

NEW FABRICATION METHOD OF POROUS Ti-6Al-4V PARTS AND SUBSEQUENT PROLIFERATION AND DIFFERENTIATION OF OSTEOBLAST CELLS

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Metallic porous materials are designed to allow the ingrowth of living tissue inside the pores and to improve the mechanical anchorage of the implant. In the present study, the production of highly porous Ti-6Al-4V parts by powder metallurgical technology and subsequently uses it in vitro bone tissue engineering is described. A space-holder method using carbamide to produce parts with porosities between 35 and 70% was used. Mechanical properties of parts were determined by compressive test. The porous parts are characterized by using scanning electron microscopy. Furthermore, study was demonstrated to investigate the effects of 3 different porosities of on proliferation, differentiation and cell-matrix interaction of mouse osteoblast-like cells, MC-3T3. Results showed the cell proliferation was significantly ($p < 0.05$) higher on 70% porous Ti-6Al-4V. However, synthesis of different types of extra cellular matrix proteins was also more abundant on 70% porous Ti-6Al-4V than 35 and 50% porous Ti-6Al-4V disk except some specific proteins. An increase in alkaline phosphate activity was significantly ($p < 0.05$) higher on 70 and 50% porous Ti-6Al-4V disk after 12 days of MC-3T3 cells incubation. The results indicated that porosity (nearly 70%) of porous Ti-6Al-4V topography affects proliferation and differentiation of osteoblast like MC-3T3 cells. The results showed that this novel process is promise to fabricate porous biomaterials for bone implants.

KEYWORDS: *Ti-6Al-4V; Porous material; Space-holder; Mechanical properties; cell proliferation*

1. INTRODUCTION

Metals and alloys are widely used for orthopedic and dental implants. Among these metallic biomaterials, titanium and its alloys which have an excellent combinations of structural and mechanical properties such as high strength to weight ratio, good corrosion resistance and superior biocompatibility with the surrounding tissue. This biocompatibility is providing in vivo long term stability of the artificial implants and scaffolds [1-4]. Much effort is currently being made to use titanium and its alloys for artificial implants and scaffolds by developing innovative processing techniques [5]. The main problem concerning these implants in orthopedic surgery is the mismatching between the elastic modulus of metallic titanium implant (110 GPa) and the elastic modulus of bone (10-30 GPa) [6, 7].

It should be mentioned that the strength and elastic modulus of titanium and its alloys can be controlled by using porous specimens with different porosity ratios. The low elastic modulus of porous titanium alloys are expected to reduce the amount of stress-shielding at the bone where the metallic part is implanted. This stress-shielding leads to bone resorption and then eventual loosening of the implant, and hence to prolong implant life time [8]. Also these porous scaffolds will be used as bone substitutes. In addition, by increasing the match of the strength and the modulus of elasticity between the bone and titanium alloys, it is expected to result in better performance of the implant bone compound.

A few methods have been developed in order to produce porous titanium and titanium alloy include sintering together of the particles [9] or plasma spraying of the powder on a dense substrate followed by the cutting of the porous layer [10], solid-state foaming by expansion of argon-filled pores [11-13] and polymeric sponge replication [14]. However, none of these conventional techniques has allowed for building parts with a completely controlled size and shape of the pore. The porous matrix must be designed to satisfy many requirements such as; the porous structure must be designed with high porosity to provide sufficient space for attachment of the new bone tissues and transport of the body fluids, the structure must be interpenetrated to allow the ingrowths of cells vascularization and diffusion of body, and the material must be of appropriate mechanical properties resist handling during implantation and in vivo loading. Furthermore, several studies suggest that surface topography and composition of titanium implants seem to be important with respect to the clinical outcome [15]. Stanford and Keller et al [16], Swart et al., [17] Davies et al [18], Schwartz et al [19], have shown that titanium implant surfaces may modulate phenotypic expression and metabolism of osteoblast cells. Osteoblast proliferation, differentiation, and maturation around titanium implants have been widely studied both in vivo and in vitro [20-22]. However, data in the literature on the biological effects of surface properties on

osteoblast proliferation and differentiation are rather contradictory and do not clearly indicate a better efficacy of a given surface as compared with surfaces with different properties.

The imperfection of the conventional techniques has encouraged the use of space-holder technique. This technique depends on mixing of the metallic powder with space holder, which can be removed without contaminating of the titanium powders was reported.

