Concise Review: The Periosteum: Tapping into a Reservoir of Clinically Useful Progenitor Cells

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Key Words. Tissue-specific stem cells • Clinical translation • Stem/progenitor cell • Bone marrow stromal cells • Tissue regeneration

ABSTRACT
Elucidation of the periosteum and its regenerative potential has become a hot topic in orthopedics. Yet few review articles address the unique features of periosteum-derived cells, particularly in light of translational therapies and engineering solutions inspired by the periosteum’s remarkable regenerative capacity. This review strives to define periosteum-derived cells in light of cumulative research in the field; in addition, it addresses clinical translation of current insights, hurdles to advancement, and open questions in the field. First, we examine the periosteal niche and its inhabitant cells and the key characteristics of these cells in the context of mesenchymal stem cells and their relevance for clinical translation. We compare periosteum-derived cells with those derived from the marrow niche in in vivo studies, addressing commonalities as well as features unique to periosteum cells that make them potentially ideal candidates for clinical application. Thereafter, we review the differentiation and tissue-building properties of periosteum cells in vitro, evaluating their efficacy in comparison with marrow-derived cells. Finally, we address a new concept of banking periosteum and periosteum-derived cells as a novel alternative to currently available autogenic umbilical blood and perinatal tissue sources of stem cells for today’s population of aging adults who were “born too early” to bank their own perinatal tissues. Elucidating similarities and differences inherent to multipotent cells from distinct tissue niches and their differentiation and tissue regeneration capacities will facilitate the use of such cells and their translation to regenerative medicine.

WHY THE PERIOSTEUM?
The periosteum’s appearance as the simple bounding envelope of the skeletal system belies its own complexity and sophistication as a composite material, mechanical structure, and biological habitat for stem cells. Despite centuries of study, the complete mechanobiology and regenerative potential of the periosteum has yet to be well understood. Remarkably, in 1742, Duhamel observed that perturbation of the periosteum results in new bone formation. After implanting silver wires beneath the periosteum of several animals, he found the wires surrounded by a bony matrix several weeks later [1]. More than a century thereafter, Ollier discovered that transplanted periosteal tissue was capable of spontaneously inducing new bone growth [2]. In the current century, the periosteum has been identified as a niche for many cells that participate in both endochondral and intramembranous ossification during prenatal development and postnatal fracture healing [3–7].

A number of studies demonstrate different clinical orthopedic applications for the periosteum, as well as its superior regenerative capacity compared with alternative tissues. The periosteum has been shown to heal large, critical-sized (unable to bridge on their own) defects in both long and flat bones (Fig. 1) [4–6, 8–10]. In addition, superior results in lumbar segmental spine fusion have been observed in human patients treated using the periosteum compared with cancellous bone [11]. In addition, the periosteum has been used for tendon-to-bone tunnel healing, resulting in larger failure loads at earlier time points than bone marrow or controls [12]. Even congenital pseudarthroses of the tibia have been treated using periosteal grafts in human patients [13]. The periosteum has found great use in enhancing bone formation for the field of dentistry and maxillofacial reconstruction as well [14–16].

Taken as a whole, elucidation of mechanisms underlying multiscale structure-function relationships in periosteal tissues, as well as the remarkable regenerative capacity of the periosteum, will provide a foundation to hone current treatment modalities and to develop new standards of care. Although the noncellular components of the periosteum should not be ignored, this review focuses on the current state of the art
of the resident cells of the periosteum, regarding the complex niche of periosteum cells, characterization of the cells themselves, and their differentiation and tissue building capacities in vitro and in vivo.

**STRUCTURE OF THE PERIOSTEUM**

The periosteum is a composite biomaterial (tissue) composed of two main layers, including an outer fibrous layer and inner cambial layer. The thin cambial layer (zone I) contains most of the cells in the periosteum. The thicker fibrous layer can be divided into a matrix layer (zone II) and a fibroblastic/collagenous layer (zone III) (Fig. 2) [17]. The periosteum is firmly anchored to the underlying bone in a prestressed state [18] via Sharpey’s fibers, which themselves make up higher order collagen structures. During natural bone growth in youth, the cambial layer of the periosteum expands along with the increasing girth and length of bones [19]. The fibrous layer, containing mostly highly organized and directional collagen fibers aligned in the direction of bone growth, expands in this way with the growth of the bone [20]. Collagen and other extracellular matrix fibers in zones II and III are responsible for much of the unique anisotropy and mechanical toughness of the periosteum tissue as a whole [18].

