

The relationship between periosteum and fracture healing

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Abstract

Fracture healing is a complex process that involves presence of osteoprogenitor cells and growth factors. Therefore, the integrity of the fracture site surrounding tissues including periosteum is necessary in order to provide the resources for bone regeneration. The purpose of this review is to organize and synthesize the relevant information regarding periosteum and fracture repair. Periosteum cells are involved in endochondral or intramembranous ossification according to the presence of a new formed cartilage. The periosteal osteoprogenitor mesenchymal cells differentiation is guided by a multitude of signaling molecules, especially bone morphogenetic protein 2 (BMP2), but also as a response to mechanical stimuli. If the periosteum is traumatized or removed, there are other osteoprogenitor cell sources as the ones located in the medullar cavity of the bones, the pericytes from the blood vessel walls as well as the undifferentiated cells from the adjacent soft tissue, muscles and fascia. However, total absence of the periosteum and lesions of the intramedullary vascular network is associated with fracture non-union. In these cases, muscular tissue surrounding the site could take over some of the cambium functions. In conclusion, there are other factors that can influence significantly fracture healing, besides periosteum.

Keywords: periosteum, bone morphogenetic protein, osteoprogenitor, fracture healing, periosteal substitutes.

Introduction

Fracture healing involves a series of mechanisms that are able in most of the case to restore the injured bone to its previous cellular structure and to regain its initial biomechanical proprieties. In order for this to happen, a series of events have to conduct involving mainly the periosteum and the soft tissues around the fracture site. In an ideal script, the periosteum and the surrounding tissues are minimally injured, but in clinical practice, the lesions are much more severe. It is important to know that there are other ways to replace part of the injured periosteum function.

The periosteum covers almost every bone of the human being. In the past, the role of the periosteum was uncertain, being defined by different theories, but nowadays it is well known the importance of the periosteum in bone blood supply and bone healing. This tissue is a natural source of pluripotent cells that can differentiate on different lines according to the structure that needs to be restored [1, 2]. However, it is not the only one involved in fracture consolidation. In fact, recent studies have reported bone regeneration without the participation of periosteal osteoprogenitor cells [3–5]. It is well known that soft tissue injuries, the presence or absence of the periosteum influence the quality of the fracture healing, but the mechanisms have not been yet completely elucidated [6–25]; additionally, other important aspects with significant impact on the fracture healing are the associated comorbidities, such as chronic kidney disease of different etiologies (on dialysis or not), diabetes mellitus,

hypertension, neoplasia, etc. [26–35]. There are some osteosynthesis techniques that offers great stability at the fracture site, but involves limited periosteum removal [36]. Knowing that periosteal substitutes can provide the resources necessary for the healing process [37–44], despite a traumatized or partially removed periosteum, will boost the confidence of the surgeon in order to intervene and to direct fracture healing mechanism by means of fracture fixations. Therefore, we reviewed the literature in order to organize and synthesize relevant information regarding fracture healing and its relationship to the periosteum, among other factors that can enhance or diminish the quality of this process.

Structural aspects of the periosteum

From the histological point of view, the periosteum has two layers, an external one that is made up mainly of fibers, an internal one that is mainly represented by cells. In 1739, Duhamel observed that fracture reduction using cerclage wire, was covered by bone matrix under the periosteal membrane. In comparison with trees, he named the inner layer of the periosteum, cambium [1]. A bit later, Ollier proved that the inner layer of the periosteum is responsible for osteogenesis after bone grafting [2]. This layer is composed of mesenchymal stem cells, osteoblasts and fibroblasts who replenish a collagen matrix. The osteoblasts are usually located near the bone cortical and surrounded by fibroblast-like cells with rich vascular and nerve networks. Along the endothelial cells of this network, there are also a significant amount of pericytes,

which can act as an auxiliary source of osteoprogenitor cells [3].

The number of fibroblast and the density of the vascular network diminish with age; therefore, the inner layer becomes thinner and very difficult to be distinguished from the outer layer. The number of osteoprogenitor cells decreases and the remaining ones become elongated, similar to the fibroblast around them.

The outer layer is composed based on the density of elastic fibers in two: a superficial, external sheet with a few fibroblast cells, elastic fibers and a collagen matrix; and a deeper, internal sheet where most of the tendon insertion of the muscles are located with a poor blood supply and high density of elastic and collagen fibers forming a network [4].

