13 Growth Factor Delivery Systems for Tissue Engineering and Regenerative Medicine

Pau Atienza-Roca, Xiaolin Cui, Gary J. Hooper, Tim B. F. Woodfield, and Khoon S. Lim

Abstract

Growth factors (GFs) are often a key component in tissue engineering and regenerative medicine approaches. In order to fully exploit the therapeutic potential of GFs, GF delivery vehicles have to meet a number of key design criteria such as providing localized delivery and mimicking the dynamic native GF expression levels and patterns. The use of biomaterials as delivery systems is the most successful strategy for controlled delivery and has been translated into different commercially available systems. However, the risk of side effects remains an issue, which is mainly attributed to insufficient control over the release profile. This book chapter reviews the current strategies, chemistries, materials and delivery vehicles employed to overcome the current limitations associated with GF therapies.

Keywords

Growth factor delivery · Tissue engineering · Delivery vehicles · Scaffolds · Biomaterials

P. Atienza-Roca · X. Cui · G. J. Hooper
T. B. F. Woodfield · K. S. Lim (✉)
Christchurch Regenerative Medicine and Tissue Engineering (CReaTE) Group, Department of Orthopaedic Surgery and Musculoskeletal Medicine, University of Otago Christchurch, Christchurch, New Zealand
e-mail: khoon.lim@otago.ac.nz

13.1 Introduction

Since tissue and organ transplantation became a widespread medical procedure, there has been a tremendous disparity between the need and the availability of organs and tissue grafts. The inherent limitations associated with organ transplantation include immune rejection, risk of disease transmission and donor-site morbidity. The tissue engineering and regenerative medicine (TERM) field, which aims to regenerate or repair tissues or organs, has emerged as an attractive strategy to overcome these issues.

In order to successfully engineer tissues in the laboratory, it is vital to firstly understand the physiological regenerative and development processes. The main components in the developmental and regenerative microenvironments are cells, extracellular matrix (ECM) and soluble signalling molecules [105]. Cells are the central unit of the tissue as they proliferate, migrate and differentiate in response to certain environmental inputs. The ECM acts as a physical support to these cells, while also providing the necessary biophysical and biochemical cues for tissue homeostasis. On the other hand, soluble signalling molecules circulate through the bloodstream and/or diffuse through interstitial fluid to modulate cellular behaviour. Thus, controlling these signals holds the potential to control cellular fate, which includes triggering or enhancing regenerative/healing processes. Growth factors (GFs)

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have been identified as soluble signalling molecules that play critical roles in both the development and regenerative processes and have been a main focus in TERM strategies.

Levi-Montalcini and Cohen firstly discovered GFs by studying the effect of sarcomas on axonal growth from chicken embryos [33], where the signalling molecule that triggered nerve growth was identified as nerve growth factor (NGF) [34]. Since then, a number of GFs which modulate many physiological processes have been discovered and applied to different regenerative medicine applications [100]. Some examples include bone morphogenetic proteins (BMP-2 and BMP-7) for bone regeneration [119] or vascular endothelial growth factor (VEGF) [67] and platelet-derived growth factor (PDGF) [139] for diabetic foot ulcers.

GFs are defined as secreted, biologically active molecules that can affect the growth and differentiation of cells. GFs act on cells by binding transmembrane receptors in a highly specific manner, which triggers a transduction cascade that generally starts with phosphorylation of the cytosolic domain of the receptor. Each GF is unique with specific roles in cellular behaviour (Table 13.1). For example, BMP-2 is essential for the maintenance of bone density [208]. It was previously reported that adult mice lacking BMP-2 showed spontaneous fractures and impaired bone repair [200]. Due to these characteristics, BMP-2 has been used in clinical settings for spinal fusion procedures and for non-union fractures [39]. A comprehensive list of these GFs with their respective unique characteristics are tabulated in Table 13.1.

In the native microenvironment, GF concentrations are usually in the nanomolar to picomolar range, where their presence is continuous and can last up to several weeks or months [9]. Initial clinical trials, which involved injection [196] or spraying [16] of GFs directly to the wound site, showed limited therapeutic effects, mainly due to the short presence of the applied GFs at the wound site. In order to better mimic the natural spatio-temporal concentrations of GFs, continuous doses of the GF were administered, causing systemic overexposure that can result in an undesirable increase in cancer risk and other side effects [53]. These results led to the realization of the need for a suitable GF delivery system, which has been the focus of many research groups in the past decades. This chapter will cover the different GF delivery approaches reported in the literature that aim to mimic key aspects of the regenerative microenvironment by controlling the spatio-temporal presence of GFs.

13.2 Design Criteria for Growth Factor Delivery Systems

The selection of an acceptable GF will not only depend on the type of organ or tissue that we are trying to regenerate, but also on the desired cell function. Some GFs are required to trigger proliferation and differentiation of cells that are already present at the site. In other cases where the cells required for healing or regeneration are absent, chemotactic GFs are able to trigger migration of cells to the wound site [106, 204]. The ECM is another key factor that needs to be considered while designing any GF delivery system, since it can modulate the effects of GFs through different mechanisms. For example, heparan sulphate [4], decorin [201], betaglycan [205], versican [70], fibronectin [127], collagen [173], vitronectin [203], SPARC (Secreted protein acidic and rich in cysteine) [19] and tenascin C [41], are ECM components that can bind GFs and modulate their diffusion and localization, further influencing their availability at the cell surface and their receptor-binding kinetics. Due to the dynamic remodeling of the ECM during regenerative processes [54, 212], it is essential to understand the interaction between GFs and the ECM for the design of an optimized delivery strategy. The interactions between cell receptor, ECM and GFs are represented in Fig. 13.1.

In order to modulate these complex interactions, mimicking different dynamic aspects of the native GF such as its localization, expression levels and expression patterns has been identified as a key design criteria for GF delivery systems. These aspects are further discussed in the sections below.