Cells that are fibroblastic in appearance make up more than 90% of the periosteum’s cell population both in vitro and in vivo. The morphology of these fibroblasts is roughest close to the bone surface, becoming flatter away from the bone. A significant subpopulation of these cells have been identified as mesenchymal stem cells (MSCs) [21–23], which are discussed below in further detail. However, the periosteum cell population is ultimately a mixed population, potentially containing fibroblasts, osteoblasts, MSCs, and pericytes (Fig. 2) [17, 24, 25]. Interestingly, fibroblasts and pericytes have been shown to exhibit morphologies, phenotypes, and differentiation capacities similar to those of MSCs [26–34]. Although the exact localization, subpopulation, and involvement of fibroblasts in periosteum function are poorly understood, pericytes have been identified as a distinct population from periosteal cells. Furthermore, pericytes may play a role in vascularization and promoting bone formation, but their contribution to periosteal bone development is currently considered to be minimal [28].

**CLINICALLY USEFUL PROGENITOR CELLS**

Stem cells have been used clinically to regenerate damaged or missing tissues, thereby restoring natural structure and function. MSCs are easily cultured, multipotent, immunoprivileged cells, making them ideal candidates for regenerative or reparative tissue engineering [35, 36]. A large body of literature describes studies using MSCs; interestingly, some ambiguity still exists in identifying a given cell as an MSC. In 2006, the International Society for Cell Therapy proposed the following criteria for identification of human MSCs, including the criteria that cells (a) remain plastic-adherent when maintained in standard culture conditions; (b) are CD105-, CD73-, and CD90-positive; (c) are CD45-, CD34-, CD14- or CD11b-, CD79a- or CD19-, and HLA-DR-negative; and (d) are able to differentiate in vitro to osteoblasts,
adipocytes, and chondroblasts [37]. Nonetheless, these criteria are insufficient for unambiguous identification of a specific cell [38, 39]. The term “mesenchymal stem cell” itself remains controversial, as nonskeletal differentiation of MSCs has not been shown conclusively in vivo and prenatal development of nonskeletal tissues does not originate from a single mesenchymal origin [40]. Furthermore, the putative equivalency of MSCs with multipotent and self-renewing cells from many different sources can lead to flawed assumptions and inapt conclusions. Although cells may exhibit similar surface markers or morphologies, the exact characteristics of cells also strongly depend on their origin and specific niches [41, 42]. Thus, the benefits of fluorescent-activated cell sorting as a tool to “purify” cells isolated from periosteum for MSC-specific markers are unclear [43]. Bone marrow-derived multipotent mesenchymal stromal cells (bMSCs) have been used to mimic the structure and healing capacity of the periosteum and its resident cells in tissue-engineered periosteum [44]. Furthermore, most studies have found periosteum-derived cells (PDCs) to be comparable to, if not superior to, bMSCs with regard to bone healing and regeneration [45–47]. For the purposes of this review, MSCs originating from the periosteum, that is, PDCs, are compared with the more conventionally used bMSCs; as such, MSCs originating from other sources are not the focus of this review [48].

Aside from the relative differentiation capacities of MSCs from various tissue sources, a number of other factors should be considered in seeking an optimal and practical source of stem cells for clinical use. Since the use of progenitor cells for regenerative medicine requires the harvesting of healthy cells from the patient prior to the main surgery, priority should be placed on minimization of patient pain and trauma while ensuring adequate cell sourcing. Many sources of mesenchymal stem cells have been identified in the body, i.e., marrow, periosteum, adipose, umbilical, muscle, etc., but the risk of donor site morbidity and the difficulty in acquiring adequate volumes of tissue to isolate MSCs remain hurdles for clinical implementation. Periosteum tissue can be removed from many convenient locations, such as areas adjacent to or resected during surgery (such as periosteum from the discarded femoral head during joint replacement surgery; see the section titled Feasibility of Banking Periosteum Tissue and Periosteum-Derived Cells at the end of this review) [49]. The regeneration of the periosteum from denuded bone has just begun to be studied [17, 25, 50]; the effect of periosteum denudation on surrounding tissues is not well understood [51], but recent studies indicate that periosteum regenerates on the surface of denuded bone in rodents (rats) [52] and higher mammals (sheep) [53], even if that bone is transplanted (without an initial patent blood supply) [53]. Furthermore, recent studies demonstrate the feasibility of using periosteum substitute implants to either augment the regenerative capacity of periosteal factors and PDCs or to replace the periosteum completely [49, 54].