☞ The periosteum and the fracture healing

Osteogenesis is possible due to the presence of mesenchymal stem cells that forms the inner sheet of the periosteum. The endochondral ossification is conducted by injury activated bone cells. After a fracture is produced, a large hematoma will form and a series of intercellular connections will be established. The chemotaxis will be activated through the release of growth factors, cytokines and interleukins [16]. The connections between the bone ends at the fracture site are established due to the activity of the periosteum. The cells located in the inner layer of the periosteum begin to proliferate and differentiate, leading to new bone formation at the distant border of the fracture site where the blood supply is adequate, and to cartilage formation at the fracture site, where the blood supply is poor [16]. Once the cartilage is formed, a neo-angiogenesis process takes place that will lead to the increase of blood supply, cartilage resorption and new bone formation by means of endochondral ossification (Figure 1).

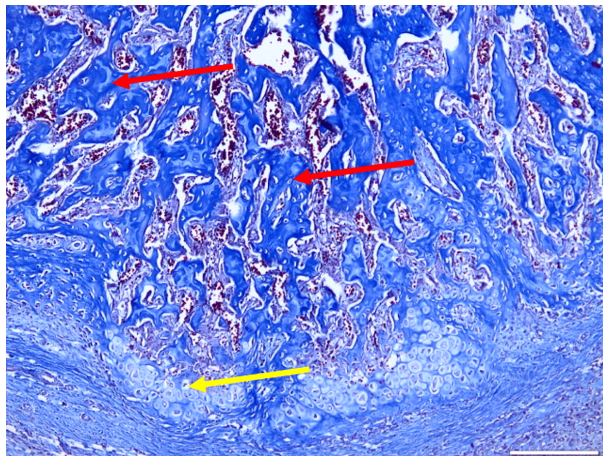


Figure 1 – Micrograph of longitudinal section through the rat fractured femur reduced with Kirschner wire revealing hyaline cartilage (yellow arrows) and new bone tissue (red arrows) that includes osteocytes (Masson's staining, $\times 100$).

This type of bone regeneration is generated by the existence of macro-movements of the bone ends at the fracture site. On the other hand, anatomical reduction and stabilization of the fracture site disables access of the periosteal cells to signaling molecules released at the

fracture site. In other circumstances, like the presence of small gaps between the bone fragments, the periosteum can contribute by means of intramembranous ossification, with generation of primary callus without cartilage formation [16].

☞ The periosteal response to signaling molecules

This tissue plays an important role in bone and cartilage regeneration due to the presence of pluripotent mesenchymal cells combined with the regular or injury stimulated production of growth factors. The periosteal osteoprogenitor cells proliferate under the influence of released platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF, FGF2) [5]. A bone fracture will trigger an inflammatory response. At first, interleukin (IL)- 1β and tumor necrosis factor-alpha (TNF- α) will be released from the macrophage, stimulate pluripotent cells differentiation into osteoclasts and determine resorption of the necrotic bone end at the fracture site. Then, this cytokines are responsible for the activation of osteoblasts and finally, for their apoptosis by means of Fas ligand (FasL)-mediated up-regulation phenomenon [6]. The same mechanism is applied to chondrocytes involved in endochondral ossification [6]. Significant amounts of vascular endothelial growth factor (VEGF) will be released by the osteoblasts and endothelial cells, inside the formed hematoma at the fracture site. This factor will determine mesenchymal cells differentiation into osteoblasts and hypertrophic chondrocytes, besides angiogenesis [7]. According to a recent experimental study on rats, VEGF is also responsible for cartilaginous resorption and soft callus transformation into primitive callus, due to a synergy with bone morphogenetic protein (BMP) 4 [8]. BMP2, 6, 7 and 9 are also involved in periosteal derived cells differentiation into osteoblasts. On the other hand, osteocytes produce sclerostin, which has the ability to block the BMP receptors type I and II and to inhibit periosteal cells differentiation into chondroblasts. Therefore, cortical bone necrosis will be associated with low levels of sclerostin due to decreasing number of osteocytes.

