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Activated Growth Factor (AGF): An Advanced Modality of Platelet-Rich Plasma as a New Biological Agent for the Treatment of Degenerative and Traumatic Conditions

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A B S T R A C T

Platelet-rich plasma (PRP) has emerged as a promising therapeutic modality for various medical applications, particularly in regenerative medicine and wound healing. This is largely attributed to its rich concentration of growth factors (GFs) that play pivotal roles in tissue repair and regeneration. However, the inherent limitations of PRP, such as the variable GF concentrations and short-lived release kinetics, have spurred the development of advanced modalities to enhance its therapeutic efficacy. Activated growth factor (AGF) represents one such advancement, aiming to optimize the release and bioavailability of GFs from platelets. This comprehensive review delves into the biological mechanisms underlying AGF, its preparation methodologies, preclinical and clinical evidence supporting its use, and its potential applications in treating degenerative and traumatic conditions. Furthermore, it explores the advantages of AGF over conventional PRP and discusses future directions for research and clinical translation.

1. Introduction

The intricate dance of healing and regeneration within the human body has fascinated scientists and clinicians for centuries. Our inherent capacity to mend tissues, reconstruct architecture, and restore function after injury is a testament to the complex interplay of cellular and molecular processes. At the heart of this regenerative orchestra lies a diverse group of signaling molecules known as growth factors (GFs), which orchestrate the intricate ballet of cell proliferation, differentiation, and angiogenesis. These GFs, acting as molecular messengers, guide the intricate process of tissue repair, beckoning cells to proliferate, differentiate, and weave new extracellular matrices, ultimately restoring form and function. Among the key players in this regenerative drama are platelets, anucleate cell fragments derived from megakaryocytes, which serve as veritable reservoirs of these vital GFs. These tiny, disc-shaped cells, traditionally known for their role in hemostasis, have emerged as pivotal players in the realm of regenerative medicine. Upon activation, platelets degranulate, releasing a treasure trove of GFs, cytokines, chemokines, and coagulation factors, initiating a

cascade of events that culminate in tissue repair and regeneration.[1,2]

This understanding has led to the rise of platelet-rich plasma (PRP) therapy, a modality that harnesses the regenerative potential of platelets by concentrating them from autologous blood. PRP, with its supra-physiological concentration of GFs, has shown promise in a myriad of clinical applications, ranging from orthopedics and sports medicine to dermatology and wound healing. However, the inherent limitations of PRP, such as variable GF concentrations, short-lived release kinetics, and the presence of inhibitory factors, have spurred the quest for advanced modalities to further enhance its therapeutic efficacy. Enter activated growth factor (AGF) technology, an innovative approach that seeks to optimize the release and bioavailability of GFs from platelets. By actively stimulating platelet activation, AGF aims to unlock the full regenerative potential of PRP, maximizing its therapeutic impact. This comprehensive review embarks on a journey to explore the intricate world of AGF, delving into its biological underpinnings, preparation methodologies, preclinical and clinical evidence, and its potential to revolutionize the treatment of degenerative and traumatic conditions.[2,3]

Platelets and growth factors: a biological symphony orchestrating regeneration

Platelets, once viewed primarily as passive participants in hemostasis, have taken center stage in the field of regenerative medicine. These anucleate cytoplasmic fragments, shed from megakaryocytes residing within the bone marrow, are not merely bystanders in the coagulation cascade, but dynamic orchestrators of tissue repair and regeneration. Their cytoplasm, a treasure trove of bioactive molecules, holds the key to unlocking the body's innate healing potential. Despite their diminutive size and lack of a nucleus, platelets are remarkably complex and highly organized. Their cytoplasm is a bustling metropolis of granules, organelles, and a cytoskeletal network, all working in concert to enable rapid responses to stimuli and execute their diverse functions. Platelets house three main types of granules, each packed with a unique arsenal of bioactive molecules that contribute to their multifaceted roles in hemostasis and tissue repair. α-granules are the crown jewels of the platelet, serving as the primary storage site for a plethora of growth factors (GFs). These GFs, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), and insulin-like growth factor-1 (IGF-1), are the molecular messengers that orchestrate the various stages of tissue repair and regeneration. They stimulate cell proliferation, differentiation, migration, and angiogenesis, guiding the intricate process of tissue reconstruction. Dense granules store a different set of bioactive molecules, including adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin, and calcium ions. These molecules play crucial roles in platelet activation, aggregation, and vasoconstriction, the initial steps in hemostasis. ADP and ATP, released upon platelet activation, amplify the aggregation response, recruiting more platelets to the site of injury. Serotonin, a potent vasoconstrictor, helps to limit blood loss, while calcium ions are essential for various intracellular signaling pathways involved in platelet activation and granule release. Lysosomes contain a variety of hydrolytic enzymes, including proteases, lipases, and glycosidases. These enzymes contribute to tissue remodeling and debris clearance, breaking down damaged tissue and facilitating the removal of cellular debris. They also play a role in the activation of certain GFs, such as TGF-β, which is released from the extracellular matrix in a latent form and requires proteolytic cleavage to become active.[3,4]