Another important factor to consider for clinical application is the time required to isolate and scale up (e.g., through proliferation) the necessary subpopulation and number of cells and to prepare the implant or tissue construct for surgical implantation. Periosteum cells proliferate much faster than most MSCs while retaining their ability to differentiate in an in vitro culture [55]. However, the starting quantity of periosteum cells may be lower, depending on situation-specific limitations in cell harvesting. Nonetheless, studies show that PDCs from elderly patients that are passaged numerous times not only fare better in producing bone or cartilage than bMSCs from a similar source, but also, surprisingly, show performance comparable to that of cells from younger patients (Fig. 3) [21, 56–59]. In summation, given the large population of aging patients requiring orthopedic surgery, PDCs demonstrate great potential to provide a more effective regenerative treatment, with a quick cell isolation turnaround time and shorter healing period than other MSCs.

**Defining the PDC**

**Source**

Because of the endogenous, dynamic mechanical, and biological (e.g., proximity to muscle and soft tissues) environment of the periosteum, periosteum tissue and cells can differ on the basis of species, sex, age, and source site [60]. Like many mechanoadapted tissues, the periosteum is a nonuniform and dynamic tissue that requires careful documentation, with particular attention to spatial and temporal reference points in experiments, as well as careful interpretation of data. The source site of the periosteum...
plays a very large role in periosteum properties. Periosteal bone formation rates differ greater than threefold throughout the skeleton [24]. Load-bearing bones, such as the tibia, have periosteum that is significantly more osteogenic than flat bones, such as the calvaria [61]. Even the medial and distal parts of the femur show inherent differences in periosteum population, which also changes with age [62]. In in vitro studies of the periosteum’s chondrogenic potential, the ilium, scapula, and tibia gave rise to chondrogenic PDCs, whereas the PDCs from the skull exhibited no signs of chondrogenesis [63]. Because of the wide range of species, source, and age of periosteum cell sources in literature, many conflicting results may be attributed to the differences of the PDCs themselves and/or inherent differences in their environment, which guide their differentiation through inductive cues.

The age of the donor from whom periosteum and resident cells are obtained is another factor that influences their biology, although interindividual differences are thought to be more significant than differences attributable to age [64]; whereas periosteum from older individuals still retain functionality (Fig. 3), younger periosteum tends to be much thicker. With increasing donor age, collagen produced by human PDCs increases, as do collagen inhibitory activities. Although old age does not seem to inhibit the regenerative properties of the periosteum, cells from older individuals behave differently than those from younger donors [58, 64]. Furthermore, the sex of the donor from whom cells are obtained is likely to affect the biology of PDCs; for example, parathyroid hormone and estrogen have been shown to affect proliferation and apoptosis of periosteum cells [65]. Compiling and summarizing previously published studies to date underscores the diverse nature of previous experiments. In some cases, previously published studies lack the detail, such as description of cell sources (species, sex, age, and site), to allow for true cross-study comparisons (Fig. 4). Ideally, all of these variables should be taken into consideration to interpret the results of a given single study in the context of cross-study comparisons.

