Review

Delivering growth factors for therapeutics

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The method by which a drug is released can have a significant effect on therapeutic efficacy. The mode of drug delivery is especially relevant when the therapeutic agent is a growth factor because the dose and spatiotemporal release of such agents at the site of injury is crucial to achieving a successful outcome. Here, we highlight delivery technologies designed to facilitate the local and controlled spatiotemporal release of growth factors through the use of biomaterials, 3D micro- or nano-particles, microspheres, gene therapy and PRGF technology. We present some of the most interesting therapeutic applications based on these approaches and, on PRGF technology in particular, in addition to the limitations, future challenges and directions of the field.

Introduction

The increasing incidence of injuries to bone, cartilage, tendon and ligament is stimulating the development of less invasive surgical procedures and accelerated treatments that can reduce morbidity while enhancing functional recovery. The fields of tissue engineering and regenerative medicine are also creating new possibilities for tissue repair. In common, all of these emerging technologies are based on the delivery of growth factors and bioactive proteins to localized orthopedic sites to trigger healing and regenerative processes.

The tissue repair process is a complex cascade of biological events controlled by numerous cytokines and growth factors that provide local signals at sites of injury; these signals regulate the mechanisms and pathways that govern wound healing and tissue regeneration [1]. In the process of bone regeneration, for example, locally produced growth factors mediate first the migration of osteoprogenitors to the defect site and subsequently the direct differentiation of osteoprogenitors towards specific cell lineages. In addition, they control cell proliferation, bone re-vascularization and the production of extracellular matrix [2]. As a consequence, scientists have realized that the successful restoration of tissue functions relies on a sequence of steps, regulated by several bioactive growth factors and proteins, that unfold in time through an orchestrated sequence of spatial changes [3].

Our progressive understanding of these aspects at a basic level is providing a more sophisticated, knowledge-based approach to the development of technologies that facilitate the controlled spatiotemporal release of bioactive factors. Furthermore, the therapeutic potential of platelets in promoting and accelerating wound healing and tissue regeneration is being recognized by the scientific and medical community [4]. Once activated, platelets secrete numerous proteins such as fibrinogen, fibronectin and vitronectin, and growth factors including platelet-derived growth factor, transforming growth factor-β, vascular endothelial growth factor (VEGF), insulin-like growth factor, hepatocyte growth factor, angiopoietins, platelet factor-4 and thrombospondin to the local milieu, and it is these factors that drive tissue regeneration mechanisms [5,6]. Realizing the potential of platelets as biological systems for growth factor delivery, researchers have devoted much effort to formulating these cells into 'therapeutic preparations' that can be clinically tested and used in varied medical disorders [7].

In this review, we summarize different delivery technologies designed to permit the local and controlled spatiotemporal release of growth factors. These technologies vary in their use of biomaterials, micro- and nano-technology, cell encapsulation and gene therapy. We also highlight the important progress that has been accomplished in the area of using platelet-based preparations for growth factor delivery. Some of the most interesting therapeutic applications of this technology are discussed, in addition to the limitations, future challenges and directions of the field.

Technologies for controlled release of growth factors

Although many recombinant growth factors and cytokines are now available for research purposes and some have also been tested in humans, the clinical experience so far has been disappointing. For example, extensive research has been accomplished in the field of bone morphogenetic proteins (BMPs) as therapeutic tools for the treatment of different orthopedic disorders [8]. Of the more than 40 BMPs identified so far, however, only recombinant human BMP-2 and BMP-7 (also known as Osteogenic protein-1) have been developed further for clinical use. The former is effective in fresh fractures [9] and interbody spinal fusion, whereas the latter has shown efficacy in the treatment of non-union fractures [10].

It is difficult to determine why only a few growth factors have been approved and commercialized for therapy in humans. One explanation might lie in the delivery strategies commonly used for growth factor release: namely, bolus injection and growth factor infusion into systemic circulation or the tissues of interest. Unfortunately, owing

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to the rapid degradation and thus low local availability of growth factors, it is likely that these delivery strategies do not meet the physiological requirements of the tissue repair processes – a possibility that might explain in part why some large-scale clinical trials have not been successful [11–13]. It also seems that any treatment aiming to mimic the natural tissue regeneration processes should not be limited to the provision of a single growth factor, but should deliver multiple agents at an optimized ratio in a specific spatiotemporal pattern.

