Volume 5, Issue 1, 2016, 342-345 **ISSN 2284-6808**

Letters in Applied NanoBioScience

www.NanoBioLetters.com

Original Research Article **Open Access Journal**

Received: 22.09.2015 / Revised: 12.10.2015 / Accepted: 20.10.2015 / Published on-line: 01.01.2016

Use of chitosan-siloxane porous hybrid scaffold as novel burr hole covers

Yuki Shirosaki [1,*], Motomasa Furuse [2], Takuji Asano [3], Yoshihiko Kinoshita [3], Toshiki Miyazaki [4], Toshihiko Kuroiwa [2]

[1]Frontier Research Academy for Young Researchers, Kyushu Institute of Technology, Japan
[2] Department of Neurosurgery, Osaka Medical College, Japan
[3]Nikkiso Co., Ltd. Japan
[4]Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Japan

*corresponding author e-mail address: ***yukis@lsse.kyutech.ac.jp***

ABSTRACT

Chitosan-siloxane porous hybrids have high potential as tissue scaffolds. This manuscript focuses on the regeneration of skull bone after a burr hole. This was done using hybrids incorporated with calcium or coated with hydroxyapatite after soaking in a phosphate solution. The specimens fitted the burr hole and the cells migrated into the pores from surrounding bone tissue. After implantation no inflammation was observed and the specimens degraded 12 months later. A coating of hydroxyapatite accelerated bone formation compared.

Keywords: *craniofacial surgery, burr hole, chitosan-siloxane porous hybrid, bone regeneration.*

1. INTRODUCTION

"Burr holes" are made to insert surgical tools during a craniotomy [1-3]. After surgery, this hole must be closed with autogenous or artificial bone graft materials. Calcium phosphate cement (CPC) is currently used for this [4-10]. However, the setting time of CPC is delayed if contact is made with cerebrospinal fluid or blood, they sometimes leak into the brain and also have a low degradation rate. Moreover, CPCs are fragile and may be broken as new bone regenerates.

Titanium plates [11,12] and some button-type materials [13-15] are also used to cover the holes. However, some ceramic buttons have the same risks as CPCs because they are fragile and titanium plates often prevent observations with magnetic resonanve imaging (MRI). In addition, titanium plate can cause thinning of the surrounding soft tissue and extrusion [16,17]. The main requirements of new skull bone graft materials are flexibility, biodegradability and acceleration of the bone regeneration.

Shirosaki *et al.*, prepared the chitosan- siloxane-γ-glycidoxypropyltrimethoxysilane (GPTMS) porous hybrids via sol-gel and freeze-drying methods [18-20]. These hybrids had high porosity and interconnected pores with around 100 μm diameter. The human osteosarcoma cell, MG63, attached, migrated and grew into the pores. Apatite deposition also occurred in the pores for hybrids containing calcium ions. The biodegradation was slow even in enzymatic solution; however, they confirmed with an *in vivo* test that they were mostly degraded within 3 months [21,22].

These results suggested that the hybrids remained until the skull regenerated.

Calcium ions and hydroxyapatite are effective at accelerating bone regeneration [23,24]. In a previous study, chitosan-siloxane hybrids incorporated with calcium chloride were soaked in alkaline phosphate solution to deposit the apatite on their surfaces [25]. The calcium ions were used for apatite formation without any loss and even after apatite deposition, the porosity remained high. In this study, three types of chitosan-siloxane porous hybrids were implanted into the burr hole and bone regeneration was observed histologically.

2. EXPERIMENTAL SECTION

2.1. Preparation of the porous hybrids.

Chitosan (0.5 g, high molecular weight, deacetylation: 79.0%, Aldrich®, USA) was dissolved in aqueous acetic acid (0.25 M, 25 mL). GPTMS (Lancaster, Lancashire, UK) and calcium chloride (Nacalai Tesque, Kyoto, Japan) were added to give a molar ratio of chitosan to GPTMS (ChG) of 1:0.5 or chitosan to GPTMS :to $CaCl_2$ (ChGCa) of 1.0 : 0.5 : 1.0. One mole of chitosan equates to one mole of deacetylated amino groups. The mixtures were stirred for 1 h at room temperature and a fraction of each resultant sol was poured into a polystyrene container and frozen at -20°C for 24 h. The frozen hybrids were then transferred to a freeze dryer (FDU-506, EYELA, Tokyo, Japan) for 12 h until dry.