**Isolation**

The method of periosteum resection is a critical aspect for proper acquisition of PDCs. As mentioned previously, younger human populations and certain species present a very thick and loose periosteum, so forceps are commonly used to peel away the periosteum from the underlying bone. However, the use of forceps can potentially result in a partial or complete loss of the cellular cambium layer by tearing through the fibrous zone II layer [81]. Aged human populations and other species present with a much thinner and fragile periosteum, and the periosteum is commonly scraped off the bone with a scalpel. For example, murine periosteum is described as being only a few cells thick, whereas chick periosteum is described as being fibrous and thick enough to be peeled using forceps [76, 82]. Although the method of scraping thin periosteum with a scalpel does ensure a more thorough removal of the cambium layer, it may result in contamination of the periosteum sample with osteoblasts and fragments of bone [81]. The best method for removing the periosteum is via the use of a periosteum elevator or similar surgical instrument [10]. Shaped like a curved chisel, this tool maintains the integrity of the periosteum tissue by cutting the Sharpey’s fibers that anchor the periosteum to the bone [81] while minimizing damage to the underlying bone, which allows for resection of both periosteal layers (cambium and fibrous layer).

Another major source of variability in PDC research is the means by which cells are isolated from the fibrous tissue. After the dissection of the periosteum, the cells are typically liberated by enzymatic digestion or cell egression (Fig. 5). Enzymatic digestion involves the use of a collagenase or similar enzyme for 1–8 hours to degrade the fibrous matrix and release the cells for culture. Isolation of cells through cell egression involves the placement of resected periosteum cambium-side down onto a culture plate and allowing the cells confined between the cambium of the periosteum and the surface of the culture plate to adhere to the plastic surface and proliferate to confluence for up to a week. Although the enzymatic digestion method is very fast, the effect of collagenase treatment on cells is not well understood. Moreover, the population of the cells includes the entire milieu of cells in the periosteum, which may be less physiologically relevant to the cell populations studied in vitro [83]. The egression method is slower for obtaining cells relative to enzymatic digestion, but allowance for egression of cells from their native environment may allow for maintenance of their physiological state and thereby may enable isolation of physiologically relevant cells without the introduction of artifacts [83]. Finally, while a wide range of isolation methods are commonly used to acquire PDCs for research (Fig. 6), further studies are needed to properly identify the differences between the cell populations resulting from implementation of different protocols.

![Figure 3. Chondrogenic capacity of periosteum-derived cells (PDCs) is retained with age. PDCs were cultured with or without TGF-β1 for 6 days, fixed, and stained with Alcian Blue. Samples are labeled with the ages of the donors, and asterisks indicate periosteum samples obtained post mortem. After [58]; used with permission. Abbreviation: TGF-β1, transforming growth factor-β1.](image-url)
Morphology

PDCs exhibit a putative morphology typical of fibroblasts (like most MSCs) that is stably maintained over many passages [58]. The observed shapes may be elongated, spindle-shaped, triangular, or cuboidal. Some studies observed uniform morphology within the population, some are able to distinguish subpopulations of cells visually, and others report a more cuboidal or polyhedral morphology during early PDC progression along chondrogenic or osteogenic lineages [69, 86]. The morphology of PDCs also varies from species to species; for instance, rabbit-derived PDCs are smaller than human PDCs under the same culture conditions [71].

PDC Phenotype and Markers

PDCs have been identified using the previously mentioned MSC markers [21–23]. Furthermore, the use of MSC surface markers to characterize (and potentially to sort) cells isolated from the periosteum provides a valuable reference tool to validate PDC populations, allowing for clear distinction between PDCs and potential “contaminant” cells, such as those from surrounding muscle, marrow, bone, cartilage, blood, and other tissues. Notwithstanding the existence of many of these potential contaminant sources, very high percentages of PDCs exhibit SH2, SH3, SH4, CD9, CD14, CD90, CD105, and CD166 and do not exhibit CD34, CD45, and CD106 [21,
84, 87, 88]. These markers drift with the increase in passage number, typically in passage 5 or greater [21, 84].

Proliferation Potential

PDCs can, to a large degree, remain undifferentiated through many passages without losing their differentiation capacities [87]. They remain viable after prolonged subcultivation and cryopreservation, do not exhibit contact inhibition, and continue to proliferate well into high densities [55, 89, 90]. They exhibit much faster proliferation rates than bMSCs and maintain their linear growth curves for more than 30 population doublings, displaying long telomeres and no signs of senescence until 80 population doublings [45, 58, 84]. Even periosteum cells from aging patients retain this high growth potency and differentiation capability, although their capacity to differentiate toward chondrogenic and adipogenic lineages diminishes with age [21, 91, 92]. In contrast, MSCs derived from bone marrow show decreasing life spans in aging donors, exhibiting telomere shortening and senescence [93, 94]. Furthermore, bMSC number and differentiation potential decreases with age as well, although reported trends between studies differ [95, 96].

