TOPIC HIGHLIGHT

WJSC 6ᵗʰ Anniversary Special Issues (2): Mesenchymal stem cells

Periosteum derived stem cells for regenerative medicine proposals: Boosting current knowledge

Concetta Ferretti, Monica Mattioli-Belmonte

Concetta Ferretti, Monica Mattioli-Belmonte, Department of Clinical and Molecular Sciences, School of Medicine, Università Politecnica delle Marche, 60126 Ancona, Italy
Author contributions: Ferretti C and Mattioli-Belmonte M equally contributed to conception and acquisition of data as well as to article drafting and revising.
Supported by Italian FIRB and PRIN project grants, No. 2010J8RYS7 and No. RBAP10MLK7
Correspondence to: Dr. Monica Mattioli-Belmonte, Department of Clinical and Molecular Sciences, School of Medicine, Università Politecnica delle Marche, Via Tronto 10/A, 60126 Ancona, Italy. m.mattioli@univpm.it
Telephone: +39-71-2206077 Fax: +39-71-2206073
Received: October 25, 2013 Revised: January 9, 2014
Accepted: April 25, 2014
Published online: July 26, 2014

Abstract

Periosteum is a thin fibrous layer that covers most bones. It resides in a dynamic mechanically loaded environment and provides a niche for pluripotent cells and a source for molecular factors that modulate cell behaviour. Elucidating periosteum regenerative potential has become a hot topic in orthopaedics. This review discusses the state of the art of osteochondral tissue engineering rested on periosteum derived progenitor cells (PDPCs) and suggests upcoming research directions. Periosteal cells isolation, characterization and migration in the site of injury, as well as their differentiation, are analysed. Moreover, the role of cell mechanosensing and its contribution to matrix organization, bone microarchitecture and bone stenght is examined. In this regard the role of periostin and its upregulation under mechanical stress in order to preserve PDPC survival and bone tissue integrity is contemplated. The review also summarized the role of the periosteum in the field of dentistry and maxillofacial reconstruction. The involvement of microRNAs in osteoblast differentiation and in endogenous tissue repair is explored as well. Finally the novel concept of a guided bone regeneration based on the use of periosteum itself as a smart material and the realization of constructs able to mimic the extracellular matrix features is talked out. Additionally, since periosteum can differentiate into insulin producing cells it could be a suitable source in allogenic transplantations. That innovative applications would take advantage from investigations aimed to assess PDPC immune privilege.

Core tip: Periosteum provides a niche for pluripotent cells. Elucidating periosteum regenerative potential is a hot topic in orthopaedics. This review discusses the state of the art of osteochondral tissue engineering rested on periosteum derived cells and suggests upcoming research directions aimed to the development of new standards of care for the maintenance of bone mass both in post-trauma healing process and in physiological turn-over.

Ferretti C, Mattioli-Belmonte M. Periosteum derived stem cells for regenerative medicine proposals: Boosting current knowledge. *World J Stem Cells* 2014; 6(3): 266-277 Available from: URL: http://www.wjgnet.com/1948-0210/full/v6/i3/266.htm DOI: http://dx.doi.org/10.4252/wjsc.v6.i3.266

INTRODUCTION

The field of Tissue Engineering and Regenerative Medicine (TERM) has burgeoned in the last decade. The term "Regenerative Medicine" was first found in a 1992 Kaiser *et al*[1] paper as "a new branch of medicine that

attempts to change the course of chronic disease and in many instances will regenerate tired and failing organ systems".

Products for regenerative medicine can consist in proteins, able to stimulate endogenous repair, living cells or even organs. Advances in regenerative medicine applications have been useful to develop new standards of care for the treatment of several diseases such as neurological, cardiovascular, metabolic (*e.g.,* diabetes), oncologic and orthopaedic disorders.

The idea of using cells to restore damaged tissue is intuitively based on their native role in tissue development and homeostasis. Cells could be delivered to the patient alone or combined with a natural or synthetic biomaterial. The interactive "diamond" concept of TERM suggests that in addition to cell type, 3D dimensional structure/architecture, mechanical/physical signals, and bioactive factors in the environment are critical and act in concert to direct tissue repair and regeneration[2]. Each of those areas is currently under dynamic investigation. In this review we will focus on cell-based therapeutic applications in skeletal tissue repair.

Mesenchymal stem cells (MSCs) represent the leading cell type for regenerative medicine purposes. They are multipotent stromal cells capable of both self-renewal and differentiation into lineages of mesenchymal tissue, including cartilage, bone, adipose tissue and skeletal muscle[3]. MSCs were originally identified in the bone marrow stroma, where they regulate key stages of haematopoiesis. Ever since, they have been isolated from other anatomical sites, such as amniotic fluid[4], Wharton's jelly[5], umbilical cord blood[6], adipose tissue[7], skin[8], synovial membrane[9], articular cartilage[10] and compact bone[11].

The main challenge in osteochondral tissue repair is the healing of critical-size defects that don't bridge on their own. They result from pathological events (*e.g.,* tumour, trauma, inflammation or congenital malformation) and can be lead to a delayed union or non-union fracture[12]. Surgical procedures employed for bone gaps treatment may be time-consuming, expensive and exposing patients to high risk of complications and discomfort[13]. To overcome these issues regenerative medicine is working to restore structure and function of damaged tissues by TERM approaches.

Since bone marrow contains osteogenic progenitors, its use was proposed to lead efficient bone regeneration and, effectively, preclinical and clinical investigations corroborated this speculation[14]. Periosteum has been identified as an intriguing niche for cells of the osteoblastic lineage as well.

Periosteum is a specialized highly vascularized connective tissue that envelopes bone surfaces (Figure 1). It is composed of an external fibrous layer containing elastic fibres and microvessels and an inner cambium layer where reside periosteum derived progenitor cells (PDPCs) that act as major players in bone development and fracture healing[13,15].
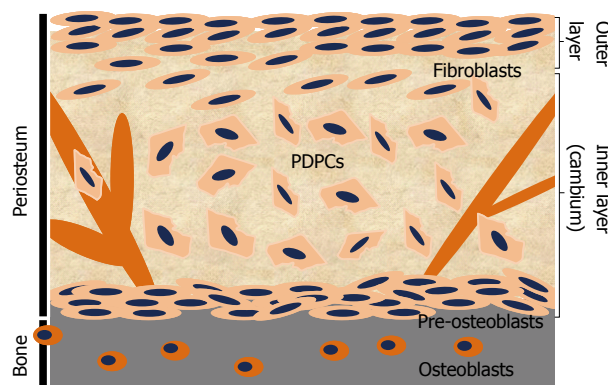

Figure 1 Schematic representation of periosteum as well as the distribution of cell populations and extracellular matrix that contribute to its biological and mechanical properties. PDPCs: Periosteum-derived precursor cells.

REGENERATIVE POTENTIAL OF PERIOSTEUM

The paramount importance of the periosteum in bone healing process was suggested since 1800 s when de Mourgues[16] discovered that transplanted periosteal tissue induced new bone growth. In 1932, Fell[17] was the first to successfully culture periosteum and in 1990s Nakahara *et al*[18] explored the osteogenic potential of PDPCs in bone tissue engineering. At the same time O'Driscoll *et al*[19] underlined the possibility to regenerate cartilage in damaged joints by periosteum transplantation.

The use of autologous periosteum graft has long been known in orthopaedic surgery. However, it's only after recent progresses that the contribution of the different sources of MSCs in bone repair, as well as their response to growth factors favouring specific differentiation processes has been examined in depth.

Periosteum as a whole have been used in thousands of orthopaedic surgeries as covering layer in autologous chondrocyte transplantation[20], in the treatment of non-union fractures[21], as a graft for reconstruction of the patellar articulation[22], or as tissue engineered bone transplant for maxillary sinus floor augmentation[23].

However, only in 2009 Colnot[24] provided direct evidence that periosteum, endosteum, and bone marrow are the major sources of skeletal stem/progenitors cells and that they differently contribute to osteogenesis and chondrogenesis. In bone healing, periosteum and endosteum both give rise to osteoblasts, whereas periosteum is the only source of chondrocytes. The distinct cellular contributions of periosteum, endosteum, and bone marrow suggested the presence of both intrinsic dissimilarities within these residing stem cell populations and differences in the tissue environment. The correct identification of *in vivo* adult skeletal progenitor sources as well as their response to nutrients, metabolites and growth factors will therefore have profound implications in cell-based therapies for the treatment of recalcitrant fractures or bone and cartilage diseases. Exploring and optimising

Table 1 Surface markers of periosteum-derived cells		
		Ref.
Minimal criteria for MSCs		
CD73	+	[13,35,79,94]
CD90	+	[13,35,79,94]
CD105	+	[13,35,79,94]
CD45	-	[13,35,79,94]
HLA-DR	-	[13,35,79,94]
CD14	-	[13,35,79,94]
CD34	-	[13,35,79,94]
Integrins		
CD29	+	[13,94]
CD49e	+	[13,94]
Adhesion molecules		
CD31	-	[13,94]
CD44	+	[13,94]
CD166	+	[13,36,94]
CD54	+	[13,94]
CD146	+	[37,38]
MHC class		
HLA-ABC	+	[13,94]
Hematopoietic markers		
CD14	-	[13,94]
CD33	-	[13,94]
CD34	-	[13,94]
CD45	-	[13,94]
CD133	-	[13,94]
Additional markers		
MSCA-1	+	[93]
CD9	+/-	[13,36,94]
CD13	+	[37,38]
STRO-1	+	[37,38]
SSEA-4	+	[37,38]
ScaI	+	[37,38]
Sox2	+	[39]
Oct4	+	[39]
Nanog	+	[39]

CD: Cluster of differentiation; HLA: Human leucocyte antigen; MSCA-1: Mesenchymal stem cell antigen 1; STRO-1: Stromal cell antigen -1; SSEA-4: Stage specific embryonic antigens 4; ScaI: Stem cell antigen I; Sox2: Sex determining region Y-box 2; Oct4: Octamer-binding 4.

the governing factors that controls PDPCs osteogenesis and chondrogenesis will be a considerable benefit. It is worth noting that periosteum meets the three primary requirements for tissue engineering: cell font, scaffold for cell retaining and delivery, as well as source of local growth factors. These peculiar features endorse its use as a whole, in autologous grafts. The injection of cell suspensions and the transplantation of cells within scaffolds have been largely employed as well[20,23,25].

