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A preliminary Study of Osseointegration in the Dental Implant Therapy *in vitro* -Culture of Mouse KUSA/A1 Cells on Titanium Plates with different Surface Modifications

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Abstract

To sustain an extensive osseointegration with optimal bone-to-implant contact (BIC) is one of the essentials for a successful oral implant therapy. In the present in vitro study, we have preliminarily elucidated that KUSA/A1 cells actively proliferated and have differentiated into osteocalcin-producing osteoblast-like cells (Obs) since day 3 of culture. We then cultured the Obs on titanium (Ti) and Ti alloy plates (discs), which have commercially available surface texture and modifications (i.e., anodic oxidization: AO, hydroxyapatite coating: HA, precision blasting: SPI) as same as the current dental implants. The histology of proliferating Obs cultured on discs (Disc-AO, Disc-HA and Disc-SPI; for 180 minutes) was investigated to study the microenvironment mimic initial osseointegration of the dental implant therapy. The present SEM of 60-min Disc-AO, Disc-HA and Disc-SPI specimens demonstrated a loose distribution of spherical-to-ovoid (10 μm in diameter, avg.) Obs migrating towards concavities of the substrate. The proliferating Obs had the bulging cell body sending out many minute filopodia and some lamellipodia to attach with the substrate; few Obs with intercellular junctions were dispersedly found. In contrast, in the 180-min Disc-AO, Disc-HA and Disc-SPI specimens, we observed the cultured KUSA/A1 cells actively proliferated and spread into flat, large polygonal (20 μm X 30 μm, approx.) Obs with several prominent lamellipodia and many dendritic filopodia to employ cell-to-disc and intercellular attachments; a few scattered small spherical-to-spindle shaped Obs were also observed interposing the large polymorphic Obs. On the other hand, the present immunohistochemistry of the attached Obs demonstrated the expression of F-actin (actin filaments of the cytoskeleton) in both 60-min and 180-min specimens, and CD51 (α V integrin) only in 180-min specimens. Hence, the present study revealed that the condition-induced KUSA/A1 cells actively proliferated and extensively adhered on different substrates yet showed similar histology. We confirmed that the present GBR engineered KUSA/A1 cells to obtain extensive cell attachment on different microtextured Ti discs within 180 min, thereby we surmised that the Obs might be further regulated to precipitate mineralization matrix for contact and distant osseointegration.

Key Words: Cell attachment, Titanium plate, KUSA/A1 cell, *in vitro*

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Introduction

Several previous studies on the dental implantology have elucidated that surface modifications with different microtextures of the implant fixture not only increased direct bone-to-implant contact (BIC) compatibility, but also acquired primary stability for immediate load bearing (Grösner-Schreiber & Tuan, 1991; Nasatzky *et al*., 2003; Di Iorio *et al*., 2005). Other studies have also demonstrated that regulation of vital reactions and consequent modeling and remodeling at the BIC interfaces might rapidly organize functional osseointegration to obtain a long-term stability of implants for the occlusal rehabilitation therapy (Grösner-Schreiber & Tuan, 1991; Groessner -Schreiber & Tuan, 1992; Swart *et al*., 1992; Davies, 1996; Chairy *et al*., 1997; Ong *et al*., 1998; Cochran, 1999; Radnal *et al*., 2000; Ong *et al*., 2001; Nasatzky *et al*., 2003; Di Iorio D *et al*., 2005; Marco *et al*, 2005; Joos *et al*., 2006; Salido *et al*., 2007; Graf *et al*., 2008; Inoue, 2008; Coelho *et al*., 2009).

