



**HAL**  
open science

# Periosteal Skeletal Stem and Progenitor Cells in Bone Regeneration

Simon Perrin, Céline Colnot

► **To cite this version:**

Simon Perrin, Céline Colnot. Periosteal Skeletal Stem and Progenitor Cells in Bone Regeneration. Current Osteoporosis Reports, 2022, 20 (5), pp.334-343. 10.1007/s11914-022-00737-8 . hal-04646470

**HAL Id: hal-04646470**

**<https://hal.u-pec.fr/hal-04646470v1>**

Submitted on 12 Jul 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Periosteal skeletal stem and progenitor cells in bone regeneration

Simon Perrin<sup>1</sup> and Céline Colnot<sup>1\*</sup>

<sup>1</sup> Univ Paris Est Creteil, INSERM, IMRB, F-94010, Creteil, France

\* Correspondence: [celine.colnot@inserm.fr](mailto:celine.colnot@inserm.fr)

## Abstract

### Purpose of review:

The periosteum, the outer layer of bone, is a major source of skeletal stem/progenitor cells (SSPCs) for bone repair. Here, we discuss recent findings on the characterization, role, and regulation of periosteal SSPCs (pSSPCs) during bone regeneration.

### Recent findings:

Several markers have been described for pSSPCs but lack tissue specificity. *In vivo* lineage tracing and transcriptomics analyses have improved our understanding of SSPC functions during bone regeneration. Bone injury activates pSSPCs that migrate, proliferate, and have the unique potential to form both bone and cartilage. The injury response of pSSPCs is controlled by many signaling pathways including BMP, FGF, Notch and Wnt, their metabolic state, and their interactions with the blood clot, nerve fibers, blood vessels and macrophages in the fracture environment.

### Summary:

Periosteal SSPCs are essential for bone regeneration. Despite recent advances, further studies are required to elucidate pSSPC heterogeneity and plasticity that make them a central component of the fracture healing process and a prime target for clinical applications.

**Keywords:** periosteum – skeletal stem/progenitor cells – bone regeneration – In vivo lineage tracing -

## Introduction

The periosteum is a thin fibrous membrane covering the outer surface of bones. It is organized in 2 layers: the outer fibrous layer, mostly composed of fibroblasts and extracellular matrix and the inner cambium layer, composed of osteoblasts and skeletal stem/progenitor cells (SSPCs). The periosteum is highly vascularized and innervated by sensory and sympathetic fibers, and hosts resident and osteal macrophages (osteomacs) [1–3]. In long bones, the periosteum is derived from the limb mesenchyme that forms the cartilage template and the surrounding perichondrium during development [4, 5]. The

periosteum plays crucial roles in bone physiology during development, growth and remodeling. Periosteal cells directly contribute to cortical bone formation and control bone growth by secreting paracrine factors such as osteocrin [6, 7]. Extrinsic factors such as mechanical loading stimulate bone formation in the periosteum during bone growth and remodeling [8–10].

The capacity of the periosteum to form bone after a fracture was first described in 1845 by Watson [11]. The periosteum displays a remarkable ability to regenerate bone and is indispensable to achieve bone healing [12]. Periosteum removal or damage at the time of fracture can cause altered healing and non-union in experimental models [13–17]. The periosteum is a key source of SSPCs for bone repair in addition to SSPCs localized within the bone marrow compartment and the adjacent skeletal muscle [18–25]. Following a bone injury, SSPCs are activated during the inflammatory phase of repair and differentiate into osteoblasts and chondrocytes. SSPC differentiation is regionalized in the fracture callus. Osteogenesis leading to intramembranous ossification occurs primarily at the periphery of the callus while chondrogenesis leading to endochondral ossification occurs in the center of the callus. The mechanical environment also influences cell differentiation as fracture stability favors intramembranous ossification while fracture instability favors endochondral ossification. The role of periosteal SSPCs in coordinating these events is still poorly understood.

In this review, we summarize recent findings on the characterization of periosteal skeletal stem/progenitor cells (pSSPCs) and their contribution to bone repair. We describe the current knowledge on pSSPC molecular regulation and interactions with the fracture environment following bone injury. Finally, we discuss the role of pSSPCs in bone repair defects and the relevance of pSSPCs in cell-based therapies.

## **Isolation and characterization of periosteal stem and progenitor cells**

Studying periosteum and pSSPCs is challenging due to the inaccessibility and thinness of the periosteum. Human pSSPCs have been isolated by scraping or peeling away the periosteum from the cortex, followed by enzymatic digestion or explant culture [26]. In animal models, similar techniques have been employed. In addition, protocols have been developed to directly isolate cells by enzymatic digestion of the bone surface or by whole bone explant culture [27–30]. Isolated pSSPCs are adherent to plastic and can be cultured *in vitro* for several passages.

SSPCs were first described in the bone marrow compartment and defined as mesenchymal stem cells based on stem/progenitor marker expression, multipotency and self-renewal capacity [31, 32]. The periosteum contains cells displaying SSPC criteria. Analysis of cell surface markers showed that pSSPCs are negative for hematopoietic and endothelial markers and express canonical mesenchymal

markers such as CD90, CD105, CD51, CD29 and Sca1 in mice and CD90, CD105, CD73 in human [20, 27, 33–35]. In 2015, Chan *et al.* identified a population of mouse skeletal stem cells from whole digested bone based on a combination of cell surface markers (CD45<sup>-</sup> TER119<sup>-</sup> TIE2<sup>-</sup> CD51<sup>+</sup> THY1<sup>-</sup> 6C3<sup>-</sup> CD105<sup>-</sup>) [36]. The SSPC populations defined by these markers were also identified in freshly isolated and primary periosteal cells from mouse and human bones [24, 25, 37, 38]. Periosteal SSPCs exhibit multipotency *in vitro* with tri-lineage differentiation capacity towards osteogenesis, chondrogenesis and adipogenesis [34, 39]. The multipotency and differentiation potential of pSSPCs was confirmed by *in vivo* ectopic transplantation experiments [40, 41]. A key feature of stem cells is their ability to self-renew, and both mouse and human pSSPCs display self-renewal potential in serial CFU or ectopic transplantation assays [22, 42].

### **Fate of periosteal stem and progenitor cells during bone repair**

The direct contribution of periosteum to bone regeneration is well established. The periosteal response to bone fracture is characterized by periosteum thickening near the site of injury within few days after fracture. Activated periosteal SSPCs proliferate and migrate at the fracture site, and participate in both intramembranous and endochondral ossification [18, 20, 25]. This ability of pSSPCs to form cartilage and bone after fracture is unique to the periosteum as SSPCs from bone marrow mostly contribute to bone and SSPCs from skeletal muscle contribute to cartilage [18, 20–23]. It remains unclear how cell fate decision of pSSPCs towards osteogenesis or chondrogenesis is regulated. Using single-cell RNAseq (scRNAseq) analyses of primary periosteal cells at steady state and 3 days post-fracture, Julien *et al.* described the steps of pSSPC activation towards the chondrogenic lineage [25]. After bone injury, periosteal SSPCs transition from a stem/progenitor state to a fibrogenic state, marked by an active extracellular matrix production and the expression of cell migration related genes. Following this transient activation step, cells undergo chondrogenic differentiation and proliferate. A recent study by Van Gastel *et al.* showed that pSSPCs can be directed towards chondrogenesis after fracture by blocking vascular ingrowth to prevent nutrient availability [43]. Activated pSSPCs with sufficient lipid intake can maintain sufficient levels of fatty acid oxidation and differentiate into osteoblasts. However, in regions with low lipid availability, reduced fatty acid oxidation leads to FOXO activation, SOX9 expression and chondrogenic differentiation [43]. Further studies are needed to determine how cell fate decisions in the periosteum are controlled by environmental factors, and whether distinct cell populations in the periosteum respond differently to signals in the fracture environment to undergo either osteogenesis or chondrogenesis.