PERIOSTEUM AS CELL SOURCE

PDPCs hold promise in osteochondral repair applications due to their ease of isolation and expansion potential. Several studies reveal periosteum as a better cell source for bone regeneration than either bone marrow or other mesenchymal cell origins. This is due to the fact that PDPCs display multipotency at single cell level[3] and a higher proliferation rate while retaining their ability to differentiate *in vitro*[26]. Furthermore, PDPCs from elderly show performances comparable to that of cells from younger subjects[3,27,28]. This may be related to telomeres stability, since *in vitro* analysis showed that after 24 population doublings telomere lengths and telomerase activity are similar to those of the parental population[3].

Harvest site, donor conditions and technical factors could affect periosteum regenerative potential: load-bearing bones have a more osteogenic periosteum than flat bones, and also inter-individual differences influence periosteum biology[29,30]. Moreover, resection methods and cell isolation procedure could affect periosteum regenerative properties as well. To this end, the use of instruments (like forceps) that can disrupt the inner cambium layer should be avoided[13]. After dissection, cells are typically obtained by egression or enzymatic digestion. Despite of isolation method, culture expanded cells retain their osteochondral potential[31,32]. Even though both techniques are commonly used, cell egression from their native environment may maintains their physiological state, without artefacts[33]. The choice of basal medium is equally important to preserve MSC characteristics and multipotent properties, even after prolonged culture *in vitro*.

Despite there is still a lack of consensus on the ideal method of culturing MSCs, it has been demonstrated that the use of DMEM-F12 preserves MSC stemness and ability to differentiate for more than 25 sub-culture passages[54].

A long-debated issue is the obtainment of a pure PDPC population, since no exhaustive markers to identify MSC populations are established. PDPCs were commonly characterized by the classic MSC antigenic profile in agreement with the minimal criteria of the International Society for Cellular Therapy (Table 1)[35]. Yet, additional efforts are required to circumvent the isolation of contaminant cells, such as fibroblasts. The use of two additive surface markers, CD166 and CD9 and the comparison of their expression levels on MSCs and fibroblasts, could address this item (Table 1). The expression of CD166 is generally higher on MSCs than on fibroblasts, while CD9 expression has the opposite pattern[36]. Moreover, MSCs with a "fibroblast-like" expression pattern (*i.e.*, low CD166 and high CD9) display a poor osteogenic differentiation[36].

Further markers enable to identify periosteum mesenchymal progenitors (Table 1) could be STRO-1, stage-specific embryonic antigen-4, ScaI and CD146, also known as melanoma cell adhesion molecule[37,38].

In addition, it could be helpful to evaluate the gene expression profile of transcription factors, such as sex determining region Y-box 2 (Sox2), octamer-binding 4 and Homeobox protein Nanog, associate to pluripotency and stemness[39].

Population enrichment for a cell-type specific surface markers by cell-sorting is recommended, too. At last, novel isolation and characterization strategies, from a heterogeneous population, are currently developing. One example is an innovative droplet-based microfluidic device as a platform for the identification and quantification of distinct cell phenotypes[30].
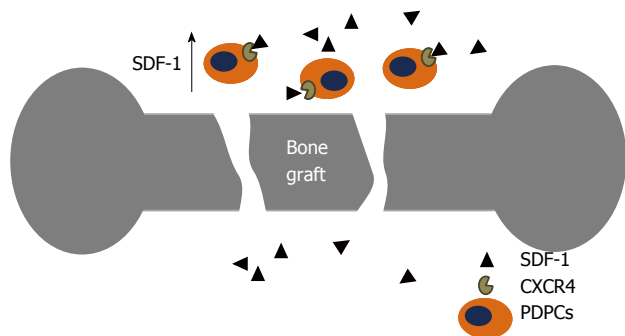
Figure 2 Stromal cell-derived factor 1/chemokine receptor 4 can recruit mesenchymal stem cells to induce fracture repair in skeletal healing. Stromal cell-derived factor 1 (SDF-1) is expresses on the periosteum of the bone graft and recruited chemochine (C-X-C motif) receptor 4 (CXCR4) expressing mesenchymal stem cells in the acute phase of bone repair. PDPCs: Periosteum-derived precursor cells.

MOLECULAR PATHWAYS IN PERIOSTEUM

The potential use of mesenchymal cells for *in situ* repair of osteochondral defects is related to their migration and homing. Understanding how MSCs migrate into tissue injured sites is therefore useful to augment cell transplantation efficiency by enhancing cell targeting.

PDPCs show a dose-dependent migratory effect under chemokine receptor ligands stimulation[40]. Interestingly, PDPCs express chemochine (C-X-C motif) receptor 4 and chemochine (C-X-C motif) receptor 5 that respectively respond to the stromal cell-derived factor 1 (SDF-1) and B cell-attractive chemokine 1 (BCA1). Osteoblasts derived from post-traumatic or osteoarthritis patients express SDF-1 and BCA1 in the bone remodelling area, indicating the potential role of these chemokines not only as chemo-attractant but also as a signaling molecule for *in situ* bone regeneration. Additional studies showed that the expression of SDF-1 is up-regulated in periosteal cells at the sites of injury and it serves as a potent chemo-attractant to recruit circulating or residing CXCR4 expressing MSCs[41], to promote their proliferation (Figure 2). Apparently, the involvement in PDPCs of the SDF-1/CXCR4 axis during bone repair has not been fully elucidated. However, SDF-1 or CXCR4 blocking clearly inhibits BMP2-induced osteogenic differentiation, probably interfering with Smads and MAP-kinase activation[40].

Bone graft integration depends on the orchestrated activation of growth factors and cytokines in both host and graft. Activation, expansion and differentiation of periosteal progenitor cells act as an essential step for successful bone remodelling. Understanding the molecular events that initiate these actions (*e.g.,* BPM2 signaling) provides insights into endogenous regeneration of periosteum and offers information for optimizing tissue engineering constructs[42].

BMP2 is a bone morphogenic protein that belongs to the transforming growth factor-beta (TGFβ) superfamily. TGFβ/BMPs signaling have widely recognized role in bone formation during mammalian development. Signaling TGFβ/BMPs transduction is performed by both canonical Smad-dependent and non-canonical Smad-indipendent [*e.g.,* p38 mitogen-activated protein kinase pathway (MAPK)] pathways. Smad and p38 MAPK pathways converge to *Runx2* gene and control mesenchymal precursor cells differentiation[43].

BMP2 is at the apex of the signaling cascade that starts periosteal progenitor proliferation and differentiation during repair and regeneration. *In vivo* studies highlight that in the absence of BMP2, periosteal progenitors remain quiescent and healing does not initiate[44]. In addition, the expression of Sox9, a chondrogenic marker is reduced as well. Thus, BMP2 is essential for the activation of periosteal progenitor cells and their subsequent differentiation along the osteo-chondrogenic lineage[44]. The relevance of BMP2 in triggering osteochondral tissue remodelling is related to its involvement in all crucial osteogenic pathways: Wnt/β-catenin cascade, Fibroblast growth factor-2 (FGF2) and Hedgehog (Hh) signaling[43]. Multiple Wnt proteins and their modulators are expressed in periosteum. Their cross-talk with Hh intermediates enhances fracture healing[42]. The role of Hh pathway in the promotion of osteogenic and chondrogenic differentiation of PDPCs in adult bone repair has been recently confirmed by *in vivo* investigations[45]. FGF2 signaling has a critical function at the early stage of fracture repair, it improves new bone volume and mineral content and it also takes part in angiogenesis[45].

BMP2 also functions as focal point for the interaction of Smad and Notch signaling during osteoblast differentiation. The latter enhances BMP-induced Alkaline Phosphatase (ALP) activity and formation of calcified nodules *in vitro*[43,44].

In-depth knowledge on BMP2 and its related signaling-pathways, hence, would provide interesting targets to promote osteochondral repair.

