Expert Opinion

Critical factors in the design of growth factor releasing scaffolds for cartilage tissue engineering

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Background: Trauma or degenerative diseases of the joints are common clinical problems resulting in high morbidity. Although various orthopedic treatments have been developed and evaluated, the low repair capacities of articular cartilage renders functional results unsatisfactory in the long term. Over the last decade, a different approach (tissue engineering) has emerged that aims not only to repair impaired cartilage, but also to fully regenerate it, by combining cells, biomaterials mimicking extracellular matrix (scaffolds) and regulatory signals. The latter is of high importance as growth factors have the potency to induce, support or enhance the growth and differentiation of various cell types towards the chondrogenic lineage. Therefore, the controlled release of different growth factors from scaffolds appears to have great potential to orchestrate tissue repair effectively. *Objective*: This review aims to highlight considerations and limitations of the design, materials and processing methods available to create scaffolds, in relation to the suitability to incorporate and release growth factors in a safe and defined manner. Furthermore, the current state of the art of signalling molecules release from scaffolds and the impact on cartilage regeneration *in vitro* and *in vivo* is reported and critically discussed. *Methods*: The strict aspects of biomaterials, scaffolds and growth factor release from scaffolds for cartilage tissue engineering applications are considered. *Conclusion*: Engineering defined scaffolds that deliver growth factors in a controlled way is a task seldom attained. If growth factor delivery appears to be beneficial overall, the optimal delivery conditions for cartilage reconstruction should be more thoroughly investigated.

Keywords: BMP, Cartilage, controlled release, growth factors, IGF, scaffolds, TGF, tissue engineering

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1. Introduction

Articular cartilage has the important functions of assuring freedom of movement of the joints and bearing loads and dissipate stresses. This unique tissue provides smooth and frictionless surfaces which, combined with its viscoelastic properties, allow the stable movement of human skeletons over our lifespan [1]. However, in many cases, articular cartilage degenerates and loses its structure and function, causing pain, loss of motion and morbidity. This is either the result of joint diseases (predominantly osteoarthritis), metabolic and genetic conditions (such as Paget's disease and Stickler syndrome), or traumatic lesions [2]. The problem is aggravated by the fact that impaired cartilage has low capacity to self-repair structural damages resulting from injuries or diseases, as has already been reported by William Hunter over two centuries ago [3].

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Considering the high prevalence of articular disorders in societies where the proportion of middle aged and elderly populations is increasing, surgeons and scientists have displayed considerable efforts to repair or regenerate this tissue [2]. Different surgical techniques have been evaluated and used to relieve patients from pain and restore the capacity of movement [4,5]. However, the success of these different therapeutic approaches has been so far limited and a traumatic complete replacement of the joint by a prosthesis is often the best compromise offered to sufferers.

Over the last decade, a novel approach bridging biotechnology and materials science has gained interest as a means of repairing deficient cartilage more efficiently [6,7]. Tissue engineering proposes to reconstruct or reconstitute tissues both structurally and functionally by combining cells, biomaterials mimicking extracellular matrix (scaffolds) and regulatory signals [8]. Different approaches have been considered for each of these aspects, for instance regarding the cell source [7,9] (chondrocytes, dedifferentiated chondrocytes or pluripotent mesenchymal stem cells), the scaffolds chemistry and architecture [10,11], or the type of growth factors to be used. The latter is of high importance as growth factors have the potency to support, induce or enhance the growth and differentiation of different cell types towards the chondrogenic lineage and orchestrate the tissue repair. However, each growth factor requires different dosages and delivery rates to the cells *in vitro* or *in vivo*. Therefore, the porous scaffolds should offer the possibility to control the release of one or more growth factors in a defined manner.

This review will report and discuss the state of the art of growth factor release from scaffolds with regards to cartilage repair, after having first introduced the cartilage healing problem and the different methods available to create porous scaffolds.

2. Cartilage structure and repair

Although the human body contains three types of cartilage (elastic, fibrous and hyaline), most current research involving porous scaffolds and growth factor release is centered on hyaline cartilage.

Hyaline cartilage is the predominant form of cartilage in the body and coats the surface of articulating joints. For this reason, it is often referred to as articular cartilage. The constituents of articular cartilage are water-containing gases, small proteins, metabolites and a high concentration of ions such as Na^+, Ca^{2+} and $C1^-$ (60 – 80% weight); chondrocytes (2% volume); and extracellular matrix (40 – 20% weight) [1,12,13]. The extracellular matrix (ECM) is mainly composed of collagen fibrils (from which over 90% is of type II), non-collagenous proteins and proteoglycans. The collagen matrix has a complex anisotropic organization which provides much of the mechanical integrity of cartilage. The proteoglycans are formed by negatively charged glycosaminoglycans polysaccharides (hyaluronic acid, chondroitin sulfate, keratan sulfate and dermatan sulfate) covalently attached to a central protein. The major proteoglycans in the cartilage (90% of the total proteoglycans) have a large number of chondroitin and keratan sulfate and are called aggrecan. They associate non-covalently with hyaluronic acid of high molecular weight to form large aggregates. Due to the high polarity of the glycosaminoglycans, proteoglycans interact strongly with water and swell. Water is drawn into the tissue because of the osmotic imbalance caused by their negative charge and mobile counter ions such as Na^+. The hydratation is restricted by the collagen fibrillar network, resulting in a swelling pressure that provides the compressive strength and elastic properties of cartilage [14,15].

Although low in number, chondrocytes continuously remodel and organize the surrounding matrix in a unique and complex anisotropic structure, as shown schematically in **Figure 1**. The cartilage can be divided into four zones from joint cavity to subchondral bone: superficial, middle, deep and calcified. The cellular organization and density varies between zones. In the superficial zone, the chondrocytes are of high density (24000 cell/mm^3 [13]), flattened and aligned parallel to the surface. In this zone, the content of aggrecan is at its lowest and collagen fibers of small diameter (20 nm) run tangential to the joint surface, thus providing resistance to the tensile forces generated in the joints. In the middle zone, cells of lower density (10300 cells/mm^3) have the typical morphology of hyaline cartilage. They are rounded and surrounded by a narrow pericellular region of low collagen fibrils content (about 2 μm) [16]. The collagen fibers, of increasing diameter, weave in an oblique fashion and the aggrecan content is higher than in the superficial zone. The deep zone consists of large and spherical cells clustered in columns (chondron, in average of 6 – 7 cells). The cell density is at its lowest (7700 cells/mm^3) but aggrecan content is maximal. Collagen fibrils of a large diameter (120 nm) are oriented in a vertical pattern, perpendicular to the joint surface. A zone of calcified cartilage follows, where chondrocytes are hypertropic and synthesize type X collagen, which can calcify the ECM. This interface provides excellent integration with the subchondral bone.

Articular cartilage contains no vasculature, nerves or lymphatic vessels. Therefore, it must remain relatively thin (2.4 mm on average [13]) to allow sufficient nutrient and waste diffusion. Under loading of the joint, the compression will cause seeping of the fluid from the matrix and redistribution within. Post-loading, the cartilage regains its original shape by resorbing the exuded fluid. These exchanges between tissue and synovial fluid allow cell sustenance.

