RESEARCH GROUP: Pharmaceutical Science & Practice

Project Title:
Developing new in vitro models of fracture healing to evaluate a novel nanoparticle controlled release system for the delivery of growth factors

Supervisor(s):
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Level: PhD

Background to the project:
Many different animal models are employed to investigate fracture healing, with a range of differences (e.g. species, anatomical site, age) used in models for each clinical scenario. In addition, there are differences in interventions (delivery systems, growth factors) that are not standardised between animal studies. This contributes to their low clinical accuracy and also makes it difficult to compare results. Overall, animal models may be hampering efforts to translate data into a strategy that will promote human bone repair.

The specialised process of fracture healing regenerates bone in a series of well-orchestrated, co-ordinated biological events that restore skeletal integrity. Bone morphogenetic proteins (BMPs) and vascular endothelial growth factor (VEGF) are known to play an important role in normal healthy bone healing. BMPs are involved in the induction of a sequential cascade which leads to chondrogenesis, osteogenesis, angiogenesis and controlled synthesis of extracellular matrix. VEGF is the main regulator of angiogenesis and has also been found to lead to osteoblast migration and differentiation [Kempen et al., 2009].

Healing of fractures can be improved by exogenous administration of these biological substances. A recent Cochrane review [Garrison et al., 2010] highlights the need for the optimisation of delivery of bioactive compounds (specifically BMP) to enhance fracture healing. Use of BMP may only be cost-effective for the most severe fractures and there are only two BMP formulations approved by the FDA for use in the US, despite the number of different growth factors and cytokines known to be required for effective healing, with at least six growth factors involved in angiogenesis alone [Risau, 1997]. It seems likely that combination therapies will more closely mimic the physiological scenario and increase the likelihood of success. However, the commercial systems used for delivery of BMPs on the market today tend to employ one human recombinant protein (either BMP-2 or BMP-7) encapsulated in a bovine collagen matrix [Vukicevic et al., 2014]. Whilst these systems should allow targeted release by nature of the precise positioning allowed by the collagen matrix, they provide an inefficient delivery system of a single compound. The devices contain around 20 times more BMP than is found in the entire human body and much of this precipitates onto the bovine matrix and induces significant inflammation locally and systemically. BMPs are
used clinically for the treatment of some fracture types, but there are no standard methods for the amount required or route of application and thus there is a need for considerable optimisation. Optimisation would permit informed decisions regarding cost and effectiveness for exogenous compounds for different fractures. The delivery system proposed has not been investigated for release of growth factors for fracture healing.

The overall aim of this project is therefore to assess the osteogenic potential of different concentrations and combinations of growth factors including BMP-2, BMP-7 and VEGF when delivered by hydroxyapatite core nanoparticles and scaffolding delivery systems.

It is hypothesised the proposed project will derive understanding to allow for informed decisions about which combinations/concentrations and delivery systems should go forward to animal testing prior to clinical trial therefore increasing the likelihood of clinical success.

**Objectives of the research project:**

**Year 1. The delivery system; Formulation and Dissolution testing,**

Hydroxyapatite core nanoparticles will be developed and loaded with various amounts of BMP and VEGF. Formulations will firstly be manufactured with model growth factors (such as BSA or OVA) to carry out initial testing of loading efficiencies, size, zeta and release studies. The optimised systems will then be loaded with the BMP and VEGF. Nanoparticles will be characterised using various techniques including size and zeta analysis and entrapment studies. The techniques gained will enable the student to work in formulation science and to appreciate that much of this research does not require the use of animals. For dissolution testing, *in vitro* release studies will be carried out into simulated body fluid to determine release rates. Samples will be analysed using HPLC and the appropriate ELISA methods. The HPLC methods to analyse the model growth factors, VEGF and BMP will be developed and validated. At this stage, only the optimised systems will be taken forward to culture testing, again allowing the student to experience an animal-free approach and to appreciate how much research may be possible before the need for animal models.

**Year 2. Two dimensional models of fracture repair; *In vitro* testing of optimised systems,**

Initially, cell lines (Saos-2 and HUVEC) will be used to establish assay methods and also to begin to collect data in a relevant human system. *In vitro* assays will employ human bone marrow stem cells, cultured according to methods established in the applicant’s lab. Cells will be treated with BMPs and VEGF alone and in combination, at various doses and times and the differentiating capacity of cells measured using various different assays for osteogenesis, chondrogenesis and angiogenesis.

**Year 3 Three-dimensional models of fracture repair; *In vitro* testing of optimised systems,**

During the third year, the student will see how we can reduce animal use by further increasing the complexity and realism of cell culture models. This year will investigate the release of the most promising bioactive compounds identified in year 2 from scaffolding systems and assess their effects on human cell differentiation. For this work package, human bone marrow stem cells will be seeded into different scaffolds and confocal imaging employed to monitor cellular infiltration and differentiation. Experiments will be repeated in the presence of the delivery systems with BMPs and VEGF, using optimum conditions derived from previous studies.

**Methods to be used:**

- Nanoparticle formulation using hydroxyapatite cores.
- Characterisation including size and zeta analysis, *in vitro* release studies and entrapment studies using HPLC and ELISA.
- *In vitro* cell culture using Saos-2, HUVEC and human bone marrow stem cells.
- Alkaline phosphatase staining, propeptide of type I collagen (PICP) via ELISA, Von Kossa staining.
- FTIR
- Glycosaminoglycan assays to measure type III collagen
- Oil red O staining to examine lipid accumulation in cells as a marker of adipocyte differentiation.
- Matrigel assay will be used to assess angiogenesis. Quantification of tubule formation and length will employ Angiosys commercial software (TCS Cellworks). The software-based analysis has been recently demonstrated to accurately quantify angiogenesis of human cells in culture [Khoo et al., 2014]. This approach has the advantage over other methods developed to quantify angiogenesis [e.g. Adini et al., 2009] as it permits straightforward, unbiased analysis of tubule length, number of tubules, number of junctions and overall area.

**Skills required of applicant:**
A 2:1 (Hons.) degree in Pharmacy, Pharmaceutical Science or closely related discipline.
Good time management skills and IT literacy.

**References:**


