Follicular Dendritic Cell-Secreted Protein (FDC-SP) Enhances **Mucointegration**

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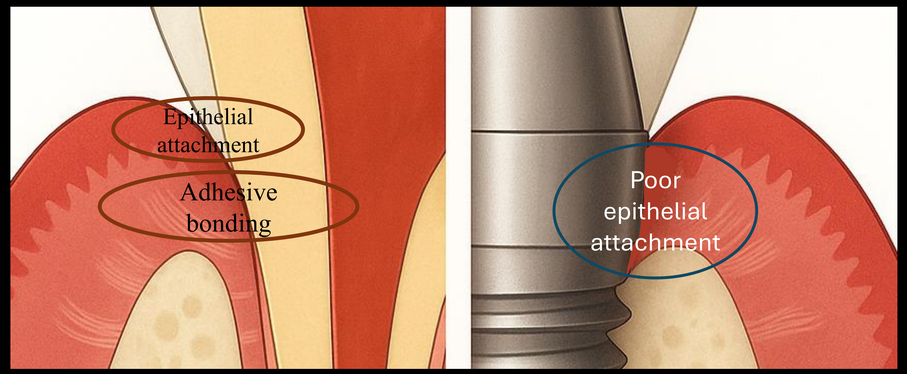
Introduction

Peri-implant long-term success requires not only stable osseointegration of the implant body but also the establishment of an effective soft-tissue seal that prevents peri-implantitis. In natural dentition, periodontal tissues provide robust biological barriers through specialized epithelial structures, including the junctional epithelium (JE) and the sulcular epithelium. However, following tooth extraction, these protective epithelial components are lost, and the healing process results in the formation of oral epithelium (OE) at the mucosal surface instead of JE(1). This structural change may compromise the soft-tissue attachment around dental implants.

Follicular dendritic cell-secreted protein (FDC-SP), originally identified as a JE-associated protein, has been reported to promote epithelial adhesion to enamel. Therefore, in this study, **we aimed to enhance soft-tissue integration at the implant transmucosal portion by promoting the adhesion of OE cells to titanium surfaces using FDC-SP**.

Comparison of Mucosa Surrounding Natural Teeth and Implants



Natural teeth possess epithelial attachment and connective attachment, but the implant mucosal penetration site only has fragile attachment.

Specific molecules are required for the adhesion between the junctional epithelium and enamel.

Junctional epithelium and Enamel

- **ODAM**
- **FDC-SP**
- **Amelotin**
- **Nephronectin**
- **laminin**

Epithelium and Connective Tissue

- **Collagen**
- **Laminin**
- **Perlecan**
- **Fibronectin**

Differences in cell adhesion and upon coating with FDC-SP

Materials Used

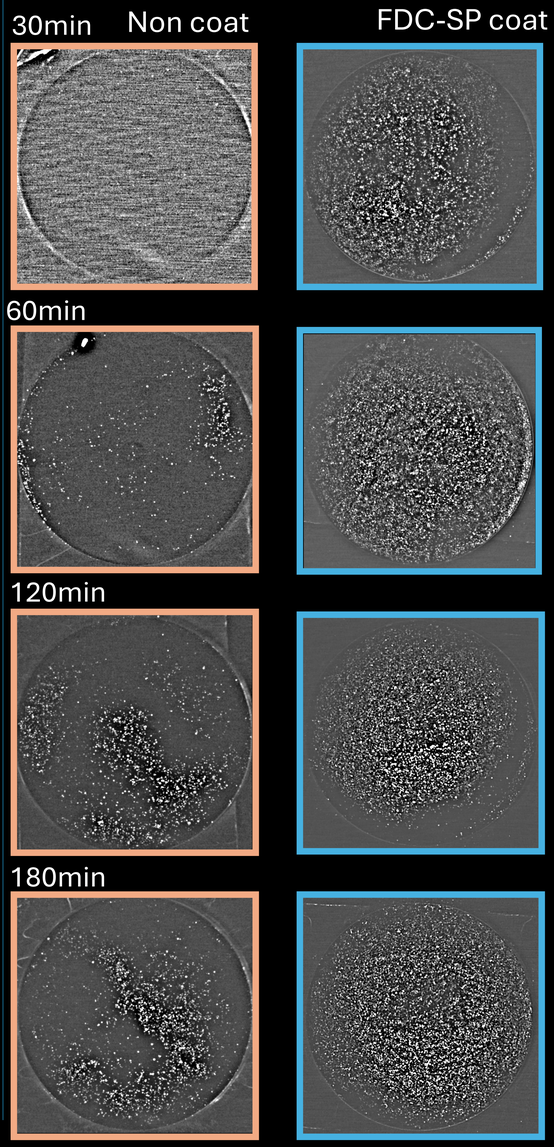
Base: Titanium disk.

