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Clinical translation of controlled protein delivery systems for tissue engineering

Kara L. Spiller and Gordana Vunjak-Novakovic*

Department of Biomedical Engineering, Columbia University, 622 West 168th Street, Vanderbilt Clinic 12-234, New York, NY 10032

Abstract

Strategies that utilize controlled release of drugs and proteins for tissue engineering have enormous potential to regenerate damaged organs and tissues. The multiple advantages of controlled release strategies merit overcoming the significant challenges to translation, including high costs and long, difficult regulatory pathways. This review highlights the potential of controlled release of proteins for tissue engineering and regenerative medicine. We specifically discuss treatment modalities that have reached preclinical and clinical trials, with emphasis on controlled release systems for bone tissue engineering, the most advanced application with several products already in clinic. Possible strategies to address translational and regulatory concerns are also discussed.

Keywords

tissue engineering; scaffolds; protein delivery; translation; regulatory pathways

1. Introduction

With the large and constantly growing numbers of patients waiting for organ transplants, the need for technologies that can regenerate new tissues is greater than ever. The field is rapidly expanding, with over two hundred companies spending over \$3.6 billion and generating over \$3.4 billion in sales as of 2011 [1]. These numbers represent increases of 1.5× and 2.7× respectively compared to four years earlier, and are projected to continue rising [1]. It is likely that products with the capability for controlled release of drugs and proteins will constitute a significant portion of the projected increase in commercialization, since regulatory approval is easier than for traditional tissue-engineered products with a cell component. Indeed, a large portion of the annual revenue in the field is owed to Medtronic's Infuse® (\$750 million), which relies on the release of bone morphogenic protein-2 to regenerate bone tissue [1].

The traditional tissue-engineering paradigm involves cell culture in three-dimensional matrices or scaffolds; the tissue is implanted directly into the body or cultivated *in vitro* for some period of time before implantation. In both cases, the scaffold serves as a structural

*Correspondence to: gv2131@columbia.edu, Phone: +1-212-305-4692, Fax: +1-212-305-2304.

and logistic template for cell attachment, maintenance of differentiated function, and assembly of functional tissues.

While significant advances have been made in the design of scaffolds, it is likely that physical signals from the scaffolds alone are insufficient to achieve full tissue regeneration. The addition of bioactive factors - drugs and growth factors - to cell culture media can enhance tissue growth, but the short half-lives of drugs and proteins in the body, their rapid clearance from the target site, and potential cytotoxicity at high doses impede their utility *in vivo*. This is why tissue engineering scaffolds with systems in place for the controlled release (CR) of bioactive factors hold great potential for providing the molecular and biological cues necessary to achieve tissue regeneration *in situ*. Although combination products like drug-eluting scaffolds, even without a cellular component, face substantial translational and regulatory hurdles, their significant advantages tissue engineering merit the effort, and products already on the market demonstrate the feasibility and utility of these sophisticated approaches [2].

In this review, we highlight the main advantages to using tissue engineering scaffolds with ability for CR of various types of proteins. We will illustrate the advantages of CR systems using examples that have progressed to pre-clinical large animal models or clinical trials (summarized in Table 1). Many of these examples are geared toward bone tissue engineering, which boasts the most commercially advanced CR systems. Gene therapy approaches are outside the scope of this review, but are also promising for tissue engineering [3, 4]. We also discuss the translational concerns and the regulatory pathways involved in the design and scale-up of CR systems for tissue engineering. Finally, we discuss the current and future needs for facilitating clinical translation of CR systems for tissue engineering.

2. Classes of CR systems for tissue engineering

Most tissue engineering approaches employ some type of three-dimensional structure to support the regeneration of new tissue (for review, [5, 6]). The addition of CR systems can render these biodegradable scaffolds bioactive and cell-instructive. The release of growth factors and cytokines can augment tissue growth or recapitulate aspects of developmental or repair processes. Proteins have been incorporated directly into porous scaffolds via surface modification or coatings. Polymeric microparticles and hydrogels can serve as injectable scaffolds.

2.1. Surface modification of scaffolds

Three dimensional, biodegradable scaffolds are often employed to guide tissue growth or regeneration. The surfaces of scaffolds can be modified with proteins through chemical conjugation or through non-covalent binding like surface adsorption, affinity binding, and ionic complexation. Modification of a biomaterial's surface provides specific control over cell interactions without affecting its bulk properties. However, the bioactivity of the protein may be reduced during modification or as a result of immobilization [7]. An immobilized protein can retain its biological function for cells in direct contact with the scaffold, but it can only diffuse into the surrounding tissue if the scaffold is degraded. Nonspecific

adsorption of protein on a scaffold via soaking often results in significant burst and rapid release [8, 9].

Proteins can be chemically conjugated to scaffolds via their primary amine groups using N-hydroxysuccinimide (NHS) or other crosslinking chemistry, or through the use of a linker molecule that is degradable or nondegradable. Incorporating heparin or heparin-rich proteoglycans into scaffolds extends the release profile of proteins with naturally high affinity for heparin such as vascular endothelial growth factor (VEGF) and bone morphogenic protein-2 (BMP2) [10], and this affinity can be augmented with the addition of heparin-binding domains [11]. Proteins have also been modified with titanium-binding motifs for attachment to titanium, one of the most widely used biomaterials [12].