Previously, we have isolated and cultured mesenchyme (e.g., dental pulp and bone marrow) - derived cells, which were engineered to activate certain mechanisms to initiate hard tissue matrix deposition in the monolayer cultures based on the guided bone regeneration (GBR) concept (Aikawa &Iwai, 2008; Kumabe et al., 2008). On the other hand, there been many studies have reported that the immature (non-inducing) KUSA/A1 (JCRB1119; Health Science Research Resources Bank (HSRRB), JPN Health Sciences Foundation, Tokyo, Japan) cells differentiated into bone matrix-producing cells exhibiting mature osteoblast phenotype within two or three days of culture in vivo (Ochi et al ., 2002; Rodriguez et al., 2007). The present in vitro study is an attempt to evaluate cell attachment and extension of cultured mouse KUSA/A1 cells on Ti discs having similar primary and secondary modifications of prevalent dental implants, therefore aims at the development of a host GBR microenvironment, which is beneficial to acquire hard tissue formation for osseointegration to ensure an optimal direct BIC of the dental implant therapy.

Materials and Methods

Preliminary study of the KUSA/A1 cell

Immature KUSA/A1 bone marrow-derived mesenchymal cells obtained from C_3H/He mouse cells (JCRB1119) in non-induction condition were distributed through the JCRB/HSRRB (Osaka, Japan). The immature osteogeneic linage cells were filtrated, centrifuged, cultured and incubated in Dulbeco's Modified Eagle's Medium (DMEM; Nacalai Tesque, Inc., Kyoto, Japan) supplemented with 20% FBS (HyClone, Logan, UT, USA) and 1% antibiotic-antimycotic agent: 125 μg/ml penicillin (Nacalai Tesque) and 200 μg/ml streptomycin (Nacalai Tesque) in cell culture 75 cm2 flasks (TPP, Switzerland) at 37℃ in humid air with 5% CO2 for 72 hours (Umezawa et al., 1992; Ochi et al., 2002; Rodriguez et al., 2007).

The immature KUSA/A1 cells (isolated with 0.25% trypsin in 1 mM EDTA soln; Nacalai Tesque) were monolayer and 3-D (Cellmatrix Type I-A; Nitta Gelatin, Osaka, Japn) cultured in the DMEM added with ascorbic acid (AA; 50 μg/ml; Sigma-Aldrich, MO, USA) and glycerol 2-phosphate disodium salt hydrate (*β*-GP; 10mM; Sigma-Aldrich) in 10% FBS for 72 hours. Subsequently, the histology, proliferation rate and differentiation of the monolayer and 3-D cultured cells were assayed and compared with PicoGreen dsDNA Quantitation Kit (Invitrogen; excitation wave length: 485 nm, emission wave length: 525 nm), Alkaline Phosphatase Substrate Kit (Bio-Rad Labs., CA, USA) and the Calcium E-test-Wako (Wako, Osaka, Japan). The intensity of visible light (wave length: 405nm) emitted by the specimens was summarized and scored using a Softmax Pro5 (Molecular Devices, CA, USA). ALPase activity expression was corrected for the total protein content (Bradford method using BSA as a standard) by the DNA amount, and was expressed as μmol/μg DNA/min (Figure 1).

Tissue engineering of KUSA/A1 cells on discs with different surface modifications

Plates (d=1cm, h=1mm; disc type; Japan Medical Materials (JMM) Physio Odontrum Implant (POI) system; JMM, Osaka) of primarily blasted-type titanium alloy (Ti-6Al-4V) those were secondarily processed with anodic oxidization (Disc-AO) or coated with hydroxyapatite (HA= $Ca_{10}(PO_4)_6(OH)_2$, 20 μm approx; Disc-HA) and JIS type 4 (99% Ti) plates (d=1cm, h=1mm; disc type; Thommen medical/ Morita, Osaka, Japan; SPI) finely blasted with Al_2O_3 sands (Disc-SPI) were supplied for the present in vitro study.

The isolated immature KUSA/A1 cells were seeded and cultured with the discs in the DMEM and exposed to AA, *β*-GP and dexametazone (100 nm) at 1.0X105 cells/ml concentration in 12-well Petri dishes (IWAKI, Tokyo, Japan), thereby the cells were induced to differentiate into mature osteogeneic cells in a humid 5% CO_2 at 37℃ (Ochi et al., 2002; Rodriguez et al., 2007).