There are two potential mechanisms of cartilage repair that rely on the depth of the lesion. Intrinsic repair concerns lesions limited to the cartilage alone, termed partial thickness or chondral defects. Such defects do not penetrate the

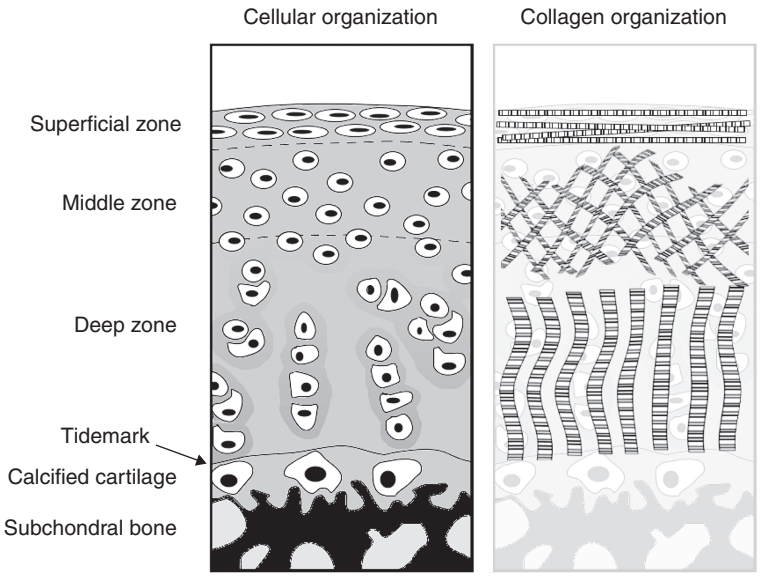

Figure 1. Schematic representation of the general structure of human articular cartilage, showing the anisotropic distribution and orientation of collagen fibers and chondrocytes within the different cartilage zones.

subchondral bone and therefore cannot be accessed by the host blood supply, macrophages or stem cells originating from the bone marrow. The repair relies solely on the limited mitotic capabilities of the chondrocytes and is rarely effective [2,5,7,17]. Conversely, extrinsic repair concerns lesions reaching the subchondral bone (full thickness or osteochondral defects). In such cases, access to mesenchymal cells and blood vessels from the bone marrow allows limited repair [18]. The defect is filled with a fibrocartilagenous tissue of heterogeneous composition and inferior mechanical competence [4]. As a result, it usually degenerates within 6 – 12 months [5,18]. Despite the poor outcome of this natural repair, it is still the basis of numerous orthopaedic treatments, such as microfracture and osteochondral drilling, which have been reviewed extensively elsewhere, together with other clinical approaches [4,19-21]. These different repair techniques may allow a temporary improvement in the patient's quality of life, but satisfactory functional results are seldom long term and clinicians are still looking for ways not merely to repair, but also to fully regenerate impaired cartilage.

A first attempt to regenerate cartilage was reported in 1989 by Grande *et al.* using a cell-based therapy: autologous chondrocyte implantation (ACI) [22]. This approach involves healthy chondrocytes being isolated and expanded *in vitro* prior to implantation in a defect sutured with a periosteal flap [23]. The positive outcome, overall, of this regenerative concept [24], has given rise to and stimulated the new field of tissue engineering in orthopedic research.

Articular cartilage tissue engineering is in general based on a central scaffolding matrix around which other strategies circumvolve, such as cells, signaling molecules and mechanical stimulation [25]. The cells of various origins are either cultured on the scaffold *in vitro* prior to implantation, or recruited from the site of implantation (in the case of an osteochondral defect). The scaffold therefore acts as an ECM where the cells can organize themselves and populate an empty space. If cells are cultured on the scaffolds, mechanical stimulation can be applied on the porous structure to orientate or fix the cells towards the cartilage phenotype [15]. Similarly, the scaffolds can contain and deliver signaling molecules such as growth factors to recruit or orientate undifferentiated cells toward the chondrogenic lineage. As this review focuses on the combination of scaffolds and growth factors, the cells and mechanical aspects of the scaffolds will not be touched upon. Excellent reviews on these topics can be found elsewhere [9,10,25,26].

3. Scaffolds for cartilage tissue engineering

3.1 General requirements

The crucial role of the scaffold in cartilage tissue engineering implies a number of requirements, based on the biological structure and repair mechanism of cartilage [4,10,21,25]. These requirements are illustrated in **Table 1**. Ideally, a porous scaffold should possess interconnected pores so that loaded or recruited cells can migrate and proliferate within the interstices. Its surface should promote cell adhesion or support chondrogenic phenotype. Biocompatibility of the scaffold material is important to avoid immunological reactions within and around the defect. Additionally, the material should degrade, to be replaced by newly formed ECM, without inducing cytotoxic, nephrotoxic or other undesirable effects due to degradation products. Physical characteristics such as compressive strength and elasticity, or

Table 1. Scaffold requirements related to the regeneration of cartilage in chondral or osteochondral defects.

Scaffold requirement	Biological basis
Biocompatibility	To allow good contact with the native tissue, cell survival, and to prevent inflammatory and immune responses
Porosity of defined size	High ratio surface/volume for effective cell seeding, cell migration, proliferation and extracellular matrix production
Inter-pore connection/permeability	Maximize nutrient/waste exchange, limit oxygen gradient and allow ingrowth of bone marrow cells in the case of ostechondral defect
Carrier for signaling molecules	Contains and release growth factors and/or cytokines in a defined and controlled way to sustain, induce or maximize cartilage formation
Cell attachment	To optimize cell seeding and to optimally retain or promote chondrogenic phenotype
Biodegradability	Allow remodeling of the newly formed tissue while avoiding inflammatory response
Structural stability and cohesion	Prevent the matrix outflow from the defect or too early deliquescence
Bonding and integration	Support integration between formed tissue and surrounding native tissue
Mechanical properties	Match the native tissue to ensure homogeneity of implant response
Structural anisotropy	Promotion of native tissue structure
Size and shape	Reproducible sizes and shapes, relevant for clinical applications
Matrix property linked to defect type or surgical application	Minimally invasive techniques, using injectable matrices solidifying *in situ* for chondral defects or preformed and stiff matrix that can be easily reshaped by the surgeon for osteochondral defects

structural stability, must also be considered in light of the surrounding cartilage or bone.

Each of these aspects of the scaffold is important to guide cell attachment, proliferation and differentiation into the tissue to regenerate [27-30], but it appears difficult to combine all of them successfully. Furthermore, most of these parameters are broadly defined and no clear consensus exists on their optimal state. Logically, the scaffold parameters should mimic as closely as possible the healthy tissue. This implies, for example, that hydrogel systems should be preferred due to their high water content and three-dimensional organization. However, porous scaffolds are still employed as they can provide mechanical properties more closely linked to the native tissue and necessary to obtain a functional tissue. In contrast, the environment that they supply to the cells is very far from cartilage and results in increased difficulties in obtaining the desired cell differentiation state. Accordingly, interesting novel approaches combine porous scaffolds and closer mimicry of the ECM structure by the fabrication of scaffolds composed of polymeric nanofibers. However, this approach also presents drawbacks regarding cell entrapment and proliferation within very tightly packed nanofibers. In summary, the optimal scaffold for cartilage tissue engineering remains to be developed. Therefore, the ability of scaffolds to act as a carrier and release system for signaling molecules, such as growth factors, appears to be of utmost significance, as it could compensate or potentiate the other parameters to achieve adequate cell proliferation or differentiation [31,32]. Recently, a number of studies has shown that the ability of scaffolds to control-release at least one biological signal is determinant of the formation of improved tissues *in vitro* and *in vivo*, despite the favorable physicochemical properties of the biomaterial [33,34]. However, ways to prepare scaffolds that combine the highest number of requirements mentioned above and release growth factors are rare. The main reason for this scarcity can be found in the association of the active molecule to the scaffold. Indeed, the active molecule must first be integrated to the scaffold, to be released in a later stage. To do so, the signaling molecules can either be incorporated directly in the scaffold matrix, or added to a prefabricated scaffold by mean of microspheres [35-37] or coatings [38,39].

