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Progress in Three-Dimensional Printing with Growth Factors

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Abstract

Incorporation of growth factors in biomedical constructs can encourage cellular activities necessary for tissue regeneration within an implant system. Three-dimensional printing offers a capacity for spatial dictation and dosage control of incorporated growth factors which promises to minimize complications from the supraphysiologic doses and burst release involved in current growth factor delivery systems. Successful implementation of three-dimensional printing with

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None.

growth factors requires preservation of the bioactivity of printed growth factors, spatial localization of growth factors within the construct architecture during printing, and controlled release of growth factors after printing. This review describes demonstrated approaches for addressing each of these goals, including direct inclusion of growth factors with the biomaterial during printing, or intermediary encapsulation of growth factors in delivery vehicles such as microparticles or nanoparticles.

Graphical Abstract

Keywords

Three-dimensional printing; growth factor; protein delivery; bioactive; tissue engineering

Introduction

Three-dimensional (3D) printing technology is being increasingly harnessed for applications ranging from commercial manufacturing to personal use. A promising application of this technology is its incorporation into medical practice, in parallel with the trend towards personalized medicine. Conceivably, the biomedical engineering field of tissue engineering could be used to create customized replacements of bodily defects with dimensions converted from medical imaging technology.¹

3D printing is a fabrication method which translates a virtual computer-aided design (CAD) model into a 3D object.² Since the first patent of this methodology by Charles Hull in 1986, 3D printing has been expanded and adapted for diverse applications.³ The primary methods of 3D printing for tissue engineering applications include extrusion printing and stereolithography.⁴ In extrusion printing, this fabrication occurs by pneumatically or mechanically dispensing material held in a cartridge through a nozzle, to deposit material in the form of strands in a layer-by-layer manner to construct the object.^{5,6} Meanwhile, stereolithography methods use focused patterns of light to solidify a liquid resin at selective locations.⁶

The fields of tissue engineering and regenerative medicine can especially benefit from this technology. Biomedical engineers wish to generate tissue that can compensate for the limitations of the body's native precursor cells to restore damaged or missing tissue.⁷ According to the classical tissue engineering paradigm, biomaterial scaffold structures, cells,

and bioactive molecules interact to encourage the growth, repair, and replacement of biological tissues. 3D printing allows for the creation of a scaffold whose outer shape conforms to the boundaries of a complex defect with high fidelity. Indeed, the aspiring role of 3D printed tissue engineering constructs for clinical practice would be translating data from imaging modalities such as computed tomography into a CAD model, which is used to create a scaffold which closely matches the defect shape.¹ 3D printing also allows specification of the inner structure of the scaffold, such as porosity and pore structure, which are essential to proper interaction of the cellular and biomolecule components with surrounding native tissue.^{8,9}

In addition to or in lieu of including autologous cells into tissue engineered constructs, a patient's native cells could be signaled to migrate, proliferate, and differentiate in an engineered scaffold through the use of bioactive molecules such as growth factors. Growth factors are physiologic polypeptides that are water-soluble and exert potent influence over many cellular functions.¹⁰ For example, bone growth can be stimulated by the interaction of bone morphogenetic protein-2 (BMP-2) or bone morphogenetic protein7 (BMP-7) with osteoblast precursors.¹¹ Meanwhile, cartilage can be induced under the direction of transforming growth factor- β (TGF- β) or connective tissue growth factor (CTGF), and vascular endothelial growth factor (VEGF) encourages vascular or neural formation.¹²⁻¹⁴

While harvesting of autologous cells requires donor site morbidity, growth factor inclusion for tissue engineering scaffolds may also incur complications by stimulating uncontrolled tissue growth, which may even encourage malignant processes.^{15,16} Therefore, being able to dictate the spatial distribution and temporal delivery of growth factors through 3D printing may help to limit their influence to desired therapeutic effects.

This article discusses the goals and challenges of 3D printing with growth factors, a review of the current progress towards these ends, experimental considerations for developing techniques of 3D printing with growth factors, and reflections on the future outlook of this field.

Main Goals and Challenges

The U.S. Food and Drug Administration has approved several therapeutic products containing growth factors.¹⁷ Such products must address certain problems with growth factor delivery. First, these bioactive molecules are needed in timeframes throughout the tissue generation process in a biomimetic match with the physiologic profile.¹⁸ However, growth factors' aqueous solubility may result in burst release, in which a large proportion of the total loaded growth factor rapidly exits the structure within the initial days after implantation.^{19,20} Growth factor functionality is then quickly negated due to clearance from the site by diffusion, or inactivation by proteolytic cleavage.^{21,22} If too little growth factor remains to be released from the structure at later timepoints, the biomolecules may not exist at sufficient concentrations to exert their intended functions.⁹

Therefore, a supraphysiologic dose of growth factors is often incorporated into existing products to ensure a sufficient active concentration, or the therapeutic products are used

clinically in settings beyond those specified in their limited regulatory approval.²³ However, these elevated dosages may cause overstimulation of tissue growth, with undesirable side effects such as inflammation and ectopic tissue growth, or alarming adverse events such as malignancy.^{16,24,25}

