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Fabrication of porous Ti/Ti-alloy scaffolds with controlled pore structures via dynamic freeze casting

2015년 8월

서울대학교 대학원
재료공학부
이 현
Fabrication of porous Ti/Ti-alloy scaffolds with controlled pore structures via dynamic freeze casting

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이 논문을 공학석사 학위논문으로 제출함
2015년 7월

서울대학교 대학원
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2015년 7월

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Abstract

Fabrication of porous Ti/Ti-alloy scaffolds with controlled pore structures via dynamic freeze casting

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As we live in elderly society, increasing demand for medical devices and implants exists. There are several medical implants which are categorized with polymer, ceramic and metal implants. Polymer implants has flexibility and some of them has biodegradability which don’t need postsurgery. However, polymer implants has low mechanical properties to be used as a hard tissue replacement. On the other hands, ceramic implants have high mechanical properties and chemical stability. But it also has some problems, which are represented by intrinsic brittleness. Because of that, ceramic implants can’t be used in parts where high stress is applied. For metallic implants, high mechanical properties and wear resistance are focused. Also, metallic implants have ductility, which can reduce concerns about broken parts. And there are biocompatible metals such as tantalum
or titanium and its alloys. Unfortunately, metallic implant is too strong for using as bone substitutes. This difference in mechanical properties causes bone near the implant to resorption, which is called “stress shielding effect”

To overcome this problem, porous structure is widely researched. Making pores in the metallic implants reduces mechanical properties to similar level of bones. The fact that cells prefer rough surface than smooth surface also support the generation of pores for implanting materials. Even more, pores can act as regeneration sites for bone, which induce more interlocking effect between implant and bone. There are several methods to make porous structure. Polymer replication, powder metallurgy, rapid prototyping and freeze casting. Especially for freeze casting methods, there are variations. General or static freeze casting, reverse freeze casting and dynamic freeze casting. Static freeze casting is mainly for very small raw powders like ceramic powders. When larger and heavier metallic powder is used, sedimentation occurs. The other two methods are solution for using metallic powders. Reverse freeze casting uses gravity. After making porous structure by sublimable vehicle, metallic powders fill the empty space. By this method, highly aligned porous metal scaffolds can be made. Dynamic freeze casting adopts rotating procedure to prevent linear dendritic growth. Rotating make growth direction of vehicle to all direction and finally generated pores have spherical shape. This method can produce porous metallic scaffold with maintained ductility.

For wider applications, we applied dynamic freeze casting to enhance
mechanical properties and functionally graded porous structure. In the first study, we enhanced mechanical properties by using Ti6Al4V powders and identified its biocompatibility by *in vitro* test. For structural analysis, with increasing contents of powder from 15vol% to 25vol%, porosity and pore size were decreased from 71% to 51% and 426 µm to 311 µm. Also mechanical properties were increased with increasing contents of powders. From 15vol% to 25vol%, yield compressive strength and stiffness were increased 76 MPa to 223 MPa and 5.0 GPa to 17.8 GPa. Yield compressive strength is higher than that of porous pure titanium scaffolds, with similar stiffness. We used pre-osteoblast cell(MC3T3-E1) for *in vitro* test and confirmed that initial cell attachment and cell viability were almost same for porous Ti6Al4V scaffolds and porous pure titanium scaffolds. For these reasons, it is successful to fabricate stronger porous metal scaffold with maintained biocompatibility.

Still there are demands for mimicking real bone structure, which is combined with porous and dense parts. So in second study, we fabricated functionally graded porous structure using compressibility and ductility of porous titanium scaffolds. We prepared different heighted rod structured scaffolds and ring structured scaffolds, longer rod is for positive and longer ring is for negative. After put rod structured scaffold into ring structured scaffold, compress them into same height. Scaffolds with bigger height are densified to some distant. In this study, 70%-50% and 70%-30% combined porous scaffold was successfully made. For
70%-50% combined structure, pore size was around 350 µm for non-compressed region and 225 µm for compressed region. Mechanical properties were also increased compared to homogeneous 70% porous scaffolds, with 79 MPa and 2.9 GPa in terms of yield compressive strength and stiffness each for positive and 87 MPa and 3.9 GPa for negative. In 70%-30% combined structure, pore size was more decreased in compressed region with around 140 µm. Also mechanical properties more enhanced to 190 MPa and 10.2 GPa for positive and 250 MPa and 14.8 GPa for negative. After compressing, there exists shape change in pores. The more compressed, the more linear shaped pores are generated. Also GFP loading was successfully done to each scaffolds. Among them, compressed region showed prolonged release.

In conclusion, wider variety of porous titanium and its alloy scaffold was generated by these two experiments. Enhanced mechanical properties and maintained biocompatibility was confirmed by porous Ti6Al4V scaffolds. And mimicking bone structure and enhancing mechanical properties by combination and compressing method was successfully done.

**Keywords:** Porous scaffold, Titanium, Titanium alloys, Dynamic freeze casting, Controlled pore structure, Functionally graded structure.
Contents

Abstract ........................................................................................................................................i

Contents ........................................................................................................................................v

List of Figures and Tables .............................................................................................................vii

Chapter 1. Introduction (Theoretical review)
  1.1. Bone graft substitutes ........................................................................................................2
  1.2. Porous metal implant as orthopedic and dental usages .................................................. 3
  1.3. The aim of this study .........................................................................................................4

