Advanced Drug Delivery Reviews xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Delivery of cellular factors to regulate bone healing ☆

Alexander Haumer [a,b], Paul Emile Bourgine [a,b], Paola Occhetta [a,b], Gordian Born [a,b], Roberta Tasso [c], Ivan Martin [a,b,*]

[a] Department of Biomedicine, University Hospital Basel, University of Basel, Switzerland
[b] Department of Biomedical Engineering, University of Basel, Switzerland
[c] Ospedale Policlinico San Martino-IST, IRCCS per l'Oncologia, Genova, Italy

ARTICLE INFO

Article history:
Received 29 September 2017
Received in revised form 8 January 2018
Accepted 13 January 2018
Available online xxxx

Keywords:
Regenerative medicine
Extracellular matrix
Extracellular vesicles
Mesenchymal stem cells
Immunomodulation
Inflammation
Bone repair
Paracrine factors
Tissue engineering

ABSTRACT

Bone tissue has a strong intrinsic regenerative capacity, thanks to a delicate and complex interplay of cellular and molecular processes, which tightly involve the immune system. Pathological settings of anatomical, biomechanical or inflammatory nature may lead to impaired bone healing. Innovative strategies to enhance bone repair, including the delivery of osteoprogenitor cells or of potent cytokines/morphogens, indicate the potential of 'orthobiologics', but are not fully satisfactory. Here, we review different approaches based on the delivery of regenerative cues produced by cells but in cell-free, possibly *off-the-shelf* configurations. Such strategies exploit the paracrine effect of the secretome of mesenchymal stem/stromal cells, presented in soluble form, shuttled through extracellular vesicles, or embedded within the network of extracellular matrix molecules. In addition to osteoinductive molecules, attention is given to factors targeting the resident immune cells, to reshape inflammatory and immunity processes from scarring to regenerative patterns.

1. Introduction

Tissue regeneration is a complex and highly dynamic process, requiring the interplay between blood and parenchymal cells, soluble mediators and extracellular matrix (ECM) molecules [1]. In the past 20 years, advances in cellular and molecular biology allowed deeper analysis and better understanding of the multi-staged wound healing process, where inflammation leads to new tissue formation and remodeling. These phases partially overlap in time and are accompanied by concomitant revascularization of the injury site together with a local and systemic defense activation, involving both the innate and adaptive immunity [2,3]. After injury, platelets or the activated-complement pathway (in absence of hemorrhage) initiate the healing cascade. This triggers the release of vasoactive mediators and chemotactic factors that attract the first cellular actors to the injury site: neutrophils, macrophages and fibroblasts [4]. These cell populations will in turn secrete a variety of factors and chemokines necessary for the priming of new tissue formation and the regulation of repair events. The timely orchestration of these processes, including primary inflammation, has revealed to be essential for an effective regeneration.

In the skeletal context, following an injury or a fracture, bone tissue has the capacity to heal without scar formation by recapitulation of the outlined wound healing phases. However, increasing severity of the trauma, comorbidities of the patient or biomechanical instability can cause an imbalance in the physiological healing cascade, leading to incomplete repair and functional failure (e.g., non-unions) [5]. In order to enhance fracture healing when compromised, cellular therapies have been extensively considered using mesenchymal stromal/stem

Abbreviations: BMP, bone morphogenetic protein; CCL, chemokine (C-C motif) ligand; CCR, chemokine receptor; CM, conditioned medium; CXCR, CXC chemokine receptors; DAMP, damage-associated molecular patterns; ECM, extracellular matrix; EV, extracellular vesicle; FGF, fibroblast growth factor; GF, growth factor; hES, human embryonic stem cells; IFN, interferon; IHH, Indian hedgehog; IGF, insulin-like growth factor; IL, interleukin; MCSF, macrophage colony-stimulating factor; MMP, matrix metalloproteinase; MSC, mesenchymal stem/stromal cell; MSC-CM, mesenchymal stem/stromal cell-conditioned medium; NLR, NOD-like receptors; OPG, osteoprotegerin; PDGF, platelet derived growth factor; PTHrP, parathyroid hormone-related protein; RANKL, receptor activator of nuclear factor kappa-B ligand; SDF, stromal cell-derived factor; TGF, transforming growth factor; TLR, toll-like receptors; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; WT, wild type.