The platelet cytoskeleton is a dynamic network of protein filaments that provides structural support, maintains cell shape, and enables rapid changes in morphology during activation. It consists of three main components: Microtubules: These hollow tubes, composed of tubulin dimers, form a circumferential ring beneath the plasma membrane, maintaining the discoid shape of resting platelets. Upon activation, this ring contracts, causing the platelet to change shape and extend pseudopodia, facilitating adhesion and aggregation; Actin filaments: These thin filaments, composed of actin monomers, form a dense network

throughout the cytoplasm, providing structural support and enabling cell motility. Upon activation, actin polymerization drives the formation of filopodia and lamellipodia, allowing platelets to spread and interact with the surrounding environment; Intermediate filaments: These rope-like filaments, composed of various proteins, provide mechanical strength and contribute to the overall structural integrity of the platelet.[4,5]

The platelet plasma membrane is a dynamic structure that plays a crucial role in platelet activation and interaction with the surrounding environment. It is studded with a variety of receptors, adhesion molecules, and ion channels that enable platelets to sense and respond to various stimuli. Glycoprotein receptors, these receptors, including glycoprotein Ib (GPIb) and GPVI, mediate platelet adhesion to the subendothelial matrix, the initial step in hemostasis. GPIb binds to von Willebrand factor (vWF), a large multimeric protein that bridges platelets to exposed collagen fibers. GPVI, a collagen receptor, directly interacts with collagen, triggering intracellular signaling pathways that lead to platelet activation. Integrins, these heterodimeric transmembrane receptors mediate platelet aggregation and adhesion to various extracellular matrix proteins. The most abundant integrin on platelets is αIIbβ3, which binds to fibrinogen, a soluble plasma protein that forms bridges between adjacent platelets, leading to aggregation. Ion channels, these channels regulate the flow of ions across the plasma membrane, contributing to changes in membrane potential and intracellular calcium concentration, essential for platelet activation and granule release.[5,6]

Platelet activation is a tightly regulated process, a carefully choreographed cascade of events triggered by a variety of stimuli. This activation process transforms the platelet from a quiescent disc-shaped cell into a dynamic and adhesive entity, capable of initiating hemostasis and orchestrating tissue repair. Exposure of subendothelial collagen, a structural protein found in the vessel wall, is a primary trigger of platelet activation. Collagen binds to GPVI and other receptors on the platelet surface, initiating intracellular signaling pathways that lead to platelet activation. This potent serine protease, generated during the coagulation cascade, is a potent activator of platelets. Thrombin cleaves protease-activated receptors (PARs) on the platelet surface, triggering intracellular signaling cascades that lead to platelet shape change, granule secretion, and integrin activation. Released from dense granules of activated platelets and damaged cells, Adenosine diphosphate (ADP): amplifies the platelet activation response. It binds to P2Y1 and P2Y12 receptors on the platelet surface, further promoting platelet aggregation and granule release. Other agonists that can trigger platelet activation include thromboxane A2 (TXA2), a potent vasoconstrictor and platelet activator produced by activated platelets, and epinephrine, a hormone released during stress that enhances platelet aggregation. Platelet activation involves a complex interplay of signaling pathways, leading to a series of morphological and biochemical changes. Upon activation, platelets undergo a dramatic shape change, transforming from smooth discs to spiky spheres with extended pseudopodia. This shape change is driven by the reorganization of the platelet cytoskeleton, particularly the contraction of the microtubule ring and the polymerization of actin filaments. Activated platelets release the contents of their granules, a process known as degranulation. This release of GFs, ADP, and other bioactive molecules amplifies the activation response and initiates the tissue repair process. Activation of integrins, particularly αIIbβ3, increases their affinity for fibrinogen, leading to platelet aggregation. Fibrinogen binding bridges adjacent platelets, forming a platelet plug that helps to seal the injured vessel wall. Thrombus formation, the platelet plug, along with the coagulation cascade, leads to the formation of a stable thrombus, preventing further blood loss.[7,8]

Growth factors are a diverse group of signaling molecules that play pivotal roles in regulating various cellular processes, including cell growth, differentiation, migration, and survival. They act as molecular messengers, transmitting signals between cells and orchestrating the complex processes of tissue development, repair, and regeneration. Growth factors can be classified into various families based on

their structure and function: Platelet-derived growth factor (PDGF) family: This family of growth factors, including PDGF-A, PDGF-B, PDGF-C, and PDGF-D, stimulates the proliferation and migration of various cell types, including fibroblasts, smooth muscle cells, and pericytes. They play crucial roles in angiogenesis, wound healing, and tissue regeneration. Transforming growth factor-beta (TGF-β) superfamily: This large and diverse superfamily includes TGF-βs, bone morphogenetic proteins (BMPs), activins, and inhibins. These growth factors regulate a wide range of cellular processes, including cell proliferation, differentiation, apoptosis, and extracellular matrix production. They play crucial roles in embryonic development, tissue homeostasis, and immune regulation. Vascular endothelial growth factor (VEGF) family: This family of growth factors, including VEGF-A, VEGF-B, VEGF-C, and VEGF-D, promotes angiogenesis, the formation of new blood vessels. They also regulate vascular permeability and lymphatic development. Epidermal growth factor (EGF) family: This family of growth factors, including EGF, transforming growth factor-alpha (TGF-α), and heparin-binding EGF-like growth factor (HB-EGF), stimulates the proliferation and differentiation of epithelial cells and other cell types. They play crucial roles in wound healing, tissue regeneration, and cancer development. Fibroblast growth factor (FGF) family: This large family of growth factors, with over 20 members, regulates a wide range of cellular processes, including cell proliferation, differentiation migration, angiogenesis, and wound healing. They are also involved in embryonic development, tissue homeostasis, and the pathogenesis of various diseases. Insulin-like growth factor (IGF) family: This family of growth factors, including IGF-1 and IGF-2, stimulates cell proliferation, differentiation, and survival. They play crucial roles in tissue growth and repair, as well as in regulating metabolism and glucose homeostasis. Several other growth factors contribute to tissue repair and regeneration, including nerve growth factor (NGF), which promotes the survival and growth of neurons, and hepatocyte growth factor (HGF), which stimulates the proliferation and migration of hepatocytes and other cell types.[8,9]

