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# Comparison of Biocompatibility of Different Titanium Materials and Compositions with Oral Keratinocytes and Fibroblasts

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## Introduction

Achieving osseointegration has become a “minimum standard” for implant survivability (1, 2). Consequently, research focus has shifted towards the soft tissue interface between an implant and/or abutment and the surrounding soft tissue due to demand for improved long-term success.

The surface properties of implants influence adhesion and differentiation of cells surrounding implants[Fig 1]. Different modifications, such as generation of nano-topographies and surface roughness, have been used to improve osteoblast cell attachment and osseointegration of oral dental implants (3). However, creating a soft tissue attachment is essential for the health and longevity of the dental implants as its responsible for protecting the implant from a bacterial invasion, alongside improving esthetic results (1, 2, 4-6). Unlike osteoblasts, fibroblasts and other epithelial cells have reduced adhesion and spreading with these cells having a higher affinity for smooth or finely grooved surfaces.

It is possible that different modifications may end up with different surface characteristics (7). The purpose of this study is to compare different titanium (Ti) materials, ranging in material composition and texturization, with Human Gingival Fibroblasts (HGFs) and Human Oral Keratinocytes (HOKs) growth rates and proliferation in vitro.

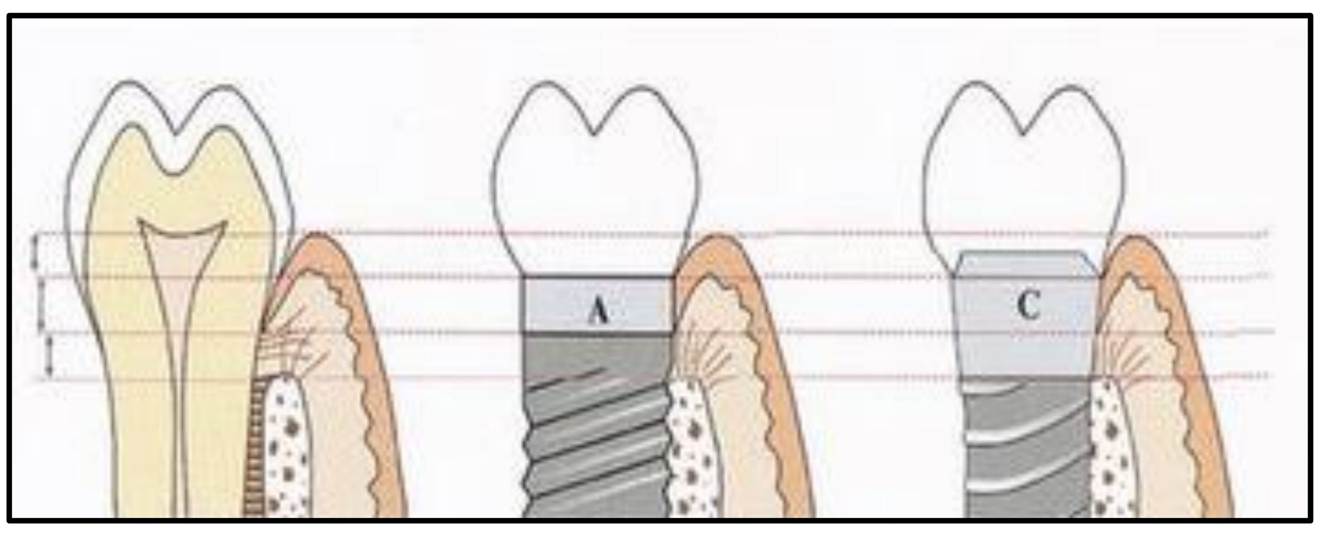