Protein: FDC-SP (After introducing the human FDC-SP plasmid into mHAT9d cells, His Tag protein was recovered and purified from the culture supernatant using cobalt resin)

Cells: Mouse oral epithelial cells (mOE-T2). Expressing red fluorescent protein tdTomato

Media: DMEM HAM/F12, Penicillin-Streptomycin, B27,FGF-2 (26ng/ml), EGF(20ng/ml)

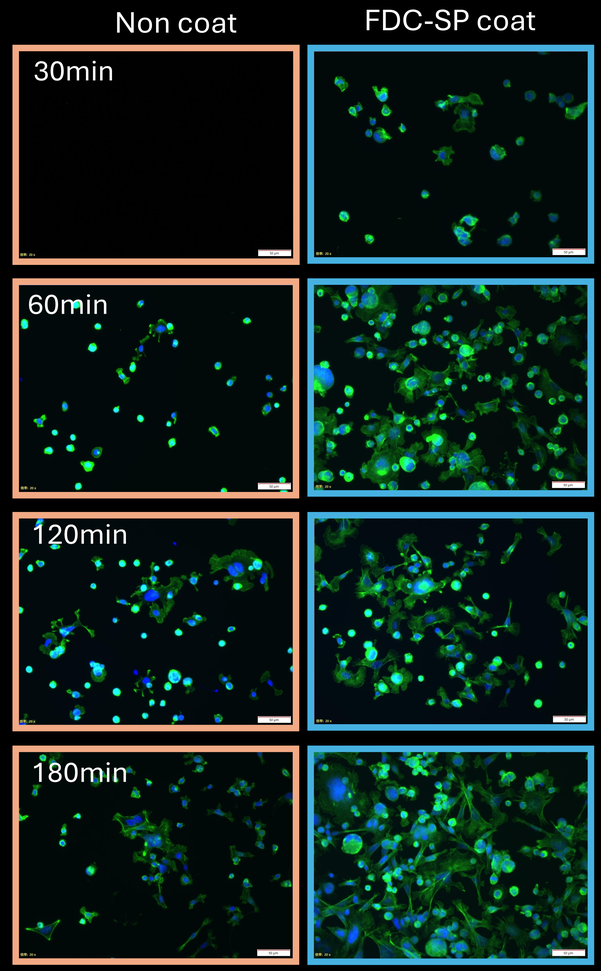
Method
FDC-SP(50 μg/ml) was applied to a titanium disc and left to stand at 4℃ for 30 minutes to coat the disc surface with protein. The control group discs were coated with PBS.
mOE-T2 cells were seeded at 5×10^5 cells/ml.
At 30 min, 60 min, 120 min, and 180 min, non-adherent cells were washed away with PBS. Adherent cells were fixed with 4% PFA, permeabilized, and stained for actin.

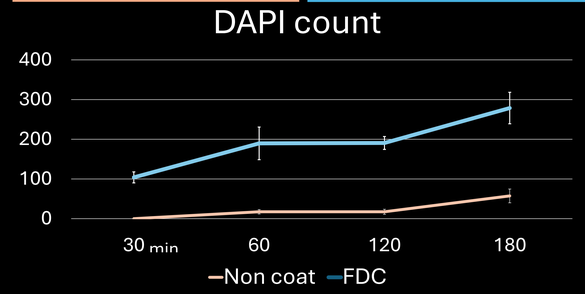


Result1

Image obtained by Fourier transform after photographing the entire titanium disc seeded with cells. White dots indicate cells remaining on the titanium disc after washing. When washed for 30 minutes, only noise is visible in the control group since no cells are present.

In the coated group, cell adhesion was observed across the entire disc surface 30 minutes after seeding. In the control group, no cells were observed at the 30-minute mark. An increase in adhering cells was observed over time.





