Ashdin Publishing
Bioceramics Development and Applications
Vol. 1 (2011), Article ID D110158, 3 pages
doi:10.4303/bda/D110158

Research Article

Enzymatically Enhanced Guided Tissue Regeneration

Jeroen J. J. P. van den Beucken, Lise T. de Jonge, Adelina S. Plachokova, and John A. Jansen

Department of Biomaterials, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
Address correspondence to Jeroen J. J. P. van den Beucken, j.vandenbeucken@dent.umcn.nl

Received 14 January 2011; Accepted 3 February 2011

Abstract In order to improve the efficacy of membranes used for guided tissue regeneration treatments, collagen membranes were surface-modified with the pro-mineralization enzyme alkaline phosphatase (ALP). ALP was surface-immobilized using electrostatic spray deposition (ESD) to preserves the biological activity of the enzyme. Initially, the optimal ALP-deposition time was assessed using ALP-activity assays and an immersion experiment with subsequent measurements on calcium deposition. These initial experiments demonstrated that optimally efficient membranes were obtained with an ALP deposition time of 30 minutes. Subsequently, ALP-coated and non-coated controls were enrolled in a cell culture experiment with primary rat bone marrow derived osteoblast-like cells. ALP-coated membranes demonstrated to have no effect on cell proliferation and morphology, but significantly accelerated the mineralization of the extracellular matrix. These results show the potential of ALP as a surface-immobilized active enzyme in biomaterial research and justify animal experimental work on this topic to obtain in vivo data.

Keywords alkaline phosphatase; coating; guided bone regeneration; electrostatic spray deposition; cell culture

1 Introduction

Guided bone regeneration (GBR) involves the application of a membrane-like barrier covering the defect to ensure that cells with the capacity to regenerate bone tissue repopulate the defect site. According to the GBR principle, cells with access to the wound space will determine the nature of the regenerated tissue [5]. As a precautionary measure to prevent collapse of the membrane, graft material (e.g. ceramic granules) can be placed in the confined area.

To date, the role of the membrane in GBR has been rather passive, i.e. the membrane only has a barrier-function. In view of new developments at the level of surface modification with surface-active biological compounds, however, the role of the membrane can be converted to an active one with stimulatory effects on tissue regeneration. For preservation of surface-activity, delicacy of the technology to immobilize biological compounds is required. Electrostatic spray deposition (ESD) represents an efficient technology for the deposition of biological compounds, as the process is highly accurate (i.e. cost-effective) and preserves functional activity of biological compounds due to relatively low ambient temperatures and fast drying [4, 7].

Recent studies on the use ESD for deposition of the pro-mineralization enzyme alkaline phosphatase (ALP) as an immobilized catalyst to increase inorganic phosphate concentrations have demonstrated to accelerate surface mineralization [1] and enhance osteogenic cell behavior in vitro [2]. Furthermore, an implantation study in rats has shown in vivo potential of ALP-coatings by significantly improving bone-to-implant contact compared to non-coated titanium controls [6].

The current study aimed to evaluate the in vitro effects of ALP-coated GBR-membranes by immersion experiments in organic phosphate-containing medium and cell culture experiments with primary rat bone marrow-derived osteoblast-like cells.

2 Materials and methods

BioGide© resorbable bilayer membranes (Geistlich Biomaterials, Geistlich Pharma AG, Wolhusen, Switzerland) were cut into 1×0.5 cm square membranes. Prior to ALP-coating, the membranes were pre-treated by magnetron sputtering with a 50 nm layer of titanium to obtain a conductive material. Deposition of ALP was carried out using a commercially available vertical ESD set-up (Advanced Surface Technology, Bleiswijk, the Netherlands) using an aqueous ALP-solution (1 mg/mL in 90:10 (v/v) water:ethanol) and standardized deposition parameters (15% relative humidity; 40 °C holder temperature; 40 mm nozzle-to-substrate distance; 0.15 mL/h flow rate; 8–11 kV applied voltage) with various deposition times (0–60 minutes).

Optimal ALP deposition time was assessed using ALP-activity assay [1] and 2-weeks immersion studies in culture medium (α-MEM, supplemented with 10% FCS, 10^{-8} M dexamethason, 10 mM β-glycerophosphate, and 50 μg/mL ascorbic acid.

