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Harnessing macrophage-mediated degradation of gelatin microspheres for spatiotemporal control of BMP2 release

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ABSTRACT

Biomaterials-based approaches to harnessing the immune and inflammatory responses to potentiate wound healing hold important promise. Bone fracture healing is characterized by an acute inflammatory phase, followed by a transition to a regenerative and repair phase. In this study, we developed genipin-crosslinked gelatin microspheres designed to be preferentially degraded by inflammatory (M1) macrophages. Highly crosslinked (>90%) microspheres allowed efficient incorporation of bioactive bone morphogenetic protein 2 (BMP2), a potent stimulator of osteogenesis in progenitor cells, via electrostatic interactions. Release of BMP2 was directly correlated with degradation of the gelatin matrix. Exposure of microspheres to polarized murine macrophages showed that degradation was significantly higher in the presence of M1 macrophages, relative to alternatively activated (M2) macrophages and unpolarized controls. Microsphere degradation in the presence of non-inflammatory cells resulted in very low degradation rates. The expression of matrix metalloproteinases (MMPs) and tissue inhibitors of MMP (TIMPs) by macrophages were consistent with the observed phenotype-dependent degradation rates. Indirect co-culture of BMP2-loaded microspheres and macrophages with isolated adipose-derived mesenchymal stem cells (MSC) showed that M1 macrophages produced the strongest osteogenic response, comparable to direct supplementation of the culture medium with BMP2. Controlled release systems that are synchronized with the inflammatory response have the potential to provide better spatiotemporal control of growth factor delivery and therefore may improve the outcomes of recalcitrant wounds.

1. Introduction

Inflammation is a key regulator of the regeneration process that helps to determine the course and extent of bone regeneration [1]. Bone defects are extensively infiltrated by immune cells that play an important role in modulating the inflammatory response via secreted factors. In particular, macrophages are key immune cells that actively participate in the evolution of the inflammatory response by shifting from an inflammatory phenotype (M1) to a regenerative phenotype (M2) as healing progresses [2,3]. The spatial and temporal balance between these two phenotypic states is a key to full functional recovery after injury (reviewed in Ref. [4]). Harnessing the inflammatory response via biomaterials-based approaches is a growing field of interest, because of the potential to regulate and accelerate tissue regeneration [5–8].

Bone fracture healing involves both anabolic and catabolic processes that work in tandem to regenerate tissue volume and promote appropriate remodeling over time [9]. These metabolic processes occur in three biological phases: inflammation, endochondral ossification and coupled remodeling (Fig. 1A). The inflammatory phase (peaking at day 1–3) results in the formation of a hematoma and recruitment of both osteoprogenitor and inflammatory cells to the wound site. The recruited inflammatory cells, mainly macrophages, then induce resident osteoprogenitor cells to differentiate, which leads to the formation of a callus during the

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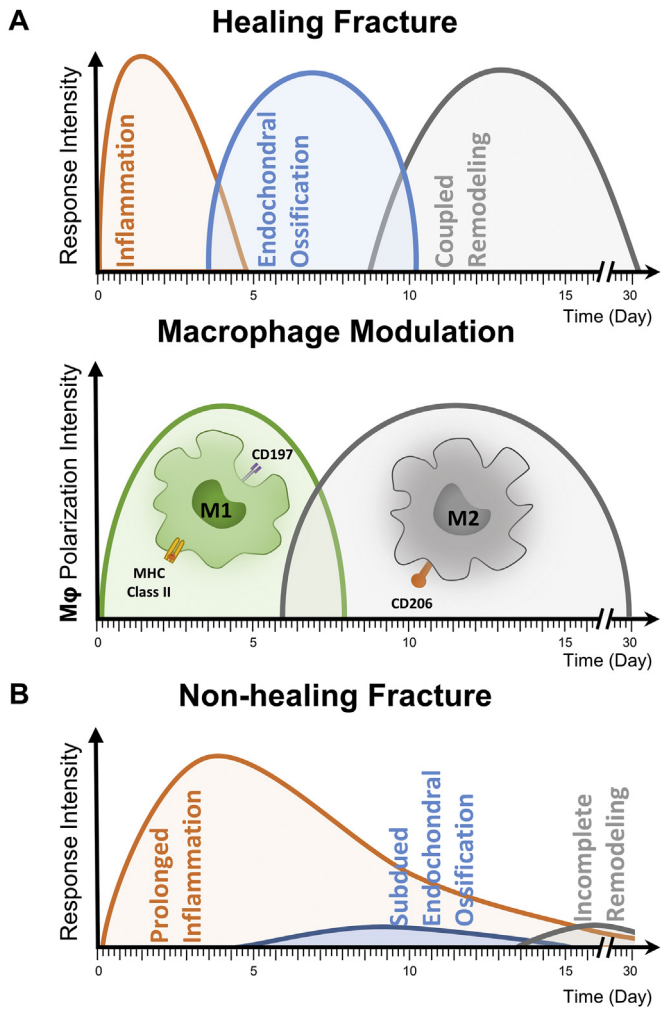