The histology of KUSA/A1 cells cultured on plates

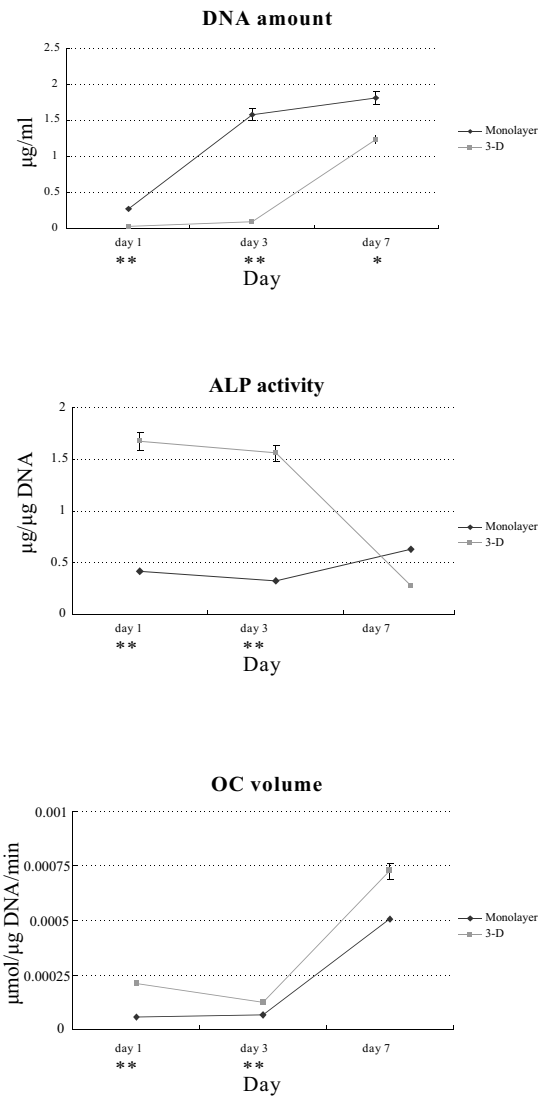
1. Scanning electron microscopy (SEM) Other 60-min and 180-min specimens were prefixed with 1% paraformaldehyde and postfixed with 1% osmium tetroxide solns., washed and atmosphere-dried, mounted on aluminum stubs and coated with gold in an Eiko IB-3 ion coater (Eiko engineering, Mito, Japan) and then prepared for the SEM. Conventional secondary electron image SEM of the specimens was examined and photographed under a Hitachi H-4000 field-emission SEM (accelerating voltage: 10 kV; Hitachi, Tokyo, Japan).
2. Immunochemical histology of KUSA/A1

cells on different surface modifications The 60- and 180-min specimens were immerse-fixed with 1% glutaraldehyde, and immunochemically stained with primary Ab: anti-CD51(αv integrin; Enzo Life Science, PA, USA) and secondary Abs: Alexa Fluor 546 goat anti-rabbit IgG (Molecular probes, OR, USA), and subsequently stained with Alexa Fluor 488 Rhodamine phalloidin (Molecular probes) following the conventional methods. Therefore, the expression of CD51 and F-actin were examined, for evaluation of cell attachment and extension of the differentiating KUSA/A1 cells distributed on the discs subject to different surface modifications, with a BIOREVO BZ-9000 fluorescence microscopy (Keyence, Tokyo, Japan).

Results

Properties of the inducing-conditioned KUSA/A1 cells

The properties of proliferative KUSA/A1 cells were preliminarily examined by phase-contrast microscope and analysis of DNA amount, ALPase activity and osteocalcin (OC)amount in the extracellular matrix (ECM). The results indicated that the KUSA/A1 cells in inducing-condition were actively proliferated and have differentiated into OC-secreting mature Obs since day 3 of culture (Figure 1).