Figure 1. Anatomy of a natural tooth & an implant tooth

## Materials & Methods

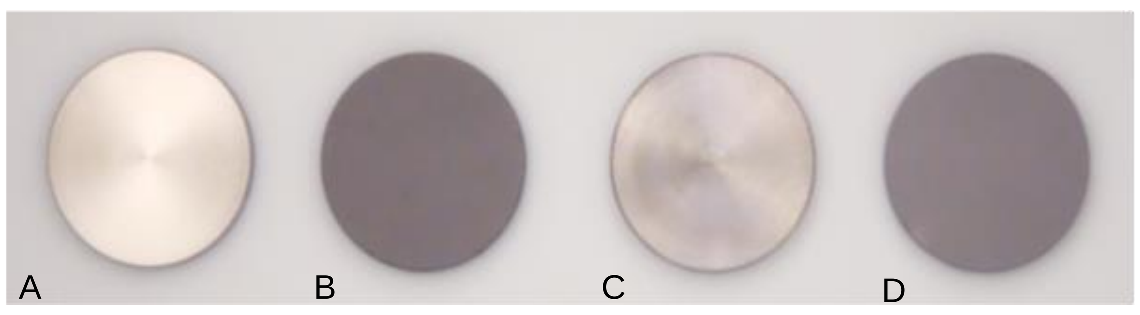


Figure 2 Tested discs with diameter at 14.5mm

Four different materials were tested:

A. Osseotite® (Commercially Pure [CP] Grade 4 Ti)

B. Machined alloy (titanium-6% Aluminum-4% Vanadium alloy [Ti-6AL-4V-Eli])

C. Osseotite® (CP Ti Grade 4 Ti)+Discrete Crystalline Deposition of CaP nanoparticles (DCD ®)

D. Machined CP Grade 4 Ti,

All materials were shaped into 14.5 mm wide and 1mm height discs.[Fig 2].

Human oral keratinocytes (Celprogen, HOK) and Human gingival fibroblasts (Innnoprot, HGF) were cultured according to manufacture Instructions [Fig 3].

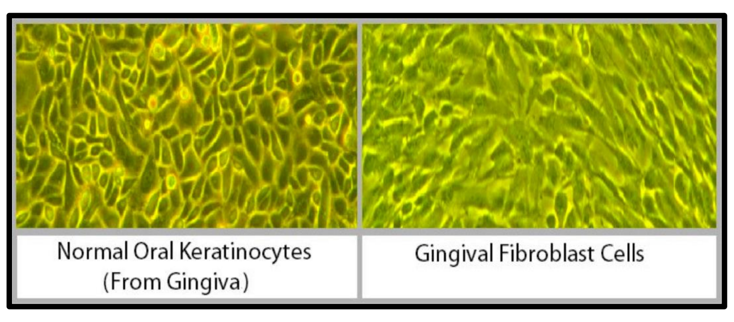


Figure 3

Early attachment and growth curve detection: cells were seeded at 2X10^4/cm^2 and Cell viability was examined by cell Counting Kit-8 (CCK-8) assay (Dojindo Cat# CK04-11) at indicated time points. Proliferation assay: Cell seeding density was 2X10^4/cm^2 and incubated for 5 days before test. Edu was added 4 hours ahead of fixation. Click-iT assay (Invitrogen, Cat# C10637) was conducted according to manufacture’s protocol. Wound healing assay: Idibi migration kit (Ibidi, Cat# 80241) was used for wound healing assay. 35,000 cells in 70ul were seeded for each chamber and incubated for 24 hours before fixed and stained with Hoechst3323 (nuclear staining).

Statistics: one-way ANOVA followed by Tukey’s multiple comparison test in GraphPad Prism 6 software program were used to analyze differences between experimental groups. P value <0.05 were considered statistically significant. Data are presented as mean ± S.E.M.