Periosteal SSPCs orchestrate another crucial step of bone repair via endochondral ossification. In the center of the callus, a site of active replacement of cartilage by bone, transdifferentiation of hypertrophic chondrocytes into osteoblasts appears to be an important source of newly formed osteoblasts [44, 45].

Through lineage tracing, Julien *et al.* showed that periosteal SSPCs exhibit a strong potential to participate in this transdifferentiation process required for bone bridging [37]. Finally, during the late stages of repair, pSSPCs can self-renew to reconstitute a new periosteum at the periphery of the callus. The *in vivo* self-renewal capacity of pSSPCs during bone repair was revealed using periosteum graft and serial fractures, showing that the periosteum maintains a pool of pSSPCs able to contribute to a subsequent injury [20].

## Genetic lineage tracing of pSSPCs during bone repair

Additional *in vivo* approaches, based on the Cre/LoxP system for genetic lineage tracing, have been instrumental to understand the endogenous role and fate of pSSPCs during bone regeneration (Table 1). Due to their mesenchymal origin, pSSPCs in long bones can be tracked from developmental stages using the limb bud mesenchymal marker PRX1. The non-inducible *Prrx1<sup>Cre</sup>* model labels not only SSPCs in the periosteum but also in bone marrow and skeletal muscle [20, 21]. In adult bone, pSSPCs still express *Prrx1* but the inducible *Prrx1<sup>CreERT</sup>* model does not allow efficient labeling of pSSPCs [20, 37, 46]. The platelet-derived growth factor  $\alpha$  (PDGFR $\alpha$ ) has been described as a marker of mesenchymal cells from various tissue origins. Studies using the *Pdgfra<sup>CreERT</sup>* model showed that PDGFR $\alpha$  marks pSSPCs with osteochondral potential during bone healing but is not restricted to the periosteum [24, 25, 47–49].

Debnath and colleagues combined the markers described by Chan *et al.* with the marker Cathepsin K (CTSK) and identified a population of pSSPCs involved in intramembranous ossification [22]. CTSK<sup>+</sup> pSSPCs, labelled by the non-inducible *Ctsk<sup>Cre</sup>* model, display self-renewing capacity and osteochondral potential in calvarial or femoral injury models. scRNAseq analysis of sorted CTSK<sup>+</sup> pSSPCs showed heterogeneity within this population, with clusters expressing chondrogenic genes (*Col2a1*, *Sox9*), osteogenic markers (*Kera*, *Alpl*), stemness markers (*Ly6a*, *Cd34*), and *Acta2* [22]. CTSK<sup>+</sup> and CD200<sup>+</sup> periosteal cells were also detected in human periosteum [22].

The protein alpha-Smooth Muscle Actin ( $\alpha$ SMA), encoded by the *Acta2* gene, labels cells in the periosteum [24, 50].  $\alpha$ SMA<sup>+</sup> periosteal cells, traced using the *Acta2<sup>CreERT</sup>* mouse line, are an heterogeneous population of osteochondroprogenitors, with self-renewing potential and required for efficient bone healing [24]. Ortinau and colleagues described a sub-population of  $\alpha$ SMA<sup>+</sup> cells, using the pIpC inducible *Mx1<sup>Cre</sup>* line [51].  $\alpha$ SMA<sup>+</sup> Mx1<sup>+</sup> pSSPCs display self-renewal potential and are required for callus formation after tibial and calvarial injury [51].

Several other markers have been identified, such as the Wnt signaling downstream target AXIN2, the receptor PDGFR $\beta$  and HOX11 [52–54]. Markers, such as Leptin receptor and Nestin, used to

characterize bone marrow self-renewing SSPCs also label cells in the periosteum [42, 55]. Tournaire *et al.* reported that Nestin-GFP<sup>+</sup> periosteal cells in Nestin-GFP transgenic mice correspond to unipotent osteoprogenitors, with limited contribution to callus formation and no stemness properties. Lineage tracing in the *Nes<sup>CreERT</sup>* model revealed an osteogenic and self-renewing potential of Nestin<sup>+</sup> periosteal cells [42, 55]. GLI1 labels populations of SSPCs, including in the periosteum, forming chondrocytes and osteoblasts after fracture [56, 57]. Osteochondrogenic markers such as SOX9 (*Sox9<sup>CreERT</sup>* model) and Osterix (*Osx<sup>Cre</sup>* model) both label populations of periosteal progenitors contributing to cartilage and bone formation after fracture [53, 58].

Overall, periosteal SSPCs populations defined by these various CRE models exhibit similar properties, such as the expression of stem/progenitor markers, osteochondral fate after fracture and self-renewing capacity. It remains unclear how these populations overlap and differ in their identity and potential, and whether the periosteum encompasses several populations of SSPCs. Moreover, no marker currently used to characterize pSSPC is fully specific to the periosteum as most of them are expressed also by cells localized in the bone marrow, skeletal muscle or by stromal cells in other tissues [21, 25, 49, 59]. Thus, marker expression is not sufficient to specifically trace periosteum-derived SSPCs and exclude the contribution of traced cells from other bone compartments during bone repair. Specifying the tissue of origin and using protocols to isolate periosteal cells without contamination from other tissues remain essential to identify pSSPCs and investigate their role in bone repair.

## **Molecular regulation of periosteal stem and progenitor cells**

During bone repair, pSSPC proliferation, migration and differentiation are governed by several signaling pathways including Bone Morphogenic Protein (BMP), Wnt, Notch, Fibroblast Growth Factor (FGF), Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor  $\beta$  (TGF $\beta$ ) and Hedgehog (HH) signaling (Figure 1). Bone Morphogenetic protein (BMP) signaling is one of the earliest pathway upregulated after fracture [25]. Increased BMP ligand and receptor expression as well as activated downstream SMAD effectors are observed in the activated periosteum at day 3 post-fracture [25, 60]. The role of BMP signaling during the early stage of bone healing was evaluated by inactivating *Bmpr1a* using the *Pdgfra<sup>CreERT</sup>* model. *Bmpr1a*-deficient periosteal SSPCs display reduced proliferation, migration and osteochondrogenic differentiation after fracture [25]. While endogenous BMP2 is required for bone healing, BMP4 and BMP7 are dispensable [61–63]. BMP2 plays a role in pSSPC fate decision, and *Bmp2* gene inactivation prevents periosteal activation and bone healing [64–66].

Notch signaling also plays a role in the early steps of bone healing before pSSPCs commit to the chondrogenic or osteogenic lineage. Notch inactivation in *Prx1*-derived cells, using *Prx1<sup>Cre</sup>* mice, causes bone non-union but Notch inactivation in chondrocytes using *Acan<sup>Cre</sup>* mice, or osteoblasts, using

*Coll.1<sup>Cre</sup>* mice, does not impact healing [67]. The crosstalk between Notch and Wnt signaling pathways is essential for the progression of the bone healing process. Whereas Notch signaling is active early to promote pSSPC activation and proliferation, the expression of Wnt pathway and target genes peaks between 5 and 10 days post-fracture [68, 69]. Inhibition of Notch signaling by Wnt signaling marks the end of the pSSPC activation phase and the initiation of osteogenic differentiation [67, 70, 71]. Wnt regulates the differentiation of SSPCs by promoting osteogenic differentiation over chondrogenesis through SOX9 repression [72]. Wnt overactivation, in *Sostdc1* knock-out mice causes premature activation of periosteal cells and overmineralized callus formation [73]. The factors regulating Wnt expression and activation remain poorly understood.