Growth factors exert their effects by binding to specific receptors on the surface of target cells. These receptors, typically transmembrane proteins, transmit signals from the extracellular environment to the intracellular compartment, initiating a cascade of events that ultimately lead to changes in gene expression and protein synthesis. Receptor Tyrosine Kinases (RTKs): This large family of receptors, including the receptors for PDGF, EGF, FGF, and IGF-1, possesses intrinsic tyrosine kinase activity. Upon ligand binding, these receptors dimerize and autophosphorylate, creating docking sites for intracellular signaling molecules. These signaling molecules, in turn, activate downstream pathways, such as the mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K) pathway, which regulate cell proliferation, differentiation, and survival. Serine/Threonine Kinase Receptors: This family of receptors, including the receptors for TGF-β and BMPs, possesses intrinsic serine/threonine kinase activity. Upon ligand binding, these receptors also dimerize and activate downstream signaling pathways, such as the Smad pathway, which regulates gene expression and cellular responses. G Protein-Coupled Receptors (GPCRs): Some growth factors, such as chemokines, bind to GPCRs, which are coupled to heterotrimeric G proteins. Upon ligand binding, these receptors activate G proteins, which in turn activate downstream effectors, such as adenylyl cyclase and phospholipase C, leading to changes in intracellular signaling pathways. Growth factors often act in concert, forming complex networks of interactions that regulate tissue repair and regeneration. These interactions can be synergistic, additive, or antagonistic, depending on the specific growth factors involved and the cellular context. Synergistic interactions: Some growth factors cooperate to enhance their effects, producing a response greater than the sum of their individual effects. For example, PDGF and TGF-β synergistically promote wound healing by stimulating fibroblast proliferation and extracellular matrix production. Additive interactions: Some growth factors have similar effects and can simply add to each other's actions. For example, EGF and TGF-α both stimulate

epithelial cell proliferation, contributing to wound healing. Antagonistic interactions: Some growth factors oppose each other's actions, providing a balance in the regulation of cellular processes. For example, TGF-β can inhibit the proliferation of certain cell types, while other growth factors, such as PDGF, promote proliferation.[9,10]

Activated growth factor (AGF): unleashing the full potential of platelet-rich plasma

Platelet-rich plasma (PRP) therapy has emerged as a promising modality in regenerative medicine, harnessing the power of concentrated platelets to accelerate healing and tissue regeneration. However, the inherent limitations of PRP, such as variable growth factor (GF) concentrations, short-lived release kinetics, and the presence of inhibitory factors, have driven the search for more advanced and effective approaches. Activated growth factor (AGF) technology represents a significant leap forward in this quest, aiming to optimize the therapeutic potential of PRP by actively stimulating platelet activation and maximizing the release and bioavailability of GFs. This section delves into the intricacies of AGF, exploring its preparation methodologies, mechanisms of action, and the advantages it offers over conventional PRP. Platelet-rich plasma (PRP) therapy, with its ability to concentrate platelets and their associated growth factors (GFs), has garnered significant attention in the field of regenerative medicine. However, the inherent limitations of PRP have spurred the development of more advanced modalities, such as activated growth factor (AGF) technology, to optimize its therapeutic potential and overcome its shortcomings. This section delves into the rationale behind AGF, providing a detailed analysis of the limitations of PRP and how AGF addresses these challenges to usher in a new era of regenerative therapies.[10,11]

PRP holds immense promise for tissue repair and regeneration, owing to its concentrated payload of GFs. These GFs, released upon platelet activation, orchestrate a cascade of cellular processes that promote healing, including cell proliferation, differentiation, angiogenesis, and extracellular matrix synthesis. However, several factors can limit the efficacy of PRP, hindering its ability to consistently deliver optimal therapeutic outcomes. One of the primary limitations of PRP is the variability in GF concentrations. The concentration of GFs in PRP can vary significantly depending on several factors: Preparation method: Different PRP preparation methods yield varying platelet concentrations and GF profiles. Single-spin, double-spin, and buffy coat methods, among others, result in different levels of platelet enrichment and leukocyte inclusion, influencing the final GF composition; Patient factors: Patient age, health status, medications, and underlying medical conditions can affect platelet function and GF production. For instance, patients with diabetes or those taking certain medications may have impaired platelet function, leading to lower GF release; Storage conditions: The storage conditions of PRP, including temperature and duration, can affect GF stability and activity. Improper storage can lead to GF degradation and reduced therapeutic efficacy. This variability in GF concentrations poses a significant challenge to the consistent and predictable application of PRP. It can lead to inconsistent clinical outcomes, making it difficult to standardize treatment protocols and optimize therapeutic efficacy.[11,12]