Table 13.1 Usage of growth factors in TERM applications

Growth factor	Main receptors	Signal transduction pathways	Role in adult tissues	Application in regenerative medicine	Clinical trials and commercially available products
BDNF [25, 66, 78]	TrkB	Ras-MAPK	Sensory neuron function		–
	LNGFR	PI3K	Cortical circuitry function	Spinal cord injury	
		PLC-γ1	Synaptic strength and plasticity	Stroke	
BMP2/BMP7 [39, 208]	BMPR-1A		Bone homeostasis and function. BMP-2: indispensable for bone fracture healing, chondrocyte proliferation and maturation. BMP7: kidney function	Spinal fusion	INFUSE® and OP-1™ implant are commercially available
	BMPR-1B	Smad1/5/8		Non-union fractures	
	BMP2-R	Ras-MAPK			
	ActR-1A	PI3K			
	ActR-2A/2B				
EGF [12, 25, 216]	EGF-R	Ras-MAPK	Injury response, homeostasis and growth in mammary gland, GI tract, nervous system. Inhibits osteogenic and chondrogenic maturation.	Diabetic foot ulcers	Diabetic foot ulcers [52]
		PI3K		Stroke	
FGF1 [11, 47, 81]	FGFR1-4	Ras-MAPK	Maintenance of vascular tone, Adipogenesis.	Peripheral ischaemia Peripheral nerve lesions	Peripheral artery disease [142], Skin burns [16], Spinal cord injury [83]
		PI3K, STAT, PLC-γ			
FGF2 (bFGF) [11, 25, 47, 81, 82, 93, 96]	FGFR1-4		Heart homeostasis and repair, vascular tone, angiogenesis, cartilage homeostasis	Periodontal defects	Periodontal defects [118], Peripheral artery disease [190], Critical limb ischemia [101], Bone lesions [87], Tracheal defects [194], Coronary artery disease [182]
				Skin wounds	
		Ras-MAPK		Bone lesions	
		PI3K		Myocardial infarction	
		STAT		Stroke	
		PLC-γ		Ligament regeneration	
				Tracheal defect repair	
FGF7 (KGF) [11, 47, 81]	FGFR1-4	MAPK, PI3K, STAT, PLC-γ	Skin repair	Oral mucosa regeneration	Kepivance® is commercially available
				Skin wounds	
HGF [56, 159]	c-Met	Gab-1	Homeostasis and regeneration of liver, lung, stomach, pancreas, heart, brain and kidney	Cirrhosis	Critical limb ischemia [137, 158], Chronic vocal cord lesions [95]
		PLC-γ		Liver regeneration	
		Ras-MAPK		Critical limb ischemia	
		PI3K		Chronic vocal cord lesions	
NGF [78, 120]	TrkA	Ras-MAPK, PI3K, PLC-γ1	Sensory neuron function	Peripheral nerve lesions	Alzheimer's disease [50, 202]
	LNGFR		Cortical circuitry function		

(continued)

Table 13.1 (continued)

Growth factor	Main receptors	Signal transduction pathways	Role in adult tissues	Application in regenerative medicine	Clinical trials and commercially available products
PDGF [1, 22, 58, 84, 117]	PDGFRα PDGFFRβ	PI3K, PLC-γ, Ras-MAPK	Postnatal angiogenesis andvascularization. Smooth muscle cell and fibroblast homeostasis.	Periodontal defects Skin wounds Bone regeneration Tissue vascularization	Periodontal defects and gingival recession [118], Diabetic foot ulcers [139]. Regranex®, Augment™ and GEM 21S® are commercially available
SDF1-α [59, 64, 171, 177]	CXCR4 CXCR7	Ras-MAPK, PI3K, JAK-STAT	Angiogenesis, mesenchymal stem cell homing during tissue repair, heart homeostasis	Revascularization Wound healing Tendon regeneration Muscle fibrosis	–
TGF-β(1-3) [110, 124, 148–150]	TβR-II TβR-I	Smad 2/3	Wound healing and skin homeostasis, bone healing, heart regeneration.	Bone regeneration Cartilage regeneration Periodontal tissue regeneration	–
VEGF-A [25, 69, 75, 166, 220, 222]	VEGFR(1-2) Nrp (1-2)	PI3K PLC-γ MAPK	Angiogenesis. Brain, liver, bone and lung homeostasis and regeneration,	Diabetic foot ulcers Bone regeneration Brain and myocardial ischemia	Diabetic foot ulcers [67]

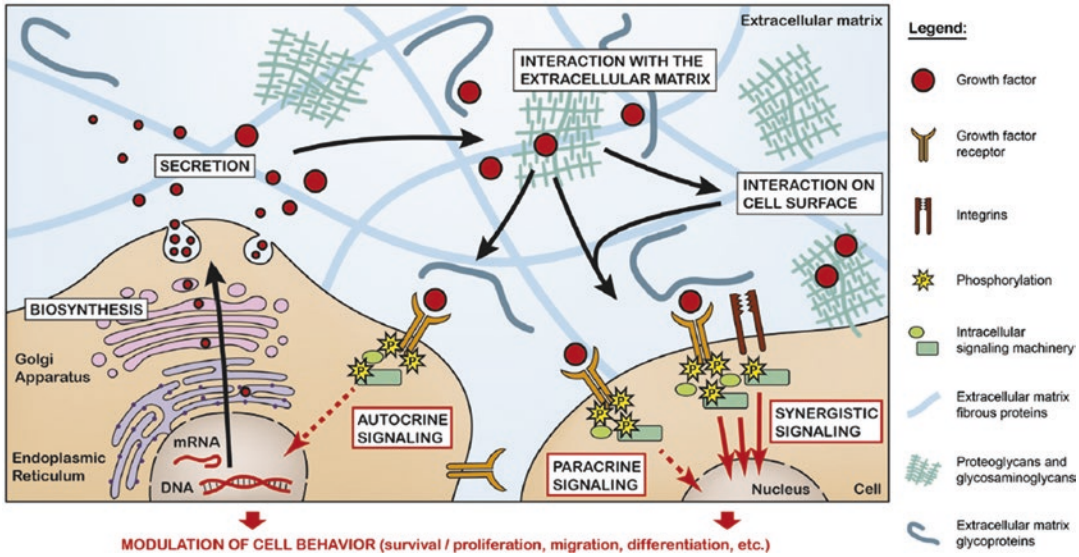

Fig. 13.1 From biosynthesis to cell receptor signalling, a growth factor's journey within the physiological ECM. After their biosynthesis, growth factors are secreted into the ECM, where they interact with ECM components before binding and activating their specific receptors. Growth factors mainly signal to cells in autocrine and paracrine fashion, to instruct their behaviour during morphogenetic processes. Complexes formed between growth factors, ECM components, and cell surface receptors may lead to additive or synergistic cell signalling events. (Reproduced from Ref. [134])

13.2.1 Localization of Delivered Growth Factors

GFs can have different effects in different tissues and cell types. For example, EGF promotes homeostasis in the GI tract [8] and mammary gland [90], but inhibits tissue maturation in the cartilage [24]. In order to achieve only the desired therapeutic outcome, the GF has to be delivered and contained spatially at the targeted tissue. Moreover, unnecessary presence of GFs in non-targeted tissues might also trigger cancer development and progression due to undesirable excessive cell proliferation [53, 68, 214]. In order to avoid these effects, major focus has been given to the study of different delivery systems that enable spatial containment of delivered GFs, such as nanoparticles or scaffolds.