This work was undertaken to develop a high porous Ti-6Al-4V parts with controllable porosity, pore size, and pore shape. A new fabrication technique, which includes the adding of space holder into the starting powder to manufacture high porous Ti-6Al-4V, was investigated. The compression tests were performed on the high porous Ti-6Al-4V samples for studying the mechanical properties. The fabricated parts were characterized by scanning electron microscopy. Furthermore, to evaluate the biological role played in bone response by surface properties of Ti-6Al-4V implants, in the present study we also have investigated cell proliferation, differentiation of MC-3T3 cells cultured on 3 different porosities of porous Ti-6Al-4V surfaces (35, 50, and 70% porosity of Ti-6Al-4V disk).

2. MATERIALS AND METHODS

2.1 Preparations of Ti-6Al-4V disks and characterization

Ti-6Al-4V powder with average size of 56 μm (supplied by SE-JONG Materials Ltd. Korea) was used in the present experimental work. The morphology of Ti-6Al-4V powder and carbamide (space holder) particles were examined by scanning electron microscope (SEM) and digital camera, respectively as shown in Figure 1. Spherical carbamide with different particle diameters (0.56, 0.8, and 1mm) was used as the present novel space holder. The weight ratios of the Ti-6Al-4V powder to the amount of space holder were calculated to obtain defined porosities of 35, 50, and 70% in the sintered compact samples. The Ti6Al-4V powders were mixed thoroughly with carbamide particles in a rolling container for one hour. To prevent the dissimilar powder from segregation a small amount of ethanol was sprinkled during blending. The powder particles adhered to the surface of the space holder. All the samples used in the present investigation were fabricated by cold compaction using a manually operated uniaxial press. The mixture was uniaxially pressed at 450 MPa into cylindrical compacts with diameter $d = 12$ mm. The height of the cylindrical samples after pressing was approximately 15 mm. The mixing of any additional lubricant is not necessary. This is of special importance for the manufacturing of Ti-4Al-6V parts, where lubricant could be an additional source of impurities in the final product. The decomposition of carbamide in the green samples was carried out in vacuum at 200°C for 2 h to

avoid the solid substance burnt $(\text{NH}_2\text{CO})_2\text{NH}$, which leads to an unacceptable increasing of impurity of the final products. The compact samples were then sintered under a vacuum of 2×10^{-3} torr. The sintering process is consisted of two steps; the first step includes holding stage at 850°C for 1 h to allow for sample outgassing; surface oxides, water molecules and contaminants volatilize off from the sample surface prior to holding at the sintering temperature. These gasses and oxides must be dissipated before final consolidation in order to prevent the samples distortion. The second step was including holding stage at 1200°C for 2 h. After sintering, some of samples were ground and cut into a thin disk with 1 to 2 mm thickness and 12 mm in diameter to evaluate the biological properties.

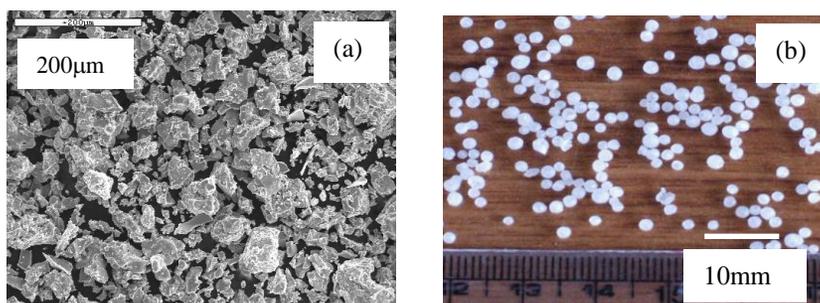


Fig. 1. Morphology of initial powders, (a) Ti-6Al-4V powders, and (b) carbamide particles.

The relative densities of green and sintered specimens were determined based on their weight and size using the theoretical density of corresponding composition. Porosity P (in percentage of volume) was calculated by measuring the apparent density ($\rho_b = \text{weight of sample}/\text{volume of sample}$) of sample by using the formula [23]:

$$P = (1 - \rho_b/\rho_s) \times 100 \quad (1)$$

where ρ_s is the density of 100% dense material. The compression tests were carried out on the specimens at room temperature with an initial stain rate of 0.5 mm/min. The microstructure of sintered parts was studied by scanning electron and optical microscopy.

2.2 Cell proliferation assay

Rat peritoneal mast cells (MC) cocultured with 3T3 fibroblasts (MC-3T3) was purchased from the Korean Cell Line Bank, Seoul, Korea). The cell lines were maintained in MEM medium (Life Technologies, Grand Island, NY) supplemented with 10% FBS, 300 g/ml L-glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. To determine the proliferation of MC3T3 cells on porous Ti-6Al-4V surfaces, we used the procedure as described by Mosmann et

