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REVIEW

Periosteum mechanobiology and mechanistic insights for regenerative medicine

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Periosteum is a smart mechanobiological material that serves as a habitat and delivery vehicle for stem cells as well as biological factors that modulate tissue genesis and healing. Periosteum's remarkable regenerative capacity has been harnessed clinically for over two hundred years. Scientific studies over the past decade have begun to decipher the mechanobiology of periosteum, which has a significant role in its regenerative capacity. This integrative review outlines recent mechanobiological insights that are key to modulating and translating periosteum and its resident stem cells in a regenerative medicine context.

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Introduction

Periosteum exhibits a remarkable regenerative capacity and has been used surgically for more than two centuries[1] to heal defects in tissues as diverse as bone and cartilage, palate, lip and gingival tissues, ocular sclera, as well as laryngeal and tracheal walls.[2] The regenerative capacity of the periosteum is inextricably tied to its mechanical and biological state[3–11] as well as periosteum's role as a niche for stem cells.[12–19] Multiscale mechanics studies have demonstrated periosteum's smart, *i.e.*, stimuli-responsive, properties, at multiple length and timescales.[3,5,6–8,9,10,16–18] In addition, recent stem cell studies implicate periosteum's mechanical environment as a major driver in guiding its resident stem cells' shape and fate.[16–26] Here we integrate these recent insights to propose mechanobiology as a key factor in modulation of periosteal stem cell niche quiescence and to discuss implications for translation in the context of regenerative medicine.

Mechanical and Permeability Properties of Periosteum

Periosteum exhibits smart mechanical and permeability properties. Smart materials respond to environmental stimuli and exhibit the capacity for a dynamic response.[9] Periosteum's intrinsic weave, comprising collagen and elastin fibers (**Figure 1c**) reinforced by collagenous Sharpeys fibers, which insert at 45 degree angle at points of attachment to the bone (**Figure 1b**), underpins its unique mechanical properties and biological capacity as a mechanically regulated stem cell niche (*cf. 'Periosteum as mechanosensor and actuator of stem cell niche*).[17,18,27,28]

Periosteal tissue is anisotropic (**Figure 2a–c**) and shows strain stiffening (**Figure 2b**).[10] The soft, hyperelastic tissue confers to bone the capacity to absorb more energy at failure compared with bone denuded of periosteum. Hence, periosteum provides a natural splint to bones under impact loads such as those incurred during trauma.[10,29]

Periosteum envelops all nonarticular bone surfaces. Dynamic mechanical loading, for example, via stance shift, results in dynamic, heterogenous strains in periosteal tissue (**Figure 3a and b**).[5] As noted above, in its native state, a multitude of Sharpey's fibers anchor periosteum to the bone. Cutting of these fibers and removal of periosteum from its natural state results in anisotropic shrinkage of the tissue[10] and a concomitant change in the stress state. For example, removal of periosteum from the anterior surface of the ovine femur results in an immediate 50% decrease in tissue area (**Figure 3e**).[3,10] On the basis of mechanical principles, the pre-stress in this tissue is calculated as 12.06–0.40 MPa longitudinally and 0.77–0.43 MPa circumferentially (with respect to long bone axes).[3]

Hence, healthy periosteum is in a state of pre-stress, resulting in significant shrinkage and stress relaxation when Sharpey's fibers are cut. A similar effect would be expected when the periosteum is lifted surgically from the surface of bone by cutting through Sharpey's fibers, although shrinkage would be limited to a degree by remaining connections to surrounding, intact periosteum (**Figure 3d**). Similarly, when periosteum is damaged or separated because of trauma, it is expected that the intrinsic stress state of the tissue changes dramatically (**Figure 3c**).[5] Recent studies implicate this stress state as a