Current techniques to prepare scaffolds of different properties and materials are presented in detail below, while their advantages and drawbacks are discussed relative to the scaffold properties and growth factor incorporation.

3.2 Materials and fabrication methods

3.2.1 Synthetic polymers

The general properties of the principal synthetic and natural polymers used in tissue engineering are summarized in **Table 2**. Within synthetic polymers, linear aliphatic polyesters such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and copolymers (PLGA) have been used, as they are biocompatible and approved by the Food and Drug Administration (FDA). By varying their copolymer ratio, the biodegradation rate and the mechanical properties can be tailored. They have already been studied for drug delivery [35,40-46] and are suitable for tissue engineering applications [47-51], as the degradation products (lactic and glycolic acids) obtained by hydrolysis are normally present in the metabolic pathways of the human body. The release rate of incorporated proteins is linked to the degradation rate of the polymer. However, their bulk degradation leads to the build-up of acidic degradation products inside the matrix, lowering the pH within the polymeric matrix. This might result in local inflammation in tissues [52] and denaturation of proteins in the matrix [53-55]. Another linear aliphatic polyester commonly used in tissue engineering is poly(ε-caprolactone) (PCL). This polymer has found many applications for its good biocompatibility and mechanical properties, but it degrades at a much lower rate than PLA, PGA and PLGA, which makes it attractive when long-term implants and controlled release applications are desired [56-59].

Another family of thermoplastic polymers that has been studied recently for drug delivery and tissue engineering is poly(ethylene glycol)-terephtalate-co-poly(butylene terephtalate) (PEGT/PBT). These polyether–ester multiblock copolymers belong to a class of materials known as thermoplastic elastomers, which exhibit good physical properties like elasticity, toughness and strength [60]. These characteristics result mainly from a phase-separated morphology in which soft, hydrophilic PEG segments are physically crosslinked by the presence of hard and semi-crystalline PBT segments at environmental temperatures. In contrast to chemically crosslinked materials, these crosslinks are reversible and will be disrupted at temperatures above their glass transition or melting point, which results in a material easy to process. This family of copolymers has proved to be of great interest for tissue engineering and drug delivery applications.

By varying the molecular weight of the starting PEG segments and the weight ratio of PEGT and PBT blocks, it is possible to tailor-make properties such as wettability [61], swelling [60,62,63], biodegradation rate [63], protein adsorption [64], mechanical properties [65,66] and release rate of embedded proteins [67]. The release mechanism is due to a combination of protein diffusion and matrix degradation, which allows zero-order release profiles over a long time period. Furthermore, PEGT/PBT block copolymers have shown to be extensively biocompatible both *in vitro* and *in vivo* [68-71] and reached clinical applications as cement stoppers and bone fillers in orthopedic surgery [72,73]. Being polyether–esters, degradation occurs in aqueous media by hydrolysis and oxidation, the rate of which varies from very low (high PBT content) to medium and high (larger content of PEGT and longer PEG segments) [60,63]. A further modulation in degradation rate and protein release profile can be achieved by substituting part or all of the terephtalate groups with succinate blocks during the copolymerization reaction [74-76].

Among the multitude of other synthetic polymers investigated for controlled release and tissue engineering applications, interesting classes are polyphosphoesters [77,78], polyphosphazenes [79-82], polyanhydrides [83] and polyorthoesters [84], as they have shown a surface erosion degradation mechanism [85,86], which is also known to affect the stability of the scaffolds in the long term to a lesser extent and to elicit a lower immune reaction. Injectable polymers are also very attractive as they can be used in minimally invasive surgery such as arthroscopy, resulting in a decrease in patient discomfort. Furthermore, they can fill irregularly shaped tissue defects [87-89], and cells and bioactive agents can easily be incorporated into them [90-92]. In particular, photopolymerizable systems like poly(propylene fumarate)-diacrylamide (PPF-DA) and poly(ethylene glycol)-diacrylamide (PEG-DA)-based polymers, or poly(ethylene oxide)-dimethacrylate (PEODM) and poly(ethylene glycol) (PEG) have been investigated, as they can entrap cells and be transdermally hardened by applying a light source [93-97]. Alternatively, chemically curable polymers (also based on PPF) have also been studied as they eliminate the need for light. In these materials, the double bonds available along the PPF backbone are crosslinked through the use of a vinyl monomer, *N*-vinyl pyrrolidinone, and an initiator, benzoyl peroxide [98-100], with minimal temperature rise. However, the incorporation of proteins and growth factors in such *in situ* polymerizable hydrogels might be hampered by the exposure to ultraviolet light and crosslinking agents, which can induce protein denaturation or aggregation and decrease the activity of encapsulated proteins [101].

3.2.2 Natural polymers

Natural polymers also offer a broad selection of materials used for tissue engineering. Since they are naturally present in the body, their biocompatibility and degradation is less problematic than with synthetic materials. These systems are typically in a gel-like phase and are easy to process. In addition, cells or biological agents can be readily incorporated during the gel formulation. Collagen, for instance, has been used for various tissue regenerations [102-106]. In particular, crosslinked collagen type I and type II scaffolds alone, or in combination with glycosaminoglycans, have been considered for bone and cartilage repair [105-108]. Collagen matrices allow the release of proteins and growth factors by diffusion, degradation of the matrix and affinity between protein and collagen [109-111]. However, their gel nature seems to prevent the cell migration within the matrix, which reduces the tissue repair [112]. A further possibility is

to use denatured collagen (gelatin) [103], fibrin [113,114] or demineralized bone matrix (DBM) [115-117]. The latter has found clinical application either as an injectable gel or as a foamy porous scaffold.

Another group of natural polymers that have been investigated for this purpose comprises polysaccharides like alginate [118,119], chitosan [120,121] and hyaluronate [122,123]. Alginate consists of two repeating monosaccharide units, L-gluronic and D-mannuronic acids, which are water soluble and jellify when exposed to calcium ions. This provides a suitable system to include growth factors directly during gel formation. The protein is then released by a mix of diffusion and matrix degradation [34,124,125]. Chitosan is structurally similar to glycosaminoglycans and is composed of β linked D-glucosamine residues. It has recently attracted more attention because of its non-toxicity, bioresorbability and wound healing abilities [126]. Furthermore, it was shown to release sensitive growth factors [33,127-132]. Hyaluronic acid is also abundantly present in the human body articulation, within the synovium fluid. However, in its natural form this material lacks some desirable properties (too high water solubility, fast resorption and tissue clearance times) to consider it as a polymer for scaffold fabrication [133]. A change of its chemical structure through an esterification reaction allows the generation of a new set of biomaterials, hyaluronates, with improved properties, increased bio-compatibility and fine-tuneable degradation rates [134]. These materials have been studied for cartilage and skin regeneration [122,135,136] and reached clinical applications for the treatment of deep skin wounds and cartilage [137,138]. Nevertheless, only a few studies consider them as a suitable candidate to release growth factors [139].

Polymers produced by micro-organisms (polyhydroxy-alkanoates) have also been recently evaluated as tissue engineering materials. These interesting polyesters are generally biodegradable and their physical properties are easily tailored [140]. In various studies, they were successfully shown as a release system for antibiotics and small molecules [141] and as scaffolds for the proliferation of chondrocytes *in vitro* [142]. However, further evaluations are still necessary with regards to their use as growth factor releasing matrices.