13.2.2 Growth Factor Expression Levels

A major challenge in designing GF delivery approaches is optimizing the concentration required for the desired therapeutic effect. A recent meta-analysis on the use of FGF-2 for periodontal defects reported that insufficient amounts of GF failed to promote bone regeneration [118]. In the same study, it was also shown that excessive concentrations of FGF-2 resulted in insignificant promotion of bone growth. Furthermore, excessive concentration of BMP-2 has been reported to promote apoptosis in osteoblasts, mesenchymal stem cells (MSCs) [79] and periosteal cells [92]. Similarly, excessive VEGF concentrations promote the formation of aberrant and hyper-permeable blood vessels [146]. Therefore, the optimal concentration of GF to be delivered onsite has to be evaluated for each specific context and delivery system.

In developmental and regenerative processes, the culmination of spatio-temporal control over GFs is the formation of concentration gradients. GFs are generally secreted from a focal spot, which can be a cluster of cells with a specific phenotype or the defined space of a regenerative process. Cells at different distances from the spot will be exposed to different concentrations of the GF, and specific concentration thresholds strictly

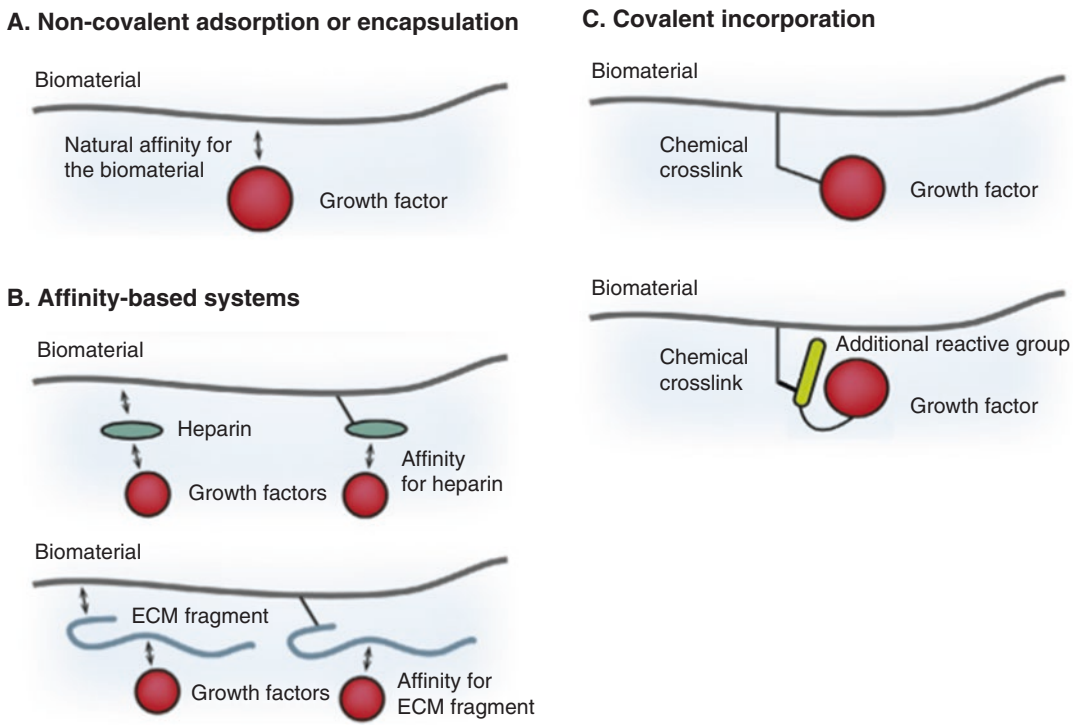

Fig. 13.2 Types of material-growth factor interaction. (**a**) Non-covalent interactions based on surface properties. (**b**) Affinity-based systems rely on natural interactions between growth factors and the extracellular matrix. (**c**) Covalent incorporation methods bind the growth to the material directly or through added functional groups or amino acids. (Adapted and modified from Ref. [134])

define spatial differentiation patterns in many stages of embryonic development [15, 154]. As individual cells are able to detect spatial differences in concentration, gradients of chemotactic GFs also represent a directional signal for cells to migrate to the wound site [6, 176] and for vascularization and innervation of tissues [78, 207]. The specific characteristics of these gradients are crucial for organized tissue formation. Thus, their adequate mimicry would be one of the pinnacles of controlled GF delivery.

13.2.3 Growth Factor Expression Patterns

The time period during which the GF is present on site is an essential parameter to achieve optimal therapeutic effects. The ordered presence and absence of specific factors corresponds to different stages of regeneration in natural processes [40, 126]. During bone regeneration, GFs that promote recruitment of MSCs and vascularization such as stromal derived factor 1 (SDF-1) and VEGF are firstly expressed. This stage is followed by the generation of a cartilaginous callus in which other GFs such as TGF-β3 are highly expressed, followed by a prolonged mineralization and remodeling phase in which expressions of TNF-α, IL-1 [126] and BMP-2 [125] are elevated. In an attempt to match these expression patterns, delaying the administration of a rhBMP-2-loaded calcium phosphate matrix for one week instead of 3 h post-surgery resulted in accelerated healing in a primate fibular osteotomy model [174]. It has also been reported that delaying the administration of an adenoviral BMP-2 vector by 5–10 days after surgery increases bone mineralization in a rat critical-size defect model [13]. It is also to be noted that the therapeutic effect of GFs is time-dependant. A 4 weeks sustained delivery of BMP-2 improves ectopic bone forma-

tion in comparison to just 5 days delivery [85], which agrees with the fact that the BMP-2 plays a major role in the long-term remodeling phase of bone regeneration [125]. These results indicate that matching specific GF expression patterns can result in improved tissue regeneration and should be taken into account in designing GF delivery systems.