DNA amount: proliferation
ALP activity: differentiation
OC (osteocalcin): mineralization
*p< 0.05
**p< 0.01

Fig. 1

Assay of the osteoinductive property of the KUSA/A1 cell. The data indicate that KUSA/A1 cells proliferate and have differentiated into mature osteoblasts since day 3 of culture.

Fine structure of the engineered KUSA/A1 cells

SEM of 60-min specimens (Disc-AO, Disc-HA and Disc-SPI) demonstrated a loose distribution of many spherical - to - ovoid (10 μm in diameter (d), avg.) differentiating Obs on the discs of different surface modifications (Figure 2). The spherical proliferating cells had a bulging cell body, the cell membrane densely distributed with many short, thin finger-like projections (filopodia), and also sent out some long-and-thicker cell processes (lamellipodia). Anchorage of the filopodia and lamellipodia onto peaks of the uneven modified Ti surfaces was observed; few filopodia and lamellipodia employed intercellular junctions. In contrast, in the 180-min specimens we observed that the KUSA/A1 Obs actively proliferated and differentiated into multilayered fully-spreading (to all sides) polygonal (20 μm X 30 μm, approx.) cells, which were obviously flattened sending out several prominent long-and-broad extensions (lamellipodia) and many filopodia to provide for cell-to-disc and intercellular attachments; scattered small spherical-to-spindle shaped Obs were also observed between the flat Obs (Figure 3).

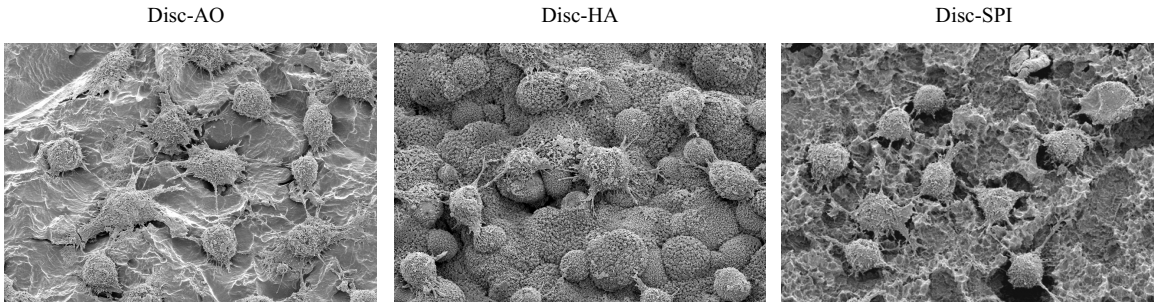


Fig. 2 SEM showing KUSA/A1 cells cultured with Disc-AO (2a), Disc-HA (2b) and Disc-SPI (2c) for 60 minutes (min). The cells migrate and attach with the substrate with extending cell processes.

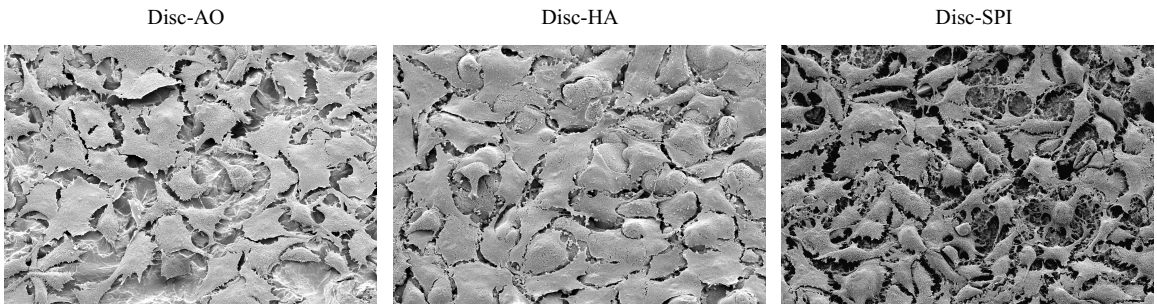


Fig. 3 KUSA/A1 cells cultured with Disc-AO (3a), Disc-HA (3b) and Disc-SPI (3c) for 180min. The cells differentiate into flat, polymorphic fully-spreading polygonal cells, which send out many broad lamellipodia, long-and-thin fillopodia and dendrites.