FGFs are expressed not only during pSSPCs activation (FGF2, FGF5, FGF9), but also during cartilage formation and maturation (FGF16, FGF18), and during ossification (FGF1, FGF17), suggesting their involvement through all stages of repair [74]. FGF2 promotes the proliferation and chondrogenic differentiation of pSSPCs through BMP2 signaling [75, 76]. By over activating FGF receptor 3 (FGFR3) signaling in *Prx1<sup>Cre</sup>* mice, Julien et al showed that FGFR3 is crucial for the differentiation of periosteum-derived chondrocytes and their ability to support cartilage-to-bone transformation during endochondral ossification [37].

In addition, PDGF-BB, TGF $\beta$  and Hedgehog are required for pSSPC proliferation and differentiation [57, 77–81]. PDGF-BB stimulates pSSPCs migration and angiotropism while inhibiting apoptosis [42, 82]. Cyclooxygenase 2 (COX2) is essential for the initiation of the periosteal response to cortical bone injury by modulating several key pathways such as HIF, PI3K-Akt and Wnt [83–85]. In sum, many signaling pathways and growth factors must act in coordination to control pSSPC activation and subsequent steps of differentiation. Whether several pSSPCs subpopulations respond to distinct molecular signals and whether regulatory networks specific to pSSPCs define their unique osteochondral potential after fracture remains to be investigated.

## **Influence of the fracture environment on periosteal stem and progenitor cells**

Bone fracture creates a major perturbation of bone tissue homeostasis with the rupture of blood vessels and nerve fibers initiating an acute inflammatory response shortly after fracture. This complex multicellular environment influences the activation and fate of pSSPCs (Figure 1). The disruption of blood vessels causes immediate bleeding and subsequent blood clot formation. The blood clot is then progressively degraded by the action of enzymes such as plasminogen (Plg) [86, 87]. Plg knock-out mice display impaired bone healing, with reduced callus, cartilage, and bone formation. Plg plays a paracrine role in the activation of pSSPCs. By cleaving the inactivated form of the matrix associated growth factor Cyr61 secreted by pSSPCs, Plg can stimulate pSSPC proliferation and migration [87].

Periosteum is essential for the revascularization of the fracture site as periosteum removal reduces blood vessel density [43]. Vascular disruption at the fracture site also causes hypoxia in the first days after injury [88]. The hypoxic environment stimulates HIF1 $\alpha$  expression by pSSPCs and the secretion of the proangiogenic factors VEGF and TSP-4 required for angiogenesis and bone healing [27, 89–91]. More research is needed to elucidate the role of the periosteum and the interplay between blood vessels and pSSPCs during fracture revascularization.

During the inflammatory phase of bone repair, immune cells are recruited at the injury site. The periosteum becomes invaded with osteomacs and activated macrophages [3]. Macrophages play a critical role in bone healing, and their depletion, using the Mafia inducible system, reduces periosteal callus formation [92]. Macrophages remove necrotic tissue at the fracture site and secrete factors involved in the recruitment and activation of pSSPCs. Gao *et al* showed that TRAP<sup>+</sup> periosteal macrophages secrete PDGF-BB, that binds to PDGFR $\beta$  expressed by pSSPCs to activate the Pi3K-Akt-CREB pathway and stimulate Periostin expression [42]. Periostin is a critical regulator of pSSPC response to injury and self-renewal [20]. Periosteal SSPCs are responsive to different chemokines. Chemokine ligand 2 (CCL2 / MCP-1) is expressed in the periosteum during the first 3 days following fracture [93]. Inactivation or inhibition of CCL2 and his receptor CCR2 delays fracture healing [93, 94]. The CCL5-CCR5 axis is necessary to induce the migration of murine and human pSSPCs [51]. *In vitro* osteogenic induction of periosteal cells can modulate macrophage polarization and promote M2 phenotype by secreting chemokines suggesting a crosstalk between macrophages and pSSPCs [95, 96].

The disruption of nerve fibers in the periosteum triggers rapid nerve sprouting from both sympathetic and sensory fibers in the first day post-bone injury. Nerve sprouting is concomitant with NGF expression in periosteal cells and macrophages, and occurs prior to revascularization [97]. Nerves subsequently regulate pSSPC activation by releasing neuropeptide calcitonin gene related peptide (CGRP), that binds to the CALCRL-RAMP1 receptor and stimulates *Osterix* expression [98]. Overall, the perturbations in the micro-environment of the periosteum following bone injury generates unique cell-cell interactions and specific signals. This environment can vary depending on the type of injury, the mechanical stimuli and additional interactions with the adjacent skeletal muscle, bone marrow and systemic factors. How changes in this complex tissue environment influence the fate of pSSPCs remains to be explored.

## **Clinical applications of periosteal stem and progenitor cells for bone repair**

As the periosteum is an essential actor of bone healing, pSSPC deficiencies can have direct consequences on repair. Metabolic dysregulation in mice with induced type 1 diabetes reduces callus formation, correlated with decreased pSSPC proliferation and osteogenic differentiation [99]. Mice with diet-induced obesity (DIO) and subsequent type 2 diabetes also exhibit impaired fracture healing [100,



101]. Periosteal SSPCs isolated from DIO mice show reduced osteochondral and adipogenic differentiation potential *in vitro* [100]. Aging is a long-known factor affecting bone repair in human and animal models partially due to a reduction of pSSPC potential and number [102, 103]. Two reports indicate abnormal extracellular matrix deposition and proliferation of periosteal cells isolated from 1-year-old mice and reduced chondrogenic potential when isolated from 2-year-old mice [104, 105]. Aging is frequently linked to osteoporosis [106]. Mice with estrogen or glucocorticoid-induced osteoporosis display an abnormal periosteal response to scratch injury, with reduced cartilage formation and maturation [107]. Overall, dysfunctions of pSSPCs are still poorly described, but could be of major interest to understand bone healing impairment.

Periosteum flap or allograft are frequently used in orthopedic surgery to promote bone repair with convincing results in animal models and in human [108–110]. Periosteal SSPCs are therefore considered for cell-based therapies. Transplanted pSSPCs can improve bone healing in aged mice, genetically induced pseudarthrosis and critical size defects [37, 108, 111]. Bone tissue engineering aims to replace autograft approaches, by using cultured SSPCs embedded in a 3D matrix containing growth factors. The choice of the optimal cell source is key for successful bone tissue engineering and the periosteum rise as a promising source of cells. Compared to other cell sources, such as bone marrow, adipose, or dental pulp derived cells, pSSPCs display higher clonogenicity, proliferation, osteogenic and chondrogenic differentiation [20, 112, 113]. Moreover, the potential of pSSPCs can be increased depending on the harvesting site and by using pre-treatment with BMP2 or FGF2 [38, 114, 115]. The development of periosteum-like matrix, that mimics the structural organization and cellular composition of the periosteum is also explored with encouraging results [116]. Growth factors can be added to the scaffold in order to stimulate endogenous pSSPCs or promote angiogenesis of the grafted bioengineered tissue (VEGF) [113, 117]. To exploit pSSPCs as an alternative source of cells for orthopedic cell-based therapies, a better understanding of their identity and the factors regulating their fate is needed.