## Results

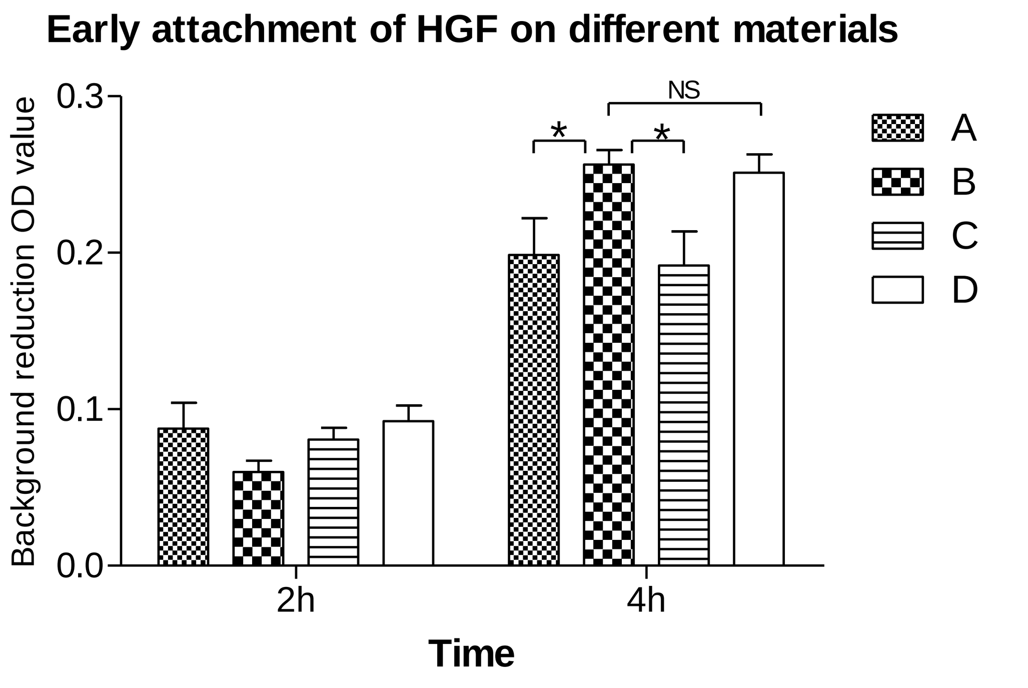


**Fig 4: Early attachment of HGF on tested materials.** No significance was detected among different materials at 2 hours. However, at the 4h interval more cells attached to discs B and D (P values); no significance was apparent between discs B and D but both discs did show more cell attachment then A and C; * *P*<0.05.