Control group: No cells were visible at 30 minutes.
At 60 minutes, a small number of actin filaments began to appear, and their spread increased over time.

Coat group: At 30 minutes, the spread was equivalent to or greater than that of the control group at 60 minutes. No significant changes were observed in the **coat group between 60 and 120 minutes, but at 180 minutes, a substantial spread of actin filaments was observed**.

Green : actin
Blue : DAPI

Three random locations on each disc were photographed, and DAPI was counted.

Many cells were consistently observed in the coat groups.

Result2

Differences in Adhesion and Proliferation Molecule Expression on FDC-SP-Coated Titanium

Green : Each heading
Blue: :DAPI

Focal Adhesion Kinase (FAK)

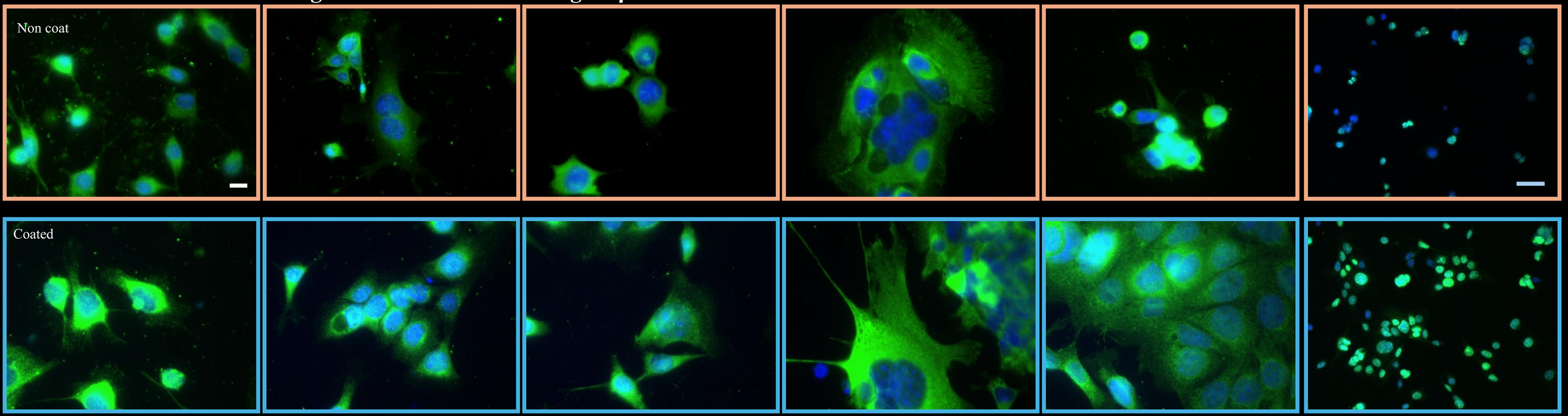
Integrin α6

Integrin β4

Vinculin

Paxillin

Ki67

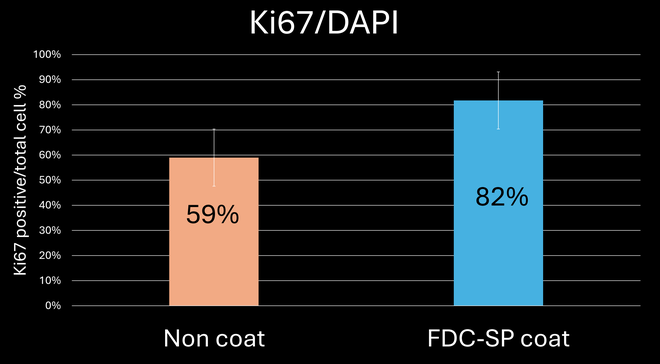


Method

3 hours after seeding, non-adherent cells were washed with PBS.
After washing, cells were cultured for 24 hours, fixed, and stained.
The white bar represents 10μm.
The light blue bar for Ki67 represents 50μm.