Although natural polymers seem suitable to prepare scaffolds of defined properties, concerns over natural polymers are still present due to the potential pathogen transmission, immune reactions, poor handling and less controlled degradability as compared to synthetic polymers.

3.2.3 Fabrication methods

Many different methods have been developed to fabricate scaffolds of various structures for tissue engineering applications. In general, the incorporation of growth factors in the scaffolds can be achieved by dispersing the protein in the polymer phase prior to scaffold processing, using two main approaches. The simplest way consists of adding the signaling molecule directly to the polymer solution or powder [45]. However, this often requires the molecule of interest to be soluble in the polymer organic solvent or at least stable at its contact. Alternatively, for molecules only soluble in aqueous solutions, a water phase containing the protein can be mixed with a polymer dissolved in an organic solvent to form a water-in-oil (w/o) emulsion [36,40]. In addition to the scaffold processing methods discussed below, this first step to associate growth factors with scaffolds might induce a loss of activity of the protein due to contact with organic solvents or to shear stresses and surface tensions in w/o emulsions [55].

Conventional scaffold fabrication techniques include fiber meshes and bonding, gas foaming, phase separation, freeze drying and particulate leaching, among others.

Fibrous non-woven, woven or knitted scaffolds can be fabricated from polymeric fibers manufactured with standard textile technologies [143]. These scaffolds, however, lack structural stability and consequently they can experience high deformations due to cells contractility and motility [144]. To improve the mechanical properties, a fiber bonding technique has been developed [145] whereby applying a heat treatment, the fibers of the scaffolds are joined at the cross-points.

In gas foaming the polymer is saturated with carbon dioxide (CO_2) at critical pressures to achieve high solubility of the gas in the polymer. When the gas pressure is brought back to the atmosphere pressure, the solubility of the CO_2 in the polymer rapidly decreases, resulting in the formation of gas bubbles or cells of variable size [40,146]. A similar approach is applied in phase separation, where a polymer solution is quickly cooled at low temperatures to generate a liquid–liquid phase separation. The solution is then quenched and a two-phase solid is formed. The solvent is removed by sublimation to fabricate the porous scaffold [45,147]. Freeze drying is slightly different than phase separation, since the polymer solution is directly frozen or freeze-dried to yield porous scaffolds [36,148].

Particulate leaching can be achieved in two ways. One consists of incorporating particles of a specific size (salt crystals or other polymeric particles with a defined shape and geometry) into a polymer solution, where the solvent used is a non-solvent for the particles. After evaporation of the solvent, a porous scaffold can be produced by leaching out the particles in a medium that is non-solvent for the polymeric scaffold [46,149-154]. Another approach, denominated compression molding, consists of mixing porogen particles with polymer granules and applying heat and pressure to melt the polymer and form a dense block. The particles are then leached as mentioned above to produce a porous scaffold [30,155,156]. This method can also be used with protein-loaded microspheres instead of polymer granules, to incorporate proteins in the scaffold. The microspheres are fused around the porogen particles (usually salt crystals) by compression and gas quenching [35,42,43].

Table 2. General properties of the principal synthetic and natural polymers used in tissue engineering.

Polymers			Biocompatibility	Biodegradability	Bulk mechanical stiffness (GPa)	Controlled release potential
Synthetic	Poly(lactic acid)	PLA	Degradation products in metabolic pathway	Bulk – 5 months to ~ 5 years	2 – 3	Small molecules
	Poly(glycolic acid)	PGA	Local inflammation	Bulk – 1 to ~ 12 months	5 – 7	Proteins
	Poly(lactic-co-glycolic acid)	PLGA	Enzymatic/Hydrolysis	Bulk – 1 to ~ 12 months	2 – 7	Growth factors
	Poly(ε-caprolactone)	PCL	Hydrolysis Minimal inflammation	Bulk – more than 3 years	0.4	Model compounds
	Poly(ethylene glycol) terephthalate-co-poly(butylene) terephtalate	PEGT/PBT	Hydrolysis Mild foreign body reaction No inflammation	Bulk – 1 month to ~ 5 years	0.01 – 0.1	Small molecules Proteins Growth factors
	Polyphospho-esters	PPEs	Hydrolysis Minimal foreign body reaction Minimal inflammation	Erosion – 1 to more than 3 years	0.4 – 0.7 180–230 (Shear)	Growth factors DNA
	Polyphosphazenes	PPAs	Hydrolysis Minimal foreign body reaction Minimal inflammation	Erosion – 1 week to more than 3 years	0.02×10^{-3} – 0.2×10^{-3}	Small molecules Proteins Growth factors
	Polyanhydrides	PAs	Hydrolysis Minimal foreign body reaction Minimal inflammation	Erosion – within 1 month	0.2×10^{-3} – 6×10^{-3}	Small molecules Proteins
	Polyortho-esters	POEs	Degradation products in methabolic pathway Minimal inflammation	Erosion – 1 week to ~ 16 months	0.012×10^{-3} – 4	Small molecules Proteins
	Poly(propylene fumarate)-diacrylates	PPF-DA	Hydrolysis Minimal foreign body reaction Mild inflammation	Bulk – 6 months to more than 3 years	0.002 – 0.12	Small molecules Proteins Growth factors
	Poly(ethylene glycol)-diacrylates	PEG-DA	Hydrolysis Minimal foreign body reaction	Bulk – 1–3 weeks	0.032×10^{-3} – 0.5×10^{-3}	Proteins DNA

Table 2. General properties of the principal synthetic and natural polymers used in tissue engineering (continued).

Polymers			Biocompatibility	Biodegradability	Bulk mechanical stiffness (GPa)	Controlled release potential
Natural	Collagen			Bulk – 1 day to ~ stable (cross-linker)	$0.1 \times 10^{-3} - 5 \times 10^{-3}$	Proteins Growth factors
	Alginate			Bulk – 1 day to 3 months	$0.01 \times 10^{-3} - 27 \times 10^{-3}$	Small molecules Proteins Growth factors DNA
	Chitosan				$3 \times 10^{-6} - 11$	Proteins Growth factors DNA
	Hyaluronate			Bulk – ~ day to 1 months	$3 \times 10^{-9} - 0.04 \times 10^{-3}$	Small molecules Proteins Growth factors
	Polyhydroxyalkanoates	PHA	Enzymatic/hydrolysis Minimal foreign body reaction Minimal inflammation	Bulk/erosion – more than 3 year	0.1 – 0.25	Small molecules Antibiotics

These fabrication techniques have been used to fabricate scaffolds for tissue engineering and drug release applications. However, a number of drawbacks can be outlined in their use for optimal control of tissue formation and protein incorporation. In particular, the pore size and shape of these matrices is often not controllable, resulting in tortuous and not completely interconnected pathways for the nutrients and biological signals that are to be released from the scaffolds. Pore tortuosity biases the distribution of viable cells within the scaffolds, which is limited mostly to 500 µm in depth [157]. Recent studies found that this phenomenon is also associated with a drop in the oxygen concentration from the outside to the center of the scaffold [158,159]. In addition, the incorporation or association of signaling molecules such as growth factors or other biological agents to the porous scaffolds is hampered by the processing conditions. In textile technologies the high temperatures involved in the manufacture can induce denaturation of the compound to be integrated. In solution-based techniques the solvents used can hinder the stability of the desired biological factor and cause aggregation and loss of activity [45,55]. In gas foaming and particulate leaching, further problems are connected to the use of pressure and heat, which might induce protein denaturation. In addition, in particulate leaching, the efficiency of the agent incorporation might be lowered during the porogen washing stage, as part of the compound is similarly washed away.

