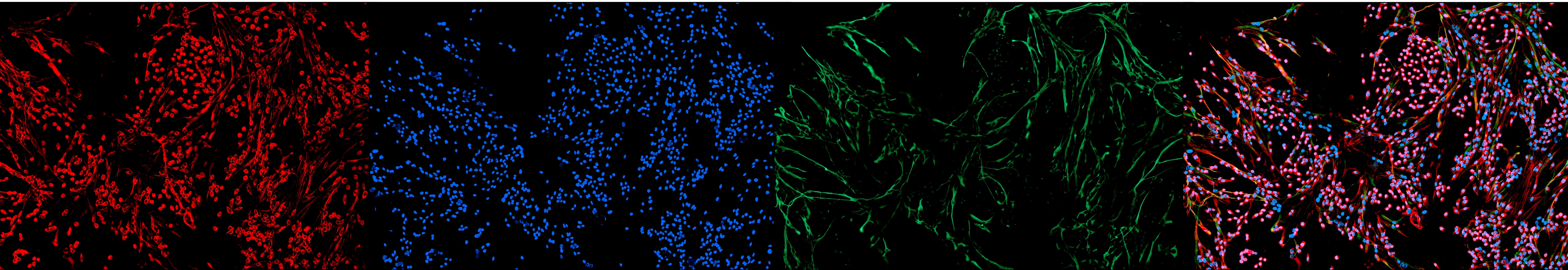


# Biocompatibility of Abutment Materials with Co-cultured Fibroblasts and Keratinocytes



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## Introduction

**W**ith nearly 2 million dental implants placed each year, and projected increases of 15% annually, implant supported restorations have been a well-accepted treatment modality to restore function and aesthetics with established early success rates (1).

**H**owever, the incidence of peri-implantitis has been steadily increasing, with estimated prevalence to be close to 22% with a range of 1-47% (1). Peri-implantitis is thought to be a bacterially induced inflammatory reaction that results in loss of implant supporting bone, which may eventually lead to implant failure. Soft tissue integration in the transmucosal zone of dental abutments creates a soft tissue seal against bacteria and preserves crestal bone level (2). The human peri-implant soft tissue (*i.e.*, oral mucosa) consists of oral keratinocytes (HOKs) and gingival fibroblasts (HGFs). HOKs comprise the stratified squamous epithelium of the periodontal tissues. HGFs are the major cell type found in the lamina propria connective tissue and play a major role in the healing and regeneration of periodontal tissue and in the soft tissue seal around the transmucosal part of dental implants (3).

**T**he surface properties of materials influence adhesion and differentiation of cells surrounding the implant abutment. Thus, the ability of different surface modifications of abutment materials to control the attachment and migration of HGFs and HOKs is advantageous (2).

**T**itanium (Ti) is the most widely used material in implant dentistry due to its established biocompatibility with peri-implant soft tissue. However, newer materials such as polymer type materials (polymethylmethacrylate [PMMA] and polyetheretherketone [PEEK]) and zirconia (Zr) have been of recent interest (4) due to their esthetic advantages and ease of chairside provisionalization (5).

**T**he aim of our study is to determine surface characteristics of abutment materials that may optimize adhesion of keratinocytes and fibroblasts, with the clinical goal of promoting early wound healing and peri-implant soft tissue stability. This study uses a co-culture system resembling the clinical environment of the peri-implant sulcus.

