



Platelet Rich Plasma (PRP) Therapy Literature Reviews

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VISIONS OF REGENERATIONS TO COME

“Sometime between the years 1934 and 1936, a random patient with a random disease visited a random doctor and for the first time in recorded human history had a better than 50:50 chance of benefiting from the encounter.”—Anonymous

The above-cited quotation still lies sequestered in the dusty archival stacks of a university library—reminiscent of critical comments on medicine by Oliver Wendell Holmes. This anecdote could be referring to any number of important events that occurred around 1934 to 1936 that profoundly affected modern musculoskeletal medicine as we know it today. For example, there was the industrial development of sulfa and penicillin antibiotics, which benefited all of medicine and humankind. Or was it the advent of Osteopathy through the insight of Andrew Taylor Still? Then, it could have been the development of Prolotherapy by George Stuart Hackett and his circle of colleagues.

It was George S. Hackett who, in that era, asked how we could better treat and heal chronic sprain injuries.¹ Hackett reached out to what little was known about wound healing at the time and came up with the pragmatic realization that **stimulating natural inflammation** could be the answer.

We now know that traumatic wound healing or tissue regeneration occurs in four phases:

- **Inflammatory Phase**, which occurs when initially injured, disrupted cells release chemical agents (i.e., so-called “growth factors”) that cause a localized inflammatory reaction. The creating of an inflammatory reaction is the first of a series of cascading events that constitute the entire healing process. Inflammation further releases more growth factors, which, in turn, cause the migration and division of inflammatory cells

needed for the phagocytosis of cellular debris, setting the stage for the next phase.

- **Proliferative Phase**, which occurs when new blood vessels form (i.e., angiogenesis) and fibroblasts migrate, proliferate, and begin depositing (regenerating) Type II collagen, resulting in the formation of so-called “granulation tissue.”
- **Maturation and Remodeling Phase**, which occurs when the Type II collagen fibers convert to Type I collagen and elastin fibers, followed by the formation of collagen fiber cross-linkage,—and
- **Re-epithelialization Phase**, which occurs when disrupted skin or surrounding connective tissue fascia is closed by scarring or regeneration, respectively.

Medical science, even in Hackett’s time, recognized inflammation as the body’s normal process for initiating the healing of the physical disruption of virtually any tissue. Such “physical disruption” might be due to regular wear-and-tear, traumatic injury, infectious disease, or degenerative disease.

Thus, Hackett surmised that injecting just a small amount of irritative substance into the location of a chronic ligament or tendon sprain injury should create an inflammatory response, which *should* ultimately stimulate the healing of the musculoskeletal injury. He chose glucose as a readily available, inexpensive, osmotic irritant—or “proliferant”—solution. As a result, Prolotherapists have been regenerating injured ligament and tendon tissue and healing chronic sprain pain and dysfunction in that fashion ever since.

In the course of applying Hackett’s practice, we have eventually come to respect the difference between ligament versus tendon injuries—as addressed in the

Literature Review of the previous issue of the *Journal of Prolotherapy*. This current review delves further into the state-of-the-science-and-art of the most cutting-edge of those therapies—Platelet-Rich Plasma Therapy—which has lately surfaced in the popular press as an excellent approach to treating especially stubborn tendon sprain injuries.

Platelet-Rich Plasma (PRP) Therapy is a particularly hot topic, nowadays—in the laboratory, the clinic, and on the street. A very recent *New York Times (NYT)* article describes how two Pittsburgh Steelers “used their own blood in an innovative injury treatment before winning the Super Bowl.”² The article goes on to cite several other sports figures who have also been successfully treated in this fashion. It refers to PRP Therapy as a means of delivering a “growth-factor cocktail” to such injuries as “tennis elbow” or “knee tendinitis” (sic).