Chapter 2. Ti-alloy based macro/micro porous scaffold with enhanced mechanical properties
  2.1. Introduction .....................................................................................................................7
  2.2. Materials and methods ..................................................................................................8
    2.2.1. Fabrication of porous Ti6Al4V scaffold ........................................................................8
    2.2.2. Characterization ..........................................................................................................8
    2.2.3. In vitro biological analysis ..........................................................................................9
    2.2.4. Statistical analysis ......................................................................................................10
  2.3. Results and discussion ....................................................................................................11
    2.3.1. Microstructure and Mechanical property ....................................................................11
    2.3.2. In vitro biological analysis .........................................................................................12
Chapter 3. Fabrication of Ti-based functionally graded porous scaffold

3.1. Introduction ..............................................................................24
3.2. Materials and methods .........................................................25
    3.2.1. Fabrication of functionally graded porous Ti scaffold ..25
    3.2.2. Characterization ..............................................................26
    3.2.3. Fabrication of protein loaded functionally graded porous Ti scaffold and its release test .........................................................27
    3.2.4. Statistical analysis .........................................................28
3.3. Results and discussion .........................................................28
    3.3.1. Structural analysis ..........................................................28
    3.3.2. Mechanical analysis ......................................................29
    3.3.3. Prolonged release observed by GFP .................................30

Chapter 4. Conclusion

4.1. Ti-alloy based macro/micro porous scaffold with enhanced mechanical properties .........................................................46
4.2. Ti-based functionally graded porous scaffold ..........................47
List of figures and tables

Figure 1. Schematic diagram of dynamic freeze casting.

Figure 2. SEM images of raw Ti6Al4V powder.

Figure 3. EDS analysis of porous Ti6Al4V scaffold, (A) analyzed area, (B) EDS spectrum, and (C) weight percentage and atomic percentage of each element.

Figure 4. SEM images of porous Ti6Al4V scaffold with different content of powders, (A)-(B) 15, (C)-(D) 20, and (E)-(F) 25vol%.

Figure 5. Micro-CT images and pore size distribution of each scaffolds. (A) 15, (B) 20, and (C) 25vol%. Scale bar = 1mm.

Table 1. Pore analysis by micro CT for each sample with different vol%

Table 2. Porosity and mechanical properties of the porous Ti6Al4V scaffold analyzed by vol% as variables(15, 20, 25 vol%).

Figure 7. SEM images of MC3T3-E1 cells after 1 day culturing on (A) Ti6Al4V 15vol%, (B) pure Ti 15vol%, (C) Ti6Al4V 25vol%, and (D) pure Ti 25vol%.

Figure 8. Cell viability measured after culturing 3 days by MTS assay on porous pure titanium and porous Ti6Al4V scaffold with 15 vol% and 25 vol%.

Figure 9. Schematic diagram of dynamic freeze casting and combination method.

Table 3. Size of scaffolds before compression and applied strain.

Figure 10. SEM images of 70%-50% combined scaffolds. (A)-(C) positive and (D)-
(F) negative. (A),(D) interfaces, (B),(E) inner parts and (C),(F) outer parts.

**Figure 11.** Micro CT images of (A) 70%-50% positive and (B) 70%-50% negative scaffold.

**Figure 12.** Pore size distribution analysis by micro CT with 70%-50% scaffolds, (A) inner part of positive, (B) outer part of positive, (C) inner part of negative, and (D) outer part of negative.

**Table 4.** Porosity and pore size of each 70%-50% scaffold with different parts.

**Figure 13.** SEM images of 70%-30% combined scaffolds. (A)-(C) positive and (D)-(F) negative. (A),(D) interfaces, (B),(E) inner parts and (C),(F) outer parts.

**Figure 14.** Micro CT images of (A) 70%-30% positive and (B) 70%-30% negative scaffold.

**Figure 15.** Pore size distribution analysis by micro CT with 70%-30% scaffolds, (A) inner part of positive, (B) outer part of positive, (C) inner part of negative, and (D) outer part of negative.

**Table 5.** Porosity and pore size of each 70%-30% scaffold with different parts.

**Figure 16.** Mechanical properties of scaffolds with homogeneous 70% porosity, combined 70%-50% scaffolds and combined 70%-30% scaffolds.

**Table 6.** Yield compressive strength and stiffness of each scaffolds.

**Figure 17.** CLSM analysis for releasing behavior, (A) 70% homogeneous porous scaffold, (B) 70%-50% combined scaffold, and (C) 70%-30% combined scaffold. Right side is initial state and left side is after 7days of release.
Chapter 1.

Introduction

(Theoretical review)
1.1. Bone graft substitute

As scientific technology and medicine develop, life expectancy of human is dramatically increased. This phenomena requires our body stronger till we become older. But our body itself also getting older, and of course getting weaker. Especially functions of our body like structural stability and strength of bone become weak. To overcome those, implant technology has emerged, which is using bone graft substitute. Autograft, allograft and xenograft substitutes are widely used. For autograft, it is most safe way among them. But autograft itself is extracted from other part of patient’s body, so limitation of supply occurs and extracting site is also constrained. Another problems are donor site complications and the pain from donor part that make patients refuse to use autograft[1, 2]. And as allograft is taken from other human body, it should take several processes, which contain elimination of proteins in the raw bone, to be used in real cases. This process lowers the ability of bone regeneration[2]. Lastly, xenograft comes from the bodies of animals. As human and animals have different immune system and proteins, immune problems of xenograft make its usage difficult.

For those reasons, synthetic bone grafts getting interests to replace autograft and allografts. Firstly, Calcium-phosphate based ceramics and cements are widely studied and used in clinical situations[3, 4]. CaP based ceramics are similar to ingredients of bones and since they are stable for thermal change, it is easy to sterilize them. But still they have some limitations. Ceramic based cements are
widely used, but for bigger scaffold, there exist mechanical problem, the brittleness. As ceramic has intrinsic brittleness, it can be broken and small particles can circulate whole body with making unwanted inflammations. Secondly, polymeric scaffold can be mimicking bone structure with pores, and it has no worry of broken. Especially for biodegradable polymers, postsurgery is even not needed. However polymer don’t show enough mechanical properties for using as a bone grafts[5]. Finally metallic scaffolds are also used in orthopedic and dental area, with great mechanical properties and wear resistance. Tantalum, titanium and its alloys are most common materials because of their great biocompatibility over other metals[6, 7].