## Conclusions

The first studies describing periosteal SSPCs *in vitro* provided limited relevance for endogenous pSSPCs functions. *In vivo* lineage tracing, using transgenic mouse models, is a valuable tool to characterize pSSPCs in their periosteal niche and study their behavior in the complex fracture environment. Different subpopulations of pSSPCs have been identified using markers such as PRX1, CTSK, and  $\alpha$ SMA. These pSSPCs share common features, including multipotency after fracture and self-renewal. It remains to be elucidated whether distinct sub-populations differ in their identity and potency or if they overlap and exhibit great plasticity based on their tissue localization and environmental signals in the fracture vicinity. Additionally, the absence of a specific marker to distinguish pSSPCs from the other SSPC sources remains a challenge. While SSPCs from the bone marrow and skeletal muscle are mostly

unipotent *in vivo*, pSSPCs are bi-potent differentiating into osteoblasts or chondrocytes. The origin of this bi-potentiality is unknown. The heterogeneity of pSSPCs populations with distinct osteochondral potential is one hypothesis. pSSPCs also evolve in a unique fracture environment at the interface of bone and skeletal muscle, exposing them to numerous biological and mechanical stimuli. Advances in single cell transcriptomics will provide new insights in the heterogeneity of pSSPCs and their responses to bone injury. The complementarity of *in vitro*, *in vivo* and transcriptomic approaches will enhance our understanding of pSSPCs and pave the way for new orthopedic cell-based therapies.

### **Acknowledgements**

We thank A. Julien and R. Marcucio for critical reading and comments. This work was supported by ANR-18-CE14-0033, ANR-21-CE18-007-01, NIAMS R01 AR072707 to C. Colnot. S. Perrin was supported by a PhD fellowship from Paris University.

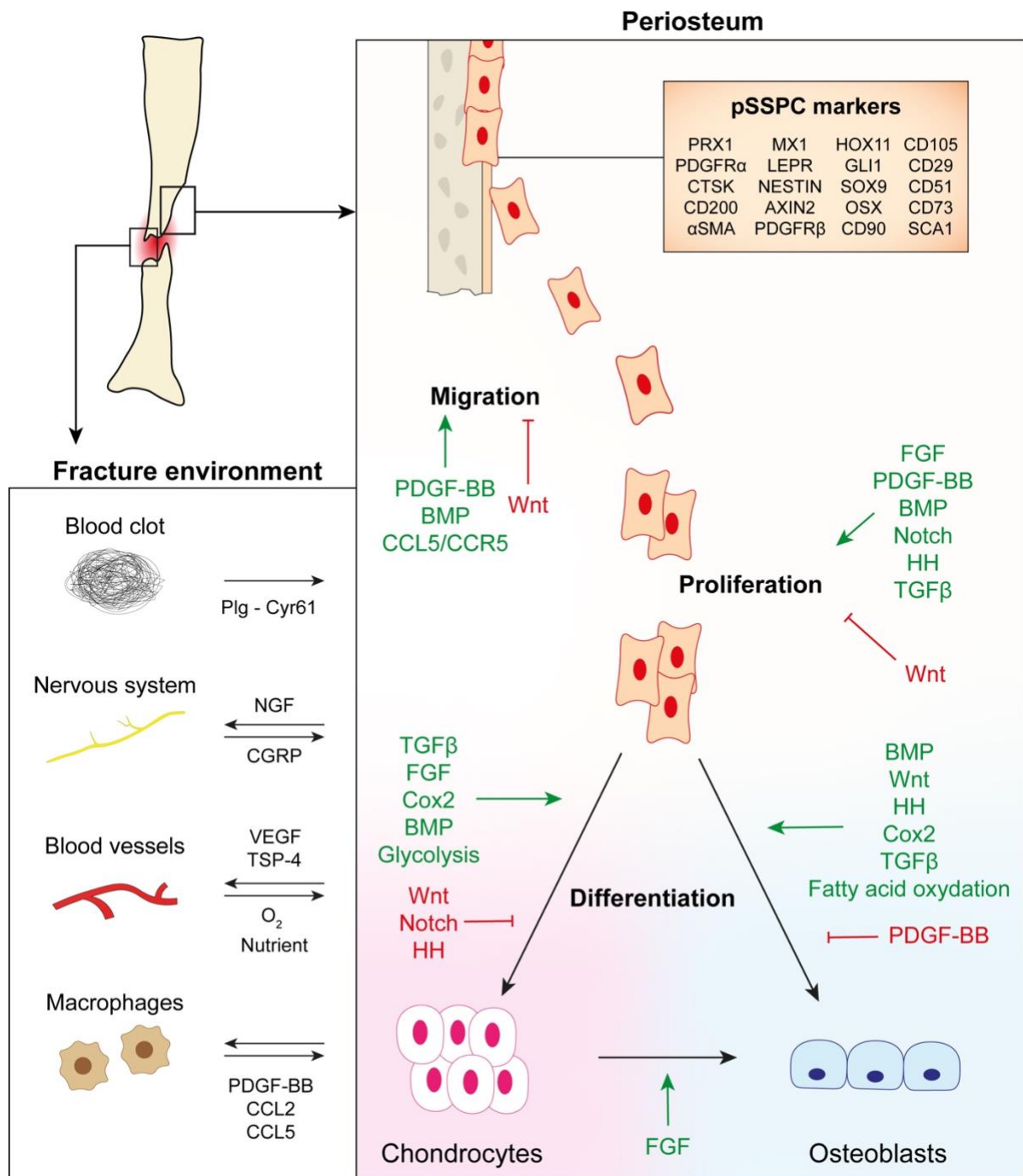
### **Competing interests**

Authors declare no competing interests.

### **Human and Animal Rights**

This article does not contain any studies with human or animal subjects performed by any of the authors.

**Figures:**



**Figure 1: Fate and regulation of periosteal stem/progenitor cells during bone repair**

After fracture, periosteal stem/progenitor cells (pSSPC), labelled by several markers (orange box) migrate to the site of injury, proliferate and differentiate into chondrocytes or osteoblasts. These steps are controlled by several signaling pathways (activation in green and inhibition in red). The fate of pSSPCs is also influenced by their interactions with the fracture environment, including the blood clot, nerve fibers, blood vessels and macrophages (left box).

**Table 1.: Markers and mouse lines labeling pSSPCs**

Markers	Mouse model	Injury model	Contribution	Comment	Used in
PRX1	<i>Prx1<sup>Cre</sup></i>	Non stabilized tibial fracture	Cartilage / Bone	Labels all SSPCs	Duchamp et al. <sup>20</sup>
	<i>Prx1<sup>CreERT</sup></i>	Non stabilized ulna fracture	Cartilage / Bone	Low cell labelling	Kawanami et al. <sup>46</sup>
PDGFR $\alpha$	<i>Pdgfra<sup>CreERT</sup></i>	Non stabilized tibial fracture	Bone / Cartilage	Labels mesenchymal cells from various tissues	Julien et al. <sup>25</sup>
		Non stabilized forelimb fracture	Bone / Cartilage		Xu et al. <sup>47</sup>
CTSK	<i>Ctsk<sup>Cre</sup></i>	Semi stabilized femoral fracture	Bone / Cartilage	Marks a subpopulation of pSSPCs and osteoclasts	Debnath et al. <sup>22</sup>
		Calvarial cortical defect	Bone		
$\alpha$ SMA	<i><math>\alpha</math>SMA<sup>CreERT</sup></i>	Semi stabilized tibial fracture	Bone / Cartilage		Matthews et al. <sup>24</sup>
	<i><math>\alpha</math>SMA-GFP</i>	Calvarial cortical defect	Bone		Ortinou et al. <sup>51</sup>
MX1	<i>Mx1<sup>Cre</sup></i>	Non stabilized tibial fracture	Bone / Cartilage	Requires plpC injection to induce Cre recombination	Ortinou et al. <sup>51</sup>
		Tibial periosteal defect	Bone		
		Tibial cortical defect	Bone		
		Calvarial cortical defect	Bone		
Axin2	<i>Axin2<sup>CreERT</sup></i>	Tibial cortical defect	Bone		Ransom et al. <sup>52</sup>
PDGFR $\beta$	<i>Pdgfrb<sup>Cre</sup></i>	Semi stabilized femoral fracture	Bone / Cartilage		Bohm et al. <sup>53</sup>
HOX11	<i>Hoxa11<sup>CreERT2</sup></i>	Non stabilized ulnar fracture	Bone / Cartilage	Restricted to zeugopod bone	Pineault et al. <sup>54</sup>
GLI1	<i>Gli1<sup>CreERT1</sup></i>	Semi stabilized femoral fracture	Bone / Cartilage	Marks bone marrow SSPCs	Shi et al. <sup>56</sup>
		Semi stabilized tibial fracture	Bone / Cartilage		Xia et al. <sup>57</sup>
Nestin	<i>Nes-GFP</i>	Semi stabilized tibial fracture	Bone	Marks bone marrow SSPCs	Tournaire et al. <sup>55</sup>
	<i>Nes<sup>CreERT</sup></i>	Tibial cortical defect	Bone	Marks bone marrow SSPCs	Gao et al. <sup>42</sup>
LEPR	<i>Lepr<sup>Cre</sup></i>	Tibial cortical defect	Bone	Marks bone marrow SSPCs	Gao et al. <sup>42</sup>
SOX9	<i>Sox9<sup>CreERT</sup></i>	Semi stabilized femoral fracture	Bone / Cartilage		He et al. <sup>58</sup>
		Rib bone resection	Bone / Cartilage	Labelling is increased when induced after fracture	Kuwahara et al. <sup>17</sup>
OSX	<i>Osx<sup>Cre</sup></i>	Semi stabilized tibial fracture	Bone / Cartilage	Labels osteogenic progenitors	Bohm et al. <sup>53</sup>