The histology of the GBR-engineered KUSA/A1 Obs attached on the 60- and 180-min Ti discs with different modifications were described as the following:

1. In the 60-min Disc-AO specimens, the anodic-oxidized Ti surface was almost smeared with a flat and smooth layer distributed with many irregular and weak wrinkles, and showed many shallow depressions with holes (i.e., pores of the porous Disc-AO) in the center. Many spherical-to-ovoid (d= 10-15 μm, approx.) Obs with a bulging cell body were found laid in the pores; they sent out some long lamellipodia and many thin filopodia to merge with the smeared surface at the margin of the depressions (i.e., focal adhesions/contacts) and for intercellular junctions (Figure 2a).

   In contrast, in the 180-min Disc-AO specimens we distinctly observed an increase in the number of Obs on the smear layer. Most of the proliferating Obs were large, flat (plate-like) and polygonal (20 μm X 30 μm approx.; polymorphic) fully-spreading cells, which extent several thin filopodia and broad lamellipodia for intercellular junctions and focal contacts with the porous Disc-AO surface; however, some interposing small spindle/ polymorphic cells were also observed (Figure 3a).

2. In the 60-min Disc-HA specimens, the coral reef-like surface was paved with many small and large hemispherical cauliflower-like elevations containing many thin needle-like crystalline structures (Figure 2b). Spherical (d= 10 μm, avg.) Obs settled among the hemispheres and sent out many tapering cellular processes of different thickness and length (extending filopodia and lamellipodia) towards the gravelly HA surface; the cell density seemed being slightly less than the Disc-AO and Disc-SPI specimens (Figure 2a & 2c).

   On the contrary, in the 180-min Disc-HA specimens, we observed that the Obs were differentiating into many flat, large and polygonal (20 μm X 30 μm approx.) Obs interposed with small polymorphic Obs. Hence, the flattened Obs provided cell-stratum focal contacts with broad the spreading lamellipodia, thinner filopodia and their dendritic cellular processes (Figure 3b).

3. In the 60-min Disc-SPI specimens, the finely blasted pure Ti had its surface microroughed showing many lacunae, small and large concavities. The spherical Obs (d= 10 μm, avg.) situated at the lacunae and sent out many long cellular processes of different thickness and length towards the spikes/peaks of the rough surface and for intercellular junctions with adjacent Obs (Figure 2c).

   In contrast, distribution of many large-and-polygonal (20 μm X 30 μm approx.) Obs was distinctly demonstrated in the 180-min Disc-SPI specimens;

interposition of few small spherica (d= 10 μm, avg.) and spindle (10 μm X 30 μm avg.) was also observed. The proper polymorphic Obs sent out several broad-and-tapered lamellipodia and thin-and-long filopodia; from the spreading cell processes they further sent out many fine (dendritic) projections for intercellular junctions and cell-substrate attachments (i.e., focal contacts) with the Disc-SPI surface (Figure 3c).