Proteins can also be physically incorporated into the main structure of scaffolds if the scaffold fabrication methods are sufficiently gentle. In this case protein release is controlled by diffusion through pores in the polymer and degradation of the polymer. For example, BMP2 was incorporated into porous poly(lactic acid) scaffolds by plasticizing the dry polymer in the presence of the protein and foaming with supercritical CO₂ [13]. Release of active protein was sustained for over 28 days *in vitro* and enhanced bone formation in a mouse segmental femur model [14].

Polymer film coatings are also used to achieve control over protein release. Coatings are formed by dipping scaffolds in aqueous polymer formulations containing proteins. Release of protein is controlled by diffusion and polymer degradation and depends on the type of polymer and the number and thickness of the layers [15]. Such coatings have been prepared from aqueous solutions of silk [16, 17] and water-in-oil emulsions with organic polymers like poly(caprolactone) and poly(lactic-co-glycolic acid) (PLGA) [18]. Coatings of calcium phosphate, in which protein release depends on electrostatic interactions with the mineral and its dissolution, are under investigation for bone tissue engineering [19, 20].

Disadvantages of polymer coatings include potential clogging of pores, inadequate adhesion to the substrate, and limitations in varying processing parameters [15, 21]. Some of these limitations can be overcome by using nanoscale thin films prepared using layer-by-layer (LbL) assembly of polyelectrolytes with alternating charges and with proteins in between [22]. The release of BMP2 was controlled over two weeks from layers of chondroitin sulfate, a negatively charged natural component of extracellular matrix, and a cationic poly(β -amino ester), and supported ectopic bone formation in an intramuscular rat model [23].

2.2. Microparticles

Encapsulation in polymeric microparticles, typically through water-in-oil-in-water double emulsions, can protect proteins from degradation and increase their stability and retention at the target site [24, 25]. Protein release, controlled primarily by diffusion through pores that form in the microparticles and degradation of the polymer, is affected by the composition and degradation profile of the polymer, the size of the particles, the dose of encapsulated protein, and polymer crosslinking. The high level of control over release profile via manipulation of fabrication parameters is a significant advantage of microparticles. Moreover, they can be engineered to release multiple proteins and they can be delivered in

combination with cells [26, 27]. Protein-loaded microspheres have also been fused into scaffolds for tissue engineering [28, 29]. Microparticles prepared from poly(lactic-co-glycolic acid) (PLGA) are approved by the FDA for the CR of multiple drugs. However, protein bioactivity during microparticle fabrication can be diminished due to contact with harsh organic solvents [30].

2.3. Hydrogels

Hydrogels are 3D crosslinked hydrophilic polymers swollen in large amounts of water, making them useful as biomimetic tissue engineering scaffolds. Most hydrogels are prepared from aqueous solutions via gentle crosslinking conditions, so proteins can be directly incorporated with virtually 100% preservation of protein bioactivity. Hydrogels can also be loaded with protein subsequent to crosslinking by soaking in protein solution [31]. The release of proteins from hydrogels depends on interactions between the protein and the polymers that comprise the hydrogel, but is generally controlled by diffusion, so that release is typically characterized by a large burst release in the first 24hrs, and it rarely lasts longer than 2 weeks [32]. Nonetheless, this method is still more effective than bolus administration, and has been used to expose chondrocytes to insulin-like growth factor-1 (IGF1) via fibrin hydrogels in osteochondral defect repair in horse [33–35].

3. Advantages of CR systems for tissue engineering

3.1. Extended bioactivity

By far the most common goal of a CR system is to extend the amount of time that encapsulated cells or cells in the vicinity are exposed to the drug. In tissue engineering, the drugs of choice are typically growth factors and cytokines. Sustained release can ensure that the lowest therapeutic dose is continuously presented to cells, resulting in greater efficiency of drug presentation, greater efficacy of its action, and decreased risk of side effects compared to bolus administration [36]. For bone tissue engineering, long-term release (4 weeks) of bone morphogenic protein-2 (BMP2) resulted in higher bone formation than short-term release (3 days) at an equivalent dose [37].

Relatively fast release of bone morphogenic protein-2 (BMP2) and BMP7 (less than 2 weeks) has proved useful for bone tissue engineering, and is at this time the most extensively studied CR system clinically, with thousands of patients treated in more than 50 clinical trials (Table 2) [38, 39]. These BMP products are recommended as alternatives to autografts, which are a “gold standard” of care, in instances where large amounts of bone are required, to facilitate healing of non-unions in long bones, or spinal fusion [38].