It is gratifying—if not somewhat humorous—that the advocates for this “new” PRP treatment describe how this “nonsurgical” therapy works by using “the body’s own cells to help it heal”—as if Prolotherapists have not been doing exactly the same thing since the mid-1930’s. And the same PRP advocates tout their noninvasive technique du jour as providing better cost-effectiveness compared to surgery, thereby making PRP Therapy hugely attractive for preferential insurance reimbursement—while standard Prolotherapy remains non-reimbursed by most healthcare insurance programs!

The truth of the matter is that Prolotherapists have been using the earliest version of PRP Therapy for years—achieving all of PRP Therapy’s basic positive attributes, albeit less potent to some degree but at a very small fraction of the cost.

The *NYT* article goes on to say that PRP Therapy “has the potential to revolutionize not just sports medicine but all of orthopedics”—possibly “obviating surgery and shortening rehabilitation.” Isn’t that one reason why Prolotherapists have been calling our style of practice “Orthopedic Medicine”—treating joint injury and dysfunction while protecting our patients, whenever possible, from more invasive, expensive, and potentially debilitating orthopedic surgery by using the nonsurgical, regenerative approach of Prolotherapy?

It is obvious that PRP Therapy is a logically next progression toward perfecting the Hackett technique for repairing extremely recalcitrant, severe ligament

and tendon tear injury. And PRP Therapy may be just technically attractive enough to catch the public’s, the physician’s (medical, osteopathic, and surgical), the dentist’s, the veterinarian’s—and the insurance company’s eye—finally!

As we mentioned in the last *JOP* literature review, Rabago, D. et. al., described a systematic review of the efficacy of four therapies for lateral epicondylitis (i.e., “tennis elbow” or sprain injury of the proximal tendon of the radial extensor muscle of the forearm).³ Those four therapies—including Platelet-Rich Plasma Therapy—are, very basically, four different types of therapy delivery systems. Each system delivers a growth factor or other therapeutic agent of some form to the injured tendon. To better understand PRP Therapy as a unique delivery system, let’s define some basic players.

First, what is a platelet? A platelet is a normal cellular component of blood. Like the normal circulating red blood cell (erythrocyte), the platelet has no nucleus. If the normal red blood cell is about eight one-thousandths of an inch in diameter, the normal platelet diameter is about one twentieth of that. Although very small, the platelet is loaded with various types of “granules” or sac-like secretory vesicles.

Secondly, what is a growth factor? A growth factor is a growth-enhancing peptide or protein that binds to receptors on a cell surface, activating cellular proliferation into more of the same cell form or differentiation (morphing) into another cellular form. In other words, a growth factor is a cell-secreted peptide or protein that promotes or increases (i.e., “up-regulates”) normal cellular functions, such as cell proliferation, differentiation, and tissue repair.⁴

According to the current literature, there are at least 16 major families of growth factors. A platelet alpha granule, alone, contains over 250 different, evolutionarily-related growth factors.⁵

There have been a large number of research-based journal articles written on the general topic of “regenerative” therapy based on the injection delivery of various sources of growth factors—some of which you will see, below. A main intention of the following literature review is to use some of those articles to familiarize the reader—both Physician and Patient—with the basic concepts and language of PRP Therapy. Also, we want to stimulate reading and increase the general level of understanding of

Prolotherapy. Please use the www.pubmed.gov website of the National Library of Medicine to access the following and other articles.

Determination of endogenous growth factors in human wound fluid: temporal presence and profiles of secretion.

Vogt PM, et. al. *Plast Reconstr Surg.* 1998 Jul;102(1):117-23.

Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing.

Eppley BL, et. al. *Plast Reconstr Surg.* 2004 Nov;114(6):1502-8.

ABSTRACT SUMMARY

These first two articles are representative of an immense volume of basic research already achieved in studying the role of growth factors in superficial, cutaneous wound healing. For example, Vogt, et. al., (1998) identified and measured the growth factors present in open skin wounds, including:

- Interleukin-1 alpha
- Platelet derived growth factor (PDGF)
- Insulin-like growth factor 1 (IGF-1)
- Transforming growth factor beta (TGFbeta)
- Basic fibroblast growth factor (bFGF)—and
- Epidermal growth factor.

