Regenerative medicine in the field of pain medicine: Prolotherapy, platelet-rich plasma therapy, and stem cell therapy—Theory and evidence

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KEYWORDS:
Regenerative medicine; Prolotherapy; Platelet-rich plasma; Stem cell therapy

The concept of "regenerative medicine" (RM) has been applied to musculoskeletal injuries dating back to the 1930s. Currently, RM is an umbrella term that has been used to encompass several therapies, namely prolotherapy, platelet-rich plasma therapy (PRP), and stem cell therapy, which are being used to treat musculoskeletal injuries. Although the specific treatments share similar concepts, the mechanism behind their reparative properties differs. Recently, treatments that possess a regenerative quality are resurfacing and expanding into the musculoskeletal field as potential therapeutic treatment modalities. RM, in the form of prolotherapy, was first used to treat tendon and ligament injuries. With the advancement of technology, RM has expanded to PRP and stem cell therapy. The expansion of different RM treatments has lead to its increase in the application for ligament and tendon injuries, muscle defects, as well as pain associated with osteoarthritis and degenerative disks. Recently, the use of ultrasound has been added to these therapies to guide the solution to the exact site of injury. We review 3 forms of RM injection: prolotherapy, PRP therapy, and stem cell therapy.

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Historically the field of interventional pain medicine has focused primarily on spinal procedures. However, musculoskeletal injuries as a whole are the most common cause of severe long-term pain and often affect psychosocial status, social interactions, and employment. With a worldwide incidence of more than 100 million musculoskeletal injuries annually, pain physicians have now begun to direct their attention to this area. Regenerative medicine is a developing field with the goal of inciting repair or replacement of pathologic tissues through the augmentation of natural processes or bioengineered means. Included in this umbrella of regenerative medicine are prolotherapy, platelet-rich plasma, and mesenchymal stem cell therapies. As increasing evidence on regenerative medicine is generated, more interventional pain physicians are looking to use this field for pain-mediated peripheral sources via percutaneous means.

Prolotherapy

The term "prolotherapy" first appeared in the medical literature during the mid-1950s and was described as a form of treatment for "incompetent structures through the generation of new cellular tissue." Introducing an irritating substance to induce healing has been used since the time of Hippocrates; however, the modern use of prolotherapy in musculoskeletal injuries can be traced back to the 1930s.
Proliferant solutions used in this form of treatment are hypothesized to induce collagen deposition through chemomodulation, as well as the "temporary neurolysis" of peripheral nociceptors through chemoneuromodulation. Modulation in this setting is purported to be mediated by multiple growth factors and cytokines. To date, the exact mechanism of prolotherapy remains elusive and has not been well defined by high-level evidence studies.

Although the exact proliferant used in prolotherapy often varies, all solutions, excluding chemotactic agents, have the universal effect of inciting local tissue irritation, which leads to an influx of inflammatory cells. Inflammation, being the first step in the wound-healing cascade, results in the end-product of fibroblast proliferation with the subsequent deposition of collagen. The different proliferants can be classified into 3 classes. Osmotic agents, which include hyperosmolar dextrose, zinc sulfate, and glycerin, act by dehydrating local cells to the point of rupture in a process referred to as "osmotic shock." Phenol, guaiacol, and punicic acid belong to the second class known as irritants and act by either directly damaging cell membranes or causing local cells to become antigenic. Chemotactic agents are the final class and include the commonly used sodium morrhuate. This class is purported to be a direct chemotactic agent to inflammatory cells, which differs from the former classes that induce inflammation indirectly. The injection protocols of these proliferants lack standardization and differ with regard to volume and concentration of proliferants used, frequency of injections, injection technique, and concurrent co-interventions.

Now more commonly referred to as "regenerative injection therapy" (RIT), prolotherapy is currently used in a wide variety of chronic pain entities. The effects on tendons and ligaments have been widely discussed in medical literature, ranging from animal studies to randomized controlled trials in humans. Animal studies have demonstrated RIT's significant advantageous effect on tendon cross-sectional area and strength. This is in contrast to animal studies regarding ligaments that have been separated by discoid. While Liu and colleagues found an increase in force failure, mass, and fiber diameter in ligaments, a more recent study showed RIT had no significant effect on fiber diameter or force failure.

