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Research Article

Artificial Bone Substitute of MGSB and Hyaluronate Hydrogels

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Abstract A novel artificial bone substitute composed of bioactive MegaGen synthetic bone (MGSB) and hyaluronate (HA) hydrogels was successfully developed for bone tissue engineering applications. HA is known to play important roles in bone regeneration due to its angiogenic and osteoconductive characteristics. Accordingly, HA hydrogel was designed to supply HA continuously for effective bone regeneration by its controlled degradation *in vivo*. Synchrotron X-ray bio-imaging clearly visualized 3-dimensional micron scale morphologies of effectively regenerated bones by the bone substitute of MGSB/Hyaluronate-Cystamine hydrogels implanted to the calvarial critical size bone defects in New Zealand white rabbits.

Keywords hyaluronate; hydrogels; calcium phosphate; bone regeneration; X-ray imaging

1 Introduction

Various artificial bone substitutes have been designed and developed for rapid and efficient bone regeneration in clinical applications [6,10,11]. Bio-OSS® is one of the representative organic bone substitutes, which has been commercialized by Geistlich Biomaterials Co. in Swiss. In contrast, MBCP® is one of the representative synthetic bones, which has been commercialized by Biomatlant Co. in France. Despite their wide applications, the bone regeneration is known to be very slow and their slow resorption to cause an invasion of fibroblast [10]. To stimulate chemotaxis and proliferation of mesenchymal stem cells for bone regeneration, osteogenic growth factors like bone morphogenic protein (BMP) have been used together with the bone substitutes [1,4,7]. BMP was encapsulated in polymer hydrogels containing bone substitutes, which significantly enhanced bone regeneration [1,4,7]. Furthermore, mesenchymal stem cells (MSC), which have been reported to differentiate to osteoblasts for rapid and efficient bone regeneration, were also encapsulated within the hybrid bone substitutes [5,8,9, 13]. However, there are many obstacles for these systems to be commercialized for clinical applications due to the high production cost of BMP and the safety issues of MSC, etc. Instead of these complicated systems, we tried to develop a novel hybrid bone substitute composed of bioactive calcium phosphate based synthetic bone and hyaluronic acid (HA) hydrogels taking advantages of the angiogenic and osteoconductive characteristics of HA [3,12].

2 Materials and methods

2.1 Synthesis of HA-Cys hydrogels

HA was dissolved in phosphate buffered saline (PBS, 0.01 M, pH = 7.4) and cystamine was added to the solution. The amount of cistamine was 20 mol% of HA repeating units. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) and 1-hydroxybenzotriazole monohydrate (HOBt), activating the carboxyl groups of HA, were dissolved in PBS and added to the mixed solution of HA and cystamine for HA-Cys hydrogel preparation. The molar amount of EDC and HOBt was 2 times of HA repeating units, respectively. The final precursor solution was mixed and incubated at 37 °C for 2 hr to complete the crosslinking reaction for HA-Cys hydrogel preparation. Then, the HA-Cys hydrogels were sealed with pre-washed dialysis membrane tube (MWCO of 7 kDa) and dialyzed against PBS for 24 hr to remove the remaining EDC, HOBt, and cystamine. Figure 1 shows the schematic representation for the preparation of HA-Cys hydrogels and the *in vivo* bone regeneration tests.

2.2 *In vivo* bone regeneration tests

New Zealand white male rabbits weighing about 2 kg were anesthetized by intramuscular injection of zoletil and rompun (v/v = 1/1, 0.1 cc/kg). The skull of each rabbit was incised and two bone defects with 9 mm diameter were made with a trephine bur (d = 8 mm). HA-Cys hydrogels described above were mixed with PBS at a volume ratio of one to one