BMP2 is considered the leader of the signaling pathway for the osteoprogenitor periosteal cells differentiation. The BMP2/transforming growth factor (TGF)- β communication is established either SMAD dependent [associated with p38 MAPK (mitogen-activated protein kinase), which converge with RUNX2 and enables cell proliferation), either SMAD independent. The role of BMP2 in osteochondral remodeling is to control most of the important osteogenic mechanisms: Wnt/ β -catenin, FGF2 and Hedgehog (Hh) signaling process. A large amount of these molecules and their modulators have periosteal origin. The Hh amplifies the healing process, while FGF2 plays an important part in volume determination and degree of callus mineralization from the beginning of the fracture repair, where it also amplifies angiogenesis [9]. Parathyroid hormone-related protein (PTHrP), which is present in large amounts in the external fibrous sheet of the periosteum, relate with receptors located inside the cambium [10] in order to stimulate osteogenesis. At this level, BMP2 increases the PTHrP levels and determines the up-regulation of the receptors [11].

At the fracture site, large amounts of cyclooxygenase type II (COX-2) and BMP7 are produced in order to increase the susceptibility of periosteal mesenchymal cells to differentiate into osteoblasts [12]. In order to do so, COX-2 up-regulates the expression of transcription factors [RUNX2, also called core-binding factor-alpha 1 (CBFA1) and Osterix (Osx)], which stands at the base of the osteoblastogenesis [13]. On the other hand, large quantities of insulin-like growth factor 1 (IGF-1), transforming growth factor-beta 1 (TGF- β 1) and FGF2 stimulates the mesenchymal pluripotent cells inside the cambium to participate in chondrogenesis [14]. Due to the periosteal cells high capability of differentiation, the periosteum has been used for regeneration of the articular femoral surface after reflection and passive movements [15].

Some of these molecules, such as recombinant human BMP2, BMP7, VEGF, parathyroid hormones and TGF- β are used in clinical applications to accelerate or to improve the fracture healing process [16].

☞ The periosteal response to mechanical stimuli

Located on the outer side of the bone, the periosteum is able to respond to mechanical forces that act at this level. Therefore, osteosynthesis methods are used to distribute loading forces at the surface of the bone in order to enhance the repairing mechanisms [36]. The native environment of the cambium osteoprogenitor cells is mechanically regulated by a combination of tension and shear forces. These cells are able to take these tensions inside through a microfilaments matrix and to transform them in signaling molecules and soluble factors in order to modulate the chondro- and osteogenesis [9, 16]. Shape deformation due to stretch forces leads to collagen fibril production, which will transform into fibrous tissue. This tissue transforms either into intramembranous bone under tension forces, either into cartilage under compression forces. When the volume of the cell is modified, it will differentiate into chondrocytes [17]. In large defects, periosteal applications of external tension forces are responsible for new bone formation [9, 16].

☞ The fracture healing in the absence of periosteum

It is certain that periosteum may play an important role in bone regeneration and development but it is not the only available mechanism. Even if the inner layer of the periosteum is able to provide the precursors of chondrocytes and osteoblasts, if the reduction of the fracture is rigid, the main osteogenic role goes to the endosteum, which is a vascular sheet of connective tissue that covers the cortical, the trabecular bone and the Haversian systems [18]. This is not only a source for osteoprogenitor cells but in most of the cases where osteosynthesis techniques are used, it is the main player in the bone regeneration process. In other cases, secondary ossification centers are responsible for intra-articular, sesamoid or carpal bone fractures consolidation, without the need of periosteal mediated endochondral ossification [16].

☞ Osteoprogenitor cell sources besides periosteal cambium

Cells located in the medullar cavity of the bones, the pericytes from the blood vessel walls [3] as well as the undifferentiated cells from the adjacent soft tissue, muscles and fascia [19] have the ability to follow osteogenic or chondrogenic differentiation lines, besides the cambium and the endosteum.

To highlight the role of the pericytes in bone healing, a recent experimental protocol revealed a significant population of osteoblast cells derived from marked pericytes after periosteum removal was performed. Therefore, this source is a viable alternative source to the periosteal inner layer [3].

The circulating stem cells or harvested from the bone marrow may be used in bioengineering in order to enhance fracture healing. Stem cells from the blood stream receive a special attention because is very easy to isolate them and it has shown great osteogenic potential [20]. Low blood supply could lead to the absence of fracture consolidation. A population of endothelial or hematopoietic osteoprogenitor cells marked CD34+ administered at the fracture site enhances the regeneration process when the periosteum is unable to participate. First, a hematoma forms and this cells migrate either from the blood vessels whose continuity was interrupted due to soft tissue damage or from bone marrow present in the intramedullary canal of the cortical bone or in cancellous bone trabeculae [20].

