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Mini-Review Article

Growth factor Containing Hydrogels for Tissue Engineering Applications

Hamid Babavalian[1], Ali Mohammad Latifi[*1], Mohammad Majid Sepantafar[2, 3], Hossein Mohammadi[4], Fatemeh Shakeri[1], Samaneh Khodi[1]

Abstract

Regenerative medicine provides substitutes for organ transplantation, which is restricted in applicability due to immune responses against allograft and the large discrepancy between the need for organs and the number of available transplantation. An artificial environment is allowing cells to induce tissue regeneration. Growth factors (GFs) play a significant role in the cell fates in their microenvironment. Nevertheless, the short half-lives of GFs and poor in vivo stability infiltration suggest that the classical routes are useless and insist on use of a drug delivery system (DDS). In this review paper, growth factor-based tissue regeneration using polymeric hydrogels is reviewed to show great potential ability of hydrogels in tissue engineering (TE).

1. Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Metallurgy and Materials Engineering, Faculty of Engineering, University of Semnan, Semnan, Iran
4. Department of biomaterials, Science and Research Branch, Islamic Azad University, Yazd, Iran

* **Corresponding Author**
Ali Mohammad Latifi
Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
E mail: amlatify@yahoo.com

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Introduction

Regenerative medicine is the field of life science and engineering which aims to direct the process of regenerating human cells, tissues or organs through controlling the biological environment [1]. Restriction factors of organ transplantation, such as immune responses against allograft and the large discrepancy between the need for organs and the number available for transplantation, would be dealt with the regenerative medicine. For instance, to retrieve therapeutic angiogenesis and ischemic tissue repair, biomaterials are widely applied as critical components in TE and DDS [2, 3]. Infusion of blends used in classical drug delivery approaches essentially lacks to target the specific cells that lead to an insufficient biological response. Modified polymer matrices are applied to deal with this problem. The emerging techniques for TE are commonly based on the localized delivery of the GFs and bioactive proteins to trigger the healing tissue process [4]. To inhibit denaturation and to control the release through polymer degradation, diffusion and external stimuli, the physical encapsulation or chemical immobilization of bioactive factors are applied [5].

With the respect to the injured site, the growth factor-based TE is divided into in vitro and in vivo categories [6]. Numerous growth factors produce local signals in the wound to control the tissue healing. Injectable biomaterials associated with controlled release of therapeutic proteins induce a temporal artificial extracellular matrix (ECM) and a depot to inhibit the protein degradation [7]. Despite the advances achieved in the GF delivery field, more investigations are needed to handle the challenges such as in vitro and in vivo experimental models and more accurate methods of characterization. Several approaches have been studied for better control over the delivery of GF release, including three dimensional micro and nanoparticles, injectable gels, composites or gene therapy (Table 1), which are mostly based on mixing the GFs with proper biomaterials by noncovalent and covalent bonds.

Viscoelasticity property and similarity to human tissues makes the polymeric systems appealing for biomaterial developments, which are developed in many fields, since the formation of first synthetic hydrogels by Wichterle and Lim in 1954 [8]. Hydrogel-based particles are noteworthy for the controlled release of protein and the co-delivery of proteins and cells [9, 10]. However, most hydrogels are extremely permeable, leading to a rapid release of the loaded proteins. Due to high water content and nontoxic polymers used in the preparation of hydrogels, they are biocompatibility and widely used in protein delivery

systems [9, 11]. Therefore, are frequently used to release GFs in a controlled and effective manner and to target the protein specifically to the wound site [12, 13]. Biomedical hydrogels that can deliver multiple GFs as well as providing an appropriate pore structure and porosity to potentially encapsulate cells are greatly potent as future therapeutic tools in TE. GFs microspheres embedded in the hydrogels are commonly applied in multimodal protein delivery [14, 15]. This review is aimed to investigate potentially GFs-based hydrogels for TE.

Table 1. Different approaches used for growth factors delivery [4].

