의학석사 학위논문

3 차원-패터닝 스마트 수화젤의 생체내 골재생 및 혈관신생능 평가

In vivo study of bone regeneration and angiogenesis by 3D-patterned smart hydrogel

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지 도 교 수　　이 부 규

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2018 년 12 월

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English Abstract

In vivo study of bone regeneration and angiogenesis by 3D-patterned smart hydrogel

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Introduction

To treat large and complex bone defects of maxillofacial area is challenging situation in clinic. In bone-tissue engineering, biomaterials alone or in combination with suitable biological and chemical factors are used to restore the bone tissue. As a carrier of cells and growth factors, we introduced matrix metalloproteinase (MMP) sensitive acrylated hyaluronic acid (HA)-based smart

hydrogel system(SHS) in previous study. In this study, we combined this SHS into 3D bioprinting technique to fabricate scaffold that can be used at various bone defect in clinical situation. And we adapted different kinds of smart hydrogel containing substance P (SP) peptide or/and bone morphogenic proten-2 (BMP-2) as a bio-inks for 3D bioprinting. Overall objective of this study is to develop the customized scaffolds which can be clinically applicable.

Materials and Methods

Subcutaneous pockets were made to the right and left of two incisions on their backs. The experimental 3D patterned smart hydrogels were implanted into the dorsal skin of each rat to the left side, and right side was set as a negative control group that nothing was grafted except pocket making. The implanted site's specimens were harvested a month after the surgery.

A full-thickness bone defect was created between frontal bone and parietal bone, using a hallow trephine bur with a 6 mm outer diameter. The defect area

was either left empty, or was filled with experimental 3D patterned smart hydrogel. The animals were sacrificed 4 weeks later under a CO2 chamber.

Results

In the smart hydrogel with SP peptide patterning group, there was a clear angiogenesis in the pocket area. Subcutaneous tissue was rather thicker than the control group. Also, much bigger vessels were infiltrated and the number of vessels was observed more than that in the only HA hydrogel group. It was clear that the smart hydrogel with SP and BMP-2 patterning group showed more bone formation than the positive control group, resulting in much smaller size of bone defect. And histologic evaluation confirmed this result. An inflammatory response was not observed around the scaffolds in any group.

Conclusion

In this study, we fabricated scaffolds using different types of smart hydrogel as a bio-ink for 3D bioprinting. This smart hydrogel scaffold can contain chemical cue that promotes angiogenesis or new bone formation. In addition, scaffold can

contain more than one type of chemical cue so that different pathways of growth promotion can occur simultaneously at the graft site successfully.

Key words: Scaffold, Bone regeneration, Hydrogel, Patterning, 3D bioprinting, Bio-ink, Bone tissue engineering, Bone morphogenic protein-2 (BMP-2), Substance P (SP)

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Introduction

Traditionally, bone fractures and defects made due to injury or disease are treated using temporary or permanent implants[1]. A major concern regarding repairing bone defects is absence of an adequate implant to promote or accelerate bone regeneration process. Bone regeneration can be promoted by grafting of biodegradable and biocompatible scaffolds on which bone is newly formed by creeping substitution from nearby adjoining living bone[2]. Many materials, including natural or synthesized bones, were adopted for bone augmentation technique. Autograft showed a lower failure rate than allograft, but autologous bone graft has limitation because of donor site morbidity and limited availability. As potential alternative to autograft, the application of allograft is restricted due to the immunogenic response. Thus, tissue engineering to handle the deficiency of tissue is becoming of increasing interest and the fabrication of scaffold for better tissue regeneration has drawn a great deal of attention.

Biomaterials alone or in combination with adequate chemical and biological cues are adopted to restore the bone in tissue engineering field[3]. In this circumstances, implantation of scaffold containing various cells or growth factors that are fit to defect have been used to accelerate bone regeneration[4,5]. Besides, presence of a bioactive material, such as growth factors promoting bone regeneration or autologous bone harvested from the patient's own body, can be helpful in bone regeneration[6,7]. There have been many researches on the effects of osteogenic supplements. When Bone Morphogenic Protein-2 (BMP-2) was incorporated with hydrogel, it was proved that bone regeneration was undoubtedly increased in bone defect model as compared with hydrogel alone[8]. And angiogenic peptides, such as substance P (SP), are also used in immobilized hydrogels to induce angiogenesis. Kohara et al.[9] confirmed that SP incorporated into hydrogel induces angiogenesis by the enhanced recruitment of angiogenic cells. Kim et al.[10] suggested that SP could promote angiogenesis through its recruitment of autologous mesenchymal stem cells (MSCs).

As a carrier of cells and growth factors without side effects such as inflammation reaction, we introduced that matrix metalloproteinase (MMP) sensitive acrylated hyaluronic acid (HA)-based SHS can be useful in bone tissue engineering in our previous studies[11,12]. This SHS can assist tissue regeneration by imitating the natural human extracellular matrix (ECM) and simultaneously containing various osteo-progenitor cells or osteo-inductive factors as well. And also, rapid ingrowth of the host vessels is critical trait for regeneration to make fully functional tissues with proper oxygen and nutrient supply[13]. Cells in regenerating tissue should be located within 200 μm around blood vessels for survival and adequate growth[14]. However, the accelerating of blood vessel network formation to defect area by using only angiogenic cues is restricted by the volume and size of the carrier system. To overcome this hurdle, diverse attempts have been made to fabricate microchannels in hydrogel that can be applied to large scale tissue regeneration. Manufactured microchannels play an important role in delivering oxygen and nutrients to the cells in the hydrogel to

improve cell viability and function. This is an important approach to surmount the limitation of hydrogel, which are difficult to use for large-scale tissue regeneration owing to limited vascularization. In our previous research, It was confirmed that fabrication of scaffold with microchannels using this SHS is a great approach to mimic the vascular structure for the regeneration of large tissue defects and all the vessels can go through along the microchannels in the matrix[15]. In this study, we adopted 3D printing techniques to fabricate microchannel structure in our scaffold.