## References

1. Chartier SR, Mitchell SAT, Majuta LA, Mantyh PW (2018) The Changing Sensory and Sympathetic Innervation of the Young, Adult and Aging Mouse Femur. *Neuroscience* 387:178–190
2. Lorenz MR, Brazill JM, Beeve AT, Shen I, Scheller EL (2021) A Neuroskeletal Atlas: Spatial Mapping and Contextualization of Axon Subtypes Innervating the Long Bones of C3H and B6 Mice. *J Bone Miner Res* 36:1012–1025
3. Alexander KA, Raggatt L, Millard S, Batoon L, Chiu-Ku Wu A, Chang M, Hume DA, Pettit AR (2017) Resting and injury-induced inflamed periosteum contain multiple macrophage subsets that are located at sites of bone growth and regeneration. *Immunol Cell Biol* 95:7–16
4. Colnot C, Lu C, Hu D, Helms JA (2004) Distinguishing the contributions of the perichondrium, cartilage, and vascular endothelium to skeletal development. *Dev Biol* 269:55–69
5. Akiyama H, Kim J-E, Nakashima K, et al (2005) Osteo-chondroprogenitor cells are derived from Sox9 expressing precursors. *Proc Natl Acad Sci U S A* 102:14665–14670
6. Kaneko S, Matsushita M, Mishima K, Takegami Y, Imagama S, Kitoh H (2020) Effect of periosteal resection on longitudinal bone growth in a mouse model of achondroplasia. *Bone Reports* 13:100708
7. Watanabe-Takano H, Ochi H, Chiba A, et al (2021) Mechanical load regulates bone growth via periosteal Osteocrin. *Cell Reports* 36:109380
8. Moore ER, Zhu YX, Ryu HS, Jacobs CR (2018) Periosteal progenitors contribute to load-induced bone formation in adult mice and require primary cilia to sense mechanical stimulation. *Stem Cell Res Ther* 9:190
9. Zannit HM, Silva MJ (2019) Proliferation and Activation of Osterix-Lineage Cells Contribute to Loading-Induced Periosteal Bone Formation in Mice. *JBMR Plus*. <https://doi.org/10.1002/jbm4.10227>
10. Cabahug-Zuckerman P, Liu C, Cai C, Mahaffey I, Norman SC, Cole W, Castillo AB (2019) Site-Specific Load-Induced Expansion of Sca-1<sup>+</sup> Prrx1<sup>+</sup> and Sca-1<sup>-</sup> Prrx1<sup>+</sup> Cells in Adult Mouse Long Bone Is Attenuated With Age. *JBMR Plus*. <https://doi.org/10.1002/jbm4.10199>
11. Watson A (1845) Observations on the Formation of Bone by the Periosteum. *Edinb Med Surg J* 63:302–307
12. Sales de Gauzy J, Fitoussi F, Jouve J-L, Karger C, Badina A, Masquelet A-C (2012) Traumatic diaphyseal bone defects in children. *Orthopaedics & Traumatology: Surgery & Research* 98:220–226
13. Garcia P, Holstein JH, Maier S, Schaumlöffel H, Al-Marrawi F, Hannig M, Pohlemann T, Menger MD (2008) Development of a Reliable Non-Union Model in Mice. *Journal of Surgical Research* 147:84–91
14. Wu X-Q, Wang D, Liu Y, Zhou J-L (2021) Development of a tibial experimental non-union model in rats. *J Orthop Surg Res* 16:261
15. Sharun K, Pawde AM, Banu S A, Manjusha KM, Kalaiselvan E, Kumar R, Kinjavdekar P, Amarpal (2021) Development of a novel atrophic non-union model in rabbits: A preliminary study. *Annals of Medicine and Surgery* 68:102558
16. Gröngröft I, Wissing S, Meesters DM, Poeze M, Matthys-Mark R, Ito K, Zeiter S (2019) Development of a novel murine delayed secondary fracture healing in vivo model using periosteal cauterization. *Arch Orthop Trauma Surg* 139:1743–1753
17. Kuwahara ST, Serowoky MA, Vakhshori V, Tripuraneni N, Hegde NV, Lieberman JR, Crump JG, Mariani FV (2019) Sox9<sup>+</sup> messenger cells orchestrate large-scale skeletal

regeneration in the mammalian rib. *eLife* 8:e40715

18. Colnot C (2009) Skeletal Cell Fate Decisions Within Periosteum and Bone Marrow During Bone Regeneration. *Journal of Bone and Mineral Research* 24:274–282

19. Murao H, Yamamoto K, Matsuda S, Akiyama H (2013) Periosteal cells are a major source of soft callus in bone fracture. *J Bone Miner Metab* 31:390–398

20. ●● Duchamp de Lageneste O, Julien A, Abou-Khalil R, Frangi G, Carvalho C, Cagnard N, Cordier C, Conway SJ, Colnot C (2018) Periosteum contains skeletal stem cells with high bone regenerative potential controlled by Periostin. *Nat Commun* 9:773

**This study provides the first evidence of the presence of skeletal stem cells within the periosteum and their enhance contribution to bone repair compared to bone marrow stromal cells.**

21. Julien A, Kanagalingam A, Martínez-Sarrà E, Megret J, Luka M, Ménager M, Relaix F, Colnot C (2021) Direct contribution of skeletal muscle mesenchymal progenitors to bone repair. *Nature Communications* 12:2860

22. ●● Debnath S, Yallowitz AR, McCormick J, et al (2018) Discovery of a periosteal stem cell mediating intramembranous bone formation. *Nature* 562:133–139

**This study identifies CTSK as a marker of pSSPCs supporting intramembranous ossification.**

23. Matsushita Y, Nagata M, Kozloff KM, et al (2020) A Wnt-mediated transformation of the bone marrow stromal cell identity orchestrates skeletal regeneration. *Nat Commun* 11:332

24. ● Matthews BG, Novak S, Sbrana FV, Funnell JL, Cao Y, Buckels EJ, Grcevic D, Kalajzic I (2021) Heterogeneity of murine periosteum progenitors involved in fracture healing. *eLife* 10:e58534