1.2. Porous metal implant as orthopedic and dental usages

Metal scaffolds have many advantages, like great mechanical properties, high wear resistance and corrosion resistance[7]. But as mechanical properties of metal implant is much higher than that of bones. This induces bone resorption near the implant, which is called “stress shielding effect” [8]. To reduce mechanical difference and increase structural similarity between metallic implant and bone, porous structure is applied. In addition to reducing mechanical difference, porous structure has other advantages. Pores in the scaffold can act as a rough surface for enhancing cell attachment. Because osteoblast-like cells tend to adhere to rough surface rather than smooth surface[9, 10]. Also pores increase surface area with
enhancing bone to implant contact[11]. Of course, when the contact area increases, interlocking should increases also.

Several methods are invented to make porous structure. In here we mainly focus on variation of freeze casting method. Freeze casting method is using the slurry with sublimable solvent vehicle and raw powders. After solidifying the slurry with temperature gradient to induce dendritic growth, sublimate the solvent vehicle while maintain the whole structure. Green body is heat treated and the porous scaffold is produced. For general freeze casting method, metal scaffold cannot be made. Because metal powder is much bigger than ceramic powder, sedimentation occurs during solidifying process. So there are two different novel strategy. First, reverse freeze casting was adopted to fabricate highly aligned porous metal scaffolds[12]. This used sedimentation phenomena as a positive way. After freezing camphene to be unidirectional, Ti/camphene slurry penetrates to the generated pores. Another method to make porous metal scaffold without sedimentation is dynamic freeze casting[13]. It applies rotation step during solidifying freeze casting vehicle. This rotation step prevents directional growth of the vehicle, which make the growth into all direction. Therefore pore shape in the produced metal scaffold is novel spherical shape. Also, by using this method, nearly no contamination of metal scaffold occurs to keep metallic ductility.

1.3. The aim of this study
In this study, we adopted dynamic freeze casting method to make porous structure in the scaffold. For the first part, we adopted titanium alloy (Ti6Al4V) powder to make porous scaffold with enhanced mechanical properties. And we certified its biocompatibility and compared with pure titanium scaffold by *in vitro* cell test. In second part, we made different heighted ring and rod shaped porous titanium scaffold and compressed them into single scaffold with same height. By varying difference between ring and rod height, we can control mechanical properties and characteristics related to pores.
Chapter 2.

Ti-alloy based macro/micro porous scaffold with enhanced mechanical properties


2.1. Introduction

For the orthopedic and dental scaffold, various materials are used. Metal implants have better mechanical properties than scaffolds with polymers and ceramics. There are some metals which have good biocompatibility [14, 15]. Especially titanium and its alloys are widely used in bone substitute materials. However, solid titanium has much higher elastic modulus than bone itself. Bone around the implant undergoes desorption, which is called stress shielding effect[8]. To overcome that problem, porous structure is arisen. In previous studies, porous titanium and its alloy scaffolds were made. For using freeze casting method, reverse freeze casting[12] and dynamic freeze casting method[13] were used for fabricating porous pure titanium scaffold. But human bone has wide range of mechanical strength and elastic modulus[16, 17]. So still the needs for enhanced strength exist. In this study, we are using Ti6Al4V powder and dynamic freeze casting method to fabricate porous scaffold with enhanced strength with sustained biocompatibility.
2.2. Materials and methods

2.2.1. Fabrication of porous Ti6Al4V scaffold

Ti6Al4V commercial powder (Sejong materials, -200mesh, Korea), commercial pure titanium (Ti) powder (−325 mesh, Alfa Aesar, Ward Hill, MA, USA) and camphene (C₁₀H₁₆, Sigma Aldrich, St.Louis, MO, USA) were used. And oligomeric polyester (HypermerKD-4; UniQema, Everburg, Belgium) was also used at 1wt.% of the slurry as a dispersant. Ti6Al4V slurry was made by 15, 20 and 25 vol% of raw powder and pure Ti slurry was also made by 15 and 25 vol% to be used for in vitro test. Mixture of them was melted at 60°C and rotated in Al mold (60mm for diameter and height) with speed of 30rpm. That mold is kept in 44°C for 12hours. When solidifying process is done, remove the green body from Al mold. And then undergo CIP process at 200MPa for 5minutes. Green body was freeze dried in vacuum chamber at -50°C to remove camphene. After freeze drying, heat treatment was done in vacuum system for temperature increasing speed at 5°C/min and held at 1250°C for 2hours.

2.2.2. Characterization

Porosity of the samples was calculated by density method. Measure the density of the sample and divide it with theoretical density of solid Ti6Al4V. The formula of this calculation is like below.
\[ P = 100 \times \left(1 - \frac{m_s/V_s}{\rho_{Ti6Al4V}}\right) \]

Where : \( m_s \)= mass of the sample, \( V_s \)= volume of the sample

\( \rho_{Ti6Al4V} \) = theoretical density of solid Ti6Al4V (4.42g/cc)

And pore structures were verified by scanning electron microscopy(SEM, JSM-6360, JEOL Techniques, Tokyo, Japan), and constitution of the samples was analyzed by EDS. (Also specimens were analyzed by micro CT(Skyscan 1173 X-ray m-tomography System, Skyscan, Kontich, Belgium) for size and distribution of pores.) Scaffolds for mechanical strength test were prepared at 8mm of diameter and 10mm of height by EDM(electric discharge method) process. And test was done with applying strain speed of 1mm/min. 3 samples were used for the test.