☞ The role of the periosteal vascular network in fracture healing

An important aspect of the fracture healing is represented by the blood support of the bone fragments involved. The bone vascularization is assured by the periosteum vascular network, being responsible for 1/3 of the cortical surface and for the surrounding soft tissues, especially muscles. The other 2/3 parts of the cortical bone are supplied by the nutritional bone artery, which forms the intramedullary network. The metaphyseal vessels represent the terminal branches of this network. There are multiple anastomoses between these two networks, therefore, when a territory is deficient, the other network is capable to provide adequate blood flow [18]. In case of hypoxia, the bone healing occurs by means of endochondral ossification with an intermediate phase represented by cartilaginous tissue formation (soft callus). This stabilizes the fracture site and permits new formed blood vessels to penetrate and to induce chondrocytes apoptosis, calcification, and then callus mineralization. In the well-oxygenated area of the fracture site, the osteoprogenitor cells differentiate into osteoblasts. Therefore, the periosteum is not just a source of pluripotent undifferentiated cells but also plays an important role in the blood supply of the bone. When the periosteum is removed, there will be an area of bone with low blood flood that needs to be compensated. This could lead to bone sequestrum and infection if the periosteum is completely detached, with no connections to the intramedullary network and surrounding traumatized tissues (Figure 2). In a murine model, the femoral periosteum was removed and the medullar cavity was reamed. After 65 days, the radiological assess-

ment revealed absence of fracture consolidation. However, the histological assessment showed a discreet osteoblastic activity. Despite these conditions of suppressed blood supply, massive bone resorption did not occurred, meaning that there were other mechanisms of bone nutrition beside periosteal and intramedullary vascular network [21]. The soft tissue surrounding the fracture site has the ability to provide osteoprogenitor undifferentiated cells in order for the regeneration process to unfold [22]. Recent studies have not been decisive regarding early use of soft tissue in order to cover fractures, but benefits were observed [23]. Using viable muscle in order to cover the fracture did not increase local blood flow significantly, rather it contained the agglutination of cells and growth factors at the fracture site [24].

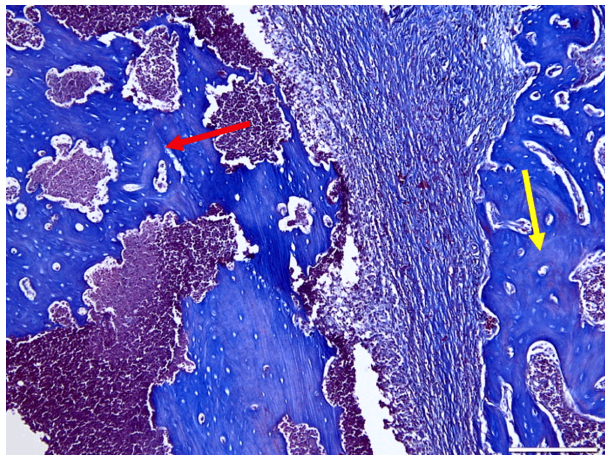


Figure 2 – Micrograph of longitudinal section through the rat fractured femur after circumferential periosteal removal was performed near the fracture site and reduced using plates and screws revealing suppuration and bone sequestrum (red arrow) and mesenchymal ossification (Masson staining, x100).

In a recent study on fractures of the rats tibia, between the avascular bone segment (periosteum was removed) and the vascular bone segment there were no significant differences regarding the resistance to torsion and shear, the energy necessary for refracturing or the degree of mineralization. Therefore, the avascular bone segment acted like a graft and healed properly.

Another study on rabbits revealed that bone grafts covered by periosteum suffered a minimal resorption at the implantation site maintaining its form. There were no osteoclasts near the receiving site. The bone graft without periosteum suffered an intense process of remodeling. These results suggest that bone grafts covered by periosteum suffer a quicker integration process without distortion of the shape [45]. However, compared with the previous study [25], the bone grafts were free transferred, having different structure and shape from the one in the receiving site. Therefore, the shape remodeling was probably a process of integration to the new site and did not influence significantly the healing process.