It is also emerging that cartilage and bone regenerative techniques are related to nuclear factor kappa β (NF-κβ)/p65 signaling, which determines the early expression of Sox9 and facilitates the subsequent chondrogenic differentiation[46,47].

MECHANOSENSING IN PERIOSTEUM

It is now well accepted that MSC differentiation and phenotypic expression can be influenced by cues from surrounding environment, both soluble (*e.g.,* cytokines and growth factors) and insoluble (*e.g.,* ECM density and stiffness). Due to its external localization on bone, periosteum is particularly sensitive to mechanical stimuli and, even in absence of other stimulations, mechanical load induces new bone formation from periosteum[48], suggesting that this is a highly specialized mechanosensitive tissue[13].

Several studies show that substrate stiffness affects cell shape thus controlling MSCs fate, including self-renewal and lineage commitment[13]. The native environment of PDPCs is mechanically regulated by a com-

bination of tension and shear. PDPCs ability to carry intracellular tension through their microfilament network controls a signaling cascade that, in turn, is responsible for the expression of soluble factors that modulate bone and cartilage growth[13].

In critical size defects, applying tensions in periosteum after surgery leads to rapid *de novo* bone healing. Therefore, mechanical signaling at the tissue level may be responsible for the start of bone regeneration at cell level[13].

Periosteum mechanobiology is probably related to its local microstructure and collagen content[13]. Some studies evidence the emerging role of periostin in the correct collagen fibrillogenesis. Periostin belongs to the matricellular proteins family and regulate cell functions and cell-matrix interaction. Periostin is expressed at high level in the periosteum during embryogenesis and it is re-expressed after mechanical stress and fracture[48]. It is also present in connective tissues subjected to mechanical stress, such as periodontal ligament, heart valves and tendons. Periostin preferential expression in collagen-rich tissues submitted to mechanical stresses (*i.e.*, periosteum) suggests it may play an essential role in bone maintenance and regeneration[48].

As matter of fact, the regulation of the periostin expression occurs by Wnt pathways; BMP2, TGFβ and retinoic acid stimulate periostin expression as well[49-51].

Through interaction with several integrins, periostin recruits and attaches osteoblasts to bone matrix and activates pro-survival signaling, by caspases inactivation, resulting in increasing bone formation[48]. In addition, periostin interacts with BMP1 to augment its deposition in the fibronectin matrix, in close proximity of lysyl oxydase, an enzyme that catalyses the collagen cross-linking[48]. At last, periostin has a binding site for glycoproteins, glycosaminoglycans and proteoglycans, suggesting a role of this protein in supporting mechanical strength in periosteum[48]. Taken together these data suggest that periostin, contributing to matrix organization, bone microarchitecture and bone strength[48], may acts as a support, thus playing a clear role in the intrinsic mechanobiology of periosteal tissue.

These insights in understanding and harnessing the innate mechanosensing of both periosteum and its cells provide a unique opportunity to induce differentiation without perturbing the biochemical environment[14].

MICRORNAS AND PERIOSTEUM

MicroRNAs (miRs) are small noncoding RNAs that have emerged as crucial post-transcriptional regulators of gene expression by either inhibiting mRNA translation or inducing mRNA degradation[52,53]. MiRs can be transcribed individually or in clusters and are encoded by introns or intergenic regions. After being transcribed, primary miRs are processed by protein complexes containing the endonuclease Drosha into the precursor miR (pre-miR), which is approximately 70 nucleotides. Pre-miR is subsequently exported to the cytoplasm[52,53]. Next, the endonuclease

Dicer further cleaves the pre-miR, resulting in the generation of the approximately 22-bp miR duplexes, which are incorporated in the RNA-induced silencing complex. One strand is then retained in the complex and becomes the mature miR, which binds to the 3' untranslated region of the target mRNA.

Hundreds of miRs have been described and currently approximately 1500 miRs are considered to be expressed in humans. Each miR binds up to several hundred complementary mRNAs, thereby modulating gene expression patterns rather than single genes. In the past decade, miRs were extensively investigated and were shown to act as key players in various critical cellular processes such as proliferation, cell cycle progression, apoptosis and differentiation.

As far as stem and progenitor cells are concerned, distinct miRs regulate their functions, modulating cell survival and homing or controlling differentiation and maturation. Additionally, experimental studies shown that miRs regulate endogenous tissue repair and might potentially be useful to enhance bone regeneration[54].

The switch between self-renewal and differentiation requires rapid widespread changes in gene expression. Since miRs can repress the translation of many mRNA targets, they are good candidates to regulate cell fate[55]. Throughout recent years extensive molecular studies have unraveled genetic and epigenetic mechanisms involved in osteoblasts differentiation and functions[54].

As mentioned above, differentiation of MSCs into the osteogenic lineage is tightly regulated by local growth factors (*e.g.*, BMPs, FGFs) that activate specific intracellular pathways, thus triggering the expression of crucial transcription factors such as Runx2 and Osterix (Osx)[54]. miRs regulate each differentiation step by targeting multiple proteins and various signaling pathways, exerting a positive or a negative effect on osteogenesis.

MiR-29b, miR148b, miR196a, miR-210, miR-2861 and miR-3960 have been reported to cause down-regulation of various inhibitors of osteoblasts differentiation, thus exerting stimulatory effects. For instance, miR-29a potentiates osteoblastogenesis by modulating Wnt signaling through a positive feedback loop[56].

On the contrary, miR138, miR-133 and miR-204 are associated with a low bone mineral density. Particularly, miR138 was shown to attenuate the ERK-dependent pathway, phosphorylation of Runx2, and Osx expression, being able to inhibit osteoblasts differentiation and bone formation by human MSCs both *in vitro* and *in vivo*[57].

Elucidating the molecular mechanisms that regulate MSC differentiation is important not only for the treatment for orthopaedic trauma, but also for regenerative medicine purposes in case of the loss of functions that naturally occurs with age. Bone homeostasis is in fact strictly related to the balance between bone deposition and resorption as well as to the correct response to mechanical forces.

MiRs act as key regulators of both bone formation and remodelling and degeneration, as well. Deregulation of miRs-mediated mechanisms is pathologically linked

to bone-related diseases, such as osteoporosis[58]. Indeed, since miRs control differentiation of osteoblast from stem cells and differentiation of osteoclasts from hematopoietic precursors[58], deregulation at these levels could affects osteoclast-related bone remodelling[58].

At present, no data are available on miRNA expression in periosteum. Therefore, profiling of miRs in PDPCs could be useful in elucidating crucial mechanisms governing pre-osteoblasts differentiation during bone development and remodelling. Moreover, advances in miR expression knowledge could also provide information on bone tissue metabolism during lifespan, with particular attention to changes related to inflammation and/or ageing.

PERIOSTEUM AND CARTILAGE REGENERATION

The chondrogenic potential of periosteum is well documented both *in vitro* and *in vivo*[19,59], in fact free autogenous periosteal grafts restore cartilage defects[60].

Immediately following cortical bone injury, periosteum undergoes a series of changes to initiate bone formation at the fracture site. Cells at the periphery of the cortex adopt an osteogenic fate whereas cells near the cortical bone junction differentiate into chondroprogenitors[42]. Chondrocytes within the fracture callus are primarily derived from the periosteum inner cambium-layer as indicates the presence of Sox-9 expressing chondroprogenitor cells in the periosteum adjacent to the fracture site[61].

The development and maturation of neochondrocytes involves several growth factors, encompassing insulin growth factor 1, TGFβ1, TGFβ3, growth differentiation factor 5 and BMP2[62]. In addition the expression of adhesion molecules, such as N-cadherin, play a role in the regulation of chondrocytic phenotype[63]. At last, for resurfacing arthoplasty in humans, periosteum has been used alone or in combination with continue passive motion to stimulate joint neochondrogenesis[62].

With aging the chondrogenic potential of periosteum decreases, as the number of chondrocytes precursors decline in the cambium layer[62]. However sub-periosteal injection of both TGFβ1[64,65] and TGFβ3 has been shown to stimulate the proliferation of PDPCs and to induce their chondrogenic differentiation[63]. Yet, a recent study showed that a subperiosteal injection of a chondroinductive growth factor mixture do not stimulate tissue differentiation of an autologous osteoperiosteal graft[66]. This suggests that the repair of cartilage defects could benefit from an *in vitro* pre-treatment of micromass PDPCs cultures with TGFβ3, which improves periosteum ability to undergo chondrogenesis and produce hyaline cartilage[66]. Quality of tissue harvest, choice and amount of appropriate stimulating molecule, time of exposure, as well as intervals between injections, may influence healing. Mechanical stimulations could affect the clinical outcome as well.

Tissue engineering approaches in cartilage tissue re-

generation could be also useful to potentiate the *in vivo* outcomes. Recently, Casper *et al*[67] showed the potential of PDPCs to infiltrate poly-epsilon caprolactone (PCL) nanofiber scaffolds in a rabbit model and the possibility to produce engineered cartilage *in vitro*. The same group has also demonstrated that the application of a directional fluid flow to periosteal explants seeded onto PCL scaffolds enhances cell proliferation, chondrogenic differentiation and organization, thus modifying the biomechanical properties of the engineered cartilage[68].

