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Cell based advanced therapeutic medicinal products for bone repair: Keep it simple?☆

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ABSTRACT

The development of cell based advanced therapeutic medicinal products (ATMPs) for bone repair has been expected to revolutionize the health care system for the clinical treatment of bone defects. Despite this great promise, the clinical outcomes of the few cell based ATMPs that have been translated into clinical treatments have been far from impressive. In part, the clinical outcomes have been hampered because of the simplicity of the first wave of products. In response the field has set-out and amassed a plethora of complexities to alleviate the simplicity induced limitations. Many of these potential second wave products have remained "stuck" in the development pipeline. This is due to a number of reasons including the lack of a regulatory framework that has been evolving in the last years and the shortage of enabling technologies for industrial manufacturing to deal with these novel complexities. In this review, we reflect on the current ATMPs and give special attention to novel approaches that are able to provide complexity to ATMPs in a straightforward manner. Moreover, we discuss the potential tools able to produce or predict 'goldilocks' ATMPs, which are neither too simple nor too complex.

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1. Introduction

The ability to create well characterized cell based advanced therapeutic medicinal products (ATMPs) is expected to play a crucial role to fulfill the needs of the ever growing demand for biologically functional bone grafts. In the year 2008 there were half a million patients in the United States in which a bone defect was repaired. This resulted in a health care burden of over $2.5 billion. It is expected that by the year 2020 these numbers have doubled [1]. It is expected of ATMPs to repair, replace and/or regenerate damaged or missing tissue, thereby complementing current therapeutic options or even provide treatments for currently untreatable pathologies in a cost-effective manner. For bone healing in particular, it is mainly expected to prevent or heal non-unions and thereby reduce the long term cost of patient care.

The development of these desired cell based ATMPs is typically based on three basic elements: biomaterials, cells and growth factors. Ideally, the chosen combination of the biomaterial and growth factors should form a biomimetic environment that drives the cells into the formation of a new functional tissue [2]. As such, the development of ATMPs is a multidisciplinary process that requires expertise from several disciplines including biology and engineering. Biology provides crucial information on the underlying molecular signaling mechanisms and understanding of cellular behavior. Engineering is then employed to mimic these processes, therefore also denominated as developmental engineering [3,4]. This can be achieved in several ways including by creating a mechanical support that provides a stimulating microenvironment via a spatiotemporal release of choice molecules and facilitates integration within the host. Moreover, fundamental knowledge in physiology, biochemistry, and biomechanics provides additional information on the design criteria of the envisioned ATMPs.

Many bio-inspired elements have been incorporated in cell based ATMPs. However, their spatial distribution has typically remained homogenous. In fact, much effort has been dedicated to distribute cells and growth factors as homogeneously as possible throughout the biomaterial [5]. This homogeneity based approach can be argued to be advantageous from the perspective that it provides a facile, elegant and industrially attractive process to produce cell based ATMPs. However, this elementary and uncomplicated approach comes at a cost. The architecture of natural tissues is not homogeneous. Instead it is characteristically marked by a systematic “heterogeneity”. In fact, it is typically rather an assembly of repetitive smaller building blocks that are present on the nano-, micro- and macro-scale, and organized into a specific tissue/anatomical structure. Regarding bone, a well known example is represented by the organization of collagen, bone marrow niche, osteons and trabeculae. Moreover, most natural tissues including bone contain a complex, repetitive and ever-finer network of blood vessels. These heterogeneous yet repetitive designs are not only of great importance to the archetypal structure of tissues, but are essential for their function. In consequence, ATMPs that are too elementary and uncomplicated are expected to result in reduced tissue behavior and display limitations with regard to tissue integration, functionality and turnover, and thus will be characterized by reduced potential clinical outcomes.

In recent years ever more sophisticated methodologies have been pioneered to give rise to the desired biological sophistication within ATMPs [6]. However, this too comes at a cost as it potentially requires the sacrifice of a straightforward approach. Specifically, the newly acquired complexity might improve the ATMP clinical performance, but reduces the clinical feasibility.

In this review, we reflect on the complexity level of current ATMPs and give special attention to novel approaches that are able to provide complexity to ATMPs in a straightforward manner. Moreover, we discuss the potential tools able to produce or predict ‘goldilocks’ ATMPs, which are neither too simple nor too complex.

2. Mechanisms of bone formation

2.1. Natural bone healing

To understand how cell based ATMPs could effectively repair or regenerate bone tissue, it is of importance to understand the natural bone healing process. In fact, bone has a remarkable potential for repair, albeit with finite capabilities. Upon fracture the bone develops a hematoma that is followed by two distinct bone formation processes (Fig. 1).

The most intuitive form of bone formation is intramembranous ossification in which the osteoblasts directly produce and deposit woven bone. This process typically takes place at the more peripheral sites of the hematoma. The newly formed woven bone contributes to the filling of the defect and thereby able to provide some mechanical stability. However, due to the unordered (or unorganized) disposition of the fiber, woven bone provides only limited mechanical support. The newly formed bone is then slowly remodeled into the strong and well organized lamellar bone via a well-orchestrated interplay of osteoclastic resorption and bone matrix deposition by osteoblasts.

The second process by which our body is able to form bone is via endochondral ossification. In this process bone is formed via a distinct intermediary cartilaginous tissue. During fracture healing, the oxygen tension within the large center mass of the hematoma strongly decreases. This activates the signaling machinery that allows cells to cope with hypoxic environments. Importantly, this response also triggers chondrogenic differentiation of residing and/or recruited progenitor cells. The newly formed cartilage is able to survive the hypoxic stress and contribute to the stabilization of the fractured bone. The cartilage continues to mature until it reaches a terminally differentiated stage, which is characterized by chondrocyte hypertrophy, cartilage catabolism, matrix calcification and angiogenesis. The infiltrating blood vessels relieve the hypoxic stress and thereby reinforce the ongoing hypertrophic differentiation as well as deliver tissue forming cells such as osteoblasts to it [7]. These cells then form lamellar bone on the recently mineralized matrix allowing the tissue to regain its original strength. The rate, quantity and quality of the various stages of bone healing are highly influenced by a myriad of factors, which include the age and health of the patients, the type of bone, the location and type of fracture, mobility of the fracture site, available blood supply and infection in or near the fracture site. As such, it can be considered that a detailed diagnostic phase followed by meticulous patient selection can play an important role in the successful development of ATMPs.

2.2. Controlling the cell fate of cell based ATMPs

Inspired by natural bone healing, cell based ATMPs for bone repair are thus optimized for either intramembranous ossification or endochondral ossification. Full mastery over the progenitor cell's osteogenic and chondrogenic differentiation processes and behaviors is therefore of the utmost importance. The cell specification and differentiation mechanisms are underpinned by a complex yet elegant interplay of several signaling pathways. These processes and interactions occur in a highly dynamic fashion in which a single signaling mechanism can even play either stimulating or inhibiting roles depending on the differentiation stage of the cell [8].

Fortunately, control over these complex processes can be achieved in a relatively simple manner as they are governed by a limited set of master regulators (Fig. 2). For example, activation of WNT and RUNX2 signaling in mesenchymal progenitor cells results in osteogenesis and subsequent intramembranous ossification [9]. WNT and RUNX2 also contribute to endochondral ossification by driving chondrocytes into hypertrophic differentiation. In short, the ATMP's cell fate can most likely be controlled by presenting a limited set of biomimetic factors that initiate a cascade of events that allow the cell's own molecular machinery to progress in differentiation status in a semi-autonomous manner.