CR systems for the release of BMP2 or BMP7 are FDA-approved and available commercially as INFUSE[®] bone graft, a BMP2-soaked collagen sponge, from Medtronic (Memphis, TN) [40–43], and OP-1, a BMP7/collagen paste from Stryker Biotech (Hopkinton, MA) [44]. INFUSE[®] has become the subject of controversy after Medtronic's other BMP-releasing product, AMPLIFY (a BMP2-releasing hyaluronic acid/ β -tricalcium phosphate scaffold) was not approved by the FDA because of adverse complications and elevated cancer risk [39] and a scathing review of Medtronic studies by prominent spine surgeons [45]. The elevated cancer risk was attributed to the high dose of BMP2, suggesting

that the risk might be mitigated and efficacy increased with a CR system that sustains a lower dose for a longer period of time, which was probed experimentally in a rat model [46, 47]. Research on these more sophisticated CR systems is ongoing [38, 48, 49].

3.2. Cellular recruitment

While the main purpose of the studies of BMP2 and BMP7 was to induce growth and tissue production by cells in the surrounding area of implantation, another advantage of CR scaffolds could be the creation of a concentration gradient that can recruit the host cells to migrate into the matrix. The release of a chemotactic protein can in principle recruit cells from both the circulation and the surrounding tissue. The most common example of this strategy is the release of angiogenic factors from implanted biomaterials in order to recruit endothelial cells and supporting pericytes to enter the area and form new blood vessels. Such a strategy has been applied clinically to coax new blood vessels into ischemic areas of myocardium.

The sustained release (4–6 weeks) of 10 μ g or 100 μ g of fibroblast growth factor-2 (FGF2) from heparin-alginate microparticles implanted into ischemic myocardial tissue resulted in a reduction in size of the ischemic area, reduction in stress perfusion defects, and decreased incidence of angina in twenty-four patients in a Phase I trial [50–52]. This system has not been studied in larger clinical trials. However, a detailed pharmacokinetic analysis of the clearance of FGF2 from the infarcted myocardium suggested that sustained delivery of FGF2 alone is unlikely to achieve long-term clinical benefit [53]. Through analytical, *ex vivo*, and animal models, it was shown that relatively low molecular weight growth factors like FGF2 are rapidly cleared from tissue through microcapillaries, which increase in number with the action of the angiogenic factors, thereby increasing their own rate of clearance and limiting the therapeutic effect [53]. Using radiolabeled FGF1 incorporated into the sustained release heparin-alginate beads, it was shown that FGF penetrated the myocardium of rabbits at maximum level at 2 days, but drastically regressed over the next week, remaining low after 31 days, even with sustained release, as a direct result of the increased vascularization [53]. Neovascularization peaked at day 8, and was 56% greater in animals receiving FGF1 compared to control animals, but decreased over the next few weeks to settle at baseline levels. The authors suggest that therapeutic efficacy of angiogenic factors might be increased by engineering drugs with lower transendothelial permeability.

The molecular factors that regulate angiogenesis are complex. Perhaps a better approach to induce neovascularization might be through the recruitment of inflammatory cells, which would then release their own complex set of biological signals to effect angiogenesis. Alginate microparticles loaded with monocyte chemoattractant protein-1 (MCP1) were incorporated into poly(glycolic acid) (PGA) mesh tubes and implanted into the inferior vena cava of SCID/bg mice [54]. The release of MCP-1 over just three days recruited host monocytes, which in turn recruited endothelial cells and supportive smooth muscle cells, and resulted in functioning venous conduits after 10 weeks. The recruitment of monocytes to tissue engineered grafts appears to explain why the implantation of human bone marrow stromal cells into SCID mice results in vascularized tissue composed entirely of mouse cells

[54], and sheds light on the mechanisms of efficacy observed in large-scale clinical trials of tissue-engineered vascular grafts formed from mesenchymal stem cells (MSCs) [55, 56].

3.3. Temporal control

To address the problem of unstable vasculature that regresses without constant stimulation by angiogenic growth factors, researchers have turned to the use of CR systems that rapidly release vascular endothelial growth factor (VEGF) followed by the delayed and sustained release of platelet-derived growth factor (PDGF) (a few weeks), a system that approximates the physiological profile observed in developmental or adult angiogenesis [57]. The VEGF is intended to recruit endothelial cells, and the PDGF is intended to recruit supporting pericytes or smooth muscle cells to stabilize newly forming blood vessels. The most common method of achieving this level of temporal control is through the use of polymeric microparticles. Because soluble proteins are hydrophilic, encapsulation in hydrophobic polymers like poly(lactic acid) (PLA) or poly(lactic-co-glycolic acid) (PLGA) limits their diffusion so that release is determined by degradation of the polymer [58]. Protein encapsulation in microparticles also increases their stability in physiological conditions, and processing parameters can be manipulated to control the rate of release [57, 59, 60]. Proteins can be encapsulated within the polymer microparticles for delayed and sustained release, or adsorbed to the surface of the microparticles for rapid release [57]. Such “sequential delivery” microspheres can be molded into scaffolds or embedded in scaffolds for hydrogels for even more control over release profile [28, 57, 61]. Embedding microparticles in hydrogels or scaffolds typically tempers the burst release and slows overall release because the drug interacts with the hydrogel matrix following its release from the microparticles [24, 62–64]. In addition, two different sets of proteins can be encapsulated in the two phases (hydrogel and microparticle), resulting in rapid release of the protein in the hydrogel and delayed, sustained release of the proteins from the microparticles [65, 66].