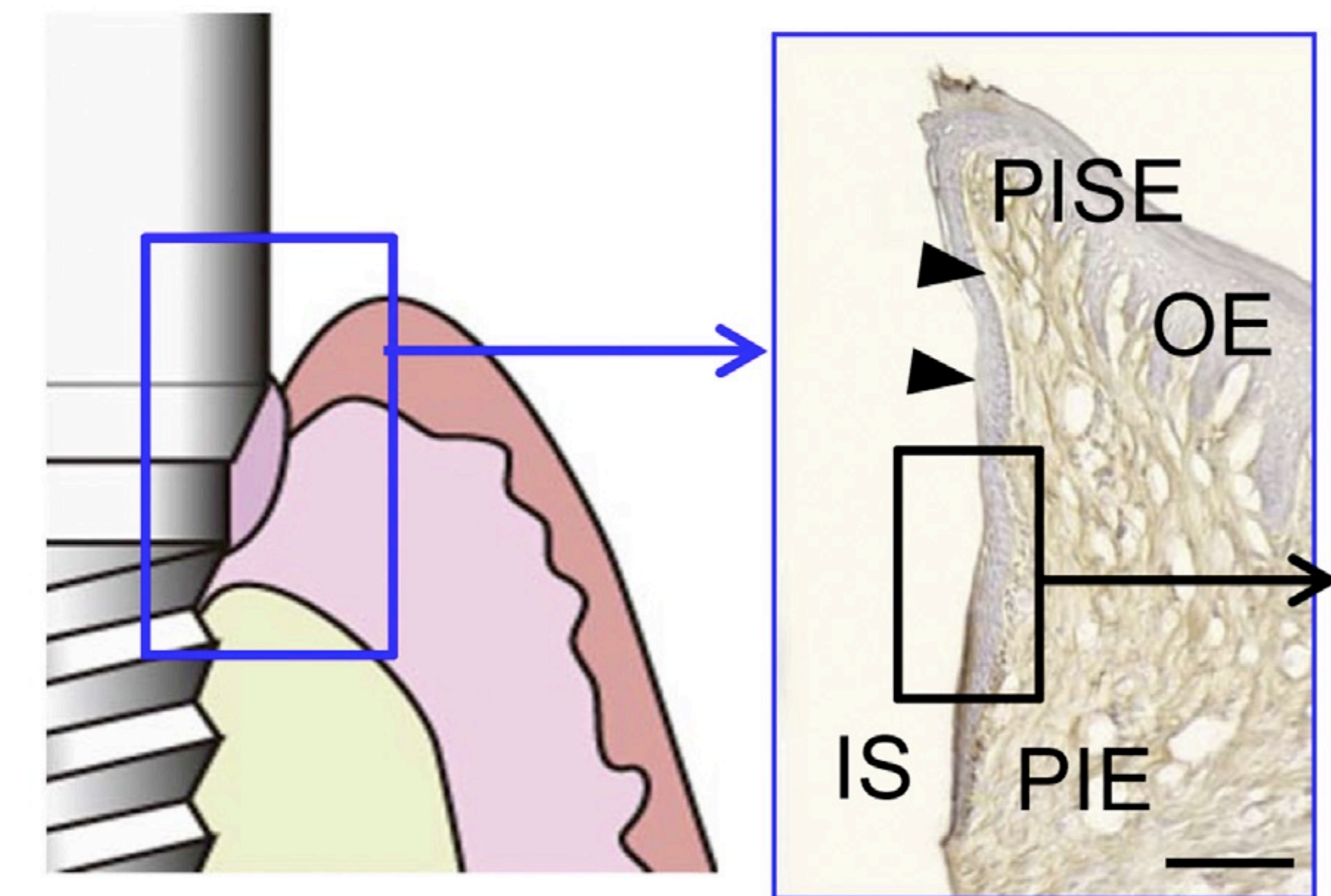


Fig.1 Soft tissue surrounding a dental implant, which has no PDL fibers, so there are only fibers attaching in a parallel manner to the implant's long axis (3).

## Materials and Methods

Six surface modifications from four abutment materials were tested: smooth titanium (TiS) as a control, rough titanium (TiR), CAD/CAM polymethylmethacrylate (PMMA), polyetheretherketone (PEEK), smooth zirconium (ZrS), and rough zirconium (ZrR). Of particular interest was to compare smooth versus rough surfaces of the same material to see if a particular surface roughness favors cellular attachment at this interface. Therefore, two different surfaces of titanium and zirconia were included.

Scanning Electron Microscope (SEM): [Fig. 3]