Different biomaterial based systems with unique properties have been designed and employed for GF delivery in order to meet these design criteria. The following section reviews the different biomaterials and respective chemistries that have been used to deliver GFs for TERM applications.

13.3 Use of Biomaterials for Growth Factor Delivery

Low biochemical stability, short circulating half-life and rapid rate of cellular internalization are limitations of delivered GFs in TERM applications. In general, combining GFs with a biomaterial is an effective approach to overcome these drawbacks. However, no single material or strategy has yet allowed the required spatio-temporal control over the delivered GFs for optimal therapeutic effect. In recent years, the convergence of different materials, chemistries and fabrication techniques has brought the field one step closer to its goal by enabling more complex release patterns, including coordinated release of different GFs. This section will provide an overview of all these strategies, focusing on the advanced materials and procedures that enable control over the outlined design criteria.

13.3.1 Incorporation Methods

GFs can be incorporated into biomaterials through different strategies. The simplest procedure involves directly submerging a material in a GF solution to facilitate the adsorption of the GF to the material [94]. For example, GFs have been adsorbed on FDA approved polymers such as poly(lactic-co-glycolic) acid (PLGA) microspheres [44] and poly(caprolactone) (PCL) scaffolds [226]. Changes in material surface roughness [163] or the addition of nanostructured features [45] can increase the overall surface area, resulting in increased GF adsorption. Another prominent strategy that involves mixing the material with the GF in a liquid phase prior to scaffold fabrication allows the fabrication of scaffolds entrapped with GFs. Common scaffold fabrication techniques include freeze drying, phase separation, molding or *in situ* polymerization [105]. One issue in these strategies is the requirement to protect the GFs from harsh conditions during these scaffold fabrication processes in order to maintain their bioactivity. For example, melt molding can expose the GFs to high temperatures whereas radical based polymerization systems can facilitate GF oxidation/denaturation [114].

13.3.2 Interaction Between Growth Factor and Biomaterial

The interaction between GF and biomaterial plays a key role in all incorporation methods, affecting not only the release profile [94, 130], but also the biological effects of the GF [130]. The different types of material-GF interaction are summarized in Fig. 13.2.

Non-covalent interactions are weaker and can be mainly hydrogen bonds [43], Van der Waals forces, ionic forces or hydrophobic interactions [42]. Modifying the surface charges, charge density [3, 60] or available functional groups [61] of the material results in different GF binding affinities and release profiles. For example, increasing the surface hydrophobicity and decreasing the isoelectric point (pI) of PLGA microspheres resulted in an increase in the amount of rhBMP-2 adsorbed, whereas changes in molecular weight did not result in any significant change [172]. Functionalizing the material surface or the polymer chains with amino [45], alkyl [27, 45] or oxygen-terminated groups [185] can increase the adsorption of rhBMP-2 and result in a longer

Table 13.2 GF delivery vehicles fabricated using various biomaterials

Delivery vehicle or combined approach	Biomaterials	GFs
Particles	PLGA	IGF-1,VEGF, BMP-2 [31, 48, 211]
	PCL-PEG-PCL	bFGF [62, 63]
	PBCA	NGF [104]
	PEG-PLGA	bFGF [227]
	Tetronic®-PCL (Heparin)	bFGF [111, 112]
	PAMAM	EGF,VEGF [5, 197]
	Phosphatidylcholine liposomes with magnetite core	BMP-2, TGF-β1 [131, 193]
	DSPE-PEG-NHS	NGF [102, 217]
	Combined lipid SLN	NGF [103]
	Poloxamer 188/HSPC/cholesterol	bFGF [228]
	Silica (MSNs)	BMP-2,FGF [225]
	Iron oxide (SPION)	EGF, BDNF [156, 178]
	Semiconductor Qdot®	BDNF, NGF [162, 218]
Scaffolds	Collagen	BMP-2, BMP-7 [100]
	PLA	BDNF [151]
	PLGA	FGF, BMP-2 [55, 223]
	Chitosan-glycerophosphate	BMP-2, Insulin [29, 180]
	Fibrin	FGF-2, VEGF-A [230]
	PEGDA-Heparin	bFGF, TGF-β, KGF, Ang1, PDGF [153, 155]
	PEG	FGF-2, PIGF-2 [129]
	GelMA	BMP-2 [7, 170]
	PEG-PLLA-PEG	TGF-β1 [109]
	B-TCP	PDGF, GDF-5, BMP-2, hGH [65, 98, 99, 191]
	Bioglass	VEGF, BMP-2 [37, 215]
	CPC	BMP-7,VEGF [165]
	Titanium	TGF-β1, BMP-2, VEGF [168, 186, 192]
Particles incorporated in scaffolds or injectable systems	OPF	TGF-β1 [71]
	PLGA, Gelatin, PPF	BMP-2 [88]
	PLGA, PEG	CNTF, NT-2 [20]
	Gelatin, OPF	TGF-β1, IGF-1 [72, 73]
	PLA, alginate	BMP-2, VEGF [86]
	PLA, chitosan	IGF-1, BMP-2 [91]
	PHBV, chitosan	BMP-2,BMP-7 [175]
Core-shell	Gelatin, PPF	BMP-2, VEGF [89]
	PLLA,PLGA	BMP2-,FGF [209]
Layer by layer	Gelatin	BMP-2 IGF-1 [161]
	OPF	BMP-2,IGF-1 [123]
Biofabrication	GelMA	VEGF [21, 157]

release time. The interaction with the functional groups will also depend on the pI of the GF, and thus each GF will have specific release profiles when incorporated in the same material based on this approach [152].

A special case in non-covalent interactions is the use of GF-binding domains from ECM molecules. These strategies are generally classified as affinity-based, as the affinity of certain GFs for these domains is significantly higher and more specific than for single chemical groups or surface charges [136]. An example of these affinity-based domains are heparin or heparan sulphate, which have been extensively used for the delivery

of specific GFs such as NGF or BMP-2 [169, 221]. Fibronectin [128] and fibrinogen [129] GF-binding domains, which bind to several GFs from the PDGF, VEGF and FGF families and some from the TGF-β family, have also been used to functionalize scaffolds. The use of affinity-based systems has been extensively studied and reported in the literature [206], and the resulting release profile, which is significantly more sustained than for other non-covalent incorporation methods, positioned them as one of the most successful GF incorporation approaches to date. However, the strategies are limited to the release of GFs that display natural affinity for these domains, and the release profile differs between different GFs due to their distinct affinities with the system. In order to further improve the therapeutic effects, some engineered GFs containing additional ECM binding domains have been studied. Genetically engineered IGF-1 including the heparin-binding (HB) domain of HB-EGF was able to interact with specific GAGs in cartilage matrix after injection to the knee [133]. Through similar techniques, collagen-binding domains were added to NGF [188] or BDNF [66], promoting their interaction with collagen scaffolds [187] and the retention of the GF at the wound site.