Figure 1. Photograph of ChGCa specimens.

The resultant porous ChG and ChGCa hybrids were then washed with NaOH (0.25 M) and distilled water to neutralize remaining acetic acid and were again lyophilized. Some ChGCa hybrids were instead soaked in aqueous Na_2HPO_4 (0.01 M, pH8.8) at 80°C for 3 days (ChGCa_HAp). These hybrids were then washed with distilled water and lyophilized. Figure 1 shows a photograph of the chitosan-GPTMS porous hybrids containing calcium chloride to use in an *in vivo* animal test.

2.2. *In vivo* animal test.

The hybrids were sterilized with γ-Ray. All surgical procedures were performed with the approval of the animal care and use committee of Osaka Medical College (No. 26044). In brief, eight adult female beagles weighting around 10 kg were each anesthetized with a mask using isoflurane (Abbott, Tokyo, Japan) in oxygen. Tracheal intubation was performed after inducing the anesthesia which was maintained via a calibrated vaporizer (TEC3; Ohmeda, UK). The beagles were cleaned and draped in a standard manner, with a longitudinal incision made over the scalp and the pericranium was lifted off with a sharp periosteal elevator. Then, four 14 mm burr holes were created to evacuate the liquefied hematoma (Figure 2). This was done in each beagle using a drill. Following evacuation, the hybrids were inserted into each burr hole. The commercial bone cement was also implanted as a control. If the hybrids did not fit the hole, the doctor cut them with scissors during surgery (Figure 3). The periosteal and skin were finally sutured. All of the above surgical procedures were performed in a sterile manner. Specimens of the parietal bones including the cranioplasty were harvested and examined histologically. After fixation in buffered 10% formaldehyde, the specimens were dehydrated in ethanol and soaked in xylene for defatting.

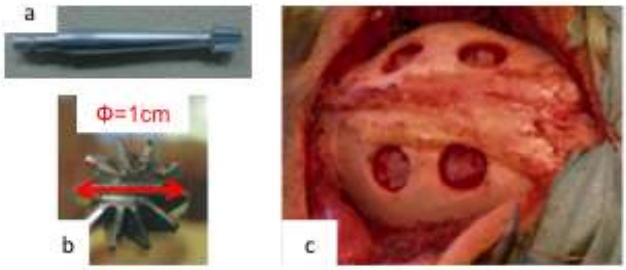

Figure 2. Photographs of a medical device for making burr holes (a, b) and intraoperative defects created in the beagle skull (c).

Figure 3. Photograph of cutting the sample with medical scissors.

Subsequently, the specimens were decalcified and thin sections were prepared by microtome and stained with hematoxyline-eosine (HE) and azan-mallory (AM).

3. RESULTS SECTION

The hybrid properties were examined in previous works [25]. The hybrids fitted the burr holes (Figure 4) and blood infiltrated the pores, but did not overflow. This means that they work as the styptic.

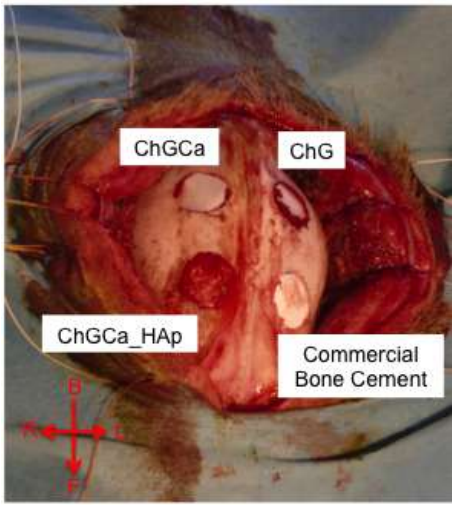

Figure 4. Photograph of pure beagle skull after implantation with the specimens

Following implantation, all beagles survived with no wound infection or automutilation. Figures 5-9 show the histological results 12 months after operation. In the case of commercial bone cement (Figure 5), the materials remained (the white area is bone cement) but this disappeared during decalcification and fibrous tissue and blood vessels were observed near the surface (→). Osteoblasts and osteoclasts were not found.