Immunohistology of the engineered KUSA/A1 cells

We examined the expression of Cd51 (αV integrin) and F-actin (actin filaments stained with Rhodamine-conjugated phalloidin) for evaluation cell extension and attachment of engineered KUSA/A1 cells on Ti Disc of different modifications (Table. 1). The results of the double-stained specimens demonstrated distinct F-actin immunoreactivity (representing the development of cytoskeleton) in all 60- and 180-specimens (Table. 1). On the other hand, CD51 immunoreactivity (relating to focal contacts of the cell-substratum attachment) was only inspected in the 180-min specimens (Table. 1)

Discussion

Many studies on the endosseous dental implantation have demonstrated that pure titanium (Ti) and Ti-alloy dental implants did not bond directly with living bone (i.e., bone-nonbonding materials; bio-inactive), while the implant (IP) surface modified with certain bioactive substrates ensured a immediately mechanical primary stability, enhanced cell attachment, improved cell biomaterial interactions (the host-to-IP response) and thereby optimized a wide bone-to-IP direct contact (BIC) - osseointegration - to acquire long-term stability of the therapy (Grössner-Schreiber & Tuan, 1991; Groessner-Schreiber & Tuan, 1992; Swart et al., 1992; Guy et al., 1993; Davies, 1996; Chairy et al., 1997; Ong et al., 1998; Cochran DL, 1999; Ong et al., 2001; Nasatzky et al., 2003; Filles et al, 2005; Marco et al., 2005; Issac et al., 2008; Graf et al., 2008; Coelho et al., 2009). Therefore, the study of BIC in the endosseous dental IP therapy has been linked to the investigation of the tissue reaction and osseointegration in the host-to-IP interfaces (Davies, 1996; Ong et al., 1998; Filles et al., 2005; Locker, 1998; Nasatzky et al., 2003; Marco et al., 2005; Inoue, 2006; Inoue, 2008; Isaac et al., 2008).

Table. 1 Immunoreactivities of F-actin and CD51 in the 60-min and 180-min specimens.

	F-actin (cytoskeleton)		CD51 (integrin)	
	60 min	180 min	60 min	180 min
Disc-AO	+	+	+	+
Disc-HA	+	+	+	+
Disc-SPI	+	+	+	+

Osseointegration begins with adhesion of non-secretory osteogenic lineage cells, which migrate and spread, proliferated and colonize, and subsequently differentiated into phenotypically mature Obs. The mature Obs showing a decrease in proliferation rate have been elucidated to be secretory cells, which elaborate extracellular matrix (ECM) to initiate osteogenesis by cell/tissue ingrowth onto the biocompatible IP surface (Grösner-Schreiber & Tuan, 1991; Davies, 1996; Boyan et al., 2003; Davies, 2003; Nasatzky et al., 2003; Meyer et al., 2005; Salido et al., 2007; Issac et al., 2008). Histology of the peri-IP microenvironment has demonstrated that shortly after settlement of the IP into the host bone drilled cavity, an inflammatory reaction was occurred to induce formation of an afibrillar (collagen-free) calcified layer, which provided a bio-mimetic scaffold and cement lines facilitating contact and distant osteogenesis. Subsequently, the peri-IP trabecular bone was gradually remodeled by a mature lamellar bone to optimize and develop functional ossoeintegration of long-term stability (Davies, 1996; Wisemann et al., 2003; Marco et al., 2005; Meyer et al., 2005; Inoue, 2006; Joos et al., 2006; Inoue, 2008).

The turnover of collagenic and non-collagenic proteins in the ECM importantly regulates adhesion at the Ob-substratum interface. For instance, the expression of osteopontin in the non-mineralized interface peaks to enhance attachment of osteogenic cells, which change in shape concomitant with differentiation and subsequently express osteocalcin gene in the mineralizing phase (Nagata et al., 1989; Davies, 1996; Jayaramen et al., 2004). On the other hand, focal contacts have been elucidated to be structures composed of extra (ECM proteins)-compartment, intra (cytoskeleton proteins, e.g., actin filaments)-compartments and interfacing specific receptor proteins (e.g., intergrins), so that the cell-ECM adhesions mechanically connect the internal actin filaments to the ECM. The formation of strong focal contacts occurs essentially in cells with low motility and is promoted by ECM proteins; motile cells with a high migration rate are cells of lower differentiation level having less adhesive structures (Anselme et al., 2000; Meyer et al., 2005; Salido et al., 2007). Integrins translate the attachment of external ligands to internal information, thereby induce adhesion, spreading, cell migration and consequently regulate cell growth and differentiation, while the cytoskeletal proteins are assumed to be the most important intracellular structure for the intracellular attachment and for signal transduction. On the other hand, changes in the differentiated phenotype of culture cells influence cell-ECM adhesions, thereby evoke changes in cell shape and cytoskeleton to affect cell metabolism (Anselme et al., 2000; Meyer et al., 2005; Salido et al., 2007).

