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Immobilization and Controlled Release of Vascular (VEGF) and Bone Growth Factors (BMP-2) on Bone Replacement Materials

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Abstract

Angiogenesis and osteogenesis are closely related processes sharing some essential mediators like VEGF (Vascular Endothelial Growth Factor) and BMP-2 (Bone Morphogenetic Protein-2). As vascularization is crucial to bone formation biofunctionalization of bone replacement materials with both of these proteins may result in enhanced bone formation. Here we will present immobilization of self prepared biologically active BMP-2 and VEGF on different bone replacement materials and their controlled release. The amount of bound protein can be steered as a function of protein concentration in the incubation solution. Desorption behaviour of proteins differs strongly depending on the type of bone replacement material used and protein ranging from 0.1-2 days for the burst phase and from 17-115 days for the sustained release phase. A combined loading and release of the growth factors is now planned.

1. Introduction

The replacement of autologous bone by bone replacement materials biofunctionalized with growth factors like BMP-2 (Bone Morphogenetic Protein-2) is a challenging goal in clinical research. Previously we have demonstrated that large amounts of BMP-2 can be immobilized on a hydroxyapatite based material (Algipore©). The released BMP-2 is highly bioactive [1,2]. VEGF (Vascular Endothelial Growth Factor) has been shown to be another important mediator for osteogenesis and angiogenesis [3-5]. Therefore the combined loading of bone replacement materials with BMP-2 and VEGF may result in accelerated and improved de novo bone formation.

2. Materials and Methods

2.1 Bone Replacement Materials

In this study we used AlgOss 100 (AlgOss, Vienna, Austria), a phycogenic material [6] and Osnatal (Tissue Processing International, Nijmegen, The Netherlands), a human material as hydroxyapatite based materials. In addition Maxresorb (Botiss, Berlin, Germany), a synthetic biphasic calciumphosphate consisting of 40% tricalciumphosphate and 60% hydroxyapatite was used. All materials are granular materials with a particle size in a range from 0.5-1.0 mm (AlgOss 100, Maxresorb) respectively 0.5-1.5 mm (Osnatal).

2.2 Preparation and Testing of BMP-2 and VEGF

BMP-2 (see [7,8]) as well as VEGF (see [9]) were self prepared by expression in E. coli. Biological activity of BMP-2 was tested with MC3T3-E1 cells by the activation of the de novo synthesis of alkaline phosphatase ($K_{0.5}$ /Kd ~ 5-20 nM) [10]. The biological activity of VEGF was determined ($K_{0.5}$ /Kd ~ 5-20 pM) with HUVEC cells in a proliferation-assay (see [11]).

2.3 Immobilization of BMP-2 and VEGF

The amounts of bound protein were determined by using radioactive labeled proteins (^{125}I-BMP-2, ^{125}I-VEGF) (see [8]). For protein immobilization the different bone replacement materials were incubated in a sodium acetate buffered (pH 4.5) solution containing BMP-2 and/ or VEGF in various concentrations (see [12]).

2.4 Desorption Experiments

After adsorption of BMP-2 and/ or VEGF the coated bone replacement material was incubated in PBS buffer (pH 7.4). At the indicated times buffer was removed, the amount of labelled BMP-2 respectively VEGF still bound on the material was quantified in a γ-counter. Before each new measurement the material is washed in PBS buffer and after measurement in the γ-counter incubated for the next period again in fresh PBS buffer (see [1,13]).

3 Results

3.1 Biological activity of self prepared BMP-2 and VEGF

Self prepared BMP-2 is pure and is highly biological active comparable to commercially available BMP-2 (Wyeth) as shown in **Fig. 1**. The $K_{0.5}$ value as a criterion for biological activity is 3.2 nM for self prepared BMP-2 and 3.1 nM for BMP-2 purchased from Wyeth.

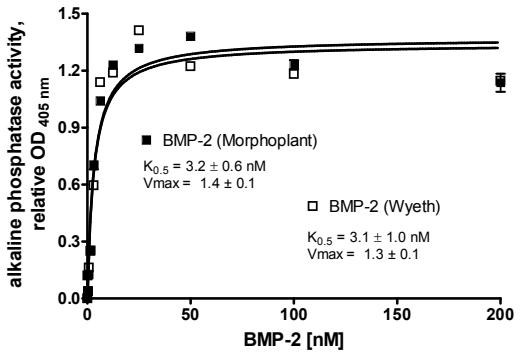

Figure 1: *Dose-activity-response curve of self prepared and commercial BMP-2 in a MC3T3-E1 cell assay [see 8]. For further details see Methods.*

The biological activity of self prepared VEGF determined by a proliferation-assay by using HUVEC cells is shown in **Fig. 2**. Also this activity is comparable to commercially available protein (Biomol, Hamburg, Germany). The $K_{0.5}$ value for VEGF is significant less than the $K_{0.5}$ for BMP-2. It is ca. 20 **pM** compared to ca. 3 **nM** for BMP-2. So also significant less VEGF compared to BMP-2 should be necessary to achieve a biological effect *in vivo*.