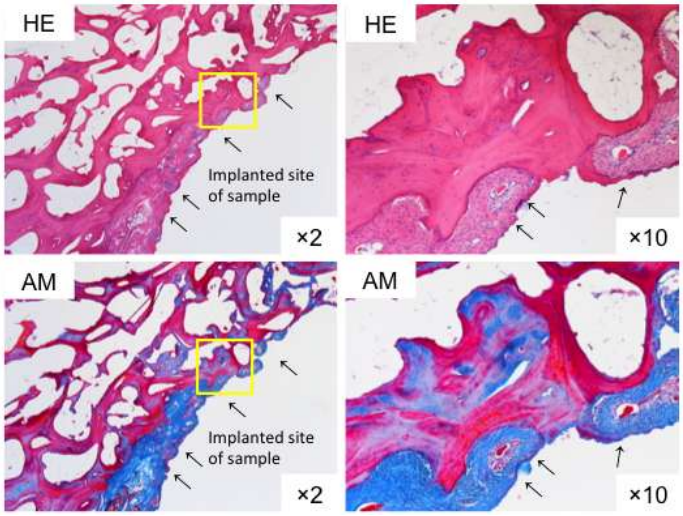

Figure 5. Light microscopic images of commercial bone cement cranioplasty 12 months after operation stained by HE (upper) and AM (lower). The right-hand images are a magnification of the left (yellow square).

The final product of the commercial bone cement was hydroxyapatite, which degraded slowly in the skull. New bone did

not regenerate at the site implanted with bone cement. In contrast, the chitosan-siloxane porous hybrid induced new bone formation in their matrix (Figures 6-8).

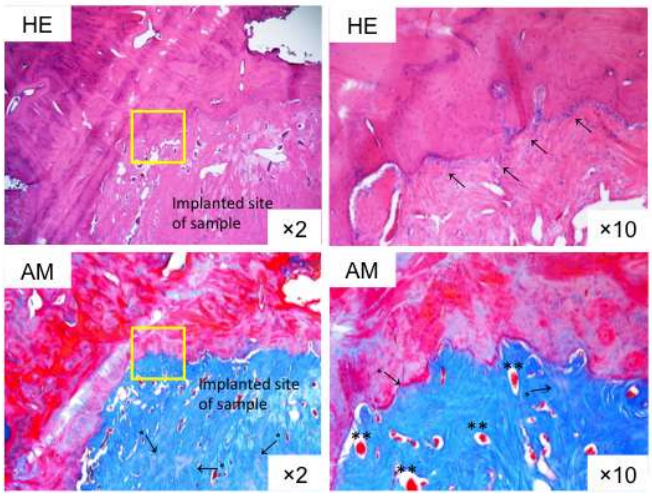

Figure 6. Light microscopic images of ChG cranioplasty 12 months after operation stained by HE (upper) and AM (lower). Images on the right images are a magnification of the left (yellow square).

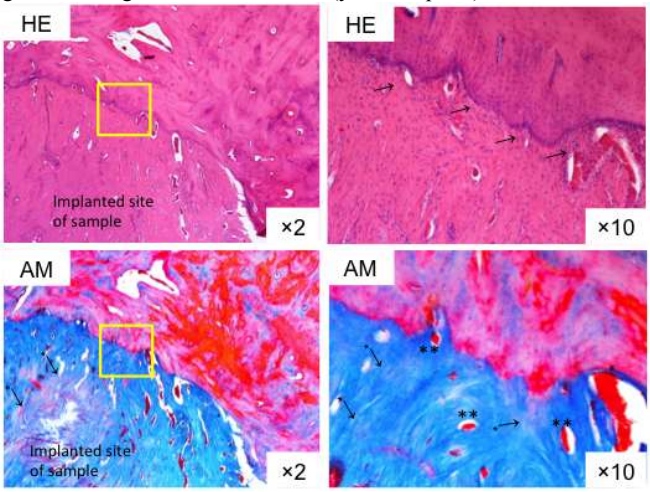

Figure 7. Light microscopic images of ChGCa cranioplasty 12 months after operation stained by HE (upper) and AM (lower). Images on the right are a magnification of the left (yellow square).

