Functionalization of scaffolds with chimeric anti-BMP-2 monoclonal antibodies for osseous regeneration

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Statement of Purpose: Bone tissue reconstruction is often necessitated by congenital anomalies, infection, trauma, and skeletal diseases. Bone tissue engineering concepts have sought to leverage advances in development of biomaterial scaffolds, signaling molecules and progenitor cells to regenerate tissues that match the physical and biological properties of natural tissues. Recent studies have demonstrated the ability of murine anti-BMP-2 monoclonal antibodies (mAb) immobilized on an absorbable collagen sponge (ACS) to mediate *de novo* bone formation, a process termed antibody mediated osseous regeneration (AMOR). The objectives of this study were to assess the efficacy of a newly generated chimeric anti-BMP-2 mAb in mediating AMOR, as well as to evaluate the suitability of different biomaterials as scaffolds to participate in AMOR.

Methods: The effects of the chimeric anti-BMP-2 mAb/BMP-2 immune complex on osteogenesis of C2C12 cells was evaluated *in vitro* through Alizarin Red staining and Western Blot analysis. In addition, chimeric anti-BMP-2 mAb was immobilized on 4 biomaterials, namely, titanium microbeads (Ti), alginate hydrogel, macroporous biphasic calcium phosphate (MBCP) and ACS, followed by surgical implantation into rat critical-size calvarial defects. Animals were sacrificed after 8 weeks and the degree of bone fill was assessed using micro-CT and histomorphometry.

Results: Results demonstrated local persistence of chimeric anti-BMP-2 mAb up to 8 weeks, as well as significant *de novo* bone regeneration in sites implanted with chimeric anti-BMP-2 antibody immobilized on each of the 4 scaffolds. Ti and MBCP showed the highest volume of bone regeneration, presumably due to their resistance to compression. Alginate and ACS also mediated *de novo* bone formation, though significant volumetric shrinkage was noted. *In vitro* assays demonstrated cross-reactivity of chimeric anti-BMP-2 mAb with BMP-4 and BMP-7. Immune complex of anti-BMP-2 mAb with BMP-2 induced osteogenic differentiation of C2C12 cells *in vitro*, involving expression of RUNX2 and phosphorylation of Smad1.

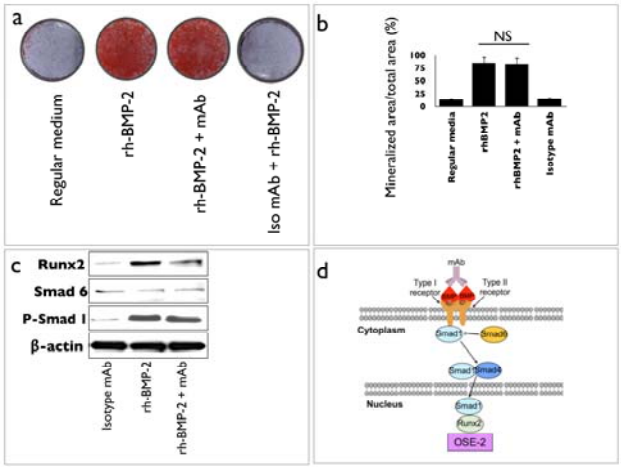

Figure 1. (a) Alizarin red staining indicating mineralized nodule formation of cultured C2C12 cells after treatment with either BMP-2 or chimeric anti-BMP-2 mAb after four weeks. (b) Quantitative analysis of the amount of alizarin staining. (c) Western blot analysis showing the effect of chimeric mAb on the levels of expression of regulators of osteogenesis in C2C12 cells. (d) Graphical summary of interactions between chimeric anti-BMP-2 mAb and the receptors (BMPR-I and BMPR-II) that mediate AMOR through the BMP signaling pathway. NS: not significant.

Conclusions: Here we report the application of chimeric anti-BMP-2 mAb immobilized on different types of scaffolds in the process of antibody mediated bone regeneration. The propose system has the ability to mediate *de novo* bone formation efficiently, as confirmed by our *in vitro* and *in vivo* studies. Furthermore, we report the cross-reactivity of the newly generated chimeric anti BMP-2 mAb with BMP-4 and BMP-7 through specificity studies. The anti-BMP-2 antibody is capable of binding to endogenous BMP-2, BMP-4 and BMP-7, leading to enhanced antibody-mediated bone regeneration. Finally, AMOR can be regulated through the BMP signaling pathway, as confirmed by the *in vitro* experiments. The present data demonstrated the ability of chimeric anti-BMP-2 mAb to functionalize different biomaterial with varying characteristics to mediate osteogenesis.

References:

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