Technology	Growth factors such as
Carrier material	IGF-1
Gene therapy	BMP-2
Cell immobilization	CNTF
Injectable hydrogel	VEGF
Polymer scaffolds	VEGF and PDGF
Microparticle	IGF-1
Composites	BMP-2

Interactions between ECM and growth factors in tissue engineering

The ECM contains varied elements like adhesive molecules, notch signal molecules and proteoglycan molecules, that bind to variety of GFs and control their activity, and provides spatial and architectural clues at various length scale as well as mechanical stiffness; therefore, it is essential to understand the biological functions and roles of GFs in the ECM [12]. The GF finally binds to the specific transmembrane receptors on the target cells and directs cellular behavior [16]. Cell destiny is influenced by chemical stability, concentration, duration and context of GFs, for example, definite GFs initiate angiogenesis, whilst the other ones induce maturation and retain the integrity of newly formed vasculature [1].

GFs can be found as a matrix bound proteins attached to ECM or soluble molecules, secreted by cells or cleaved from the matrix through enzymes [18]. Soluble GFs have slow diffusion rate and short biological half-lives (for example; 3 min for basic fibroblast growth factor (bFGF)), because they are degraded quickly and deactivated by any chemical and physical degrading reactions happening in the body temperature such as, enzyme. These properties trigger the soluble GFs to act in a diffusible fashion and to display a local short-range diffusion through the ECM, not endocrine fashion, which maintains the activity of GFs in the ECM [19]. Some of the GFs are commercially applied in the human body due to the bolus injection and GF infusion into the systemic circulation of desired tissue [4].

Unfortunately, the rapid degradation and low local availability of GFs causes adverse side effects and these delivery approaches do not encounter the physiological requirements of the tissue repair process [20]. Multiple GF deliveries should be occurred in an optimized ratio and spatio-temporal pattern to imitate the natural tissue regeneration process. Additionally, the large size of GFs, poor adsorption and degradation by proteolytic enzymes in the acidic condition limits the bolus injection, systemic intravenous and oral administrations, respectively.

A DDS must be utilized to make GFs perform efficiently in the body. For example, the GF secretion will be controlled at a desired site of action in the presence of an appropriate carrier and prevents the proteolysis in vivo studies for a long period from days to week in TE purposes, which must be followed by carrier degradation in the body after the complete release of GFs [6]. Generally, the GFs and factors associated with the application such as concentration, spatial-temporal gradients and the combination of GFs affect the tissue regeneration [12]. Cells also interact with adjacent cells via junctional structures which are beyond the scope of this review. The vehicles for GFs delivery take the physical forms of porous scaffolds, microspheres and micro-or nanocapsules and the release profile of a GF or bioactive factor can be adjusted through handling the physical and chemical properties such as, porosity, pore size, degree of cross-linking and degradation rate [21]. Consequently, the systems can be designed to yield differential profiles of GF release and different spatial gradients, leading to the release of GFs in response to the specific signal from the microenvironment, which rather relates to therapeutic neovascularization [4]. It is recently reported that appropriate polymer systems triggering the sequential temporal release of different GFs shows more stable blood networks than the vehicles bringing the GFs concurrently [22].

In addition to the identification of GF and its ability to diffuse through the ECM, the target cell number, the type of receptors and the intracellular signal transduction influence on the delivery of a certain message to distinct cells. For example, an identical GF can transmit different instructions depending on the receptor and the cell type [23]. In vivo application of GFs in solution form results in several issues such as severe side effects, because of high initial concentrations in injecting large doses, and the degradation of GFs occurring through various ways including denaturation, oxidation or proteolysis [24, 25]. Two well-defined pathways are proposed to present GFs; the chemical immobilization of GF into the matrix, involved the chemical binding between the polymer and the tissue, and the physical encapsulation of GFs in the delivery system, attained by encapsulation and diffusion release of GFs from the substrate into the surrounding tissue. The efficiency of GF delivery is developed by three dimensional patterning of scaffolds. The detail about the pathways is reviewed elsewhere [26]. The chemical modification can modulate the biodegradability of synthetic matrix, its biofunctional features and enhance the efficacy of GF delivery by the modulation of protease [27]. Four strategies are presented for GF release; including direct loading, covalently binding, carrier systems and electrostatic interaction [28]. Although, the direct loading is the easiest way to add GFs and peptides to polymer matrix, the incorporation of proteins into a matrix without modification leads to a rapid release during the primary

swelling phase. Eventually, followed by the prolonged release of a certain amounts of proteins which delayed by the gel network [29]. Since the protein release rate is commonly diffusion-controlled via aqueous channels inside the hydrogel, is not expected to have a controlled release of protein during a prolonged time [30].

