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Review

Bone remodelling *in vitro*: Where are we headed? -A review on the current understanding of physiological bone remodelling and inflammation and the strategies for testing biomaterials *in vitro*

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ABSTRACT

Bone remodelling is a dynamic process required for the maintenance of bone architecture in response to the changing mechanical needs. It is also a vital process during the repair of bone tissue following injury. Clinical intervention in terms of autografting or allografting is often required to heal bone injuries where physiological healing fails.

The use of biomaterials as alternatives to autografts and allografts has spurred a significant research interest into further development of biomaterials for better clinical outcomes. Unfortunately, many biomaterials fail to make it to the clinic or fail after implantation due to the inconsistencies observed between *in vitro* and *in vivo* studies. It is therefore important to mimic the *in vivo* situation as closely as possible in an *in vitro* setting for testing biomaterials. The current *in vitro* models focus mostly on investigating the behaviour of osteoblast progenitors with the biomaterial under development as well as assessing the behaviour of osteoclasts, endothelial cells *etc*. However, the sequence of events that take place during bone healing or remodelling are not incorporated into the current *in vitro* models.

This review highlights our current understanding of the physiological bone remodelling and the bone healing process followed by strategies to incorporate both the physiological and pathophysiological events into an *in vitro* environment. Here, we propose three strategies for the assessment of biomaterials for bone, which includes; (1) testing biomaterials in the presence of immune cells, (2) testing biomaterials for osteogenesis, and (3) testing biomaterials in the presence of osteoclasts followed by osteoblasts to recapitulate the physiological events of bone resorption prior to bone formation. The focus of this review is to discuss the third strategy in details as the first two strategies are currently incorporated into a majority of *in vitro* experiments.

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1. Introduction

Autologous bone grafts are considered the gold standard for the treatment of bone defects since they possess osteoconductive (stimulates bone cells to grow on its surface), osteogenic (contains cells involved in bone formation) and osteoinductive (stimulates progenitor cells to differentiate into bone cells) properties [1]. However, the use of autografts is limited by drawbacks such as donor-site morbidity, limited availability, pain and prolonged hospitalization and rehabilitation. An alternative to autologous bone grafting is the use of allografts, where bone tissue is harvested from other humans (typically cadavers). The advantage of using allografts over autografts is that they eliminate donor site morbidity and can be available in sufficient quantities. However, allografts do not provide the necessary osteoinductive signals, carry a risk of infection and immune rejection [2]. The limitations associated with the use of autografts and allografts have motivated the development of a wide variety of synthetic tissue engineered bone biomaterials in the recent years. Using these biomaterials eliminates the risk of disease transmission, reduces the number of surgical procedures, reduces the risk of infection or immunogenicity, and there is an abundant availability of these biomaterials [3]. These biomaterials are designed to utilize the body's natural biological response to tissue damage in conjunction with engineering principles. Therefore, a biomaterial intended for clinical application must undergo rigorous testing *in vitro* in an environment that mimics body's natural responses. In the case of bone, remodelling is the main biological process at play during bone repair.

Bone remodelling relies on a dynamic equilibrium between bone resorption and formation [4,5], which makes the designing of a viable and accessible model to study the impact of bone remodelling on biomaterials in an *in vitro* setting, a challenging problem. One of the main problems faced when developing biomaterials for bone regeneration is testing the ability of the biomaterial to be remodelled in sync with the natural healing process or in other words, the ability of the biomaterial to retain its physical properties and possess enough strength and stiffness until *in vivo* tissue ingrowth has replaced the slowly degrading biomaterial matrix [6]. A current practice to test the efficacy of biomaterials for bone is to examine its biocompatibility, mechanical properties, structural architecture and more recently, its bioresorbability [7] Biocompatibility is by far one of the most extensively researched areas for biomaterials [8]. One of the standard ways for testing biocompatibility of biomaterials is to assess the potential of the biomaterial to support cell viability, proliferation and whether or not it would incur an inflammatory response in the host [9,10]. Whilst this is vital for the success of the implanted biomaterial *in vivo*, these tests only relate to one aspect of the bone remodelling and healing process *i.e.*, bone formation. Biomaterials are often not accurately assessed for their bioresorbability in an environment that mimics bone resorption. Despite the current understanding of the bone remodelling process, there are no standard *in vitro* assays to assess the regenerative potential of biomaterials to form bone in a culture system that represents the *in vivo* situation. Therefore, there is no consensus as to which culture system or *in vitro* model can best assess a biomaterial's capacity to form bone. A key requirement for future bone biomaterial development and clinical translation of promising therapies would be for researchers around the world to adopt similar criteria for testing biomaterials prior to *in vivo* testing, to draw accurate conclusions regarding the biomaterial under development.