In the quest for better control over growth factor release, several technologies are being explored. Delivery of growth factors by means of 3D micro- or nanoparticles, injectable gels, composites or gene therapy are but a few examples (Table 1). Many of these approaches are based on combining the growth factors with appropriate biomaterials by either noncovalent or covalent bonding. Such a combination of polymers and growth factors can provide controlled release into the local microenvironment to yield desirable concentrations over a period ranging from days to months. Frequently used polymers for the release of bioactive factors include synthetic materials such as poly(glycolic acid), poly(lactic acid) and their copolymers [14]; nitrocinnamate-derived polyethylene glycol hydrogel systems [15]; and natural polymers such as alginate [16], gelatin [17] and collagen [18].

Vehicles for growth factor delivery take the physical forms of porous scaffolds, microspheres, and micro- or nano-capsules. The release profile of a bioactive factor can be altered either by manipulating the properties of the polymer [19] or by adjusting physical and chemical properties of the vehicle such as porosity, pore size, tortuosity, degree of cross-linking and degradation rate. As a result, systems can be designed to produce differential profiles of growth factor release, distinct spatial gradients of factors and even the release of growth factors in response to specific cues from the cellular microenvironment [20].

Such approaches might be particularly relevant to therapeutic neovascularization. Indeed, it has been reported that, although VEGF can induce endothelial cell proliferation and migration, thereby facilitating a survival signal until immature vessels become stabilized, the spatiotemporal delivery of VEGF with other agents such as angiopoietins, platelet-derived growth factor or basic fibroblast growth factor might provide a more stabilized and mature vasculature. Furthermore, tight control on the release ratio of angiogenic to anti-angiogenic factors might optimally regulate this biological process. Recently, tailored polymer systems that facilitate the sequential temporal release of various growth factors have been shown to induce more stable blood vascular networks than vehicles that introduce the factors simultaneously [21].

In other situations, it can be desirable to release the bioactive agent over long periods of time. In cell microencapsulation technology, cells can be genetically modified to release the therapeutic factor and then encapsulated in 3D alginate scaffolds [22–24]. These scaffolds are coated with a semipermeable immunobarrier that exerts a double protective function: namely, immuno-isolating the transplanted tissue from host's immune response, and protecting the host from any biological risk. As long as the cells are viable within the polymer scaffolds, the long-term '*de novo*' production of growth factors and neurotrophic factors is feasible. The use of cells immobilized in macrodevices such as hollow fibers has also been explored in the treatment of central nervous system diseases [25].

Preparation rich in growth factors

An exciting alternative to the formulation and use of platelets as growth factor and protein reservoir units is 'preparation rich in growth factors' (PRGF) [26], an autologous plasma product that is enriched in platelets. After activation, PRGF facilitates the local release of several growth factors and bioactive proteins that modulate the processes of wound healing and tissue engineering [27]. An advantage of this type of preparation is that it is easily and rapidly obtained from a patient's own blood and thus, because the donor and receptor should be the same, the immunological concerns are theoretically circumvented. Moreover, leukocytes can be eliminated from PRGF to avoid the pro-inflammatory effects of the proteases and acid hydrolases contained within white blood cells.

Methods of preparing platelet-rich preparations commonly use commercially available thrombin derived from bovine plasma, which triggers platelet activation through the thrombin receptor present on the platelet surface. This use of thrombin has been associated with the development of antibodies to clotting factors V and XI and thrombin, however, resulting in life-threatening coagulopathies [28]. To address this issue, our group and others have proposed the use of calcium chloride [26] or TRAP [29] to activate the platelet preparations more safely. Furthermore, by regulating the processing technique and centrifugation parameters, among other variables, it is possible to control the concentration of platelets and therefore the dose of platelet-derived growth factors. More importantly,

Table 1. Different technologies used for growth factor delivery

Technology	Growth factor	Application	Refs
Carrier material	IGF-I	Fibrin hydrogel used to repair articular cartilage defect in horse	[42]
Gene therapy	BMP-2	Adenoviral gene transfer of BMP-2 to enhance fracture healing in a established sheep model	[43]
Cell immobilization	CNTF	Cells expressing CNTF entrapped in hollow fibers for the treatment of human retinal degeneration (phase I clinical trial)	[44]
Injectable hydrogel	VEGF	Binary molecular weight alginate gel formulation for the treatment of ischemic disease in mice	[45]
Polymer scaffolds	VEGF and PDGF	Sequential delivery of VEGF and PDGF from PLG scaffold systems in severely ischemic hindlimbs in mice	[16]
Microparticle	IGF-I	IGF-I delivery from PLGA microspheres for new bone formation in two defect models of ovine long bones	[46]
Composites	BMP-2	PPF/TCP composites releasing BMP-2 for bone regeneration in segmental defects in rats	[47]

Abbreviations: IGF-I, insulin-like growth factor; BMP-2, bone morphogenetic protein-2; CNTF, ciliary neurotrophic factor; VEGF, vascular endothelial growth factor; PDGF, platelet derived growth factor; PLG, poly(lactide-co-glycolide); PLGA, poly(lactide-co-glycolide acid); PPF/TCP, poly(propylene) fumarate/tricalcium phosphate.

adjusting the platelet concentration will also permit final regulation of the growth factor ratio because platelet-rich preparations contain a mixture of bioactive agents deriving from both platelets and plasma. For example, insulin-like growth factor-I, which might induce the late-stage differentiation and activity of osteoblasts [30], is principally found in plasma.