3D bioprinting techniques have been drawing attention because of the usefulness of guaranteed interconnectivity and solid freeform fabrication. 3D printing can construct scaffolds by using currently used medical images (such as computerized tomography scan, X-ray and magnetic resonance imaging) using computer-aided design. Custom, patient-specific design and high structural complexity are some of the significant benefits of 3D printing making it very useful for tissue engineering[16,17]. 3D bioprinting is a technology to fabricate

constructs from living cells with or without a carrier material in a layer by layer manner. The material that is printed is referred to as a "bio-ink," which can be characterized as an ink formulation allowing printing of living cells[18,19].

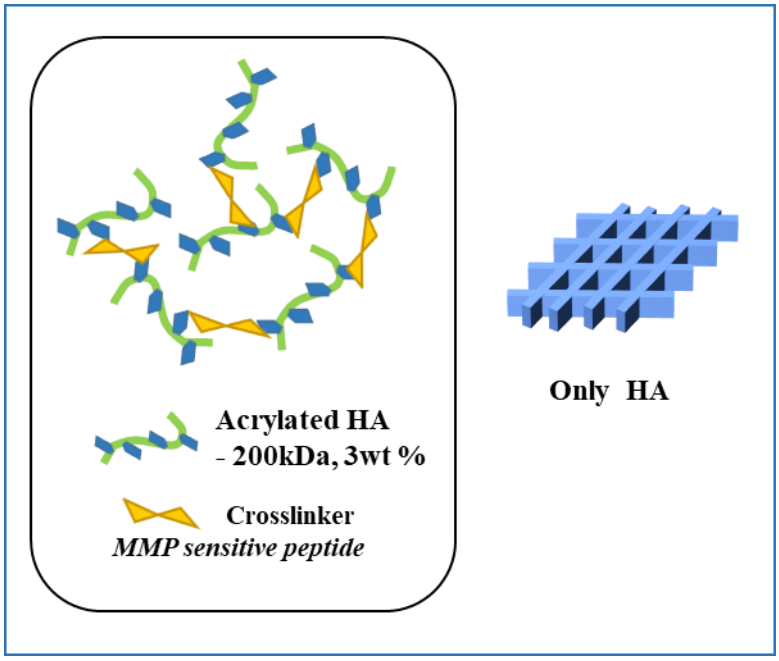
In this study, we adapted different kinds of smart hydrogel mentioned above as a bio-ink for 3D bioprinting. We prepare two kinds of smart hydrogel with BMP-2 and/or SP peptide patterning. Using these different kinds of smart hydrogel as a bio-ink, we made a 3D bioprinted scaffolds in a layer by layer fashion. Overall objective of this study is to develop the customized scaffolds which can be clinically applicable. Angiogenesis and bone regeneration are assessed 4 weeks after grafting our scaffold to Sprague Dawley (SD) rats.

Material and Methods

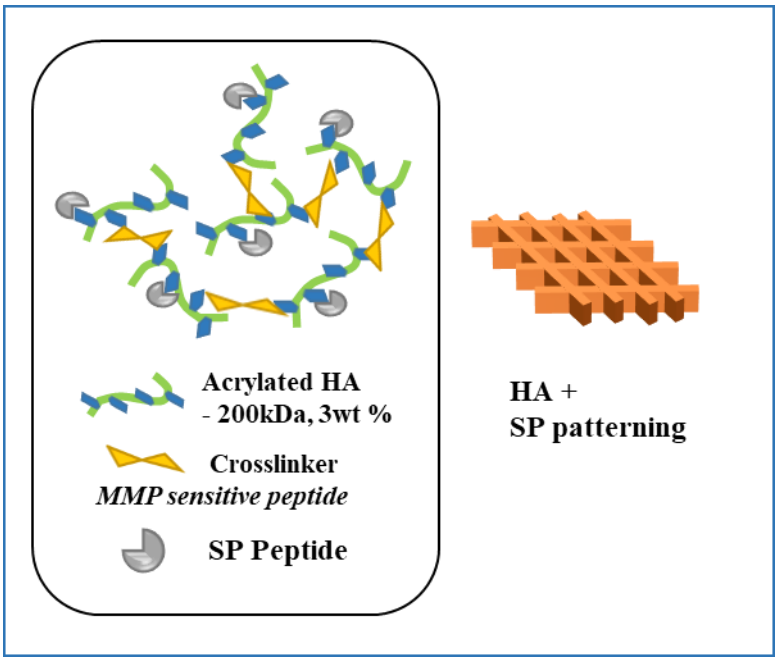
Preparation of HA smart hydrogel

The acrylation of HA followed that of a previous study[8]. Synthesized acrylated HA (200 kDa, 3 wt. %) was liquefied in a 0.3 M TEA-buffered solution. An MMP-

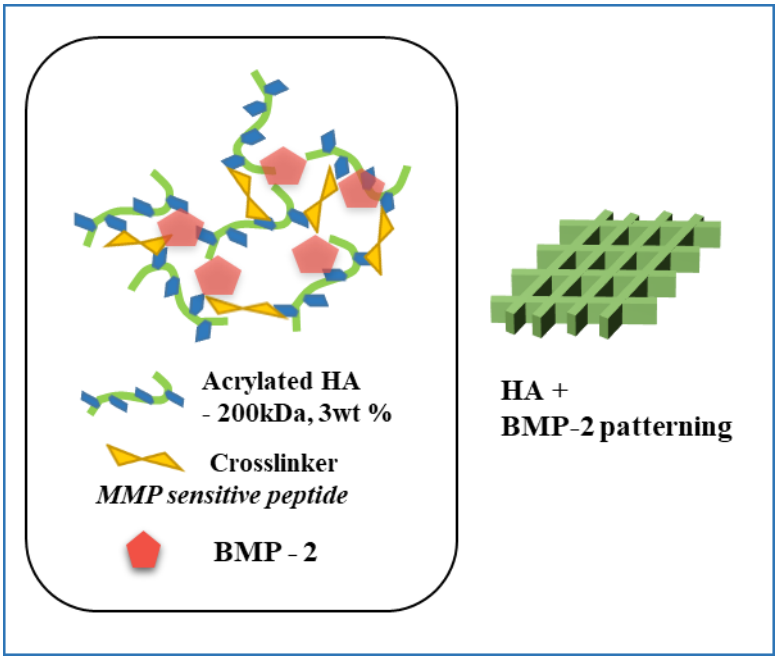
sensitive peptide was superinduced to an acrylated HA solution as a cross-linker with the same molar ratio for gel preparation. The HA hydrogel was formulated via a Michael-type addition reaction (Fig. 1A). smart hydrogel was invented by which SP was immobilized in 20% molar ratio to acrylated HA and BMP-2 was incorporated into a HA hydrogel at a concentration of 20 μg / ml. (Fig. 1B, 1C)