al [23]. The assay is dependent on the cellular reduction of MTT [3-(4, 5 dimethylthiazol-2-yl)- 2, 5- diphenyltetrazolium bromide] (Sigma Co., USA), by the mitochondrial dehydrogenase of viable cells, to a blue formazan product, which can be measured by a spectrophotometer. Environment for cell cultural conditions were taken from previously published report [24]. Ti-6Al-4V samples (disks) were sterilized by subsequent cleaning with ethanol in an ultrasonic bath, and then sterile PBS and sterile water. Titanium discs were placed on the bottom of 24-multiwell dishes and plated with MC-3T3 at a density of 2×10^4 cells/cm². MC-3T3 cells were harvested from culture flask by trypsin and plated on porous Ti-6Al-4V disks, and the numbers of viable cells were determined at 24, 48, 72, and 96 h by the MTT assay. Porous Ti-6Al-4V disks were transferred into 24-well plates and MTT (5 ng/mL) was added; disks were then incubated for 4 h at 37°C. The dye was eluted from the porous Ti-6Al-4V disks with acidified isopropanol, and optical density was measured by a spectrophotometer (Beckman DU-40, Milano, Italy) at 570 nm. Porous Ti-6Al-4V disks without cells and medium alone were used as negative controls.

2.3. Alkaline phosphatase measurements

Alkaline phosphate (ALP) was determined with p-nitrophenylphosphate as a substrate. MC-3T3 cells plated on porous Ti-6Al-4V disks as described above cell proliferation assay were analyzed on the 2nd, 6th, and 12th days of culture. Cells were scraped into 500-mL ice-cold harvest buffer (10 mM Tris HCl, pH 7, 4, 0.2% NP-40, and 2 mM phenylmethylsulfonyl fluoride, PMSF). Enzymatic activity was measured by an automatic analyzer (Hitachi 747, Boehringer Mannheim, Indianapolis, IN, USA). The results were expressed as UI/(enzyme activity)/104 cells.

2.4. Insoluble extracellular matrix (ECM) measurements

MC-3T3 cells were plated on titanium surfaces as described above cell proliferation assay and cultured for up to 96 h. At given times (6, 24, 48, 72, 96 h), cells were fixed on titanium surfaces by 50% (vol/vol) methanol-acetone for 10 min at room temperature and air-dried. Disks were incubated with calcium-and magnesium-free phosphate-buffered saline (PBS)/0.5% bovine serum albumin (BSA), for 2 h at 4°C, then filled with 50 mL of one of the following rabbit anti antisera: anti-collagen I (CoI), fibronectin (FN), and vitronectin (VN), (Chemicon, Temecula, CA, USA), in PBS/0.5% BSA and 0.2% Tween 20 and allowed to react for 1 h at room temperature. Plates were then washed with PBS, filled with 50 mL horseradish peroxidase-conjugated anti-rabbit IgG in PBS, 0.2% Tween 20, allowed to react for 1 h, washed again with PBS, and filled with 150 mL of 1 mg/mL o-phenylenediamine, 0.006%

hydrogen peroxide, 0.1 M citrate buffer, pH 5.0. After 30 min of incubation, the absorbance at 450 nm was measured by a spectrophotometer. Titanium disks without cells and disks coated with purified extracellular matrix (ECM) were used as controls. To evaluate the effects of serum on ECM deposition by MC-3T3 cells, we used serum-starved cells, cultured in MEM medium supplemented with 10% FBS, 300 .g/ml L-glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin in an incubator at 37 °C, as controls.

2.5. Cell–matrix interaction

The procedure for cell-matrix interaction was taken from previously described report [25]. Briefly, Ti-6Al-4V titanium-alloy disks were sterilized by subsequent 46 cleaning with ethanol in an ultrasonic bath, and then sterile PBS and sterile water. Titanium discs were placed on the bottom of 24-multiwell dishes and plated with MC 3T3 at a density of 2×10^4 cells/cm². The cellular constructs were maintained with MEM medium supplemented with 10% FBS, 300 g/ml L-glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin in an incubator at 37 °C, and the cell culture medium was changed every 3 days. Cell constructs were harvested at 12, 24, 48 and 52 h fixed with 4% glutaraldehyde for 1 h at room temperature, dehydrated through a series of graded alcohol solution, and then air-dried overnight. Dry cellular constructs were sputter coated, and observed by SEM.

2.6. Statistical analysis

Results were calculated as mean \pm standard division (SD). Statistical analysis was performed by the Student t test, and the differences were considered significant with "p" values less than 0.05.