2.2.3. In vitro biological analysis

Two kinds of samples (15vol% and 25vol% for porous Ti6Al4V and porous pure Ti) were prepared to evaluate biological properties by in vitro test using MC3T3-E1 cells (ATCC, CRL-2593; Rockville, MD). All samples were sterilized by autoclave and UV radiation. For the cell attachment test, 8Φ in mm with 1mm in height at each condition was used. Each samples were put in 4well plate. For the cell proliferation test, same size 3 samples with each condition was prepared.
MC3T3-E1 cells were seeded on each samples at a density of $1 \times 10^4$ cells/ml and cultured in a Minimum Essential Medium (α-MEM, Welgene Co, Ltd., Korea) containing 5% Fetal Bovine Serum (FBS, Life Technologies, Inc., USA) and 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin, GIBCO, Grand Island, NY) in a humidified incubator with 5% CO$_2$ at 37 °C. After 3 days of culturing, the morphology of cells was observed using scanning electron microscopy. Prior to the observation, the cells were fixed with 2.5% glutaraldehyde for 10 min followed by dehydration in graded ethanol (70, 95, and 100%). The samples were immersed in hexamethyldisilazane for 10 min then air dried. After 3 days of culturing, cell viability was examined using MTS assay (CellTiter 96 Aqueous One Solution, Promega, USA).

2.2.4. Statistical analysis

The data are presented as the mean ±SE of mean.
2.3 Results and discussion

2.3.1. Structural and mechanical analysis

As illustrated in figure 1, dynamic freeze casting method was adopted to fabricate porous Ti6Al4V scaffold. Morphology and the size of raw Ti6Al4V powder is shown in figure 2. Their size is around 30-50 µm. Microstructure of each porous scaffolds are shown in figure 3. As vol% of the slurry increases, porosity decreases. And also there is a change in pore size. Table 1 lists porosity and pore size of the fabricated scaffolds. Porosity was decreased 71% to 51% and Pore size was also decreased 426±99 µm to 311±66 µm as vol% increases 15 to 25. Of course reduced concentration of freezing vehicle causes reduced porosity. But for pore size, as concentration of powder increases in the slurry, there are less space for camphene nucleation and growth during solidification.

To measure the mechanical properties of each scaffolds, 15 to 25 vol%, compressive strength test was done. In figure 6 and Table 2, higher strength was obtained by using alloy powder [13]. As initial contents of Ti6Al4V powder increases from 15vol% to 25vol%, yield compressive strength is increased with 76±2 MPa to 223±12 MPa and stiffness also increased by 5.0±0.5 GPa to 17.8±1.5 GPa. Structural difference is not that much. So this phenomena is caused by composition of alloy itself. In many literatures, generally alloying induces change in mechanical properties [18]. This is also applied to titanium and its alloys. As titanium becomes Ti6Al4V, elastic modulus is almost maintained. But for yield
compressive strength and ultimate tensile strength, Ti6Al4V is stronger than pure titanium as 120-130% [14]. This is also applied to the porous scaffold. Therefore, stiffness is around same but strength is enhanced.

2.3.2. In vitro biological analysis

For in vitro test, we chose scaffolds with 15vol% and 25vol%. As shown in figure 7, MC3T3-E1 cells are well attached on all specimens. This proves that Ti6Al4V is not harmful for initial attachment of cells. If Ti6Al4V emits cytotoxic elements, cells could not attach to the surface and eventually died. It matches to the other researches over biocompatibility of Ti6Al4V and its usage as a biomaterial [19, 20].

In order to obtain a quantitative biological property, cell proliferation was measured by MTS assay (Figure 8). As seen in cell attachment test, cells proliferate well on each porous scaffolds. The value between porous Ti6Al4V scaffold and porous pure Ti scaffold has no significant difference. Cell viability figure is 0.20±0.02 and 0.20±0.04 for porous pure Ti with 15vol% and 25vol%. For porous Ti6Al4V scaffold, 0.25±0.05 and 0.21±0.04 with 15vol% and 25vol%. Therefore it is also confirmed for longer biocompatibility.
Figure 1. Schematic diagram of dynamic freeze casting.
Figure 2. SEM images of raw Ti6Al4V powder.
Figure 3. EDS analysis of porous Ti6Al4V scaffold, (A) analyzed area, (B) EDS spectrum, and (C) weight percentage and atomic percentage of each element.
Figure 4. SEM images of porous Ti6Al4V scaffold with different content of powders, (A)-(B) 15, (C)-(D) 20, and (E)-(F) 25vol%.
Figure 5. Micro-CT images and pore size distribution of each scaffolds. (A) 15, (B) 20, and (C) 25vol%. Scale bar = 1mm.
Table 1. Pore analysis by micro CT for each sample with different vol% 

<table>
<thead>
<tr>
<th>Vol%</th>
<th>Porosity(%)</th>
<th>Pore size(µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>71±1</td>
<td>426±99</td>
</tr>
<tr>
<td>20</td>
<td>61±1</td>
<td>312±66</td>
</tr>
<tr>
<td>25</td>
<td>51±1</td>
<td>311±66</td>
</tr>
</tbody>
</table>
Figure 6. Mechanical properties of porous Ti6Al4V scaffold with different Ti6Al4V powder vol%, as compared to porous pure titanium scaffolds[13].
Table 2. Porosity and mechanical properties of the porous Ti6Al4V scaffold analyzed by vol% as variables (15, 20, 25 vol%).

