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Review article

Overcoming translational challenges – The delivery of mechanical stimuli *in vivo*

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ABSTRACT

Despite major medical advances, non-union bone fractures and skeletal defects continue to place significant burden on the patient, the clinicians and the healthcare system as a whole. Current bone substitute approaches are still limited in effectiveness and to date no adequate bone substitute material has been developed for routine clinical application. Tissue engineering presents a novel approach to tackling this clinical burden and developing an acceptable solution for the treatment of skeletal defects. Over the past three decades the field has evolved to appreciate the key biological, material and physical parameters influencing the development of a cell-based tissue engineered therapy and to create associated technologies to exploit such parameters. In recent years a number of therapies have started progressing along the pre-clinical pipeline to build a case for regulatory approval and ultimately clinical adoption. However, little emphasis has been given to the translational challenges faced when moving from "bench-to-bedside". One particular challenge lies in the delivery of functional mechanical stimuli to implanted cell populations to activate and promote osteogenic activities. This review introduces novel bio-magnetic approaches to overcoming this challenge.

Contents

Abbreviations: BMP, bone morphogenetic protein; CSBD, critical sized bone defect; ECM, extracellular matrix; FGF, fibroblastic growth factor; HA, hydroxyapatite; hADMSCs, human adipose derived mesenchymal stem cells; BM-hMSCs, human bone marrow derived mesenchymal stem cells; MNP, magnetic nanoparticles; MSCs, mesenchymal stem cells; PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic acid); PLLA, poly lactic acid; PRP, platelet-rich plasma; PTH, parathyroid hormone; RGD, arginylglycylaspartic acid; SPIONs, superparamagnetic iron oxide nanoparticles; TCP, tricalcium phosphate; TGF-β, transforming growth factor – beta; VEGF, vascular endothelial growth factor.

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1. Introduction

Regenerative medicine is a pioneering field which aims to develop novel strategies to replace and regenerate human cells, tissues and organs in order to restore normal function (Mason and Dunnill, 2008). The field thrives on the cross collaboration of multiple disciplines to develop tissue engineering approaches to achieve these goals. It harnesses the tools and knowledge developed by material scientists, molecular biologists, engineers and clinicians for the design and development of cellular therapies to treat a broad range of diseases and conditions. This field offers an alternative therapeutic approach to meeting a vast proportion of orthopaedic needs and has therefore generated significant interest in the orthopaedic field over the past 30 years (Wimpenny et al., 2012; Amini et al., 2012)

Skeletal bone defects as a result of trauma (excessive force to the skeleton), tumour resection and disease (osteoporosis) demands clinical intervention to encourage repair and regeneration of the damaged tissue. Treating fractures is very much dependant on the type and location of the fracture with the level of treatment ranging from simple immobilisation of the fractured bone or in more severe cases, surgery (Gaston and Simpson, 2007). Fracture healing mechanisms are well documented and understood to efficiently repair the damaged bone with minimal scar tissue formation in the majority of cases (Amini et al., 2012). Under normal circumstance, good bone repair is achieved as a result of these mechanisms within 4–6 weeks of treatment. However, in 5–10% of cases, defects fail to reach unions within 6 months and consequently develop into clinically defined non-union bone fractures (Gaston and Simpson, 2007; Gothard et al., 2014a). Impaired healing can be attributed to disturbed mechanical or biological environment, infection and poor vascularisation (Gómez-Barrena et al., 2015; Gothard et al., 2014a).

Non-union bone fractures represent a significant clinical challenge with substantial socio-economic implications both in terms of therapeutic cost and the broad age range of people afflicted (Gothard et al., 2014a; Pape and Pufe, 2010; Salgado et al., 2004; Meng et al., 2013; Flierl et al., 2013). Around 3.6% of the UK population (of 64 million) will suffer from bone fractures within their lifetime, with 850,000 new fractures cases recorded each year in the UK (Gothard et al., 2014a; Mills and Simpson, 2013). This figure is expected to increase with the ever ageing population due to the increased prevalence of fractures associated with osteoporosis in people over the age of 50 (1 in 3 women and 1 in 5 men) (Kanis et al., 2012). As mentioned, 5–10% of these fractures will fail to repair and will progress to non-union bone fractures. The cost to the NHS of treating non-unions ranges between £7000 and £79,000 per person depending on severity (Mills and Simpson, 2013), with an average treatment cost of between £15.5K and £17.2K, assuming a 'best-case scenario' (Kanakaris and Giannoudis, 2007). This of course contributes to the current £2bn per year burden of treating bone fractures to the NHS. A thorough table highlighting all associated direct and indirect costs relating to non-union treatments has been published by Kanakaris and Giannoudis (Kanakaris and Giannoudis, 2007).