Single-cell lineage analysis has also been performed on PDCs. Three separate populations of cells are found in PDCs: (a) pre-committed osteogenic, (b) committed osteogenic, and (c) un-committed cells [97]. Whereas heterogeneity is observed in single-cell clonal populations, PDCs are clonogenic, independent of donor age, and exhibit steady marker expression and growth rate up to 30 population doublings [59].

Differentiation Potential

Well-established biochemical agents have been used to induce differentiation of bMSCs in vitro. To induce osteogenesis, ascorbic acid, β-glycerophosphate, and dexamethasone are used in a medium containing fetal bovine serum (FBS) [98]. To induce chondrogenesis, high cell density cultures are used with transforming growth factor-β in a serum-free medium. Lastly, to induce adipogenesis, dexamethasone, insulin, isobutylmethykanthine, and indomethacin are used in a medium containing FBS [99]. PDCs have also shown single-cell, in vitro potential for osteogenic, chondrogenic, adipogenic, and myogenic differentiation [59].

MSCs are typically capable of committing to osteogenic, chondrogenic, and adipogenic lineages [23, 58, 76]. However, although the modulation and regulation of gene expression for embryonic MSCs during mesenchymal condensation have been mapped extensively and bMSCs closely follow those trends [97], it has yet to be shown conclusively that PDCs follow these same spatiotemporal patterns.

Periosteum cells also respond differently to osteoinductive agents than other MSCs do in vitro. One study compared the effects of bone morphogenetic protein-2 (BMP2) and dexamethasone on alkaline phosphatase (ALP) expression by bMSCs and PDCs. Depending on culture confluence, either BMP2 or dexamethasone exhibits a larger effect on bMSC ALP expression. However, in combination, BMP2 and dexamethasone show an additive effect; in contrast to dexamethasone, BMP2 increases ALP expression in PDCs, and the combined treatment is less effective than BMP2 alone [79]. On the basis of the results of this study, bMSCs and PDCs may possess distinct biochemical differentiation pathways. bMSCs and PDCs have also been shown to have different osteogenic, adipogenic, and chondrogenic potential. Interestingly, some studies using unsorted PDCs and bMSCs found bMSCs to have a slightly higher osteogenic and chondrogenic capacity than PDCs [47, 100]. Another study showed similar osteogenic potential between the two sources of pluripotent cells [46]. However, another study using PDCs and bMSCs selected for CD90 and against CD11b and CD45
found PDCs to have higher osteogenic, chondrogenic, and adipo-
genic capacities than bMSCs [88]. Taken together, these sometimes
contradictory data from different studies that use different subjects
and protocols underscore the need to standardize PDC protocols to
maximize cross-study comparison, as well as to use caution in inter-
preting data, which will be described in more detail below. Although
definitive conclusions are difficult to draw from these myriad stud-
ies, it is important to note the differences in fate determination
between bMSCs and PDCs. Although bMSCs and their behavior are
much better characterized, standardized, and analyzed, much care
must be taken in applying conclusions regarding bMSCs to PDCs, as
bMSCs and PDCs may have distinct differentiation mechanisms and
innate differences attributable to their distinct skeletal niches.

Mechanosensitivity

In addition to biochemical induction of lineage commitment, re-
cent studies have shown stem cells to exhibit exquisite mecha-
nosensitivity, including lineage commitment independent of bio-
chemical factors [101–107]. bMSCs have been differentiated
using surface morphology or mechanical strain alone [108, 109].
Harnessing the innate mechanosensitivity of both stem cells and
orthopedic tissues provides a unique opportunity to induce dif-
ferentiation without need to perturb the biochemical environ-
ment or genome [101]. Specifically, mechanoinduction of stem
cells may become a preferred method for clinical applications,
because it lacks the unknown or unwanted downstream or side
effects of biochemical additives or genetic approaches, it is much
easier to control both spatially and temporally than biochemical
signals, and it can be used in rehabilitation treatments and phys-
tical therapy to promote healing [60, 101, 110]. Furthermore, by
attempting to recapitulate certain aspects of prenatal develop-
ment, scientists hope to harness nature’s paradigms to create de
novo native and functional tissue [101].