<table>
<thead>
<tr>
<th></th>
<th>Porosity(%)</th>
<th>Compressive strength(MPa)</th>
<th>Stiffness(GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 vol%</td>
<td>71±1</td>
<td>76±2</td>
<td>5.0±0.5</td>
</tr>
<tr>
<td>20 vol%</td>
<td>61±1</td>
<td>130±10</td>
<td>11.6±2.4</td>
</tr>
<tr>
<td>25 vol%</td>
<td>51±1</td>
<td>223±12</td>
<td>17.8±1.5</td>
</tr>
</tbody>
</table>
Figure 7. SEM images of MC3T3-E1 cells after 1 day culturing on (A) Ti6Al4V 15vol%, (B) pure Ti 15vol%, (C) Ti6Al4V 25vol%, and (D) pure Ti 25vol%.
Figure 8. Cell viability measured after culturing 3 days by MTS assay on porous pure titanium and porous Ti6Al4V scaffold with 15 vol% and 25 vol%
Chapter 3.

Fabrication of Ti-based functionally graded porous structure
3.1. Introduction

As porous metal scaffolds become widely used, there are still demands for more mimicking bone structure, which has porous central core and denser outer part. Because as structure of the scaffold becomes more similar to that of bones, reduced “stress shielding effect” is expected [21, 22]. So, in recent researches, gradient structure was adopted by several methods[23]. As a solution to that, researchers used metal and polymer combined structure[24] and mix metal with high elastic modulus(Ti) and low elastic modulus(Mg)[25]. Also some rapid prototyping method was used for gradient structure[26, 27]. Especially for freeze casting method, it is really challenging to make porosity gradient in specific shape. As it uses slurries, during melting and solidifying process, all ingredients are mixed. In this study, we use intrinsic ductility of porous titanium scaffold by dynamic freeze casting to fabricate graded porous structure [13]. Shaping two different from of scaffold, which are ring and rod shape. And assemble the into single scaffold by compressing through one direction(z-axis) [28]. This results in two parts with different porosity and pore size, which we call gradient porous structure.

For porous scaffold, drugs like rhBMP or tetracycline hydrochloride can be loaded and released[28-31]. Compressing process after drug loading prolong the release time by capturing functional proteins. Particularly in this two body combination system, two different types of drugs can be loaded and those drugs can be released simultaneously. To specify this concept preliminary, we loaded
GFP (Green Fluorescent Protein) in the scaffold and see the change by confocal laser scanning microscopy (CLSM).

3.2. Materials and methods

3.2.1. Fabrication of functionally graded porous Ti scaffold

Commercial pure titanium (Ti) powder (~325 mesh, Alfa Aesar, Ward Hill, MA, USA) and camphene (C₁₀H₁₆, Sigma Aldrich, St. Louis, MO, USA) were used. And oligomeric polyester (HypermerKD-4; UniQema, Everburg, Belgium) was also used at 1wt.% of the slurry as a dispersant. Ti powder slurry was made by 15vol% for fabricating 70% porous structure. Mixture of them was melted at 60°C and rotated in Al mold (60mm for diameter and height) with speed of 30rpm. That mold is kept in 44°C for 12hours. When solidifying process is done, remove the green body from Al mold. And then undergo CIP process at 200MPa for 5minutes. Green body was freeze dried in vacuum chamber at -50°C to remove camphene. After freeze drying, heat treatment was done in vacuum system for temperature increasing speed at 5°C/min and held at 1300°C for 2hours. After sintering, machined the bulk sample with 2 types, which we call positive when the rod is longer and negative the opposite, and 2 different heights. Precise sample size and applied strain are indicated in Table 2. And then combine them by z-axis to make interlocking
between two different specimens occur. Compressing strain followed the equation in the literature [28]:

$$ F.P. = 1 - \left( \frac{1 - I.P.}{1 - \varepsilon} \right) $$

Where: $F.P.$ = final porosity of the scaffold,

$I.P.$ = initial porosity of the scaffold,

$\varepsilon$ = strain applied to the scaffold with z-axis

### 3.2.2. Characterization

Porosity of the samples was calculated by density method. Measure the density of the sample and divide it with theoretical density of solid pure titanium. The formula of this calculation is like below.

$$ P = 100 \times \left( 1 - \frac{m_s/V_s}{\rho_{Ti}} \right) $$

Where: $m_s$ = mass of the sample, $V_s$ = volume of the sample

$\rho_{Ti}$ = theoretical density of solid Ti (4.51g/cc)

And pore structures were verified by scanning electron microscopy(SEM, JSM-
6360, JEOL Techniques, Tokyo, Japan), and specimens were analyzed by micro
CT(Skyscan 1173 X-ray m-tomography System, Skyscan, Kontich, Belgium) for
size and distribution of pores. Scaffolds for mechanical strength test were prepared
at 12.1mm of diameter and 10mm of height after compressing process. And test was
done with applying strain speed of 1mm/min. 3 samples were used for the test.

3.2.3. Fabrication of protein loaded functionally graded porous Ti
scaffold and its release test

Green fluorescent protein was chosen because it is possible for visualize
distribution and gradual changes. 50µg/ml concentration of green fluorescent
protein (GFP) was used for loading in each ring and rod shaped scaffolds. After
dipping the scaffolds in the solution, put them in the vacuum chamber for 30mins
and leave them in the solution for overnight to make GFP penetrate into the
scaffolds. And then the scaffolds were washed with phosphate buffered saline (PBS)
twice, and dried. Dried scaffolds were compressed to be 12.1mm in diameter and
2mm of height. Put the scaffold in vials which contains 2ml of PBS. The vials are
kept in 37°C for 7days. At every measurement, medium was substituted with clean
one. Variation was checked by CLSM (CLSM, Zeiss-LSM510, Carl Zeiss Inc., NY,
USA) and UV spectrophotometer (UV-1700, Shimadzu, Japan). For UV
spectrophotometry, wavelength of 220nm was selected for measuring.
3.2.4. Statistical analysis

The data are presented as the mean ±SE of mean.

