Protamine Based Polyelectrolyte Coated Micro-carriers Of Recombinant Human Bone Morphogenetic Protein 2 Enhanced Posterolateral Spinal Fusion In Rats

Tao Hu, MD1, Raymond Wing Moon Lam, PhD1, Ming Wang, MD2, Soo Yein Toh, B.Sc1, Sunny Akogwu Abbah, MD.PhD1, James Cho-Hong Goh, PhD1, Jun Li, PhD3, Kishore Bhakoo, PhD4, Simon Cool, PhD5, Hee-Kit Wong, FRCS1,2.
1Department of Orthopaedic Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, 2Department of Orthopaedic Surgery, National University Hospital, Singapore, Singapore, 3Department of Bioengineering, National University of Singapore, Singapore, Singapore, 4Singapore Bioimaging Consortium, Agency for Science, Technology and Research, Singapore, Singapore, 5Institute of Medical Biology, Agency for Science, Technology and Research, Singapore, Singapore.

Disclosures:

Introduction: Recombinant human bone morphogenetic protein 2 (rhBMP-2) is regarded as the most potent bone inducing growth factor. However, large amounts are required for consistency in clinical outcome, which leads to significant complications, such as seroma and heterotopic bone formation. This has been attributed in part to poor modulation of in vivo release from absorbable collagen carriers. The purpose of this study is to explore a new protamine based polyelectrolyte complex (PEC) alginate microbeads carrier to control the delivery, and enhance the biological ability of low-dose rhBMP-2 in posterolateral spinal fusion application.

Methods: A shell of alternating FDA-approved polycation (protamine) and polyanion (heparin) was fabricated on a strontium alginate microbead (core) template. Clinical grade rhBMP-2 was incorporated on the outermost layer by heparin specific binding motif. Formation of the core/shell multilayered microbeads was confirmed by fluorescence with FITC tagged heparin, TRITC tagged protamine and RITC tagged avidin (Model protein), and examined with laser scanning confocal microscopy. Bone inductive ability of the carrier vehicle was evaluated using a rat posterolateral spinal fusion model. There were three study groups. Group 1: protamine-PEC microbeads with 500ng rhBMP-2; Group 2: Absorbable Collagen Sponge with 500ng rhBMP-2; Group 3: no rhBMP-2 group as negative control.
All the animals were euthanized at 6 weeks. The quality of fusion was evaluated using manual palpation and mean fusion score, micro-CT and histological staining (H&E stain, Masson’s Trichrome stain).

Results: Confocal laser scanning microscopy images showed that TRITC-labeled protamine, RITC-labeled heparin and FITC-labeled avidin was coated in a sequential manner without serious diffusion into the core. After six weeks implantation, rats receiving PEC microbead treatment had better solid fusion, as determined by manual palpation and mean fusion score (P<0.05). Micro-CT images showed localized mineralized bone tissue deposition in Group 1 (Figure A). However, unsatisfactory bone formation was observed in Groups 2 and 3 (Figure B, C). Histological staining results confirmed the results of Micro CT, and revealed woven bone formation, as well as mature bone marrow tissue centrally in Group 1 (Figure D, G), whilst in Groups 2 and 3, no osseous union was observed between the two transverse processes, and fibrous tissue was mainly observed around the scaffold (Figure E,F,H,I) indicating failure of posterolateral spinal fusion.

Discussion: The protamine based PEC microbeads system in this study is based on the high affinity of heparin to act as in vivo reservoir for many growth factors, including the BMPs. From current study, solid fusion is achieved at 1/20 of conventional dose of rhBMP-2. This new carrier presented dramatically superior osteoinductive activity than collagen sponge at low-dose of rhBMP-2.

Significance: Protamine based PEC microbeads system amplified in vivo activity of rhBMP-2 in posterolateral spinal fusion and could be an alternative carrier for BMP-2 and other heparin binding growth factors.

Acknowledgments: This work was financially supported by Biomedical Research Council, Agency for Science, Technology and Research (A*STAR), Singapore. T.H thanks National University of Singapore for the NUS Graduate Scholarship.

References:
ORS 2014 Annual Meeting
Poster No: 0675