Among the novel scaffold fabrication techniques currently available, rapid prototyping systems appear to be the most promising to satisfy the many requirements of a porous scaffold. They can process a wide number of biomaterials [10,160,161] in a custom-made shape and with matching mechanical properties in comparison with the specific application considered [162-164]. The outcomes are three-dimensional (3D) scaffolds that normally possess fine tuneable porosity, pore size and shape, and have a completely interconnected pore network, which allows a more proficient cell migration and nutrient perfusion than scaffolds built using conventional techniques [157,158]. Within rapid prototyping systems that show encouraging results, 3D fiber deposition (3DF) has lately been investigated to fabricate custom-made scaffolds and to modulate their mechanical properties for tissue engineering applications [65,66,165]. Although scaffolds fabricated with conventional techniques can still be shaped with custom-made molds, it is more difficult to control their mechanical properties, pore size, shape and interconnectivity, resulting in nutrient limitations and cell apoptosis in the center of the construct, as previously explained. Briefly, 3DF is a fused deposition modeling (FDM) technique, where a molten polymeric filament is extruded from a CAM controlled robotic unit on a stage. Filaments are deposited to form a layer and a porous scaffold is built using a layer-by-layer strategy, following a CAD pattern. Many other FDM tools have been developed to fabricate scaffolds, comprising also multi-dispensing

systems that allow the deposition of different materials at the same time to produce constructs with different physico-chemical properties [166]. This possibility is appealing to study the release of multiple compounds from a single scaffold and to exploit the different interactions of the polymers with different cell populations in order to regenerate a more complicated hierarchical structure. However, FDM techniques retain the disadvantage of applying high temperatures during fabrication. Thus, the direct incorporation of a biological factor remains problematic.

Other direct printing technologies include solid free form (SFF) techniques like 3D printing™, selective laser sintering (SLS) and laser ablation (LA). 3D printing was one of the first rapid prototyping devices to be developed for tissue engineering applications. Here, a 3D scaffold is fabricated by depositing in a CAD/CAM-controlled manner a jet of solvent on top of a polymer powder-bed. The solvent binds the powder, thus forming patterned fibers and building the scaffold layer by layer [167,168]. In a similar way, selective laser sintering consists of projecting a laser beam on a polymeric powder-bed. The laser beam sinters the powder due to the local increase of the temperature above the glass transition temperature of the polymer. The porous scaffolds are still fabricated using CAD/CAM [169,170]. Laser ablation works in the opposite way, as from a solid block of material the porous structure is formed through the fusion of the material hit by the laser beam in specific locations [171]. If the ablation process is conducted in all three directions, a scaffold can be built. These techniques allow the fabrication of periodic structures with well-defined, controlled and completely interconnected porosity, but still have as disadvantages the use of solvents or the production of heat (although here localized to the spot where the laser beam hit the polymer, as in SLS and LA), which will affect or compromise the direct incorporation of proteins. A promising modification of SLS that can release active compounds like ribonuclease is surface selective laser sintering (SSLS) [172], although ribonuclease is known to be an exceptionally stable enzyme.

Biocompatible and biodegradable photosensitive polymers that can be used in rapid prototyping techniques like stereolithography have also been investigated. Stereolithography is normally used to produce a negative replica that is filled typically with ceramic or metallic slurries and burnt away during sintering [173]. This step still includes the use of high temperature. Therefore, the use of photosensitive polymers in this system would allow the direct fabrication of the scaffold. Incorporation of any biological compounds, however, depends on their sensibility towards the light source used to start the polymerization (typically UV or blue light).

As can be seen from the different scaffold preparation methods introduced, the incorporation of growth factors in the scaffold matrix is problematic due to potential denaturation by the preparation process. A schematic representation of the different potential causes of protein denaturation by the various preparation methods is given in **Figure 2**. The difficulty is to combine a scaffold of defined design and properties with a controlled release system for growth factors. A possible way to circumvent this difficulty consists of dissociating the scaffold preparation step from protein incorporation. This can be done simply by adsorbing the active molecule onto a scaffold surface by soaking. Alternatively, this can be done by applying growth factor-loaded microspheres [33,129] or polymer coatings [38,39] to prefabricated scaffolds of defined properties or by incorporating microspheres or liposomes in hydrogels [113,174]. Microspheres and coatings are usually prepared using water-in-oil-in-water (w/o/w) or w/o emulsion techniques. In both cases, a w/o emulsion containing the growth factor is prepared and either applied to a prefabricated scaffold or poured in a stirred second water phase to form a w/o/w emulsion. Upon solvent evaporation, a polymeric coating or microspheres are formed. The latter are then introduced in a scaffold. Although this method means that more steps are involved during the scaffold preparation, these approaches allow better control of both the scaffold properties and growth factor stability.

4. Growth factor release from scaffolds for cartilage tissue engineering

Growth factors are polypeptides involved in the cellular communication system [175]. They transmit signals that modulate cellular activity, by either inhibiting or stimulating proliferation, differentiation, migration, or gene expression [176]. In general, growth factors are pleiotropic, meaning that the same growth factor may act on different cell types to induce similar or distinct effects. In addition, different growth factors can induce the same effect for a given cell type (redundancy). They exert their effect on target cells either in an endocrine (released in the bloodstream), paracrine (diffusion to nearby target cell) or autocrine (source and target cell are the same) fashion. They initiate their action by binding to specific receptors located on the target cell membrane [177]. When a sufficiently large number of receptors has been activated, a signal transduction process takes place that results in a specific cellular activity [178]. Consequently, growth factor effects are concentration- and time-dependent. Hundreds of growth factors have been described and grouped by homology in families and superfamilies [179]. Some have been more extensively characterized and are now readily available by means of recombinant technology, which allows a thorough investigation of their potential in various tissue engineering applications.

Growth factors are usually produced by cells as inactive or partially active precursors. These precursors are often more stable than the active molecule. Upon proteolytic cleavage or binding to ECM molecules, the growth factors are activated