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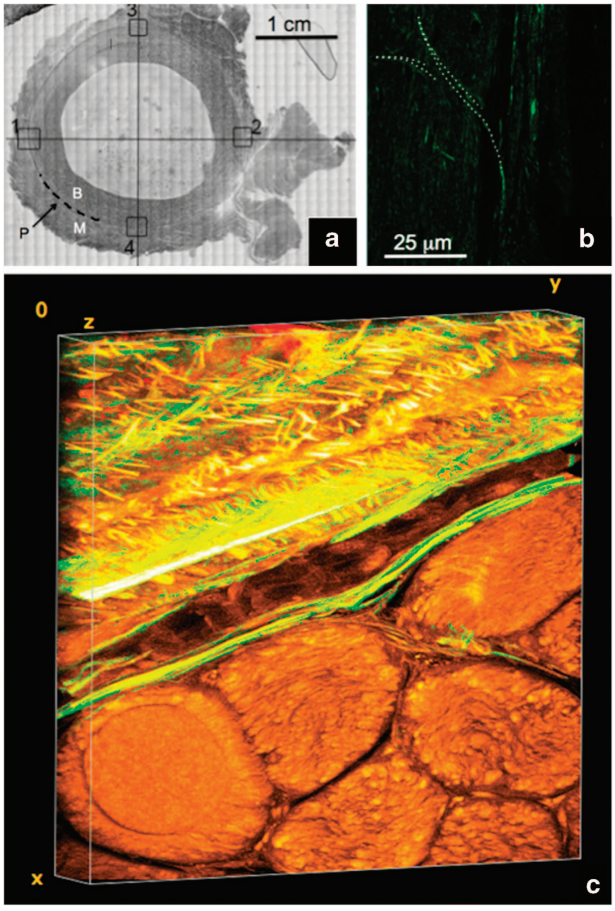

Figure 1 Periosteum, a complex weave of structural protein fibrils including elastin and collagen, covers and attaches to all nonarticular bone surfaces via higher order, collagenous Sharpey's fibers. (**a**) In the mid-diaphyseal cross-section of the sheep femur, periosteum and bone are contiguous, with the periosteum (P) forming a membrane interface between muscle (M) and bone (B). (**b**) Sharpey's fibers exhibit fluorescence typical for collagen (green autofluorescence), and insert at an ~45 degree angle into bone surfaces, with higher density of fiber connections at bone ends (metaphysis) compared with the mid-diaphysis. (**c**) Periosteum's intrinsic weave comprises mainly structural proteins, including elastin fibers, which confer elasticity, and collagen fibers, which confer toughness, to the composite tissue. Using second harmonic imaging protocols, these proteins can be imaged and rendered in three dimensions. Adapted from Whan *et al.*[27] and Ng *et al.*[28]

trigger to activate stem cell egression from the otherwise quiescent periosteal stem cell niche (*cf.* below '*Periosteum as mechanosensor and actuator of stem cell niche'*).[17,18]

Periosteum is a putative gatekeeper for molecular cross-talk between bone and muscle. After trauma, factors from soft tissues such as muscle influence bone healing via the periosteum, for example, through transport of cells and molecular factors (paracrine and endocrine) between muscle and bone.[9,30–33] A recent study demonstrated direction- and flow rate-dependent permeability in periosteal tissue; permeability was significantly higher in the bone to muscle direction than *vice versa*, under high flow rates such as those expected to occur at traumatic impact.[9] This surprising finding may provide a mechanism for a unique molecular signaling and transport cascade triggered at the time of trauma (immediate increase from the bone to the muscle)[9] as well as a mechanism for recruitment of factors from the muscle to the bone via changes in fluid balance due to subsequent edema and associated microhemodynamic changes.[30] An understanding of the nano-microfluidics and functional barrier properties intrinsic to periosteum is needed to understand the full complexities of this smart tissue membrane and its implications for tissue healing and regenerative medicine (*cf.* '*Future directions'*).

Periosteum as Mechanosensor and Actuator of Stem Cell Niche

The periosteum serves as a niche for mesenchymal stem cells.[11–13,15,34,35] Periosteum provides one of the larger niches for stem cells in the body, when one considers other adult mammalian stem cell niches including bone marrow, skin/hair follicle, intestine, neuron and testis.[36,37] Similar to other stem cell niches comprising stem cells, supporting niche cells, extracellular matrix (ECM) and soluble factors, periosteum's stem cell niche is connected by the circulatory and nervous systems.[37,38] Periosteum is replete with blood vessels as well as sympathetic and sensory nerve fibers, whose distribution in the different layers of the tissue has been shown to vary in normal and osteoporotic rats.[39]