Hydrogel structure and their mechanism of release

Hydrogels absorb a numerous amounts of water and, whereas, remain insoluble in an aqueous solution because of the chemical or physical cross linking of their polymeric networks. As a delivery vehicle, hydrogels can simultaneously encapsulate cells and bioactive molecule and several gel systems to facilitate a closely control of release characteristics by the systematic changes in physical and chemical structure [31]. Hydrophilic hydrogels involves remarkable physicochemical properties to be applied in drug delivery. For instance, the lack of hydrophobic interactions in hydrogels, which inhibits the denaturation of these species, makes them outstanding candidates to encapsulate biomacromolecules [32]. Moreover, gel formation usually occurs at ambient temperature and organic solvents are hardly required using synthetic and natural polymers. In-situ gelatin with cell and drug encapsulation abilities more separates hydrogels from the other hydrophobic polymers [33].

Naturally hydrogels involve several beneficial characteristic such as inherent biocompatibility and biodegradability, although, induce inflammatory responses and lacks sufficient mechanical properties. On the other hand, synthetic hydrogels usually have well-defined structures, but do not own the bioactive characteristic. Mesh size of the hydrogels is influenced by factors including (i) the degree of cross linking in gel; (ii) chemical structure; and (iii) external stimuli such as temperature and pH. Mesh size, ranges from 5 to100 nm in the swollen state for biomedical hydrogels, is critical to determine the physical properties of hydrogels including mechanical strength and the diffusivity of releasing molecule [34]. The size scales, that are larger than most small-molecule drugs, prevent the drug diffusion to be delayed in swollen hydrogel matrices. However, the hydrodynamic radii of macromolecules, such as peptides and proteins, can endure the release from swollen hydrogels [31]. A favorite rate of macromolecule diffusion can be obtained by change in the structure and the mesh size of swollen hydrogels [35]. Large molecular weights and three dimensional structures make the effective delivery as a challenge.

The inclination of biomolecules to extremely short plasma circulation times and rapid renal clearance results multiple daily injections, leading to a high doses and may induce local toxicity and immune responses. Unlike hydrogels which can be used as injectable matrices, solid scaffolds usually require more invasive delivery route. Solid scaffolds with typically porous structures are fabricated by methods such as, solvent casting, particulate leaching, electrospinning, gas foaming and rapid prototyping [36. 37, 38, 39, 40]. A significant difference of hydrogels from solid scaffold is their three dimensional matrices fabricated from hydrophilic polymers with a high water quantity, which distinctively makes hydrogel macroscopically solid, while behave such an aqueous solution on a microscopic scale. Therefore, the diffusion of molecular species from the hydrogel only depends on the space between crosslinked polymer components, but, it would be hard to control the rate of release [41].

Several natural polymers including gelatin, collagen, fibrin, hyaluronic acid, alginate, chitosan and dextran, which are interestingly similar to the components of ECM, and several synthetic polymers including poly(ethylene oxide) (PEO), poly(acrylic acid) (PLA) and poly(vinyl alcohol) (PVA) are applied to provide hydrogels [42]. The encapsulation release of bioactive materials is a common property of hydrogels in controlled release, in addition to several distinctive properties such as stimuli responsiveness that can be tailored into hydrogel networks during construction [31].