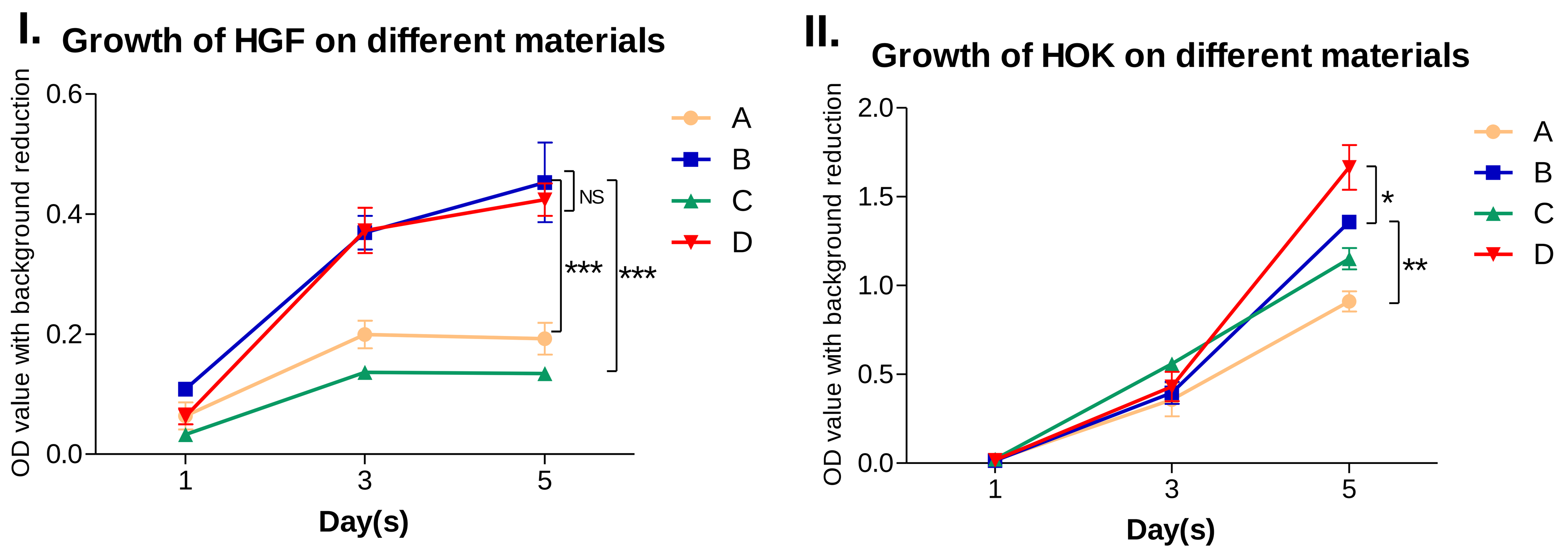


**Figure 5: Cell growth on tested materials.** HGF (I.) and HOK (II.) growth on different martials were evaluated by CCK8. The results showed that (a) in HGF, disc B and D had more cell growth at day 5 compared with disc A and C(P<0.001); but no significance was detected between B and D. (b) in HOK, disc D had the most cell growth, however, disc B is better than disc A (P<0.01) and no significance between disc B and C.