They and others have identified the basic roles of these growth factors, such as:

- Interleukin-1 alpha—found to be specifically linked to the stimulatory Inflammatory Phase of healing
- Transforming growth factor beta—found to be linked to the matrix formation of the Maturation and Remodeling Phase—and
- Epidermal growth factor—found to be linked to the Re-epithelialization phase.

Eppley, et. al., (2004) went a step further and measured the degree of concentration of platelets and growth factors in PRP. They reported platelets as being concentrated up to 8-fold. Various growth factors, including PDGF, TGFbeta, and vascular endothelial growth factor (VEGF), were found to be concentrated from 3- to 6-fold.

JOP COMMENTARY

Understanding the science of wound repair or healing has been at the forefront of medical interest since the days of Hippocrates with the earliest recognition of the classic inflammatory signs of *rubor* (redness), *calor* (warmth), *tumor* (swelling), and *dolor* (pain).⁶ Since Hippocrates, most of the initial research on healing understandably addressed the healing of cutaneous (skin) wounds. With a continued stream of discoveries based on the advent of the latest analytical tools at the molecular and submolecular level, there has been a burst of recent activity identifying and understanding the sequential roles of the various growth factors involved in wound repair of all tissues—not just cutaneous tissue. More specific basic research focused on the healing of ligaments and tendons is exemplified by the following articles. ■

The roles of growth factors in tendon and ligament healing.

Molloy T, et. al. *Sports Med.* 2003;33(5):381-94.

Independent and additive stimulation of tendon repair by thrombin and platelets.

Virchenko O, et. al. *Acta Orthop.* 2006 Dec;77(6):960-6.

Low molecular weight heparin impairs tendon repair.

Virchenko O, et. al. *J Bone Surg Br.* 2008 Mar;90(3):388-92.

ABSTRACT SUMMARY

Molloy, et. al., (2003) address specific growth factors encountered in ligament and tendon healing. After reading about the factors found in superficial wounds, above, these should appear familiar, including:

- Platelet derived growth factor (PDGF)—produced shortly after tendon injury, stimulates production of other growth factors such as IGF-1, and is involved in the tissue remodeling phase of healing.
- Insulin-like growth factor 1 (IGF-1)—present in the early inflammatory phase.
- Transforming growth factor beta (TGFbeta)—present in the inflammatory phase.
- Basic fibroblast growth factor (bFGF)—present in the late inflammatory phase, stimulates angiogenesis, and regulates cellular migration and proliferation.

- Vascular endothelial growth factor (VEGF)—present after the inflammatory phase stimulating angiogenesis (neovascularization).

Listed with the factors, above, are various general roles that each factor plays in the process of ligament or tendon repair.

Virchenko, et. al., (2006) introduce the concept that the blood coagulation substance, thrombin, plays an additional role in tendon repair, which is not, yet, well understood. The second Virchenko article (2008) supports the first in showing that continuous heparin (anti-thrombin) treatment significantly impairs tendon wound healing by making thrombin unavailable.

JOP COMMENTARY

Ligament and tendon wound healing is a complicated series or cascade of interlinked, molecular events intertwined with the, likewise complex, coagulation (i.e., hemostasis) cascade. Platelets,—well known components of the hemostasis cascade—are now equally well known to be involved in ligament and tendon repair. Thrombin (another well-known hemostasis component), also, demonstrates properties in wound healing that are similar to those of known growth factors, although the exact nature of thrombin's role in healing yet remains to be fully understood. It can be said that thrombin causes the PRP injection to clot. That clot may act as a biological “scaffolding” or physical infrastructure upon which the healing may progress more readily.

