The Basic Biology of Platelet Growth Factors

Basic Biology:

Since 1990, medical science has recognized several components in blood which are part of the natural healing process and if added to wounded tissues or surgical sites as a concentrate have the potential to accelerate healing. These specific components in blood include platelet derived growth factor (PDGF) and transforming growth factor beta (TGFß), both of which are contained within the alpha granules of platelets, and fibronectin and vitronetin, which are cell adhesion molecules found in plasma, and fibrin itself.

Platelet Biology:

Platelets are living but terminal cytoplasmic portions of marrow megakareocytes. They have no nucleus for replication and will die off in five to nine days. Prior to this understanding of their role in wound healing they were only thought to contribute to the hemostatic process, where they adhere together to form a platelet plug in a severed vessel and actively extrude several initiators of the coagulation cascade. We now know that they also actively extrude the growth factors involved with initiating wound healing. These growth factors, also called "cytokines" are proteins each of about 25,000 Daltons molecular weight. They are stored in the alpha granules in platelets. In response to platelet to platelet aggregation or platelet to connective tissue contact, as occurs in injury or surgery, the cell membrane of the platelet is "activated" to release these alpha granules. The alpha granules release these growth factors via active extrusion through the cell membrane. Complete growth factors are not released by platelet disruption or fragmentation. Instead, these growth factors are actively extruded through the cell membrane where histones and carbohydrate side chains are added to complete their unique chemistries and make them "active" growth factors.

Platelet Derived Growth Factor (PDGF):

Platelet Derived Growth Factor is the evolutionary sentinel growth factor that initiates nearly all wound healing. It exists in three dimeric forms: **PDGFaa, PDGFbb, and PDGFab**. Each form is active but the specific role of each one has not been determined as yet. Upon release in its active form it attaches to a specific kinase receptor on a target cell. These receptors are transmembrane receptors. The PDGF occupation of their extra-membrane portion of the receptor site causes activations in the sub-membrane intracytoplasmic area. Specifically, this activation causes a high energy phosphate bond activation **(kinase activation)** of a signal protein bound to the cytoplasmic projection of the transmembrane receptor. When this signal protein is "activated" by the high energy phosphate it is also cleaved off the transmembrane receptor. The now activated signal protein floats within the cytoplasm and into the nucleus. Within the nucleus this signal protein will trigger the expression of various genes.

Platelet derived growth factors' main functions are to stimulate cell replication **(mitogenesis)** of healing capable **stem cells** and what are also called pre-mitotic partially differentiated **osteo-progenitor cells** which are also part of the connective tissue-bone healing cellular composite. It also stimulates cell replication of endothelial cells. This will cause budding of new capillaries into the wound **(angiogenesis)**, a fundamental part of all wound healing. In addition, PDGF seems to promote the migration of perivascular healing capable cells into a wound and to modulate the effects of other growth factors.

Transforming Growth Factor-beta (TGFß):

The so-called "super family" of TGFßs numbers about forty- seven, and includes all of the well-published bone specific morphogen growth factors of the 13 known Bone Morphogenic Proteins (BMPs). The type of TGFß found in platelets is **TGFß1** and **TGFß2**, which are the more generic connective tissue growth factors involved with matrix formation (i.e. cartilage and bone matrix as well as vascular basal lamina matrix.) Transforming growth factor beta 1 and 2, also about 25,000 Daltons of molecular weight, are found in the alpha granules of platelets, and are actively extruded in response to the effects of tissue injury or surgery on platelets.
Their mechanism of cellular stimulation through a transmembrane receptor, kinase activation of a signal protein, and that signal protein's expression of various gene sequences, is the same as that described for PDGF. Cells which are activated by TGFß1 or TGFß2 include fibroblasts, endothelial cells, osteoprogenitor cells, chondroprogenitor cells, and mesenchymal stem cells. If a fibroblast is "activated" it will undergo cell division and produce collagen. An endothelial cell will be stimulated to produce new capillaries. An osteoprogenitor cell will further differentiate and produce bone matrix. A chondroprogenitor cells will further differentiate and produce the matrix for cartilage. A mesenchymal stem cell will be stimulated to mitose so as to provide the large population of wound healing cells needed for completion of healing.

Fibronectin and Vitronectin:

Both of these are proteins called cell adhesion molecules. As part of cellular proliferation and migration particularly seen in bone and cartilage healing, cells move to new positions to lay down their products such as bone or cartilage. Related to bone, this is termed **osteoconduction**. These cells move via a process of endocytosis in which they pinch in a portion of their cell membrane into vesicles at their tail end. These vessels are transported through the cytoplasm to their front end where they are re-incorporated into the cell membrane surface on the front end and therefore the cell moves in a creeping fashion. This movement must take place on a framework. If the framework has reversible binding sites on it or structures into which a cell membrane may invaginate, so much the better. Fibronectin and vitronectin also seem to be able to provide a foothold or grip for cells as they move. Whether this is through reversible binding to the cell membrane or its surface texture is unknown at this point.

Fibrin:

Like fibronectin and vitronectin, fibrin is derived from plasma and contributes to cell mobility in the wound. The role of fibrin, which is a cross linked protein derived from the fibrinogen in plasma, is not only to serve as a scaffold or surface for cell migration, but to entrap platelets. As a cross linked protein where the crosslinking occurs as part of the clotting process it entraps platelets as well as red blood cells. This insures a random distribution of platelets throughout the wound and therefore the growth factors they contain. Through these recognized components in blood, natural wound healing is initiated, directed, and controlled. As a young science, blood component concentrates has not been completely studied. In fact, only a small percentage of
the knowledge base is known today. Clinically, adding enriched platelets and plasma to various clinical systems has shown acceleration of bone graft healing and maturation of the graft, acceleration of skin graft healing and its maturation, and enhanced hemostasis in bone and soft tissue defects. The clinical applications of blood component concentrates sometimes referred to as Platelet Rich Plasma (PRP) will be discussed related to the medical and dental disciplines in which they have proven efficacy in the following sections.