In addition to biotechnological regulation during PRGF formulation, the ratio of growth factors in platelets and subsequent therapeutic effects can be manipulated by pharmacological means. For example, Wallace *et al.* [31] demonstrated in striking fashion that orally administered platelet-rich preparations accelerated the healing of gastric ulcers through the presentation of VEGF; interestingly, they suggested that the content of pro- versus anti-angiogenic factors in platelets might be regulated by several drugs, including ticlopidine, NSAIDs and thrombopoietin, which can alter the content of some growth factors relative to others. This possibility might have important clinical considerations because shifts in the serum levels of pro- versus anti-angiogenic factors can influence the healing of colon and stomach ulcers and have been suggested to be significant in the repair of joint injuries in arthritis [32].

The versatility of PRGF technology is also gaining the attention of the scientific and medical community. In effect, by controlling the elaboration protocol of the platelet-rich preparation or by combining the latter with biomaterials, different formulations with distinct therapeutic potential can be obtained. As a consequence, therapeutic alternatives based on growth factors are being churned out. For example, a liquid formulation enriched in plasma- and platelet-derived growth factors can be easily obtained from a patient's blood after platelet activation. This liquid preparation might be used as a conventional eye-drop and cell culture media [33,34]. It might also be potentially used in surgery, or particularly in dentistry to bioactivate dental implant surfaces by creating a biologically active nanomembrane on the titanium surface of the implant [35]. This approach accelerates osseointegration of the dental implants, improving their initial stability and consequently ensuring their future success.

When the goal is to maintain the growth factors at the site of implantation and to retain them from an excessive initial burst of release, however, the scaffold-like PRGF might be the best option. Simply by controlling the activation process of the platelets, it is possible to obtain a 3D fibrin scaffold from which more controlled delivery of the growth factors can be achieved. Tighter control over growth factor pharmacokinetics and biodistribution can also be obtained by combining the scaffold-like preparation with different natural and synthetic biomaterials such as collagen, calcium sulfate and polycaprolactone composites [36]. These biomaterials carry the opposite charge to growth factors, and thus form ionic complexes with them; therefore, release of the growth factors can be retarded and controlled depending on the strength of the ionic interaction.

Interestingly, these scaffold-like preparations not only act as carriers for growth factors and proteins, but also allow cellular infiltration and subsequent integration of the newly formed tissue within the native one. Because these fibrin scaffolds are biocompatible, noncytotoxic and nonimmunogenic to prevent adverse effects on recruited cells and neighboring tissue, their combination with isolated cells and growth factors has opened the door to several tissue engineering approaches, especially for bone regeneration but also for cartilage and periodontal tissue engineering [37].

Other interesting examples of potential approaches and formulations obtained from this technology include the

Table 2. Selected examples of platelet-based technology

Objective	Therapeutic approach	Results so far	Refs
Gastric ulcer healing	Orally administered human PRP (3×10^8 ptl/mL) every 12 h to rats	Treatment with platelet rich plasma significantly accelerated ulcer healing	[31]
Chronic ulcer treatment	Controlled pilot trial to evaluate the effectiveness of PRGF in the treatment of chronic cutaneous ulcers	At 8 weeks, the mean percentage of surface healed in the PRGF group was significantly higher than in the control group (73% versus 21.4%) ($P < 0.05$).	[38]
Bone regeneration in sinus floor augmentation	PRP (1×10^6 ptl/mL) mixed with autologous bone applied in patients	Bone densitometric values and bone amount were higher in sites treated with PRP	[48]
Distraction osteogenesis of the long bones	Combination of bone marrow cells and PRP was assayed in patients	The femoral lengthening showed significantly faster healing than the tibial lengthening by the bone marrow cell and PRP transplantation	[49]
Nerve regeneration	PRP and fibrin sealant applied in a controlled animal study in rats	The best results were obtained when the nerves were sutured and PRP was added to the sutures, showing a neurotrophic effect.	[50]
Repair of chronic elbow tendinosis	Buffered PRP was injected into the common extensor or flexor tendon	At final follow-up (mean 25.6 months), PRP patients reported 93% reduction in pain compared with before the treatment	[51]
Repair of anterior cruciate ligament	Collagen and PRP (1×10^6 ptl/mL) mixture used to fill the ACL site at the time of suture repair in pigs	Collagen-PRP mixture resulted in significant improvements in load at yield, maximum load, and linear stiffness at 4 weeks post-treatment	[52]
Expansion of mesenchymal stem cells (MSCs)	Platelet lysates obtained from PRP as cell culture media	MSCs maintained their osteogenic, chondrogenic, and adipogenic differentiation properties and their immuno-suppressive activity	[33]
Dry eye symptoms	Eighteen consecutive patients with symptomatic dry eye were treated with topical PRP	Symptoms improved significantly in 89% of the 18 patients. Improvement on lachrymal meniscus and conjunctival hyperemia were observed.	[53]