In human trials, Reeves and Hassanein showed significantly improved ligamentous stability and knee flexion in patients with anterior cruciate ligament laxity 36 months after hyperosmolar dextrose injections. Case reports have also demonstrated magnetic resonance imaging verified repair of complete anterior cruciate ligament and Achilles tendon tears following a series of RIT, without surgical intervention. In addition, RIT has been documented as a favorable treatment modality in overuse syndromes such as lateral epicondyle tendinopathy, Achilles tendinosis, coccygodynia, chronic groin pain, and plantar fasciitis. The benefits of intra-articular RIT have been assessed in multiple joints. Reeves and Hassanein have reported significant reduction in pain, as well as radiologic improvement, in patients with knee osteoarthritis (OA). Patients with finger OA also showed significant improvements in pain and range of motion following hyperosmolar dextrose injections compared to controls at 6 months. A randomized controlled trial of the sacroiliac joint demonstrated significant improvement in pain after intra-articular RIT. Although the pain was not statistically significant in comparison with intra-articular steroid injection, the RIT group had a longer period of pain relief.

Studies for the use of RIT in neck and back pain have been widely investigated. Its use for cervical-mediated pain has been promoted, with retrospective studies demonstrating improvement in pain and function following whiplash injuries. High-level evidence regarding nonspecific low back pain has been less congruent. Complicated by limitations in methodology and the heterogeneity of clinical protocols, studies of RIT on low back pain have been difficult to interpret collectively. However, the response of leg pain secondary to moderate-to-severe lumbar degenerative disk disease appears promising in the case series by Miller and colleagues. This uncontrolled study showed statistically significant pain improvements in patients treated with 25% dextrose, suggesting RIT may have an indication in this specific patient population.

Despite a minimal volume of robust evidence to support its use in musculoskeletal pathologies, RIT's overall safety profile and strong anecdotal evidence leads many physicians to continue using this treatment modality for a wide array of chronic pain entities.

Platelet-rich plasma therapy

In the search for better modalities in the nonsurgical treatment of musculoskeletal injuries, platelet-rich plasma therapy (PRP) has recently been thrust to the forefront of public attention. Initially used clinically in the fields of cardiothoracic and maxillofacial surgery in the late 1980s and early 1990s, PRP has since been adopted into the field of musculoskeletal medicine. The concept behind its use as a nonsurgical treatment modality is to place it directly into areas of soft tissue pathology, via percutaneous injection, to facilitate tissue regeneration. Healing is theorized to occur secondary to the PRP's ability to augment the recruitment, proliferation, and differentiation of cells involved in the regeneration of injured tissue. These actions are purported to be mediated by numerous growth factors and bioactive proteins secreted by PRP's platelets following activation, in a process known as degranulation. The exact mechanism by which PRP acts to augment the endogenous healing process remains elusive despite extensive research.

PRP by its strictest definition is an autologous sample of blood with a concentration of platelets above the physiological baseline. Following the extraction of autologous venous blood with a large-gauge needle to prevent prema-
ture platelet activation,53 platelets are separated from other blood components and further concentrated.50 This occurs through a centrifuge process, in which platelets are able to be isolated from the other cell components of blood based on their physiological size.50 The further concentrating of platelets occurs with subsequent centrifuge cycles.54 Although some authors suggest a minimal platelet concentration to be "therapeutic,"48,52,55 studies with lesser concentrations have shown good results.52,56 Numerous PRP preparation centrifuges are available for clinical use, with each system producing a slightly variable end product. Differences include platelet concentration, presence of leukocytes, and total amount of PRP produced.49,50,57 The administration protocol of PRP currently remains nonstandardized. Although historically "activated" by exogenous substances, evidence may now suggest that PRP may be activated endogenously by type I collagen in vivo.58 This degranulation method may reduce the loss of platelet-secreted bioactive mediators and also the risk of activator agent-induced complications such as coagulopathy.45,50,51

