Creating new functional biomaterials: construction and production of Bone Morphogenetic 2-ELP hybrid proteins

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Bone morphogenetic protein 2 (BMP-2) is a potent osteoinductive cytokine from the TGF-β superfamily that triggers the development of stem cells into osteoblasts. Its therapeutic interest has led to the development of various production systems for recombinant variables of BMP-2. Production has been achieved in expression systems ranging from animal cells to bacteria, but is always associated with three major drawbacks: low production rates (in animal cells), low activity (bacterial cells) and low solubility due to aggregation in inclusion bodies (bacterial cells) [1].
In this study we have developed a strategy to overcome the low production levels as well as the insolubility of BMP-2 in *E. coli* by fusing it with an elastin like polymer (ELP). This recombinant ELP, based on repetitions of the main monomer VPAVG, displays no measurable cytotoxicity [2] and exhibits thermoresponsive properties as well as hysteresis behaviour [3]. By exploring this thermal responsiveness we are able to purify the fusion protein using a simple and low cost method and thus avoid expensive chromatographic techniques. The mature human-BMP2 domain was cloned in frame to the N-terminus of the (VPAVG)n (n = 60 or 220) polymers. The production of the genetic constructs was achieved in *E. coli* BL21 (DE3) with Lysogeny Broth (LB) supplemented with lactose for auto-induction. Purification of the hybrid BMP2:(VPAVG)n polymers was accomplished by exploring the thermal responsiveness of the ELP tail. Physical and chemical characterization as well as bioactivity studies of both constructs are currently in progress.

1. Bessa P. C., Casal M., Reis R. L. (2008) “Bone morphogenetic proteins in tissue engineering: the road from laboratory to the clinic, part I (Basic concepts)”, Journal of tissue engineering and regenerative medicine 2, 1-13;

2. Bessa, P., Machado, R., Nürnberger , S., Dopler, D., Banerjee, A., Cunha, A. M., Rodríguez-Cabello, C., Redl, H., van Griensven, M., Reis, R.L., Casal, M. 2010. Thermoresponsive self-assembled elastin-based nanoparticles for delivery of BMPs. *Journal of Controlled Release* 142: 312–318.

3. Machado R., Ribeiro A., Padrão J., Silva D., Nobre A., Teixeira J., Arias F., Cunha A., Rodroguez-Cabello J., Casal M. (2009) “Exploiting the natural ocurring elastin: construction, production and characterization of a recombinant thermoplastic protein-based polymer”, Journal of Nano Research, 6, 133-145;