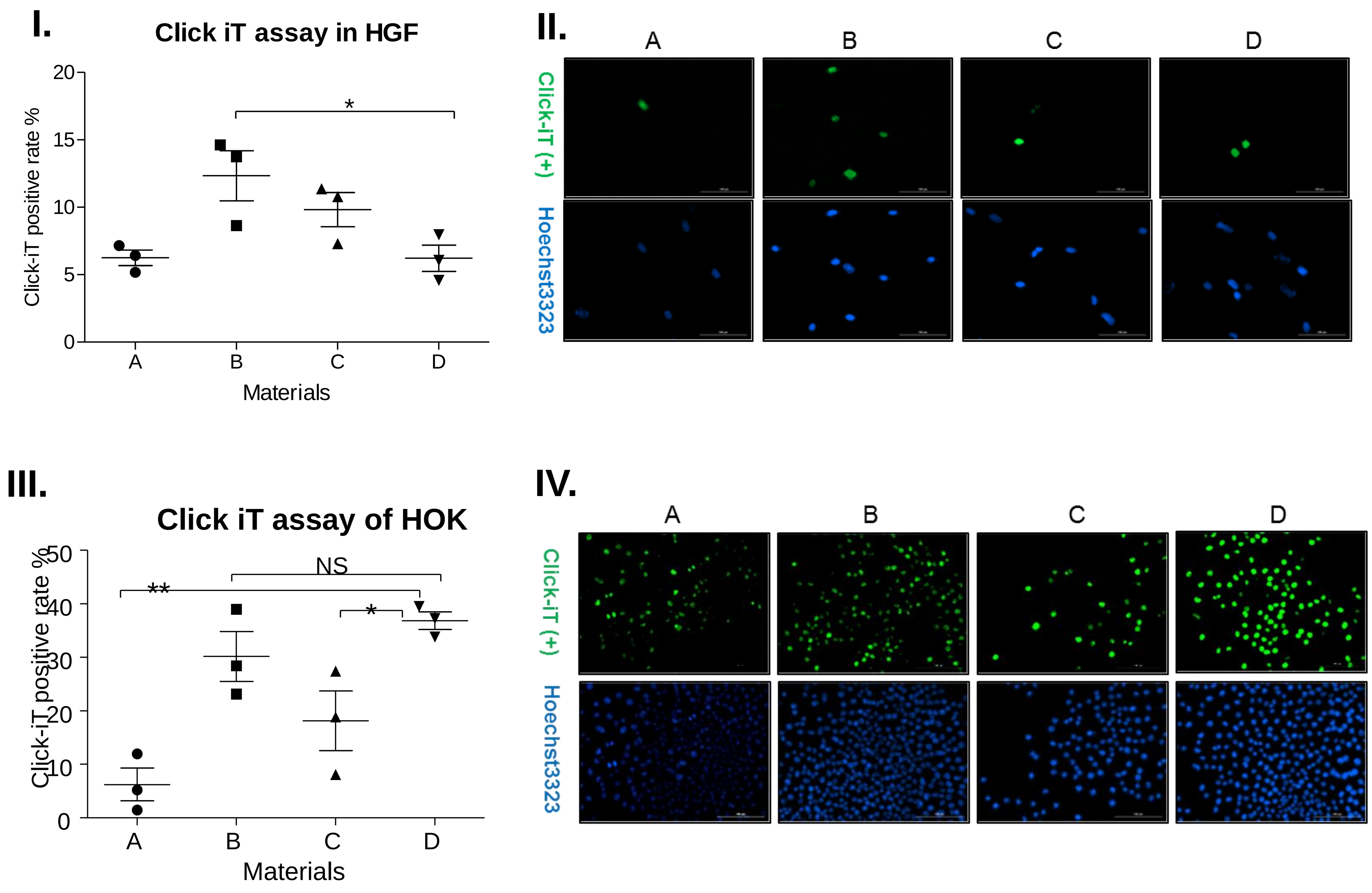


**Figure 6: Click-iT assay for HGFs and HOK.** HGF (I and II) and HOK (III. and IV) proliferation on different materials were examined by Click-iT assay. Click it positive (Green) staining indicated proliferation positive cells. Proliferation rate% was calculated by # Click-iT positive X100/# Hoechst3323. Disc B had more proliferation rate than Disc D in HGF. However, Disc B has similar proliferation rate with Disc D in HOK (no significance).

