Preparation, characterization and *In Vivo* evaluation of a combination delivery system based on hyaluronic acid/jeffamine hydrogel loaded with PHBV/PLGA blend nanoparticles for prolonged delivery of Teriparatide

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Learning Objectives

1. Develop novel CDS for prolonged delivery of Teriparatide with the aim of reducing the frequency of injection.
2. Design a CDS with more desirable release profile in comparison to NPs and hydrogel alone.
3. Formulate an effective Teriparatide delivery system and preserve the biological bioactivity.

Introduction: Osteoporosis is identified as the most common cause of bone loss particularly in the elderly. Among the different therapeutic options, Teriparatide is the only therapeutic agent which stimulates bone formation. There are only few studies available on Teriparatide sustained release formulation. In the current study, biodegradable PHBV/PLGA blend nanoparticles (NPs) containing Teriparatide were loaded in hyaluronic acid/jeffamine (HA-JEF ED-600) hydrogel to prepare a combination delivery system (CDS) for prolonged delivery of Teriparatide.

Methods: The CDS was prepared by adding Teriparatide loaded PHBV/PLGA NPs to HA-JEF ED-600 hydrogel simultaneously to crosslinking reaction. Swelling behaviour, crosslinking efficiency and rheological characterization of HA-JEF ED-600 hydrogel were evaluated. The percentage of NPs incorporation within the hydrogel, loading capacity and morphology of Teriparatide loaded CDS were examined. The release profile of Teriparatide from CDS was investigated and compared with PHBV/PLGA NPs and HA-JEF ED-600 hydrogel. Teriparatide stability was examined by intrinsic fluorescence and circular dichroism spectroscopy. Cell toxicity was evaluated by MTT assay. LD_{50} was determined according to Miller and Tainter method. *In vivo* performance of CDS containing Teriparatide was evaluated by determining serum calcium level in mice.

Results: The CDS showed crosslinking efficacy of 89.9% with a swelling ratio of 178.0%. The rheological studies demonstrated that the viscoelasticity properties shifted from viscous material in HA gel to elastic material in hydrogel. The percentage of NPs incorporation within the hydrogel was figured out to be 86% with 2.31% loading capacity. Intrinsic fluorescence and circular dichroism spectroscopy have proved that Teriparatide is stable after processing. The release profile represented 63% Teriparatide release from CDS within 50 days with lower burst release compared to NPs and hydrogel. MTT assay disclosed no sign of reduction in cell viability. LD_{50} of Teriparatide loaded CDS was 131.8 mg/kg. *In vivo* studies demonstrated that Teriparatide loaded CDS could effectively increase serum calcium level after administration in mice.

Conclusion: Favourable results in the current study introduced CDS as a promising candidate for controlled delivery of Teriparatide and pave the way for future investigations in the field of designing prolonged delivery systems for other peptides and proteins.