Effects of the orientation of anti-BMP2 monoclonal antibody immobilized on scaffold in antibody mediated osseous regeneration

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Statement of Purpose: Recently, we have shown that anti-BMP2 monoclonal antibodies (mAbs) can trap endogenous BMP ligands and thus provide BMP inductive signals for osteo-differentiation of progenitor cells. Here, it was hypothesized that the effectiveness of this strategy requires the availability of the anti-BMP-2 mAb antigen binding sites.

Methods: *In vitro* testing included a flow cytometric assay to determine the binding of immune complex between anti-BMP-2 mAb attached to protein G-coupled microbeads and rhBMP-2 to C2C12 cells. Next, protein G was used as a linker to the anti-BMP-2 mAb Fc region to absorbable collagen sponge (ACS) and implanted into rat calvarial defects. The ability of ACS/protein G/anti-BMP-2 mAb immune complex to mediate bone formation was compared to that of ACS/protein G/isotype control mAb or ACS/anti-BMP-2 mAb. Moreover, the biomechanical strength of bone regenerated by ACS/protein G/anti-BMP-2 mAb immune complex was compared to the strength of the regenerated bone by ACS/anti-BMP-2 mAb or ACS/protein G/isotype mAb control group.

Results: Results demonstrated higher binding of the anti-BMP-2/BMPs to C2C12 cells, when the mAb was initially attached to protein G microbeads. After 8 weeks, micro-CT and histomorphometric analyses revealed increased bone formation within defects implanted with ACS/protein G/anti-BMP-2 mAb compared with defects implanted with ACS/anti-BMP-2 mAb ($p<0.05$) or isotype control mAb ($p<0.05$). Immunohistochemical staining confirmed increased BMP-2, -4, and -7 expression in presence of ACS/protein G/anti-BMP-2 mAb *in vivo*. Our biomechanical analysis showed that scaffold immobilized with protein G/anti-BMP-2 mAb regenerated significantly stronger bone in comparison to anti-BMP-2 mAb. The negative control group, protein G/isotype mAb, did not promote bone regeneration and exhibited significantly lower mechanical properties ($p<0.05$).

Conclusions: Altogether, the results of this study demonstrated that application of protein G as a linker to immobilize anti-BMP-2 mAb, increased *in vitro* binding of the anti-BMP-2 mAb/BMP immune complex with BMP-receptor positive cell, as well as increased strength and amounts of *de novo* bone formation *in vivo*.