☆ Funding: This work was supported by the People Programme (Marie Curie Actions) of the European Union 7th Framework Programme FP7/2007-2013/under REA grant agreement No. 607868 (iTERM, to A. Scherberich), by the Swiss National Science Foundation (Grant number NBM 1579, to Prof. Ivan Martin) and by the Italian Ministry of Health ("Young Investigator Grant" - GR-2013-323 02357519).

* Corresponding author at: Department of Biomedicine, University Hospital Basel, Laboratory for Tissue Engineering, Hebelstrasse 20, Basel 4031, Switzerland.
E-mail addresses: alexander.haumer@usb.ch (A. Haumer), paul.bourgine@usb.ch (P.E. Bourgine), paola.occhetta@usb.ch (P. Occhetta), gordian.born@unibas.ch (G. Born), ivan.martin@usb.ch (I. Martin).

https://doi.org/10.1016/j.addr.2018.01.010
0169-409X/

cells (MSCs). These cells have been identified as progenitor population differentiating into cells directly involved in the replacement of skeletal tissue at the injury site. [6].

Autologous MSCs have been tested in several pre-clinical studies and even used in clinical cases (keywords "mesenchymal stem cells" and "bone" yield 264 results on clinicaltrial.gov), but major drawbacks still hamper the use of such procedures in the routine treatment of challenging bone defects [7]. First, the enhancement of clinical outcome could not be demonstrated to be repeatable. This may be related not only to the patient-to-patient heterogeneity of bone defects and of autologous MSCs, but also to the variability of protocols for MSCs isolation and preparation [8]. Second, the production of MSCs for clinical use is extremely complex and costly, such that demonstration of cost-effectiveness cannot be provided. Many groups have tried to avoid high costs and complex logistics linked to good manufacturing practice-based approaches by directly applying MSCs intraoperatively [9,10]. However, the success of this method was strictly related to the concentration and engrafting capacity of the injected cells and the absence of an experimental control group receiving a placebo treatment contributed to the weakness of the reports.

Despite promising results, the mechanisms by which MSCs exert beneficial effects towards a damaged tissue remain unclear even in seemingly successful studies. The scientific hypothesis underlying the above mentioned studies relied on the notion that MSCs exert their functions by directly replacing damaged cells. However, proper labeling and tracking of donor cells in vivo suggested that therapeutic effects provided by implanted MSCs are often short-lived, and most times related to dynamic paracrine interactions between MSCs and endogenous cells [11,12]. These experimental findings contributed to a new concept, whereby MSCs not only participate to direct tissue replacement, but also indirectly orchestrate the repair cascade by secretion of soluble "regenerative" factors [13–15]. Together with increasing evidences of their participation to all phases of tissue repair, MSCs progressively emerged as a pivotal cell population "paracrinally" modulating the endogenous environment and the immune response [16,17].

Leveraging the paracrine regulatory role of MSCs offers the possibility to develop a standardized and cost-effective clinical procedure. This approach would bypass the use of living cells by harnessing their so-called "secretome", representing the combination of secreted structural and bioactive molecules [18,19]. However, the exploitation of this scenario requires the suitable engineering of delivery strategies, in order to efficiently prime/modulate inflammation, tissue formation and remodeling events. In this context, beyond the injection of soluble factors, extracellular vesicles (EVs) and the ECM have been proposed as delivery vehicles.

In this manuscript, we will first review the successive phases of bone healing and the associated regulatory molecules. A special emphasis is given to those at the interplay with the immunological system. We will then describe how restoration of such processes in cases of impaired healing can be addressed by delivery of factors produced by MSCs. We report that such pro-regenerative factors can be delivered through cell-free approaches as soluble mediators, channeled through EVs or coupled within engineered ECMs.

2. Key regulators of bone healing

Bone healing occurs through the classical healing cascade characterized by the inflammatory, the repair and the remodeling phases. In parallel, a progressive revascularization of the injury site develops from the very first days after injury. While these phases occur successively, they also partially overlap in time. However, each phase is distinguishable by the stage-specific tissue status, as well as the cellular and molecular factors involved (Fig. 1). Deciphering the role and timing of actions of such factors during bone morphogenetic developmental events can be source of inspiration for tailoring new delivery strategies prompting bone fracture healing [20,21].