Fig. 1. Role of inflammatory cells in normal and pathological fracture healing. A) Normal fracture healing involves phases characterized by inflammation, endochondral ossification, and coupled tissue remodeling. The phenotype of local macrophages changes from pro-inflammatory (M1) to pro-reparative (M2) as bone repair proceeds. B) In non-healing fractures, the progression to the endochondral ossification is delayed or inhibited, such that new bone is not regenerated.

endochondral phase (day 5–10). The transition from inflammatory to a tissue regeneration phases is assisted by a concomitant shift in local macrophage phenotype from pro-inflammatory toward pro-repair. It has been shown that osteoinductive signals are maximally beneficial when applied during the late inflammatory period that precedes the endochondral phase [9], making this the optimal therapeutic window for delivering osteoinductive factors. Appropriately-timed cytokine delivery, therefore, can lead to enhanced progenitor cell commitment, which leads to increased callus size and stiffness, as well as subsequent remodeling to mature ossified tissue [9].

Each year in the United States, more than half a million patients receive bone defect repairs, out of which over 100,000 cases are due to nonunion fractures that rarely heal without secondary intervention [10,11]. Non-healing fractures often show an early or even an extended inflammatory phase (Fig. 1B), but are typically characterized by an impaired endochondral phase, due to inadequate blood supply or a poor cellular or cytokine response [1]. Various strategies have been explored for augmenting bone regeneration in recalcitrant fractures and in patients with limited healing capacity. While autografts remain the most common clinical option, they are of limited availability and can cause donor-site morbidity and pain [12–14]. Allogeneic bone grafts are an alternative, but are hampered by issues of compatibility and disease transmission [15–18]. Engineered bone substitutes made from natural and synthetic [19,20] polymers are being developed, but a key issue is that these materials by themselves are not naturally osteoinductive. During resorption and remodeling, these materials are generally replaced by dense collagenous tissue which does not provide the mechanical stability and functionality of native bone. For these reasons, there is great interest in potentiating the development of new mature bone through the release of osteoinductive factors.

Bone morphogenetic protein 2 (BMP2) is a member of the transforming growth factor beta (TGF-β) super-family of proteins and has been shown to be highly osteoinductive through its effects on progenitor cells [21,22]. This process unfolds via a characteristic pathway of receptor activation that leads to specific signaling cascades involved in osteogenesis [23]. BMP2 has been shown to induce bone formation in both ectopic and orthotopic sites in animal models [24,25] as well as in clinical trials [26,27]. It is particularly essential for the initiation of fracture repair [24] and consequently has been used as a therapeutic agent to accelerate bone regeneration. However, the efficacy of BMP2 therapy is highly dependent on the method of delivery to the target site. Systemic delivery is inefficient and topical administration often results in washout of the protein from the target site [28]. BMP2 delivery from biomaterial scaffolds has been investigated, but rapid hydration or dissolution of the carrier can similarly result in washout [29]. To compensate for this loss, BMP2 is often administered at high concentrations, which increases the risk of bone overgrowth, heterotopic ossification and other complications [30–32]. High doses of BMP2 have also been associated with systemic inflammation and the generation of reactive oxygen species, which can lead to serious consequences [33,34]. Therefore, there is a need for methods to both spatially and temporally control the release of BMP2, to maximize its therapeutic effect at doses that are safe.

The strategy in the present study was to create a BMP2 delivery system that is responsive to the inflammatory environment, resulting in efficient and spatiotemporally controlled initiation of the endochondral phase of bone regeneration. We developed crosslinked gelatin microspheres that sequester BMP2 and which are designed to be delivered in a minimally invasive manner to sites of bone injury (Fig. 2A). These microspheres are preferentially degraded by macrophages of the inflammatory phenotype, which enables the release of BMP2 during the optimal therapeutic window for initiating the transition to the endochondral phase during fracture repair (Fig. 2B). The degradation of microspheres and the release kinetics of BMP2 release were characterized using enzymatic treatment and by culturing with inflammatory (M1) and regenerative (M2) macrophages in both two- and three-dimensional culture systems. Also, the mechanism by which M1 macrophages more aggressively degrade microspheres was examined and was compared to M2 macrophages and other non-inflammatory cells commonly implicated in fracture healing. Finally, we show that inflammatory macrophage-mediated release of BMP2 from gelatin microspheres has the effect of promoting osteogenic differentiation in mesenchymal stem cells. Such biomaterial-based approaches can be used to synchronize growth factor release with the inflammatory response, and thereby promote faster and more complete bone regeneration in hard-to-heal defects.

2. Material and methods

2.1. Cell isolation and culture

Macrophages were derived from monocytes isolated from the