The release profile for delivery systems that use non-covalent incorporation is generally characterized by an initial burst release [77]. The observed burst release profile has been suggested to have a role in early post-implantation complications [23, 199]. In order to reduce or eliminate burst release, protein immobilization to the matrix through covalent incorporation has been extensively studied [130]. It has been reported that the release of GFs conjugated to the biomaterial is then dependent on the materials' degradation profile. Moreover, it is possible to have further precise control over the GF release profile by adding features such as protease-cleavable sequences to the material [57] or to the GF-material linkage [46]. Aside from improved control over the GF release profile, presentation of covalently bound GFs to cells can result in a differentiated response in comparison to soluble GFs by inhibiting the internalization of the GF-receptor complex [80]. Covalent incorporation can also be used for patterning GF [116], including the formation of gradients in a material. Several GFs have been covalently incorporated in biomaterials for different applications, leading to improved functions such as endothelial cell proliferation [30], osteoblast adhesion to titanium implants [179], or even bone formation *in vivo* [219]. Common reactions for GF immobilization include carbodiimide coupling [121], photo-polymerization methods such as phenyl azide-based [219] or acrylate-based [116], and also click chemistry [115, 135]. One of the main limitations of these approaches is poor control over the exact reaction site of the GF, which can lead to disruption of the receptor-binding domain [130]. In order to improve the therapeutic effects of covalently incorporated GFs, some studies have engineered growth factors containing functional groups [144] or amino acids [189] at specific sites that do not overlap with the receptor-binding domain. Overall, covalent incorporation shows great potential for GF delivery as it offers higher control over the presentation and the release profile of GFs.

13.3.3 Delivery Vehicles for Growth Factor Administration

Biomaterials used for GF delivery can be fabricated into different types of vehicles, such as particles or scaffolds. Each delivery vehicle poses favourable characteristics and is adaptable to specific therapeutic strategies or administration procedures. A comprehensive list of different GF delivery vehicles is tabulated in Table 13.2 below.

13.3.3.1 Particle Systems

Particle systems, which can be in the range of <1 μm for nanoparticles or <1000 μm for microparticles, have been used to deliver GF for TERM applications [138]. The particle size affects the rate of GF release due to different surface-to-volume ratios and intracellular uptake [147].

Nanoparticles

Nanoparticles (NPs) can infiltrate deeper into tissue via capillaries and epithelial lining due to their small sizes, improving the transport properties and pharmacokinetic prolife of drugs *in vivo*. They are generally highly soluble and display low immunogenicity [107]. Targeted delivery of GFs to specific tissues can be achieved using surface functionalized NPs or using electromagnetic fields [224]. Surface functionalization can also enable NPs to cross the blood-brain barrier (BBB), which is not possible for other delivery systems without invasive procedures [51]. In general, NPs can be classified into polymeric, lipidic and inorganic depending on their composition.

Polymeric NPs can be fabricated as nanospheres, nanocapsules, micelles and dendrimers, all of which have been studied for GF delivery. PLGA is the most studied material to form nanospheres and nanocapsules, and it has been used for IGF-I [48], VEGF [31] and BMP-2 release [211]. Functionalization of PLGA nanoparticles with different concentrations of heparin has also been used to form an affinity-based system, where increasing the heparin concentration resulted in longer term release [31]. Low frequency ultrasound was combined with bFGF-loaded PLGA NPs to increase microvessel permeability for targeted skeletal muscle angiogenic therapy [26]. Apolipoprotein E (ApoE) was adsorbed to poly(butylcyanoacrylate) (PBCA) NPs in order to cross the BBB through an ApoE receptor-mediated response. NGF was adsorbed to the PBCA NP surface and then delivered to rats by intraperitoneal injection. Symptoms of scopolamine-induced amnesia were reduced after the administration, indicating targeted delivery to the brain [104]. Some polymeric NP systems have also been able to achieve long-term release: a heparin-conjugated Tetronic®-PCL micellar system was used for bFGF delivery, showing long-term delivery up to 2 months [111, 112].

Lipid based NPs that have been used for GF delivery are mostly liposomes and solid lipid nanoparticles (SLNs) [10]. Liposomes are closed vesicles formed by bilayers of hydrated phospholipids which enclose an aqueous core [35]. The main advantages of these formulations are their inherent low toxicity and scalable production methods [17]. Phosphatidylcholine liposomes loaded with magnetite particles were used for bone and cartilage regeneration after loading with BMP-2 [131] or TGF-β1 [193] respectively. Both tissues were targeted by magnetic induction [131, 193]. Despite their flexibility, liposome nanoparticles display low GF loading capacity and low stability due to enzymatic degradation, leading to a short release [224]. Other types of lipid NPs with different conformations have been used in order to overcome these issues. For example, a lecithin anionic nanolipid core was loaded with VEGF and covered by a Pluronic F-127 shell. The system showed increased stability in comparison to liposome systems, and a sustained release of VEGF for more than 30 days. The release period was extended by increasing the lecithin/Pluronic F-127 ratio, presumably due to changes in the ionic charge that enabled stronger interactions with VEGF [145]. Recently, an SLN system has been conjugated with heparin and loaded with NGF for neuronal differentiation. The release could be tuned by changing the composition of the solid core, where using stearylamine resulted in a faster release than using esterquat, and increasing the amount of cholesterol resulted in slower release [103].

