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Review

Uncovering the periosteum for skeletal regeneration: The stem cell that lies beneath

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ABSTRACT

The cartilage- and bone-forming properties of the periosteum have long since been recognized. As one of the major sources of skeletal progenitor cells, the periosteum plays a crucial role not only in bone development and growth, but also during bone fracture healing. Aided by the continuous expansion of tools and techniques, we are now starting to acquire more insight into the specific role and regulation of periosteal cells. From a therapeutic point of view, the periosteum has attracted much attention as a cell source for bone tissue engineering purposes. This interest derives not only from the physiological role of the periosteum during bone repair, but is also supported by the unique properties and marked bone-forming potential of expanded periosteum-derived cells. We provide an overview of the current knowledge of periosteal cell biology, focusing on the cellular composition and molecular regulation of this remarkable tissue, as well as the application of periosteum-derived cells in regenerative medicine approaches. **This article is part of a Special Issue entitled "Stem Cells and Bone".**

Contents

Introduction

The osteogenic potential of the periosteum, a thin vascular membrane that covers the external surface of bone except for the articular

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surfaces of the long bones, was described for the first time in 1742 by Henri-Louis Duhamel du Monceau [1]. His findings were later confirmed by Louis Xavier Ollier, who showed that upon treatment of bone fractures the integrity of the periosteum must be retained to achieve successful healing [2].

Subsequently, research has revealed that the periosteum consists of two layers (Fig. 1): an outer fibrous layer containing fibroblasts dispersed in between collagen fibers, and an inner cambium layer which contains skeletal progenitor cells and osteoblasts, and is highly vascularized and innervated [3]. The periosteum serves as an attachment site for tendons, ligaments and muscles. In addition, periosteal blood vessels deliver 70–80% of the blood supply to the bone cortex [4]. Furthermore, progenitor cells in the cambium layer continuously give rise to osteoblasts to allow appositional bone growth as well as cortical bone modeling and remodeling in concert with osteoclasts. The astounding potential of the periosteum is however mainly revealed after bone fracture, when periosteal progenitor cells undergo an impressive expansion, followed by differentiation into osteoblasts and chondrocytes, but predominantly the latter. This process forms the cartilaginous fracture callus that undergoes hypertrophy, followed by replacement by bone and eventual remodeling to restore the original shape of the bone [5,6]. This remarkable property of the periosteum has elicited extensive research into the use of periosteum-derived cells for regenerative approaches, and preclinical studies demonstrating the potential of these cells in the treatment of non-healing bone fractures and large bone defects are becoming available.

In this review, we summarize recent findings expanding our insight into the cellular composition and molecular regulation of the periosteum, and provide an overview of regenerative medicine approaches using periosteum-derived cells, as well as clinical attempts to mimic the periosteum for improved fracture healing.