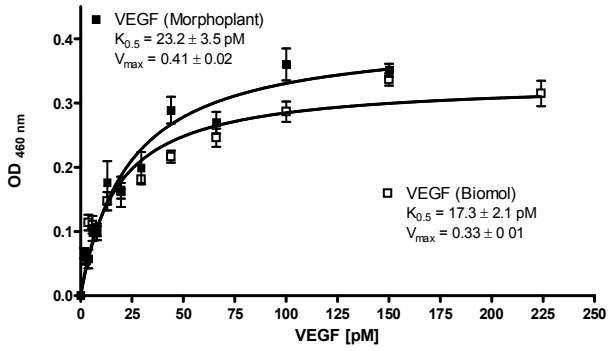

Figure 2: *Dose-activity-response curve of self prepared and commercial VEGF in an HUVEC cell based proliferation assay. For further details see Methods.*

3.2 Immobilization of BMP-2 and VEGF

As shown in **Table 1** the amount of bound BMP-2 can be steered by varying the protein concentration in the incubation solution. By using 0.3 mg BMP-2/ ml we are able to bind ca. 2 mg BMP-2/ g granules. All three tested bone replacement materials bind similar amounts of BMP-2.

Table 1: *Immobilization of ^{125}I-BMP-2 on different bone replacement materials*

	Immobilized BMP-2 [mg/g]		
	AlgOss 100	Osnatal	Maxresorb
c_0 = 0.1 mg/ml	0.81 ± 0.01	0.57 ± 0.04	0.77 ± 0.01
c_0 = 0.3 mg/ml	1.72 ± 0.04	1.92 ± 0.18	2.22 ± 0.01

The amount of immobilized VEGF also depends on the concentration of the used protein solution and increases dose-dependent. However in contrast to BMP-2 there are clear differences between the three used materials: AlgOss 100 binds over tenfold more VEGF than Osnatal (see **Table 2**). Up to 370 µg VEGF can be immobilized per g AlgOss 100.

Table 2: *Immobilization of ^{125}I-VEGF on different bone replacement materials*

	Immobilized VEGF [µg/g]		
	AlgOss 100	Osnatal	Maxresorb
c_0 = 10 µg/ml	86.5 ± 4.7	8.1 ± 0.6	38.1 ± 1.1
c_0 = 50 µg/ml	368.2 ± 1.5	29.9 ± 0.6	82.1 ± 3.2

3.3 Desorption of BMP-2 and VEGF

For biological functionality of growth factors like BMP-2 and VEGF it is essential that they are released over a defined period of time depending on their biological function. The bound protein should be released from the surface over several days or weeks in biologically active form.

The release of the immobilized proteins occurred in form of a two phase exponential decay: an initial burst-phase is followed by a prolonged second phase. **Fig. 3** shows the release of BMP-2 from different bone replacement materials. Osnatal loses ca. 35% of immobilized protein within the first two days of desorption. Half-lives for the second phase of BMP-2 release lie in a range from 33 up to 115 days for all three materials so bone formation can be stimulated over several weeks respectively months.

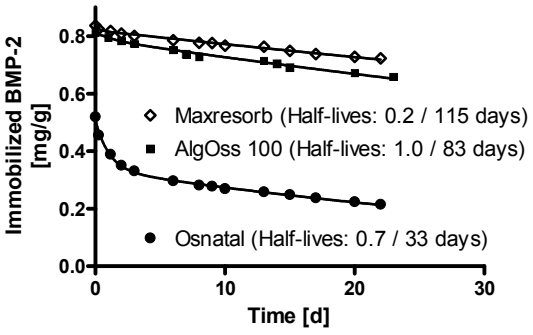

Figure 3: *Release of ^{125}I-BMP-2 from different bone replacement materials (adsorption: c_0 = 0.1 mg/ml)*

In **Fig. 4** the release of immobilized VEGF from different bone replacement materials is demonstrated. Half-lives for the second slower phase of desorption lie in a range from 17 to 38 days and are lower compared to the release of BMP-2. All three tested materials show a pronounced burst-phase. Interestingly BMP-2 and VEGF differ strongly in desorption behaviour from AlgOss 100: VEGF is released with a pronounced burst-phase whereas BMP-2 is released slowly and constant.