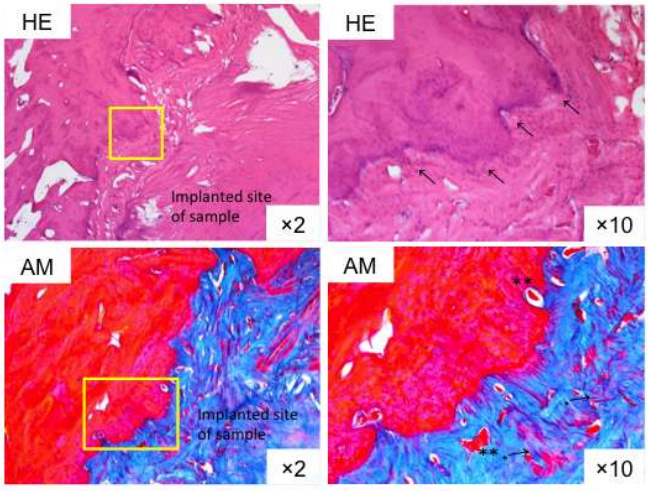

Figure 8. Light microscopic images of ChGCa_HAp cranioplasty 12 months after operation stained by HE (upper) and AM (lower). Images on the right are a magnification of the left (yellow square).

Every hybrid degraded and disappeared within 12 months of implantation. Even in the case of ChG, many osteoblasts were on the hybrid (→) and many blood vessels (**) with havers canals formed in the new tissue (Figure 6). The osteoid tissue formed (blue) and some calcifications were found (light pink, *→), as shown in with AM staining images. In a previous study [18], the MG63 cells were cultured into the chitosan-siloxane porous hybrids *in vitro*. The cells were attached and migrated into the interconnected pores and grew well. The hybrids also promoted alkaline phosphatase activity and the mineralization of human bone marrow cells because of silicon ion release [26]. The *in vitro* cell behavior matched the results of the *in vivo* test. Typically, a pore size of around 100-400 μm is proposed as optimal for osteoconduction [27] and pore sizes of greater than 300 μm are recommended to enhance bone formation via vascularization [28]. There are some reports that a pore sizes smaller than 100 μm is not sufficient for cell migration; however in this work, the chitosan-siloxane hybrids induced new bone and blood vessel formation even at a pore size of around 100 μm. This means that the chitosan-siloxane hybrid has the potential to promote bone regeneration with its microstructure, surface structure, and the released element.

Incorporation of calcium ions (ChGCa) accelerated bone formation (Figure 7). Osteoblasts were also present at the interface of the pre-existing bone and newly formed osteoid tissue. Calcifications were found in the osteoid tissue more easily than in ChG. Coating of hydroxyapatite (ChGCa_HAp) showed excellent bone formation compared with other cases (Figure 8). Similarly, osteoblasts exist along the pre-existing bone and many blood vessels were formed in the osteoid tissue. Azan staining showed that strong calcifications were found in the osteoid tissue as shown in Figure 9.

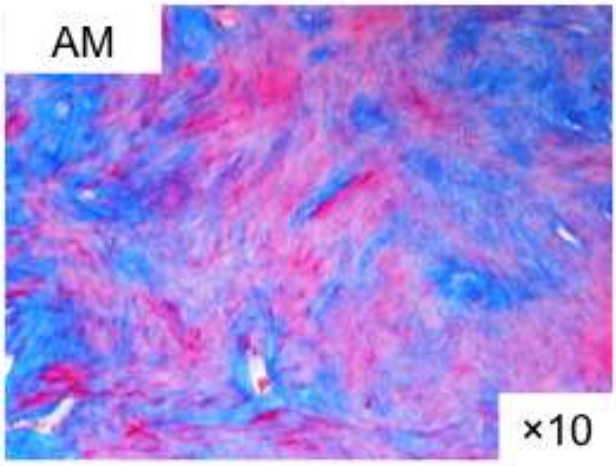

Figure 9. Light microscopic images of ChGCa_HAp cranioplasty 12 months after operation stained by AM.

Unfortunately, osteoclasts were not seen. Soluble silica and calcium ions released from the bioactive glass stimulated bone growth [23]. ChGCa also released silicon species and calcium ions [25]. Their synergy promoted the more active calcification of ChGCa compared with ChG. The proliferation and differentiation of osteoblasts depends on the calcium phosphate reactivity [24]. The dissolution rate and early bone formation are linked and free calcium and inorganic phosphates have an influence on bone formation. ChGCa_HAp has needle-like and low crystalline apatite deposits on the surface of pores. The low crystalline apatite can dissolve easily *in vivo*. This means that the apatite on the ChGCa_HAp dissolved and the calcium and phosphate ions released and then accelerated bone formation. In the present work, we could not find osteoclasts around the new bone. The osteoclasts participate in the degradation of material. this case, we observed them only 12 months after implantation and the hybrids

had already disappeared. The osteoclasts may be present in the early stages after implantation.