Acrylated HA
- 200kDa, 3wt %
Crosslinker
MMP sensitive peptide
Only HA

A


Acrylated HA
- 200kDa, 3wt %
Crosslinker
MMP sensitive peptide
SP Peptide
HA +
SP patterning

B



C

Figure 1. Schematic images of 3D-patterned smart hydrogel. A. Molecular structure of the smart hydrogel, B. molecular structure of the smart hydrogel with SP peptide patterning, C. molecular structure of the smart hydrogel with BMP-2 patterning.

Fabrication of a scaffold using the 3D bioprinting technique

The smart hydrogel was carefully injected into the glass syringe (Hamilton syringe, 50 ㎕) so that trapped air do not occur in the syringe. smart hydrogel in the glass syringe was heated in the 80°C hot plate for 10 minutes to achieve proper viscosity for 3D printing. And we cool down the glass syringe containing the heated smart hydrogel in the freezer for 10 minutes, because glass syringe was heated together at the same time during hot plate heating. If the glass syringe does not cool down, gelation of smart hydrogel continues with the temperature of the glass syringe itself during 3D printing procedure, making it clog the nozzles of 3D printer. Two glass syringes with different type of smart hydrogel were located on each printer head and the following distance of coordinates were measured: 1. Position of the Z-axis at the end point of each nozzle to compensate its difference. 2. Position of the X and Y-axis of each head of printer. And then, to fabricate scaffold with microchannel in a layer by layer fashion using different type of bio-inks, G-code was coded reflecting X, Y, Z-axis coordinate distances of each head.

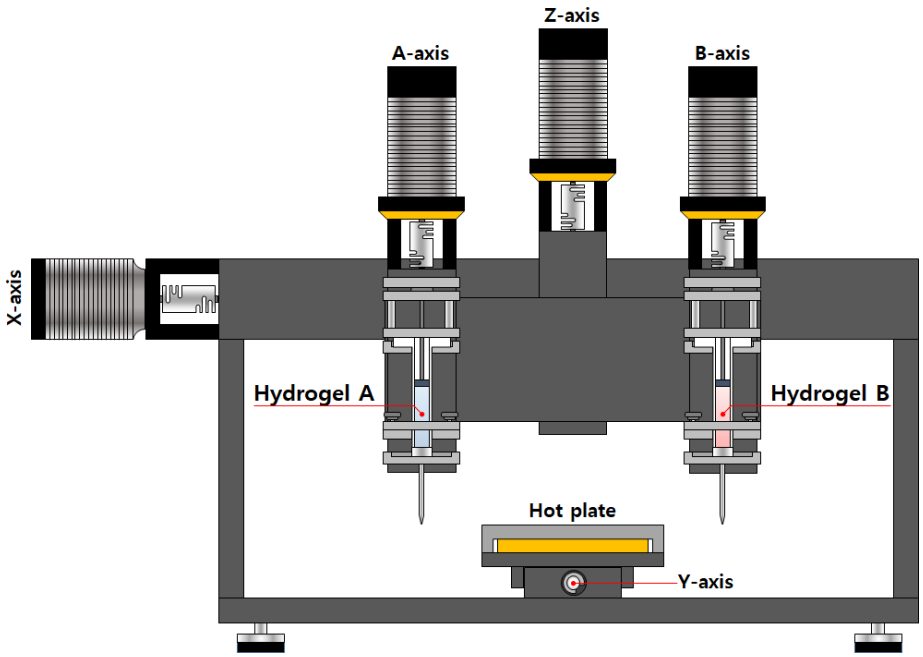
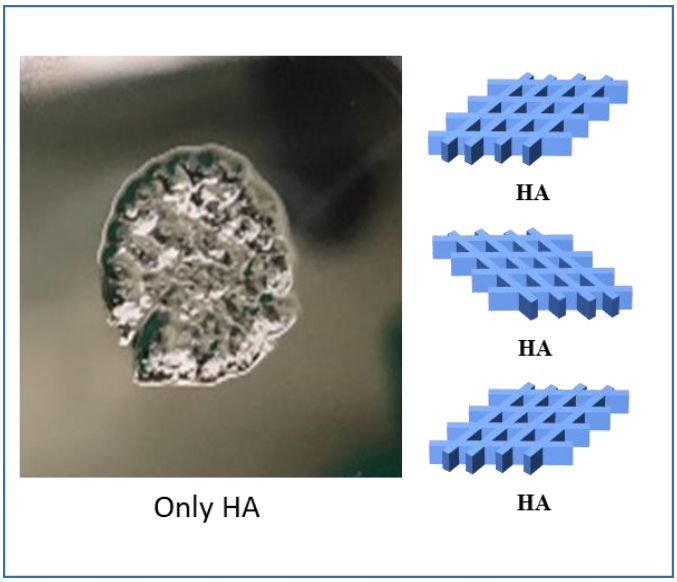


Figure 2. Schematic image of 3D bioprinting machine to fabricate scaffold using different kinds of smart hydrogel(A, B) in a layer by layer fashion.