Traditionally, non-union bone fractures have been treated by debridement of the non-union site and fixed by internal plates, non-reamed or intramedullary nails either with or without the application of an external fixation systems (Kanakaris and Giannoudis, 2007). These approaches are highly invasive and associated with risk of infection and damage to the blood supply. Scaffold guided regeneration approaches on the other hand are also considered to be viable therapeutic options for non-unions (Meng et al., 2013). It is well accepted that autologous bone graft (bone taken from the patient) is the gold standard treatment for non-union fractures. The success of this approach is highly dependent on the quality of bone harvested and limited by quantity and donor site morbidity (Zimmermann and Moghaddam, 2010). Allogeneic bone grafts involve the use of cadaveric bone to treat the patients defect and is an alternative approach to autologous grafts originally designed to overcome the limitations associated with autologous approaches. The pitfalls of this approach however lie with the risk of immune rejection, pathogen transmission, batch variability and potential limited supply in the future with the increasing ageing population and consequent increase in demand (Zimmermann and Moghaddam, 2010). Further to this, the rate and success of tissue integration is far lower with allogeneic specimens further limiting its routine application. Ceramics (*e.g.* calcium phosphate ceramics) and metals (*e.g.* stainless steel and titanium or titanium alloys) have also been suggested and researched as alternatives but neither option completely fulfils the requirements of a suitable and functional bone graft substitute (Salgado et al., 2004; Nascimento et al., 2007). Metals tend to exhibit poor integration with the surrounding native bone while ceramics are often brittle and have very low tensile strength. It is therefore believed that regenerative medicine and tissue engineering would contribute to the development of a functional clinical solution.

This review will broadly address the fundamental tissue engineering approaches required to develop a suitable bone substitute along with the pre-clinical translational challenges encountered. We attempt to highlight novel approaches that can be applied to overcome certain translational challenges related to direct *in vivo* mechanical stimulation of implanted cell populations to enable progression towards the clinic.

2. Bone

As humans, we begin life with 270 individual bones in our body. This forms our skeleton and serves to support our body weight, promote movement while providing mechanical protection to vital organs (Huang and Ogawa, 2010). As we grow and mature our primary infantile bones are replaced with secondary more mature bone to cope with the changing mechanical environment encountered as developing human beings (Papachroni et al., 2009). The skeleton is in fact a highly metabolically active organ that undergoes lifelong and continuous bone remodelling to maintain structural integrity and bone homeostasis (Hadjidakis and Androulakis, 2006; Raisz, 1999; Rucci, 2008). Bone is a complex structure made up of collagen type-1 constituting the majority of the non-mineralised organic component of the tissue along with a collection of other proteins, proteoglycans, glyosaminoglycans and glycoproteins (Stevens, 2008). Hydroxyapatite contributes to the inorganic mineral component of bone and along with the organic elements collectively make up the extracellular matrix (ECM). The nanocomposite structure is integral to the required compressive strength and high fracture toughness of bone and is maintained by the bone cells – osteoblasts, osteoclasts and osteocytes (Amini et al., 2012; Salgado et al., 2004). Bone can be classified as being either cortical or cancellous in nature, each differing slightly in their architecture with cortical bone appearing to have a compact element while cancellous bone having more of a trabecular appearance. These differences consequently impact on mechanical properties of the specific type of bone. In addition bone consists of a highly connected cellular network made up of sensory osteocytes and effector osteoblasts and osteoclasts, the coordinated function of which govern and mediate lifelong bone remodelling and skeletal homeostasis (Huang and Ogawa, 2010; Rucci, 2008).

Bone remodelling is responsible for altering the intricate architecture of bone to dynamically adapt to fluctuating mechanical needs (in accordance with Wolfs law) facilitating bone growth and repair of small microfractures (Hadjidakis and Androulakis,