In order to generate 3D artificial cartilage resembling native articular one, a recirculating flow-perfusion bioreactor, which simultaneously offer shear stress and hydrodynamic pressure, was also developed and, in presence of periosteum/PCL constructs, good ECM composition, cell distribution and mechanical properties were obtained[59].

PERIOSTEUM AND BONE HEALING

In fracture healing, periosteum is the major responsible for bridging the callus formation and participating to endochondral and intramembranous ossifications.

Steps of fracture bone repair have been well summarize by Shapiro[69]. After fracture, cells from the inner cambium layer of periosteum proliferate and differentiate: at the periphery of the fracture the inner layer arranges a collar of bone by intramembranous ossification; nearer to the fracture site the cambium layer produces a mass of cartilage around the fracture location that, subsequently, undergoes to endochondral ossification[69]. Osteoblastic potential of periosteum differs not just with age but also by location: calvaria periosteum showed less osteogenic potential than tibia ones[29,70].

Even though the use of periosteal autografts for the treatment of bone fractures is a well-established procedure[21,51], only recently it was demonstrated that autologous periosteal precursor cells cultured on a 3D matrix are responsible to promote the healing of a distal femur atrophic non-union[71]. Unfortunately, autografts are not always feasible, also due to donor-site morbidity, and alternatives have to be sought. Indeed, the use of allografts for the treatment of critical sized bone defects remains a challenge. Allografts avoid donor site pain and morbidity and fill the need for large volumes of graft materials[72]. Yet, clinical evidences showed that where periosteum orchestrates bone remodelling, allograft healing ability is lower if compared to autograft[73]: allografts exhibit minimal engraftment and a 60% failure rate 10-years-post-transplantation[74,75].

Alternatives to the use of native periosteum for critical size defects healing could be hence hypothesized. For instance, when periosteum contains too few PDPCs or has been damaged, it is possible to create a tissue engineered periosteum (TEP)[13]. At present, few studies have well characterized TEP mechanical properties. Therefore, this approach is currently intended only for use in oral applications, where TEP would experience less mechani-
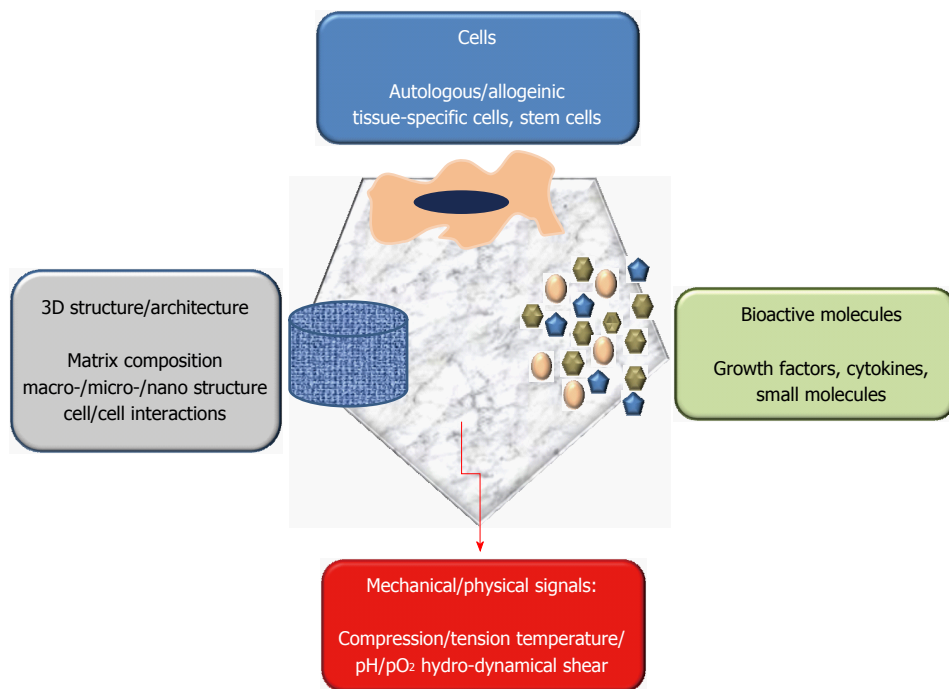
Figure 3 Interactive "diamond" concept of Tissue Engineering and Regenerative Medicine suggests that in addition to cell type, 3D dimensional structure/ architecture, mechanical/physical signals, and bioactive factors in the environment are critical and act in concert to direct tissue repair and regeneration. Cell activity is dynamically regulated by the other key cornerstones of the diamond.

cal strain than in a dynamically loaded environment (*i.e.*, femur)[13].

It is been a long time since the need to realize constructs that reproduce the intrinsic properties of autogenous bone, by culturing PDPCs *ex-vivo* and subsequently seeding into a natural or synthetic scaffold, has emerged[33]. The success of this approach is strictly related to the use of an appropriate material able to improve PDPC differentiation, with a corrected structure/topography and able to provide adequate support for nutrients and growth factors[2] (Figure 3).

For the development of an engineered tissue, elucidating the steps that can enhance PDPC osteogenic differentiation is advantageous as well[76]. In mesenchymal stromal cells this involves the following processes: cell proliferation, cell migration-aggregation and cell differentiation with the dynamic expression of osteogenic transcription and growth factors[77]. Moreover, early MSC osteogenic differentiation is characterized firstly by a proliferative burst, including the formation of nodule-like structures, accompanied by the expression of ALP.

To replicate this differentiation profile, PDPC culture conditions reproducing these key events are required. It has been widely demonstrated that under osteogenic conditions, PDPCs express mRNAs for bone markers (*e.g.*, collagen type Ⅰ, osteopontin and osteocalcin), whilst in a chondrogenic environment they display chondrogenic markers such as collagen type Ⅱ and aggrecan[76]. Moreover, the addition of foetal bovine serum (FBS) and dexamethasone (Dex) to the culture media has a positive effects on osteocalcin and ALP expression, in the early differentiation stages[78]. For the expression of the main transcription factors governing osteogenesis and

hence differentiation towards a mature osteoblast, the subsequent combination of trans-retinoic acid (atRA), FBS, Dex and BMP2 is required[78]. At last, also vascular endothelial growth factor (VEGF) plays a role in osteogenesis and it is express in human normal periosteum as well as in periosteum after fracture healing: the addition of VEGF to a basal culture medium enhance PDPC osteoblastic differentiation. That was corroborated by our results as well[79].

In bone tissue engineering approach, scaffolds are generally used as temporary substitutes of the original tissue after injury. As well-known, 3D scaffolds should be tolerated by the body, provide cell attachment, migration and proliferation, allow for biochemical signaling and possess a bone-like stiffness and degradation rate commensurate to bone healing[2,80]. Canonical classification includes natural and synthetic scaffolds. Natural scaffolds such as chitosan, collagen, gelatine, fibrin glue and hyaluronic acid show several advantages, such as an ECM-like chemistry and structure, the presence of cell-adhesive sequences and a resorbability driven by enzymes, with the production of non-toxic easily excreted molecules. Natural materials are also often used as drug carries for their aptitude to retain growth factors that encourage cellular migration and proliferation[81]. Drawbacks in their use include limited availability, low mechanical resistance and potential immunogenicity[80]. In this respect synthetic scaffolds display many advantages, encompassing easy modulation of chemical and mechanical properties, biodegradability and avoidance of infections or immunogenicity.

Hydroxyapatite or its analogues (including natural bone matrix) are the most popular inorganic components

for bone replacement, due to their chemical similarity to the mineral component of mammalian bone[80]. Collagen/demineralised bone powder scaffold combined with PDPCs has been proposed as a potential tool for bone tissue engineering[82]. In our experience scaffolds with an increased amount of inorganic phase were able to modulate stem cells behaviour[11] as well as periosteal-derived stem cells osteogenic properties[83]. The rationale for the use of Calcium Phosphate biomaterials and the evaluation of their bone forming capacity in the presence of PDPCs has been recently summarized by Roberts *et al*[84].

Modern bone regenerative medicine strategies aim to "take lesson from Nature" in scaffold development. To this respect a chitosan-heparin coating acting as a synthetic periosteum was recently proposed for the improvement of bone allografts outcomes[85]. Several biomaterials, such as naturally derived acellular matrices, commercially available collagen-based sponges and synthetic polymers[86-88] have also been investigated as periosteum mimicking. These materials improve cell localization but show an inadequate cell survival[86-88]. Instead, the use of hydrogels, which emulate mechanical properties and hydration of the native periosteum ECM, seems a promising approach. Hydrogels may be properly tailored for correct degradation, inclusion of biomolecules and cell-adhesion ligands in order to elicit a specific cell functions[85]. It has been shown that hydrogel-based tissue engineered periosteum enhance osteoblast progenitor cells infiltration, bone callus formation and allograft biomechanical stability[72,73].

Besides, peculiar surgical techniques have been used as a tool for mimicking periosteum. Since 1986 Masquelet[89] developed a simple method to reconstruct long bone defects based on the insertion of a cement spacer that maintains the space for bone reconstruction and promotes the formation of a synovium-like membrane. This induced membrane (IM) prevents the graft resorption and favours its re-vascularization. Moreover, the membrane acts as an *in situ* growth factors delivery system, which is capable of enhancing bone graft healing[89].