Freeman et al. extended the sequential delivery of growth factors to include a third protein [67]. By incorporating the proteins into alginate-sulfate hydrogel, which can bind multiple heparin-binding proteins, the timing of release of the three proteins was dependent on their equilibrium binding constants with heparin, so that VEGF was released first followed by PDGF and lastly by transforming growth factor- β 1 (TGF β 1). After subcutaneous implantation in rats, the sequential release of the three factors over an eight-day period resulted in the formation of larger, more mature blood vessels than the simultaneous release of the three factors, prepared using non-sulfated alginate. This increase in vascularization persisted at 3 months for the sequential release scaffolds, while blood vessels regressed in groups treated with instantaneous release [67]. Despite the large numbers of publications regarding the sequential delivery of multiple growth factors for therapeutic angiogenesis [57, 67–70] and the failure of monotherapeutic strategies in multiple clinical trials [51, 52, 71–73], this strategy has not yet been studied in pre-clinical or clinical studies.

3.4. *In situ* differentiation of progenitor cells

For many applications, recruitment of cells from the surrounding tissue is inadequate for functional regeneration of the new tissue. The use of progenitor cells in combination with a biomaterial offers the possibility to regenerate tissues, *in vitro* and *in vivo*. Differentiation of

progenitor cells requires biological signals, and can be induced by the addition of appropriate cytokines to cell culture media. However, for applications in which time is of the essence, like acute trauma, CR systems can provide the important differentiation factors to progenitor cells within biomaterials so that they can be implanted more quickly. Dramatic neovascularization was observed in a murine model of hindlimb ischemia when cord-blood derived endothelial progenitor cells were co-injected with a mixture of PLGA microspheres containing the angiogenic factors VEGF, hepatocyte growth factor, and angiopoietin-1 [27]. The level of perfusion was significantly higher when all three proteins were delivered than when cells were delivered alone or with just two of the proteins.

Allowing the new tissue to develop within the body may also enhance integration with surrounding tissue [74]. For example, rabbit MSCs were seeded onto porous gelatin-chondroitin-hyaluronate scaffolds containing TGF β 1-loaded gelatin microspheres and implanted into full thickness cartilage defects autologously in rabbits [75]. A comparison group of MSCs was pre-cultivated with TGF β 1 for 7 days and then seeded on scaffolds with blank microspheres prior to implantation. The release of TGF β 1 over 30 days *in vivo* resulted in enhanced chondrogenic differentiation and cartilage repair by the MSCs, with more integrated cartilage tissue than that produced by pre-differentiated MSCs.

3.5. Potential for “on demand” release kinetics

The significant level of control over protein release profile that can be achieved by CR systems allows the realization of more nuanced objectives, such as response to local microenvironmental stimuli, the coupling of scaffold degradation with new tissue formation or the modulation of protein delivery through the application of external stimuli. For example, pH-responsive hydrogels based on poly(N-isopropylacrylamide) and poly(acrylic acid) were designed to extend the release profile of angiogenic growth factors in the acidic environment of ischemic tissue and to dissolve upon restoration of physiological pH [76].

A central tenet of tissue engineering is that the newly forming tissue should replace the degrading biomaterial, preferably at the same rate. CR systems can be designed to achieve this goal by physically coupling the degradation of the scaffold and release of stimulatory factors with cellular ingrowth through cell-mediated degradation. Hydrogels were formed from a mixture of macromers containing cell-adhesive peptides, sites that were degradable by matrix metalloproteinases (MMPs), and VEGF, so that degradation and release of the protein occurred only when MMP-secreting cells were present [77–79]. This cell-mediated release of VEGF led to enhanced reperfusion of ischemic hind limbs in mice when the hydrogels were injected into the area and polymerized *in situ* [79]. The presence of the adhesive ligands were required for vascularization, showing the importance of the matrix composition, and the VEGF-containing hydrogels performed better than bolus administration of VEGF, indicating that sustained release can increase drug efficacy [79]. This method is also under investigation for the release of brain-derived neurotrophic factor (BDNF) for nerve regeneration [80] and the release of BMP2 for bone regeneration [81, 82].

Similarly, BMP2 was incorporated into biomimetic coatings of calcium phosphate (CaP) on porous scaffolds, so that its release was controlled by osteoclast degradation of the coating [83–85]. Titanium alloy discs were immersed in concentrated simulated body fluid to inhibit

crystal growth, resulting in the formation of a thin layer of amorphous CaP that could serve as a seeding surface for the subsequent addition of a crystalline CaP layer containing BMP2 [83]. The release of BMP-2 in an ectopic ossification site (subcutaneous implantation) in rat was mediated by foreign body giant cells and then by osteoclasts after 3 weeks. The gradual, cell-mediated release of BMP2 over a few weeks using this method resulted in enhanced bone formation and integration with surrounding tissue, and reduced coverage by foreign body giant cells, compared to coated discs with adsorbed BMP2. After 5 weeks, the discs were almost completely surrounded by new woven bone. In contrast, discs coated with CaP but with only adsorbed BMP2 released the protein over a few days, resulting in limited bone formation after one week that was completely resorbed by the fifth week [83]. This coating technique was also used for cell-mediated liberation of BMP2 from porous titanium scaffolds implanted into the maxillae of adult miniature pigs [84]. Interestingly, in this orthotopic model, the presence of BMP2 caused an overall *decrease* in new bone volume, attributed to an overly high dose (13 and 23 µg/implant) that stimulated bone resorption activities of osteoclasts. With a lower dose (5.8 µg), the sustained release of BMP2 caused enhanced bone formation compared to burst release in a subcutaneous implantation model in rat, even in a mechanically unstable situation that normally inhibits osteogenesis [85].