PDCs inhabit a multilayered fibrous tissue that is anchored
to the underlying bone in a prestressed natural state via
Sharpey’s fibers [18, 60]. The periosteum is an exquisitely
mechanically sensitive tissue. Increased loading of a limb
causes rapid changes in gene expression, resulting in bone
formation via the periosteum [111, 112]. Gross mechanical
manipulation of the periosteum, such as Duhamel’s place-
ment of silver wires, extraperiosteal saline injections, surgical
release of the periosteum, or guided tissue fabrication adja-
cent to the periosteum stimulates periosteal hypertrophy,
DNA synthesis, cell proliferation, and bone growth [1, 67, 109,
113, 114]. Furthermore, direct mechanical stimulation, such as dy-
namic fluid pressure, induces PDC proliferation and chondrogenesis
[115, 116]. Physiological strains induced by four-point bending elicit
an osteogenic response in PDCs, but no response in MSCs [73].
Changes in mechanical environment also correlate strongly with ar-
eas of new bone formation or cell proliferation derived from the
periosteum [18, 117, 118].

In addition to direct mechanical effects, substrate and sur-
face conditions strongly influence PDC biology. PDC prolifera-
tion is enhanced on fibrin substrates, but reduced in three-dimen-
sional (3D) scaffolds [70]. However, PDCs become more osteo-
genic on 3D scaffolds compared with two-dimensional scaffolds
[109]. The behavior of PDCs in 3D environments may provide
mechanistic clues for the rapid, proliferative, woven bone forma-
tion observed in hematoma-filled, critical-sized defects surrounded
in situ by intact periosteum [10]. PDCs also become more osteo-
genic on smooth, machined titanium surfaces compared with
rough, acid-etched titanium surfaces (Fig. 7A) [74, 120]. In contrast,
bMSCs become more osteogenic on acid-etched titanium surfaces
than smooth, machined surfaces (Fig. 7B) [74]. These contrasting
observations strongly suggest that, in addition to their inherent dif-
fferences in biochemical pathways, PDCs and bMSCs also possess
different mechanotransduction pathways and means of mechan-
adaptation in response to changes in their biophysical (mechanical)
environment.

Taken together, these data support an overarching hypo-
thesis that bMSCs and PDCs exhibit distinct structure-function rela-
tionships and differentiation capacities that are attributable to
their distinct niches. Specifically, given the spatiotemporal cues
inherent to their native habitats, one would expect bMSCs to be
more responsive to biochemical gradients and signals found in
the marrow niche. Similarly, one would expect PDCs to be more
responsive to mechanical signals found in the periosteal niche
[18, 60, 117, 121–123]. Hence, for regeneration of mechanically
active, musculoskeletal tissues, PDCs may offer more robust re-
generation potential than bMSCs.

In Situ Tissue Regeneration

Extensive studies have used PDCs in combination with various scaf-
gfolds to generate in situ bone tissue for fracture healing or bridging
of critical-sized defects (Fig. 1) [124–129]. Fewer studies have im-
plemented PDCs successfully for in situ cartilage generation [130].
Although still inconclusive as to whether PDCs outperform bMSCs in
bone tissue generation in vivo, the current state of the art indicates
that PDCs demonstrate an ability to regenerate tissue that is at least
comparable to that of bMSCs [15, 45, 46].

