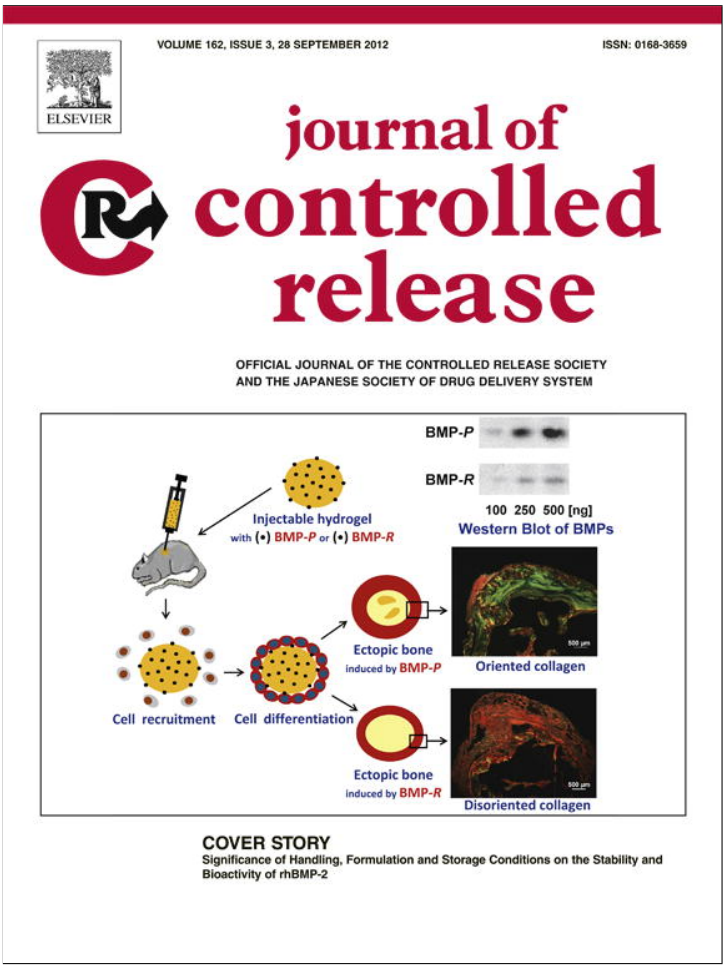

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Cover story

Significance of handling, formulation and storage conditions on the stability and bioactivity of rhBMP-2

Several biological and pharmacological proteins such as growth factors are extensively used for numerous regenerative medicine applications. The stability and efficacy of these therapeutic proteins are very much dependent on proper handling and storage conditions. However, little attention is paid on these aspects while working with such sensitive molecules, which significantly affect the outcome, rendering variable *in vivo* results. The significance of these factors is demonstrated by Dr. Varghese and his team in this issue using a well-known clinically used growth factor, namely recombinant human Bone Morphogenetic Protein-2 (rhBMP-2) [1]. This growth factor is approved by European and American authorities for specific bone regeneration applications in human [2]. For example, Medtronic offers InductOs®, which is a basic collagen matrix (of animal origin) soaked in BMP-2 (12 mg). Recently, clinical use of this protein has been questioned as such high doses were shown to have several undesired side effects [3]. Understanding the stability of this protein for bone tissue regeneration is therefore very important to develop safe therapeutics.

Dr. Varghese and his colleagues compared BMP-2 stability and bioactivity obtained from two commercial sources, InductOs® (BMP-*P*) and R&D systems (BMP-*R*) [1]. Both of these proteins are obtained from genetically engineered Chinese hamster ovary (CHO) cells. The *in vitro* experiments using BMP-2 from different sources suggest that they indeed possess significant variance in their bioactivity. The clinically approved BMP-*P* exhibited superior stability, as compared to BMP-*R* at physiological pH. The BMP-*P* remained stable at pH 7.4 as well as 4.7 as determined by dynamic light scattering experiments. The BMP-*R*, however, revealed rapid time dependent aggregation behavior. Similar behavior was also observed with respect to the adhesive nature of the protein with polypropylene Eppendorf tubes. Interestingly, in glass vial both proteins showed significant stability, suggesting a simple solution while storing BMPs for long time.

The *in vitro* differences were also reflected in the *in vivo* experiments, in a rat ectopic model with injectable hyaluronic acid (HA) hydrogel as BMP carrier. After 7 weeks post-implantation larger bone volume was observed with BMP-*P* group as compared to the BMP-*R* case. Another astounding difference was observed with respect to the level of oriented collagen observed in these two groups. Natural orientation of collagen matrix has been attributed to the mechanical loading and remodeling of the bone resulting in 'organized' bone tissue [4]. The polarized light microscopy images of the ectopic tissue surprisingly showed a new facet for collagen formation, which can be directly related to protein stability rather than mechanical activation. These experiments highlight the influence of handling and storage conditions on the therapeutic function of this protein that has been overlooked before. The study by Dr. Varghese and his group advises caution in comparing results from different experiments and warrantees careful assessment of handling and storage conditions while handling therapeutic proteins for biological applications. It is important to know that seemingly irrelevant experimental factors can significantly affect the experimental outcomes.

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