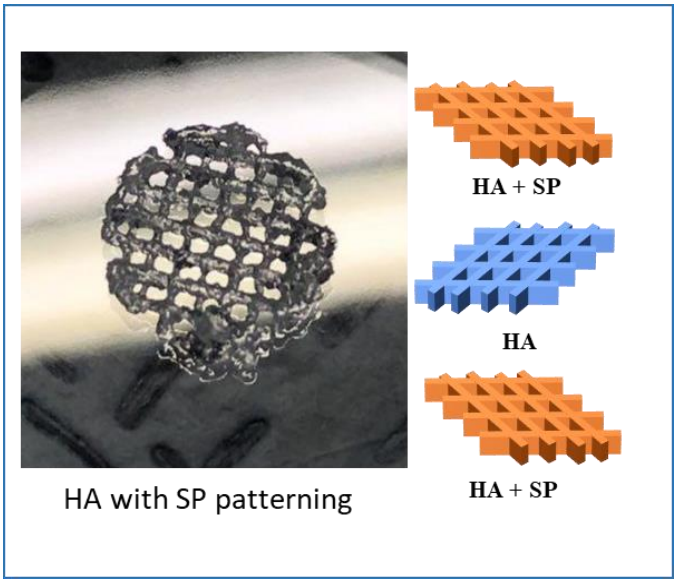
In vivo rat subcutaneous pocket model for evaluation of angiogenesis

We conducted experiment procedures following the Guidelines for the Care and Use of Laboratory Animals provided by the Asan Medical Center institutional review board. 6 Male Sprague Dawley (SD) rats (8-week-old, average weight 180g) were obtained from OrientBio (Sungnam, Kyungkido, Korea). All rats were housed in cages with free access to food and water. Anesthetic induction was done by intraperitoneal injection of Zoletil inj. (20-40mg/kg) mixed with Rompun inj. (10mg/kg). The back of each rat was shaved and disinfected with a routine antiseptic solution. A local anesthetic (1% lidocaine with epinephrine 1:100,000) was injected for hemostasis and to reduce pain. Subcutaneous pockets were made to the right and left of two incisions on their backs. The experimental 3D patterned smart hydrogels (Fig.3A, 3B) were implanted into the dorsal skin of each rat to the left side, and right side was set as a negative control group that nothing was grafted except pocket making. Total 2 rats were used for this subcutaneous pocket model study (Fig. 4). After creating the defects and grafting

the scaffolds, 4-0 black silk was used to suture the overlying skin. Routine antibiotics and analgesics were prescribed postoperatively to prevent infection and release pain after surgery. The implanted site's specimens were harvested a month after the surgery. The implanted scaffolds were retrieved and fixed in 10% formalin.



A



B

Figure 3. Schematic and optic image of scaffolds used in subcutaneous pocket model. A. Positive control group : Only HA hydrogel group. B. smart hydrogel with SP peptides patterning

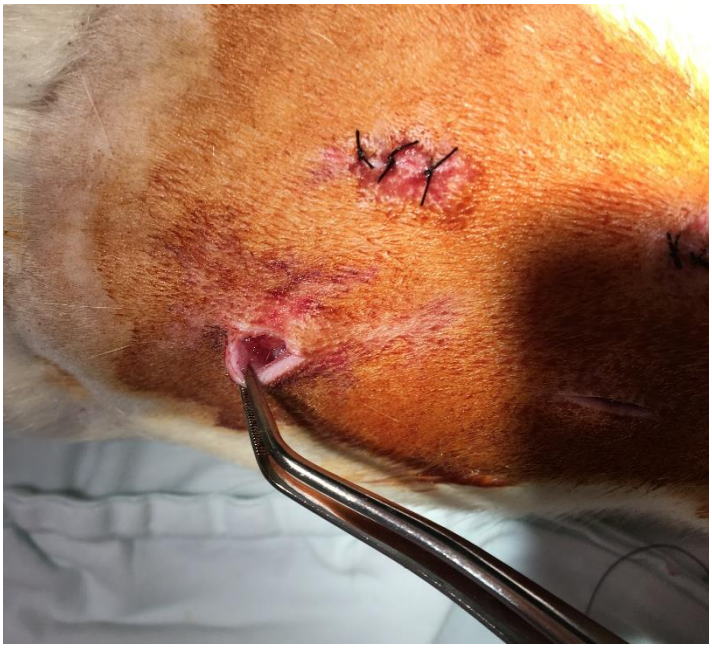


Figure 4. Surgery for creating subcutaneous pocket model.

In vivo rat calvarial defect model for evaluation of bone regeneration

Male SD rats (n = 3), aged 8 weeks, were used for this experiment. Same anesthetic induction and local anesthetic was done as in vivo subcutaneous pocket model experiment. Scalp of each rat was shaved and disinfected with a routine antiseptic solution. A sagittal incision was done using a No. 15 blade along the sagittal suture, and the periosteum was elevated. Overlying tissue was dissected to expose the cranial bone. A full-thickness bone defect was created between frontal bone and parietal bone, at the central part of the cranial bone, using a hallow trephine bur with a 6 mm outer diameter without a dura perforation (Fig. 5). The defect area was either left empty, or was filled with each hydrogel. According to experiment design, in the negative control group, we grafted nothing after making calvarial bone defect. In the positive control group, we graft only HA hydrogel scaffold (Fig. 3A) at the defect to compare the effects of smart hydrogel scaffold with BMP-2 and SP peptide patterning (Fig. 6). The animals were sacrificed 4 weeks later under a CO2 chamber.

Figure 5. Surgery for creating calvarial defect model.

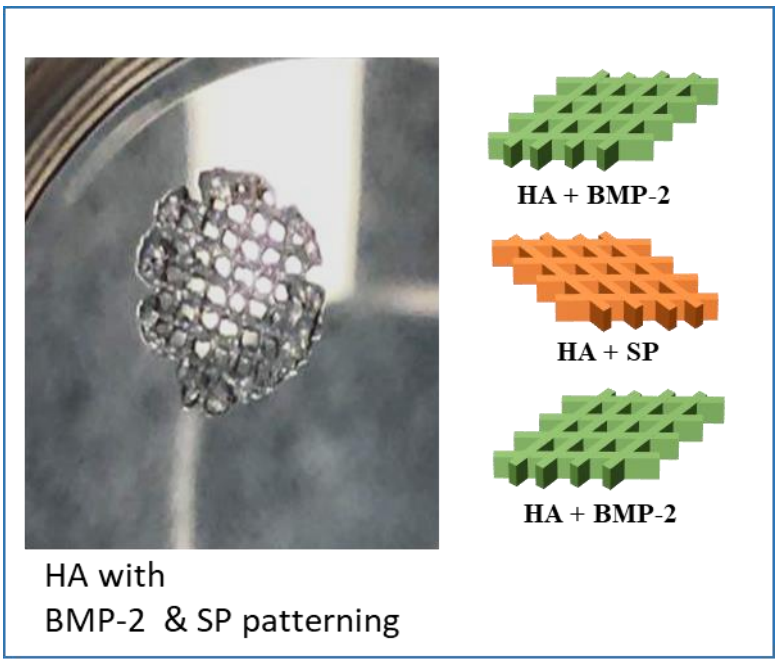


Figure 6. Schematic and optic image of scaffold used in calvarial defect model.