3. RESULTS AND DISCUSSION

3.1. Preparations of Ti-6Al-4V disks and morphology study

The procedure used during this work is basically simple: mixing space-holder with Ti-6Al-4V powder, cold compaction, then decomposition the space-holder in a relatively low temperature, and finally sintering to obtain a porous Ti-6Al-4V body. Figure 2 shows the structure of the parts which presence two kinds of pores. The first type is micropores with a size of several micrometers, as can be seen in Figure 2 a. This type of pores was found between the particles of Ti-6Al-4V resulting from shrinkages that occurred during sintering process. The second type is macropores that arise as a result of space-holder removal (Figure 2 b, c, and d). The size of macropores mainly depends on the size of the space-holder ranging from 0.560 mm to 1.0 mm. The structure of samples pressed with 35, 50, and 70% of space-holder with size 0.8 mm and sintered at 1200°C

for 2h were used is shown in Figure 2 b, c, and d. It was found that the macropores remain nearly unchanged during the sintering process. In addition the Ti-6Al-4V alloy foams with 70% porosity displayed an interconnected porous structure resembling bone (Figure 2 e). The lower porosity resulted in less interconnected pores (Figure 2 b). Obviously, the parts have been a good homogenous of the pores. Also, it can be noticed that some of pores do not have a spherical shape. The main reason is that the row particles of the space-holder have this defect.

The chemical analysis results of organic residuals (oxygen, carbon, and nitrogen) after the different processing steps are shown in Figure 3. It can be seen that the contents of oxygen, carbon and nitrogen remain nearly unchanged after removal of space-holder. After sintering, all the concentration of carbon, oxygen, and nitrogen are increased slightly. The reason for this behavior is probably attributable to residual contaminants in the furnace.

3.2. Mechanical characterization

To achieve a functionally satisfying implant in practical applications, porous scaffold design needs to consider both the strength and the Young's modulus. The elastic modulus of bone ranges from 0.1 to 20 GPa; and the compressive strength ranges from 2 to 200 MPa [26]. To avoid stress shielding the elastic moduli of bone and implants should be as similar as possible, which may lead to bone resorption and even necrosis.

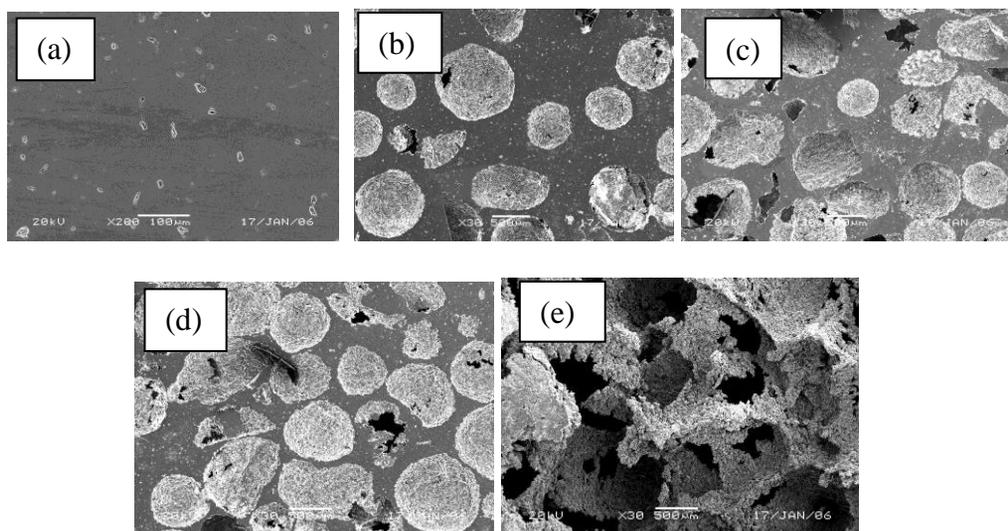


Figure 2. SEM micrograph of porous Ti-6Al-4V with 0.8 mm pore size sintered at 1200°C for 2h in vacuum atmosphere. a) micropores, b) 35% porosity, c) 50% porosity, d) 70% porosity, and e) interconnected porous

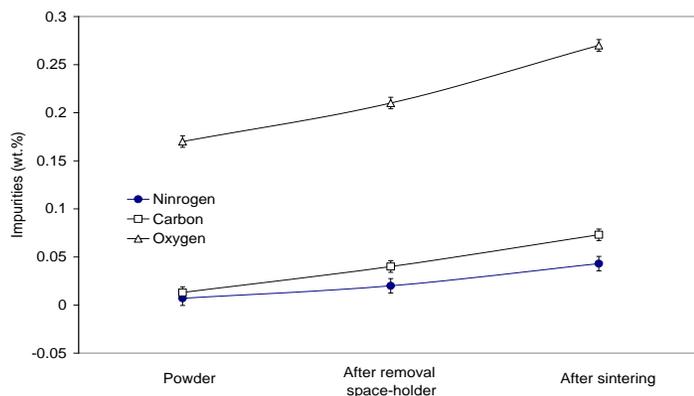


Figure 3. Chemical analysis of Oxygen, Carbon and nitrogen contents in Ti-6Al-4V parts after processing steps.