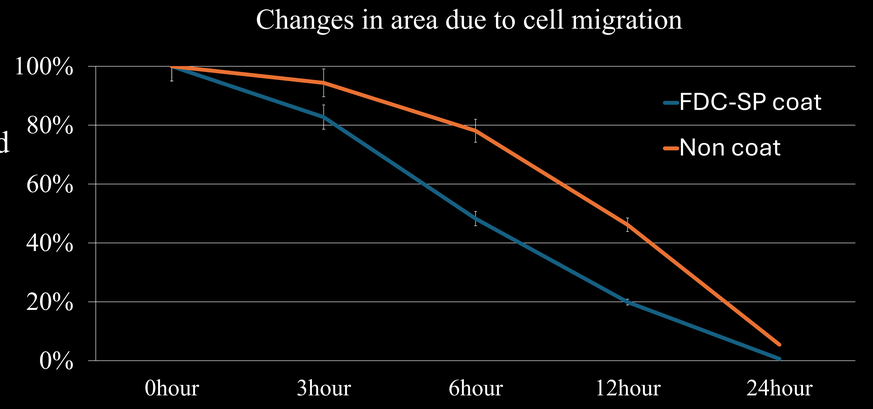
Result

FAK, integrin α6β4, vinculin, and paxillin showed strong expression in the coat group.
The Ki67-positive reaction also showed a significant increase in the coat group.



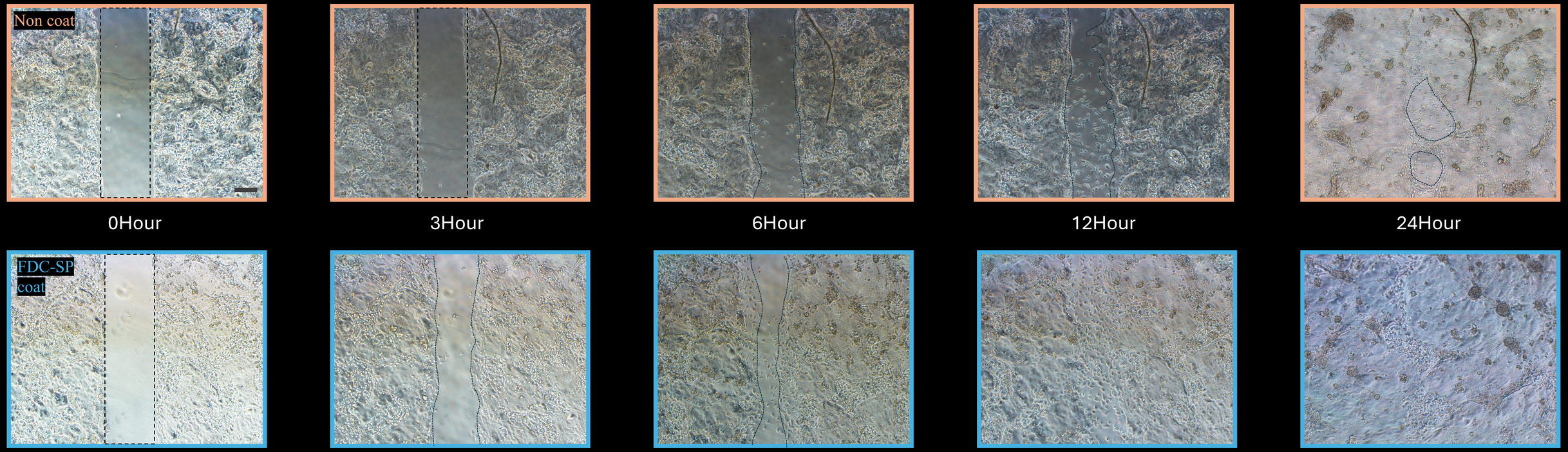
Evaluation of Migration Capacity Using FDC-SP Coating

Coat a 3.5cm plastic dish with FDC-SP.
After coating, dry the dish and place a silicone mold with 500μm gaps. Seed 70μL at a concentration of 4×10^5 cells/ml into the mold.
Culture until cells confluent within the mold (48 hours).
After mold removal, observe 5 random locations at 0h, 3h, 6h, 12h, and 24hMeasure area using the Wound Healing Size tool in ImageJ and average the values.
Set the area at 0h as 100% and consider complete wound closure as 0%.
The bar in the image represents 200 μm.



Result3

Both coated and uncoated surfaces approached 0% within 24 hours.
Images taken at 6 and 12 hours show cells migrating into the gaps on the coated surface, whereas the uncoated surface maintained the shape of the gaps with sparse cell migration.