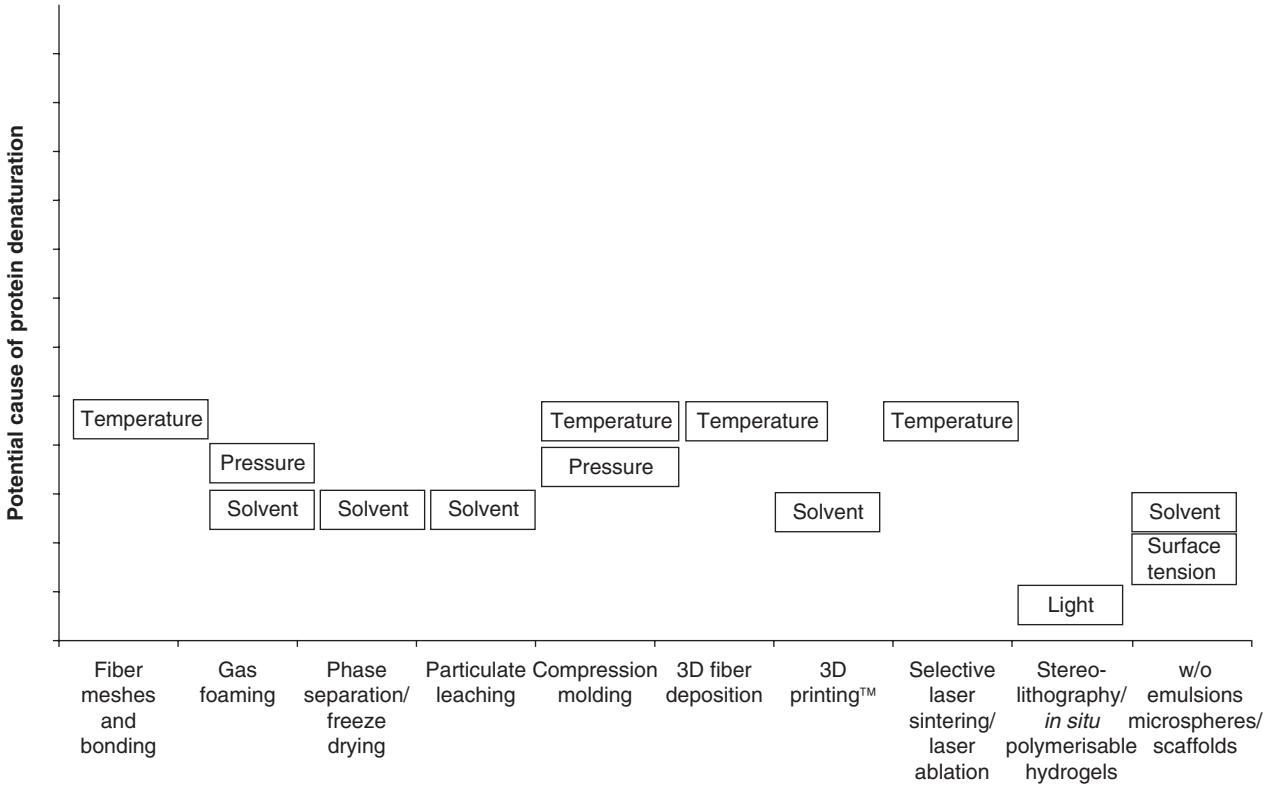

Figure 2. Potential causes of protein denaturation intrinsic to various scaffold preparation methods.

Table 3. Growth factors of interest for articular cartilage regeneration.

Regulatory effects	Growth factor	Ref.
Chondrogenic differentiation of progenitor cells	TGF-β_1	[196-200,249]
	IGF-1	[250]
	BMPs	[223-226,251-253]
Chondrocyte proliferation	TGF-β_1	[201-204]
	IGF-1	[174,201,208, 211]
	FGF	[254,255]
Matrix synthesis	TGF-β_1	[192-195,256-258]
	BMPs	[218-221,230]
	IGF-1	[212,213,259]

and rapidly degraded. In general, their biological half-life time is short (in the range of minutes) [175,180]. This important factor, combined with the potential toxicity of the growth factors at systemic level, naturally led to the sustained release of these proteins to enhance their efficacy. In combination with a porous scaffold, this approach offers a localized supply of signaling molecules aiming to enhance the proliferation or differentiation of cells towards the desired phenotype *in vivo*. With regard to cartilage, several growth factors that have regulatory effects on cartilage metabolism have been identified and are summarized in **Table 3**.

To better understand their potential, a brief description of the most relevant growth factors for cartilage regeneration is provided below.

4.1 TGF-β_1

TGF-β_1 is a 25 kilodalton (kDa) homodimeric protein, a member of a superfamily of over 100 different related proteins, which include the bone morphogenetic proteins (BMPs) and growth and differentiation factors (GDF) [181]. In addition to TGF-β_1, two highly homologous isoforms (TGF-β_2 and TGF-β_3, 70 – 80% sequence homology) have been identified in all mammalian species and are less abundant in the body [182,183]. Although the three different isoforms have been investigated for cartilage regeneration, TGF-β_1 was the first to be discovered and is the most extensively studied.

Most cells can express TGF-β_1 receptors and secrete TGF-β_1. As a result, its cellular activities are numerous and play an important role in cell proliferation and differentiation, bone formation [111,184,185], angiogenesis [186,187], neuroprotection [188] and wound repair [181,189,190]. The half-life of TGF-β_1 in the body is short (< 30 min [175,187,191]) due to a rapid binding to ECM components, which activates, inhibits or buffers its activities [175]. Of great interest for cartilage tissue engineering, TGF-β_1 controls the production of ECM by stimulating the synthesis of

collagens, fibronectin [192,193] and proteoglycans [194,195] and it has positive effects on cartilage differentiation and repair, as detailed in **Table 3** [114,196-204]. Nevertheless, TGF-β_1 can also induce undesirable side effects such as inflammatory responses and osteophyte formation in articular cartilage defects if present in the knee joint for prolonged periods [205].

4.2 IGF-1

IGF-1 is a 70 amino acids polypeptide structurally related to insulin. It is synthesized primarily in the liver under the regulation of growth hormone. IGF-1 controls the DNA synthesis of multiple cell types including chondrocytes and accounts for most of the chondrocyte stimulating activity found in serum [179,206]. The half-life of IGF-1 in the body is short (10 – 12 min) [207]. To provide long-term growth stimulation, it associates with IGF binding proteins (IGFBPs) which form a more stable reservoir of the growth factor. A number of these binding proteins are secreted by chondrocytes to regulate IGF-1 activity [208] and seem to be associated with components of the chondrocytes' pericellular matrix [209,210].

In addition to its positive effect on chondrocytes proliferation [201,211], IGF-1 increases proteoglycan and collagen type 2 synthesis [212-214]. *In vivo*, it has been reported to enhance the chondrocyte-based repair of osteochondral defects [215].

4.3 BMPs

Bone morphogenetic proteins, originally identified as inducers of bone and cartilage formation in ectopic tissues [216], compose a subfamily of the TGF superfamily. Almost 30 members have been identified that regulate the growth and differentiation of chondroblasts and osteoblasts [217]. For example, BMP-2, BMP-4 and BMP-7 maintain chondrocyte phenotype and stimulate proteoglycan synthesis in culture [218-222]. Many BMPs can direct mesenchymal stem cells towards the chondrogenic lineage, as was demonstrated for BMP-2, -3, -4 and -9 [223-226]. Recently, BMP-2 and -7 were demonstrated to be the most effective in this respect [227,253].

In vivo, BMP-2 was shown to be effective in regenerating hyaline cartilage in osteochondral defects, with or without autologuous chondrocytes [228,229]. However, BMP-2 might induce the formation of osteophytes (due to dose and length of exposure) and appears to be less potent than TGF-β_1 in promoting proteoglycan synthesis in the joint [230].

4.4 Scaffolds, growth factor release and interest for cartilage regeneration

The short half-life of IGF-1, TGF-β_1 and BMP-2, and the potential side effects of the latter two, combined with their chondrogenic potential, make these growth factors promising candidates for sustained delivery from porous scaffolds in cartilage tissue engineering. To reach this goal, different methods and materials have been evaluated. A summary is given in **Table 4**. The majority of the efforts to design drug delivery systems for cartilage applications were conducted with TGF-β_1. It should be noted that numerous papers describe the release of BMPs from scaffolds. However, these studies have been conducted in view of bone tissue engineering applications, which are outside the scope of this review.