Compressive test was conducted on Ti-6Al-4V disk with a porosity ranged from 35 to 70% to evaluate the mechanical properties. The compressive strength and Young's Modulus for the Ti-6Al-4V disk with pore size 0.8 mm is shown in Figure 4. It can be seen that the Ti6Al-4V disk showed the typical deformation behavior of metallic foams under compressive loading. Generally both the Young's modulus and the peak stress increased with a decrease in porosity. The compressive stresses of the Ti-6Al-4V disk with porosity 35, 50, and 70% were 400, 225, and 40 MPa, respectively. The corresponding Young's moduli were found to be 19, 14, and 11 GPa, respectively. It can be seen that the mechanical properties of the foam with porosity 70% were very close to those of the natural bone.

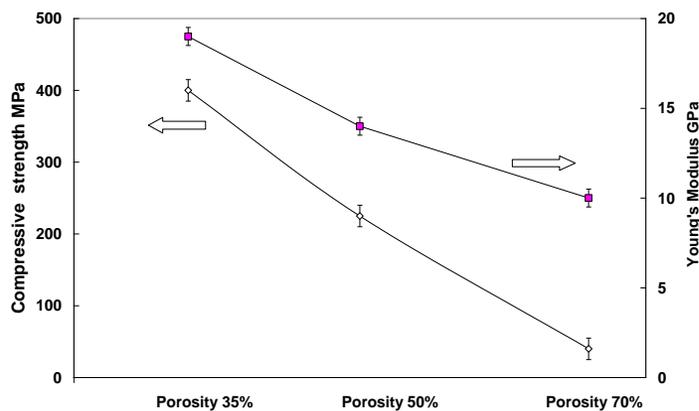


Figure 4. The Compressive strength and Young's Modulus for the Ti-6Al-4V disk with pore size 0.8 mm.

3.3. Cell proliferation assay

Mechanisms related to bone formation around titanium dental implants involve sequential events such as cell attachment, proliferation, differentiation, and deposition of bone matrix [16-19]. Several reports indicate that the surface characteristics of titanium implants are able to modify cellular biological functions [19, 20, 22]. Nevertheless, studies on the effects of surface topography on osteoblast proliferation, differentiation, and maturation are rather contradictory. In the present study, we have investigated the influence of 3 different types of porous Ti-6Al-4V surfaces (35%), (50%), and (70%) on the in vitro proliferation, differentiation and cell matrix interaction of MC-3T3 cells.

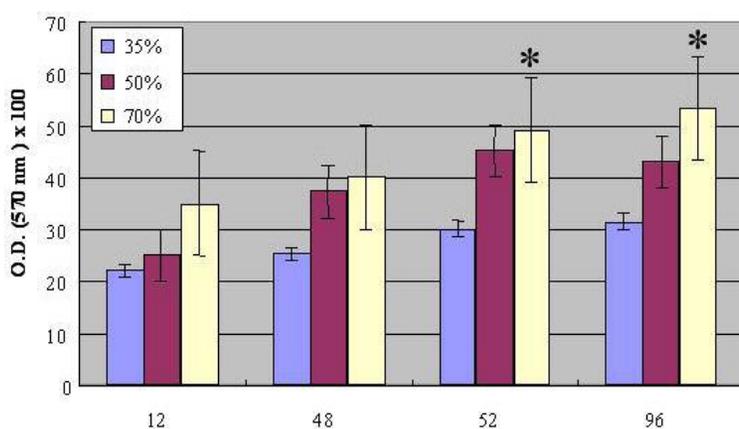


Figure 5. MC-3T3 cell proliferation on Ti-6Al-4V disk surfaces (mean. SD, N=12) determined By the MTT assay.

Figure 5 shows the cell proliferation was higher on 70 % than on 35 and 50 %, porous Ti-6Al-4V surfaces, in agreement with the observations of Martin et al. [27]. Although, cell survival on the different porous Ti-6Al-4V surfaces may also be regulated through apoptotic mechanisms but cellular apoptosis did not analysis. However, simple microscopic observation shows there was not significant difference ($p > 0.05$) in cell death rates among cells cultured on the 3 different porosity porous Ti-6Al-4V surfaces (data not shown). Analysis of these data, therefore, confirms that the lower number of cells observed on 35% porous Ti-6Al-4V surfaces was indeed due to a lower proliferation of MC-3T3 cells on this type of surface rather than to an increased cell death rate.