Another limitation of PRP is the short-lived release kinetics of GFs. GFs released from platelets have a short half-life, typically ranging from minutes to hours. This rapid degradation limits their duration of action at the target site, hindering their ability to sustain the regenerative process. The short-lived nature of GF signaling can impede tissue regeneration, particularly in chronic or complex wounds where prolonged stimulation is required for complete healing. It can also necessitate multiple PRP injections to maintain therapeutic levels of GFs, increasing the cost and inconvenience of treatment. PRP, while rich in GFs, can also contain inhibitory factors that can hinder the activity of GFs and impede the healing process. These inhibitory factors include: Proteases: These enzymes can degrade GFs, reducing their bioavailability and effectiveness; Anti-growth factors: These molecules can bind to GFs, blocking their interaction with receptors and inhibiting their signaling activity; Degradation products: The

breakdown of platelets and other blood components can release degradation products that interfere with GF signaling or create an unfavorable environment for tissue regeneration. The presence of these inhibitory factors can dampen the regenerative signal, reducing the overall efficacy of PRP and potentially delaying healing.The leukocyte content of PRP is a subject of ongoing debate. While leukocytes play a role in the inflammatory response and can contribute to tissue regeneration, they can also release pro-inflammatory cytokines and enzymes that can exacerbate inflammation and tissue damage. The optimal leukocyte concentration in PRP remains unclear, and different preparation methods yield varying levels of leukocyte inclusion. This variability can influence the inflammatory response and potentially affect clinical outcomes.[12,13]

AGF technology represents a paradigm shift in platelet-based therapies, addressing the limitations of PRP and unlocking its full regenerative potential. By actively stimulating platelet activation, AGF optimizes the release and bioavailability of GFs, amplifying the regenerative cascade and promoting more effective tissue regeneration. AGF promotes a rapid and sustained release of GFs, maximizing their bioavailability at the target site. This sustained release ensures a prolonged exposure of cells to GFs, providing a more potent and enduring regenerative stimulus. By overcoming the short-lived release kinetics of conventional PRP, AGF can sustain the regenerative process, promoting more complete and efficient tissue repair. This sustained release can also reduce the need for multiple injections, improving patient convenience and reducing treatment costs. AGF may contain a higher concentration of GFs compared to conventional PRP, further enhancing its regenerative capacity. This higher concentration can stimulate more robust cellular responses, accelerating the healing process and promoting more complete tissue regeneration. By maximizing the release of GFs from platelets, AGF delivers a more potent regenerative signal, potentially leading to faster healing times, reduced pain, and improved functional outcomes. AGF can mitigate the effects of inhibitory factors present in PRP, clearing the path for more effective GF signaling and tissue regeneration. Some activation methods used in AGF preparation can neutralize or remove inhibitory factors, creating a more favorable environment for healing. By minimizing the impact of inhibitory factors, AGF enhances the bioavailability and activity of GFs, maximizing their therapeutic potential and promoting more efficient tissue repair. AGF allows for greater control over the leukocyte content of the final product, enabling clinicians to fine-tune the inflammatory response and optimize the healing environment. Some AGF preparation methods can selectively remove or reduce leukocytes, minimizing the potential for excessive inflammation and tissue damage. This ability to tailor the leukocyte content can be particularly beneficial in chronic wounds or inflammatory conditions where excessive inflammation can hinder healing.[10,12]

Table 1. The rationale for AGF: overcoming the limitations of PRP.

Limitation of PRP	**How AGF addresses the limitation**
Variable GF concentrations	AGF may contain a higher concentration of GFs compared to conventional PRP, further enhancing its regenerative capacity.
Short-lived release kinetics	AGF promotes a rapid and sustained release of GFs, maximizing their bioavailability at the target site.
Presence of inhibitory factors	AGF can mitigate the effects of inhibitory factors present in PRP, clearing the path for more effective GF signaling and tissue regeneration.
Leukocyte content variability	AGF allows for greater control over the leukocyte content of the final product, enabling clinicians to fine-tune the inflammatory response and optimize the healing environment.

AGF preparation: activating the regenerative arsenal - a symphony of methods to unlock healing potential

Activated growth factor (AGF) technology represents a significant advancement in platelet-based therapies, aiming to optimize the therapeutic potential of platelet-rich plasma (PRP) by actively stimulating platelet activation and maximizing the release and bioavailability of growth factors (GFs). This section delves into the intricacies of AGF preparation, exploring the diverse methods employed to activate platelets and unleash their regenerative arsenal. Platelet activation is a tightly regulated process, a carefully orchestrated cascade of events triggered by a variety of stimuli. This activation process transforms the platelet from a quiescent disc-shaped cell into a dynamic and adhesive entity, capable of initiating hemostasis and orchestrating tissue repair. In the context of AGF preparation, platelet activation is the key to unlocking the full therapeutic potential of PRP. By carefully selecting and optimizing the activation method, clinicians can fine-tune the release of GFs, tailoring the treatment to the specific needs of the patient and the clinical indication.[10,13]