Inorganic nanoparticles such as mesoporous silica NPs (MSNs), quantum dots (QDs) or metallic NPs have also been applied to GF delivery. In general terms, inorganic nanoparticles excel due to their easy handling and their physical properties. MSNs are used due to their high surface area and porosity [210]. For example, BMP-2 has been covalently grafted to the MSNs surface through an aminosaline linker, while dexamethasone was loaded in the nanopores to form a dual delivery system. The combination resulted in synergistic induction of bone formation in an *in vivo* ectopic model [229]. It has also been shown that the release kinetics can be tuned by controlling the porosity of the nanoparticles, where increased porosity leads to faster release [74], or by coating them with PEG, resulting in increased release time [14]. Magnetite NPs have been combined with other types of NPs, includ-

ing MSNs [160] and liposomes [131, 193], in order to provide them with magnetic properties that enable guided targeting using an external magnetic field. Other magnetic NPs can also be directly incorporated with GFs, such as superparamagnetic iron oxide nanoparticles (SPIONs), which enabled targeting specific areas of the brain using a magnetic field after adsorption of BDNF to their surface [156]. The main drawbacks of metal-based nanoparticles are their poor degradability and their tissue accumulation. Thus, their long-term toxicology should be further evaluated [210]. QDs have fluorescent properties that can be used to track conjugated molecules. Conjugation of BDNF [218] and NGF [162] with QDs enabled tracking of the GF after internalization by neurons [218] and PC12 cells [162], which was used to monitor its receptor internalization dynamics. In the field of bone regeneration, calcium phosphate nanoparticles have also been studied due to their high biocompatibility and bioactivity [18].

Overall, NP delivery systems represent a promising approach for GF delivery. One of the most important advantages of NP systems is the possibility of intravenous administration, which positions them as the least invasive GF delivery method. The specific properties of different NPs provide great advantages such as targeted delivery, enhanced MRI contrast or tracking of the NPs. Other systems such as MSNs can be used as a sequential delivery system, and complex NPs can be synthesized in order to combine the advantages of different nanostructured materials. On the other hand, aspects such as long-term toxicity and tissue accumulation of NPs should be further investigated before advancing to the clinical field.

Microparticles

The use of microparticles (MPs) generally results in a lower cellular uptake and tissue penetration in comparison to NPs due to their larger sizes [147]. On the other hand, their increased volume results in higher drug loading capacity, slower release and ease of production. These characteristics enable a longer-term release, which can be extended by increasing the particle size [28]. The materials used to generate MPs for GF release include naturally derived polymers such as gelatin [149, 150], alginate [122], and chitosan [164], as well as synthetic polymers such as PLGA [167]. As MPs adaptability to intravenous administration is low in comparison to NPs, most of the applications require the formation of a scaffold through microsphere fusion [143] or being incorporated in a solid scaffold [49] or an injectable hydrogel [38].

13.3.3.2 Scaffold Systems

Biomaterial scaffolds can be incorporated with GFs and implanted at the damaged area to achieve local release [114]. Scaffold systems can be classified as solid scaffolds or hydrogels depending on their composition.

Solid scaffolds are typically porous matrices fabricated by techniques such as solvent casting, gas foaming, particulate leaching, electrospinning or rapid prototyping [105]. These systems can be classified as organic or inorganic. Due to their mechanical properties and inherent tissue compatibility, inorganic scaffolds such as ceramic, bioglass or titanium play an important role in regenerative medicine [108]. Calcium phosphate-based systems excel due to their compositional similarities to the native bone ECM, and thus they have been extensively studied for GF delivery [18]. Most commonly used calcium phosphate materials include hydroxyapatite and TCP scaffolds with different porosities, which have been used for BMP-2 delivery resulting in positive effects ([65, 99, 191]. The incorporation of GFs within TCP to treat bone defects has resulted in different commercially available products. Therapeutic Goods Administration (TGA, Australia) and Health Canada have approved the safety of utilization of tricalcium phosphate (TCP) as scaffold to deliver PDGF (Augment™ Bone Graft; BioMimetic Therapeutics, Franklin, TN). Different clinical trials have concluded that PDGF-BB [2, 132, 140, 184, 195] and FGF-2 [32] loaded in β-TCP resulted in improved bone regeneration in periodontal osseous defects [118]. Clinical trials using β-TCP as scaffold to deliver GDF-5 for sinus lift augmentation in 2010 [98] and for periodontal defects in 2012 [213] also yielded posi-

tive results. Other calcium phosphates have also been studied for GF delivery. Mesoporous bioglass scaffolds have been fabricated to load VEGF, and the addition of pores resulted in more than 90% loading efficacy and extended release profile while retaining VEGF bioactivity [215]. Sumner et al employed a titanium scaffold to deliver TGF-β1 and BMP-2 in a dog humerus model, resulting in improved integration [186]. In another study, VEGF and antibacterial peptides were bound to titanium scaffolds, resulting in increased cell attachment and reduced bacterial growth [192].

Polymeric solid scaffolds have also been extensively studied for GF delivery. Homo- and copolymers of lactide and glycolide (like PLGA or PLLA) have been widely used due to their degradation into lactide and glycolide, which can enter into metabolic pathways [76]. The physical properties of these polymers can be altered by varying the ratio of lactide/glycolide, molecular weight or crystallinity [183], which directly influence the release profile of GFs. For example, PLA scaffolds have been loaded with BDNF by entrapment for spinal cord injury applications [151] while PLGA has been loaded with BMP-2 for bone regeneration [55]. Affinity-based systems have also been generated by conjugating heparin to the surface of PLGA scaffolds. FGF was incorporated in the scaffolds, resulting prolonged release and stimulation of vascularization *in vivo* [223].

Hydrogel scaffolds are one of the most successful and versatile GF delivery approaches, and the major proof of that are the commercially available products [100]. A collagen hydrogel loaded with BMP-2 (INFUSE®-BMP-2; Medtronic, Minneapolis, MN) has been approved by the FDA for treatment of degenerative disc disease. Another similar design using type I collagen matrix to encapsulate BMP-7 (OP-1™ Putty; Olympus Biotech Corporation, Hopkinton, MA) is also approved for fractures of long bones and lumbar fusion procedures. Furthermore, PDGF impregnated in a hydrogel (REGRANEX®, BioMimetic) has been approved for diabetic ulcer treatment. However, an increased rate of mortality secondary to malignancy was detected in patients treated with high amounts of REGRANEX® [53], which clearly shows the need for optimized controlled delivery systems.

Synthetic hydrogels such as poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG) are biologically inert, but have well-controlled and reproducible physical and chemical properties and no risk of disease transmission. These characteristics are of special interest for clinical translation and mass production. As an example, PEG has been crosslinked using thiol-ene chemistry [198]. This combination enabled high control over the mesh size and the degradation time, where decreased mesh size and increased degradation time led to longer-term release for up to 60 days. Naturally-derived hydrogels have higher batch-to-batch variation, but they hold the potential to interact with cells and undergo cell-mediated degradation. Most widely used naturally-derived hydrogels include fibrin, collagen, gelatin, chitosan, alginate and hyaluronic acid. As an example, tyraminated hyaluronic acid crosslinked using horseradish peroxidase (HRP) has been studied as an injectable system for protein delivery, showing increased release time by increasing the crosslinking density through changes in HRP concentration [113]. Fibrin sealants have been used for controlled release of FGF-2 and VEGF-A, enhancing blood reperfusion after myocardium infarction or limb ischemia [230].