3.3. Results and discussion

3.3.1. Structural analysis

In previous study, pores of compressed porous titanium scaffold lose its initial round shapes and becomes irregular or linear shapes[28]. For combined scaffold, this phenomena is also verified. Static region, which means not compressed, has its intrinsic pores with round shape and size. But compressed region is not.

For the first, the 70%-50% scaffold, SEM images of cross section is represented in figure 10. Pores in the compressed region has irregular shape and reduced sizes. This can be seen through micro CT(figure 11). There are significant change of density over compressed parts. As shown in figure 12 and Table 3, inner part of positive scaffold, which is compressed part, has 52% of porosity and pore size with 242±92 μm. Otherwise outer part has 71% of porosity and pore size with 348±109 μm. Likewise, negative scaffold has similar trends. In figure 12 and Table
3, information of negative scaffold is also shown. Inner part of negative scaffold has 72% of porosity and pore size with 350±83 µm. Otherwise outer part, which is compressed part in negative scaffold, has 52% of porosity and pore size with 215±85 µm.

As more drastic decrease in porosity, which is 70%-30% scaffold, there is bigger difference in porosity and pore size. In the SEM images of the 70%-30% combined scaffold in figure 13, pores with strain become more linear and the wall between pores are thicker than non-compressed pores and pores in 50% porosity. For 30% parts, the difference is clearly shown in micro CT images also (figure 14). In Table 4, quantitative analysis was done for each part. Inner part of positive scaffold, it has reduced porosity with 29% and 160±65 µm of pore size. But in outer part of it, porosity maintains to 71%, and pore size also stand still 328±89 µm. For negative scaffold, inner part is not compressed, so it has 70% of porosity and 298±73 µm of pore size. However, in outer part, porosity and pore size decreased to 28% and 123±57 µm. All of this combination was successfully done by ductility of the porous scaffold itself. As dynamic freeze casting doesn’t use any binders or possible contamination sources, intrinsic metallic ductility is maintained. And during compressing, outer pores can act as mechanical interlocking sites. This also make combination possible without severe cracks or delaminations.

3.3.2. Mechanical analysis
For combined functionally graded porous structure, it is expected to have enhanced mechanical properties[28]. Simply it is because there are more dense part when it is compared to homogeneous porous structure. Further, it comes from interlocking effect between two combined parts. This expectation is proved in figure 16. Compared to scaffolds with homogeneous porosity of 70%, all combined scaffolds has higher mechanical properties. Also, for the higher strained scaffolds (combined with lower porosity), the stronger mechanical properties is obtained. Yield compressive strength and stiffness values for 70%-50% scaffolds are 79.2±1.4 MPa, 2.9±0.2 GPa for positive and 87.0±4.7 MPa, 3.9±1.1 GPa for negative scaffold. And increased mechanical properties are obtained for 70%-30% scaffolds with 190.3±13.0 MPa, 10.2±1.1 GPa for positive and 250.6±17.7 MPa, 14.8±1.1 GPa for negative scaffolds. When it is compared to homogeneous 60% and 50% porous scaffolds,

3.3.2. Prolonged release observed by GFP

After GFP loading in the separate scaffolds, compressed scaffolds have trapped GFP inside. As time goes by, GFP in more porous part come out earlier and faster. However, GFP in compressed region releases slower. When the scaffolds are analyzed by CLSM, there are differences between homogeneous 70% scaffolds and combined ones. In figure 17, before scaffolds are immersed in PBS, all specimen glitters green light. But after 7 days, intensity of green light decreased through all
specimens. Among them, compressed region has higher intensity of green light. This is because trapped GFP releases slowly and steady. This difference release behavior can be applied to further applications. If the scaffolds need fast antibiotics release and steady release of growth factor, just put growth factor in the scaffolds which are compressed later and put antibiotics in the other region. From this method, we can adjust scaffolds’ characteristics to their incision sites.
Figure 9. Schematic diagram of dynamic freeze casting and combination method.
Table 3. Size of scaffolds before compression and applied strain.

<table>
<thead>
<tr>
<th></th>
<th>Ring height (mm)</th>
<th>Rod height (mm)</th>
<th>Strain (ε)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% (diameter 12.1mm)</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>70%-50% positive</td>
<td>10</td>
<td>17</td>
<td>0.41</td>
</tr>
<tr>
<td>70%-50% negative</td>
<td>17</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>70%-30% positive</td>
<td>10</td>
<td>23</td>
<td>0.57</td>
</tr>
<tr>
<td>70%-30% negative</td>
<td>23</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>
Figure 10. SEM images of 70%-50% combined scaffolds. (A)-(C) positive and (D)-(F) negative. (A),(D) interfaces, (B),(E) inner parts and (C),(F) outer parts.
Figure 11. Micro CT images of (A) 70%-50% positive and (B) 70%-50% negative scaffold.
Figure 12. Pore size distribution analysis by micro CT with 70%-50% scaffolds, (A) inner part of positive, (B) outer part of positive, (C) inner part of negative, and (D) outer part of negative.
Table 4. Porosity and pore size of each 70%-50% scaffold with different parts.