Hydrophilicity of hydrogels prevents the host immune response and decreases phagocytic activities in vivo condition which leads to the increase of delivery circulation time [43]. Hydrogels can also contribute in scaffolding in TE applications, such as cartilage and nerve TE [44, 45, 46,]. The slight gelling conditions and in situ polymerization abilities of hydrogels allow the simultaneous encapsulation of cells and GFs. In order to provide the necessary signals for cell migration, differentiation and angiogenesis ECM production, the release of encapsulated GFs need to be controlled by several routes including: (i) Diffusion-controlled (ii) Swelling-controlled and (iii) Chemically-controlled [47]. Diffusion-controlled, modeled by Fick's law of diffusion with constant or variable coefficients, is the most valid mechanism to illustrate drug release from hydrogels [34]. Swelling-controlled release occurs in a diffusion condition faster than hydrogel swelling. The modeling involves moving boundary conditions where molecules are released at the interface of swollen hydrogels [48]. Chemically-controlled release illustrates the release by reactions in a delivery matrix, most commonly, the cleavage of polymer chains, and reversible or irreversible reactions that take place between the polymer network and releasable drug.

Under definite conditions, the rate of drug release would be controlled by the surface or the bulk erosion of hydrogels [49]. The rate and mode of drug release from hydrogel matrices are influenced by the geometry of hydrogel-based system, materials selection and network fabrication. For instance, drug diffusion coefficient, the most prominent variable, is influenced by the molecular size of the drug, characteristics of the polymer network, hydrogel, the incorporation of ionic group, stimuli-responsiveness and crosslinking density [50].

The majority of hydrogel systems depend on the moderate release of physical encapsulation process, a clear advantage over more sophisticated release process, which is the frequent practical methods to control the release, such as active local GF delivery in TE. Loading is completed by the incubation of preformed gel with the protein or by addition of the protein to the hydrogel forming monomers [31]. Both features of the polymeric network and the protein effect on the release mechanism,

for instance, when the hydrogel pores are larger than the hydrodynamic radius of the protein, the driving force is diffusion that depends on the protein size and the water-content of the gel, called free volume [34]. On the other hand, swelling or erosion (bulk or surface) render the release in presence of hydrogels pores smaller than the protein diameter. In general, the majority of gel matrices show diffusion controlled release, following Higuchi's kinetics, suggesting that the release is proportional to the square root of time [51]. The efficiency of release profile was mainly confirmed for the delivery of several GFs for TE applications [52]. The diffusional spatio-temporal GF verified effective not only for bone regeneration, but also for engineered tissues such as, blood vessels [53]. Both the diffusion via matrix and the hydrogel degradation rate influence on the protein release rate from the hydrogels. Crosslinking density of the polymer network can adjust the release kinetics of proteins from hydrogels. In this case, the ability of synthetic polymers to adjust their chemical structure for a modular release is of their beneficial, whereas, natural polymer networks can be adjusted to some extent through changing polymer concentration and crosslink density [54].

A significant advantage of hydrogels materials compared to natural polymers is being static such that the delivery from the static matrices is initiated by the passive diffusion or coupling with the rate of matrix resorption occurring independently, in preference to offering specific signals for molecular interaction with released GFs, or interaction with the cells in the targeted tissue [55]. However, delivery systems are mainly designed to perform under static conditions, environments such as many tissues are mechanically dynamic, therefore, and the release of GFs could be controlled by mechanical signals. Mechanical compression such as an increased pressure inside the gels leads to the release of unbound molecules. Moreover, upon gel relaxation, GF bound to the hydrogel dissociates, which achieves the pool of soluble drug available for release by subsequent compression [56].