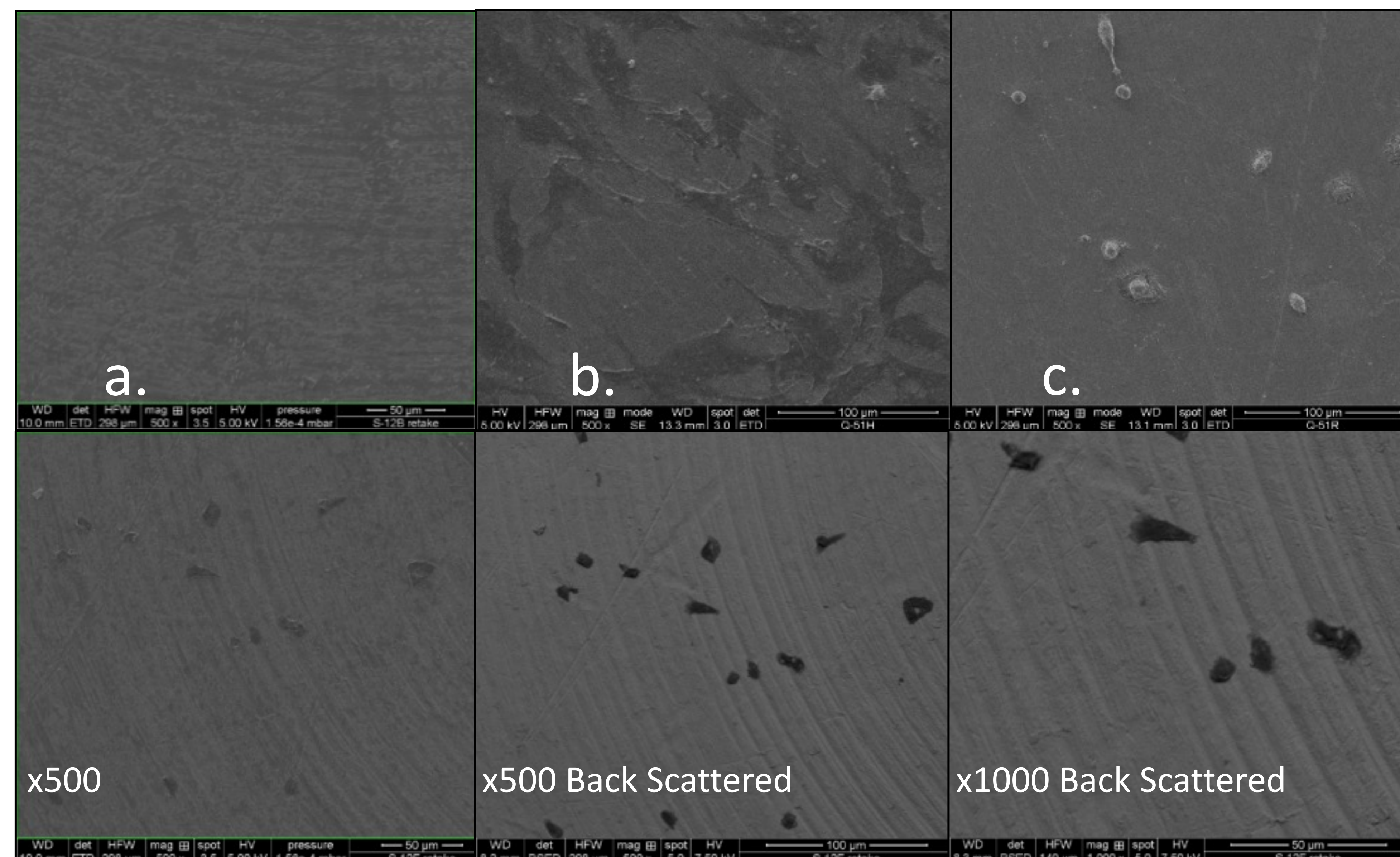


Fig.3 Examples of SEM images of disc surfaces after incubation in culture medium. Control disc (3a) with no cellular attachment. Disc with only HGF cells (3b). Disc with only HOK cells (3c).

Proliferation of co-cultured cells: Cell early attachment and growth curve were examined by Cell Counting Kit-8 (CCK-8). Proliferation of co-cultured cells at different time points was quantified using Click-iT assay [Fig 4].