3.4. Cell growth and morphology

Several studies suggest that differentiation toward an osteoblastic phenotype correlates with a decrease in cell proliferation and an increase in alkaline phosphatase (ALP) activity as well as in extracellular matrix (ECM) production

[28]. In particular, in MC-3T3 cells, ALP activity can be considered as an osteoblastic phenotypic marker and, thus, an index of osteoblastic differentiation [29, 30]. Indeed, in the MC-3T3 cells, when proliferation slows, the expression of osteoblastic functions, including ALP and collagen production, increases [31]. Analysis of our data (Figure 6) shows that the increase in ALP activity was related to surface morphology of the matrix, being the highest when the cells were cultured on 75% porous Ti-6Al-4V surfaces. Collagen I (CoI), fibronectin (FN), vitronectin (VN), and tenascin (TN) are among the components of the ECM produced by osteoblasts during differentiation, and CoI is the major component of bone connective tissue [32]. The analysis of ECM production in MC-3T3 cells demonstrated that the synthesis of CoI, FN, and VN, varied depending on the porosity of porous Ti-6Al-4V surface, Figure 7 (a to c). Particularly, the matrix was deposited on 75% porous Ti-6Al-4V primarily in the first hour of culture (6-24 h), whereas matrix deposition on 35% porous Ti-6Al-4V and 50% porous Ti-6Al-4V surfaces took place progressively between 6 and 96 h. Among the 3 different porous Ti-6Al-4V surfaces, 75% porous Ti-6Al-4V displayed the higher induction of ECM synthesis and organization, proving to be the best surface for ECM production by osteoblast-like MC-3T3 cells. However, among 3 different ECM, Col production onto the 70% porous Ti-6Al-4V was not significantly different with 35 and 50% porous Ti-6Al-4V after 96 h. In the case of FN and VN, cells synthesized significantly higher on 70% porous Ti-6Al-4V disks surfaces compared on 35 and 50% porous Ti-6Al-4V disks. Therefore our study shows that for better production of ECM component depends on the surface characteristics for specific proteins. However, production of ECM correlated with receptors for ECM components. In agreement with data on ECM production, we found a higher expression of these receptors may be found on highly porous materials compared with surfaces of less porous materials. In fact, MC-3T3 cells proliferate better on 75% porous Ti-6Al-4V, while 35 and 50% porous Ti-6Al-4V surfaces promote their differentiation toward an osteoblastic phenotype only for some specific ECM. Therefore, analysis of our data suggests that 75% porous Ti-6Al-4V surfaces may favor a better biological outcome of dental implants *in vivo* since they seem to induce differentiation toward an osteoblastic phenotype, enhancing bone healing and the long-term maintenance of osseointegration. Further studies are needed to support this hypothesis.

3.5. Cell matrix interaction

For tissue engineering, only surface compatibility is not sufficient. It is important to have a three-dimensional structure of scaffold for cell attachment, growth, and migration. SEM micrographs reveal that the micro observation on the cellular response to this structure. In this study, cell matrix interaction (cell

spreading) was evaluated in terms of SEM micrograph observation. The larger the coverage area of the cytoplasm and nucleus by wide spreading cell membrane is, the better the cell spread (grow). From the observation of cell spreading shown in Figure 8 a-b and c-d), the adhesion cells on the porous titanium disk showed the morphology with out a distinct spreading appearance. These cell appearance represents the poor cell adhesion between cells and mats [33].

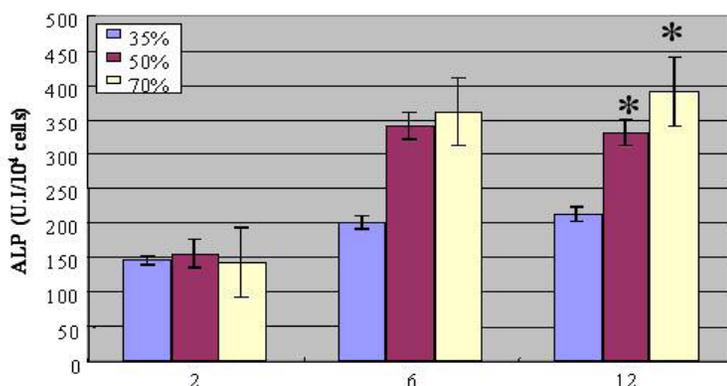


Figure 6. Alkaline phosphatase (ALP) activity (mean + SD, n = 12) of MC-3T3 cells on Ti-6Al-4V disk surfaces determined by spectrophotometric analysis at different time points. *p < 0.05, (70%) and (50%) vs. (35%) surfaces