Periosteum is a dual layered composite tissue comprising an outer fibrous layer of ECM (predominantly collagen with some elastin[27]) and an inner cambium layer where its mesenchymal stem cells, referred to as periosteum-derived stem cells (PDCs), reside.[35] As noted above, collagenous Sharpey's fibers, which insert at an ~45 degree angle to bone surfaces, physically anchor periosteum to bone surfaces and maintain the tissue in a state of tensional pre-stress *in situ* (*cf*. *Mechanical and permeability properties of periosteum*). Interestingly, measures of periosteal cambium and fibrous layer thickness and cellularity in human tibiae and femora from aged donors show no correlation with age or body mass, although the cambium layer of the predominant bending axis of the tibia exhibits greater thickness and cellularity than that of the femur.[35] In osteoporotic rats, metaphyseal periosteum thickness and cellularity are increased over normal, age-matched controls.[39] Gross changes in layer organization, thickness and cellularity are visible in mid-diaphyseal periosteum in amputated and contralateral or proximal long human bones of the lower extremity.[35] Hence, periosteum's stem cell niche may provide an ideally situated interface to serve as a mechanosensor as well as an actuator for healing via cellular and molecular trafficking.

The periosteum's regenerative power has been harnessed clinically to heal critical-sized bone[4,13,40,41] and cartilage[42–44] defects. Periosteal resection or lifting leads to the separation of the Sharpey's fibers, which then abruptly alters tissue stress state[5] and results in anisotropic tissue shrinkage.[10] Changes in periosteum's mechanical stress state correlate with rapid mobilization of PDCs to injury site, and PDC-driven tissue repair.[4,6,45] Interestingly, in a study correlating predominant stress states (magnitude and mode of loading) to volumes of tissue generated in critical-sized bone defects, early tissue genesis correlates significantly to the maximal net change in strain (above *circa* 2000–3000 microstrain, in tension or compression), rather than strain magnitude *per se*, providing further evidence that changes in cell shape may drive mechanoadaptation by progenitor cells. Furthermore, mechanical loading has been linked to gene expression pattern

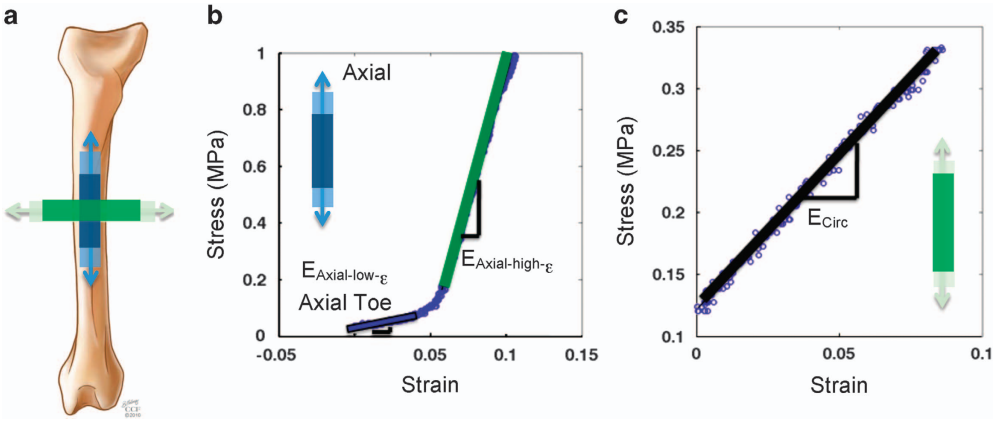

Figure 2 Mechanical properties of periosteum depicted using an example from studies on samples from the anterior periosteum of sheep femora. Stress and strain (ε) were measured in the axial (blue) and circumferential (green) directions. (**a**–**c**) Periosteum stiffness (*E*) is highly anisotropic; E_{Axial} is significantly different from $E_{circumferential}$. (**b**) When loaded in the axial direction, periosteum exhibits strain stiffening with low, linear elasticity at strains up to *circa* 0.04 and high, linear elasticity at strains above *circa* 0.06. (**c**) When loaded in the circumferential direction, periosteum exhibits linear elasticity, with $E_{axial\text{-}low\text{-}\varepsilon} < E_{circ} < E_{axial\text{-}high\text{-}\varepsilon} \sim$. Adapted from McBride *et al.*[10]