Recently, Cuthbert *et al*[90] investigated the morphology, molecular properties and gene expression pattern of IMs from patients undergoing large bone defects surgery, showing that IMs share strong architectural similarities, vascular features and growth factor expression of periosteum[90]. Moreover, cells expanded from IMs revealed a mRNA profile similar to PDPCs[90]. Cuthbert *et al*[90] thus, provided evidences that the IM technique generates a dynamic periosteum-like structure, offering important insights into new bone regeneration approaches. Nevertheless, further studies are required to establish if this surgical technique could be suitable for all bone regeneration applications despite of the nature of disease, the lesion site and the patient-related features.

PDPCS IN ORAL AND MAXILLOFACIAL TISSUE ENGINEERING

Periosteum has found great use in enhancing bone for-

mation in the field of dentistry and maxillofacial reconstruction[91,92]. Even though human jaw periosteal cells (JPC) are a promising source for the engineering of cell-based osseoinductive grafts in oral surgery[93], their harvesting and subsequent characterization is not particularly easy. Specific surface markers can facilitate the isolation of a cell pure population, while an accurate analysis of the gene expression profile can allow a detailed comprehension of the JPCs.

In the last years, several markers have been suggested to enrich the osteogenic progenitor cell fraction from the entire JPCs population. Among these, particular attention has received mesenchymal stem cell antigen-1 (MSCA-1) and CD166. MSCA-1$^+$ enriched JPCs have an higher osteogenic potential compared with MSCA-1, as well as CD166$^+$ respect to CD166$^{-[93]}$. Magnetic-activated cell sorting isolation technology was also recommended for increasing recovery and purity of rare MSCA-1$^+$ cells from jaw periosteum[93].

The high osteogenic potential of MSCA-1$^+$ cell fraction is strictly related to the expression of specific markers, such as lipoprotein receptor-related protein 6 (LRP-6), a key component of the WNT receptor complex. MSCA-1$^+$/LRP-6$^+$ also induce an high expression of stanniocalcin 1 (STC-1) and of tissue inhibitor of metalloproteinases-4 (TIMP-4)[93]. STC-1 is involved in endochondral and intramembranous bone formation while TIMP-4 is tangled in ECM remodelling during JPCs osteogenesis[93].

In spite of PDPCs derived from periosteum, other sources of stem cells such as dental pulp[94,95] and periodontal ligament[95] have been proposed for dentistry applications. Harvest morbidity and patient acceptance should affect the final choice of the appropriate cell source for regenerative medicine purposes.

Cutting-edge applications
The great plasticity of mesenchymal stromal cells, due to their ability to differentiate into multiple lineages, makes them good candidates for *in vivo* regeneration innovative procedures. The use of allogeneic MSCs in regenerative medicine is also encouraged by their immunosuppressive and immunomodulatory features.

MSCs derived from different sources have been studied for the generation of Insulin-Producing Cells (IPCs) in the treatment of type 1 diabetes. Kim *et al*[96] examined the differentiation in IPCs of MSCs isolated from different sources: bone marrow, adipose tissue, Wharton's jelly and periosteum. Even though cultured under similar conditions, only IPCs derived from PDPCs showed a significant increase in insulin secretion under glucose stimulation[96].

These results indicate the periosteum as a suitable source of multipotent progenitor cells that could be employed in allogenic transplantations.

However, even if MSC immune privilege is well known for cells derived from bone marrow, umbilical cord blood and adipose tissue, no studies confirm that

PDPCs have similar properties. Therefore, this aspect needs to be further investigated in order to accomplish PDPCs innovative applications[96].

CONCLUSION

Small bone defects can be bridged with conventional grafting[97], whilst bone regeneration in large bone defects is challenging and several factors (*i.e.*, defect site and patient related factors) may affect treatment outcomes. The healing of large size defects, hence, looks into tissue engineering strategies, including the use of exogenous stem cells, growth factors and bioactive scaffolds[2,98]. Recently relevant breakthroughs in designing and creating bone substitutes have been achieved. After the sophisticated approaches combining biomaterials/stem cell constructs the concept of a guided bone regeneration has received attention: the use of smart, bioactive-induced membranes started gaining momentum.

Scientific word is therefore going on with investigations understanding molecular basis of cell/tissue endogenous repair, as well as, improving scaffold design. To this end, periosteum will offer new intriguing cues for further investigation.

Periosteum plays a key role in ECM architecture and cell cytoskeletal reorganization under mechanical stress, by the activation of the mechanosensing signaling. The comprehension of cell molecular mechanisms associated with mechanosensing and cell intrinsic repair abilities has underlined a critical role of periostin. Its expression is up-regulated in the presence of mechanical stress in order to preserve bone tissue integrity and function. Periostin up-regulation leads to the activation of specific pathways that support cell survival. It also ensures a correct collagen fibrillogenesis and matrix organization, opening intriguing perspective in designing future strategies for bone tissue regeneration.

In addition, a further characterization of cellular epigenetic mechanisms miRs related is encouraged: directing the mRNAs expression, miRs affect pivotal differentiation pathways and could therefore represent important targets in promoting osteochondral regeneration.

Finally, considering periosteum dynamic response to environmental and mechanical stimuli, two strategies have been pursued: the use of periosteum itself as a "smart material" (*i.e.*, TEP) and the realization of constructs (*e.g.*, chitosan-heparin coating and PEG-hydrogels) able to mimic the ECM features of this tissue. At present TEP constructs are not tuned for the repair of a dynamically loaded environment such as long bones.

Taken together, the data highlight periosteum involvement in bone anabolic pathways and suggest novel TERM approaches in osteochondral tissue repair. Moreover, a deeper understanding of the molecular basis of cell mechanosensing, as well as of microRNA involvement in PDPC differentiation responses, could be useful for the development of new procedures for the maintenance of bone mass both in post-trauma healing process and in physiological turn-over (therefore preventing osteoporosis).

REFERENCES

1 **Kaiser LR**. The future of multihospital systems. *Top Health Care Financ* 1992; **18**: 32-45 [PMID: 1631884]

2 **Giannoudis PV**, Einhorn TA, Marsh D. Fracture healing: the diamond concept. *Injury* 2007; **38** Suppl 4: S3-S6 [PMID: 18224731 DOI: 10.1016/S0020-1383(08)70003-2]

3 **De Bari C**, Dell'Accio F, Vanlauwe J, Eyckmans J, Khan IM, Archer CW, Jones EA, McGonagle D, Mitsiadis TA, Pitzalis C, Luyten FP. Mesenchymal multipotency of adult human periosteal cells demonstrated by single-cell lineage analysis. *Arthritis Rheum* 2006; **54**: 1209-1221 [PMID: 16575900 DOI: 10.1002/art.21753]

4 **Antonucci I**, Iezzi I, Morizio E, Mastrangelo F, Pantalone A, Mattioli-Belmonte M, Gigante A, Salini V, Calabrese G, Tetè S, Palka G, Stuppia L. Isolation of osteogenic progenitors from human amniotic fluid using a single step culture protocol. *BMC Biotechnol* 2009; **9**: 9 [PMID: 19220883 DOI: 10.1186/1472-6750-9-9]

5 **Troyer DL**, Weiss ML. Wharton's jelly-derived cells are a primitive stromal cell population. *Stem Cells* 2008; **26**: 591-599 [PMID: 18065397 DOI: 10.1634/stemcells.2007-0439]

6 **Buzzi M**, Alviano F, Campioni D, Stignani M, Melchiorri L, Rotola A, Tazzari P, Ricci F, Vaselli C, Terzi A, Pagliaro PP, Cuneo A, Lanza F, Bontadini A, Baricordi OR, Rizzo R. Umbilical cord blood CD34(+)cell-derived progeny produces human leukocyte antigen-G molecules with immunomodulatory functions. *Hum Immunol* 2012; **73**: 150-155 [PMID: 22178696 DOI: 10.1016/j.humimm.2011.12.003]

7 **Konno M**, Hamabe A, Hasegawa S, Ogawa H, Fukusumi T, Nishikawa S, Ohta K, Kano Y, Ozaki M, Noguchi Y, Sakai D, Kudoh T, Kawamoto K, Eguchi H, Satoh T, Tanemura M, Nagano H, Doki Y, Mori M, Ishii H. Adipose-derived mesenchymal stem cells and regenerative medicine. *Dev Growth Differ* 2013; **55**: 309-318 [PMID: 23452121 DOI: 10.1111/dgd.12049]

8 **Orciani M**, Di Primio R. Skin-derived mesenchymal stem cells: isolation, culture, and characterization. *Methods Mol Biol* 2013; **989**: 275-283 [PMID: 23483402 DOI: 10.1007/978-1-62703-330-5_21]

9 **Gullo F**, De Bari C. Prospective purification of a subpopulation of human synovial mesenchymal stem cells with enhanced chondro-osteogenic potency. *Rheumatology* (Oxford) 2013; **52**: 1758-1768 [PMID: 23804221 DOI: 10.1093/rheumatology/ket205]

10 **Short B**, Wagey R. Isolation and culture of mesenchymal stem cells from mouse compact bone. *Methods Mol Biol* 2013; **946**: 335-347 [PMID: 23179842 DOI: 10.1007/978-1-62703-128-8_21]