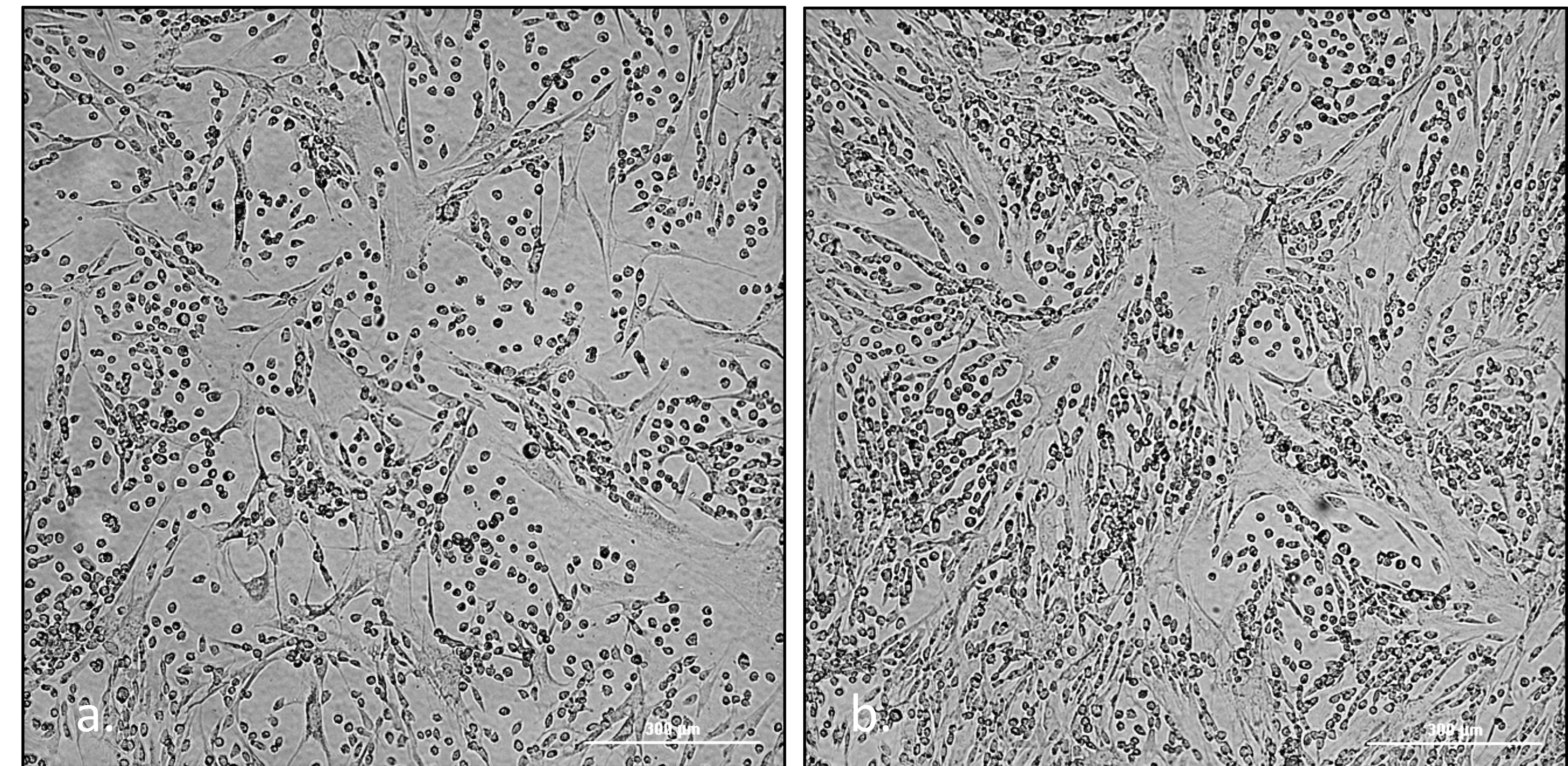


Fig.4 These cells have different morphology so they could be differentiated even in a co-culture environment. Different ratios of the epithelial cells to fibroblasts, as well as their confluence, were tested as part of the study. This image shows an example of what was seen microscopically after 24 (4a) and 48 (4b) hours with 100% confluence of co-cultured cells in a 1:1 ratio.

## Results

**Migration Assay:** A cell migration assay was used to examine the clinical goal of promoting early wound healing and peri-implant soft tissue stability. Figure 5 shows examples of the migration assay from time zero (5a) to 24 hours of cell migration (5b), which represents wound healing in a clinical environment.