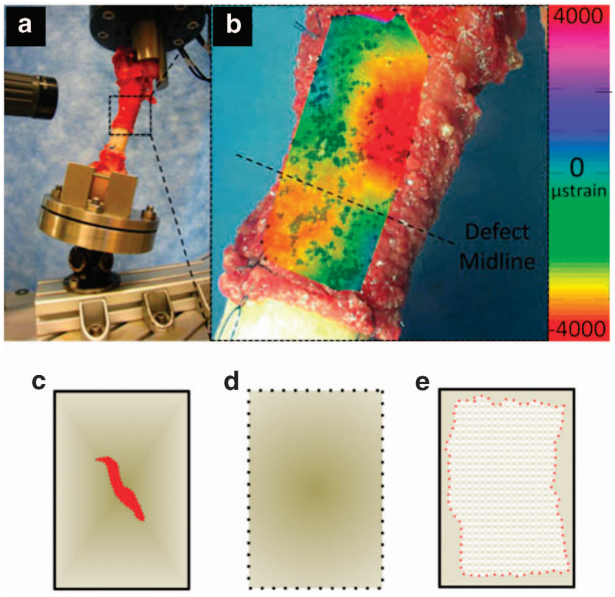

Figure 3 The intrinsic stress of periosteal tissue emerges from nano- to microscopic structure of the tissue and impacts the function of the tissue at multiple length scales, from nano to meso. Depicted schematically. (**a**, **b**) Effects of mechanical loading, for example, imbued through stance shift, are observable from the tissue to the cellular length scales as heterogenous strain distributions (colors, from negative compressive strains to positive tensile strains). Adapted from McBride *et al.*[5] (**c**) If the periosteum is damaged through trauma (red area), intrinsic tissue stresses are expected to redistribute locally, depicted schematically as a gradient in color. (**d**) Periosteal lifting cuts through the Sharpey's fibers and disconnects the periosteum from the bone surface. Even if the periosteum tissue itself is not cut, it is expected that the stress state of the tissue will shift, with stress relaxation in areas further from intact boundaries with Sharpey's fibers, depicted schematically as a gradient in color. (**e**) Intact periosteum is pre-stressed *in situ*. When a piece of periosteum is removed from the bone, with cutting of Sharpey's fibers in the process, the tissue shrinks anisotropically, indicative of its biaxial pre-stress state, depicted schematically in the overlay with red dotted edges.

changes favoring an increased PDC proliferation.[46] A better understanding of factors linking mechanical cues and activation of PDC's niche is imperative for unlocking the periosteum's remarkable regenerative power.

Recently, we demonstrated quantitatively that loss in periosteal pre-stress leads to significant changes in the fibrous layer's ECM architecture, observed as a significant increase in the degree of collagen crimp (**Figure 4a–d**). Loss of tissue pre-stress is accompanied by an immediate increase in nuclear rounding within the cambium layer of the periosteum, where the PDCs reside, which persists for several days (**Figure 4e**).[17,18] Our working hypothesis is that the periosteum acts as a mechanosensor and actuator of the stem cell niche, where changes in periosteum's baseline stress state modulate the quiescence of its stem cell inhabitants, triggering the rapid cell egression and homing to sites of injury observed in preclinical ovine studies as well as differentiation and tissue genesis.[2,4–9,11,47]

PDC Response to Shape- and Volume-Changing Stresses

The putative role of shape and volume-changing stresses on stem cell differentiation has become increasingly proven over the past decade. When subjected to controlled shape- and volume-changing stresses[24] or patterns of cell adhesion proteins typical for epithelial or mesenchymal tissue templates,[14] mesenchymal stem cells exhibit patterns of gene expression typical for nascent lineage commitment. Efforts are underway to define libraries of such mechanical cues[24] or patterns of adhesion proteins[14] that can be used as a reliable and effective means to induce stem cell differentiation toward the specific types of tissues.[25] Periosteum as well as endosteum and bone marrow are the main sources of skeletal stem cells.[13,16,19,48] PDCs are particularly useful for *in vitro* bone regeneration study because they remain multipotent and are able to proliferate at the high rate while still *in vitro*.[49,50]