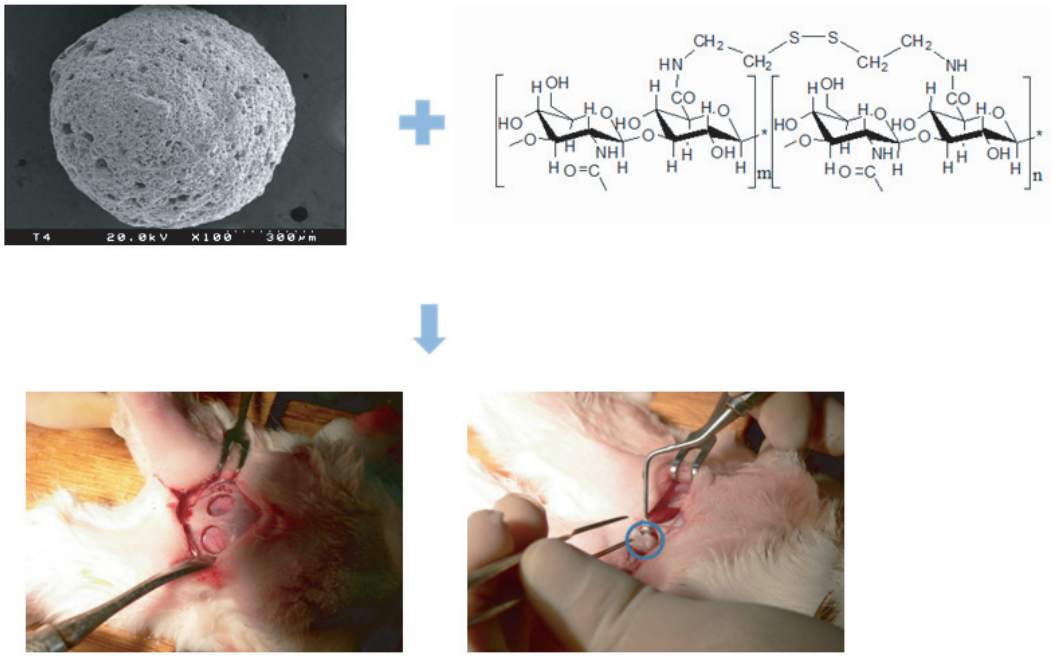

Figure 1: Schematic representation for *in vivo* bone regeneration test using a biphasic calcium phosphate of MGSB/Hyaluronate-Cystamine (HA-Cys) hydrogels implanted to the calvarial critical size bone defects in New Zealand white rabbits.

and homogenized completely with a homogenizer (T-18 basic, IKA, Tokyo, Japan) at 8,000 rpm for 5 min. The prepared microhydrogel was mixed with MGSB, which was inserted into the calvarial critical size bone defect of the rabbit. The rabbits were sacrificed in 4 weeks to assess the bone regeneration in comparison with a control group (no treatment). The regenerated bone defect samples were fixed with 10% formalin for 2 days and decalcificated with 10% EDTA for 2 ~ 3 weeks.

2.3 Synchrotron X-ray microtomography

Microtomography was performed on the International Consortium of Phase Contrast Imaging and Radiology 7B2 synchrotron X-ray microscopy beamline at the Pohang Light Source [2]. The experimental geometry and the detector position in particular were selected to emphasize the refraction-based mechanism. The regenerated bone sample was typically placed 200 ~ 400 mm from the detector to achieve the best contrast. The sample was mounted on a high precision motor-controlled stage with rotational, tilting, and translational resolutions of 0.002°, 0.0009°, and 250 nm, respectively. After passing through the sample, the transmitted X-ray beam was converted by a scintillator to visible light, reflected by a silicon wafer, and then magnified by an optical lens. The detector system (Nihon Kessho Koogaku Co., Ltd.) was consisted with a thin $CdWO_4$ cleaved single crystal ($30 \times 30 \times 0.3\,mm^3$) scintillator and a CCD camera. A microscopic objective lens magnified the image displayed on the scintillator before it was captured by the CCD. After magnification, the image was captured by the image acquisition system. Several images were averaged into one image at every 0.9° increment of rotation. This process was repeated 200 times, which took about less than 1 hr. The field of view was tunable by adapting different magnification lens with 1600 × 1200 pixels. The image set was reconstructed by four parallel computers equipped with a reconstruction algorithm. Reconstructed slices were composed of 1600 × 1600 pixels in the X and Y directions. Vertically stacked 2D slices were constructed into volume-rendered 3D images using Amira software.