**This study characterizes the  $\alpha$ SMA+ population within the periosteum.**

25. ●● Julien A, Perrin S, Martínez-Sarrà E, Kanagalingam A, Carvalho C, Luka M, Ménager M, Colnot C (2022) Skeletal stem/progenitor cells in periosteum and skeletal muscle share a common molecular response to bone injury. *J of Bone & Mineral Res jbmr*.4616

**This study uses single cell transcriptomics to describe how pSSPCs respond to bone injury.**

26. Roberts SJ, van Gastel N, Carmeliet G, Luyten FP (2015) Uncovering the periosteum for skeletal regeneration: the stem cell that lies beneath. *Bone* 70:10–18

27. van Gastel N, Torrekens S, Roberts SJ, Moermans K, Schrooten J, Carmeliet P, Luttun A, Luyten FP, Carmeliet G (2012) Engineering vascularized bone: osteogenic and proangiogenic potential of murine periosteal cells. *Stem Cells* 30:2460–2471

28. Perrin S, Julien A, Duchamp de Lageneste O, Abou-Khalil R, Colnot C (2021) Mouse Periosteal Cell Culture, in vitro Differentiation, and in vivo Transplantation in Tibial Fractures. *BIO-PROTOCOL*. <https://doi.org/10.21769/BioProtoc.4107>

29. Declercq HA, De Ridder LI, Cornelissen MJ (2005) Isolation and Osteogenic Differentiation of Rat Periosteum-derived Cells. *Cytotechnology* 49:39–50

30. Wang Q, Huang C, Zeng F, Xue M, Zhang X (2010) Activation of the Hh Pathway in Periosteum-Derived Mesenchymal Stem Cells Induces Bone Formation in Vivo. *The American Journal of Pathology* 177:3100–3111

31. Bianco P (2014) “Mesenchymal” stem cells. *Annu Rev Cell Dev Biol* 30:677–704

32. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8:315–317

33. Sacchetti B, Funari A, Michienzi S, et al (2007) Self-Renewing Osteoprogenitors in Bone Marrow Sinusoids Can Organize a Hematopoietic Microenvironment. *Cell* 131:324–336

34. De Bari C, Dell’Accio F, Vanlauwe J, et al (2006) Mesenchymal multipotency of adult human periosteal cells demonstrated by single-cell lineage analysis. *Arthritis Rheum* 54:1209–1221

35. Stich S, Loch A, Park S-J, Häupl T, Ringe J, Sittinger M (2017) Characterization of single cell derived cultures of periosteal progenitor cells to ensure the cell quality for clinical application. *PLoS ONE* 12:e0178560
36. Chan CKF, Seo EY, Chen JY, et al (2015) Identification and Specification of the Mouse Skeletal Stem Cell. *Cell* 160:285–298
37. ● Julien A, Perrin S, Duchamp de Lageneste O, Carvalho C, Bensidhoum M, Legeai-Mallet L, Colnot C (2020) FGFR3 in Periosteal Cells Drives Cartilage-to-Bone Transformation in Bone Repair. *Stem Cell Reports* 15:955–967  
**This study reveals the important role of periosteum-derived chondrocyte transdifferentiation into osteoblasts during bone healing.**
38. Groeneveldt LC, Herpelinck T, Maréchal M, Politis C, van IJcken WFJ, Huylebroeck D, Geris L, Mulugeta E, Luyten FP (2020) The Bone-Forming Properties of Periosteum-Derived Cells Differ Between Harvest Sites. *Front Cell Dev Biol* 8:554984
39. Choi Y-S, Noh S-E, Lim S-M, Lee C-W, Kim C-S, Im M-W, Lee M-H, Kim D-I (2008) Multipotency and growth characteristic of periosteum-derived progenitor cells for chondrogenic, osteogenic, and adipogenic differentiation. *Biotechnol Lett* 30:593–601
40. Isogai N, Landis WJ, Mori R, Gotoh Y, Gerstenfeld LC, Upton J, Vacanti JP (2000) Experimental use of fibrin glue to induce site-directed osteogenesis from cultured periosteal cells. *Plast Reconstr Surg* 105:953–963
41. Eyckmans J, Luyten FP (2006) Species specificity of ectopic bone formation using periosteum-derived mesenchymal progenitor cells. *Tissue Eng* 12:2203–2213
42. Gao B, Deng R, Chai Y, et al (2019) Macrophage-lineage TRAP+ cells recruit periosteum-derived cells for periosteal osteogenesis and regeneration. *Journal of Clinical Investigation* 129:2578–2594
43. ●● van Gastel N, Stegen S, Eelen G, et al (2020) Lipid availability determines fate of skeletal progenitor cells via SOX9. *Nature* 579:111–117  
**This study highlights the role of revascularization and the involvement of cell metabolism in pSSPCs fate decision.**
44. Zhou X, von der Mark K, Henry S, Norton W, Adams H, de Crombrughe B (2014) Chondrocytes Transdifferentiate into Osteoblasts in Endochondral Bone during Development, Postnatal Growth and Fracture Healing in Mice. *PLoS Genet* 10:e1004820
45. Hu DP, Ferro F, Yang F, Taylor AJ, Chang W, Miclau T, Marcucio RS, Bahney CS (2017) Cartilage to bone transformation during fracture healing is coordinated by the invading vasculature and induction of the core pluripotency genes. *Development* 144:221–234
46. Kawanami A, Matsushita T, Chan YY, Murakami S (2009) Mice expressing GFP and CreER in osteochondro progenitor cells in the periosteum. *Biochemical and Biophysical Research Communications* 386:477–482
47. Xu J, Wang Y, Li Z, et al (2022) PDGFR $\alpha$  reporter activity identifies periosteal progenitor cells critical for bone formation and fracture repair. *Bone Res* 10:7
48. Uezumi A, Ikemoto-Uezumi M, Tsuchida K (2014) Roles of nonmyogenic mesenchymal progenitors in pathogenesis and regeneration of skeletal muscle. *Front Physiol* 5:68
49. O'Rourke M, Cullen CL, Auderset L, Pitman KA, Achatz D, Gasperini R, Young KM (2016) Evaluating Tissue-Specific Recombination in a Pdgfr $\alpha$ -CreERT2 Transgenic Mouse Line. *PLoS ONE* 11:e0162858
50. Matthews BG, Grcevic D, Wang L, Hagiwara Y, Roguljic H, Joshi P, Shin D-G, Adams DJ, Kalajzic I (2014) Analysis of  $\alpha$ SMA-Labeled Progenitor Cell Commitment Identifies Notch Signaling as an Important Pathway in Fracture Healing. *J Bone Miner Res* 29:1283–1294