<table>
<thead>
<tr>
<th>Combination</th>
<th>Part</th>
<th>Porosity(%)</th>
<th>Pore size(μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (70%-50%)</td>
<td>Outer</td>
<td>71</td>
<td>348±109</td>
</tr>
<tr>
<td></td>
<td>Inner</td>
<td>52</td>
<td>242±92</td>
</tr>
<tr>
<td>Negative (70%-50%)</td>
<td>Outer</td>
<td>52</td>
<td>215±85</td>
</tr>
<tr>
<td></td>
<td>Inner</td>
<td>72</td>
<td>350±83</td>
</tr>
</tbody>
</table>
Figure 13. SEM images of 70%-30% combined scaffolds. (A)-(C) positive and (D)-(F) negative. (A),(D) interfaces, (B),(E) inner parts and (C),(F) outer parts.
Figure 14. Micro CT images of (A) 70%-30% positive and (B) 70%-30% negative scaffold.
Figure 15. Pore size distribution analysis by micro CT with 70%-30%
scaffolds, (A) inner part of positive, (B) outer part of positive, (C) inner part of negative, and (D) outer part of negative.

Table 5. Porosity and pore size of each 70%-30% scaffold with different parts.

<table>
<thead>
<tr>
<th>Combination</th>
<th>Part</th>
<th>Porosity(%)</th>
<th>Pore size(µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (70%-30%)</td>
<td>Outer</td>
<td>71</td>
<td>328±89</td>
</tr>
<tr>
<td></td>
<td>Inner</td>
<td>29</td>
<td>160±65</td>
</tr>
<tr>
<td>Negative (70%-30%)</td>
<td>Outer</td>
<td>28</td>
<td>123±57</td>
</tr>
<tr>
<td></td>
<td>Inner</td>
<td>70</td>
<td>298±73</td>
</tr>
</tbody>
</table>
Figure 16. Mechanical properties of scaffolds with homogeneous 70% porosity, combined 70%-50% scaffolds and combined 70%-30% scaffolds.
Table 6. Yield compressive strength and stiffness of each scaffolds.

<table>
<thead>
<tr>
<th>Combination</th>
<th>Yield compressive strength (MPa)</th>
<th>Stiffness (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% only</td>
<td>55.7±2.3</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>70%-50% positive</td>
<td>79.2±1.4</td>
<td>2.0±0.3</td>
</tr>
<tr>
<td>70%-50% negative</td>
<td>87.0±4.7</td>
<td>2.1±0.6</td>
</tr>
<tr>
<td>70%-30% positive</td>
<td>190.3±13.0</td>
<td>4.2±0.2</td>
</tr>
<tr>
<td>70%-30% negative</td>
<td>250.6±17.7</td>
<td>4.8±0.2</td>
</tr>
</tbody>
</table>
Figure 17. CLSM analysis for releasing behavior, (A) 70% homogeneous porous scaffold, (B) 70%-50% combined scaffold, and (C) 70%-30% combined scaffold. Right side is initial state and left side is after 7 days of release.
4.1. Ti-alloy based macro/micro porous scaffold with enhanced mechanical properties
Porous Ti6Al4V scaffold was successfully fabricated by dynamic freeze casting method with Ti6Al4V powder. As alloyed powder itself has higher yield strength and ultimate tensile strength, porous scaffold itself also has higher yield strength. But it has still similar stiffness with porous pure Ti scaffold. This makes porous scaffold usages for wider range. Because as bone has various mechanical strength to each parts, we can adopt metal scaffold to stronger part with porosity is almost same. And with biological analysis, cells can attach and proliferate with similar trend in pure Ti and Ti6Al4V scaffolds. This means bone regeneration through pores are almost same with each scaffolds. Therefore porous Ti6Al4V scaffolds can widen the capability of metal implant usages.

4.2. Fabrication of Ti-based functionally graded porous scaffold

Combined structure with different porosity can be made by compressible
porous Ti scaffold with its intrinsic ductility. As mimicking the structure of bone, stress shielding effect can be reduced. Also, enhanced mechanical properties with this combination method make it possible to wider range of implantation. And for its unique structure, porous metal scaffold can adjust to its implanting site. For inner porous scaffold, it is more similar to the structure of bone. Outer porous scaffold can be used in the region which needs earlier bone formation from outer side. In this study, just 50% and 30% of porosity was generated by two body compressing. So there are still possibilities for lower porosity with different objectives and more combination parts can be adopted like three or more parts. Therefore, this combination method has great potential as wider functionally graded structure for porous scaffolds.

References


[9] Nishimura N, Kawai T. Effect of microstructure of titanium surface on the behaviour of osteogenic cell line MC3T3-E1. Journal of Materials Science:


초록

52
우리가 살아가면서 사회가 고령화 사회로 접어들어, 의료기기 및 임플란트에 대한 요구가 증가하고 있다. 고분자, 세라믹 그리고 금속으로 구분되는 여러 의료용 임플란트가 있다. 고분자 임플란트는 신축성을 가지고 있으며, 몇몇은 생분해성도 가지고 있어 추가수술이 필요하지 않다. 그러나 고분자 임플란트는 기계적 물성이 낮아 경조직 대체에는 쓰일 수가 없다. 반면 세라믹 임플란트는 높은 기계적 물성과 화학적 안정성을 가지고 있으나 본질적인 메질성으로 대표되는 문제를 가지고 있다. 그것 때문에, 세라믹 임플란트는 강한 압력이 가해지는 부분에는 쓰일 수가 없다. 금속 임플란트의 경우, 높은 기계적 물성과 강한 내마모성으로 주목받고 있으며, 금속 임플란트는 연성을 가지고 있어 부리지는 부분에 대한 걱정을 줄일 수 있다. 그리고 탄날륨과 티타늄 및 티타늄 합금과 같은 생체적합성을 가진 금속 역시 존재한다. 안타깝게도 금속 임플란트는 그 자체로 골 대체제로서 쓰이기에는 너무 강하다. 이런 기계적 물성의 차이가 임플란트 주위의 뼈가 녹아버리는 "응력 차폐 현상"을 유발한다.