Histological evaluation

The animals were sacrificed 4 weeks after surgery under a CO2 chamber. The operative field at the calvaria was removed from the skull and it was decalcified by incubating in EDTA solution (7%, pH ¼ 7.0) for 3–4 days changing 2 days after fixing in 10% formalin overnight, dehydrated in 70% ethanol and embedded in paraffin. For histochemical analysis, paraffin sections were done in the midline of calvarial defect, 2 sections in each groups, and they were fixed for 10 min with xylene and stained with H&E (Sigma). Digital images from stained sections were taken by means of a transmission and polarized light Axioskop Microscope, Olympus BX51 (Olympus corporation, Tokyo, Japan).

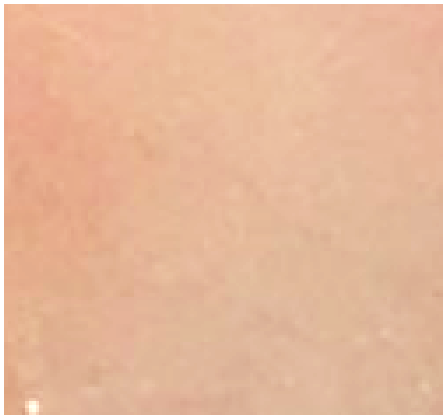
Results

Angiogenesis analysis

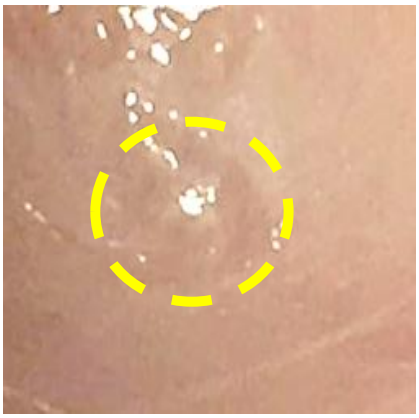
To make a biomimetic hydrogel, HA, which is one of the major components of ECM, was acrylated, and MMP-sensitive peptides degradable by cell-secreted

MMP were used as a cross-linker[12]. Angiogenic peptide SP was immobilized on the acrylated HA to induce angiogenic activity in the hydrogel. 3D bioprinting technique was used to fabricate microchannels in the scaffold. To assess angiogenesis and vascularization, simple visual inspection and histological analysis were done.

As shown in Fig. 7B, in the only HA hydrogel group, no noticeable difference was observed visually. But, in the smart hydrogel with SP peptide patterning group (Fig. 7C), there was a clear angiogenesis in the pocket area(marked by red triangle). To confirm this visual inspection histologically, hematoxylin and eosin (H&E) staining was performed. As shown in Fig. 8B, in the only HA hydrogel group, subcutaneous tissue was rather thicker than the negative control group (Fig. 8A) and small vessels were observed in images. In the smart hydrogel with SP peptides patterning group (Fig. 8C), almost same pattern was observed, but the difference is that infiltrated vessel was much bigger and the number of vessels was more than that of only HA group.



A



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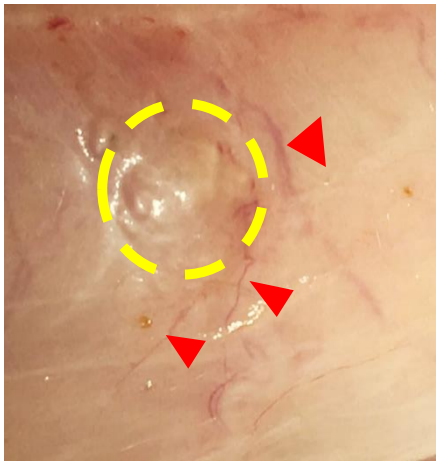
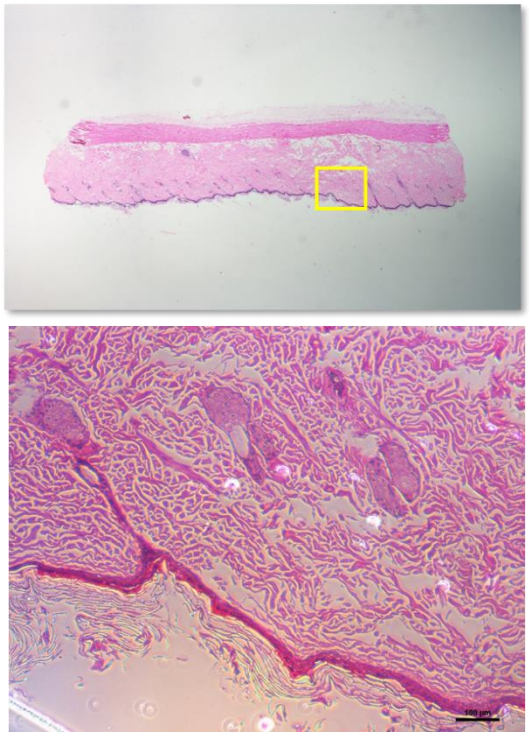