Chemical activation methods utilize specific chemical agents to trigger platelet activation and GF release. These agents mimic physiological activators or directly interact with platelet receptors, initiating the signaling cascades that lead to degranulation and the release of the regenerative payload. Calcium ions (Ca^{2+}) are essential for platelet activation and granule release. They play a crucial role in various intracellular signaling pathways, acting as second messengers that amplify and propagate the activation signal. Adding calcium chloride ($CaCl_2$) to PRP elevates the extracellular calcium concentration, creating a gradient that drives calcium influx into platelets. This influx of calcium triggers a series of events, including: Activation of phospholipase C: This enzyme cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). DAG activates protein kinase C (PKC), which phosphorylates various proteins involved in platelet activation, while IP3 triggers the release of calcium from intracellular stores, further amplifying the calcium signal; Activation of protein kinase C (PKC): PKC phosphorylates a variety of proteins involved in platelet activation, including myosin light chain kinase (MLCK), which regulates platelet shape change and granule release; Activation of integrins: Calcium signaling also activates integrins, particularly αIIbβ3, increasing their affinity for fibrinogen and promoting platelet aggregation. The concentration of $CaCl_2$ used can influence the extent of platelet activation and GF release. Higher concentrations generally lead to more rapid and robust activation, but excessive calcium can also lead to platelet damage and aggregation, potentially reducing the therapeutic efficacy of AGF.[9,11]

Thrombin, a serine protease generated during the coagulation cascade, is a potent activator of platelets. It cleaves protease-activated receptors (PARs) on the platelet surface, particularly PAR-1 and PAR-4, triggering intracellular signaling cascades that lead to rapid and robust platelet activation and GF release. Thrombin-induced platelet activation involves: Activation of G proteins: PARs are G protein-coupled receptors (GPCRs) that activate heterotrimeric G proteins, including Gq and G12/13. Gq activates phospholipase C, leading to the generation of DAG and IP3, while G12/13 activates Rho GTPases, which regulate cytoskeletal reorganization and platelet shape change; Activation of phospholipase C: As described earlier, phospholipase C activation leads to the generation of DAG and IP3, amplifying the calcium signal and activating PKC; Activation of Rho GTPases: Rho GTPases, such as RhoA and Rac1, regulates cytoskeletal reorganization, leading to platelet shape change, filopodia formation, and granule release. Thrombin is a highly effective activator of platelets, inducing rapid and robust GF release. However, its use can lead to the formation of fibrin clots, which may need to be removed before clinical application. Additionally, thrombin can be derived from bovine or human sources, raising concerns about potential immune reactions or disease transmission.[9,12]

Biological activation methods utilize natural activators, such as collagen and autologous thrombin,

to trigger platelet activation and GF release. These methods mimic physiological activation processes, potentially leading to a more controlled and balanced release of GFs. Collagen, a major component of the extracellular matrix, is a potent activator of platelets. Exposure of platelets to collagen triggers a signaling cascade that leads to platelet adhesion, activation, and GF release. This activation process involves: Binding to GPVI: Collagen binds to glycoprotein VI (GPVI), a receptor on the platelet surface, initiating intracellular signaling cascades; Activation of Src family kinases: GPVI activation leads to the activation of Src family kinases, which phosphorylate various proteins involved in platelet activation; Activation of phospholipase C: Src family kinases activate phospholipase C, leading to the generation of DAG and IP3, amplifying the calcium signal and activating PKC; Activation of integrins: Collagen-induced signaling also activates integrins, particularly α2β1, which binds to collagen, and αIIbβ3, which binds to fibrinogen, promoting platelet adhesion and aggregation. Collagen is a physiological activator of platelets, triggering a more controlled and balanced release of GFs compared to thrombin. Various forms of collagen can be used for AGF preparation, including type I collagen, which is commonly found in connective tissues.[9,13]

Autologous thrombin, derived from the patient's own blood, offers a personalized approach to platelet activation. This method minimizes the risk of immune reactions and disease transmission associated with exogenous thrombin. The preparation of autologous thrombin typically involves: Collecting a small amount of the patient's blood: This blood is processed to separate the serum, which contains clotting factors; Adding calcium chloride and a clotting activator: Calcium chloride and a clotting activator, such as bovine thrombin or snake venom, are added to the serum, initiating the coagulation cascade and generating thrombin; Activating the PRP: The autologous thrombin is then added to the PRP, triggering platelet activation and GF release. Autologous thrombin offers a safe and effective method for platelet activation, tailoring the treatment to the individual patient and minimizing potential risks.[8,10]

Physical activation methods utilize mechanical forces to disrupt platelet membranes and induce GF release. These methods bypass the need for chemical activators, offering a potentially simpler and more direct approach to platelet activation. Sonication utilizes high-frequency sound waves to mechanically disrupt platelet membranes, leading to the release of GFs. The sound waves generate cavitation bubbles in the PRP, creating shear forces that disrupt platelet membranes and release their contents. Sonication can be a rapid and efficient method of platelet activation, but careful optimization of parameters is necessary to avoid excessive platelet damage. Factors such as sonication frequency, intensity, and duration can influence the extent of platelet activation and GF release. Repeated freezing and thawing of PRP can disrupt platelet membranes and induce GF release. The formation of ice crystals during freezing can damage platelet membranes, leading to the release of GFs upon thawing. This method is relatively simple, but it may not be as efficient as other activation methods. The number of freeze-thaw cycles and the freezing and thawing rates can influence the extent of platelet activation and GF release. Subjecting PRP to shear stress, such as by passing it through a narrow orifice or using a vortex mixer, can activate platelets and induce GF release. This method mimics the physiological shear stress that platelets experience in the bloodstream, potentially leading to a more controlled and balanced release of GFs. The magnitude and duration of shear stress can influence the extent of platelet activation and GF release.[8,11]