Different strategies have been designed in order to obtain the benefits of synthetic and natural polymers in the same scaffold. In a comprehensive study, gelatin or heparin were crosslinked to PEG diacrylate (PEGDA) and the composites were used for incorporation of bFGF, TGFβ, KGF, angiopoietin-1 (Ang1) and PDGF. In general, the heparin conjugated PEGDA resulted in a longer GF release profile, which was different for each GFs due to differences in their interaction with heparin [153, 155]. In another study, the GF-binding domain of fibrin was incorporated in a PEG hydrogel. Co-delivery of FGF-2 and

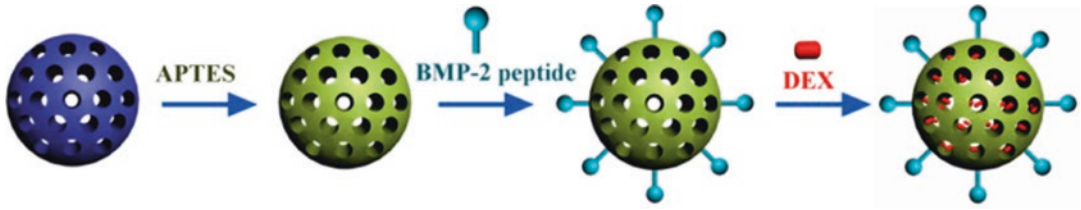

Fig. 13.3 Mesoporous silica nanoparticles were incorporated with two different bioactive components. Firstly, MSNs were functionalized with an amino group by treatment with APTES. BMP-2 was covalently linked to the amino groups through carbodiimide chemistry, and Dexamethasone was incorporated into the MSN pores by surface adsorption. (Reproduced from Ref. [229])

PlGF-2 using these gels enhanced skin wound healing [129]. On the other hand, modification of naturally-derived polymers with functional groups that enable controlled crosslinking is also a generalized strategy. These modifications enable tailoring the crosslinking density and mesh size, providing higher control over the release profile. As an example, gelatin undergoes gelation at temperatures under 35 °C. This process is not adequate for applications requiring high control over the network characteristics. Thus, gelatin functionalization with methacryloyl (GelMA) has been used for different applications that demand high control over the crosslinking density [97], including the generation of scaffolds for BMP-2 encapsulation [7, 170]. Increasing the degree of functionalization of GelMA results in decreased mesh sizes, increasing the release time of GFs such as BMP-2 [141].

Both hydrogels and solid scaffolds are the most successful platforms for GF delivery, as shown by the amount of commercially available products and clinical trials performed to date. The ability to spatially deliver GFs at the wound site by implantation of the scaffold or injection followed by *in situ* crosslinking is the most important advantage of these platforms.

13.3.3.3 Combined Approaches in GF Delivery

The combination of different materials and platforms allows several advantages in GF delivery applications. Firstly, it enables coordinated delivery of GFs by incorporating them in different materials or through different methods [9]. Secondly, combining different materials that can be independently modified increases the tailorability of the release profile.

Multiple incorporation strategies can be used in the same material in order to deliver different GFs with independent release profiles. For example, encapsulation of PDGF in PLGA microspheres, followed by surface adsorption of VEGF and generation of a scaffold by gas foaming-particulate leaching, resulted in a burst release of VEGF and a prolonged PDGF release [181]. In a different study, BMP-2 was covalently grafted to the surface of MSNs through an aminosaline linker while dexamethasone (DEX) was incorporated in the nanopores, obtaining short-term DEX release profile and a longer-term BMP-2 release profile (Fig. 13.3) [229].

MPs or NPs can be further incorporated into a scaffold (Fig. 13.4). TGF-β1 loaded gelatin particles have been immobilized in oligo poly(ethylene glycol) fumarate (OPF) and resulted in a reduction of the burst release. The release time could be further increased by increasing the molecular weight and the crosslinking time of the OPF hydrogel [71]. Encapsulation of NT-3 in PLGA MPs and inclusion of these MPs in a ciliary-neurotrophic factor (CNTF) loaded hybrid hydrogel resulted in a rapid CNTF release and a more sustained NT-3 release. Increasing the crosslinking density of the hydrogel phase resulted in increased release time from weeks to months [20]. Also, gelatin MPs encapsulated in OPF have been used for coordinated and tailorable delivery of TGF-β1 and IGF-1 with the aim of cartilage regeneration [72, 73]. Further examples include BMP-2-loaded

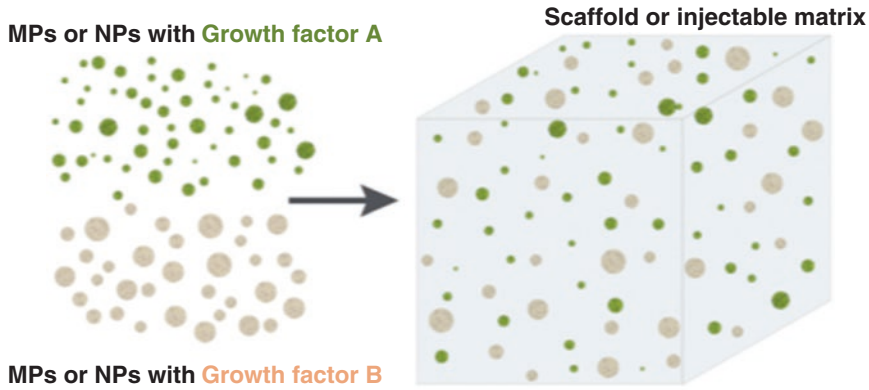

Fig. 13.4 Materials with different release characteristics are used to generate MPs or NPs, enabling high control over the release profile of one or more growth factors. These particles can be incorporated into a matrix in order to generate a scaffold or an injectable composite material. (Adapted from Ref. [9])