When the cells adhere onto the specimen surface, the cell spread out and become flattened on the metal surfaces [34]. This is good index to the quality of cell growth in assessing the biocompatibility of implant materials. In this study, the disk having less porosity had not good cell spreading than the disk having high porosity (70%) after the 52 h of culture. cell seeded on the porous scaffold (70%) are found to have appropriate interaction and better cell spreading with their environment based on the following observation: (1); the cells maintain a normal phenotypic shape, suggesting that cells function biologically within this structure, (2); the cells favor this structure, so they adhere onto the surface, and proliferate on the porous network, packing the structure surface with the cells after 52 h of culture, and (3); these cells spread out the cytoskeletal actin fiber and integrate with the surface of surrounding pores to form a cellular network, Figure 8 C and F. Furthermore, the reason behind the better cell spreading onto the porous matrix might be contact guidance phenomenon derived from the influence of material surface topography on the actin cytoskeleton, focal adhesion, and microtubule of the cells or from the biomechanical equilibrium between cells and materials surface. From this evidence, it indicates that porous structures of the disk positively promote cell-matrix interactions.

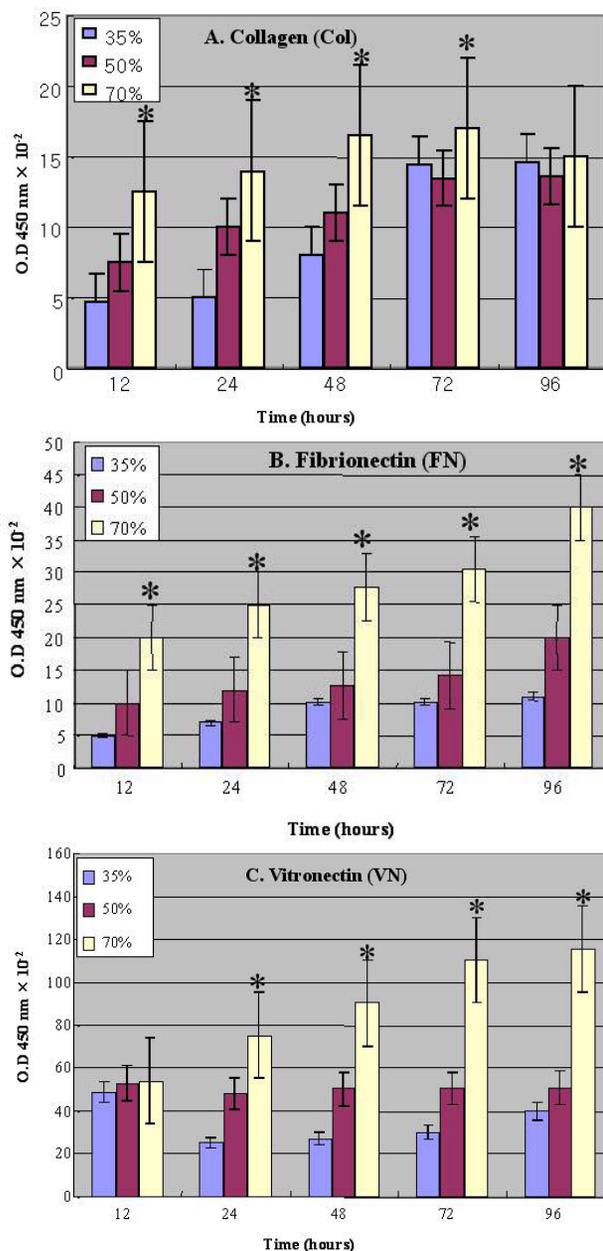


Figure 7. Synthesis of extracellular matrix (ECM) proteins and integrin expression in MC-3T3 cells on porous Ti-6Al-4V disk surfaces. ECM protein synthesis (mean \pm SD, $n = 12$) determined by enzyme-linked immunoassay in situ at different time points: (A) collagen I (CoI), (B) fibronectin (FN), and (C) vitronectin (VN). * $p < 0.05$, titanium disk (70%) vs. (50%) and (35%) porosity surfaces.