4. CONCLUSIONS

The chitosan-siloxane porous hybrids were very flexible and could easily be cut with surgical scissors to fit the burr holes. The blood infiltrated the pores but did not overflow. No inflammation occurred in the 12 months after implantation and the hybrids degraded and disappeared completely. New bone formation occurred at the site of implantation and there were many osoteoblasts and blood vessels with havers canals. Hydroxyapatite coating accelerated the calcification. The chitosan-siloxane porous hybrids could be used to repair burr holes after cranioplasty.

5. REFERENCES

[1] C. Kilincer, O. Simsek, M.K. Hamamcioglu, T. Hicdonmez, S. Cobanoglu, Contralateral subdural effusion after aneurysm surgery and decompressive craniectomy: case report and review of the literature, *Clin. Neurol. Neurosurg.*, 107, 5, 412-416, **2005**.

[2] C.G. Bénar, J. Gotman, Modeling of post-surgical brain and skull defects in the EEG inverse problem with the boundary element method, *Clin. Neurophysiol.*, 113, 1, 48-56, **2002**.

[3] T. Kawagushi, S. Fujita, K. Hosoda, Y. Shose, S. Hamano, M. Iwakura, N. Tamaki, Multiple burr-hole operation for adult moyamoya disease, *J. Neurosurg.*, 84, 3, 468-476, **1996**.

[4] M.J. Provenzano, K.P.J. Murphy, L.H. Riley III, Bone cements: Review of their physiochemical and biochemical properties in percutaneous vertebroplasty, *Am. J. Neuroradiol.*, 25, 7, 1286-1290, **2004**.

[5] J.M. Kuemmerle, A. Oberle, C. Oechslin, M. Bohner, C. Frei, I. Boecken, B.von Rechenberg, Assessment of the suitability of a new brushite calcium phosphate cement or cranioplasty – an experimental study in sheep, *J. Craniomaxillofac. Surg.*, 33, 1, 37-44, **2005**.

[6] M. Dujovny, A. Aviles, C. Agner, An innovative approach for cranioplasty using hydroxyapatite cement, *Surg. Neurol.*, 48, 3, 294-297, **1997**.

[7] L.C. Chow, Next generation calcium phosphate-based biomaterials, *Dent. Mater. J.*, 28, 1, 1-10, **2009**.

[8] R.Verheggen, H.A. Merten, Correction of skull defects using hydroxyapatite cement (HAC) – Evidence derived from animal experiments and clinical experience, *Acta. Neurochir.*, 143, 9, 919-926, **2001**.

[9] D.J. Verret, Y. Ducic, L. Oxford, J. Smith, Hydroxyapatite cement in craniofacial reconstruction, *Otolaryngol. Head Neck Surg.*, 133, 6, 897-899, **2005**.

[10] A. Moreira-Gonzaiez, I.T. Jackson, T. Miyawaki, K. Barakat, V. DiNiks, Clinical outcome in cranioplastry: critical review in long-term follow-up, *J. Craniofac. Surg.*, 14, 2, 144-153, **2003** .

[11] S. Mukherjee, B. Thakur, I. Haq, S. Hettige, A.J. Martin, Complications of titanium cranioplasty – a retrospective analysis of 174 patients, *Acta. Neurochi.*, 156, 5, 989-998, **2014**.

[12] J.J. Kuttenberger, N. Hardt, Long-term results following reconstruction of craniofacial defects with titanium micro-mesh systems, *J. Craniomaxillofac. Surg.*, 29, 2, 75-81, **2001**.

[13] H. Kashimura, K. Ogasawara, Y. Kudo, K. Yoshida, A. Sugawara, A. Ogawa, A newly designed hydroxyapatite ceramic burr-hole button, *Vasc. Health Risk Manag.*, 6, 105-108, **2010**.

[14] H.V. Easwer, A. Rajeev, H.K. Varma, S. Vijayan, R.N. Bhattacharya, Cosmetic and radiological outcome following the use of synthetic hydroxyapatite porous-dense bilayer burr-hole buttons, *Acta. Neurochi.*, 149, 5, 481-486, **2007**.

[15] S.W. Low, Y.J. Ng, T.T. Yeo, N. Chou, Use of OsteoplugTM polycaprolactone implants as novel burr-hole covers, *Singapore Med. J.*, 50, 8, 777- 780, **2009**.

