involves extracellular matrix including laminin-5 and integrin, an adhesion protein present on the cell surface. In the present study, Mirror-polished Ce-TZP/Al<sub>2</sub>O<sub>3</sub> showed higher expression of laminin-5 and integrin $\beta$ 4 than Machine-polished, indicating that Mirror-polished surfaces may be superior in obtaining epithelial adhesion. Further studies are needed to elucidate the detail of how surface properties contribute to the formation of the junctional epithelium in vivo.
- The cell–material interactive mechanism underlying bio-activation on mirror-polished surfaces needs to be fully clarified. Clarification of how interfacial signal transmission from material to cell results in epithelial adhesion on mirror-polished Ce-TZP/Al<sub>2</sub>O<sub>3</sub> surfaces would be of great interest.

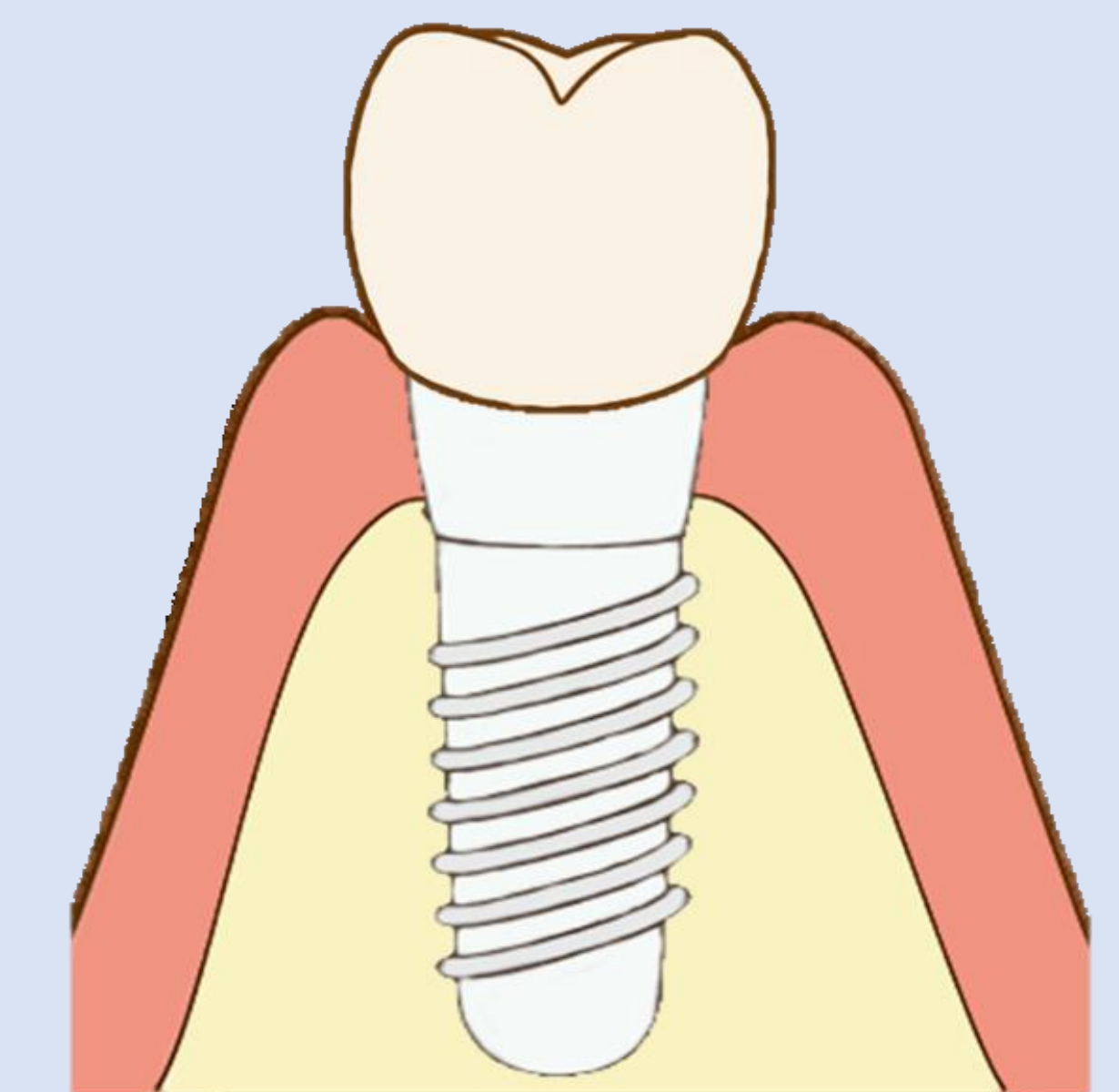
## Conclusion

### MrZr compared to MZr ...

- ① Enhanced the proliferation of JE-1
- ② Increased gene expression of Laminin-5 and Integrin  $\beta$  4
- ③ Improved the adhesion strength of JE-1



This study suggested that Ce-TZP/Al<sub>2</sub>O<sub>3</sub> with a mirror-polished surface might achieve a **stronger epithelial sealing**.



## Reference

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