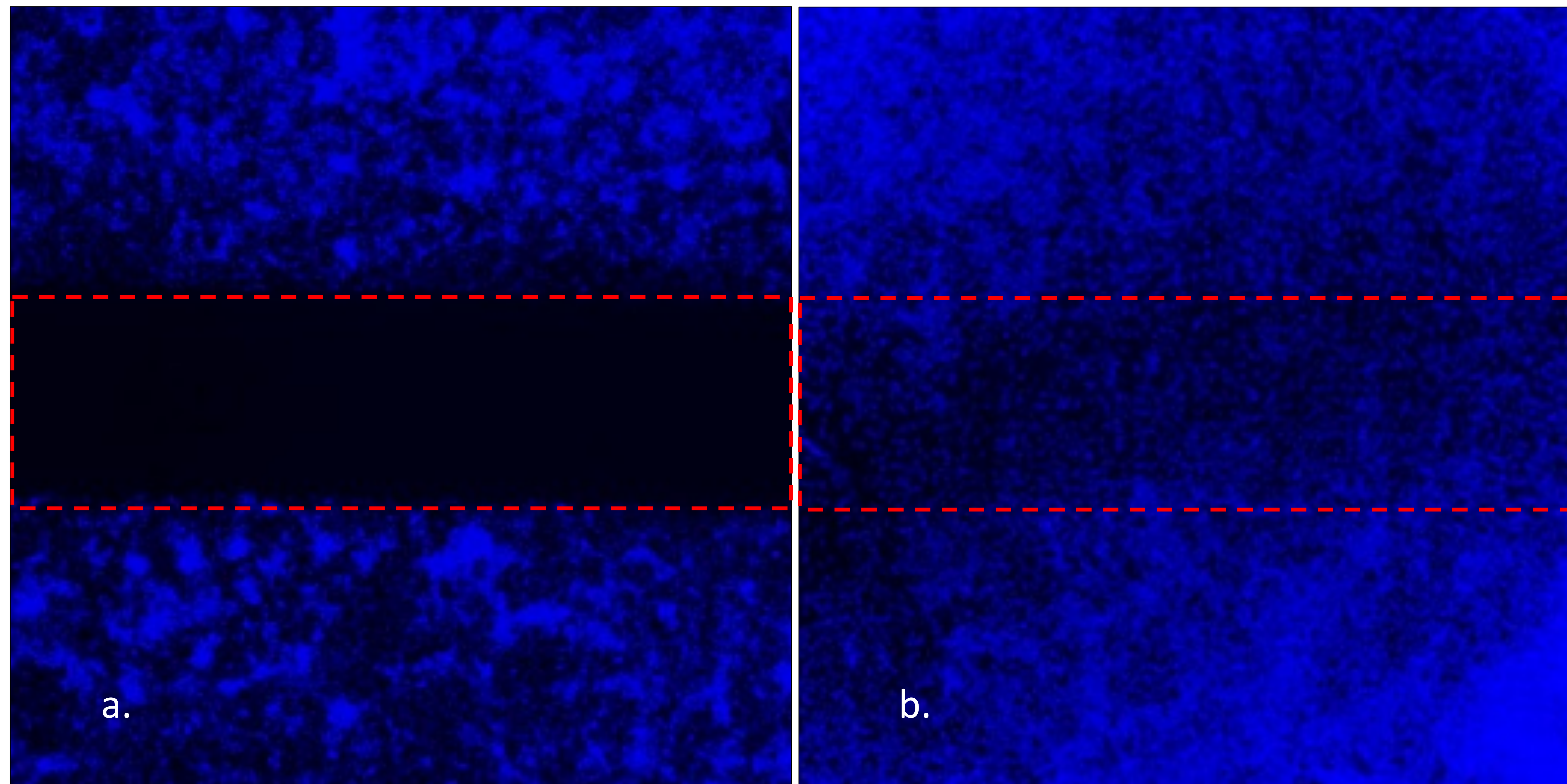


Fig.5 Example of the migration assay used in which cell migration was visible after 24 hours.

**PEEK** had the greatest cell proliferation with  $OD_{450} = 2.3618$ , while rough Zr was second with  $OD_{450} = 1.9402$ . Smooth Ti, rough Ti, and smooth Zr had comparable proliferation. With smooth Ti serving as a negative control, our findings would indicate that these materials may not have favorable surface topographies for cell proliferation [Fig 6].