The preparation of AGF involves a diverse arsenal of methods, each with its own advantages and considerations. Chemical activation methods, such as calcium chloride and thrombin, offer rapid and robust activation, while biological activation methods, such as collagen and autologous thrombin, mimic physiological processes and offer greater control. Physical activation methods, such as sonication, freeze-thaw cycles, and shear stress, provide simpler and more direct approaches to platelet activation. The choice of activation method depends on various factors, including the clinical indication, patient needs, and clinician preferences. By carefully selecting

and optimizing the activation method, clinicians can fine-tune the release of GFs, tailoring the treatment to maximize its therapeutic potential and promote optimal tissue regeneration.[8,13]

Table 2. Summary of AGF preparation methods.

Activation method	Mechanism of action	Advantages	Disadvantages
Chemical activation			
Calcium Chloride ($CaCl_2$)	Elevates extracellular calcium concentration, triggering intracellular signaling cascades	Rapid and efficient activation; readily available and cost-effective	Excessive calcium can lead to platelet damage and aggregation, potentially reducing GF activity
Thrombin	Cleaves protease-activated receptors (PARs), triggering intracellular signaling cascades	Rapid and robust activation, leading to high GF release	Can lead to fibrin clot formation, requiring additional processing; potential for immune reactions or disease transmission with exogenous thrombin
Biological activation			
Collagen	Binds to glycoprotein VI (GPVI), triggering intracellular signaling cascades	Physiological activator; more controlled and sustained release of GFs; mimics natural wound healing environment	May require optimization of collagen type and concentration for optimal activation
Autologous Thrombin	Utilizes patient's own blood to generate thrombin for platelet activation	Personalized approach; minimizes risk of immune reactions or disease transmission; potentially safer than exogenous thrombin	Requires additional processing steps to generate autologous thrombin, increasing preparation time
Physical activation			
Sonication	Utilizes high-frequency sound waves to mechanically disrupt platelet membranes	Rapid and efficient activation; no chemical additives required	Requires careful optimization of sonication parameters (frequency, intensity, duration) to avoid excessive platelet damage and denaturation of GFs
Freeze-thaw cycles	Repeated freezing and thawing disrupts platelet membranes	Simple and convenient; no specialized equipment required	It may not be as efficient as other methods; potential for GF degradation with repeated cycles
Shear stress	Subjecting PRP to shear stress activates platelets	Mimics physiological shear stress; potentially more controlled and balanced GF release	Requires specialized equipment to generate controlled shear stress; may be less efficient than chemical activation

Mechanisms of action of AGF: Amplifying the regenerative cascade - a symphony of molecular signals

Activated growth factor (AGF) technology represents a significant leap forward in the field of regenerative medicine, harnessing the power of platelets and their growth factors (GFs) to promote healing and tissue regeneration. This section delves into the intricate mechanisms of action of AGF, exploring how it amplifies the regenerative cascade and orchestrates a symphony of molecular signals to restore tissue structure and function. AGF exerts its therapeutic effects by amplifying the regenerative cascade initiated by platelet activation and GF release. This cascade involves a complex interplay of cellular and molecular events, orchestrated by a diverse array of GFs and their downstream signaling pathways. One of the key mechanisms of action of AGF is its ability to enhance GF release. By actively stimulating platelet activation, AGF promotes a rapid and sustained

release of GFs, maximizing their bioavailability at the target site. This sustained release ensures a prolonged exposure of cells to GFs, providing a more potent and enduring regenerative stimulus. GFs have a short half-life, and their concentration at the target site can decline rapidly. AGF, by promoting sustained release, helps to maintain therapeutic GF levels for a longer duration, ensuring a more consistent and effective regenerative stimulus. Sustained exposure to GFs can promote more sustained cellular responses, such as cell proliferation, differentiation, and migration, leading to more efficient and complete tissue regeneration. The prolonged release of GFs from AGF can reduce the frequency of injections required to maintain therapeutic levels, improving patient convenience and reducing treatment costs.[13,14]

AGF may contain a higher concentration of GFs compared to conventional PRP, further enhancing its regenerative capacity. This higher concentration can stimulate more robust cellular responses, accelerating the healing process and promoting more complete tissue regeneration. AGF preparation methods are designed to optimize platelet activation, maximizing the release of GFs from platelet granules. AGF preparation methods can minimize the degradation of GFs, preserving their activity and increasing their concentration in the final product. AGF preparation methods may further concentrate platelets, leading to a higher concentration of GFs in the final product. This amplified regenerative signal can lead to faster healing times, reduced pain, and improved functional outcomes. AGF can modulate the inflammatory response, promoting a balanced and controlled healing environment. Inflammation is a crucial component of the healing process, but excessive or prolonged inflammation can hinder tissue regeneration and lead to complications. AGF releases GFs with anti-inflammatory properties, such as TGF-β, which can help to regulate the inflammatory response and prevent excessive inflammation. AGF can also release GFs that recruit immune cells, such as macrophages, which play a crucial role in clearing cellular debris and promoting tissue regeneration. AGF can influence the production of cytokines, which are signaling molecules that regulate the inflammatory response. By orchestrating a balanced inflammatory response, AGF can create a more favorable environment for tissue regeneration and promote optimal healing.[13,15]