2.1. The role of the immune compartment in bone healing

Upon bone fracture, disruption of the vascular supply results in hematoma formation at the injury site, with initiation of the acute inflammatory response [22–24]. The initial inflammatory phase, reaching a peak 24 h post-injury [25,26], plays a pivotal role in the response to injury, since it initiates the repair cascade by stimulating angiogenesis, attracting and promoting MSC differentiation, and enhancing ECM deposition [27–29]. These features result from the tight interaction of molecular factors and resident progenitor cells in interplay with a well-orchestrated immune response. The local inflammation is induced in response to damage-associated molecular patterns (DAMPs, or alarmins) that bind specific receptors such as Toll-like Receptors (TLRs) and NOD-like receptors (NLRs). Distinctive for this early phase is a surge of a vast variety of proinflammatory cytokines, such as interleukin (IL)-1, lL-6, tumor necrosis factor-α (TNF-α), receptor activator of nuclear factor kappa-B ligand (RANKL) and macrophage colony-stimulating factor 1 (MCSF-1) as well as transforming growth factor-β (TGF-β) superfamily members including bone morphogenetic protein (BMP)-2, -4, -5, and -6 [27,30]. During the initial inflammatory phase, short-lived inflammatory cells, such as polymorphonuclear neutrophils, are recruited to the site of injury where the fibrin clot acts as a scaffold for the invading cells. These acute-phase inflammatory cells then recruit more long-lived monocytes and macrophages by secretion of chemokines such as monocyte chemoattractant protein 1 (i.e. C-C motif chemokine 2 (CCL2)) and IL-6 [24,31,32]. These signals, together with the danger signals TLRs and NLRs, activate tissue-resident macrophages and promote the expression of various cytokines and chemokines, such as IL-6, IL-1β, IL-1 receptor, type 1 (IL-1R1) ligands and CCL2, which direct the myeloid cell response. Macrophages are key players in different phases of bone regeneration [33]. Two distinct macrophage populations act in this phase of the healing cascade and influence the bone formation pathways: while bone tissue resident macrophages (osteomacs) appear to play an important role in intramembranous ossification, pro-inflammatory macrophages, recruited to the site, affect the endochondral bone formation route [24,34]. The varied functions of macrophages during bone tissue regeneration are realized through the tremendous plasticity of these cells. Throughout the normal healing process, macrophages adopt phenotypes ranging from a pro-inflammatory or "M1"

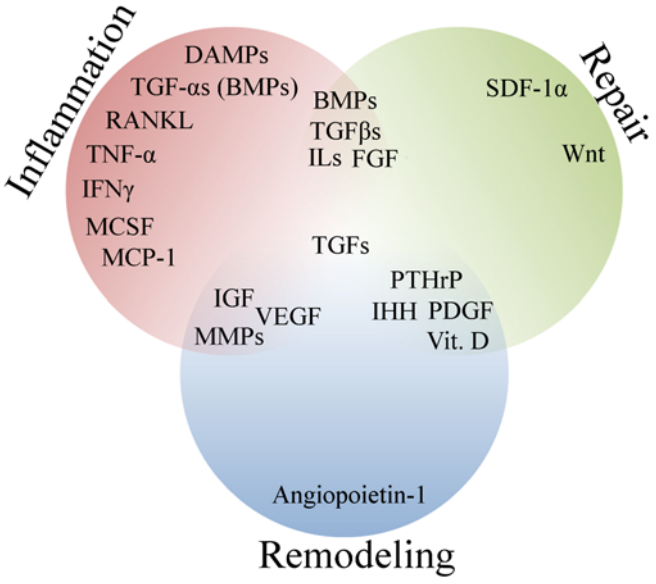

Fig. 1. Key factors involved in the bone healing phases, based on the current status of knowledge. The regulatory function of a factor to a specific phase of bone healing is represented by its proximity to the external part of one of the three circles, displaying inflammation, repair and remodeling. Positioning of the factor at the interfaces among the circles indicates overlapping functions.

Please cite this article as: A. Haumer, et al., Delivery of cellular factors to regulate bone healing, Adv. Drug Deliv. Rev. (2018), https://doi.org/10.1016/j.addr.2018.01.010