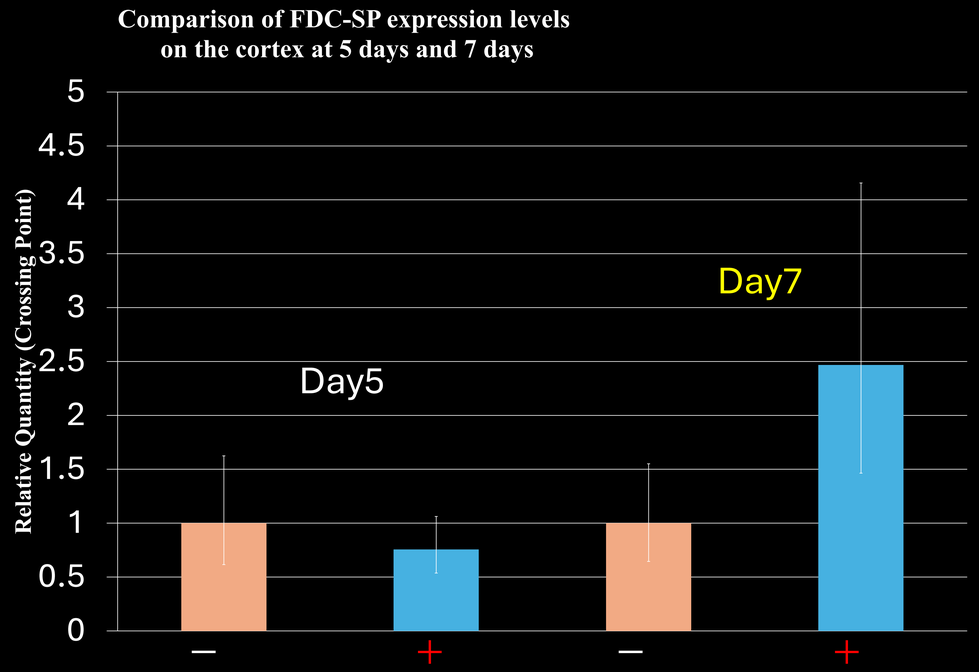
Evaluation of FDC-SP Expression via Long-Term Culture on the Surface

Metod

After coating titanium discs with FDC-SP, they were incubated at 4° C for 30 minutes and washed with PBS.mOE-T2 cells were seeded at 5 × 10^5 cells/ml. After 30 minutes, non-adherent cells were washed away with PBS, and adherent cells were analyzed by RT-qPCR at 5 and 7 days.

Result4

On day 5 of culture, FDC-SP expression was not detected in either the coated or uncoated groups, but by day 7, expression increased only in the coated group.



Conclusion

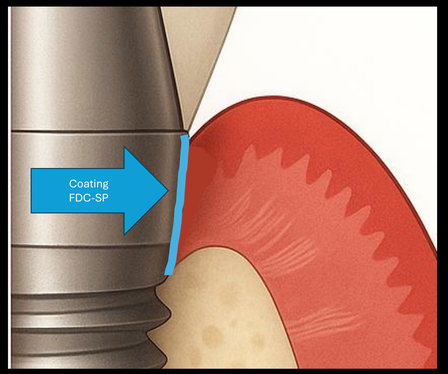
Coating with FDC-SP significantly enhanced the initial adhesion of oral mucosal epithelial cells to titanium. It was also demonstrated that adhesion-related proteins showed increased expression due to FDC-SP. Furthermore, enhanced cell growth capacity was observed. Cell migration ability was also observed to increase with FDC-SP coating. Furthermore, culturing on the coated surface for 7 days induced FDC-SP expression in oral mucosal epithelial cells, which normally do not express it.

Discussion

Based on these findings, it is thought that OE cells begin to secrete FDC-SP themselves by receiving FDC-SP from external sources, thereby enhancing adhesion and migration.

However, these studies have not yet elucidated the receptors involved or the pathways through which this phenomenon occurs. We aim to clarify these points in future research.

In the future, coating titanium abutments used at the implant mucosal penetration site with FDC-SP is expected to improve the attachment of peri-implant mucosal epithelium, thereby contributing to the prevention and treatment of peri-implant mucositis.



References
(1):Comparative analyses of the soft tissue interfaces around teeth and implants: Insights from a pre-clinical implant model Xue Yuan, Xibo Pei , Jinlong Chen , Yuan Zhao , John B Brunski , Jill A Helms