11 **Gigante A**, Cappella M, Manzotti S, Cecconi S, Greco F, Di Primio R, Mattioli-Belmonte M. Osteoinduction properties of different growth factors on cells from non-union patients: in vitro study for clinical application. *J Biol Regul Homeost Agents* 2010; **24**: 51-62 [PMID: 20385071]

12 **Petrochenko P**, Narayan RJ. Novel approaches to bone grafting: porosity, bone morphogenetic proteins, stem cells, and the periosteum. *J Long Term Eff Med Implants* 2010; **20**: 303-315 [PMID: 21488823]

13 **Evans SF**, Chang H, Knothe Tate ML. Elucidating multiscale periosteal mechanobiology: a key to unlocking the smart properties and regenerative capacity of the periosteum? *Tissue Eng Part B Rev* 2013; **19**: 147-159 [PMID: 23189933 DOI: 10.1089/ten]

14 **Chang H**, Knothe Tate ML. Concise review: the periosteum: tapping into a reservoir of clinically useful progenitor cells. *Stem Cells Transl Med* 2012; **1**: 480-491 [PMID: 23197852 DOI: 10.5966/sctm.2011-0056]

15 **Knothe UR**, Dolejs S, Matthew Miller R, Knothe Tate ML. Effects of mechanical loading patterns, bone graft, and

proximity to periosteum on bone defect healing. *J Biomech* 2010; **43**: 2728-2737 [PMID: 20673900 DOI: 10.1016/j.jbiomech.2010.06.026]

16 **de Mourgues G**. [Léopold Ollier, 1830-1900, the father of orthopedic surgery]. *Rev Chir Orthop Reparatrice Appar Mot* 1979; **65** Suppl 2: 2-3 [PMID: 158783]

17 **Fell HB**. The Osteogenic Capacity in vitro of Periosteum and Endosteum Isolated from the Limb Skeleton of Fowl Embryos and Young Chicks. *J Anat* 1932; **66**: 157-180.11 [PMID: 17104365]

18 **Nakahara H**, Bruder SP, Goldberg VM, Caplan AI. In vivo osteochondrogenic potential of cultured cells derived from the periosteum. *Clin Orthop Relat Res* 1990; (**259**): 223-232 [PMID: 2208860 DOI: 10.1097/00003086-199010000-00032]

19 **O'Driscoll SW**, Fitzsimmons JS. The role of periosteum in cartilage repair. *Clin Orthop Relat Res* 2001; (**391 Suppl**): S190-S207 [PMID: 11603704 DOI: 10.1097/00003086-200110001-00019]

20 **Brittberg M**, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994; **331**: 889-895 [PMID: 8078550 DOI: 10.1056/NEJM199410063311401]

21 **Doi K**, Sakai K. Vascularized periosteal bone graft from the supracondylar region of the femur. *Microsurgery* 1994; **15**: 305-315 [PMID: 7934797 DOI: 10.1002/micr.1920150505]

22 **Hoikka VE**, Jaroma HJ, Ritsilä VA. Reconstruction of the patellar articulation with periosteal grafts. 4-year follow-up of 13 cases. *Acta Orthop Scand* 1990; **61**: 36-39 [PMID: 2336949 DOI: 10.3109/17453679008993062]

23 **Schmelzeisen R**, Schimming R, Sittinger M. Making bone: implant insertion into tissue-engineered bone for maxillary sinus floor augmentation-a preliminary report. *J Craniomaxillofac Surg* 2003; **31**: 34-39 [PMID: 12553924 DOI: 10.1016/S1010-5182(02)00163-4]

24 **Colnot C**. Skeletal cell fate decisions within periosteum and bone marrow during bone regeneration. *J Bone Miner Res* 2009; **24**: 274-282 [PMID: 18847330 DOI: 10.1359/jbmr.081003]

25 **Ossendorf C**, Kaps C, Kreuz PC, Burmester GR, Sittinger M, Erggelet C. Treatment of posttraumatic and focal osteoarthritic cartilage defects of the knee with autologous polymer-based three-dimensional chondrocyte grafts: 2-year clinical results. *Arthritis Res Ther* 2007; **9**: R41 [PMID: 17451597 DOI: 10.1186/ar2180]

26 **Bruder SP**, Jaiswal N, Haynesworth SE. Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J Cell Biochem* 1997; **64**: 278-294 [PMID: 9027588]

27 **Lim SM**, Choi YS, Shin HC, Lee CW, Kim DI. Isolation of human periosteum-derived progenitor cells using immunophenotypes for chondrogenesis. *Biotechnol Lett* 2005; **27**: 607-611 [PMID: 15977065 DOI: 10.1007/s10529-005-3625-5]

28 **Koshihara Y**, Honda Y. Age-related increase in collagen production in cultured human osteoblast-like periosteal cells. *Mech Ageing Dev* 1994; **74**: 89-101 [PMID: 7934211 DOI: 10.1016/0047-6374(94)90101-5]

29 **Bilkay U**, Tokat C, Helvaci E, Ozek C, Zekioglu O, Onat T, Songur E. Osteogenic capacities of tibial and cranial periosteum: a biochemical and histologic study. *J Craniofac Surg* 2008; **19**: 453-458 [PMID: 18362726 DOI: 10.1097/SCS.0b013e318052fe3d]

30 **Srisa-Art M**, Bonzani IC, Williams A, Stevens MM, deMello AJ, Edel JB. Identification of rare progenitor cells from human periosteal tissue using droplet microfluidics. *Analyst* 2009; **134**: 2239-2245 [PMID: 19838410 DOI: 10.1039/b910472k]

31 **Nakahara H**, Bruder SP, Haynesworth SE, Holecek JJ, Baber MA, Goldberg VM, Caplan AI. Bone and cartilage formation in diffusion chambers by subcultured cells derived from

the periosteum. *Bone* 1990; **11**: 181-188 [PMID: 2390376 DOI: 10.1016/8756-3282(90)90212-H]

32 **Nakahara H**, Goldberg VM, Caplan AI. Culture-expanded human periosteal-derived cells exhibit osteochondral potential in vivo. *J Orthop Res* 1991; **9**: 465-476 [PMID: 2045973 DOI: 10.1002/jor.1100090402]

33 **Hutmacher DW**, Sittinger M. Periosteal cells in bone tissue engineering. *Tissue Eng* 2003; **9** Suppl 1: S45-S64 [PMID: 14511470 DOI: 10.1089/10763270360696978]

34 **Pal R**, Hanwate M, Jan M, Totey S. Phenotypic and functional comparison of optimum culture conditions for upscaling of bone marrow-derived mesenchymal stem cells. *J Tissue Eng Regen Med* 2009; **3**: 163-174 [PMID: 19229888 DOI: 10.1002/term.143]

35 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]

36 **Halfon S**, Abramov N, Grinblat B, Ginis I. Markers distinguishing mesenchymal stem cells from fibroblasts are down-regulated with passaging. *Stem Cells Dev* 2011; **20**: 53-66 [PMID: 20528146 DOI: 10.1089/scd.2010.0040]

37 **Covas DT**, Panepucci RA, Fontes AM, Silva WA, Orellana MD, Freitas MC, Neder L, Santos AR, Peres LC, Jamur MC, Zago MA. Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts. *Exp Hematol* 2008; **36**: 642-654 [PMID: 18295964 DOI: 10.1016/j.exphem.2007.12.015]

38 **Russell KC**, Phinney DG, Lacey MR, Barrilleaux BL, Meyertholen KE, O'Connor KC. In vitro high-capacity assay to quantify the clonal heterogeneity in trilineage potential of mesenchymal stem cells reveals a complex hierarchy of lineage commitment. *Stem Cells* 2010; **28**: 788-798 [PMID: 20127798 DOI: 10.1002/stem.312]

39 **Dadheech N**, Srivastava A, Belani M, Gupta S, Pal R, Bhonde RR, Srivastava AS, Gupta S. Basal expression of pluripotency-associated genes can contribute to stemness property and differentiation potential. *Stem Cells Dev* 2013; **22**: 1802-1817 [PMID: 23343006 DOI: 10.1089/scd.2012.0261]

40 **Stich S**, Loch A, Leinhase I, Neumann K, Kaps C, Sittinger M, Ringe J. Human periosteum-derived progenitor cells express distinct chemokine receptors and migrate upon stimulation with CCL2, CCL25, CXCL8, CXCL12, and CXCL13. *Eur J Cell Biol* 2008; **87**: 365-376 [PMID: 18501472 DOI: 10.1016/j.ejcb.2008.03.009]

41 **Ito H**. Chemokines in mesenchymal stem cell therapy for bone repair: a novel concept of recruiting mesenchymal stem cells and the possible cell sources. *Mod Rheumatol* 2011; **21**: 113-121 [PMID: 20830500 DOI: 10.1007/s10165-010-0357-8]

42 **Colnot C**, Zhang X, Knothe Tate ML. Current insights on the regenerative potential of the periosteum: molecular, cellular, and endogenous engineering approaches. *J Orthop Res* 2012; **30**: 1869-1878 [PMID: 22778049 DOI: 10.1002/jor.22181]