The effects of PRP have been extensively documented for a variety of tissue types, in a vast number of different laboratory and clinical settings. Despite the uncertainty of its intrinsic healing mechanism and administration protocol differences, the early evidence of PRP's use for musculoskeletal-mediated pain entities has been encouraging. In vitro and animals studies have demonstrated positive results in numerous types of tissue injuries that frequently plague patients including muscle,59 tendon,60,61 meniscus,62 ligament,63 cartilage,64-66 osteochondral surfaces,67 nerve,68,69 and intervertebral disks.70,71 Although anecdotal evidence exists to support its percutaneous clinical administration for muscle,72-74 and acute ligamentous pathology,50,75 high-level evidence is mostly void. Pain generated from connective-tissue of the foot has had minimal investigation. A pilot study of plantar fasciitis reported nearly 80% of patients showed improvement at 1 year following ultrasound-guided PRP injections.76

OA occurs in up to 9.6% of men and 18% of women over 60 years of age and is expected to be the fourth leading cause of disability within the next 10 years.1 Local anesthetic and corticosteroids historically used for treatment are now being called into question, due to their purported detrimental effects on structure and inability to affect the natural history of OA.77-81 The use of PRP as a treatment modality has gained recent interest and may be a possible future candidate to treat this condition. Two recent pilot studies, including 1 involving over 100 degenerative knees, showed data suggestive of pain reduction, improved knee function, and improvement in quality of life at 1 year following intra-articular PRP injection.82,83 Although long-term follow-up demonstrated a decline in pain control, pain improvement remained above baseline.84

The use of PRP for chronic tendinopathies has been investigated, with pilot studies showing advantageous effects stemming from its percutaneous administration at various anatomical locations.85-87 Perhaps 1 of the most compelling pieces of literature to support the use of this treatment modality in tendinopathy has been described in a level 1 evidence study by Peerbooms and colleagues. The authors evaluated the response to intratendinous injections of either PRP or corticosteroids in patients with refractory lateral epicondyle tendinopathy. The results showed a significant difference in pain control and function in favor of the PRP group at 26- and 52-week follow-ups.88

Much like RIT, the overly theme that precludes strong comparison of studies in the current medical literature is the lack of randomized controlled trials with protocol standardization. Future studies need to address questions regarding PRP, such as possible long-term side effects associated with administration, how storage of PRP affects efficacy, and how the presence of leukocytes effects different tissue subtypes. Procedural standardization also needs to be addressed in terms of injection technique, postinjection protocol, and concurrent use of nonsteroidal anti-inflammatory drugs.

**Stem cell therapy**

Adult tissues often have the ability to repair and regenerate following injury. To date, the exact mechanism of repair is poorly understood; however, it is hypothesized to occur through the proliferation and differentiation of cells that ultimately restore tissue functionality. One possible explanation is found within nonhemopoietic progenitor cells found in pathologic tissue, as well as cell reservoirs at other locations, which may help to provide this reparative capability.89 These regenerative-capable cells are commonly referred to as mesenchymal stem cells (MSCs), or bone marrow stromal cells.89,90 MSCs are purported to be multipotent cells, with the capability of replicating as undifferentiated cells91 and also multilineage differentiation in response to local cell signaling pathways.89,90,92-95

Over 40 years ago, bone marrow was the first location from which stem cells were isolated.92,95,96 Since that time, stem cells have been found in a variety of tissue types including adipose97-99 and synovium, among others.4,97,100 Differences in marker gene expression between marrow-derived MSCs and MSCs from other sources have been demonstrated. However the effects of these differences have not been fully delineated.94,101 Conflicting evidence currently exists regarding the differentiation efficacy of MSCs obtained from different stem cell reservoirs, such as marrow and adipose tissue.101,102 In addition, there is controversy surrounding the quality of stem cells derived from bone marrow or adipose MSCs with respect to growth kinetics, cell senescence, and multilineage differentiation potential.101-102 These differences in MSCs and mesenchymal-like cells have prompted the International Society for Cellular Therapy to create minimum criteria for MSCs based on surface marker expression, activity in culture, as well as differentiation potential.103

Evidence suggests that in response to injury, MSCs are first mobilized from perivascular niches into peripheral cir-
With subsequent migration into damaged tissues.\textsuperscript{90} Although the exact reparative mechanism of MSCs is uncertain, early evidence suggested that these cells may differentiate into native cells of the injured tissue. More recent evidence suggests MSCs may also induce healing through a paracrine-like effect. This includes the mobilization of MSCs to specific injured tissues, followed by the ability for these reparative cells to secrete bioactive factors that induce a regenerative microenvironment known as “trophic activity.”\textsuperscript{90,107}