This review aims to discuss the problems faced in mimicking the bone remodelling and bone healing processes *in vitro*, how close we have come to doing so and the future strategies for biomaterial testing.

2. Physiological bone remodelling

Bone remodelling is a series of well-orchestrated biological events that are regulated by complex interactions between the various cell types found in bone primarily osteoblasts, osteoclasts and osteocytes. Each cell type has a specific role to play during the different stages of the remodelling process. Unlike modelling, which involves either resorption or formation (but not both) in any given area, bone remodelling always follows a set sequence of events; namely the activation of cells, leading to resorption and then the formation of bone [11–13]. The process of remodelling is orchestrated by two main cell types: osteoclasts, specialised for bone resorption, and osteoblasts, responsible for bone formation, which arrange themselves in a discrete anatomic structure called the basic multicellular unit (BMU) [14]. Osteoclasts are derived from hemopoietic precursors supplied by the bone marrow and the penetrating blood vessels, whereas osteoblasts are derived from mesenchymal stem cells (MSCs) in the bone marrow. These precursor cells differentiate within the BMU to fully functional resorbing osteoclasts and synthesizing osteoblasts [15].

Our current understanding of the bone remodelling process is that it consists of five stages, namely activation, resorption, reversal, formation and termination [16] (Fig. 1) which are described below:

Activation: During this first stage of the remodelling process, biochemical (*e.g.*, secretion of hormones such as oestrogen or parathyroid hormone) or mechanical (resulting from damage to bone or change in loading) signals induce the bone lining cells (quiescent osteoblasts) to release cytokines such as RANK-L (receptor activator of NFkB) and M-CSF (Monocyte-colony stimulating factor). These factors then recruit and activate osteoclast precursors to initiate bone resorption [17–19]. Once osteoclast precursors are recruited, they differentiate into multinucleated osteoclasts and attach to the surface of bone [20].

Resorption: Once osteoclasts are attached to the bone surface, they secrete hydrogen ions and acid phosphatases which reduce the pH of the bone resorbing compartment, and resorb the bone mineral. Enzymes such as matrix metalloproteinases and members of the cathepsin family are also secreted during this phase to digest the organic components of the bone matrix [21,22]. This results in the formation of resorption pits on the surface of bone undergoing remodelling, after which, the osteoclasts migrate away and undergo apoptosis [23].

Reversal: During this phase, a group of mononuclear cells that line the resorptive lacunae differentiate into macrophages and remove the remaining debris to initiate the reversal process. After the macrophages clear off the resorptive lacunae, bone formation begins [24].

Formation: During the next 4–6 months, pre-osteoblasts are recruited and differentiate towards osteoblasts to deposit the proteinaceous bone matrix termed osteoid. Osteoid is gradually mineralised to form new bone. Osteoblasts continue to deposit new bone until they become quiescent as a single cell layer on top of the newly formed mineralised matrix. At this point, some osteoblasts will undergo apoptosis whereas others become buried in the newly formed matrix as osteocytes [20].

Termination: When the same amount of bone is formed as was being initially resorbed, the remodelling process is finished. Because of the morphology of the remodelling BMU, where the osteoblast teams trail behind osteoclast teams and the entire structure moves as a unit, the resorption and formation processes are said to be coupled to one another. Coupling is a strictly controlled process in remodelling, ensuring that where the bone is removed, new bone will be restored [4,20].

The understanding of the physiological bone remodelling process is of critical importance with regards to the design and development of a biomaterial for it to completely reconstruct the damaged tissue. The next section of this review will discuss the sequence of events that take place upon biomaterial implantation.

3. Primary biological response upon biomaterial implantation

To assess the potential of a biomaterial to successfully form bone, it is important to understand the primary biological response that the biomaterial would be subjected to. Upon implantation, a biomaterial, regardless of its composition and structure, will be immersed in a bodily environment containing different blood cells, proteins and inflammatory