Interestingly, whereas periosteum alone has shown the ca-
pacity to generate bone de novo, even in the absence of bone
matrix, bone matrix exhibits osteoinductive effects on perioste-
um-derived bone formation [10, 131]. Furthermore, in vitro
studies have demonstrated the presence of vascular endothelial
growth factor isoforms in PDCs; angiogenesis may further modu-
late PDC differentiation and bone formation [85, 132]. These
data suggest that PDCs are affected synergistically by the sur-
rrounding environment during the spatiotemporally dynamic
processes of tissue genesis and angiogenesis; these are critical
factors to consider in designing and optimizing tissue engineer-
ing scaffolds to deliver physical and chemical cues to MSCs
seeded within [90, 133]. These issues are also key for the persis-
tence and maturation of nascent tissue [49].
enabled them to bank their tissues. Banking of periosteum and periosteum-derived cells may provide new avenues to address this current limitation. Given the increasing population of elderly (aged 65 or older) in the U.S. (12.9% of the current population; 20% of the population in 2030), “the number of people affected by osteoarthritis . . . and therefore the number of total hip arthroplasties will . . . increase” significantly [136]. In hip replacement surgery, the femoral head and part of the neck are resected to accommodate the neck of the implant. Hence, periosteum from the femoral neck and tissues resected during joint replacement and other orthopedic surgeries may present an untapped source of patients’}

Figure 7. Periosteum-derived cells indicate more osteogenic differentiation on machined titanium surfaces (A), and bone marrow-derived multipotent mesenchymal stromal cells indicate more osteogenic differentiation on acid-etched titanium surfaces (B). Shown is the expression of bone extracellular matrix-related genes analyzed by polymerase chain reaction analysis visualized with ethidium bromide staining and by reverse transcription-polymerase chain reaction for the expression time course for each gene. Genes are normalized to GAPDH expression. Bars indicate SD. Adapted from [74, 120]; used with permission. Abbreviations: AE, acid-etched surface; ALP, alkaline phosphatase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; M, machined surface.
own stem cells once routine banking protocols can be established.

**CONCLUSION**

When these data are taken together in comparison with bMSCs, the periosteum demonstrates excellent potential as a comparable if not superior source of cells for clinical orthopedic tissue engineering and regenerative medicine. Current areas of ongoing research involve assessment of periosteal mechanical properties and structure in order to define the in vivo mechanical environment of the PDCs [18, 60, 117, 137]. This will help reveal the endogenous mechanical and biophysical signals that PDCs experience during physiological activities and traumatic injuries. Also, the regrowth of the periosteum over bone after its removal is poorly understood yet is imperative to the progression of the use of PDCs in clinical orthopedic applications [53]. Furthermore, the design of artificial periosteum, tissue-engineered periosteum, or periosteum substitute [16, 44, 49, 54, 128, 138] has become an active research arena in the past 5 years. Different cells are being tested on periosteum-like membranes, and PDCs are being tested on different scaffolds as well, in an attempt to mimic and maximize the regenerative potential of pluripotent cells. Although many promising clinical applications and clinical studies are also under way using PDCs, variance in and/or contradictory data, due to a lack of reference protocols and/or poor characterization of the periosteum tissue and periostium-derived cells, may stymie further advances in the field. Also, when considering the voluminous data sets currently available and continuing to grow, it is important not to lose sight of the vagaries of cell behavior in different contexts, such as MSC or PDC behavior in vitro versus in vivo [139]. Further studies are needed, using a variety of approaches to ensure progress in clinical application, and documentation of protocols is key. Also, although stem cell mechanics (including mechanobiology and mechanotransduction) has become an active field of study, relatively few published studies have addressed PDCs’ mechanosensitivity and mechanoadaptation. Such studies could broaden the indications for use of PDCs clinically; for example, physical therapy protocols could be defined to facilitate tissue regeneration and healing by stem cells [60]. Furthermore, a robust comparison of bMSC and PDC mechanobiology is sorely needed to expand and tailor the use of these distinct cell types in cell-based regenerative medicine therapies. Although bMSCs are a relatively known entity compared with PDCs, it is becoming clear that knowledge of bMSCs may not be readily applicable to different pluripotent cell populations derived from different tissue sources. By bringing these differences and similarities to light, the scientific community will better understand how to maximize the use of stem cells in general and PDCs in particular, facilitating translation to regenerative medicine.

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**AUTHOR CONTRIBUTIONS**

H.C.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; M.L.K.T.: conception and design, data analysis and interpretation, manuscript writing, final approval of manuscript, provision of study material or patients, financial support.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

The authors indicate no potential conflicts of interest. The intellectual property behind the periosteum substitute membrane described (from the corresponding author’s laboratory, MKT) has been declared through an Invention Disclosure and is pending patent. This does not change MKT’s adherence to the journal’s best practices and publishing guidelines.
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