Most of the methods evaluated to combine growth factor delivery and supporting structures are based on the use of hydrogels of diverse materials. This approach is interesting as it allows the encapsulation of cells easily in the releasing matrix prior to implantation, which can be done optimally by non-invasive injection [37,174]. However, the limited mechanical properties and stability of hydrogels might ultimately hamper their use in articular cartilage repair [25]. With regards to the mechanically more sound porous structures, the association of growth factors is usually achieved by separating the scaffold preparation step from the protein incorporation, in order to reduce the detrimental effect of scaffold processing on protein. Growth factor loaded microspheres or liposomes were incorporated in hydrogels or prefabricated scaffolds [114,129,174,231] and polymer coating applied on compression molding scaffolds [39] or, more often, prefabricated matrices were soaked with growth factor solutions [139,232,233]. Although adsorption of the growth factors by soaking seems the easiest and least harmful approach, it limits the possibility to control the release of the growth factors from the scaffolds. With this approach, the affinity of the protein for the substrate regulates almost entirely desorption or diffusion profiles. In addition, it was demonstrated that adsorption could also result in protein denaturation [234]. Another affinity-based approach that uses heparin as a preferential binding site for growth factors is of interest. For instance, the conjugation of heparin to PLGA nanospheres allowed a sustained release of active FGF over 3 weeks [235]. This release could be further modulated by the incorporation of heparin or of functionalized nanospheres within hydrogels such as fibrin [236]. Although this approach is seldom used to prepare scaffolds with more relevant growth factors for cartilage applications; recent reports underline its potential *in vivo*. For example, TGF-β_3 bound to heparin within a thermo-reversible hydrogel improved the proliferation rate and amount of cartilage-associated ECM proteins produced by encapsulated rabbit mesenchymal stem cells [237].

It is interesting to note that the activity of the released protein from the scaffolds, although an essential factor to take into consideration, is not always evaluated in the published literature or if it is, is done in an incomplete way. This renders evaluation of the scaffolds' processing methods difficult.

Even so, most studies confirmed the potential of growth factor release. *In vitro*, the sustained delivery of TGF-β_1 and IGF-1 supported cartilage repair and maintenance. The fast

Table 4. Strategies to release growth factors from scaffolds and resulting delivery rates.

Growth factor	Scaffold type	Growth factor incorporation	Materials	Advantages	Disadvantages	*In vitro/ in vivo*	Release rate (80% completion, in days)	Remaining activity	Cells added?	Ref.
TGF-β_1	Hydrogels	Soaking	Gelatin Dextran	Easy Harmless Injectability	Limited control of release Low mech. properties	*In vivo* *In vitro*	1 – 14 2	n.a 70 – 80%	No No	[261] [262]
		Dispersion in hydrogel solution	Alginate	Easy Harmless Sustained delivery	Limited control of release Low mech. properties	*In vivo*	> 5	n.a	No	[34]
	Composite hydrogel – microspheres	W/o/w	PEO-PLGA	Injectability Controlled release	Surface tensions/solvent Low mech. properties	*In vitro*	15	n.a	Bovine chondrocytes	[174]
		Soaking	OPF-gelatin	Easy Harmless Injectability	Low mech. properties	*In vitro* *In vivo*	3 – 24 –	n.a	Bovine chondrocytes No	[37,238,260] [244]
	Composite hydrogel – liposome	Liposome	Fibrin	Sustained release	Limited control of release Surface tensions/solvent	*In vivo*	25	n.a	No	[113,114]
	Freeze dried scaffold	Soaking	Collagen	Easy Harmless	Low mech. properties	*In vivo*	8 – 35	n.a	No	[111]
	Composite freeze dried scaffold – microspheres	W/o/w	Collagen and/or chitosan–chitosan	–	Surface tensions/solvent Limited control of release	*In vitro*	1 – 3	n.a	Porcine and leporid chondrocytes	[33,129]
	Compression molded	Polymer coating	PEGT/PBT	Sustained and controlled release	Surface tensions/solvent	*In vitro* *In vivo*	12 – 50 12	85% 85%	No No	[39] [245]

Mech: Mechanical; N.a: Not available; OPF: Oligo(poly(ethylene glycol) fumarate); PEO: Poly (ethylene oxide); Ref: References; w/o/w: water-in-oil-in-water emulsion.

Table 4. Strategies to release growth factors from scaffolds and resulting delivery rates (continued).

Growth factor	Scaffold type	Growth factor incorporation	Materials	Advantages	Disadvantages	*In vitro/ in vivo*	Release rate (80% completion, in days)	Remaining activity	Cells added?	Ref.
IGF-1	Decellularized bladder	Soaking	Collagen	Easy Harmless	Limited control of release	*In vivo*	1	n.a	No	[232]
	Composite hydrogel – microspheres	W/o/w	PEO-PLGA	Injectability Controlled release	Surface tensions/solvent Low mech. properties	*In vitro*	5 – 15	n.a	Bovine chondrocytes	[174]
	Composite hydrogel – liposome	Liposome	Fibrin	Injectability	Surface tensions/solvent	*In vivo*	?	n.a	No	[114]
BMPs	Phase separated	Dispersion in polymer solution	PLGA	Controlled release	Solvent	*In vitro*	6	n.a	No	[263]
	Hydrogel	Soaking	Gelatin	Easy Harmless Injectability	Limited control of release Low mech. properties	*In vivo*	1	n.a	No	[233]
	Phase separated/salt leached	Soaking	Hyaluronate	Easy Harmless	Limited control of release	*In vitro*	10	n.a	Murine cells	[139]
	Helistat ©	Soaking	Collagen	Easy Harmless	Limited control of release	*In vivo*	5 – 10	n.a	Leporid chondrocytes	[228,246]
	Composite hydrogel – liposome	Liposome	Fibrin	Injectability	Limited control of release Surface tensions/solvent	*In vivo*	–	n.a	No	[114]
	Microspheres	W/o/w	PLGA	Controlled release	Surface tensions/solvent	*In vivo*	10	n.a	No	[264]

Mech: Mechanical; N.a: Not available; OPF: Oligo(poly(ethylene glycol) fumarate); PEO: Poly (ethylene oxide); Ref: References; w/o/w: water-in-oil-in-water emulsion.

release of TGF-β_1 over 1 or 3 days from chitosan microspheres being embedded in collagen or chitosan scaffolds induced the proliferation and glycosaminoglycans production of co-encapsulated chondrocytes over 21 days [33,129]. Similarly, the release over 7 days from gelatin microparticles embedded in hydrogels allowed the multiplication of chondrocytes over 28 days with maintenance of their phenotype [37]. Such approaches could be of interest for the regeneration of chondral defects as they allow the preparation of the cell-containing scaffolds prior to implantation. A more elaborate strategy consists of embedding gelatin microparticles simultaneously acting as porogen and TGF-β_1 delivery system, in hydrogels without cells. Due to the natural presence of collagenase in the injured knee, the particles release the growth factor while being digested. The voids created by the degraded gelatin microspheres allow the ingrowth of progenitor cells [238]. Similar to TGF-β_1, the release of IGF-1 over 5 days from PLGA microspheres co-encapsulated with chondrocytes in a hydrogel induced the proliferation and enhanced the glycosaminoglycans production of chondrocytes embedded in a hydrogel [174].