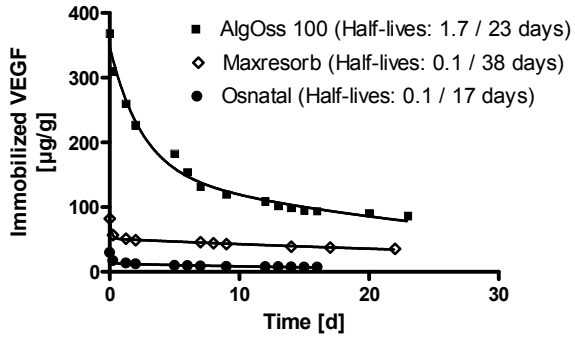

Figure 4: *Release of ^{125}I-VEGF from different bone replacement materials (adsorption: c_0 = 50 µg/ml)*

4 Conclusion

Bone (BMP-2) and vascular (VEGF) growth factors were self prepared in biological active form and used for coating experiments with human (Osnatal), synthetic (Maxresorb) and phycogenic (AlgOss) bone replacement materials. Radioactive labelling of proteins allowed for identification of adsorption and desorption behaviour. Therefore we were able to determine the amounts of immobilized BMP-2 respectively VEGF. All three used materials bind similar amounts of BMP-2 but there are significant differences in the adsorption of VEGF. Work is in progress for the preparation of a carrier for both growth factors and the simultaneous release of the factors in rates and amounts that comply to the sequences lain down by nature during bone healing.

5. References

[1] Zurlinden, K., Laub, M., & Jennissen, H. P. (2005) *Materialwiss. Werkstofftech. (Mat. Sci. Engineer. Technol.)*, **36,** 820-827.

[2] Zurlinden, K., Laub, M., Spassova, E., Moser, D., Schopper, C., Russmüller, G., Ewers, R., & Jennissen, H. P. (2010) *Biomed. Tech.*, **55 (Suppl. 1),** 28-30.

[3] Senger, D. R., Galli, S. J., Dvorak, A. M., Perruzzi, C. A., Harvey, V. S., & Dvorak, H. F. (1983) *Science*, **219,** 983-985.

[4] Nieves, B. J., D'Amore, P. A., & Bryan, B. A. (2009) *Biofactors*, **DOI: 10.1002/biof.46,** published online.

[5] Mayr-Wohlfart, U., Waltenberger, J., Hausser, H., Kessler, S., Gunther, K. P., Dehio, C., Puhl, W., & Brenner, R. E. (2002) *Bone*, **30,** 472-477.

[6] Spassova, E., Gintenreiter, S., Halwax, E., Moser, D., Schopper, C., & Ewers, R. (2007) *Materialwiss. Werkstofftech.*, **38,** 1027-1034.

[7] Jennissen, H. P., Zumbrink, T., Chatzinikolaidou, M., & Steppuhn, J. (1999) *Materialwiss. Werkstofftech. (Mat. Sci. Engineer. Technol.)*, **30,** 838-845.

[8] Laub, M., Chatzinikolaidou, M., & Jennissen, H. P. (2007) *Materialwiss. Werkstofftech. (Mat. Sci. Engineer. Technol.)*, **38,** 1020-1026.

[9] Zhang, X., Potty, A. S., Jackson, G. W., Stepanov, V., Tang, A., Liu, Y., Kourentzi, K., Strych, U., Fox, G. E., & Willson, R. C. (2009) *J. Mol. Recognit.*, **22,** 154-161.

[10] Wiemann, M., Rumpf, H. M., Bingmann, D., & Jennissen, H. P. (2001) *Materialwiss. Werkstofftech. (Mat. Sci. Engineer. Technol.)*, **32,** 931-936.

[11] Rui, J., Dadsetan, M., Runge, M. B., Spinner, R. J., Yaszemski, M. J., Windebank, A. J., & Wang, H. (2012) *Acta Biomater.*, **8,** 511-518.

[12] Jennissen,H.P. & Zurlinden,K.(2008) Process for Producing Particles Loaded with Growth Factors, and the Particles Obtained in this Way. PCT Publ.No. WO2009056567, [EP2209505], pp. 1-21 (+ 3 Figs.). European Patent Office, Munich.

[13] Chatzinikolaidou, M., Lichtinger, T. K., Müller, R. T., & Jennissen, H. P. (2010) *Acta Biomater.*, **6,** 4405-4421.