C

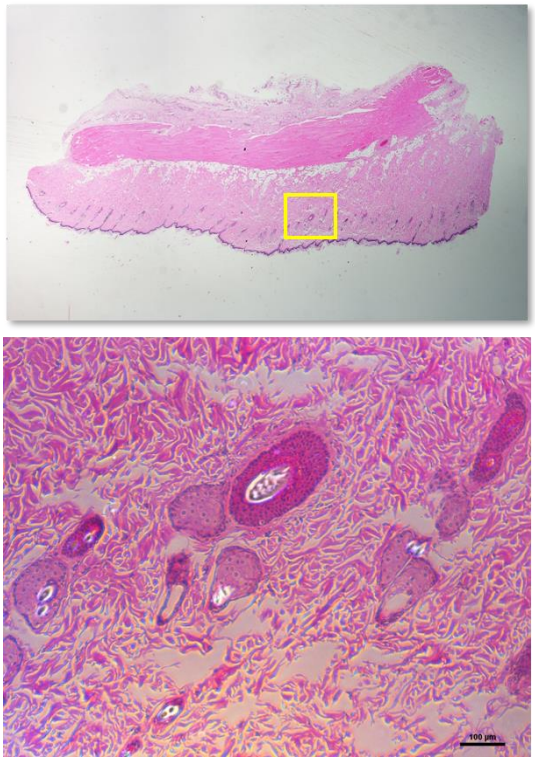
Figure 7. Image of grafted site after 4 week in subcutaneous pocket model. A. Negative control group. B. Positive control group : Only HA hydrogel group. C. smart hydrogel with SP peptide patterning group.

CONTROL



A

Only HA



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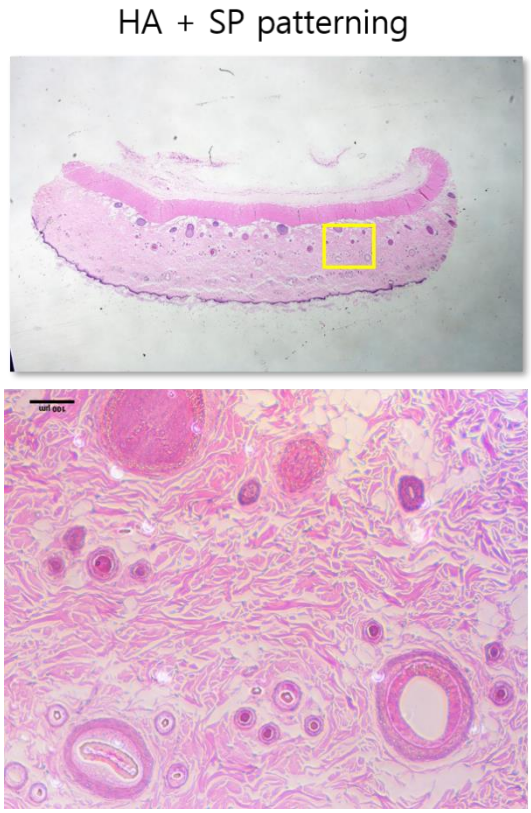


C

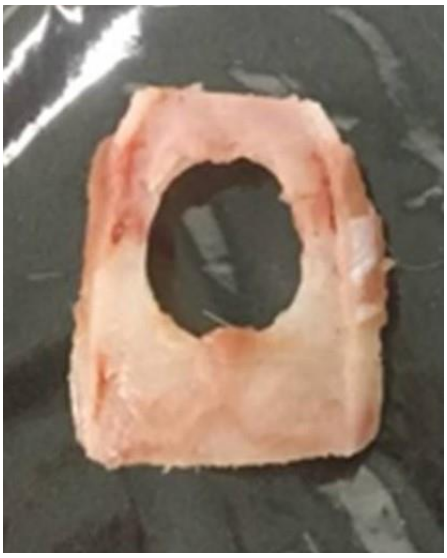
Figure 8. Hematoxylin and eosin-stained image after 4 weeks in subcutaneous pocket model. A. Negative control group. B. Positive control group: only HA hydrogel group. C. smart hydrogel with SP peptide patterning group.

Bone formation analysis

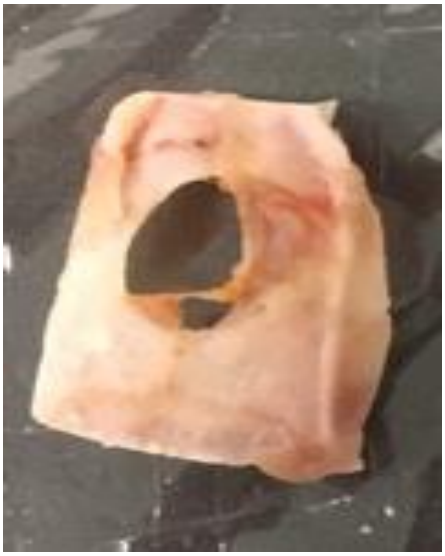
To investigate the bone formation of the scaffolds using smart hydrogel bio-ink, visual inspection and histological analysis were done. SD rat of Negative group of this experiment died the next day after surgery. So, specimen of two groups were available for this analysis. As shown in Fig. 9A and 9B, noticeable difference of bone formation was observed visually, showing different pattern and defect size of bone formation area. In the positive control group (Fig 9A, only HA hydrogel group), bone formation pattern was observed evenly around the bone defect margin. On the other hand, in the smart hydrogel with SP and BMP-2 patterning group (Fig. 9B), bone formation pattern was tilted to one side around the bone defect. But it was clear that the smart hydrogel with SP and BMP-2 patterning group showed more bone formation than the positive control group, resulting in much smaller size of bone defect.

To confirm these outcomes histologically, hematoxylin and eosin staining was done. An inflammatory response was not observed around the scaffolds in any

group of this experiment. Shown in Fig. 10A, in the only HA hydrogel group, there is not much new bone formation along the periphery of the defect. In contrast, in smart hydrogel with SP and BMP-2 patterning group (Fig. 10B), noticeable amount of new bone was found along the periphery of the defect.



