EXPERT OPINION

Meeting current musculoskeletal health demand through deeper insights into tissue homeostasis and regeneration

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The burden of chronic musculoskeletal disorders is challenging and prompts therapeutic advancements. The notion that chronic conditions such as osteoarthritis and tendinopathy are linked to deficient healing by failure of one or several of the cellular/molecular processes involved is gaining ground. Alterations underpinning disruption of healing mechanisms that contribute to the development of chronic musculoskeletal pathologies include unresolved inflammation, abnormal angiogenic status, alterations in paracrine communication, decline in stem cell functioning and inability to maintain homeostasis in the extracellular matrix compartment. The complexity of failed healing may be challenged with interventions that target multiple biological processes such as cell therapies and/or platelet-rich plasma.

Keywords: cell therapies, osteoarthritis, platelet-rich plasma, tendinopathy, tissue healing

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1. Introduction

In the next two decades, all countries in the developed world will have to meet the medical needs and the economical implications of the growing musculoskeletal health demand at an affordable price. The situation was partly foreseen at the beginning of the millennium, when the United Nations/World Health Organization launched the international collaborative movement named the 'Bone and Joint Decade' (2002 – 2011) to address the need for research and novel therapies. Among musculoskeletal ailments, osteoarticular conditions and tendinopathies, which affect both the elderly and young adults, are common, and cause severe long-term pain and chronic impairment, impacting quality of life and favoring the adoption of sedentary ways of living.

The problem is getting worse since evolving data point to an increased incidence of the risk factors including aging, traumatic sports injuries and metabolic diseases. Knee osteoarthritis (OA) illustrates this tendency, as it is no longer a condition of the elderly. Currently, the estimated median age of knee OA diagnosis is 55 years, the incidence being higher among obese (1.02% per year vs 0.37% per year for non-obese males) [1]. Total knee replacement is now more prevalent than rheumatoid arthritis, and nearly as prevalent as congestive heart failure [2]. Nearly 1.5 million of those with primary total knee replacement are in their 50s – 60s, and therefore prone to costly revision surgery and other long-term complications. Not only morbidity but also financial costs are of great consequence. Critical progress on novel biological interventions could meet pressing health needs, reduce costs and make patients to resume an active healthy lifestyle.

However, the scope for new therapeutic interventions is limited by our insufficient understanding of chronic musculoskeletal diseases. Over the past decade, the

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notion that chronic musculoskeletal conditions such as OA and tendinopathy are linked to deficient healing by failure of one or several of the cellular/molecular processes involved has gained ground. In this editorial, we suggest that conceptual progress in our understanding of the biology of failed healing mechanisms may eventually help in developing superior therapies.

2. Emerging concepts in non-healing mechanisms: hallmarks of tissue repair and its failure

Healing may be hindered by repetitive mechanical stress without sufficient time to heal, as postulated for overuse tendinopathies. Also, joints such as the knee or the hip suffer cyclic stresses and strains that can provoke continual traumatic microdamage to bone, cartilage and/or meniscus. If these areas of microdamage are not repaired, they may grow and accumulate into frank, macroscopic damage. Prolonged failed healing triggers an adaptive response that involves cellular and molecular changes that further exacerbate the inability of the tissue to cope with stressors. Non-healing with time progressively deteriorates critical structural and functional features.

The complexity of this problem is highlighted by the main hallmarks associated with failed healing and could be used to categorize available molecular and cellular knowledge. To achieve conceptual progress, similar abstractions were recently performed in cancer [3] and aging research [4].

Alterations underpinning disruption of healing mechanisms that help to acquire a chronic musculoskeletal pathology include at least five features: unresolved inflammation, abnormal angiogenic status, alterations in paracrine communication, decline in stem cell functioning and inability to maintain homeostasis in the extracellular matrix (ECM) compartment. Although tissue/organ-specific features exist regarding pathophysiology and healing potential of OA and tendinopathy, they share common features of degenerative diseases. Tissue/organ degeneration is consequence of some failure in healing mechanisms. Therefore, novel insights in these mechanisms may provide basis for novel treatment development.

2.1 Unresolved inflammation

When tissue is stressed, inflammation is a crucial mechanism to restore tissue homeostasis. Resolution of inflammation involves timely arrest of leukocyte infiltration and adequate polarization of macrophages. Discrepancies in these processes have been reported at some stage of the disease in OA and in tendinopathy. In fact, immune activation is present in the early phases of development of OA. Moreover, progression is attributed to modifications in Toll-like receptors (TLRs), in particular TLR-2 and TLR-4 [5]. Toll signaling through binding endogenous danger-activating molecular patterns (DAMPs) (i.e., molecular fragments of the ECM, intracellular molecules released to the extracellular environment on cell damage) is involved in the control of tissue repair and its failure, but the detailed mechanisms involved are not yet described. Additionally, the presence of activated T cells in knee/hip OA patients confirms the involvement of immunological/inflammatory components.