External control over release from scaffolds and hydrogels has been achieved through the use of stimuli-responsive polymers that swell or degrade in response to externally introduced drugs or the application of light, ultrasound, or a magnetic or electric field [86]. Kloxin et al. prepared PEG-based hydrogels with photolabile groups that released the peptide RGDS upon application of light [87]. By using light to release the RGDS from the hydrogels at day 10, the temporal profile seen in developmental chondrogenesis, encapsulated hMSCs upregulated glycosaminoglycan production and cartilage-specific genes *in vitro*, compared to persistently expressed RGDS.

Externally controlled CR systems hold significant promise for advancing tissue engineering approaches, but none have yet passed the prototype stage, as logistical issues for implementing in a clinical setting are still being worked out. Methods to externally control protein release using noninvasive methods such as near-infrared light and ultrasound are under investigation [88–90]. Given that ultrasound can be used for imaging and has been shown to enhance tissue regeneration in animal models [91], its use in controlling protein delivery is particularly appealing.

4. Translation concerns

There are many design issues to consider in the clinical translation of all tissue engineering devices, such as the foreign body response, whether the mode of application is practical, and whether the device represents a substantial enough improvement over existing devices that clinicians will wish to adopt it. A few major challenges in the translation stage of product development that seem to be especially important for tissue engineering strategies that include a CR component are outlined below. At every stage of translation, a detailed analysis of the effects of the released growth factors must be conducted. In addition, clinically relevant cell sources must be studied to prevent age-, animal-, or disease-specific

effects like the well-documented species-specificity of chondrocytes [92] or their decrease in responsiveness to IGF1 with age and disease [93].

4.1. Animal models

The first challenge comes in translating *in vitro* results to *in vivo* results. Despite numerous *in vitro* studies showing synergistic effects of IGF1 and TGF β 1 on chondrogenesis within hydrogels [31, 94, 95], this synergy did not translate into full-thickness cartilage defects in rabbits [96]. TGF β 1 loaded into the hydrogel phase was released rapidly, while IGF1 loaded into gelatin microspheres released more slowly, which approximates the physiological profile observed in chondrogenic differentiation of MSCs [97]. The authors suggested that the lack of agreement between *in vitro* and *in vivo* studies might be due to the complex environment seen in wound healing, where these growth factors exert a wide spectrum of effects.

Similarly, it is important to choose an appropriate, functional animal model for pre-clinical studies, especially considering that tissue engineering therapies will most likely be required in diseased tissues. The sequential delivery of VEGF and BMP2 showed improvements in ectopic bone formation in subcutaneous implantation, but VEGF had no beneficial effect compared to BMP2 alone in orthotopic bone defects in rats, dogs, and pigs [66, 98–100]. The fact that angiogenic factors have been shown to be beneficial for osteogenesis *in vitro* and in other rodent models [101–103] suggests that the interplay between angiogenic factors and BMP2 is complex and depends on the local environment. CR devices should thus be tested in multiple animal models at early stages of development [104].

In adipose tissue engineering, some studies showed that CR of angiogenic factors from biomaterials in the epigastric pedicle in rats caused substantial neovascularization that in turn drove the formation of large amounts of adipose tissue, while other studies showed that substantial neovascularization was *not* accompanied by adipose tissue formation when the CR systems were placed next to the femoral pedicle, which has less of a source of adipocytes [105]. Apparently conflicting results can be obtained using slightly different models, further emphasizing the need to evaluate CR devices in multiple animal models.

4.2. Clinical trials

In translating from large animal models to Phase I and larger clinical trials, the selection of the patient population can be the difference between successful CR devices and failure. The struggles of angiogenesis trials illustrate this point. Despite substantial preclinical evidence showing improvements in revascularization in several animal models of myocardial infarction or ischemic disease, as well as success in Phase I trials, no clinical improvement was achieved from angiogenic growth factor therapy in more than 25 Phase II and Phase III trials involving over 2500 patients [73].

It is likely that angiogenic therapy has not been proven efficacious in large clinical trials because they were not administered in a sustained release formulation, so that any newly formed blood vessels regressed after an initial improvement [53, 86]. However, other issues with clinical trial design are apparent. Many of the trials were restricted to the “no option”

population of patients who have exhausted standard treatment strategies, and therefore might be inherently less responsive to attempts at neovascularization [106]. However, choosing a less severe population would face considerable challenges by institutional review boards and the U.S. Food and Drug Administration (FDA).