Basic research delving into the intricacies of wound healing has served as a natural spring board for developing application of those basic understandings to real world medical and surgical problems. If basic science has shown the importance of the delivery of growth factors to injured ligament, muscle, and tendon tissues, then how can those factors be delivered most effectively facilitate tissue healing? The following articles reflect such delivery applications to a couple of other-than-ligament-tendon tissue injury issues. ■

Intervertebral disc regeneration using platelet-rich plasma and biodegradable gelatin hydrogel microspheres.

Nagae M, et. al. *Tissue Eng.* 2007 Jan;13(1):147-58.

Effects of intramyocardial injection of platelet-rich plasma on the healing process after myocardial infarction.

Li XH, et. al. *Coron Artery Dis.* 2008 Aug;19(5):363-70.

ABSTRACT SUMMARY

Nagae, et. al., (2007) report on the delivery of PRP-impregnated, biodegradable, gelatin hydrogel microspheres to a rabbit model of intervertebral disc degeneration. The experimental PRP group showed significant healing of the disc degenerative process.

Li, et. al., (2008) describe the delivery of thrombin-activated PRP to a rat model of myocardial infarction (i.e., coronary heart attack). The thrombin-PRP injection resulted in the improvement of several parameters that demonstrated enhanced myocardial remodeling and accelerated myocardial healing.

JOP COMMENTARY

These two articles represent the relatively few existing articles relating to the application of PRP Therapy to musculoskeletal tissue injuries other than ligaments and tendons. As shown in these two articles, wounds need not be just traumatic—they may also be due to wear-and-tear degeneration or a vascular accident. Although there is currently only a smattering of study on the application of PRP technique to such tissues, this literature does provide evidence that PRP Therapy is an extremely potent healing remedy—when delivered in an effective way. ■

Platelet-rich plasma stimulates porcine articular chondrocyte proliferation and matrix biosynthesis.

Akeda K, et. al. *Osteoarthritis Cartilage.* 2006 Dec;14(12): 1272-80.

Buffered platelet-rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation.

Mishra A, et. al. *Tissue Eng Part C Methods.* 2009 Feb 13. [Epub ahead of print]

ABSTRACT SUMMARY

Akeda, et. al., (2006) present an “in-vitro” (i.e., laboratory counter-top) model in which porcine chondrocytes (mature pig cartilage cells) were grown in culture media and PRP was introduced into the culture media. While, the cells remained structurally and molecularly unchanged, including their proteoglycan (e.g., glucosamine) and collagen molecular types, cell proliferation and glucosamine-collagen synthesis were enhanced.

Mishra, et. al., (2009) present an interesting in vitro model whereby mesenchymal stem cells were grown in culture media enhanced with either PRP or non-enhanced (control) media. The PRP-treated cells demonstrated increased proliferation and the development of chondrogenic (cartilage precursor cell) molecular markers.

JOP COMMENTARY

These articles introduce another relatively new concept that is becoming a “household” phrase: **tissue engineering**. If we consider ligaments or tendons as having limited regenerative capacity due to their relative lack of blood vessels and regenerative fibroblasts, certainly articular cartilage tissue is even more limited. The tissue engineering approach uses a natural or synthetic “scaffolding” upon which, in these two cases, chondrocytes (articular cartilage cells) or primitive stem cells are carried and nurtured, enabling cellular multiplication (growth) and regeneration of new tissue in the laboratory—or in the outpatient clinic.

As mentioned above, if clotted, PRP can provide a natural infrastructural scaffolding, which is, by design, rich in growth factors. PRP clots when mixed with thrombin, and can be injected into a patient’s site of articular cartilage defect or the complex can be precisely implanted by arthroscopy—rather than necessitating an open operation for implanting the regenerating cell-scaffold complex!