### Coculture of HOK and HOF 48h

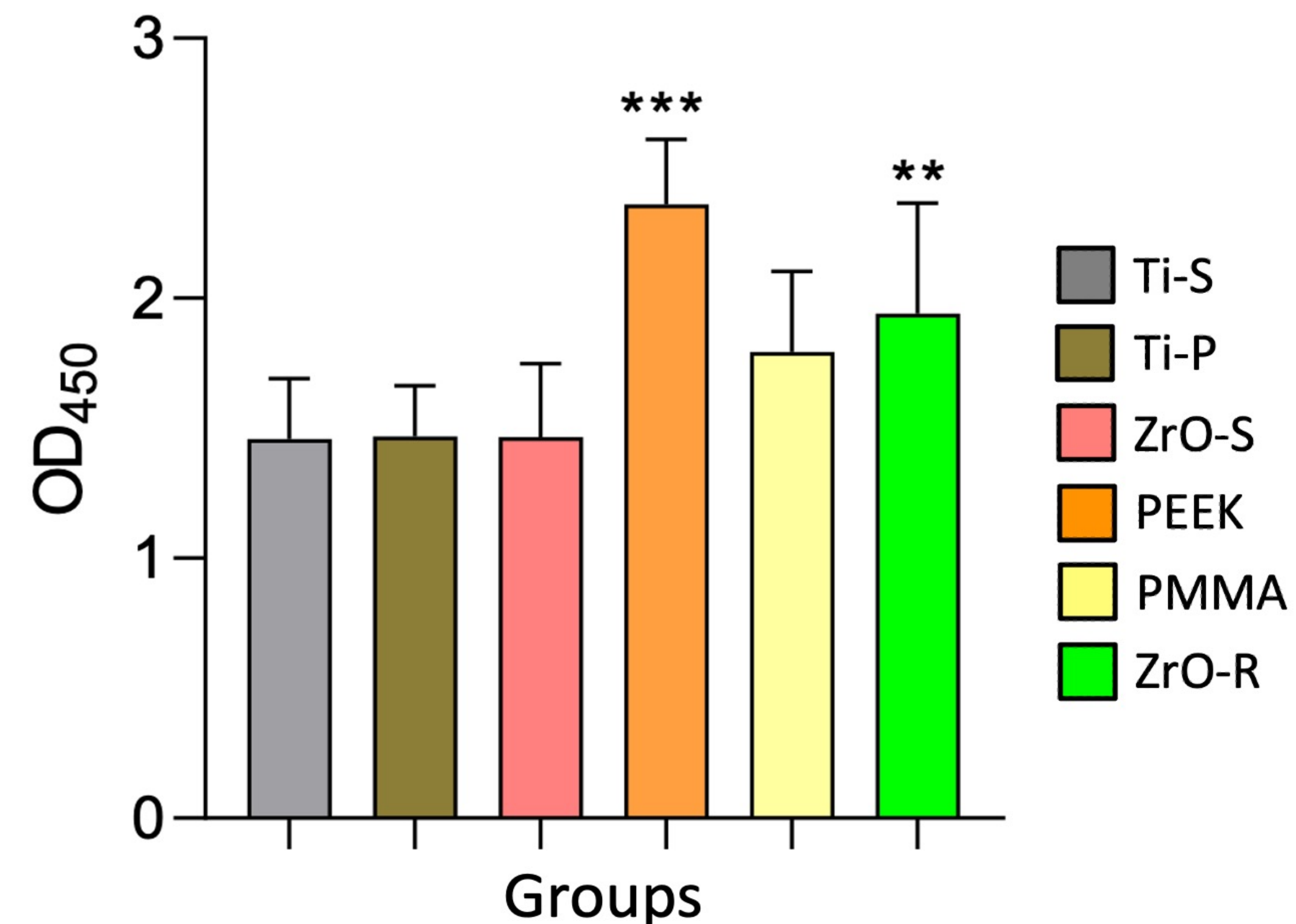


Fig.6 Preliminary results of cell proliferation after 48 hours using an absorbance microplate reader. Our findings show that PEEK had significantly greater proliferation than any other tested materials ( $P < 0.001$ ). Rough Zr had second greatest proliferation with statistical significance of  $P < 0.01$ . Thus, our results would indicate that these two materials may support greater compatibility with the co-culture system at this time point.

## Conclusions

The aim of this study was to simultaneously and directly compare cell attachment by using gingival fibroblasts and oral keratinocytes to evaluate the initial attachment and early cell growth on materials with different surface topographies. Based on our preliminary findings, PEEK could be considered a favorable material as a temporary abutment [Fig 7].