Hydrogel-based drug delivery system

In this section, we will investigate different hydrogel-based DDS for TE. In addition to permeability, the molecular transport of nutrients and wastes in which are not vital for cell survival in the hydrogels, can readily be declined by the co-delivery of proteins and cells .The variation of cross linking density mostly renders a change on the mechanical properties of hydrogels, including *mechanical* stiffness which is required to be decoupled from the variation of the permeability [57, 58]. Various GFs incorporated into a hydrogel matrix are delivered by microparticles of acidic and basic gelatin, including the independent release of bone morphogenic protein 2 (BMP-2) or insulin-like growth factor I (IGF-1) carried on by glycidyl methacrylated dextran (Dex-GMA)/gelatin matrix [59]. Spatio-temporal control over the delivery of GFs both increases the tissue regeneration and avoids unpleasant and potentially adverse-effect elsewhere than the target which can be achieved from hydrogels by (i) direct and (ii) indirect delivery approaches. Direct release can be accomplished by several approaches including physical encapsulation, non-covalent binding and covalent immobilization to the delivery system using enzyme-degradable linkers and double carriers, in which the release of protein loaded micro or nanospheres in hydrogels is obtained by diffusion and/degradation mechanisms. Indirect approaches rely on gene therapy and cell transplantation. The gene therapy is performed by the expression of desired protein that is to be delivered into the target tissue, whereas, the cell transplantation is developed by encapsulating the specific proteins secreted by cells in a hydrogel [60, 62]. Typically, diffusion, swelling, erosion, external stimuli mechanisms or their combinations could control the drug release from the hydrogels [31].

Synthetic-based polymers

It is reported that the concentration of polymer and the yield of neuritis stimulated from retinal explants affect the in vitro release of ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) from poly(ethylene glycol) (PEG) and acrylated PLA (PLA-b-PEG-b-PLA) hydrogels [62]. Hydrogels reported by Hubbell et al. have been extensively investigated for GF delivery due to easily modification of the network via macromer chemistry and solution concentration [63, 64].

For example, PEG/polyesters based on PLGA-PEG-PLGA triblock hydrogels were applied to release TGF-β1, as a slow releasing drug reservoir for wound healing that showed a considerable re-epithelization, and porcine growth hormone (pGH) and Zn-pGH for 10-14 days in vitro [65, 66, 67,]. Another type of hydrogel that is based on thermosensitive PEG-based networks is formed upon gelation of p(HPMAm-lac)-PEG-p(HPMAm-lac) triblock copolymers, its cartilage TE ability is proved by releasing proteins [68]. A decrease in myocardial infarction is achieved by a control over the release of platelet-derived growth factor BB (PDGF-BB), stromal cell-derived factor-1 (SDF-1) and IGF-I using peptide-based hydrogels [69-71]. Stimuli-responsive hydrogels are engineered to release GF upon adding a drug; such as developed gyrase subunit B (*GyrB*) coupled by coumermycin that is responsive to aminocoumarin antibiotic, novobiocin to release VEGF [72]. Furthermore, the hydrogels consisting of multiarm vinyl sulfone-terminated PEG, a monocysteine containing adhesion protein, and a matrix metalloproteinases (MMP) are investigated for TE, such as the release of VEGF by MMP [73].

Phelps et al. provided an incorporation of VEGF, enzyme degradable sites and arginine-glycine-aspartic acid (RGD) cell adhesive ligands and reported the system as a directive scaffold and a growth delivery vehicle [74]. The risk of immune response and viral and bacterial contaminations with the use of natural polymers can be overcome by synthetic polymers; however, immune response is obviously stimulated by the most synthetic polymers, thus the proteins incorporation results in a harsh environment leading to inactive and inappropriate proteins for oral drug administration. Thus, a strong decision cannot be made [75]. Protein was loaded on a chemically crosslinked p(HPMAm-lac)-PEG-p(HPMAm-lac) hydrogel prepared

by thermogelling together with photo polymerization and showed a diffusion mechanism of release [76]. VEGF-conjugated, biofunctionalized PEG-peptide hydrogels could release VEGF only upon a local cellular demand and a controlled induction of angiogenesis [55]. Degradable PEG hydrogel networks by protease and light can be used to degrade PEG-based polymers to release the GFs and induce migration cells, however, the light is only applicable in prototypes because the range of applied wavelength or electric fields for activation is not suitable for in vivo applications [77,78]. The release rate of GFs is also influenced by the dynamic mechanical environment in delivery site, thus, mechanical loading can drive the bioactive molecules delivery (e.g. heart TE) [79]. Dextran hydrogel networks were applied to release bFGF with a close to first-order kinetics in 28 days and the release of proteins did not show a burst-effect [80].