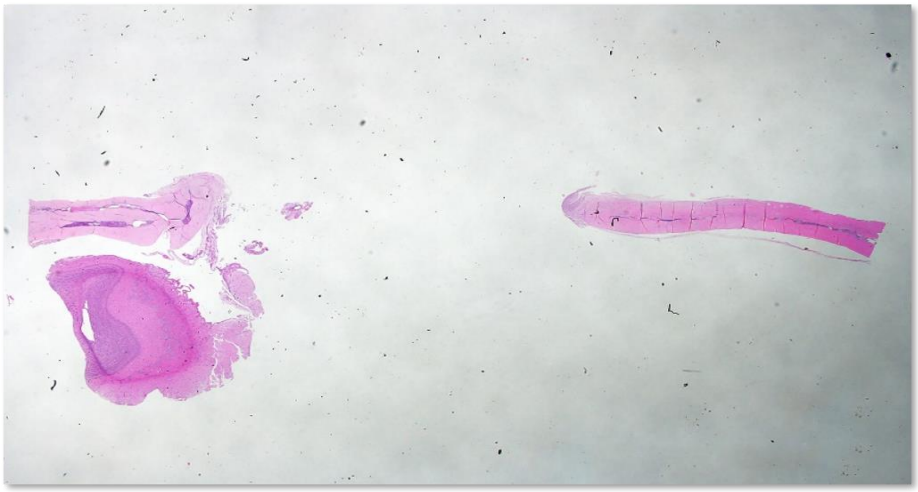
A



B

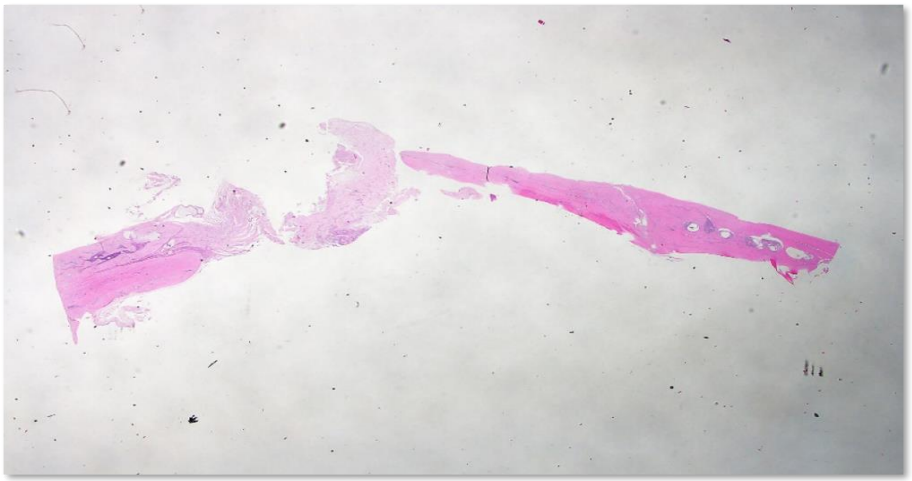
Figure 9. Image of specimen after 4 weeks in calvarial defect model. A. Positive control group: only HA hydrogel group. B. smart hydrogel with SP and BMP-2 patterning group.

Only HA



A

HA with BMP-2 + SP patterning



B

Figure 10. Hematoxylin and eosin-stained image after 4 weeks in calvarial defect model. A. Positive control group: only HA hydrogel group. B. smart hydrogel with SP + BMP-2 patterning group.

Discussion

As shown in Fig. 9B, new bone formation was tilted to one side of the defect. Our scaffold's mechanical property was too soft, so it makes it hard to handle and position this scaffold to the bone defect exactly. Taking this into consideration, it is likely that scaffold was grafted in a skewed state at the time of the surgery. This is probably why the result that bone formation pattern was tilted to one side of bone defect is so. So it needs to be mechanically reinforced so that it is enough to maintain initial form until operator handle or graft it to the bone defect. Mechanical properties can be modulated by varying molecular weight (MW) of HA and weight percent (wt %) of hydrogel, but one of the main hurdles that researchers have encountered in applying bioprinting technology is that hydrogel exists as either precursor fluid solutions with insufficient mechanical properties, or polymerized hydrogels that if printed can clog nozzles or become broken up during extrusion process. According to Skardal and Atala[20], to succeed in bioprinting effectively, a biomaterial must meet 4 basic

requirements: 1) the appropriate mechanical properties to allow deposition, 2) the ability to hold its shape as a component of a 3D structure after deposition, 3) the capability for user control of the 2 prior characteristics, and 4) a cell friendly and supportive environment at all phases of the bioprinting procedure. Our scaffold has met those requirements with a certain amount, but it seems that improvement is needed to have a better material quality which is satisfactory for both mechanical property and printability. Enhancing mechanical property by increasing wt % of hydrogel results in low printability of materials that can clog nozzles during printing process. Many research have explored various hydrogel formulations to overcome these bioprinting problems including cell spheroid printing into hydrogel substrates[21,22], temporal control of hydrogel stiffness using photopolymerizable methacrylated HA and gelatin[23], PCL/alginate struts coated with alginate-based bioink to reinforce the structure[24], and recently rapid polymerizing ultraviolet light (UV)-initiated crosslinking[25]. We can try this procedure by making a slight change in the wt % composition of our smart

hydrogel or adapt one of those methods.

In addition, scaffold was so transparent that it was not easy for the researcher to verify that scaffold was properly located after placing scaffold to the bone defect. Therefore, it will be helpful to make this scaffold somewhat pigmented or opaque so that operator can easily recognize it is in right position or maintaining its intact form. Even though wt% composition of hydrogel may not be changed due to technical limitations, making this scaffold opaque is not difficult matter and this simple change will be very helpful when this scaffold is handled by surgeon in clinical situation.

In calvarial defect model study, we originally set up three groups for this study: Negative control group, Positive control group (only HA hydrogel), smart hydrogel with SP and BMP-2 patterning group. But rat set as a negative control group, died next day after surgery. So we can compare our scaffold's osteogenic potential to positive control group only. But positive control group barely showed new bone formation. It is expected to have results similar to negative control

group. So we estimate that there was probably no difference between the two groups.

Treating large bone defects of maxillofacial area is challenging situation in clinical practice. New approaches are needed to solve challenging problem. Nowadays, most commercialized product for graft are particle and putty type. These types of grafting material are difficult to be used for complex bone defects, because complex anatomy of surgical field and continuous bleeding make these types of material difficult to mold and maintain the shape that surgeon wants. We fabricated customized 3D bio-printed scaffold that was fitted to these complex defects favorably. By doing so, there was no need for fixation procedure. And also, these scaffolds can contain different kinds of chemical cues at once by using different types of bio-ink and printing them in a layer by layer fashion. Chemical cues inside scaffold slowly release as MMP secreted in our human body degrade this scaffold. And then, it promotes angiogenesis and osteogenesis in the bone defect. Another huge advantage is that we can choose

any chemical cues or peptides which we want to deliver to the defect by incorporating them into smart hydrogel. During experiment period, the scaffold was maintained in the graft position and there was no inflammation or side effect. Therefore, using this biocompatible and degradable material, producing the customized scaffolds containing growth factors is useful in clinical routine.