43 **Chen G**, Deng C, Li YP. TGF-β and BMP signaling in osteoblast differentiation and bone formation. *Int J Biol Sci* 2012; **8**: 272-288 [PMID: 22298955 DOI: 10.7150/ijbs.2929]

44 **Chappuis V**, Gamer L, Cox K, Lowery JW, Bosshardt DD, Rosen V. Periosteal BMP2 activity drives bone graft healing. *Bone* 2012; **51**: 800-809 [PMID: 22846673 DOI: 10.1016/j.bone.2012.07.017]

45 **Wang Q**, Huang C, Zeng F, Xue M, Zhang X. Activation of the Hh pathway in periosteum-derived mesenchymal stem cells induces bone formation in vivo: implication for postnatal bone repair. *Am J Pathol* 2010; **177**: 3100-3111 [PMID: 20971735 DOI: 10.2353/ajpath.2010.100060]

46 **Wang X**, Wang Y, Gou W, Lu Q, Peng J, Lu S. Role of mesenchymal stem cells in bone regeneration and fracture repair: a

review. *Int Orthop* 2013; **37**: 2491-2498 [PMID: 23948983]

47 **Caron MM**, Emans PJ, Surtel DA, Cremers A, Voncken JW, Welting TJ, van Rhijn LW. Activation of NF-κB/p65 facilitates early chondrogenic differentiation during endochondral ossification. *PLoS One* 2012; **7**: e33467 [PMID: 22428055 DOI: 10.1371/journal.pone.0033467]

48 **Merle B**, Garnero P. The multiple facets of periostin in bone metabolism. *Osteoporos Int* 2012; **23**: 1199-1212 [PMID: 22310955 DOI: 10.1007/s00198-011-1892-7]

49 **Horiuchi K**, Amizuka N, Takeshita S, Takamatsu H, Katsuura M, Ozawa H, Toyama Y, Bonewald LF, Kudo A. Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. *J Bone Miner Res* 1999; **14**: 1239-1249 [PMID: 10404027 DOI: 10.1359/jbmr.1999.14.7.1239]

50 **Eijken M**, Swagemakers S, Koedam M, Steenbergen C, Derkx P, Uitterlinden AG, van der Spek PJ, Visser JA, de Jong FH, Pols HA, van Leeuwen JP. The activin A-follistatin system: potent regulator of human extracellular matrix mineralization. *FASEB J* 2007; **21**: 2949-2960 [PMID: 17449718 DOI: 10.1096/fj.07-8080com]

51 **Wen W**, Chau E, Jackson-Boeters L, Elliott C, Daley TD, Hamilton DW. TGF-ß1 and FAK regulate periostin expression in PDL fibroblasts. *J Dent Res* 2010; **89**: 1439-1443 [PMID: 20940356 DOI: 10.1177/0022034510378684]

52 **Heinrich EM**, Dimmeler S. MicroRNAs and stem cells: control of pluripotency, reprogramming, and lineage commitment. *Circ Res* 2012; **110**: 1014-1022 [PMID: 22461365 DOI: 10.1161/CIRCRESAHA.111.243394]

53 **van Rooij E**. The art of microRNA research. *Circ Res* 2011; **108**: 219-234 [PMID: 21252150 DOI: 10.1161/CIRCRESAHA.1 10.227496]

54 **Taipaleenmäki H**, Bjerre Hokland L, Chen L, Kauppinen S, Kassem M. Mechanisms in endocrinology: micro-RNAs: targets for enhancing osteoblast differentiation and bone formation. *Eur J Endocrinol* 2012; **166**: 359-371 [PMID: 22084154 DOI: 10.1530/EJE-11-0646]

55 **Mathieu J**, Ruohola-Baker H. Regulation of stem cell populations by microRNAs. *Adv Exp Med Biol* 2013; **786**: 329-351 [PMID: 23696365 DOI: 10.1007/978-94-007-6621-1_18]

56 **Kapinas K**, Kessler CB, Delany AM. miR-29 suppression of osteonectin in osteoblasts: regulation during differentiation and by canonical Wnt signaling. *J Cell Biochem* 2009; **108**: 216-224 [PMID: 19565563 DOI: 10.1002/jcb.22243]

57 **Eskildsen T**, Taipaleenmäki H, Stenvang J, Abdallah BM, Ditzel N, Nossent AY, Bak M, Kauppinen S, Kassem M. MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells in vivo. *Proc Natl Acad Sci USA* 2011; **108**: 6139-6144 [PMID: 21444814 DOI: 10.1073/pnas.1016758108]

58 **van Wijnen AJ**, van de Peppel J, van Leeuwen JP, Lian JB, Stein GS, Westendorf JJ, Oursler MJ, Im HJ, Taipaleenmäki H, Hesse E, Riester S, Kakar S. MicroRNA functions in osteogenesis and dysfunctions in osteoporosis. *Curr Osteoporos Rep* 2013; **11**: 72-82 [PMID: 23605904 DOI: 10.1007/s11914-013-0143-6]

59 **Tarng YW**, Huang BF, Su FC. A novel recirculating flow-perfusion bioreactor for periosteal chondrogenesis. *Int Orthop* 2012; **36**: 863-868 [PMID: 21674291 DOI: 10.1007/s00264-011-1291-x]

60 **Korkala O**, Kuokkanen H. Autogenous osteoperiosteal grafts in the reconstruction of full-thickness joint surface defects. *Int Orthop* 1991; **15**: 233-237 [PMID: 1743838 DOI: 10.1007/BF00192300]

61 **Murao H**, Yamamoto K, Matsuda S, Akiyama H. Periosteal cells are a major source of soft callus in bone fracture. *J Bone Miner Metab* 2013; **31**: 390-398 [PMID: 23475152 DOI: 10.1007/s00774-013-0429-x]

62 **Malizos KN**, Papatheodorou LK. The healing potential of the periosteum molecular aspects. *Injury* 2005; **36 Suppl 3**: S13-S19 [PMID: 16188544 DOI: 10.1016/j.injury.2005.07.030]

63 **Mara CS**, Sartori AR, Duarte AS, Andrade AL, Pedro MA, Coimbra IB. Periosteum as a source of mesenchymal stem cells: the effects of TGF-β3 on chondrogenesis. *Clinics (Sao Paulo)* 2011; **66**: 487-492 [PMID: 21552678 DOI: 10.1590/S180 7-59322011000300022]

64 **Reinholz GG**, Fitzsimmons JS, Casper ME, Ruesink TJ, Chung HW, Schagemann JC, O'Driscoll SW. Rejuvenation of periosteal chondrogenesis using local growth factor injection. *Osteoarthritis Cartilage* 2009; **17**: 723-734 [PMID: 19064326 DOI: 10.1016/j.joca.2008.10.011]

65 **Olivos-Meza A**, Fitzsimmons JS, Casper ME, Chen Q, An KN, Ruesink TJ, O'Driscoll SW, Reinholz GG. Pretreatment of periosteum with TGF-beta1 in situ enhances the quality of osteochondral tissue regenerated from transplanted periosteal grafts in adult rabbits. *Osteoarthritis Cartilage* 2010; **18**: 1183-1191 [PMID: 20633683 DOI: 10.1016/j.joca.2010.06.003]

66 **Gotterbarm T**, Breusch SJ, Vilei SB, Mainil-Varlet P, Richter W, Jung M. No effect of subperiosteal growth factor application on periosteal neo-chondrogenesis in osteoperiosteal bone grafts for osteochondral defect repair. *Int Orthop* 2013; **37**: 1171-1178 [PMID: 23503670 DOI: 10.1007/s00264-013-182 7-3]

67 **Casper ME**, Fitzsimmons JS, Stone JJ, Meza AO, Huang Y, Ruesink TJ, O'Driscoll SW, Reinholz GG. Tissue engineering of cartilage using poly-epsilon-caprolactone nanofiber scaffolds seeded in vivo with periosteal cells. *Osteoarthritis Cartilage* 2010; **18**: 981-991 [PMID: 20434575 DOI: 10.1016/j.joca.2010.04.009]

68 **Tarng YW**, Casper ME, Fitzsimmons JS, Stone JJ, Bekkers J, An KN, Su FC, O'Driscoll SW, Reinholz GG. Directional fluid flow enhances in vitro periosteal tissue growth and chondrogenesis on poly-epsilon-caprolactone scaffolds. *J Biomed Mater Res A* 2010; **95**: 156-163 [PMID: 20540101 DOI: 10.1002/jbm.a.32830]

69 **Shapiro F**. Bone development and its relation to fracture repair. The role of mesenchymal osteoblasts and surface osteoblasts. *Eur Cell Mater* 2008; **15**: 53-76 [PMID: 18382990]

70 **Uddströmer L**. The osteogenic capacity of tubular and membranous bone periosteum. A qualitative and quantitative experimental study in growing rabbits. *Scand J Plast Reconstr Surg* 1978; **12**: 195-205 [PMID: 368969 DOI: 10.3109/02844317 809012995]

71 **Funk JF**, Matziolis G, Krocker D, Perka C. [Promotion of bone healing through clinical application of autologous periosteum derived stem cells in a case of atrophic non-union]. *Z Orthop Unfall* 2007; **145**: 790-794 [PMID: 18072048 DOI: 10.1055/s-2007-965686]