Thus, PRP technology can be very useful by providing a bio-scaffolding within which injectable, tissue-engineered, autologous cartilage cells may be introduced into a wound space to proliferate. There is a significant volume of research directed toward applying PRP Therapy to the problems of joint articular cartilage degeneration—as seen so often in wear-and-tear osteoarthritis. Further stem cell research is likely to bring us to the next major threshold of discovery in understanding and employing this elegant extension of the standard Hackett Prolotherapy model. ■

Augmented bone regeneration activity of platelet-rich plasma by biodegradable gelatin hydrogel.

Hokugo A, et. al. *Tissue Eng.* 2005 Jul-Aug;11(7-8):1224-33.

Benefit of percutaneous injection of autologous platelet-leukocyte-rich gel in patients with delayed union and nonunion.

Bielecki T, et. al. *Eur Surg Res.* 2008;40(3):289-96. [Epub 2008 Feb 15]

ABSTRACT SUMMARY

Hokugo, et. al., (2005) investigated PRP’s ability to enhance bone repair in a rabbit model. The PRP was incorporated into a gelatin hydrogel. This PRP delivery system was applied topically to rabbit ulna bone defects. They observed that growth factors, such as PDGF and TGFbeta, were released directly from the PRP and more slowly released from the hydrogel as it degraded. Successful bone regeneration and bone defect healing resulted.

Bielecki, et. al., (2008) present an application of PRP by injection of autologous platelet-leukocyte-rich gel to delayed-union and nonunion fracture patients—as opposed to employing standard orthopedic surgical open grafting procedures. All delayed union cases demonstrated successful union after an average of 9.3 weeks. The nonunion group demonstrated 13/20 successful unions after an average of 10.3 weeks.

JOP COMMENTARY

There appears to be even more advanced interest in the clinical literature regarding the application of PRP technique to facilitate bone healing, especially in regard to filling large traumatic or post-surgical defects. Again, these studies demonstrate the powerful effect of Platelet-Rich Plasma when used to facilitate tissue repair—essentially for any musculoskeletal tissue. All it takes is the proper delivery system.

SUMMARY

So, the **delivery system** is what it is all about. That is delivering the growth factors and the cellular building blocks (stem cells or mature tissue cells) to the right place at the right time.

Since the 1930s and George S. Hackett’s initial trials, Prolotherapy has been on the cutting edge of modern tissue regenerative therapy and providing that delivery system on-call, any time, any place. As practiced by Hackett

and his followers, Prolotherapy has consistently provided the most basic, inexpensive, effective delivery of the most fundamental wound repairing stimulants or proliferants. Doesn't that fit the definition of "efficacious?" Standard Prolotherapy is both clinically **efficient** and **effective**.

All along, Hackett's Prolotherapy has been the natural forerunner of today's more advanced PRP Therapy! Whenever a Prolotherapy needle penetrates into an injured ligament or tendon enthesis (i.e., the anchoring site of ligament or tendon attachment to bone), a very small, bleeding wound occurs at the needle point. That is why it has always been effective to "pepper" an injection site with numerous, small, gentle needle stabs—to create multiple, tiny wounds, essentially recreating the original sprain injury.

Needle wounding physically disrupts cells and causes cellular release of cellular and tissue-derived growth factors—both healing-specific and hemostatic-specific. Minute needle-wound bleeding results in multiple, equally minute clots immediately occurring at those wound sites—each clot being a local accumulation of circulating platelets, thrombin, and red and white cells. Already released growth factors activate those platelets, other circulating cells, and local tissue cells, all of which release more growth factors and stimulate an inflammatory reaction in a cascading, crescendo fashion.

While performing the minute wounding at needle point, a small amount of proliferant solution is, also, injected into the injury site. This glucose-based, osmotically-active, irritative proliferant causes even more local, physical cellular disruption with the release of more growth factors—causing even further Inflammatory Phase activity.