In the same study [174], the opportunity to combine the release of different growth factors from the same supporting structure was investigated by mixing IGF-1 and TGF-β_1-containing PLGA microspheres. This approach appeared promising, as the two growth factors had synergistic effects on the enhancement of chondrocyte proliferation and on the maintenance of their phenotype. Taking a broader perspective, it is likely that the release from scaffolds of different growth factors with different release profiles would be beneficial. For instance TGF-β_1 or BMP-2 could be released in a first step to induce chondrogenic differentiation of progenitor cells, while IGF-1 release over longer time periods would maintain and enhance the obtained phenotype at a later stage. Other methods have been considered for this purpose. For example, mixing two populations of gelatin microparticles releasing IGF-1 and TGF-β_1 within an hydrogel or adsorbing TGF-β_1 to the hydrogel directly allowed the independent control of the release profiles of the two proteins [239]. More recently, PLGA microspheres releasing TGF-β_1 and IGF-1 were bound together with dichloromethane vapor to create a scaffold releasing the proteins sequentially over 70 days [240]. Another reported approach consists of applying multiple gelatin coatings containing BMP-2 and IGF-1 on flat surfaces to control the release of each growth factor independently by diffusion through the superposed layers [241,242]. Similarly, the successive coating of PEGT/PBT copolymers containing different model proteins on prefabricated compression-molded scaffolds allowed a tailored and independent release [243]. However, these techniques remain preliminary and still have to be tested in relevant articular cartilage defects.

In vivo, the beneficial effect of growth factor sustained release has also been demonstrated. In rabbit osteochondral defects, the release of TGF-β_1 over at least 5 days from alginate microparticles [34] or a release of BMP-2 within 10 days from collagen sponges were evaluated [228,229]. An improvement of the tissue repair after 6, 12 or 24 weeks was measured, in comparison to defects filled with unloaded matrix or left empty. BMP-2 delivery showed a similar cartilage restoration as compared to the implantation of autologuous chondrocytes in the defect. This indicates the potency of the released growth factors to differentiate progenitor cells present at the implant site, which may eliminate the need of an extra cell source. Similarly, in minipig chondral defects, a 25-day release of TGF-β_1 or BMP-2 from fibrin hydrogels induced successful healing by differentiating migrating synovial cells [113,114]. However, recent studies in rabbit ostechondral defects with scaffolds releasing TGF-β_1 at similar concentrations showed either only a limited improvement of cartilage restoration [244], or no improvement when release was over 12 days [245]. The same negative result was found in chondral defects exposed to IGF-1-releasing liposomes, possibly because of incorrect dosage, a release rate that was not evaluated, or to the lack of suitable progenitor cells [113].

Although the delivery of growth factors from supporting scaffolds appears overall to be beneficial, different aspects of the release still need to be examined to further enhance cartilage regeneration, especially *in vivo*. For instance, the amount of growth factor released is vital to achieve the optimal result while avoiding side effects. This parameter has been evaluated with different TGF-β_1 concentrations released in osteochondral and chondral defects and revealed a concentration dependency of chondrogenesis between 200 and 900 ng/ml. Above 900 ng/ml, adverse effects such as osteophytes formation, synovitis and cartilage erosion were observed [34,114]. However, such studies were not performed with other growth factors and the optimal dose ranges of BMP-2 and IGF-1, for example, are still unknown for chondral or osteochondral defects. In addition, the integrity and activity of the released growth factor is seldom considered or evaluated. Often, if the protein released from a scaffold still elicits a biological response *in vitro* or *in vivo*, it is considered as fully active, even though only a small part of the protein might actually be active. Considering the high potency of growth factors and their high costs, this point requires more attention. An exact determination of the ratio of protein effectively active would allow the further selection of the most suitable scaffold preparation methods. Optimally, the release of highly active growth factors would permit the reduction of the amount of growth factor needed for a similar effect and a more precise management of potential side effects. Finally, the influence of growth factor release rate on cartilage reconstruction has rarely been investigated. This is surprising as research focusing on the controlled release of BMP-2 from porous scaffolds for bone tissue engineering clearly showed that this parameter was as important as dosage [246-248]. Recently, we evaluated the influence of release profiles of TGF-β_1 from porous scaffolds

on the chondrogenic differentiation of bone mesenchymal stem cells *in vitro*. The most effective stimulation was found for a burst delivery of the growth factor. Although this should be further confirmed *in vivo*, it suggests that a sustained release over days might not be necessary to induce cartilage formation, which would additionally minimize side effects. The influence of the release rate for BMP-2 and IGF-1 should also be evaluated to better understand the requirements for an optimal scaffold delivery system. Such knowledge would allow more effective improvement of the regeneration of articular cartilage by mean of supporting structure and growth factor release.

5. Conclusions and future considerations

In current cartilage tissue engineering research, the role of the scaffold is crucial. Different strategies and approaches have been considered, both cell-based and cell-free. Over time, the requirements of the scaffolds have been defined and refined, and many materials, processing methods and designs are now available. However, even though positive results have been obtained, none of these techniques have so far resulted in a complete and functional repair.

In parallel, over recent years various growth factors have been identified that regulate cartilage homeostasis and induce the chondrogenic differentiation of progenitor cells. Logically, the use of these signaling molecules in combination with scaffolds is being investigated for cartilage tissue engineering. The local release of selected growth factors from scaffolds is aimed at attracting pluripotent cells, stimulating their differentiation and maintaining their acquired phenotype – which has shown great potential.

Although this concept seems logical and appealing, the intrinsic properties of growth factors limit the number of materials and preparation methods that can be used to prepare growth factor releasing scaffolds. In addition, the physiological mechanisms of growth factors should be taken into account. More than just sustained, the release from scaffold should be precisely controlled, as cells react in a concentration- and time-dependent manner to growth factors. Optimally, the delivery of multiple growth factors should mimic the endogenous profile of growth factor production during tissue morphogenesis or repair. Therefore, a greater understanding of the required therapeutic doses and release kinetics will be important to obtain the benefits resulting from the association of growth factors and scaffolds.

6. Expert opinion

Tissue engineering is a very exciting and promising field of research. It holds the potential not simply to treat the symptoms of a disease, but to recreate tissues and ultimately organs. The strength and crucial innovation of tissue engineering lies in its multidisciplinary approach. To regenerate tissues, the tissue engineer has to be as pluripotent as the cells that he uses, and has to combine and apply the knowledge of molecular and cellular biology, material and pharmaceutical sciences and clinical experience. This multidisciplinary nature might also be the biggest weakness of tissue engineering, as only a few can be expert in all these research fields. Focus on only a few of the aspects involved (such as a heavy accent on engineering and less on the biology) is probably why over the past decade, tissue engineering (in our case of cartilage) has not delivered the results expected, especially clinically. To bring it closer to clinical applications, the key would be to build strong multidisciplinary teams and focus on integration of all the different research aspects. However, this is not often sought.

The controlled release of growth factors from porous structures is a good example. The concept to deliver to the cells the right molecular signal at the right dose and over the right period seems obvious. However, the application is less straightforward. In most cases, the controlled delivery of a protein is considered by most researchers to be something that does not require much effort, as something already well under control, and it is not rare to hear during research discussions: 'why not release this growth factor from the scaffold?'. However, as this review underlines, it is not so easy.

First, the incorporation of a growth factor within a well-defined scaffold is not straightforward. Growth factors are labile proteins, designed within the body to be degraded rapidly. This makes them even more susceptible to denaturation by heat, solvent contact or even light. This first difficulty explains the lack of evaluation of the remaining bioactivity of growth factors in most studies.

Once incorporated to the scaffold, the controlled and defined release of growth factors is difficult to obtain. In most cases, a single burst delivery completed within days is considered as sustained and therefore sufficient. This is, however, probably the most important point to consider. Each growth factor has a different physiological mechanism of action and this specific mechanism should result in a specific release profile, capable of inducing cells or tissue in the right direction. But this 'most effective' release profile is not known and is little investigated in cartilage tissue engineering.

Important knowledge for the researcher to design and use the controlled release of growth factors in the most efficient way is lacking. A more thorough investigation of the release requirements for each growth factor within a specific application will undeniably be crucial for the future success of cartilage tissue engineering.

Declaration of interest

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