Especially when the tissue does not have inherent self-regenerating ability due to pathologic condition or impaired general condition, this scaffold system can be much more valuable for tissue regeneration. Diabetes mellitus (DM) is a chronic metabolic disease that global prevalence of diabetes among adults over 18 years of age has risen from 4.7 % in 1980 to 8.5 % in 2014[26]. Wound healing deficiency is also a characteristic of diabetic patients, involving several physiologic factors: reduced or impaired angiogenic response, growth factor production, bone healing[27]. And osteomyelitic condition including osteoradionecrosis (ORN) or mediation-related osteonecrosis of the jaw (MRONJ) is a serious complication in patients treated with radiation therapy or

antiresorptive medications[28,29]. The pathogenesis of these diseases is not clearly understood and it can be seen as a decrease in healing activity at an affected site[30]. Conventional treatment is based mostly on symptomatic therapy, and consists of sequestrectomy, debridement, and surgical resection of the affected bone. All these approaches aim at bone regeneration, but it is not very successful due to impaired healing ability and usually results in large bone defect. Some researchers reported that growth factors like BMP-2 or MSCs can promote tissue regeneration in damaged tissue with an impaired healing ability[31,32]. Given these reports, it is expected to be effective using smart hydrogel scaffold in these pathologic, compromised conditions because our SHS can deliver growth factors or chemical cues without side effect. Though a synergic regeneration potential can be expected with MSCs and BMP-2, the effect of scaffold containing these factors in the specific pathologic conditions (DM, ORN, MRONJ) has not been investigated. It needs to be explored after this preliminary study.

Conclusion

In this study, we fabricated scaffolds using different types of smart hydrogel as a bioink for 3D bioprinting. This SHS can contain any chemical cue that promotes angiogenesis or new bone formation by immobilizing them into acryl group or incorporating into hydrogel. In addition, by adapting layer by layer fashion printing technique, scaffold made of this SHS can contain more than one type of chemical cue so that different pathways of growth promotion can occur simultaneously at the graft site.

To use this method for clinical applications, further study including more specimens, long-term follow-up, quantitative data analysis should be necessary to confirm the osteogenic and angiogenic potential after transplantation of 3D patterned smart hydrogel.

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국문 요약

3 차원-패터닝 스마트 수화젤의 생체내 골재생 및 혈관신생능 평가

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연구 배경

임상에서 구강악안면영역의 크고 복잡한 뼈 결손부위를 치료하는 것은 매우 어려운 문제이다. 조직공학분야에서는 생체 재료 단독 혹은 생체 재료와 적절한 생물학적, 화학적 요인들을 결합하여 뼈 조직을 회복시키는데 사용하고 있다. 세포와

성장인자의 전달체로서, 이전 연구에서 Matrix metalloproteinase (MMP) sensitive acrylated hyaluronic acid (HA) 기반의 스마트 수화젤을 소개하였다. 이번 연구에서는 이 스마트 수화젤을 3D 바이오 프린팅 기술과 결합하여, 임상상황에서의 다양한 골 결손 부위에 적용 가능한 지지체를 제작하였다. Substance P (SP) 와 Bone Morphogenic Protein-2 (BMP-2) 를 각각 포함하는 다른 종류의 스마트수화젤을 3D 프린팅을 위한 바이오잉크로 사용하였다.

연구 방법

SD 쥐의 등 좌, 우측 두 곳에 절개를 가하여 피하조직에 포켓을 만들어주었다. 이번 연구에서 제작한 지지체를 쥐 등의 좌측 포켓에 이식하고, 우측 포켓은 음성대조군으로 설정하여 아무것도 이식하지 않았다. 수술 1 달 뒤 이식한 부위의 표본을 채취하였다. 외부 직경이 6 mm 인 트레핀 버를 사용하여 전두골과 두정골 사이 가운데 전층골결손을 생성하였다. 결손 부위에 실험 계획에 따라 스마트 수화젤로 제작한 지지체를 이식한 군과 그렇지 않은 군으로 나누어 비교 평가 하였다.

연구 결과

SP 패터닝 스마트수화젤 그룹의 이식한 부위에서 명확한 혈관신생이 관찰되었다. 대조군에 비해 피하조직이 두꺼운 양상을 보였고, 양성대조군으로 설정한 어떤 성장인자도 패터닝되지 않은 수화젤을 이식한 그룹과 비교했을때 더 큰 혈관이 침투한 것과 더 많은 수의 혈관이 신생 된 것을 조직학적으로 확인하였다.

두개골결손 모델에서, SP 와 BMP-2 패터닝 스마트 수화젤 그룹의 이식한 부위에서도 대조군에 비해 더 많은 골 생성이 일어나 골결손 부위의 크기가 훨씬 작아진 것을 확인할 수 있었다. 결과 중 어떤 그룹에서도 염증 반응은 관찰되지 않았다.

결론

이번 연구에서는 바이오 프린팅에 사용될 바이오 잉크로서, 1 가지 이상의 다른 종류의 스마트 수화젤을 사용하여 지지체를 제작하였다. 이 스마트 수화젤로 제작한 지지체는 연구자가 원하는 성장인자를 선택적으로 지지체에 포함시킬 수 있는 장점이 있다. 게다가 이 지지체는 1 가지 이상의 성장 인자를 포함할 수 있기 때문에, 성장을 촉진시킬 수 있는 다른 종류의 기전을 동시에 성공적으로 촉진시킬 수 있음을 증명하였다.

중심단어: 지지체, 골재생, 수화젤, 패터닝, 3D 프린팅, 바이오잉크, 골조직공학, 골형성단백질-2 (BMP-2), P 물질 (Substance P)