Thus, standard Prolotherapy causes an enhanced Inflammatory Phase (IP) reaction to ensue. Ultimately, IP-generated growth factors stimulate ligament or tendon fibroblastic cells to lay down (i.e., regenerate) new Type II collagen fibers in the subsequent Proliferative Phase—which is followed by the Maturation-Remodeling and Re-epithelialization healing phases. Ligament and tendon sprain injury healing is the ultimate result with diminished pain and restored function—all this occurring without any PRP necessarily being performed.

So, the difference between standard Prolotherapy and PRP Therapy is just a matter of degree—and the possible provision of an infrastructural scaffolding to fill in a structural void. The increased concentration of platelets and, thus, increased concentration of platelet-delivered growth factors simply makes PRP Therapy appear to be

a more potent treatment, especially for repairing a severe tendon sprain injury involving a significant tear or gross (versus microscopic) tissue defect at the enthesis.

As borne out by Rabago, D., et. al., however, it is yet to be determined what the real difference is between standard Prolotherapy and PRP Therapy. In their systematic review, they were not able to discern a significant clinical difference between the four therapeutic delivery systems over the long haul. Clearly, more study is needed to answer the question of differential long-term effectiveness and safety between standard Prolotherapy and PRP Therapy. Regardless, Prolotherapists and Prolotherapy patients have all along been "back to the future" in the arena of tissue regeneration and healing.

A PRACTICAL NOTE

PRP Therapy is surely here to stay. It will become even more technically embellished and refined, supported by other high-tech modalities, such as ultrasound-based needle guidance. As such, it will also remain much more expensive than routine, standard Prolotherapy, requiring more technological capital and personnel investment. Thus, PRP will garner a relatively higher price tag for insurance reimbursement and on the fee-for-service market.

Currently, PRP Therapy is enjoying a typical "high-tech hype." It is the musculoskeletal treatment *du jour*. An energizing supplement to this PRP high-tech hype is that PRP is often advertised as being supported by ultrasound needle guidance and is enjoying reimbursement by healthcare insurance companies.

But, the chief inherent danger in "high-tech" therapies is that the given procedure often becomes "low-touch" and relatively very expensive. Because of the "*du jour*" popularity amongst physicians and its insurance coverage attractiveness amongst patients, PRP Therapy could unnecessarily and unwisely supplant standard Prolotherapy in the treatment of the minimal to moderately severe ligament and tendon sprain injuries.

In a healthcare economy in which the United States spent \$1.6 trillion on healthcare in 2008, we need to abate the current burgeoning rate of total healthcare costs. If we continue our current rate of spending, we will have a healthcare economy by 2015 in which those costs will equal 20% of the GDP—or worse!⁷

Therefore, from the aspect of practical healthcare management aimed at practicing cost-effectiveness and common sense, PRP Therapy **should not** be considered

the panacea for treating **all** sprain injuries. Most minor to moderately severe sprain injuries of ligaments or tendons will respond to standard Prolotherapy just as quickly and at much less a healthcare cost—compared to the greater cost of PRP Therapy. In addition, PRP is a significantly more painful treatment than standard Prolotherapy.

Therefore, PRP Therapy should be reserved for the “too-hard” sprain injury cases for which standard Prolotherapy is less than adequate—especially the refractory tendinoses with significant tears. Continue to employ standard Prolotherapy for the minimal to moderately severe cases that are obviously responding. Just because it is an attractive “state-of-the-art” therapy does not mean that PRP Therapy need become an ever-pervasive “state-of-the-mind” option. “High-tech-Low-touch” often supplants “Low-tech-High-touch” therapies—often to the patient’s and the economy’s disadvantage. ■

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Errata: Correction regarding the first Literature Review article in JOP Volume I, Issue 2: For the sake of absolute accuracy, any reference to the Rabago, et. al, article as a ‘meta-analysis’ should, instead, have been as a ‘systematic review.’ A meta-analysis requires pooling of data. Since, Rabago, et al, could not pool their data, their report is a systematic review. This is a small but important distinction in describing their analytical statistical approach.

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