[16] G.Z. Sanus, T. Tanriverdi, M.O. Ulu, A.M. Kafadar, N. Tanriover, F. Ozien, Use of cortoss as an alternative material in calvarial defects: the first clinical results in cranioplasty, *J. Craniofac. Surg.*, 19, 1, 88-95, **2008** .

[17] S.H. Yoon, J.S. Burm, W.Y. Yang, S.Y. Kang, Vascularized bipedicled pericranial flaps for reconstruction of chronic scalp ulcer occurring after cranioplasty, *Arch. Plast. Surg.*, 40, 4, 341-347, **2013**.

[18] Y. Shirosaki, T. Okayama, K. Tsuru, S. Hayakawa, A. Osaka, Synthesis and cytocompatibility of porous chitosan-silicate hybrids for tissue engineering scaffold application, *Chem. Eng. J.*, 137, 1, 122-128, **2008**.

[19] Y. Shirosaki, T. Okayama, K. Tsuru, S. Hayakawa, A. Osaka, In vitro bioactivity and MG63 cytocompatibility of chitosan-silicate hybrids, *Int. J. Mater. Chem.*, 3, 3A, 1-7, **2013**.

[20] Y. Shirosaki, K. Tsuru, S. Hayakawa, A. Osaka, Biodegradable chitosan-silicate porous hybrids for drug delivery, *Key Eng. Mater.*, Vols. 361-363, 1219-1222, **2008**.

[21] S. Amado, M.J. Simões, P.A.S. Armada da Silva, A.L. Luís, Y. Shirosaki, M.A. Lopes, J.D. Santos, F. Fregnan, G. Gambarotta, S. Raimondo, M. Fornaro, A.P. Veloso, A.S.P. Varejão, A.C. Maurício, S. Geuna, Use of hybrid chitosan membranes and N1E-115 cells for promoting nerve regeneration in an axonotmesis rat model, *Biomaterials*, 29, 33, 4409-4419, **2008**.

[22] M.J. Simões, A. Gärtner, Y. Shirosaki, R.M. Gil da Costa, P.P. Cortez, F. Gartnër, J.D. Santos, M.A. Lopes, S. Geuna, A.S.P. Varejão, A.C. Maurício, In vitro and in vivo chitosan membranes testing for peripheral nerve reconstruction, *Acta. Med. Port.*, 24, 1, 43-52, **2011**.

[23] I.D. Xynos, A.J. Edgar, L.D.K. Buttery, L.L. Hench, J.M. Polak, Ionic products on bioactive glass dissolution increase proliferation of huan osteoblasts and induce insulin-like growth factor II mRNA expression and protein synthesis. *Biochem. Biophys. Res. Commun.*, 276, 2, 461-465, **2000**.

[24] F. Barrère, C.A. van Blitterswijk, K. de Groot, Bone regeneration: molecular and cellular interactions with calcium phosphate ceramics, *Int. J. Nanomed.*, 1, 3, 317-332, **2006**.

[25] Y. Shirosaki, K. Okamoto, S. Hayakawa, A. Osaka, T. Asano, Preparation of porous chitosan-siloxane hybrids coated with hydroxyapatite particles, *BioMed. Res. Int.*, Article ID 392940, 6 pages, **2014** .

[26] Y. Shirosaki, K. Tsuru, S. Hayakawa, A. Osaka, M.A. Lopes, J.D. Santos, M.A. Costa, M.H. Fernandes, Physical, chemical and in vitro biological profile of chitosan hybrid membrane as a function of organosiloxane concentration, *Acta. Biomater.*, 5, 1, 346-355, **2009**.

[27] L.A. Cyster, D.M. Grant, S.M. Howdle, F.R.A.J. Rose, D.J. Irvine, D. Freeman, C.A. Scotchford, K.M. Shakesheff, The influence of dispersant concentration on the pore morphology of hydroxyapatite ceramics for bone tissue engineering, *Biomaterials*, 26, 7, 697-702, **2005**.

[28] V. Karageorgiou, D. Kaplan, Porosity of 3D biomaterial scaffolds and osteogenesis, *Biomaterials*, 26, 27, 5474-5491, **2005**.

6. ACKNOWLEDGEMENTS

This work was supported in part by the programmes in the "Improvement of Research Environment for Young Researchers (Kojinsenbatsu)", Ministry of Education Culture, Sports, Science and Technology (MEXT), and the Foundation for the Promotion Ion Engineering.