In addition, studies have shown considerable fluctuations in physiological measurements such as myocardial perfusion in patients with coronary artery disease, and ankle-brachial index and transcutaneous partial oxygen pressure in patients with peripheral artery disease, which further complicates measures of improvement [106, 107]. The identification of biomarkers of neovascularization would aid in measuring outcomes in clinical trials [106]. Angiogenesis trials in particular have been compounded by a considerable placebo effect, leading to the recommendation to conduct trials only in a double-blinded and randomized manner [73, 106].

Clinical trials of CR devices must also carefully evaluate potential side effects, especially cancer. Obviously proteins that are designed to promote growth might lead to the growth of tumors. Although there is no evidence that the release of BMP2 results in *de novo* tumor formation, Medtronic's experience with AMPLIFY highlights the need to carefully screen patients for history of cancer or undiagnosed tumors.

4.3. Cost

Many experienced researchers agree that cost can be the main obstacle to translation. It has been estimated that the development of a CR device for tissue engineering could cost \$50–\$800 million over eight years from preclinical animal models to regulatory approval [104]. Good avenues of support to begin the translation stage include grants from the SBIR program of the NIH and from the Coulter Foundation, which provides funding for translational projects in biomedical engineering at 16 universities in the United States [108]. However, this funding is nowhere near that required to develop a tissue engineering product, so private investors are also required.

Finally, because patent protection is limited to 20 years from initial submission, it is important that new technologies are translated quickly and efficiently, in order to ensure favorable potential returns on investment [104]. Large companies are unlikely to buy new technologies directly from universities, so the greatest chance of success is held by small companies, usually university spin-offs, that can take the technology from the university through key animal studies to the start of clinical trials [109].

4.4. Regulatory approval

In order to market a medical device or drug in the U.S., it must be approved by the Food and Drug Administration (FDA). Classification, which determines its regulatory pathway, depends on its intended use, primary mode of action (PMOA), and level of risk (Fig. 1). Medical devices are classified as Class I (low risk, external to the body), Class II (more complicated, more risk), or Class III (substantial risk; implanted in the body or life-sustaining). Class I and II devices are either exempt from FDA approval or can achieve clearance through the premarket notification 510(k) pathway.

A tissue engineering scaffold is usually considered a Class II or III medical device if the primary intended purpose is not through chemical action, but the release of a drug or biologic or the presence of cells constitutes an additional designation as a “combination product” [110]. Tissue engineering scaffolds that do not contain a CR component are primarily reviewed by the FDA’s Center for Devices and Radiological Health (CDRH), while combination products are handled by the Office of Combination Products (OCP) and are referred for review by the Center for Biologic and Evaluation Research (CBER) or the Center for Drug Evaluation and Research (CDER) [111]. The OCP decides which pathway the product will follow for approval, which affects all aspects of product development including preclinical and clinical testing [112].

The major determinant of which of the largely parallel pathways is most appropriate is the biomaterial’s primary mode of action (PMOA): devices will eventually require a premarket approval (PMA), drugs will require a new drug application (NDA), and biologics (growth factors, cytokines, etc.) will require a biologic license application (BLA) (Fig. 1). The PMA/NDA/BLA is the most stringent level of market approval required by the FDA.

To facilitate FDA approval of a tissue engineering scaffold, with or without a CR system, preclinical animal studies and biocompatibility tests should be conducted and meticulously documented according to International Standard organization (ISO) 10993 guidelines, with short-term, intermediate-term, and long-term time points of evaluation [109]. The American Society for Testing and Materials (ASTM) International Committee is also in the process of developing standards for testing tissue-engineered products [113]. For a detailed description of regulatory considerations for tissue engineers at the preclinical stage, please see Lee et al. [111].

Clinical trials conducted under an investigational device exemption (IDE) or as an investigational new drug (IND) must be approved by an institutional review board (IRB) and the FDA [114]. An IDE/IND is also required to study products “off-label” in applications for which they are not FDA-approved. With these preliminary levels of approval, products are exempt from quality systems beyond normal design control or laws regulating shipping of the device.

To obtain a PMA/NDA/BLA, clinical trials must demonstrate that the device is safe and effective for its intended use. If clinical trials conducted outside the United States are to be used in support of a PMA/NDA/BLA, they must be conducted in accordance with IDE regulations and the ethical standards set by the Declaration of Helsinki [115]. Clinical trials should also be registered with the ClinicalTrials.gov data bank. In addition to successful completion of Phase III clinical trials, good manufacturing practices (GMP) must be in place to mass produce the biomaterials [109]. GMP documentation includes the Design History file with the detailed scaffold design, and the Device History File documenting the manufactured biomaterials [109].

It can be difficult to conduct studies of CR systems for tissue engineering with a large enough number of patients to garner the level of statistical significance that the FDA is accustomed to seeing in large drug trials [104]. It is recommended to involve regulatory

bodies early in the design of preclinical and clinical trials to generate highly focused aims, to ensure that all requirements are met, and to avoid having to repeat studies [104, 111]. Some have recommended that the development of CR systems for tissue engineering follow a modular approach, in which approval for the scaffold is obtained separately from the biological component in order to reduce risk and increase the likelihood that a large company will pick up the technology and take it through a PMA [109]. A clear understanding of the relationship of the device to currently approved products will expedite the review process, especially if some components of the product can be cleared through a 510(k) pathway.