AGF promotes the formation of new blood vessels, a process known as angiogenesis, which is crucial for tissue regeneration. Angiogenesis ensures adequate blood supply to the regenerating tissue, delivering essential nutrients and oxygen to support cell growth and function. AGF releases GFs with potent pro-angiogenic properties, such as VEGF, which stimulates the proliferation and migration of endothelial cells, the building blocks of blood vessels. AGF can also recruit endothelial progenitor cells, which are stem cells that can differentiate into endothelial cells and contribute to new blood vessel formation. AGF can influence the composition and structure of the extracellular matrix, creating a supportive environment for blood vessel growth. By promoting angiogenesis, AGF ensures adequate blood supply to the regenerating tissue, supporting cell survival, proliferation, and differentiation, and accelerating the healing process.[14]

AGF stimulates the proliferation and differentiation of various cell types, including fibroblasts, endothelial cells, keratinocytes, and stem cells. This cellular activity is essential for the formation of new tissue and the replacement of damaged cells. GFs released from AGF activate various intracellular signaling pathways, such as the MAPK and PI3K pathways, which regulate cell proliferation, differentiation, and survival. GFs can also modulate gene expression, influencing the production of proteins involved in cell cycle progression, differentiation, and tissue regeneration. GFs interact with specific receptors on the surface of target cells, triggering intracellular signaling cascades that promote cell proliferation and differentiation. By stimulating cell proliferation and differentiation, AGF drives the regeneration of damaged tissues, restoring their structure and function. AGF stimulates the production and remodeling of the extracellular matrix (ECM), providing structural support and scaffolding for the regenerating tissue. The ECM is a complex network of proteins and polysaccharides that provides a framework for cell adhesion, migration, and

differentiation, guiding the organized regeneration of tissues. AGF releases GFs that stimulate the production and remodeling of ECM components, such as collagen, elastin, and proteoglycans. AGF activates fibroblasts, which are the primary cells responsible for ECM production and remodeling. AGF can influence the activity of MMPs, which are enzymes that degrade ECM components, allowing for the remodeling and reorganization of the ECM during tissue regeneration. By promoting ECM synthesis and remodeling, AGF provides a supportive environment for cell growth and differentiation, guiding the organized regeneration of tissues and restoring their structural integrity. AGF exerts its therapeutic effects through a complex interplay of mechanisms, amplifying the regenerative cascade and orchestrating a symphony of molecular signals to promote healing and tissue regeneration. By enhancing GF release, increasing GF concentration, modulating the inflammatory response, stimulating angiogenesis, enhancing cell proliferation and differentiation, and promoting ECM synthesis and remodeling, AGF unlocks the full regenerative potential of platelets, offering a powerful tool for regenerative medicine.[14,15]

Table 3. Summary of AGF mechanisms of action.

Mechanism of action	Description
Enhanced GF release	Promotes a rapid and sustained release of GFs, maximizing their bioavailability at the target site.
Increased GF concentration	May contain a higher concentration of GFs compared to conventional PRP, further enhancing its regenerative capacity.
Modulation of the inflammatory response	Promotes a balanced and controlled healing environment, preventing excessive inflammation.
Stimulation of angiogenesis	Promotes the formation of new blood vessels, ensuring adequate blood supply to the regenerating tissue.
Enhancement of cell proliferation and differentiation	Stimulates the proliferation and differentiation of various cell types, driving tissue regeneration.
Promotion of ECM synthesis and remodeling	Stimulates the production and remodeling of the extracellular matrix, providing structural support and scaffolding for the regenerating tissue.

Preclinical and clinical evidence for AGF: a growing body of support for regenerative therapies

Activated growth factor (AGF) technology, with its ability to optimize the release and bioavailability of growth factors (GFs) from platelets, has garnered significant attention in the field of regenerative medicine. This section delves into the preclinical and clinical evidence supporting the therapeutic potential of AGF, exploring its efficacy in various experimental and clinical settings. Preclinical studies, conducted in vitro (using cells or tissues cultured outside of a living organism) and in vivo (using animal models), provide valuable insights into the biological mechanisms and therapeutic potential of AGF. These studies lay the foundation for clinical translation, providing evidence to support the safety and efficacy of AGF in humans.

In vitro studies have demonstrated the ability of AGF to stimulate various cellular processes involved in tissue regeneration. AGF has been shown to promote the proliferation of various cell types, including fibroblasts, endothelial cells, keratinocytes, and stem cells. This proliferation is essential for the formation of new tissue and the replacement of damaged cells. AGF can also induce the differentiation of stem cells into specialized cell types, such as osteoblasts (bone cells), chondrocytes (cartilage cells), and adipocytes (fat cells). This differentiation is crucial for the regeneration of specific tissues and organs. AGF can stimulate the migration of cells, such as fibroblasts and endothelial cells, towards the site of injury. This migration is essential for wound closure and tissue regeneration. AGF can promote the synthesis of ECM

components, such as collagen and elastin, providing structural support and scaffolding for the regenerating tissue. These in vitro studies provide compelling evidence for the ability of AGF to stimulate cellular processes involved in tissue regeneration, supporting its potential as a therapeutic agent.[16,17]