Cell migration requires a dynamic interaction between the cell, its substrate and its cytoskeleton. Filopodia (cytoplasm supported with long F-actin bundles) and lamellipodia (cytoplasm supported with a F-actin meshwork; due to extension of plasma membrane to acquire a distinct large surface area of the cells) are actin-rich structures to facilitate cell migration (Anselme et al., 2000; Zhu et al., 2006). Fine structure studies have demonstrated that Obs cultured on the smooth IP surface grew to have stress fibers running in all directions and thin filopodia, while Obs cultured on rough surface grew to be flat polygonal cells having long filopodia and defined wide lamellipodia with enhanced expression of stress fibers coupling with form focal adhesions; the actin stress fibers transmitted forces to the substrate (Anselme et al., 2000; Jayaraman et al., 2004; Diener et al., 2005; Salido et al., 2007). In the present study, we preliminarily confirmed that the osteogenic linage KUSA/A1 cells in inducing-conditioned were actively proliferated and differentiating into Obs to show high OC activity since day 3 of culture; dexamethazone reduces cell proliferation and induces cell differentiation, while *β*-GP enhances mineral formation (Umezawa et al., 1992; Ochi et al., 2002; Meyer et al., 2005; Rodriguez et al., 2007). On the other hand, the SEM and immunohistology of the αv integrin and F-actin expression indicated that the engineered KUSA/A1 cells on the Ti discs with different modifications (i.e., Disc-AO, Disc-HA and Disc-SPI) migrated/ dispersed along the bioactive interface with active participation to bind, initiate and establish clear focal adhesions (Boyan et al., 2003; Diener et al., 2005; Zhu et al., 2006; Salido et al., 2007; Issac et al., 2008).

The response to the surface micro-topography and chemistry of the substrate were mediated (e.g., by integrins) and essentially affected not only adhesion, spreading and signaling, but also ECM synthesis and tissue morphogenesis that were dependent on cytoskeletal organization of the mature Obs (Anselmen, 2000; Lange et al., 2002; Boyan et al., 2003; Jayaraman et al., 2004; Lenhert et al., 2005; Salido et al., 2007). Some studies in the literature have stressed that Obs were sensitive to uneven the bioactive surface of IPs; deep grooves and micro-roughness of the IP surface accelerated migration of Obs and increased bone-to-IP contact compared to smooth surfaces. Because the modified surfaces provided cell adhesion and *vice versa* the Obs cytoskeletal organization supported active roles governing an appropriate osteogenic surface for cell migration, certain autocrine and paracrine regulations of osteogensis at the cell-substratum interface occurred (Grösner-Schreiber & Tuan, 1991; Davies, 1996; Anselme et al., 2000; Nasatzky et al., 2003; Salido et al., 2007; Graf et al., 2008; Coelho et al., 2009). There has a study indicating that the porous anodic-oxidized Ti surface induces cell adhesion by providing

favorable a substratum for filopodia to stabilize, while the nano-HA coating microrough surface enhances the spreading of well-developed filopodia and lamellipodia to acquire high mobility of the migrating Obs; cells with a low motility had strong focal contacts while motile cells had less adhesive structures with the substrate, and an intermediate level of attachment force induces a maximal migration rate (Meyer et al., 2005). On the other hand, an *in vitro* study have found that microgrooved surfaces modulated primary cell-surface interactions to acquire higher attachment and adherence strength, while smooth surfaces provided microenvironment for optimal cell reactions of the Obs at the cell-substratum interface (Filles et al., 2005).