4.5. Scale-up and manufacturing

Scale-up and manufacturing practices are easier for tissue engineering products that do not contain a cellular component, but the presence of a system for the CR of proteins can make sterilization difficult. Proteins in a CR system are typically susceptible to damage during terminal sterilization methods like steam, dry heat, and ethylene oxide. Sterility must therefore be validated for all source materials and they must be processed using aseptic conditions that require special in-process controls and testing [111].

Tissue engineered medical products standards (TEMPS) are being prepared by subcommittees within Committee F04 of the American Society for Testing and Materials International (ASTM). FDA Guidances for Industry are available for Tissue Guidance to inform manufacturing processes [116]. Quality control during scale up will depend on a clear understanding of the biology and mechanisms of action of the product.

5. Current and Future Needs

CR systems hold enormous potential to stimulate tissue growth and stem cell differentiation in order to regenerate damaged tissues. To improve the success rate of clinical translation, several issues must be addressed. In terms of design of CR systems, it is vital that the dose and rate of release of drugs and proteins are carefully evaluated in multiple animal models. At this stage, it is no longer helpful to simply incorporate a protein in a scaffold and compare it to scaffolds without drug. Doses that are too high may cause unwanted side effects or change the balance of the catabolic and anabolic effects of the protein [84].

Similarly, the effects of release rate and duration must be carefully characterized, and, if possible, matched to the physiological profile seen in normal development or repair. The design of stimuli-responsive or bio-responsive CR systems may aid in this endeavor. There is a need for a simple way to monitor in vivo release kinetics [25]. Many studies analyze blood levels of radiolabelled proteins, which do not consider the local release profile and distribution.

Methods that visualize released and remaining drug can be combined with mathematical modeling to better describe the CR profile [53]. Finally, the complexity of physiological development indicate the need for more sophisticated CR systems that can control the delivery of multiple proteins in precisely defined spatiotemporal profiles. For this reason it

may be more useful to recruit a cell type that can direct subsequent repair efforts, as was demonstrated by the recruitment of monocytes that then enhanced vascularization [54].

At the translation stage, it is important to seek advice early from clinicians, industry, and regulatory officials, to make sure that studies are carefully designed to make regulatory approval as smooth as possible. Careful selection of patient populations, methods of evaluation, and study endpoints is essential to achieving adequate levels of statistical significance and determining if there are any dangerous side effects. It is extremely time-consuming and costly to translate CR systems for tissue engineering to the market, but successful products already on the market (such as Medtronic's Infuse®) show that it is possible and can be quite profitable.

6. Conclusions

CR systems for tissue engineering can be used to extend the bioactivity or presentation of drugs and proteins, recruit cells from the surrounding area, control the temporal presentation of drugs and proteins, induce *in situ* differentiation of stem cells, and physically couple scaffold degradation and new tissue growth. Many sophisticated systems are being studied at the preclinical and clinical levels, especially for bone and therapeutic angiogenesis. Challenges to translation include high cost, difficulties in recruiting appropriate and large enough patient populations for clinical trials, and complex regulatory pathways. More careful analysis of the technologies and more strategic business models may improve the successful translation of CR systems for tissue engineering [104].

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Figure 1.
FDA regulatory path for tissue-engineering devices in regenerative medicine

Table 1

Preclinical and clinical studies of controlled release systems for tissue engineering

Advantage of Controlled Delivery	Example Technology	Target Tissue	Animal Model	References
To extend bioactivity of drug	BMP2 and BMP7 from collagen sponges	Bone	Clinically applied for multiple bone defects	see Table 2
	PDGF-BB from β -tricalcium phosphate particles	Bone	Clinically applied for bone defects from periodontal disease	171, 172
	BMP2 and/or VEGF, PDGF or FGF from various hydrogels and scaffolds	Bone	Cranial defect in rats and pigs, femoral defects in rats, osteoporotic vertebral bodies in sheep	46,47, 99 ^a , 100 ^a , 117–124
	BMP7 from PLGA microspheres, carboxymethyl cellulose	Bone	Osteoporotic vertebral bodies in sheep	125
	VEGF and PDGF from brushite-chitosan scaffold	Bone	Intermedullary bone defects in rabbits	126
	bFGF from gelatin microspheres and collagen scaffolds	Bone	Alveolar bone defects in dogs	127
	IGF1 from fibrin	Cartilage	Cartilage defects in horses	33–35
	IGF1 from hyaluronan	Osteochondral tissue	Osteoarthritic temporomandibular joints in rabbits	128
	PDGF from collagen gel	Ligament	Collateral ligament injury in rats	129
	VEGF and IGF1 in alginate hydrogel	Muscle	Ischemic hindlimbs in mice	36
	BDNF in PNIPAAm-PEG hydrogels	Nerves	Spinal cord injuries in rats	130
	bFGF, FGF1, or FGF2 from gelatin or alginate microspheres in collagen scaffold	Adipose tissue	Rat vascular pedicle/fat pad	105 ^b , 131, 132
To recruit cells	FGF2 from alginate microbeads to recruit endothelial cells	Angiogenesis	Clinical trials	51, 52
	MCP1 from alginate microbeads in scaffolds to recruit monocytes	Angiogenesis	Subcutaneous in immunodeficient mice	54
	IGF1 from hydrogels to recruit chondrocytes	Cartilage	Subcutaneous in athymic mice	62
	TGF β 1 to recruit MSCs	Cartilage	Subperiosteally in rabbits	133
	SDF1 α from PEGylated fibrinogen to recruit stem cells	Myocardial infarct	Myocardial infarction in mice	134
	SDF1 α from PLGA scaffolds to recruit stem cells	Modulation of inflammatory response	Subcutaneous in mice	135
	VEGF, SDF1 and BMP-6 from microdelivery system for stem cell recruitment and differentiation	Bone	Subcutaneous in rats	136
For temporal control	Sequential delivery of VEGF then PDGF from PLGA microspheres and scaffolds	Angiogenesis	Subcutaneous in rats; ischemic hindlimbs in mice; myocardial infarction in rats	57, 68, 69, 137
	Sequential delivery of VEGF, PDGF, and TGF β 1 from alginate hydrogels	Angiogenesis	Subcutaneous in rats	67