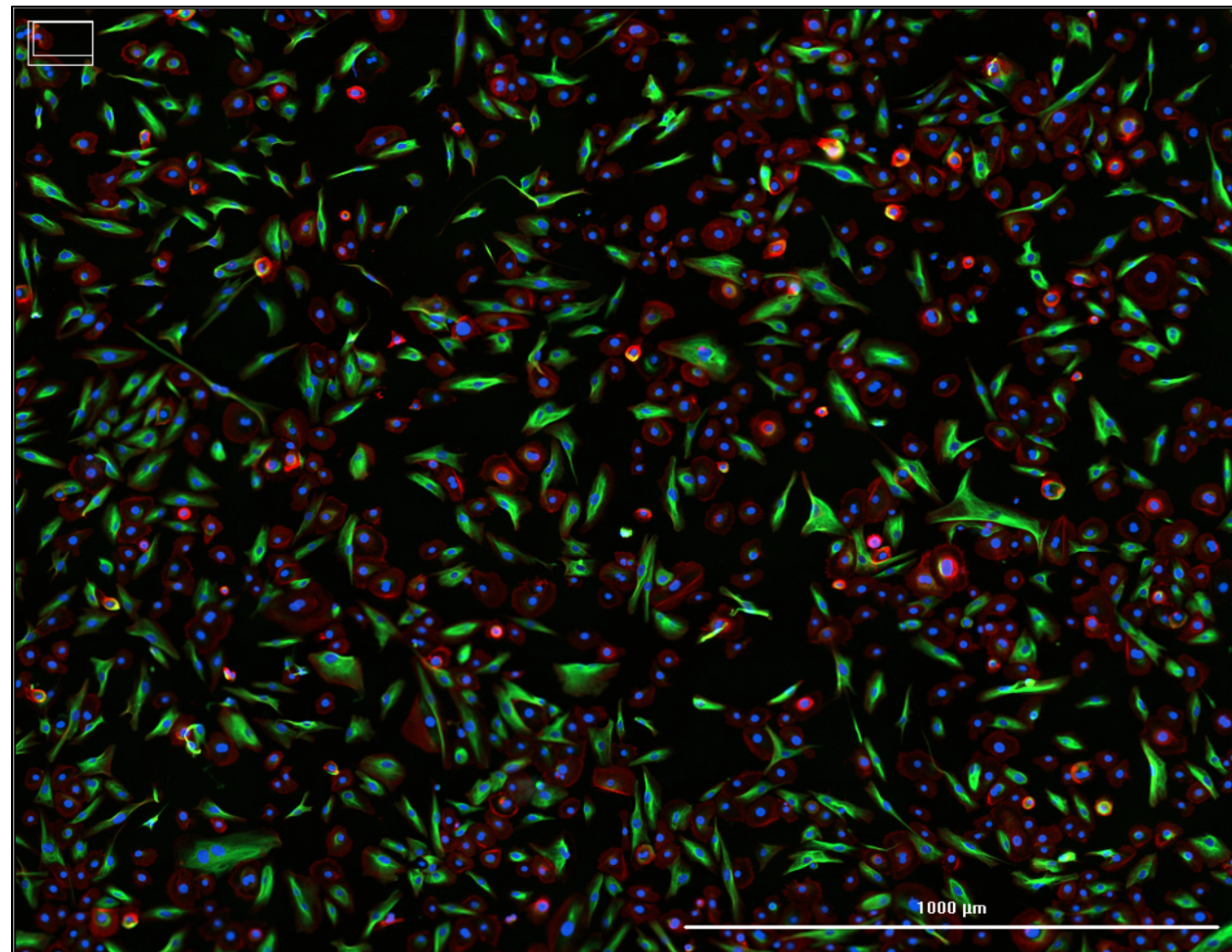


Fig.7 Superimposed image of a preliminary sample that was stained with actin, Hoechst, and vimentin that shows cytoskeleton, nuclei, and mesenchymal cells, respectively, using new cell lines. This image was obtained using human telomerase reverse transcriptase (hTERT) cells of immortalized gingival fibroblasts and gingival keratinocytes in a 3:1 ratio in 100% epithelial medium for 4 days.

## Future Direction

Our next steps include: 1. introducing a bacterial component to our experiments, which would more accurately represent the clinical environment, and 2. identifying the abutment materials that could enhance soft tissue integration by serving as a seal against bacterial invasion to help preserve bone level for long-term maintenance of dental implants [Fig 8 and 9].