Natural-based Polymers

SDF-1, a naturally occurring chemokine that is rapidly over expressed in response to tissue injury, was delivered by an alginate hydrogel patch with purified recombinant SDF-1 [81]. In a study, VEGF as a polymer was successfully delivered with an improved control over release kinetics by a cross-linking alginate microparticle in which Zn^{2+} was applied instead of Ca^{2+} [82]. Hennink et al. developed a dextran based delivery system, where a dextran backbone is derivatized with hydroxylethyl methacrylate (HEMA) moieties, potentially suitable to deliver IL2, hgH, GF and cytokine in a controlled style. It was reported that native proteins were released by diffusion/swelling [83, 84]. Himestra et al. synthesized dextran-peptide bioconjugates, dextran vinyl sulfone conjugates (dex-VS) and tetrafunctional mercapto poly (ethylene glycol) (PEG-4-SH) polymers. The release of bFGF from the hydrogels showed a diffusional release and a specific level of tailor ability [80]. Sun et al. synthesized a hydrogel derived by Dextran-allyl isocyanate-ethylamine (Dex-AE) with varying degrees of substitution by which an increased swelling and VEGF release rate were observed.Furthermore, an increase in the size and the number of newly formed functional vessels was observed by the release of multiple angiogenic GFs [85]. In another study, hybrid hydrogels comprising glycidyl methacrylated dextran and gelatin, processed into microspheres, have been developed to deliver GFs, including BMP-2 and IGF-1 [86]. Park et al. prepared crosslink thermosensitive hyaluronic acid/Pluronic composite hydrogels that released human growth hormone (hGH) with kinetics associated with the mass erosion [87]. A photopolymerized hydrogels comprising glycidyl methacrylate modified hyaluronan hydrogels was prepared to release BMP-2 and/or VEGF from their matrices for bone regeneration in situ. It was shown that the amount of formed mineralized tissue is increased by the co-delivery of an angiogenic molecule (VEGF) in conjunction with an osteoinductive molecule (BMP-2) [88]. Crosslinked PEG diacrylate/thiolated hyaluronan hydrogels were investigated for the delivery of multiple GFs (VEGF and/orAng-1) in both presence and absence of heparin. It was found that greater neovascularization was existed when the hydrogels were loaded with both GFs [89].

The physical and chemical instability of proteins are challenging issues in drug delivery [90]. Therefore, injectable protein pharmaceuticals are preferred over oral administration, because in the oral drug delivery system, drug must be protected due to the harsh environment produced by pH. To handle the issue, natural based polymers are used to design pH-sensitive hydrogels in oral drug delivery application. To prepare a hydrogel with high porosity, George et al, prepared alginate-guar gum hydrogels by freeze-casting method and used BSA as a model protein drug to study controlled drug delivery. Lower and higher amounts of the drug release were shown in pH~1.2 and pH~7.4, respectively [75]. In a study by Lee et al. a biomaterial containing alginate hydrogels increased the release rate of VEGF both in vitro and in vivo conditions [79]. It was revealed that gelatin hydrogels incorporated bFGF with low water content possess a low release in vivo [91, 92]. It also enhanced bone defect regeneration after 21 week implantation compared to free gelatin hydrogel which showed no bone formation [93]. In other study, it was reported that the controlled release of TGF-β1 accelerated bone repair from the gelatin hydrogel compared to the free TGF-β1 [94].

Conclusion

Noteworthy advancement has been made up to date both in the understanding of GF biology and the way microenvironment controls the cellular response, and in the development of polymeric hydrogels to control the delivery of GFs to stimulate tissue regeneration. The concentration and gradient of a GF in a tissue regulates the cellular response. The dynamic nature of these interactions provides spatio-temporal control over GF release is a vital issue to obtain a desired effect. Although major advances have been made in the field of GF delivery, much work lies ahead. For instance, the improvement of proper in vitro and in vivo experimental models and more strategies of characterization can facilitate progress within the field.

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