By virtue of inhabiting the highly active and mechanosensitive environment of the periosteum, PDCs are constantly exposed to different degrees of deviatoric (shape changing) and dilatational (volume changing) stresses. These stresses occur simultaneously to the biaxial pre-stress intrinsic to periosteum's collagen–elastin fibrous network, from early stages of development and growth[7,51] and throughout life.[3,10] Hence, the local mechanical milieu of PDCs is highly dynamic and sensitive to the pre-stress state of the periosteum.[5,17,18] The cytoskeletal networks of PDCs organize and reassemble, regulating their intracellular tension and controlling cytoskeletal-related chemical pathways that maintain bone homeostasis and growth.[6,25,52]

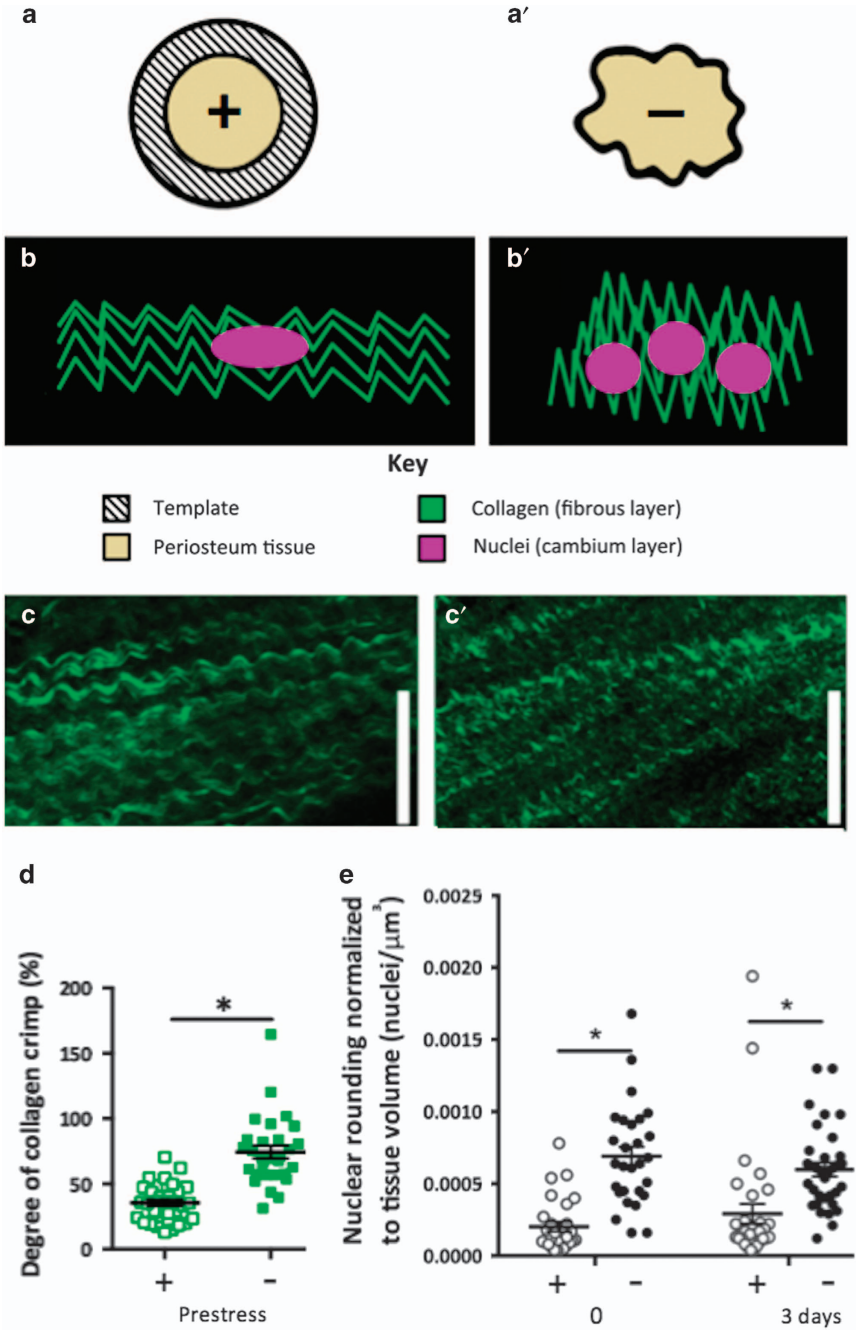

Figure 4 Recent studies implicate periosteum as a mechanosensor and actuator of the stem cell niche. (**a**, **a′**) Release of periosteum's intrinsic mechanical pre-stress (i.e., with pre-stress, +, maintained by annular template, and without pre-stress, −) leads to changes in collagen structure (**b**, **b′**, **c**, **c′**,**d**) within the fibrous layer and nuclear shape of cells within the cambium layer (**b**, **b′**, **e**). Such changes in nuclear shape have been shown to up- and downregulate gene expression typical for nascent lineage commitment of stem cells.[20-25] Figure adapted from Yu *et al.*[17,18]

Many different proteins and pathways have been shown to be involved in PDCs' response to mechanical forces and their differentiation. For instance, periostin, which regulates periosteal interaction with the ECM, has a pivotal role in this process. Periostin levels increase during embryogenesis and when cells are exposed to stress, suggesting a role for periostin in bone mechanoadaptation.[53] Moreover, periostin is capable of binding to glycoproteins, glycosaminoglycans and proteoglycans, implicating a central role for periostin in regulating the mechanical environment of ECM.[53] Furthermore, the expression level of periostin is regulated by the Wnt signaling pathway, TGFβ, BMP2 and retinoic acid.[54–56] Mechanisms by which stress modulates the expression of these pathways,[47] these pathways' interactions and their role in maintenance of PDCs' bone-generating capacity are still under investigation.

In sum, the tissue-generating capacity of PDCs is sensitive to their local mechanical environment; however, underlying mechanisms of transduction are yet to be elucidated. Given the high mechanosensitivity of the stem cells to shape- and volume-changing stresses, it will be crucial to study how these