3 Results and discussion

Figure 1 shows a schematic representation for HA-Cys hydrogels and *in vivo* bone regeneration tests. HA was dissolved in phosphate buffered saline (PBS, 0.01 M, pH 7.4) and mixed with cystamine dihydrochloride. The amount of cystamine was 20 mol% of HA repeating units. HA-Cys hydrogels were prepared by the addition of 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide (EDC) and 1-hydroxybenzotriazole monohydrate (HOBt) in PBS. For *in vivo* bone regeneration tests, two bone defects with a diameter of 9 mm were made on the skull of New Zealand white male rabbits. HA-Cys hydrogels described above were completely homogenized and mixed with MGSB,

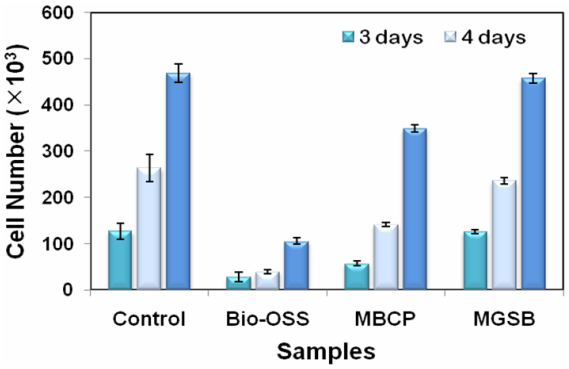

Figure 2: MTT test results for the cytotoxicity of bone substitutes of Bio-OSS®, Micro-Macroporous Biphasic Calcium Phosphate (MBCP®), and MegaGen Synthetic Bone (MGSB) by measuring the MC3T3-E1 cell proliferation after incubation for 3 to 5 days.

which was inserted into the calvarial critical size bone defect of the rabbit. After bone regeneration for 4 weeks, the recovered bone defect area was investigated by the non-destructive synchrotron X-ray tomographic analysis in comparison with the control sample.

Figure 2 shows the MTT test results of bone substitutes. MGSB resulted in the least cytotoxicity followed by MBCP® and Bio-Oss®. The results reflect that osteoblasts can easily attach to the MGSB with a micro-porous structure contributing for effective bone regeneration. From the results, MGSB was used for the preparation of artificial bone substitute using HA hydrogels.

Figure 3 shows the 3D reconstructed images of tomographic data for bone substitute samples. The volume-rendering images of MGSB/HA-CYS hydrogels showed the 3D micron-scale morphologies of regenerated bone plates. The synchrotron 3D X-ray images clearly visualized that the bone regeneration by MGSB/HA-CYS hydrogels was more effective with a better interconnection to the MGSB than the control. Bone is reported to be regenerated by the deposition of calcium phosphate which was carried by adjacent blood vessels. This novel approach would be successfully applied to investigate the bone regeneration process *in vivo* as a non-destructive method and contribute for the development of artificial bone substitutes for clinical applications.

4 Conclusions

A novel artificial bone substitute consisted with MGSB and HA hydrogels was successfully developed for effective bone regeneration. We could observe 3D micron scale morphologies of regenerated bones by the artificial bone substitute of MGSB and HA-Cys hydrogels via synchrotron X-ray imaging. This novel approach would be successfully applied for the development of artificial bone substitutes.

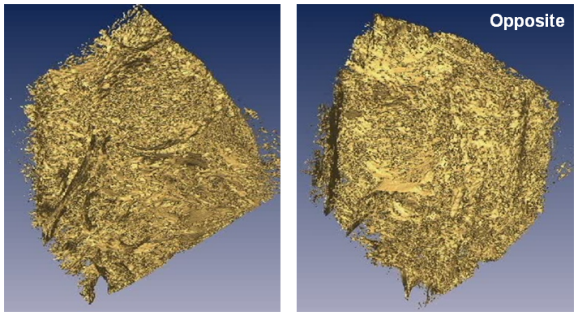

Figure 3: Synchrontron X-ray images of regenerated bones by MGSB/HA-CYS hydrogels in the calvarial critical size bone defects of New Zealand White rabbits in 4 weeks.

