Enhancement of Bone Tissue Formation by Alginate Gel-Assisted Cell Seeding into a Porous Ceramic Scaffold

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Statement of Purpose: For successful cell-based tissue engineering, the cooperation among three basic components is required: a 3D scaffold, appropriate cells, and growth factors as stimuli for differentiation. Scaffolds from calcium phosphate group, such as β-tricalcium phosphate (β-TCP) and hydroxyapatite, have been used widely due to their excellent biocompatibility, resorbability, osteoconduction and osteointegration. However, it has been observed that bone formation in β-TCP and other ceramic scaffolds primarily occurred non-uniformly; with non-uniform cell seeding and nutrient flow as the most probable reasons. In this study, the potentiality of an alginate gel-assisted cell seeding method in improving seeding efficiency, cell proliferation, and osteogenic differentiation on β-TCP scaffolds for bone tissue engineering was investigated. The cell-seeded scaffolds were implanted in Sprague-Dawley rats to assess their capability for *in vivo* bone induction.

Methods: Porous β-TCP scaffolds were prepared via the gel-sponge method, reported previously [1, 2]. Scaffolds were seeded with MG-63 cells by three methods: 1) conventional seeding (CS), 2) alginate gel-assisted (GS), and 3) GS with BMP-2 growth factor (GSB). There were 3×10^5 cells seeded per scaffold in either 900 μL DMEM or in a mixture of 450 μL DMEM and 450 μL of 1w% sodium alginate (Pronova VLVG, Novamatrix) solution per scaffold, with 50 ng/mL BMP-2 added to the cell solution before seeding for GSB samples. Cell solutions were added to scaffolds dropwise, incubated for 4 hours, and crosslinked in 0.2 M $CaCl_2$ solution (GS+GSB). All samples were immersed in standard media and incubated at 37°C with 5% CO_2 with regular media changes. Seeding efficiency was determined by quantifying the cells remaining in the wells using a hemacytometer after the four-hour incubation. Cell proliferation was assessed with the alamarBlue assay (Invitrogen) after a one-week incubation. Osteocalcin protein was assayed after one week *in vitro* cell culture by staining with an osteocalcin primary antibody (Abcam) and FITC secondary (Abcam) following standard methods. Release of BMP-2 from β-TCP–alginate gel scaffolds was quantified over a 13-day period by a Sandwich ELISA BMP-2 immunoassay (Quantikine, DBP200). For SEM observation, one week *in vitro* cultured samples were fixed, dehydrated, critical point dried, and sputter coated. For *in vivo* trials, mesenchymal stem cells (MSCs) were harvested from rats and seeded in the scaffolds. CS, GS and GSB samples seeded with MSC were prepared for *in vivo* study using similar methods to *in vitro* samples, with an untreated β-TCP scaffold was used as a control. Cell-scaffold samples were implanted into an ectopic location in female Sprague-Dawley rats. After eight weeks, the rats were sacrificed according to the UW IACUC procedures. The harvested *in vivo* samples were prepared according to common histology practices and stained with hematoxylin and eosin (H&E) and Masson's trichrome.

Results: The alginate-gel assisted seeding system showed significant improvement in seeding efficiency with a β-TCP scaffold. Seeding efficiency for conventional method was 59.4 ± 11.3% (CS sample), it increased to 95.7 ± 5.3% (GS sample) and 95.3 ± 6.3% (GSB samples) as shown in Figure 1. This indicates that alginate gel assisted in retaining the cells. There was greater population of MG-63 cells in both GS and GSB samples compared to CS samples after one week cell culture (658,125 ± 74,434, 1,002,903 ± 114,210 and 1,019,070 ± 122,507 for CS, GS and GSB respectively). Osteocalcin stained *in vitro* samples indicated greater osteocalcin present in GS and GSB samples than in CS samples. BMP-2 release rates from alginate gel remained relatively consistent, exhibiting a linear trend of the cumulative release profile, releasing 52.6% of dose over a 2 week period. SEM imaging showed the morphology of MG-63 cells after one week of cell culture. These results imply that cells seeded in the alginate-gel seeding β-TCP scaffold were retained within the scaffolds and showed enhanced osteogenic activity. The osteogenic activity of the *in vivo* implants was evaluated with H&E and Masson's trichrome stains. Cells were found throughout all of the samples. Mineralized bone matrix was observed in the control sample, demonstrating the osteogenic activity of the scaffold. Comparing among CS, GS and GSB samples, existence of bone matrix was more observed in both GS and GSB samples. These results confirm the efficacy of alginate gel-assisted cell seeding for enhancing *in vivo* osteoconductivity and tissue development.

Conclusions: The efficiency of alginate gel-assisted method in improving bioactivity of ceramic scaffolds for tissue engineering was investigated. The *in vitro* results demonstrated an enhanced seeding efficiency and osteogenic activities of MG-63 cells compared to the conventional seeding method. The *in vivo* results using MSCs also showed improvement in osteogenic activities with gel-assisted seeding system including growth factor. In addition, alginate gel showed the capability to control the release of growth factor BMP-2. Therefore, it can be concluded that the alginate gel-assisted cell seeding method has the potential to enhance bone regeneration and can be investigated for use with other tissue engineering applications.

References:

1 Ramay HRR. Biomat. 2003;24:3293-3302.

2 Ramay HR. Biomat. 2004;25:5171-5180.