72 **Hoffman MD**, Benoit DS. Emerging ideas: Engineering the periosteum: revitalizing allografts by mimicking autograft healing. *Clin Orthop Relat Res* 2013; **471**: 721-726 [PMID: 23179118 DOI: 10.1007/s11999-012-2695-7]

73 **Hoffman MD**, Xie C, Zhang X, Benoit DS. The effect of mesenchymal stem cells delivered via hydrogel-based tissue engineered periosteum on bone allograft healing. *Biomaterials* 2013; **34**: 8887-8898 [PMID: 23958029 DOI: 10.1016/j.biom aterials.2013.08.005]

74 **Hornicek FJ**, Gebhardt MC, Tomford WW, Sorger JI, Zavatta M, Menzner JP, Mankin HJ. Factors affecting nonunion of the allograft-host junction. *Clin Orthop Relat Res* 2001; (**382**): 87-98 [PMID: 11154010 DOI: 10.1097/00003086-200101000-00 014]

75 **Mankin HJ**, Hornicek FJ, Raskin KA. Infection in massive bone allografts. *Clin Orthop Relat Res* 2005; (**432**): 210-216 [PMID: 15738824 DOI: 10.1097/01.blo.0000150371.77314.52]

76 **Gruber R**, Mayer C, Bobacz K, Krauth MT, Graninger W, Luyten FP, Erlacher L. Effects of cartilage-derived morphogenetic proteins and osteogenic protein-1 on osteochondrogenic differentiation of periosteum-derived cells.

Endocrinology 2001; **142**: 2087-2094 [PMID: 11316776 DOI: 10.1210/en.142.5.2087]

77 **Kärner E**, Bäckesjö CM, Cedervall J, Sugars RV, Ahrlund-Richter L, Wendel M. Dynamics of gene expression during bone matrix formation in osteogenic cultures derived from human embryonic stem cells in vitro. *Biochim Biophys Acta* 2009; **1790**: 110-118 [PMID: 19007861 DOI: 10.1016/j.bbagen.2008.10.004]

78 **Roberts SJ**, Chen Y, Moesen M, Schrooten J, Luyten FP. Enhancement of osteogenic gene expression for the differentiation of human periosteal derived cells. *Stem Cell Res* 2011; **7**: 137-144 [PMID: 21763621 DOI: 10.1016/j.scr.2011.04.003]

79 **Ferretti C**, Borsari V, Falconi M, Gigante A, Lazzarini R, Fini M, Di Primio R, Mattioli-Belmonte M. Human periosteum-derived stem cells for tissue engineering applications: the role of VEGF. *Stem Cell Rev* 2012; **8**: 882-890 [PMID: 22622690 DOI: 10.1007/s12015-012-9374-7]

80 **Murphy WL**, Peters MC, Kohn DH, Mooney DJ. Sustained release of vascular endothelial growth factor from mineralized poly(lactide-co-glycolide) scaffolds for tissue engineering. *Biomaterials* 2000; **21**: 2521-2527 [PMID: 11071602 DOI: 10.1016/S0142-9612(00)00120-4]

81 **Busilacchi A**, Gigante A, Mattioli-Belmonte M, Manzotti S, Muzzarelli RA. Chitosan stabilizes platelet growth factors and modulates stem cell differentiation toward tissue regeneration. *Carbohydr Polym* 2013; **98**: 665-676 [PMID: 23987397 DOI: 10.1016/j.carbpol.2013.06.044]

82 **Thitiset T**, Damrongsakkul S, Bunaprasert T, Leeanansaksiri W, Honsawek S. Development of collagen/demineralized bone powder scaffolds and periosteum-derived cells for bone tissue engineering application. *Int J Mol Sci* 2013; **14**: 2056-2071 [PMID: 23337204 DOI: 10.3390/ijms14012056]

83 **Gentile P**, Mattioli-Belmonte M, Chiono V, Ferretti C, Baino F, Tonda-Turo C, Vitale-Brovarone C, Pashkuleva I, Reis RL, Ciardelli G. Bioactive glass/polymer composite scaffolds mimicking bone tissue. *J Biomed Mater Res A* 2012; **100**: 2654-2667 [PMID: 22615261 DOI: 10.1002/jbm.a.34205]

84 **Roberts SJ**, Geris L, Kerckhofs G, Desmet E, Schrooten J, Luyten FP. The combined bone forming capacity of human periosteal derived cells and calcium phosphates. *Biomaterials* 2011; **32**: 4393-4405 [PMID: 21421268 DOI: 10.1016/j.biomaterials.2011.02.047]

85 **Almodóvar J**, Mower J, Banerjee A, Sarkar AK, Ehrhart NP, Kipper MJ. Chitosan-heparin polyelectrolyte multilayers on cortical bone: periosteum-mimetic, cytophilic, antibacterial coatings. *Biotechnol Bioeng* 2013; **110**: 609-618 [PMID: 22903591 DOI: 10.1002/bit.24710]

86 **Xie C**, Reynolds D, Awad H, Rubery PT, Pelled G, Gazit D, Guldberg RE, Schwarz EM, O'Keefe RJ, Zhang X. Structural bone allograft combined with genetically engineered mesenchymal stem cells as a novel platform for bone tissue engineering. *Tissue Eng* 2007; **13**: 435-445 [PMID: 17518596 DOI: 10.1089/ten.2006.0182]

87 **Schönmeyr B**, Clavin N, Avraham T, Longo V, Mehrara BJ. Synthesis of a tissue-engineered periosteum with acellular dermal matrix and cultured mesenchymal stem cells. *Tissue Eng Part A* 2009; **15**: 1833-1841 [PMID: 19125645 DOI: 10.1089/ten.tea.2008.0446]

88 **Zhang X**, Xie C, Lin AS, Ito H, Awad H, Lieberman JR, Rubery PT, Schwarz EM, O'Keefe RJ, Guldberg RE. Periosteal progenitor cell fate in segmental cortical bone graft transplantations: implications for functional tissue engineering. *J Bone Miner Res* 2005; **20**: 2124-2137 [PMID: 16294266 DOI: 10.1359/JBMR.050806]

89 **Masquelet AC**. Muscle reconstruction in reconstructive surgery: soft tissue repair and long bone reconstruction. *Langenbecks Arch Surg* 2003; **388**: 344-346 [PMID: 13680234]

90 **Cuthbert RJ**, Churchman SM, Tan HB, McGonagle D, Jones E, Giannoudis PV. Induced periosteum a complex cellular scaffold for the treatment of large bone defects. *Bone* 2013; **57**: 484-492 [PMID: 23954755 DOI: 10.1016/j.bone.2013.08.009]

91 **Taba M**, Jin Q, Sugai JV, Giannobile WV. Current concepts in periodontal bioengineering. *Orthod Craniofac Res* 2005; **8**: 292-302 [PMID: 16238610 DOI: 10.1111/j.1601-6343.2005.00352.x]

92 **Tobón-Arroyave SI**, Domínguez-Mejía JS, Flórez-Moreno GA. Periosteal grafts as barriers in periradicular surgery: report of two cases. *Int Endod J* 2004; **37**: 632-642 [PMID: 15317567 DOI: 10.1111/j.1365-2591.2004.00855.x]

93 **Olbrich M**, Rieger M, Reinert S, Alexander D. Isolation of osteoprogenitors from human jaw periosteal cells: a comparison of two magnetic separation methods. *PLoS One* 2012; **7**: e47176 [PMID: 23094035 DOI: 10.1371/journal.pone.0047176]

94 **Lohberger B**, Payer M, Rinner B, Kaltenegger H, Wolf E, Schallmoser K, Strunk D, Rohde E, Berghold A, Pekovits K, Wildburger A, Leithner A, Windhager R, Jakse N. Tri-lineage potential of intraoral tissue-derived mesenchymal stromal cells. *J Craniomaxillofac Surg* 2013; **41**: 110-118 [PMID: 22898339 DOI: 10.1016/j.jcms.2012.06.001]

95 **Orciani M**, Di Primio R, Ferretti C, Orsini G, Salvolini E, Lazzarini R, Mattioli Belmonte M. In vitro evaluation of Mesenchymal stem cell isolation possibility from different intraoral tissues. *J Biol Regul Homeost Agents* 2012; **26**: 57S-63S

96 **Kim SJ**, Choi YS, Ko ES, Lim SM, Lee CW, Kim DI. Glucose-stimulated insulin secretion of various mesenchymal stem cells after insulin-producing cell differentiation. *J Biosci Bioeng* 2012; **113**: 771-777 [PMID: 22425523 DOI: 10.1016/j.jbiosc.2012.02.007]

97 **Keating JF**, Simpson AH, Robinson CM. The management of fractures with bone loss. *J Bone Joint Surg Br* 2005; **87**: 142-150 [PMID: 15736731 DOI: 10.1302/0301-620X.87B2.15874]

98 **Knothe UR**, Springfield DS. A novel surgical procedure for bridging of massive bone defects. *World J Surg Oncol* 2005; **3**: 7 [PMID: 15691380 DOI: 10.1186/1477-7819-3-7]