stresses affect the shape and volume of PDCs as well as intracellular pathways responsible for PDC-mediated tissue genesis and differentiation.

Future Directions

Periosteum is a remarkable, smart material whose mechanobiology provides a putative means to regulate stem cell quiescence. While the past decade of research has led to myriad discoveries of mechanisms underlying periosteum's mechanobiology and regenerative capacity, further studies are needed to elucidate the tissue, its cellular constituents and mechanisms of PDC-mediated healing. Recent advances in multiscale live imaging will be key to tie changes in tissue mechanobiology to PDC egression, homing and tissue genesis.[17,18]

The intricate composite structure of the periosteum (*cf. Periosteum as mechanosensor and actuator of stem cell niche*) suggests that it might also function as a type of membrane bioreactor, through the multiscale electro–chemo–mechanical coupling behavior of its constituents. Previous theoretical investigations of other musculoskeletal tissues with similar characteristics[57] have uncovered complex, yet fundamental emergent properties. Such properties may also be found in the periosteum because of tight junctions between PDCs,[14] ion gradients and mass transport through an electrolyte solution, and local electrostatics.[58] Negatively charged aggrecans[59] induce the presence of an electrical double layer potential in tight junction nanopores, which could explain the directional dependent and selective permeability characteristics of periosteal tissue.[9,14] Within this framework, our working hypothesis is that Sharpey's fibers represent sites where these electromechanical phenomena would be less evident and flow would be solely limited to hydraulic resistance, describing the site specificity of periosteum permeability observed in experimental measurements.[57]

Computational modeling is a promising avenue to explore such multiscale electro–chemo–mechanical behavior of the periosteum in combination with the aforementioned multiscale live imaging techniques.[8,60] Multiphysical models combining electrohydrodynamics, chemical transport and electrostatics[61,62] will assist us in understanding the role of periosteum as a functional barrier capable of selectively allowing the passage of particular molecules and adjusting its permeability and directionality.

Conflict of Interest

The authors declare no conflict of interest.

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