Nevertheless, the topography of micro-textured surfaces is not considered to be a factor affecting the mobility of focal adhesions (Diener et al., 2005). In conclusion, the present study demonstrated that focal adhesions and intercellular connections were acquired with well-developed long filipodia and broad lamellipodia of the fully-spread attached KUSA/A1 cells within 180 minutes of culture on Disc-AO, Disc-HA and Disc-SPI samples; no distinct histological differences of the differentiating Obs were found in the Ti discs with different microtextures. The results elucidated that anodic-oxidized, hyroxyapatite-coated and precision-microroughed Ti disc (Disc-AO, Disc-HA and Disc-SPI samples) surfaces substantially enhanced attachment, migration and active proliferation of KUSA/A1 cells in the present GBR microenvironment. We then predict that the bone marrow-derived KUSA/A1 cells attached on the substrate surface might be regulated to differentiate into mature Obs, and hence create a microenvironment conducting contact osseointegration within first three days using the present GBR tissue-engineering methods. Further studies to clarify distant osseointegration, bone modeling and remodeling following contact osseointegration of the implant-tissue interfaces should be the next steps in programming of the GBR tissues.

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人為培養環境內 (in vitro) 牙科植體骨整合的初步研討-鼠KUSA/A1細胞在不同表面處理的鈦板上的培養生長

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摘要

為了獲得成功的牙科植體治療，確實良好而廣泛的骨整合及植體-骨骼間接觸為必要條件。在現今人工培養環境中的研究，我們初步証明了KUSA/A1細胞從培養的第三天；即大量繁殖並分化為分泌骨鈣素的類成骨細胞。我們在以下三種鈦及鈦合金圓盤上培養類成骨細胞：(1) A.O.即陽極氧化處理。(2) Ha：羥磷灰石覆蓋。(3) S.P.I：精密噴砂處理。這三種植體的表面處理與現今普及的商用植體是一樣的。我們在培養了180分鐘後，研討了這三種植體上衍生的類成骨細胞的組織學像，用以探究微環境中模擬牙科植體骨整合的成效 。 在培養60分鐘時，我們採樣這三種檢體，在掃描電子顯微鏡下，可發現鬆散的球狀到卵圓狀類成骨細胞散佈著(平均直徑10 μm)，且向植體表面凹陷處移動。這些增生中的類成骨細胞有著腫脹的胞體，並伸出許多微小的絲狀偽足和一些片狀胞足與植體表面接觸， 一些具有細胞間鍵結的類成骨細胞也零星散佈著。相對的，在培養180分鐘後，採樣檢體，我們發現培養中的KUSA/A1細胞大量繁殖並變為平坦大而多角形(大約20 μm$\times$30 μm)，具有明顯的片狀胞足與樹突狀偽足的類成骨細胞與植體表面及其它細胞互相連結；在這些多形性類成骨細胞間，亦可見零星散佈著小而

關鍵字：細胞附著、鈦板、Kusa/A1 細胞、體外實驗

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圓形到紡錘形的類成骨細胞，另一方面，在免疫組織化學方面，在培養了60分鐘與180分鐘的檢體中發現附著於植體表面的類成骨細胞顯示出肌動蛋白質的存在(F-actin, actin filaments of cytoskeleton)，而穿透性細胞膜接受器蛋白質(α v integrin)(CD51)只在培養了180分鐘檢體中發現 。 因此，在這次研討中揭示：因不同植體表面誘導衍生的KUSA/A1細胞皆能在不同植體表面緊密附著並大量增生，而其組織學像皆相似 。

我們確信在這次的骨引導再生中於培養180分鐘內使得KUSA/A1細胞能在不同表面處理的鈦板上緊密附著，因此我們臆測類成骨細胞能進一步的受控用於骨整合中骨-植體接觸面及遠處基質的骨化作用。