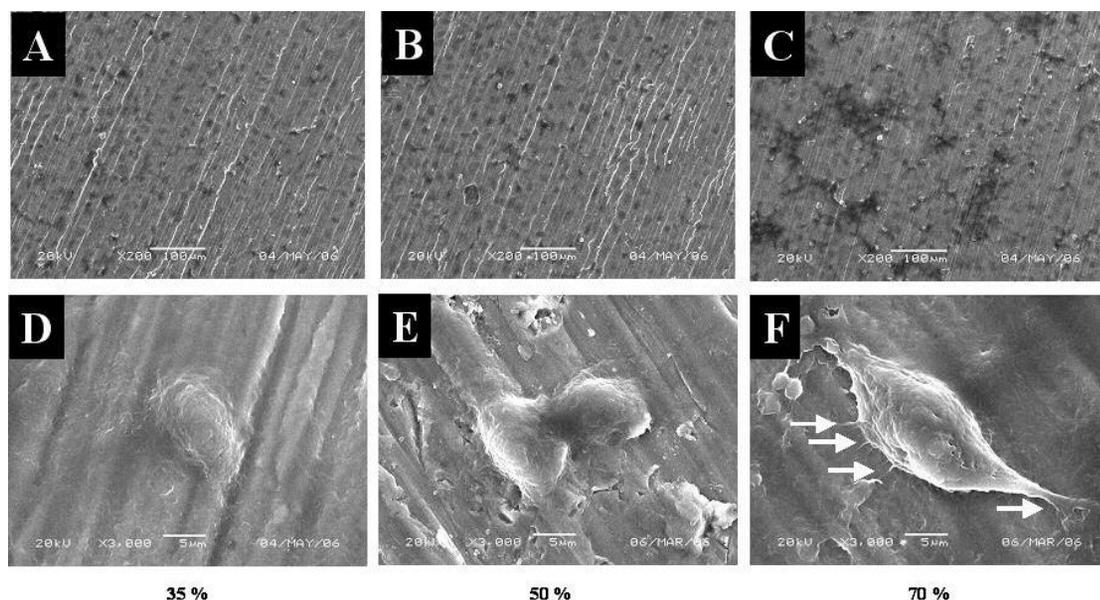


Figure 8. SEM micrographs of Ti-6Al-4V disk with different porosity (A-D 35%, B-E 50%, and C-F 70%) after 52 h cell incubation showing cell proliferation and spreading morphology. Arrow indicates the phenomenon of contact guidance with the disk surface. Upper (A-C) and lower (D-F) panel is low and high magnification, respectively.

4. CONCLUSIONS

Space-holder processes are attractive for the production of highly porous metals. Advantages they offer over other processing routes include precision and uniformity in the size, the volume fraction and the shape of pores across large samples, coupled with wide latitude in selection of the material the foam is made from. Porous Ti-6Al-4V with controlled porosity ranged from 35 to 70%, have been fabricated by a space-holder and powder metallurgy method. Furthermore, our preliminary results from in vitro show that there is perfect correlation between cell proliferation and differentiation on the porosity of 3 different porous Ti-6Al-4V. In fact, MC-3T3 cells proliferate better on 70% porous Ti-6Al-4V, while 35 and 50% porous Ti-6Al-4V promotes their differentiation towards osteoblastic phenotype only for some specific ECM. Therefore, analysis of our data suggests that mild porosity (nearly 70%) of porous Ti-6Al-4V may favor a better biological outcome of dental implants in vivo, since they seem to induce differentiation towards an osteoblastic phenotype, enhancing bone healing and long term maintenance of osseointegration.

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طريقة جديدة لإنتاج أجزاء عالية المسامية من سبيكة التيتانيوم بغرض استخدامها في التطبيقات الطبية واختبار تطور ونمو خلايا بناء العظام بداخلها

يتم تصميم المواد المعدنية والتي تستخدم كبداية للعظام الطبيعية بمسامية عالية وذلك كي تسمح للخلايا الحية بالنمو داخلها وكذلك كي تسمح بمرور السوائل خلالها. وفي هذه الدراسة فقد تم تصنيع أجزاء عالية المسامية من سبيكة التيتانيوم باستخدام تكنولوجيا متالوروجيا المساحيق وحافظ الفراغات وتم اختبار هذه الأجزاء في وسط يشبه الجسم الحي. وقد استخدمت مادة اليوريا ذات حبيبات كروية الشكل كحافظ للفراغات وذلك لصنع أجزاء بنسب مسامية 35 و 50 و 70% وقد تم تحديد الخواص الميكانيكية لهذه الأجزاء بإجراء اختبار الضغط. كما تم فحص التركيب البنائي للأجزاء باستخدام الماسح الإلكتروني.

وقد تم في هذا البحث دراسة تأثير نسب المسامية علي عملية انتشار وتفاعل خلايا بناء العظم للفئران. وقد أظهرت النتائج أن نمو وانتشار الخلايا كان أكبر بصورة واضحة للعينات ذات المسامية العالية (70%) وكذلك فقد وجد انه تم تخليق أنواع كثيرة مختلفة من البروتين للعينات ذات المسامية 70% عن العينات ذات المسامية الأقل (35 و 50%) وقد تم رصد زيادة في نشاط الفوسفات القاعدي للعينات ذات نسب المسامية 50 و 70% بعد وضعها في حاضن خلايا من MC-313.

وقد خلص البحث الي ان هذا الاسلوب الجديد لصناعة اجزاء معدنية ذات نسب مسامية عالية يعتبر اسلوب واعدا جدا لصناعة الاجزاء التعويضية للعظام الطبيعية.