Modulating Osteogenesis of Mesenchymal Stem Cells by Modification of Growth Factor Availability

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Statement of Purpose: Growth factors play an important role in controlling the proliferation and differentiation of osteoprogenitor cells. In this in vitro study, four growth factors (VEGF, IGF-1, FGF-2 and BMP-2) were selected to explore the effects of growth factors on osteogenesis of mesenchymal stem cells (MSCs). The addition of growth factors and neutralizing antibodies according to a previously documented specific release pattern in vitro (profiled addition) were compared with a constant, daily addition (constant addition), with respect to the effects on bone formation. We hypothesized that the profiled addition of specific growth factors would yield higher expression of bone-related markers compared with constantly applied growth factor.

Methods: Osteoprogenitors were harvested from the femora of C57BL mice, male, 10-12 weeks old, using an established protocol. The cells were cultured in osteogenic media over a 4-week period. Progression of osteogenesis was monitored by markers for cell proliferation, production of osteocalcin (OC), alkaline phosphatase (ALP-2), and Von Kossa staining. Two different protocols were applied to deliver growth factors: *profiled addition:* applying by mimicking documented release profiles of the growth factors (**Fig. 1**); *constant addition :* applying constantly at 10x of the highest concentration of the expression level the growth factors. Profiled addition started two days before and ended two days after the peak of the expression. Cells without addition of growth factors were used as control. For the constant addition, the final concentrations of VEGF, IGF-1, FGF-2 and BMP-2 were 12 ng/ml, 15 ng/ml, 300 ng/ml and 1.4 ng/ml, respectively. The same protocols were also applied to neutralize the growth factors. The ratio of antibodies to growth factor was 10:1, which should neutralize 90% of the released growth factor. The final concentrations of neutralizing antibodies used for constant addition were 90 ng/ml, 292 ng/ml, 2370 ng/ml and 4.77 ng/ml for VEGF, IGF-1, FGF-2 and BMP-2, respectively. One-way ANOVA and Tukey's Post Hoc were conducted using SPSS 14.0.

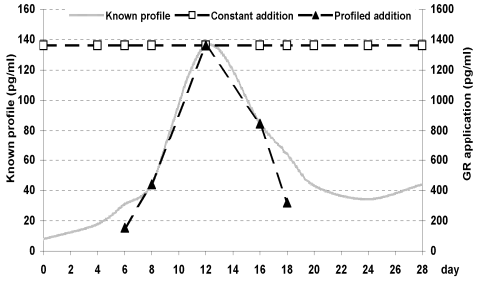

Fig. 1. The administration protocols of constant and profiled addition. The right Y-axis (growth factor delivery) is **10 times** higher than the left Y-axis (documented release level).

Results:

Addition of VEGF and BMP2 increased MSC proliferation Profiled but not constant addition of VEGF increased MSC cell number by 1.9 fold ($p < 0.05$, Fig. 2). Similarly, constant addition of BMP-2 stimulated cell proliferation by 50% ($p < 0.05$). All other groups of VEGF and BMP2 did not affect MSC proliferation; neither did the groups with added IGF-1 or FGF-2.

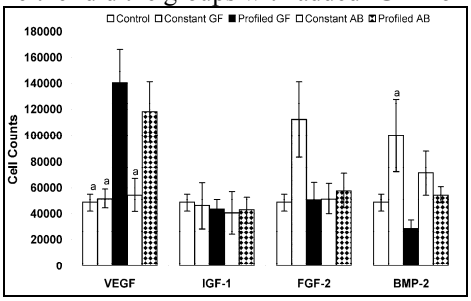

Fig. 2. Profiled addition of VEGF and constant addition of BMP-2 increased proliferation of MSCs. a. $p < 0.05$ vs. profiled addition, One-Way

Addition of IGF-1, FGF2 and BMP-2 modified ALP-2 activity of MSCs Compared with the control group, both constant and profiled addition of FGF-2 decreased ALP-2 activity of MSCs by 92% and 77%, respectively ($p < 0.05$, Fig. 3). Similarly, constant and profiled application of IGF-1 decreased ALP-2 activity by 52% and 69%, respectively, but no statistical significance was reached. Profiled addition of antibodies for IGF-1, FGF-2 and BMP-2 decreased ALP-2 levels by 95%, 89% and 77%, respectively ($p < 0.05$, Fig. 3). Constant addition of IGF-1 and BMP-2 antibodies also decreased ALP-2 activity by 88% and 78%, respectively ($p < 0.05$).

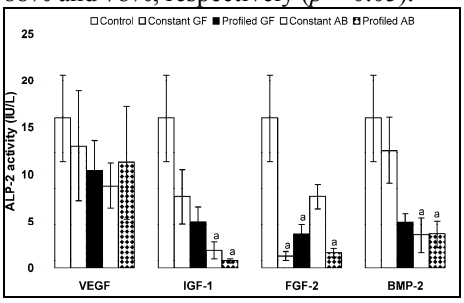

Fig. 3. Neutralization antibodies of IGF-1 and BMP-2, either by constant or profiled addition, decreased ALP-2 activities of MSCs. a. $p < 0.05$ vs. control.

Conclusions:

The aim of this study was to enhance osteogenesis of MSCs by the addition of exogenous growth factors and antibodies according to the known expression profile of these molecules in vitro. Selective, temporally specific addition of growth factors, such as BMP-2 and VEGF appeared to enhance osteogenesis. Drug delivery devices that facilitate profiled availability of specific growth factors may provide optimized strategy to enhance osteogenesis.

Acknowledgement
Supported in part by the Ellenburg Chair in Surgery at Stanford University and the Khosla/Santosh Foundation.