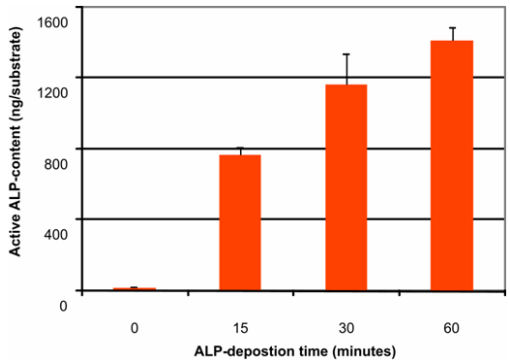

Figure 1: Active ALP-content of membranes coated with ALP for up to 60 minutes. Bars represent mean ± standard deviation ($n = 3$).

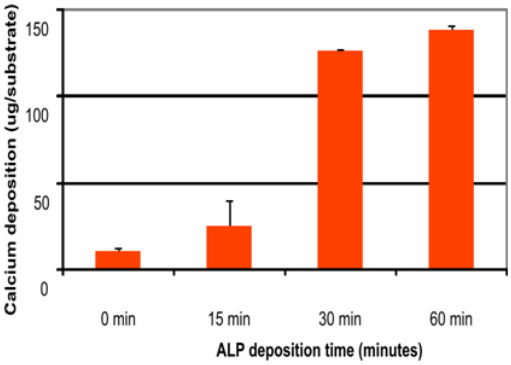

Figure 2: Calcium deposition onto membranes coated with ALP for up to 60 minutes after a 2-week immersion period in culture medium. Bars represent mean ± standard deviation ($n = 3$).

Cell culture experiments were carried out with ALP-coated membranes and non-coated controls in two individual runs. For each run, cells were freshly isolated from the femora of male Wistar rats and pre-cultured for 1 week in culture medium, as described previously [3]. Subsequently, cells were seeded at a density of 40.000 cells/membrane and cultured for up to 24 days. Cell behavior was evaluated by assessing proliferation (protein BCA assay), differentiation (ALP-activity assay), mineralization (calcium assay), and cell morphology (scanning electron microscopy).

3 Results and discussion

Membrane optimization

ALP was deposited using ESD for up to 60 minutes, after which ALP-activity was assessed. Figure 1 shows a linear increase in ALP-activity with increasing ALP deposition time.

Subsequently, ALP-coated and non-coated control membranes were immersed in culture medium for a period of 2 weeks. The deposition of calcium onto the membranes during this immersion period is presented in Figure 2, showing a non-linear increase with ALP deposition time.

Together, the results on active ALP-content and calcium deposition demonstrate that a ALP deposition time of 30 minutes is optimal.

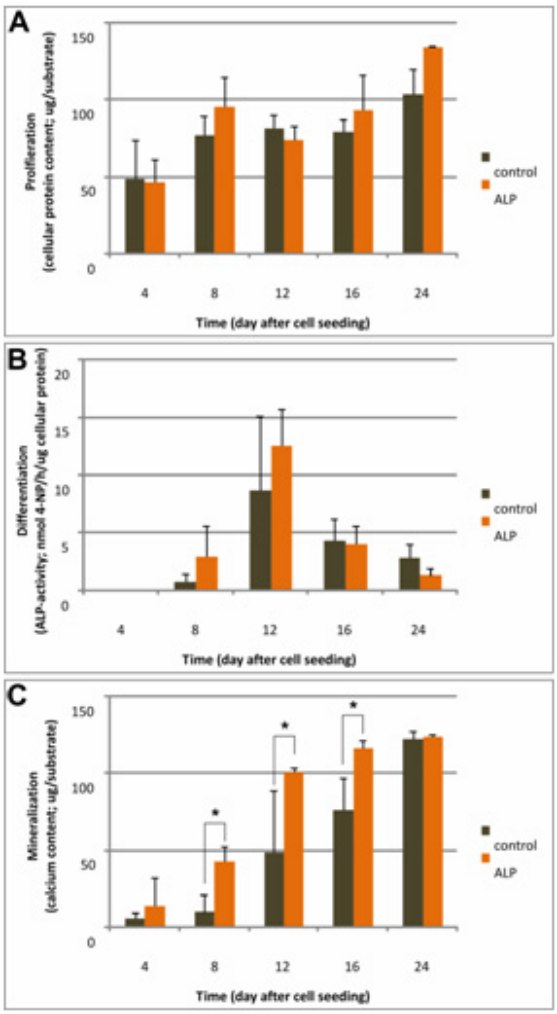

Figure 3: Proliferation (A), differentiation (B), and mineralization (C) of osteoblast-like cells cultured on ALP-coated and non-coated control membranes for up to 24 days. Bars represent mean ± standard deviation ($n = 3$). ⋆ indicates statistically significant difference ($P < .05$).