51. ●● Ortinau LC, Wang H, Lei K, et al (2019) Identification of Functionally Distinct Mx1+ $\alpha$ SMA+ Periosteal Skeletal Stem Cells. *Cell Stem Cell* 25:784-796.e5  
**This study identifies  $\alpha$ SMA+ Mx1+ cells as a population of pSSPCs required for bone healing.**
52. Ransom RC, Hunter DJ, Hyman S, et al (2016) Axin2-expressing cells execute regeneration after skeletal injury. *Sci Rep* 6:36524
53. Böhm A-M, Dirckx N, Tower RJ, et al (2019) Activation of Skeletal Stem and Progenitor Cells for Bone Regeneration Is Driven by PDGFR $\beta$  Signaling. *Developmental Cell* 51:236-254.e12
54. Pineault KM, Song JY, Kozloff KM, Lucas D, Wellik DM (2019) Hox11 expressing regional skeletal stem cells are progenitors for osteoblasts, chondrocytes and adipocytes throughout life. *Nat Commun* 10:3168
55. Tournaire G, Stegen S, Giacomini G, Stockmans I, Moermans K, Carmeliet G, van Gestel N (2020) Nestin-GFP transgene labels skeletal progenitors in the periosteum. *Bone* 133:115259
56. Shi Y, He G, Lee W-C, McKenzie JA, Silva MJ, Long F (2017) Gli1 identifies osteogenic progenitors for bone formation and fracture repair. *Nat Commun* 8:2043
57. Xia C, Ge Q, Fang L, et al (2020) TGF- $\beta$ /Smad2 signalling regulates enchondral bone formation of Gli1<sup>+</sup> periosteal cells during fracture healing. *Cell Prolif*.  
<https://doi.org/10.1111/cpr.12904>
58. He X, Bougioukli S, Ortega B, Arevalo E, Lieberman JR, McMahon AP (2017) Sox9 positive periosteal cells in fracture repair of the adult mammalian long bone. *Bone* 103:12–19
59. Kramann R, Schneider RK, DiRocco DP, Machado F, Fleig S, Bondzie PA, Henderson JM, Ebert BL, Humphreys BD (2015) Perivascular Gli1+ Progenitors Are Key Contributors to Injury-Induced Organ Fibrosis. *Cell Stem Cell* 16:51–66
60. Yu YY, Lieu S, Lu C, Miclau T, Marcucio RS, Colnot C (2010) Immunolocalization of BMPs, BMP antagonists, receptors, and effectors during fracture repair. *Bone* 46:841–851
61. Tsuji K, Cox K, Bandyopadhyay A, Harfe BD, Tabin CJ, Rosen V (2008) BMP4 Is Dispensable for Skeletogenesis and Fracture-Healing in the Limb. *Journal of Bone and Joint Surgery* 90:14–18
62. Tsuji K, Cox K, Gamer L, Graf D, Economides A, Rosen V (2010) Conditional deletion of BMP7 from the limb skeleton does not affect bone formation or fracture repair. *J Orthop Res* 28:384–389
63. Salazar VS, Capelo LP, Cantù C, et al (2019) Reactivation of a developmental Bmp2 signaling center is required for therapeutic control of the murine periosteal niche. *eLife* 8:e42386
64. Chappuis V, Gamer L, Cox K, Lowery JW, Bosshardt DD, Rosen V (2012) Periosteal BMP2 activity drives bone graft healing. *Bone* 51:800–809
65. Tsuji K, Bandyopadhyay A, Harfe BD, Cox K, Kakar S, Gerstenfeld L, Einhorn T, Tabin CJ, Rosen V (2006) BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. *Nat Genet* 38:1424–1429
66. Wang Q, Huang C, Xue M, Zhang X (2011) Expression of endogenous BMP-2 in periosteal progenitor cells is essential for bone healing. *Bone* 48:524–532
67. Wang C, Inzana JA, Mirando AJ, Ren Y, Liu Z, Shen J, O’Keefe RJ, Awad HA, Hilton MJ (2016) NOTCH signaling in skeletal progenitors is critical for fracture repair. *Journal of Clinical Investigation* 126:1471–1481
68. Zhong N, Gersch RP, Hadjiargyrou M (2006) Wnt signaling activation during bone regeneration and the role of Dishevelled in chondrocyte proliferation and differentiation. *Bone* 39:5–16
69. Kim J-B, Leucht P, Lam K, Luppen C, Ten Berge D, Nusse R, Helms JA (2007) Bone regeneration is regulated by wnt signaling. *J Bone Miner Res* 22:1913–1923



70. Novak S, Roeder E, Sinder BP, Adams DJ, Siebel CW, Grcevic D, Hankenson KD, Matthews BG, Kalajzic I (2020) Modulation of Notch1 signaling regulates bone fracture healing. *J Orthop Res* 38:2350–2361
71. Lee S, Remark LH, Josephson AM, et al (2021) Notch-Wnt signal crosstalk regulates proliferation and differentiation of osteoprogenitor cells during intramembranous bone healing. *npj Regen Med* 6:29
72. Minear S, Leucht P, Miller S, Helms JA (2010) rBMP represses Wnt signaling and influences skeletal progenitor cell fate specification during bone repair. *J Bone Miner Res* 25:1196–1207
73. Collette NM, Yee CS, Hum NR, Murugesu DK, Christiansen BA, Xie L, Economides AN, Manilay JO, Robling AG, Loots GG (2016) *Sostdc1* deficiency accelerates fracture healing by promoting the expansion of periosteal mesenchymal stem cells. *Bone* 88:20–30
74. Schmid GJ, Kobayashi C, Sandell LJ, Ornitz DM (2009) Fibroblast growth factor expression during skeletal fracture healing in mice. *Dev Dyn* 238:766–774
75. Nakajima F, Nakajima A, Ogasawara A, Moriya H, Yamazaki M (2007) Effects of a Single Percutaneous Injection of Basic Fibroblast Growth Factor on the Healing of a Closed Femoral Shaft Fracture in the Rat. *Calcif Tissue Int* 81:132–138
76. van Gastel N, Stegen S, Stockmans I, Moermans K, Schrooten J, Graf D, Luyten FP, Carmeliet G (2014) Expansion of Murine Periosteal Progenitor Cells with Fibroblast Growth Factor 2 Reveals an Intrinsic Endochondral Ossification Program Mediated by Bone Morphogenetic Protein 2. *Stem Cells* 32:2407–2418
77. Moore ER, Mathews OA, Yao Y, Yang Y (2021) *Prx1*-expressing cells contributing to fracture repair require primary cilia for complete healing in mice. *Bone* 143:115738
78. Wang Q, Huang C, Zeng F, Xue M, Zhang X (2010) Activation of the Hh pathway in periosteum-derived mesenchymal stem cells induces bone formation in vivo: implication for postnatal bone repair. *Am J Pathol* 177:3100–3111
79. Huang C, Tang M, Yehling E, Zhang X (2014) Overexpressing Sonic Hedgehog Peptide Restores Periosteal Bone Formation in a Murine Bone Allograft Transplantation Model. *Molecular Therapy* 22:430–439
80. Miyaji T, Nakase T, Iwasaki M, Kuriyama K, Tamai N, Higuchi C, Myoui A, Tomita T, Yoshikawa H (2003) Expression and distribution of transcripts for sonic hedgehog in the early phase of fracture repair. *Histochem Cell Biol* 119:233–237
81. Doherty L, Yu J, Wang X, Hankenson KD, Kalajzic I, Sanjay A (2019) A PDGFR $\beta$ -PI3K signaling axis mediates periosteal cell activation during fracture healing. *PLoS ONE* 14:e0223846
82. Wang X, Matthews BG, Yu J, Novak S, Grcevic D, Sanjay A, Kalajzic I (2019) PDGF Modulates BMP2-Induced Osteogenesis in Periosteal Progenitor Cells. *JBMR Plus* 3:e10127
83. Huang C, Xue M, Chen H, Jiao J, Herschman HR, O’Keefe RJ, Zhang X (2014) The Spatiotemporal Role of COX-2 in Osteogenic and Chondrogenic Differentiation of Periosteum-Derived Mesenchymal Progenitors in Fracture Repair. *PLoS ONE* 9:e100079
84. Xie C, Ming X, Wang Q, Schwarz EM, Guldberg RE, O’Keefe RJ, Zhang X (2008) COX-2 from the injury milieu is critical for the initiation of periosteal progenitor cell mediated bone healing. *Bone* 43:1075–1083
85. Yukata K, Xie C, Li T-F, et al (2018) Teriparatide (human PTH1–34) compensates for impaired fracture healing in COX-2 deficient mice. *Bone* 110:150–159
86. Bravo D, Josephson AM, Bradaschia-Correa V, et al (2018) Temporary inhibition of the plasminogen activator inhibits periosteal chondrogenesis and promotes periosteal osteogenesis during appendicular bone fracture healing. *Bone* 112:97–106
87. Wang L, Yao L, Duan H, et al (2021) Plasminogen Regulates Fracture Repair by Promoting the Functions of Periosteal Mesenchymal Progenitors. *J Bone Miner Res* 36:2229–