이 문제를 해결하기 위해서, 다공성 구조가 폭넓게 연구되고 있다. 금속 임플란트 내에 기공을 만드는 것은 기계적 물성을 낮추어 뼈와 비슷하게 할 수 있고, 세포가 매끈한 표면보다 거친 표면을 선호한다는 사실 또한 이식용 재료 내의 기공의 생성을 지지한다. 더욱이 기공은 뼈
의 재생 장소로서 역할을 할 수 있으며, 이는 뼈와 임플란트의 맞물림을
유도한다. 이러한 다공성 구조를 제조하는데는 여러 방법이 있다. 고분자
구조 복제법, 분말 야금, 동결 주조법 그리고 동결 주조법이 그것이다. 동결
주조법의 경우, 여러 변형이 있는데, 고정식 동결 주조, 역방향 동결 주
조, 그리고 동적 동결 주조법이 있다. 고정식 동결 주조법은 주로 세라믹
분말과 같은 매우 작은 분말을 주로 이용하고 금속 분말과 같은 큰 분말
에서의 충분리가 일어난다. 다른 두 방법은 금속 분말을 이용하는데에 대
한 해결방법이 될 수 있는데, 우선 역방향 동결 주조법은 중력을 이용한
다. 승화 가능한 매체를 이용하여 다공성 구조를 만든 후 금속 분말이 기
공을 채운다. 이 방법을 통해서 정렬된 기공을 가진 다공성 금속 스카플
드를 제조할 수 있다. 동적 동결 주조법은 회전을 도입하여 승화성 매체
의 직선적인 수지상 성장을 방해한다. 회전을 통하여 매체의 성장을 전방
향으로 유도하여 최종적으로 구형의 기공을 생성하게 한다. 이 방법은 연
성을 유지한 다공성 금속 스카플드의 제조를 가능하게 한다.

더 폭넓은 응용을 위하여, 우리는 기계적 물성의 증가와 기능적
단계 기공 구조를 만드는데 동적 동결 주조법을 적용하였다. 첫 연구에서
는 Ti6Al4V 분말을 이용하여 기계적 물성을 증가시켰으며, 세포 실험을
통해 생체적합성을 확인하였다. 분말의 부피적 함량이 15% 에서 25%
로 증가함에 따라 기공률 및 기공의 크기가 71% 에서 51%, 426 µm 에
서 311 µm 로 감소함을 확인하였다. 기계적 물성 역시 분말 포함량의 증가에 따라 증가하였다. 부피적 함량이 15%에서 25%로 증가함에 따라, 압축량도 강도 및 강성이 76 MPa에서 223 MPa로, 5.0 GPa에서 17.8 GPa로 증가하였다. 이는 기존의 다공성 티타늄 스캐폴드의 기계적 물성보다 증가한 수치이다. 세포 실험을 위하여 조골세포(MC3T3-E1)를 이용하였으며, 초기 세포의 부착성과 세포의 생존 능력이 다공성 Ti6Al4V 스캐폴드와 다공성 티타늄 스캐폴드에서 유사함을 확인하였다. 이런 근거에서 생체적합성을 유지하면서 더 강한 다공성 금속 스캐폴드의 제조가 성공적으로 이루어졌다는 것을 확인하였다.

다공체 분야에 있어서, 여전히 다공성 부분 및 밀도가 높은 부분이 혼재하는 실제 뼈의 구조를 모사하는데 대한 요구가 존재한다. 그래서 두번째 연구에서는 다공성 티타늄 스캐폴드의 연성을 이용하여 기능적 단계 기공 구조를 제조하였다. 서로 다른 높이를 가진 고리 형태 및 막대 형태의 스캐폴드를 준비하였고, 막대 형태가 긴 경우에는 positive, 고리 형태가 긴 경우는 negative로 표현하였다. 막대 구조의 스캐폴드를 고리 형태의 스캐폴드 내에 집어넣은 후, 같은 높이가 될 때까지 압축하였다. 두 경우에 대하여 밀집화 되는 정도는 같았다. 본 연구에서 기공률 70%-50% 와 70%-30% 가 합쳐진 다공성 스캐폴드가 성공적으로 제조되었다. 70%-50% 혼재 구조의 경우, 압축되지 않은 부분에서는 약
350 µm, 압축 된 부분에서는 225 µm 정도의 기공 크기를 가진다. 기계적 물성 역시 균일한 70% 다공성 스캐폴드에 비하여 증가하였는데, positive 구조에서는 압축항복강도가 79 MPa 이고, 강성이 2.9 GPa 으로 나타났으며, negative 구조에서는 위와 같은 물성이 87 MPa 과 3.9 GPa 으로 나타났다. 70%~30% 혼체 구조의 경우, 기공 크기는 압축된 부분에서 더욱 감소하여 약 140 µm가 되었다. 기계적 물성 역시 더 증가하여 positive에서는 190 MPa 와 10.2 GPa, negative에서는 250MPa 와 14.8 GPa 로 나타났다. 압축 이후, 기공의 형태에도 변화가 있었다. 더 많이 압축될수록, 기공의 형상이 더욱 직선적으로 변화하였다. 녹색형광단백질의 담지 역시 가능하였고, 압축된 부분에서 더 오랫동안 지속적인 방출이 가능함을 확인하였다.

결론적으로, 이 두 가지 실험을 통해서 더 다양한 다공성 티타늄 및 티타늄 합금의 쓰임이 가능해졌다. 다공성 Ti6Al4V 스캐폴드에서는 증대된 기계적 물성 및 유지된 생체적합성이 확인되었다. 그리고 조합 및 압축 방법을 통하여 뼈의 구조 모사 및 기계적 물성의 증대가 이루어졌고, 약물의 담지 가능성을 확인하였다.
주요어: 티타늄, 티타늄 합금, 동적 동결 주조법, 제어된 기공구조, 기능적 단계 구조

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