Cell culture experiments

Cell culture experiments were carried out using two experimental substrates: ALP-coated membranes (30 minutes ALP deposition time) and non-coated controls in two individual runs. Both runs showed similar results and the results of one run are presented below.

Cell proliferation (Figure 3(A)) showed an increase during cell culture for both experimental groups.

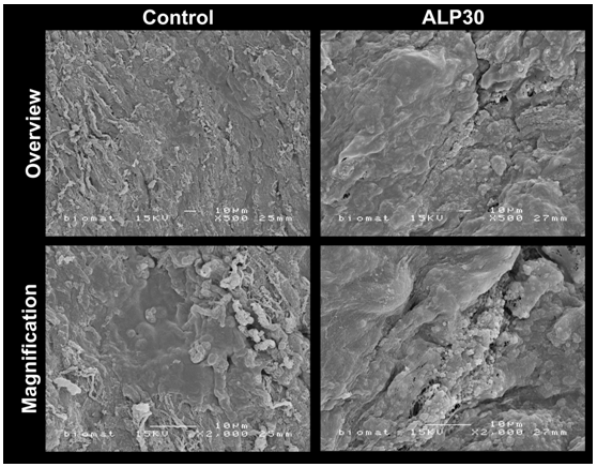

Figure 4: Scanning electron microscopy images of ALP-coated and non-coated control membranes after 16 days of cell culture with osteoblast-like cells.

Osteoblast-like cell differentiation showed an increase till day 12 of cell culture, after which ALP-activity decreased. No statistically significant differences were found for cell proliferation and differentiation between the experimental groups at individual time points ($P > .05$). In contrast, ALP-coated membranes significantly accelerated mineralization of osteoblast-like cells compared to non-coated controls at days 8, 12, and 16 of cell culture ($P < .05$). After 24 days of cell culture, mineralization was equal for both experimental groups.

Scanning electron microscopy (Figure 4) confirmed the calcium measurement, showing an higher extent of deposition of calciumphosphate nodules on ALP-coated membranes compared to non-coated controls.

4 Conclusions

The membrane optimization experiments demonstrate that the optimal ALP deposition time is found at 30 minutes. These ALP-coated membranes significantly accelerate mineralization during osteoblast-like cell culture compared to non-coated control membranes without affecting cell proliferation, differentiation, and morphology. Consequently, the data of the present study justify continuation of experimental work on ALP-coated membranes towards animal experiments.

Acknowledgment This work was supported by a grant from Osteology Foundation (Lucerne, Switzerland; grant #07-015).

References

[1] L. T. de Jonge, S. C. G. Leeuwenburgh, J. J. J. P. van den Beucken, J. G. C. Wolke, and J. A. Jansen, *Electrosprayed enzyme coatings as bioinspired alternatives to bioceramic coatings for orthopedic and oral implants*, Adv Funct Mater, 19 (2009), 755–762.

[2] L. T. de Jonge, J. J. van den Beucken, S. C. Leeuwenburgh, A. A. Hamers, J. G. Wolke, and J. A. Jansen, *In vitro responses to electrosprayed alkaline phosphatase/calcium phosphate composite coatings*, Acta Biomater, 5 (2009), 2773–2782.

[3] C. Maniatopoulos, J. Sodek, and A. H. Melcher, *Bone formation in vitro by stromal cells obtained from bone marrow of young adult rats*, Cell Tissue Res, 254 (1988), 317–330.

[4] V. N. Morozov and T. Y. Morozova, *Electrospray deposition as a method to fabricate functionally active protein films*, Anal Chem, 71 (1999), 1415–1420.

[5] S. Nyman, *Bone regeneration using the principle of guided tissue regeneration*, J Clin Periodontol, 18 (1991), 494–498.

[6] C. Schouten, J. J. van den Beucken, L. T. de Jonge, E. M. Bronkhorst, G. J. Meijer, P. H. Spauwen, and J. A. Jansen, *The effect of alkaline phosphatase coated onto titanium alloys on bone responses in rats*, Biomaterials, 30 (2009), 6407–6417.

[7] I. Uematsu, H. Matsumoto, K. Morota, M. Minagawa, A. Tanioka, Y. Yamagata, et al., *Surface morphology and biological activity of protein thin films produced by electrospray deposition*, J Coll Interface Sci, 269 (2004), 336–340.