88. Lu C, Saless N, Hu D, et al (2011) Mechanical Stability Affects Angiogenesis During Early Fracture Healing. *Journal of Orthopaedic Trauma* 25:494–499
89. Stegen S, Deprez S, Eelen G, Torrekens S, Van Looveren R, Goveia J, Ghesquière B, Carmeliet P, Carmeliet G (2016) Adequate hypoxia inducible factor 1 $\alpha$  signaling is indispensable for bone regeneration. *Bone* 87:176–186
90. Andrés Sastre E, Maly K, Zhu M, et al (2021) Spatiotemporal distribution of thrombospondin-4 and -5 in cartilage during endochondral bone formation and repair. *Bone* 150:115999
91. Street J, Bao M, deGuzman L, et al (2002) Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. *Proc Natl Acad Sci U S A* 99:9656–9661
92. Raggatt LJ, Wullschlegel ME, Alexander KA, Wu ACK, Millard SM, Kaur S, Maugham ML, Gregory LS, Steck R, Pettit AR (2014) Fracture Healing via Periosteal Callus Formation Requires Macrophages for Both Initiation and Progression of Early Endochondral Ossification. *The American Journal of Pathology* 184:3192–3204
93. Ishikawa M, Ito H, Kitaori T, Murata K, Shibuya H, Furu M, Yoshitomi H, Fujii T, Yamamoto K, Matsuda S (2014) MCP/CCR2 Signaling Is Essential for Recruitment of Mesenchymal Progenitor Cells during the Early Phase of Fracture Healing. *PLoS ONE* 9:e104954
94. Xing Z, Lu C, Hu D, Yu Y, Wang X, Colnot C, Nakamura M, Wu Y, Miclau T, Marcucio RS (2010) Multiple roles for CCR2 during fracture healing. *Disease Models & Mechanisms* 3:451–458
95. He F, Umrath F, Reinert S, Alexander D (2021) Jaw Periosteum-Derived Mesenchymal Stem Cells Regulate THP-1-Derived Macrophage Polarization. *IJMS* 22:4310
96. He F, Umrath F, von Ohle C, Reinert S, Alexander D (2021) Analysis of the Influence of Jaw Periosteal Cells on Macrophages Phenotype Using an Innovative Horizontal Coculture System. *Biomedicines* 9:1753
97. Li Z, Meyers CA, Chang L, Lee S, Li Z, Tomlinson R, Hoke A, Clemens TL, James AW (2019) Fracture repair requires TrkA signaling by skeletal sensory nerves. *Journal of Clinical Investigation* 129:5137–5150
98. Zhang Y, Xu J, Ruan YC, et al (2016) Implant-derived magnesium induces local neuronal production of CGRP to improve bone-fracture healing in rats. *Nat Med* 22:1160–1169
99. Doherty L, Wan M, Kalajzic I, Sanjay A (2021) Diabetes impairs periosteal progenitor regenerative potential. *Bone* 143:115764
100. Marin C, Tuts J, Luyten FP, Vandamme K, Kerckhofs G (2021) Impaired soft and hard callus formation during fracture healing in diet-induced obese mice as revealed by 3D contrast-enhanced computed tomography imaging. *Bone* 150:116008
101. Brown ML, Yukata K, Farnsworth CW, Chen D-G, Awad H, Hilton MJ, O’Keefe RJ, Xing L, Mooney RA, Zuscik MJ (2014) Delayed Fracture Healing and Increased Callus Adiposity in a C57BL/6J Murine Model of Obesity-Associated Type 2 Diabetes Mellitus. *PLoS ONE* 9:e99656
102. Lu C, Miclau T, Hu D, Hansen E, Tsui K, Puttlitz C, Marcucio RS (2005) Cellular basis for age-related changes in fracture repair. *J Orthop Res* 23:1300–1307
103. Clark D, Nakamura M, Miclau T, Marcucio R (2017) Effects of Aging on Fracture Healing. *Curr Osteoporos Rep* 15:601–608
104. O’Driscoll SWM, Saris DBF, Ito Y, Fitzimmons JS (2001) The chondrogenic potential of periosteum decreases with age. *J Orthop Res* 19:95–103
105. Yukata K, Xie C, Li T-F, et al (2014) Aging periosteal progenitor cells have reduced

regenerative responsiveness to bone injury and to the anabolic actions of PTH 1-34 treatment. *Bone* 62:79–89

106. Demontiero O, Vidal C, Duque G (2012) Aging and bone loss: new insights for the clinician. *Therapeutic Advances in Musculoskeletal* 4:61–76

107. Zhang H, Shi X, Wang L, et al (2018) Intramembranous ossification and endochondral ossification are impaired differently between glucocorticoid-induced osteoporosis and estrogen deficiency-induced osteoporosis. *Sci Rep* 8:3867

108. Barastegui D, Gallardo-Calero I, Rodriguez-Carunchio L, Barrera-Ochoa S, Knorr J, Rivas-Nicolls D, Soldado F (2021) Effect of vascularized periosteum on revitalization of massive bone isografts: An experimental study in a rabbit model. *Microsurgery* 41:157–164

109. Harhaus L, Huang J-J, Kao S-W, Wu Y-L, Mackert GA, Höner B, Cheng M-H, Kneser U, Cheng C-M (2015) The vascularized periosteum flap as novel tissue engineering model for repair of cartilage defects. *J Cell Mol Med* 19:1273–1283

110. Abed PF, El Chaar E, Boltchi F, Bassir SH (2020) The Novel Periosteal Flap Stretch Technique: A Predictable Method to Achieve and maintain Primary Closure in Augmentative Procedures. *J Int Acad Periodontol* 22:11–20

111. Hassibi H, Farsinejad A, Dabiri S, Vosough D, Mortezaeizadeh A, Kheirandish R, Azari O (2020) Allogenic Bone Graft Enriched by Periosteal Stem Cell and Growth Factors for Osteogenesis in Critical Size Bone Defect in Rabbit Model: Histopathological and Radiological Evaluation. *Iran J Pathol* 15:205–216

112. Amler A-K, Dinkelborg PH, Schlauch D, et al (2021) Comparison of the Translational Potential of Human Mesenchymal Progenitor Cells from Different Bone Entities for Autologous 3D Bioprinted Bone Grafts. *IJMS* 22:796

113. Ho-Shui-Ling A, Bolander J, Rustom LE, Johnson AW, Luyten FP, Picart C (2018) Bone regeneration strategies: Engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives. *Biomaterials* 180:143–162

114. Bolander J, Herpelinck T, Chaklader M, Gklava C, Geris L, Luyten FP (2020) Single-cell characterization and metabolic profiling of in vitro cultured human skeletal progenitors with enhanced in vivo bone forming capacity. *Stem Cells Translational Medicine* 9:389–402

115. Bolander J, Ji W, Leijten J, Teixeira LM, Bloemen V, Lambrechts D, Chaklader M, Luyten FP (2017) Healing of a Large Long-Bone Defect through Serum-Free In Vitro Priming of Human Periosteum-Derived Cells. *Stem Cell Reports* 8:758–772

116. Dai K, Deng S, Yu Y, Zhu F, Wang J, Liu C (2022) Construction of developmentally inspired periosteum-like tissue for bone regeneration. *Bone Res* 10:1

117. Wu L, Gu Y, Liu L, et al (2020) Hierarchical micro/nanofibrous membranes of sustained releasing VEGF for periosteal regeneration. *Biomaterials* 227:119555