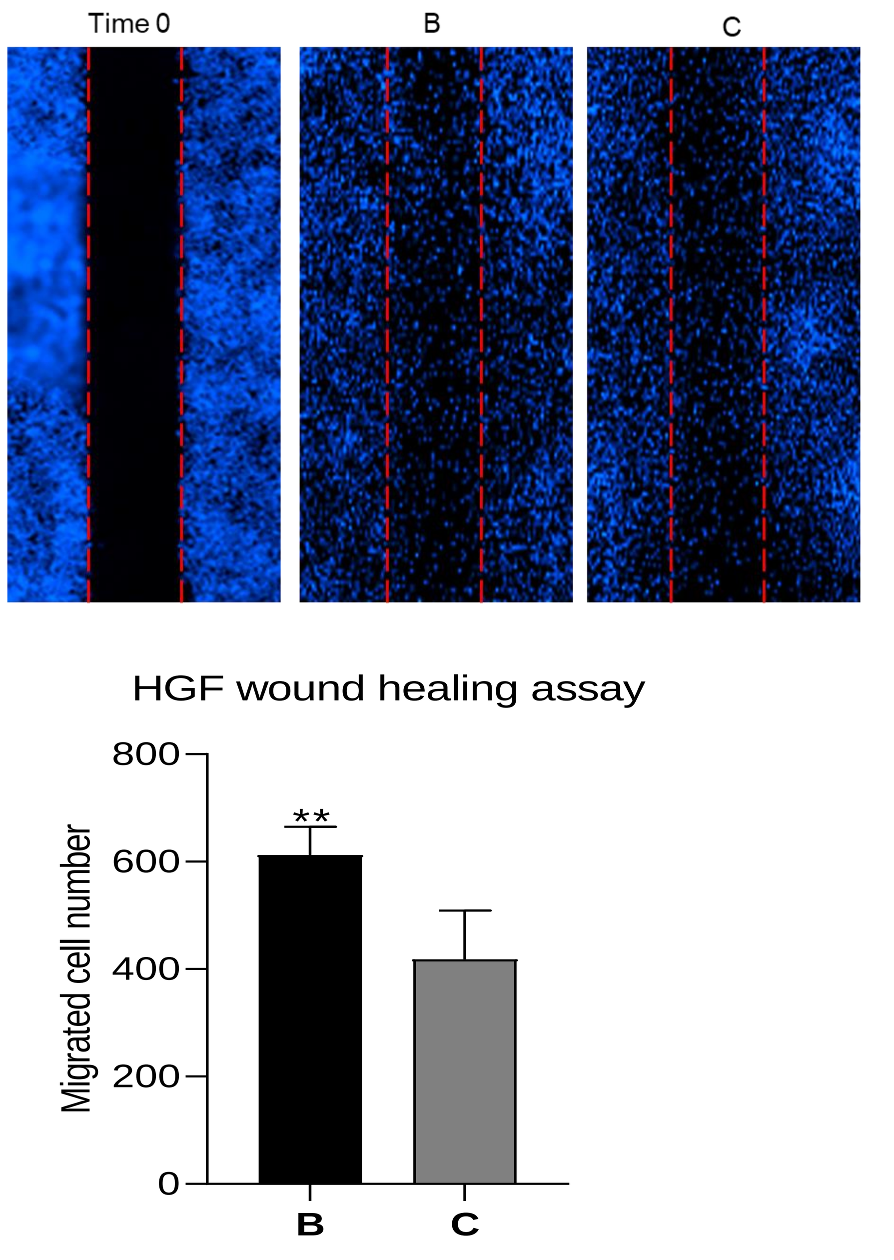


**Figure 7: Wound healing assay for HGFs after 18h.** Time 0 was defined as the insert removal. All other samples were incubated for 18h before cell fixation with hoechst3323 staining to check cell migration. Each blue dot stands for single cell nuclear. Area included by red dash line indicates gap for cell migration. Disc B appear more efficient for cell migration compared to Discs C. Further quantification is needed for confirmation.

**The results showed : (1) more cells attached to disc B and D at 4h in HGF (No significant difference was found in HOK early attachment ); (2) growth of HGF on discs B and D (no significance between both) were better than disc A and C. However, disc D was ideal for growth of HOK; (3) best proliferation of HGF was found in disc B while for proliferation in HOK, no significant different between B and D; (4) According to HGF early attachment and growth pattern, Disc B (D) had a better biocompatibility of HGF than disc C (A). Our pre-test of wound healing confirmed that Disc B provided a faster surface of wound healing.**

## Discussion

The results from this experiment showed better affinity of Machined CP Grade 4 Ti (D) and Machined alloy (Titanium-6% Al-4V; B) with human oral keratinocytes and human gingival fibroblasts compared to Grade 4 Ti Osseotite (A) and grade 4 Ti Osseotite with DCD (C).

An ideal abutment should allow for rapid fibroblast and epithelial cell proliferation and attachment. The surface topography has a strong influence on the soft-tissue cell behavior, whereas fibroblasts initially appears to adhere better on smooth substrate surfaces but show rapid proliferation on rough surfaces, epithelial cells favor smooth surfaces for both adhesion and proliferation (8). As indicated from the early attachment, growth curve, and proliferation data fibroblast cells showed increased affinity and proliferation on smooth surfaces.

Although further optimization of material modification is needed, validating data with repeated experimentation and increased sample size should be recommended as continuation of this project and preclinical study with animal model for implant material test is suggested.

Conclusion: among tested materials, machined alloy Ti-6AL-4V-Eli has the best biocompatibility of fibroblast.

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