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References

[1] N. Aebli, H. Stich, P. Schawalder, J. C. Theis, and J. Krebs, *Effects of bone morphogenetic protein-2 and hyaluronic acid on the osseointegration of hydroxyapatite-coated implants: an experimental study in sheep*, J Biomed Mater Res A, 73 (2005), 295–302.

[2] S. Baik, H. S. Kim, M. H. Jeong, C. S. Lee, J. H. Je, Y. Hwu, et al., *International consortium on phase contrast imaging and radiology beamline at the Pohang Light Source*, Rev Sci Instrum, 75 (2004), 4355–4358.

[3] R. A. Carano and E. H. Filvaroff, *Angiogenesis and bone repair*, Drug Discov Today, 8 (2003), 980–989.

[4] T. M. G. Chua, S. J. Warden, C. H. Turner, and R. L. Stewartb, *Segmental bone regeneration using a load-bearing biodegradable carrier of bone morphogenetic protein-2*, Biomaterials, 28 (2007), 459–467.

[5] J. I. Dawson and R. O. C. Oreffo, *Bridging the regeneration gap: stem cells, biomaterials and clinical translation in bone tissue engineering*, Arch Biochem Biophys, 473 (2008), 124–131.

[6] O. Gauthier, E. Goyenvale, J. M. Bouler, J. Guicheux, P. Pilet, P. Weiss, et al., *Macroporous biphasic calcium phosphate ceramics versus injectable bone substitute: a comparative study 3 and 8 weeks after implantation in rabbit bone*, J Mater Sci Mater Med, 12 (2001), 385–390.

[7] R. E. Jung, F. E. Weber, D. S. Thoma, M. Ehrbar, D. L. Cochran, and C. H. F. Hämmerle, *Bone morphogenetic protein-2 enhances bone formation when delivered by a synthetic matrix containing hydroxyapatite/tricalciumphosphate*, Clin Oral Implants Res, 19 (2008), 188–195.

[8] J. M. Kanczler, P. J. Ginty, J. J. A. Barry, N. M. P. Clarke, S. M. Howdle, K. M. Shakesheff, et al., *The effect of mesenchymal populations and vascular endothelial growth factor delivered from biodegradable polymer scaffolds on bone formation*, Biomaterials, 29 (2008), 1892–1900.

[9] J. Kim, I. S. Kim, T. H. Cho, K. B. Lee, S. J. Hwang, G. Tae, et al., *Bone regeneration using hyaluronic acid-based hydrogel with bone morphogenic protein-2 and human mesenchymal stem cells*, Biomaterials, 28 (2007), 1830–1837.

[10] M. A. W. Merkx, J. C. Maltha, H.-P. M. Freihofer, and A. M. Kuijpers-Jagtman, *Incorporation of three types of bone block implants in the facial skeleton*, Biomaterials, 20 (1999), 639–645.

[11] M. M. Stevens, R. P. Marini, D. Schaefer, J. Aronson, R. Langer, and V. P. Shastri, *In vivo engineering of organs: the bone bioreactor*, Proc Natl Acad Sci, 102 (2006), 11450–11455.

[12] J. Street, M. Bao, L. deGuzman, S. Bunting, F. V. Peale, N. Ferrara, et al., *Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover*, Proc Natl Acad Sci, 99 (2002), 9656–9661.

[13] A. R. Vaccaroa, P. G. Whang, T. Patelc, F. M. Phillips, D. G. Andersona, T. J. Albert, et al., *The safety and efficacy of OP-1 (rhBMP-7) as a replacement for iliac crest autograft for posterolateral lumbar arthrodesis: minimum 4-year follow-up of a pilot study*, Spine J, 8 (2007), 457–465.