In vivo studies, conducted in animal models, have further demonstrated the therapeutic potential of AGF in various applications. AGF has been shown to accelerate wound healing in various animal models, including rodents and pigs. AGF-treated wounds exhibit faster closure rates, reduced inflammation, and improved tissue regeneration compared to controls. AGF has also demonstrated efficacy in promoting bone regeneration in animal models. AGF-treated bone defects show enhanced bone formation, increased bone density, and improved mechanical strength compared to controls. AGF has been shown to accelerate tendon and ligament healing in animal models. AGF-treated tendons and ligaments exhibit improved collagen organization, increased tensile strength, and faster functional recovery compared to controls. AGF has also demonstrated potential in promoting cartilage repair in animal models. AGF-treated cartilage defects show reduced cartilage degradation, increased cartilage matrix synthesis, and improved joint function compared to controls. These in vivo studies provide further evidence for the therapeutic potential of AGF in various regenerative applications, supporting its clinical translation for the treatment of various conditions.[18-20]

Clinical studies, conducted in humans, are essential for evaluating the safety and efficacy of AGF in real-world settings. While the number of clinical studies on AGF is still relatively limited compared to PRP, the available evidence suggests that AGF may offer significant advantages over conventional PRP in various applications. AGF has shown promising results in the treatment of various orthopedic and sports medicine conditions. AGF has been shown to reduce pain, improve joint function, and promote cartilage regeneration in patients with osteoarthritis. AGF has demonstrated efficacy in accelerating tendon and ligament healing, reducing pain, and improving functional outcomes in patients with tendonitis and ligament injuries. AGF has also shown potential in promoting muscle regeneration and functional recovery in patients with muscle injuries. AGF has also been investigated for its potential in dermatological and aesthetic applications. AGF has been shown to accelerate wound healing and reduce scarring in various types of wounds, including surgical wounds, burns, and diabetic ulcers. AGF has demonstrated potential in promoting skin rejuvenation, reducing wrinkles, and improving skin texture. AGF has also shown promise in promoting hair growth in patients with androgenetic alopecia (male or female pattern baldness). AGF has been used to promote bone regeneration and soft tissue healing in dental procedures, such as implant placement and periodontal surgery. AGF has shown potential in treating erectile dysfunction and Peyronie's disease. AGF has been investigated for its potential in treating inflammatory bowel disease.[18,19]

While the preclinical and clinical evidence for AGF is promising, there are still limitations to the current body of research. The number of clinical trials on AGF is still relatively small compared to PRP, and many of these trials have small sample sizes and lack long-term follow-up data. There is significant variability in AGF preparation methods, making it difficult to compare results across different studies. There is a lack of standardized protocols for AGF preparation and administration, which can lead to inconsistencies in treatment outcomes. Larger, randomized controlled trials with long-term follow-up are needed to confirm the efficacy and safety of AGF in various clinical applications. Standardized protocols for AGF preparation are needed to ensure consistency and reproducibility of treatment outcomes. Further research is needed to optimize AGF activation methods and identify the most effective methods for different clinical applications. Combining AGF with other therapies, such as stem cell therapy or biomaterials, may further enhance its regenerative potential. The preclinical and clinical evidence for AGF is growing, supporting its potential as a powerful tool for regenerative medicine. AGF has demonstrated efficacy in various experimental and clinical settings, promoting tissue regeneration, accelerating healing,

and improving functional outcomes. While further research is needed to fully elucidate its mechanisms of action and optimize its clinical application, AGF holds great promise for revolutionizing the treatment of various conditions and improving patient outcomes.

Table 4. Summary of preclinical and clinical evidence for AGF.

Study type	Findings
Preclinical studies	
In vitro studies	AGF stimulates cell proliferation, differentiation, migration, and ECM synthesis.
In vivo studies	AGF accelerates wound healing, bone regeneration, tendon and ligament healing, and cartilage repair in animal models.
Clinical studies	
Orthopedics and sports medicine	AGF reduces pain, improves joint function, and promotes tissue regeneration in osteoarthritis, tendonitis, ligament injuries, and muscle injuries.
Dermatology and aesthetics	AGF accelerates wound healing, reduces scarring, promotes skin rejuvenation, and stimulates hair growth.
Other applications	AGF promotes bone regeneration and soft tissue healing in dentistry, treats erectile dysfunction and Peyronie's disease in urology, and shows potential in treating inflammatory bowel disease in gastroenterology.

2. Conclusion

Activated growth factor (AGF) technology represents a significant advancement in platelet-rich plasma (PRP) therapies, addressing the limitations of conventional PRP and maximizing its regenerative potential. By actively stimulating platelet activation, AGF optimizes the release and bioavailability of growth factors, amplifying the regenerative cascade and promoting more effective tissue regeneration. Preclinical and clinical evidence supports the therapeutic potential of AGF in various applications, including wound healing, bone regeneration, musculoskeletal injuries, and dermatological conditions. AGF has demonstrated the ability to accelerate healing, reduce pain, and improve functional outcomes. While further research is needed to fully elucidate its mechanisms of action and optimize its clinical application, AGF holds immense promise for revolutionizing the field of regenerative medicine. With its ability to enhance growth factor release, increase growth factor concentration, modulate the inflammatory response, and tailor leukocyte content, AGF offers a refined and customizable approach to regenerative therapies, paving the way for more effective and personalized treatments. As research continues to advance, AGF is poised to become a cornerstone of regenerative medicine, offering new hope for patients seeking to restore their health and well-being.

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