The ability to properly formulate the platelets and growth factors in novel formulations has stimulated the research and use of this type of preparations in a wide range of medical fields. Abbreviations: ptl/mL, platelets per millilitre; ACL, anterior cruciate ligament; MSCs, mesenchymal stem cells.

Table 3. Can growth factors be delivered for therapeutics?

Technology	Challenges
Entrapment of growth factors in microspheres	Scale-up. Ensure the long-term stability of the encapsulated growth factors. Biosafety.
Cell microencapsulation	Scale-up and biosafety. Storage protocols. Standardization of the technology.
Gene therapy	Scale-up and biosafety. Careful analysis of the benefits and risks.
Polymeric microchips	*In vivo* efficacy and biosafety.
PRGF technology	Establishing the dosing for each therapeutic treatment. Combination with biomaterials to control temporal release of growth factors.
Composites	Biosafety and biocompatibility. Efficacy to release growth factors in a spatiotemporal manner.

combination of PRGF with hydrocolloid or alginate-based dressings for the treatment of chronic ulcers [38], the interaction of PRGF with augmentation biomaterials used in dentistry or orthopedic surgery to facilitate their handling and manipulation, and the fabrication and use of an elastic and hemostatic fibrin. This biocompatible fibrin, which can be obtained after total retraction of the platelets has occurred, is an excellent tool with which to seal post-extraction sockets in dentistry and to promote the full epithelialization of soft tissues.

The ability to prepare platelets and growth factors in novel formulations has stimulated the research and use of this type of preparation in varied medical fields including dentistry, oral implantology, orthopedics, ulcer treatment, eye disorders and tissue engineering, among others [7,39]. Table 2 summarizes some of the most interesting recent therapeutic applications of platelet-based technology. Of particular note is the use of PRGF in surgery to accelerate the reconstruction and repair of musculoskeletal tissues. For example, activated PRGF can be injected among the ruptured tendon fibers after a tendon has been sutured. Applying this surgical approach in six injured athletes, we observed a significant acceleration in functional recovery as compared with a matched group that underwent conventional surgery [40]. Encouraging results have also been reported on the use of PRGF in arthroscopic surgery of anterior cruciate ligament and avulsion of articular cartilage [41].

Concluding remarks

Developing versatile therapeutic formulations that can control the local dose and spatiotemporal release of growth factors to the injured tissue is essential to achieving a successful outcome. Continual progress in our understanding of the basic biology of tissue repair and regeneration will provide important information for the further design of such systems. In the past few years, several technologies have been developed to induce the continuous and controlled release of therapeutic agents through the use of various biomaterials, 3D micro- or nanoparticles, microspheres, gene therapy, fabrication of composites, injectable gels, gene therapy and PRGF technology. The final goal of these technologies is to provide a more physiological release of growth factors to meet the needs of the injured tissue.

Although major advances have been made in the field of growth factor delivery, much work lies ahead (Table 3). For example, the development of more appropriate *in vitro* and *in vivo* experimental models and more accurate methods of characterization will facilitate progress in the field. The growth of micro- and nano-technologies must be realized by optimizing scale-up processes and by ensuring their safety and efficacy. Indeed, these novel technologies are likely to improve existing conventional approaches to patient care. The progress of gene therapy will ultimately demand patient acceptance and careful consideration of the social and economic consequences of genetic testing and therapy.

In the field of PRGF technology, elucidating the molecular complexity of these products, identifying both the essential growth factors that determine the fate of a specific tissue and the criteria to establish the dosing, improving the pharmacokinetics and biodistribution of the growth factors released, and developing tailored products for each pathological situation are but a few of the challenges. The ability to address each of these challenges will lead to a future where growth factors will be delivered solely to the place where they are needed and only at the levels and time at which they are required, thereby improving the benefit to individuals suffering from severely injured tissues.

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