Details about the role of DAMPs (also known as alarmins) such as high mobility box group 1, heat shock and S100 proteins, hyaluronic fragments, uric acid, nucleic acids and mitochondrial components, in tendinopathy are emerging [6]. TLR binding is the preliminary step to activate various intracellular pathways including NF-κB signaling and ensuing release of inflammatory cytokines. Subsequent deregulation of key proinflammatory molecules (IL-1β, TNF-α) may prolong the inflammatory phase and delay healing. In addition, this mechanism has repercussion for ECM homeostasis (another hallmark of non-healing tissues) because NF-κB-activated cells can be involved in MMP-mediated ECM damage.

2.2 Abnormal angiogenic status

Normal healing is characterized by an angiogenic stage in which blood vessels proliferate to cope with the nutrient demand of proliferating cells. This is followed by downregulation in cell number and regression of blood vessel. The latter is fine-tuned by pro- and anti-angiogenic factors that either promote or inhibit angiogenesis. Cyclic loading of human tendon cells maintains an increased expression and activity of angiogenic factors, and this has been proposed as a feature of tendinopathy. Fibroangiogenic features are manifested in some tendinopathies and in the synovium in early phases of OA [7]. Moreover, angiogenesis-related cytokines have been proposed as sensitive markers for early tendon degeneration. The current paradigm proclaiming that, unlike other tissues, tendon and cartilage cannot follow the classical sequence of tissue healing mechanisms because of the lack of innervation and vascularization is eclipsed by their secretory paracrine capability. In fact, tenocytes and chondrocytes synthesize soluble mediators that can affect the migration of several populations of cells, including innate immune cells and cells involved in angiogenesis.

2.3 Alterations in paracrine communication, dynamic reciprocity

The concept of dynamic reciprocity, that is, crosstalk between adjacent tissues, helps in understanding how modifications in the biological microenvironment will affect neighboring cells through paracrine signaling [8,9]. Similarly, the concept applies to current understanding of OA as a disease of whole organ, involving not only the cartilage but also the synovium, menisci, subchondral bone, fat pad and other soft tissues. In this context, dynamic reciprocity means that injury starting in the cartilage will elicit a response of the whole joint with preponderance of some tissues over others, that is, the synovium or the subchondral bone in the early response to injury.

Similarly, tendon cells are no longer considered in isolation, because close structures such as the paratenon or synovium are strongly involved in tendon functioning.

2.4 Decrease in the regenerative capacity of stem cells

The success of tissue repair also depends on the functioning of stem cells. Results obtained after heterochronic parabiosis, that is, exposure of an aged organism to a youthful systemic environment, show that failed healing could be attributed not to the stem cells but to the aging process [10]. Identifying systemic differences between old and young may help in achieving molecular control over the niche composition and adequate stem cell activation.

2.5 Dysregulation of the ECM compartment

Adaptation to sustained tissue stress also involves cell-extrinsic mechanisms that regulate several aspects of the ECM compartment (i.e., ECM remodeling, cell positioning, cell–ECM interactions). However, the use of pro-anabolic products osteogenic protein 1, fibroblast growtth factor 18, inducible nitric oxide synthase inhibitor or risedronate (for maintaining bone support) or products that inhibit catabolic enzymes have been discontinued due to the safety and/or efficiency failures [11].

3. Biological interventions

Reversing non-healing mechanisms represents a therapeutic opportunity [12]. But, given their complexity and our insufficient knowledge, even halting the progression of the disease can be a reasonable target. Currently, pharmacological interventions aim at targeting pathways common to inflammation, that is, components of the NF-κB, and MAPK pathways. Alternatively, catabolic cytokines and proteases (inhibitors of IL-1b, MMPs, aggrecanases) have been examined as structure-modifying treatments. Pain reduction was the goal of mAbs to NGF including tanezumab and fulranumab, but safety issues have arrested this development [13].

Instead, the complexity of failed healing may be challenged with interventions that target multiple mechanisms, such as cell therapies and platelet-rich plasma (PRP) therapies. These treatments are distinct from those delivering the classic drug that triggers a single target, in that they modify the system in several dimensions. Both are attractive options to harness natural healing resources, and direct non-healing conditions, toward healing and restoration of tissue homeostasis [14,15].

Both therapeutic approaches exploit the endogenous regenerative capacity, but detailed mechanism of action for PRP or mesenchymal stem cells (MSCs) has not been discovered yet.