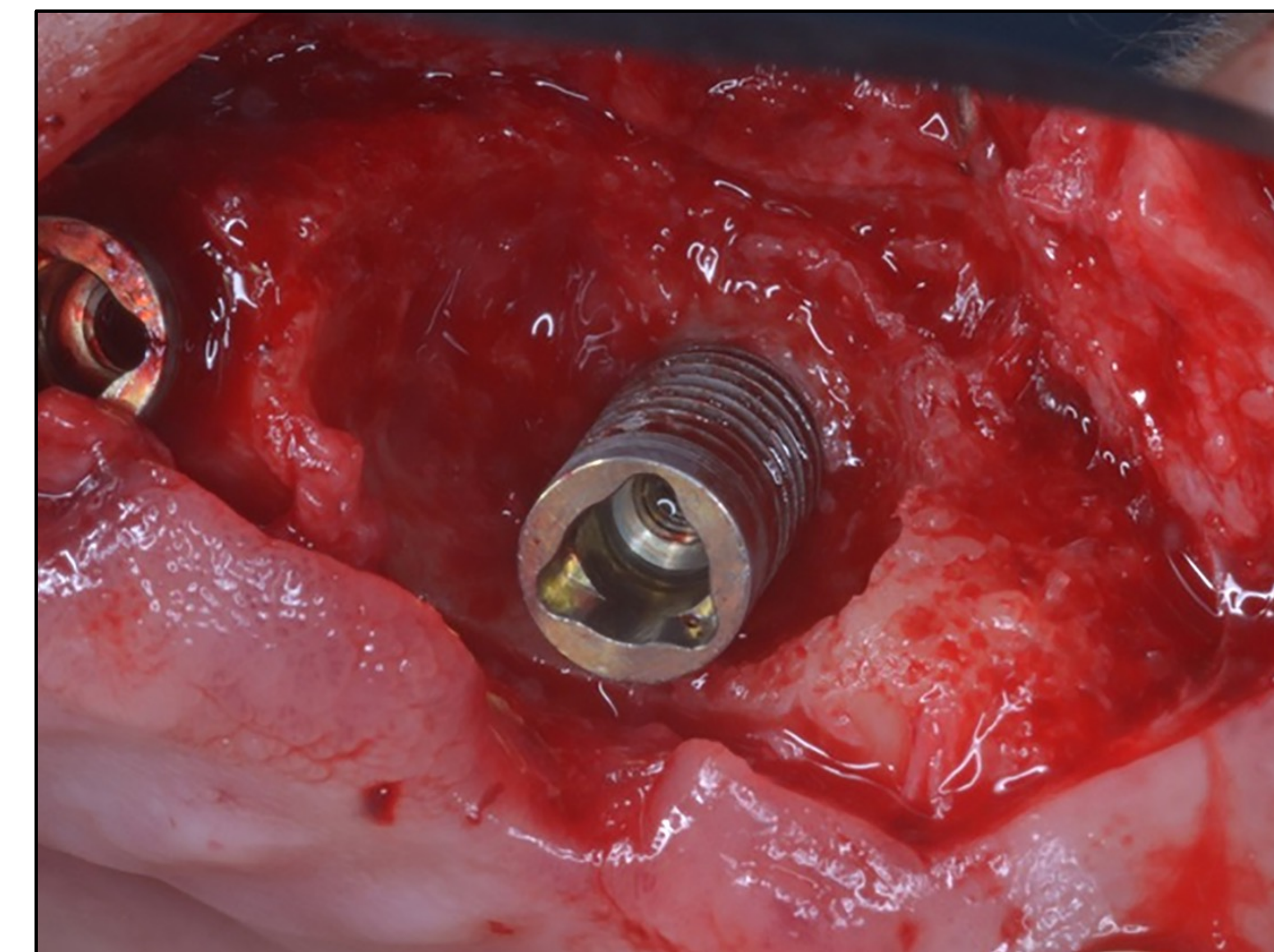


Figure 8: Implant affected by peri-implantitis, showing circumferential bone loss.

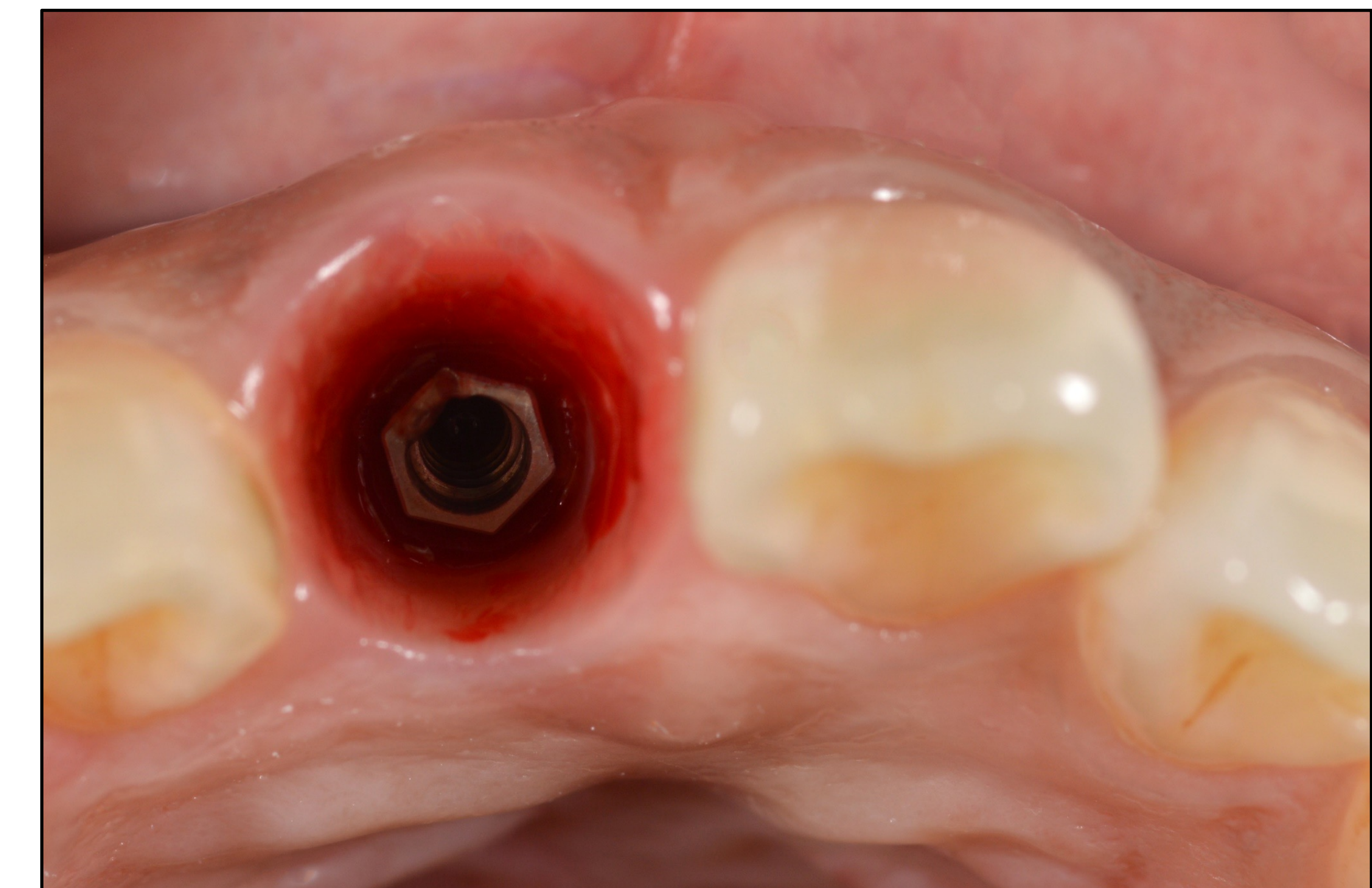


Figure 9: Stable peri-implant soft tissue at transmucosal level compared to contralateral natural tooth.

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