The periosteum during skeletal development, growth and aging

Developmental origin of the periosteum

Embryonic bone formation can proceed through either the intramembranous or the endochondral pathway. During intramembranous ossification, mesenchymal stem/progenitor cells will condense and differentiate into osteoprogenitor cells expressing runt-related transcription factor 2 (RUNX2) and osterix (OSX), which in turn give rise to osteoblasts, the mature bone-forming cells [7]. During this process, undifferentiated progenitor cells remain at the bone periphery, forming the periosteum. These cells will drive further appositional growth of the bones by giving rise to new osteoblasts [8]. The formation of an entire bone by the direct differentiation of progenitors to osteoblasts is rather the exception and is responsible for the formation of only a few bones, such as the flat bones of the skull. In contrast, most bones develop through endochondral ossification, a process in which initially a cartilage template is laid down, that is later replaced by bone [9]. More precisely, during the development of the long bones, mesenchymal stem/progenitor cells expressing paired-related homeobox gene 1 (PRX1) will condense and give rise to chondrocytes, which express the transcription factor sex determining region Y box 9 (SOX9) [10,11], in the inner part of the condensation, while at the periphery undifferentiated cells remain to form a perichondrium. As the chondrocytes proliferate and the cartilage anlage grows, cells in the center will stop proliferating, enlarge and become hypertrophic. These hypertrophic chondrocytes will mineralize their surrounding matrix and attract blood vessels, chondroclasts and osteoprogenitor cells [9]. In addition, they play an important role in directing adjacent perichondrial cells to become osteoblasts and form a mineralized bone collar, thus converting the perichondrium into periosteum. Several molecular signals have been shown to link the development of the cartilage anlage with that of the surrounding perichondrium/periosteum, including the Indian hedgehog/parathyroid hormone-related protein, bone morphogenetic protein (BMP) and fibroblast growth factor (FGF) signaling pathways [9,12–14]. The crucial role of the periosteum during further bone development is highlighted by the fact that the periosteal layer will not only serve as the source of osteoprogenitor cells that invade the cartilage template to form the primary ossification center [15], but it will also contribute to the radial growth of the long bones by continuously producing mature osteoblasts from periosteal progenitor cells [16].

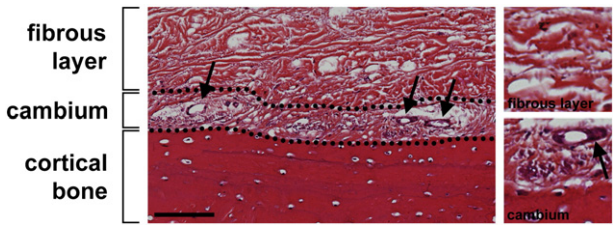

Fig. 1. Histological analysis of the periosteum. H&E stained section of human fibular bone showing cortical bone with periosteum. The periosteum can be divided into an outer fibrous layer and an inner cambium layer, which is highly cellular and contains numerous blood vessels (arrows). Scale bar = 100 μm.

The periosteum during skeletal growth and aging

Periosteal bone apposition is a cardinal feature of skeletal growth. Long bones grow wider as they grow taller, and there is extensive individual variation in this process [16,17]. One of the most striking determinants of periosteal apposition in humans is gender. There is little difference between the sexes in the extent of periosteal apposition until puberty, when periosteal expansion markedly accelerates, especially in men when sexual dimorphism appears [16–18]. The skeletal gender differences in radial bone growth are traditionally attributed to the actions of sex steroids. In the periosteum, estrogens promote the expansion of early osteoblast progenitors but inhibit their differentiation by osteogenic agents such as parathyroid hormone (PTH) or BMP2, thus limiting periosteal bone expansion [19,20]. Androgens in contrast stimulate both the proliferation of early periosteal osteoprogenitors and their differentiation to mature osteoblasts. Current evidence further indicates that in addition to differences in sex steroid secretion, skeletal sexual dimorphism also depends on gender-related differences in growth hormone/insulin-like growth factor 1 signaling and sensitivity to mechanical loading [18].

At the completion of longitudinal growth, periosteal apposition will decrease to no more than a few millimeters per year and, together with continued endosteal resorption, result in thinning of the cortical shell [16,17]. Biomechanical analyses suggest that these changes in bone size have important implications for the determination of fracture risk in the elderly. In addition to many factors that affect adult periosteal bone apposition, including mechanical forces, nutrients, endocrine factors and lifestyle, an important gender difference remains during adulthood and aging. Men experience a greater rate of periosteal growth with aging, leading to a superior ability to maintain bone strength and protection against age-related fracture risk. This may again be related to differences in sex steroid levels, as orchidectomy reduces and ovariectomy increases periosteal bone formation in adults [21]. Since periosteal and endosteal/trabecular osteoprogenitor cells seem to respond differently to sex steroids as well as antiresorptive and anabolic osteoporotic drugs [3], targeting the periosteum with anabolic agents such as PTH or androgens may prove a potent approach for fracture risk reduction in osteoporotic patients or the elderly.