These interventions stem from two concepts namely, providing competent cells, seeking engraftment and restoration of tissue function and/or modifying the biochemical microenvironment by providing a pool of signaling molecules involved in early healing. Most cell therapies in cartilage disease involve *ex vivo* expansion of chondrocytes or MSCs derived from adipose or bone marrow tissue and local delivery in the joint. Stem cell therapies may offer advantages over chondrocyte transplantation. Different cell sources, including fat pad, bone marrow, adipose tissue, the synovium and peripheral blood stem cells, are being tested. Improved OA symptoms have been reported in few randomized studies using autologous stem cells [16-18]. Stem cell research is fueled not only by the academia but also by the industry. Currently, off-the shelf allogeneic stem cell products, that is, Cartistem and Cartiform are tested for localized cartilage defects and meniscus regeneration [19]. However, developments are slow because of the high regulatory burden that applies for these cell products. Meanwhile, stem cell tourism is growing as patients with joint conditions seek through internet for alternative stem cell treatment in unregulated clinics round the world.

Investigational cell therapies for tendon have used skin cells (because of their transdifferentiation capacity) or tenocytes; alternatively, the efficacy of MSCs is being examined. In all cases, human clinical trials support the biosafety of the approach but the efficacy is not obvious; hence, discussion on stem cell therapies must reflect clinical research reality as well as the costs.

Recent research in rejuvenation indicated that the age of the host dictates the success of the approach [10]. The failure of triggering regenerative mechanisms is not attributed to the stem cells but to an old niche, that is, the environment that regulates cell fate. Recent data indicate that the niche can be revitalized by administration of determined molecular signals. For example, calibration of Notch, Wnt and TGF-β/Smad signaling pathways recovers the deficiency of repair of old muscle [10].

PRP, an autologous plasma fraction of peripheral blood, is the simplest regenerative medicine intervention that has rapidly extended to multiple medical fields, mainly because of its ease of use and biosafety. The concept is that PRP induced healing by providing a molecular milieu for regeneration. In this context, PRP aims to reactivate failed healing mechanisms, likely providing signaling factors that help to resume inflammation and angiogenesis and facilitate the recovery of ECM homeostasis. As these products are largely safe, with an advantageous balanced risk–benefit, clinical applications have preceded exhaustive basic research. Thereafter, most studies were directed to examining clinical outcomes rather than identifying the precise biochemical mechanisms underlying the effects of PRP. Nevertheless, intra-articular injections of PRP are more efficacious than hyaluronic acid. In tendinopathies, PRP injection has potential to improve pain and function, and the challenge is to identify patients for whom the therapy is indicated [20].

4. Expert opinion

The economic and social burden of musculoskeletal disorders is challenging and calls for therapeutic advancements.

Biological interventions in their actual form cannot meet the clinical demand for long-lasting treatment. Identifying hallmarks may open up many avenues worth being explored in non-healing mediated dysfunction.

Because an immune response occurs as the earliest response to tissue stress, it may drive non-healing mechanism participating in the onset of tendinopathy or OA. However, current understanding is limited by insufficient knowledge regarding leukocyte transmigration code, or how to achieve adequate macrophage polarization for inflammation to be resumed. In some disease phenotypes, healing is inadequate because of a misbalanced angiogenic status. Potentially instructive aspects of failed healing such as alterations in paracrine signaling are useful for constructing hypothesis where therapeutic developments could be based. Future investigations will need to unravel molecular and cellular features as indicators of the various non-healing hallmarks. There is growing awareness of the benefits of using biomarkers that measure and evaluate normal and pathological healing and responses to therapeutic interventions.

Novel technologies are making it possible to deal with the intrinsic complexity of regenerative mechanism. Groundbreaking advances in technologies for profiling gene expression patterns and protein functionality have provided deeper insights. However, current investigation unveil a good deal of yet-to-be-understood complexity, and our limitations in elucidating how to fit the molecular details within the intricate patterns of failed healing limit the development of translational approaches. Algorithms to predict multiple biological facets such as protein interactions in normal and failed healing using structural data and spatial and temporal gene expression hold promise in the near future. Additionally, we need to investigate population dynamics and individual cell functions within the different compartments of the body *in situ*. However, technologies aimed at this are still expensive, limited and cumbersome.

Refinement of regenerative medicine therapies should be framed in practice-oriented approaches with crucial clinical implications entailing improvements in symptoms, and arresting or even driving backward the progressive deterioration. From clinical trials, we must obtain reliable data of effectiveness parameters that can have crucial clinical implications in the short- (improvement of symptoms) or long-term events (percentage of regenerated/degenerated tissue).

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents, received or pending, or royalties.

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