According to other studies, the periosteal grafts have different osteogenic potential depending on the area of harvesting [1].

☞ The role of muscles in fracture healing

The interaction between surrounding muscles and

bone can offer the necessary support in order for a fracture to heal, despite the absence of the periosteum.

Generically, it is considered that soft tissue integrity is important in bone regeneration, but the mechanisms are not completely elucidated. Until recently, the muscles were considered to be responsible for the blood supply of the fracture site. This aspect was revealed by Harry *et al.* in an experimental study regarding the covering of opened tibial fractures with musculocutaneous flaps and fasciocutaneous flaps [24]. In the group where muscles were included, the healing was faster. However, recent studies revealed an important cell component and paracrine function of the muscles that are involved in osteogenesis [46]. New bone formation from the undifferentiated cells of the muscles was first reported by Urist [47]. Recent experimental studies on different species showed the trend of forming a more bulky and dense callus at the level of bone–muscle interface, either because of the muscle osteogenic capacity, either because it provides the perfect environment in order for the ossification process to take place [48].

Muscle derived cells and satellite muscle cells [37] activated by BMP2 are able to differentiate into bone cells [40]. C2C12 myoblast after being infected with a retrovirus, differentiated into osteoblasts that expressed surface osteocalcin [49]. Muscle derived stromal cells can be recruited and transformed into osteoprogenitor cells after exposure to low levels of TNF- α [39]. Liu and *et al.* proved that MyoD myogenic cell line had an important role in bone regeneration, but were not incorporated where the periosteum was intact [38]. Therefore, these cells can be involved as secondary osteoprogenitor cell source when the cambium cannot provide them [50].

Next to the cellular component, the muscles are able to produce more than 200 proteins, including myostatin, BMPs, osteonectin, IL-1, -4, -6, TNF- α , IGF-1 [50]. According to an experimental study, muscle derived IGF-1 local applications accelerated the bone formation of a bone defect [51]. On the other hand, myostatin is responsible for the inhibiting role on the bone healing and muscle development, growth and regeneration [41]. Therefore, the level of myostatin is directly connected with the severity of the muscle lesions. In this matter, neutralization molecules proved to be useful in order to improve bone and muscle regeneration [41]. On the other hand, osteonectin plays an important role in bone matrix production, in collagen fibril network assembly and osteoblasts proliferation [52]. This molecule is produced by the injured and regenerated myotubes, proportionally with the size of the muscle lesion [52]. In summary, the muscular tissue is able to provide osteoprogenitor cells, stability and blood supply at the fracture site in the absence of the periosteum.

☞ Artificial periosteal substitutes

According to studies, the complete removal or compromising of the periosteum increases the risk of fracture non-union [10, 15, 21]. Therefore, artificial membranes that resemble the periosteum were created in order to enhance fracture healing. In an experimental study, a bio-artificial nanofibrous periosteum was used in order to cover femoral fracture after periosteum was removed.

The results showed that this technique provided reasonable biomechanical resistance and improved pain management [42]. Another study showed that polyvinyl alcohol nanofiber was a suitable reservoir for osteogenic factors and played an important role in bone regeneration [43]. According to Caridade *et al.*, polysaccharide-based membranes impregnated with BMP2 can act as an artificial periosteum scaffold in fracture healing [44]. Taking into consideration that standalone injuries of the periosteum are very rare, and usually are associated with severe soft tissue destruction, bioengineering of the periosteal substitutes represents a valid option for future research.

Conclusions

The periosteum provides osteoprogenitor mesenchymal cells and 1/3 of the bone cortical blood flow, making it important for the bone regeneration process. BMP2 and other signaling molecules have the ability to interact with the periosteum and surrounding soft tissue in order to initiate the healing mechanisms in fractures. Understanding these pathways may serve in future research of targeted therapies in order to facilitate bone regeneration. Experimental studies have shown that total removal of the periosteum associated with an injured intramedullary vascular network leads to fracture non-union, but limited periosteal removal may not affect significantly fracture consolidation. In current practice, fractures are usually associated with soft tissue and periosteum injuries. Therefore, muscle covering, less aggressive hemostasis, preservation of the intramedullary blood flow, use of artificial periosteum, and local administration of synthesized growth factors represents alternatives in order to obtain the best results after fracture repair.

Conflict of interests

The authors declare no conflict of interests.

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