Based on their augmenting effects, physicians are now harvesting these cells for clinical application. Following the aspiration from autologous bone marrow, MSCs are typically isolated from other marrow cells and concentrated in a series of steps. The process of isolating MSCs, which are a small portion of marrow’s nucleated cell fraction, occurs most often through their adhesion to plastic in tissue culture.\textsuperscript{4} Evidence by Hernigou and colleagues has suggested that a minimum number of progenitor cells per volume is needed in order for percutaneous injections to be efficacious in tissue repair.\textsuperscript{108} Since bone marrow aspiration contains an MSC concentration well below this suggested “minimum,” the isolated MSCs are further cultured for multiple passages to induce cell expansion.\textsuperscript{4} A passage includes the removal of the isolated cells from culture, with replacement into culture media with new nutrients and growth factors for further cell expansion. This total process typically takes several weeks from the time of aspiration until the final product is administered to the patient.\textsuperscript{4,109}

Literature shows acute skeletal muscle injury in humans leads to the mobilization of MSCs into vascular circulation.\textsuperscript{105} Further studies have demonstrated the stepwise progression of bone marrow–derived MSCs into eventual multinucleated muscle fibers.\textsuperscript{110} In the use of tendons, intratendinous therapy induces histologic and biomechanical improvements in the early stages of tendon healing following injury.\textsuperscript{111} Recent evidence by Mirsha and colleagues also suggests other regenerative medicine modalities such as PRP may enhance the effect of MSCs. The authors of this study showed a statistically significant enhancement in MSC proliferation when exposed to PRP in vitro compared to controls.\textsuperscript{112}

MSCs’ effect on cartilage defects and OA has been well studied and reported. Their ability to differentiate into chondrocytes has been well documented, with evidence suggesting that the use of dexamethasone in micro doses may direct differentiation toward a chondrogenic lineage.\textsuperscript{4} Full-thickness cartilage tear repairs are purported to occur solely through the actions of MSCs.\textsuperscript{113} However, MSCs from osteoarthritic marrow are found to have reduced proliferation rates, reduced chondrogenic activity, and an increase predisposition towards osteogenic differentiation.\textsuperscript{114,115}

These limitations to repair have led clinicians to search for a treatment modality to augment the healing of cartilage through percutaneous intervention. Animal studies have shown evidence of both morphologic and histologic improvements in cartilage healing, as well as meniscus regeneration, following the treatment of intra-articular injected MSCs suspended in hyaluronic acid.\textsuperscript{116,117} Percutaneous injection of bone marrow–derived MSCs in a patient with knee OA yielded a 95% reduction in pain and magnetic resonance imaging evidence of increased meniscus and cartilage volume.\textsuperscript{4}

A surge of interest has been noted in recent medical literature regarding the use of regenerative medicine to combat intervertebral degenerative disk disease. Cells expressing stem cell markers have been identified in intervertebral disks.\textsuperscript{118} MSCs have been 1 of the primary targets of cell therapy in the treatment of disk degeneration, with positive effects seen in animal and in vitro studies. MSCs have shown a superior ability to produce extracellular matrix compared to more terminally differentiated cells. Other effects of MSCs include the ability to differentiate into disk-like cells, prevent disk cell apoptosis, limit disk cell senescence, and retard the local immune system. Theoretically these effects should play a role in preventing the overall degenerative process of intervertebral disks.\textsuperscript{119} The clinical use of MSCs for degenerative disk disease is scant in current literature; however, a recent case study demonstrated significant pain reduction following the percutaneous placement of MSCs into lumbar degenerative intervertebral disks.\textsuperscript{109} This evidence may suggest further clinical trials are needed to determine the role of these cells as a future treatment mobility for intervertebral degenerative disk disease.

Although increasing data are being generated to support the use of MSCs in musculoskeletal injuries, most studies to this point have been conducted using in vitro and animal models. As some authors have appropriately suggested, the effects of MSCs may differ in clinical use given the physiological differences in cellular composition and mechanical loading within species.\textsuperscript{120} Many questions and unverified theories still remain regarding the clinical production, use, and placement of these naturally occurring regenerative cells.

Conclusions

Regenerative medicine is a new intriguing concept on the horizon in the field of pain medicine. Although continued research is greatly needed to determine efficacy and safety profiles, early evidence may foreshadow its future use in clinical practice.

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