Advantage of Controlled Delivery	Example Technology	Target Tissue	Animal Model	References
	Sequential delivery of IGF1 then HGF from alginate hydrogel	Angiogenesis	Acute myocardial infarction in rats	70
	Sequential delivery of TGF β 1 then IGF1	Cartilage	Osteochondral defects in rabbits	96 ^c , 138
	Sequential delivery of VEGF and BMP2 (and vice versa) from PLGA microspheres in gelatin hydrogels and from alginate/PLA scaffolds	Bone	Subcutaneous implantation compared to bone defects in rats and dogs; segmental femur defects in mice	25, 66 ^a , 14
	Sequential delivery of BMP2 then BMP7 from poly(ϵ -caprolactone) scaffolds	Bone	Iliac crest defects in rats	139
For in situ differentiation of progenitor cells	Dexamethasone from PLGA microspheres	Cartilage	Subcutaneous in mice	60
	TGF β 1 from gelatin microspheres in gelatin-HA-chondroitin scaffold	Cartilage	Osteochondral defects in rabbits	75
	bFGF from microspheres in collagen scaffold	Nerves	Cerebral ischemia/ stroke model in rats	140
	VEGF from PLGA microspheres	Angiogenesis	Ischemic hindlimbs in mice	27
Potential for on-demand release kinetics	MMP-sensitive release of VEGF from PEG-based hydrogels	Angiogenesis	Subcutaneous in rats	77, 79
	MMP-sensitive release of BMP2 from PEG-based hydrogels	Bone	Cranial defects in rats	141
	Inflammatory cell- and osteoclast-mediated release of BMP2 and VEGF	Bone	Subcutaneous in rats; cranial defects in mice	83, 84 ^d , 85, 142, 143
	MMP-sensitive release of BDNF from HA hydrogels	Nerve	Spinal cord injuries in rats	80
	External control of VEGF then S1P via hollow fibers	Angiogenesis	Subcutaneous in mice	144
	Photolabile links to release RGD	Cartilage	<i>in vitro</i>	87

^aThese studies showed the importance of appropriate animal models (VEGF and BMP2 were synergistic ectopically but not orthotopically).

^bThis study showed that different results can be obtained at different sites of implantation in the same animal model.

^cThis study showed that *in vitro* results do not always translate to *in vivo* results.

^dThis study showed that increasing the dose of BMP2 can impact the balance between bone formation and bone resorption.

Table 2

Clinical trials of BMP2 and BMP7 release for bone tissue engineering.

Protein	Carrier	Product	Company	Clinical target	Outcome	References
BMP-2	Collagen sponge	INFUSE®	Medtronic (Memphis, TN)	Long bone fractures	FDA approval for open tibial fractures	41, 145–148
BMP-2	Collagen sponge	INFUSE®	Medtronic (Memphis, TN)	Oral and maxillofacial reconstruction	FDA approval for sinus and alveolar ridge augmentation	40
BMP-2	Collagen sponge	INFUSE®	Medtronic (Memphis, TN)	Spinal fusion	FDA approval for anterior lumbar interbody fusion	43, 149–154
BMP-2	Collagen/calcium phosphate	Compression-resistant matrix (CRM); MASTERGRAFT; MBCP	Medtronic (Memphis, TN)	Spinal fusion	FDA approval for bone void filler	155–159
BMP-7	Collagen sponge	OP-1, Osi-graft	Stryker (Kalamazoo, MI)	Long bone fractures	FDA approval for long bone nonunions	44, 160–164
BMP-7	Collagen sponge +/- paste-thickening agent	OP-1, OP-1 Putty	Stryker (Kalamazoo, MI)	Spinal fusion	FDA approval of OP-1 putty for posterolateral spinal fusion	165–170