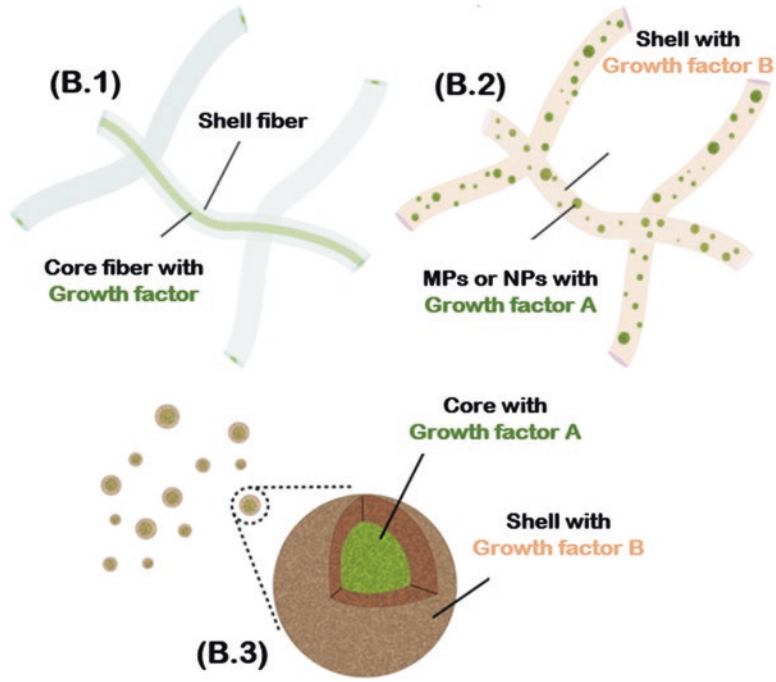

Fig. 13.5 Representation of core-shell approaches. Materials with different release characteristics are used to generate scaffolds with an internal core and an external shell, enabling high control over the release profile of one or more growth factors. Core-shell approaches can take the form of fibres, particles or scaffolds. (Adapted from Ref. [9])

PLA microspheres incorporated in VEGF-loaded alginate hydrogels [86] and IGF-1 encapsulated in gelatin microspheres loaded into chitosan scaffolds containing BMP-2 [91], both resulting in enhanced bone regeneration. Other strategies used to combine different materials for coordinated GF delivery include Layer-by-layer and core-shell approaches (Fig. 13.5 and 13.6).

In the past decade, emerging biofabrication approaches that generate complex scaffolds following a layer-by-layer automated deposition technique have also been studied for GF delivery. This automated high resolution approach offers a superior level of control over the spatial distribution of the materials in each single layer, dictating the scaffold architecture. Byambaa et al. bioprinted a scaffold with similar architectural features as bone using bioinks consisting of VEGF covalently conjugated to GelMA through carbodiimide chemistry. The GelMA-VEGF

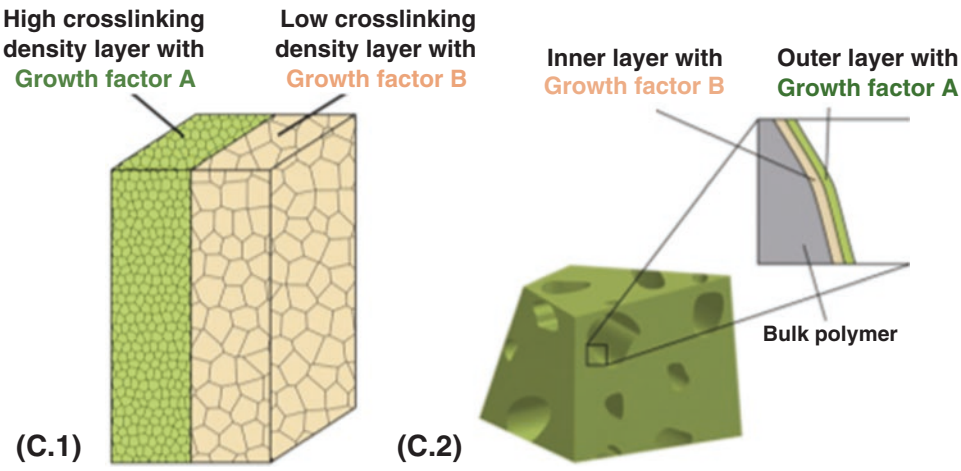

Fig. 13.6 Representation of Layer-by-layer approaches. Materials with different release characteristics are used to generate layered scaffolds or coatings, enabling higher control over the release profile of one or more growth factors. (Adapted from Ref. [9])

regions of the scaffold resulted in increased endothelial cell proliferation and tubulogenesis [21]. In a different study, VEGF-loaded GelMA MPs with different crosslinking densities were bioprinted in a Matrigel®/alginate bioink, showing increased release time by increasing the crosslinking density of GelMA. GelMA MPs with a more sustained delivery resulted in increased bioactivity in *in vitro* 3D cultures, and the presence of VEGF-releasing particles resulted in increased vascularization *in vivo* [157]. The possibilities of fine tuning the release profile expand if a coaxial systems is used for rapid prototyping [36], enabling the combination of both core-shell and biofabrication approaches. These examples showcase the potential of biofabrication to generate complex biomimetic scaffolds that include spatially- and temporally-controlled GF release systems for both tissue regeneration and *in vitro* modelling.

13.4 Conclusions and Future Perspectives

Although several GFs have been identified as signalling molecules that play important roles in developmental and regenerative processes, the use of GFs as therapeutics agents has yet made significant progress in the clinic. One major issue that still persists is the lack of suitable GF delivery systems that achieve optimal therapeutic effect while avoiding side effects. It was identified that the ideal GF delivery system should meet key design criteria such as being able to deliver the GF to a localized site, as well as mimicking the native GF expression levels and patterns during a typical tissue regenerative process.

A number of commercially available GF products exist in the market, but showed limited clinical success with potential side effects, further highlighting the need for development of more advanced delivery systems. The spatial and temporal control over the GF release profile from these delivery systems is highly desired. Various biomaterials, incorporation methods and fabrication techniques have been developed and employed for GF delivery. Although these delivery platforms often pose desirable characteristics, they are usually only adapted to the release of one specific GF. In the native regenerative microenvironment, several GFs work concurrently with different expression levels and profiles, synergistically facilitating the desired cellular behaviour. With our current understanding of this basic biological phenomena and the limitations of the current GFs delivery vehicles, it

is recommended that the field moves forward with combinatorial approaches that enable orchestrated release of multiple GFs.

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