Terminology:

Platelet Rich Plasma (PRP): is the only scientifically correct term for a concentration of autologous platelets greater than the peripheral blood concentration suspended in a solution of autologous plasma. Other terms such as Platelet Concentrates (PC), Autologous Platelet Gel (APG), and Plasma Very Rich in Platelets (PVRP) have been applied to the same biologic material and although are not the correct terms, are acceptable for clinical usage.

Safety of PRP:

Because it is autologous, PRP avoids the risk of transmissible diseases such as HIV, Hepatitis B, C, or D, and other blood borne pathogens. Because it is used topically in and on top of a wound in a clotted fashion, it never reenters the individual's circulation. It is therefore safe when clot accelerators such as bovine thrombin are used or when PRP is added to other materials such as bovine collagen, gelfoam, PLA-PGLA constructs, etc.We believe the use of growth factors has taken our practice to the next quantum leap. We have been using growth factors to enhance our results with our stem cell and PRP treatments for some time now. Growth factors are signaling molecules that are typically secreted at the site of repair by many different cell types including platelets, stem cells and fibroblasts. These growth factors bind to the cell membrane and start the cascade of DNA synthesis, mitosis and cell repair. Some of these growth factors affect the stem cell environment or as it is sometimes called the "stem cell niche". It will make the environment much more favorable to the various stem cells.

The technical term for the various growth factors is cytokines. Cytokines—from the Greek cyto- (“cell”) and kinos (“movement”)—are tiny cell-signaling protein molecules secreted by various cells. Constituting a category of signaling molecules utilized widely in intercellular communication, cytokines may be classified as peptides, proteins, or glycoproteins. You might think of the cytokines as the bodies mobile phone system. They can be the difference between success and failure. Cytokine imbalance is responsible for most disease processes.

Cytokines activity is mostly local, but in some cases they impact the entire body. There are several groups of Cytokines or growth factors. These growth factors can act in three main ways. The first is that it can act in an endocrine fashion. This means that the growth factors may affect cells in a distant area. This is similar to our bodies own endocrine system. For instance, our pituitary gland located in our head affects cells in many different parts of the body. The next aspect concerns paracrine action which means that the factors may affect neighboring cells. The last aspect is an autocrine action meaning that the factors affect the surrounding cells. Interestingly enough stem cells work in a very similar fashion. One should think of the cytokines as architects of cell repair. The repair occurs mainly at the cellular level. The area surrounding the cells is called the extracellular matrix.

We must realize that there are both good and bad cytokines. If you have a symptomatic osteoarthritis the chances are that your symptoms are caused by bad cytokines. Two bad cytokines that comes to mind are Interleukin 1 (IL-1) and Interleukin 6 (IL-6). These bad cytokines cause pain, swelling, and typically cell death. When you are causing cell death you are in what we call a catabolic state. This means you are breaking down tissue causing destruction of the cells and eventually failure of the system. Another important bad cytokine is called Tissue Necrosis Factor or TNF. TNF will cause cell death and block repair.

In Regenerative Stem Cell Therapy, we are trying to counteract these bad cytokines by manipulating good cytokines that come from both stem cell and PRP injections. We are to turn the tide and get the joint or tendon into an anabolic state. An anabolic state means we are now building up tissue.

Three very important good cytokines are Interleukin-1-Antagonist (AIL-1 also called IL-1A), Interleukin 10, and IGF-1. They can override catabolic action of the bad cytokines. What these growth factors actually do is try to modulate or diminish the inflammatory response. We now know this is one of the most important jobs of mesenchymal stem cells. If a significant inflammatory response is present than repair is jeopardized. Realize that the reduction of inflammation is more on a cellular level and thus non-steroidal anti-inflammatories will not achieve this goal.

In addition to modulating the inflammation, the good cytokines actually help repair the tissue. There are many good cytokines that can accomplish repair. I would have to write a book to discuss them all at length. What I would do is give a brief synopsis of what is probably the more important ones but realize they are all important.

One of these factors is Transforming Growth Factor or TGF. TGF is important in collagen synthesis and tissue repair. This is a major repair growth factor. TGF appears to stimulate macrophages (a form of white blood cell) to secrete various growth factors. It also directs the macrophages and other cells to areas needing repair. Finally, it stimulates angiogenesis (which means that it forms blood vessels). Another important factor is Fibroblast Growth Factor or FGF. FGF helps organize connective tissue. FGF's functions are similar to TGF but it seems more involved with stimulation of new growth of blood vessels.

Insulin Growth Factor or IGF-1 is the active form of Human Growth Hormone. This is a multi-talented growth factor. It is very important in cell division etc. It is so important that we are giving it as an intra-articular (joint) injection or an injection into a tendon. IGF-1 is found in PRP and stem cells. It is secreted by the liver thru stimulation of Growth Hormone. Studies show that IGF-1 stimulates the glucose uptake by cells thus supplying them with more energy. It also inhibits protein catabolism (destruction). It is also believed that it affects cell receptors which drive a stem cell toward the formation of cartilage tissue. It seems to block many of the interleukin inflammatory pathways.

One more important growth factor is called Vascular Endothelial Growth Factor or VEGF. VEGF is important in that it helps to establish a blood supply where typically there is no blood supply. This is a very important concept in stem cell science.

Up until now these specific factors were extremely expensive and sometimes had significant side effects. Previously, it was almost impossible to obtain these growth factors. They are now available and are OK with the FDA and are extremely safe if used properly. The trick is to learn to use them in the proper dosage regimen and combination.