Given these complications, a crucial goal for the development of therapeutic products which deliver exogenous growth factors is minimizing the dose of loaded growth factor molecules.^{26,27} This consideration is especially important in light of a systematic review suggesting that risk of malignancy is growth factor dose-dependent.²⁸ Therefore, the control over growth factor quantity and distribution within a tissue engineering scaffold afforded by 3D printing makes this technology an exciting strategy for optimizing growth factor dosage.^{29,30}

Nevertheless, the use of this technology is not without its own difficulties. As growth factors are proteins, their activity depends on the maintenance of secondary and tertiary structural conformations, to ensure proper recognition by their respective cell membrane receptors and the induction of subsequent cellular responses.³¹ The engineering challenge lies in the sensitivity of growth factors to conformational disruption, denaturation, and loss of bioactivity under conditions commonly employed for 3D printing, including high temperatures and organic solvents.⁶ A necessary research objective is thus the synthesis of a 3D printed biomaterial scaffold including growth factors with preserved bioactivity.

Printing growth factors—or delivery vehicles containing them—at definitive and strategic locations within a scaffold can assist with the minimization of dosage. Considering the geometry of the scaffold and the structural organization of the desired future tissue, growth factors can be incorporated into the scaffold only at sites advantageous for interactions with the cells whose activities the growth factors will direct.³² Once successfully incorporated, the growth factors must be released from the scaffolds or otherwise allowed exposure to the membrane receptors of these cells at key times in the chronology of tissue development, which may require differential and long-term growth factor release in a pattern unique to the tissue type and the growth factors themselves.³³

Therefore, three goals are necessary for the successful employment of 3D printing to incorporate growth factors into tissue engineering scaffolds: (I) preservation of growth factor bioactivity, (II) specific spatial localization of printed growth factors within the scaffold, and (III) control of subsequent release of growth factors from the scaffold. Strategies to accomplish each of these goals may, and ultimately must, overlap. For example, encapsulating growth factors in delivery vehicles which are subsequently printed can enable protection of growth factors from harsh printing conditions by the surrounding microparticle or nanoparticle barrier. Meanwhile, the location of delivery vehicles and their growth factor contents can be precisely established and maintained by embedding the vehicles within the scaffold structure. Finally, tuning the delivery vehicle material's degradation would determine the rate of growth factor release.

General strategies for 3D printing with growth factors that have been proposed or attempted include mixing the growth factors into the solution of material which is to be 3D printed, or encapsulating growth factors in delivery vehicles which are then 3D printed with the

construct material.^{14,30} Table 1 summarizes the manner in which these strategies can accomplish the three goals described above, as the following content of this article will discuss in further detail.

The current work does not include the numerous strategies for introducing growth factors and their delivery vehicles to scaffolds after scaffold fabrication by 3D printing, such as by immersion in a growth factor solution or injection of such a solution into scaffold pore channels.^{34,35} This scope is defined given the dissimilarity of considerations for these methods compared to direct printing with growth factors. For example, in terms of bioactivity preservation, post-fabrication techniques bypass 3D printing conditions; for spatial localization and release, these methods employ different mechanisms of dosage control and delivery.^{36–38} Similarly, this review also excludes techniques of inkjet printing which are useful for modifying the surfaces of scaffolds fabricated by other solid free-form methods.⁶

Current Progress

Studies describing direct 3D printing of growth factors with biomaterial scaffolds began to appear in the literature within the past decade. After the first few years of introductory publications, the quantity of articles on this topic has steadily increased, as shown in Fig. 1. The predominant 3D printing technique investigated for this purpose is extrusion printing, with twenty-four of the twenty-eight studies employing this method, while the other four studies feature stereolithography.

We now present a survey of the existing literature for 3D printing with growth factors as of October 2018 and the status of progress towards the goals of preservation of growth factor bioactivity and localization of growth factors within scaffolds during printing, as well as controlled release of growth factors from scaffolds after printing. These articles were found using the search terms “print growth factor,” “print bioactive molecule,” “print biomolecule,” and “print cytokine” in the Web of Science and PubMed online databases. Summaries of the articles discussed including the applications, material systems, and 3D printing methods involved are provided in Tables 2 and 3.

(I) Bioactivity Preservation

As physiologic proteins, growth factors can lose their secondary and tertiary structures—And therefore their functions—under inhospitable conditions, such as those usually required for 3D printing. In order to preserve the bioactivity of growth factors during 3D printing, either printing conditions must be adapted to non-denaturing ranges, or printed growth factors must be protected from exposure to an otherwise denaturing environment of high temperatures or organic solvents.^{6,39,40}

Preservation Strategy 1: Non-Denaturing Printing Conditions—Several different biomaterials have been studied for use in 3D printing of tissue engineering scaffolds, yet many of these materials must be printed under conditions that potentially threaten the bioactivity of incorporated growth factors.⁶ Importantly, the selection of natural polymers may enable printing in milder, more physiologic settings. Growth factors can be printed in

collagen or gelatin at or below room or body temperature.^{12,32,41,42} Additionally, for bone tissue engineering applications, calcium phosphate cement pastes can be printed by extrusion at room temperature and physiologic pH with non-organic solvents.⁴³ The loaded growth factor can thus be printed with the scaffold materials while retaining bioactive functionalities such as induction of cell gene expression and extracellular matrix (ECM) formation.^{12,32}

To incorporate growth factors into scaffolds composed of biomaterials requiring high temperatures for printing, multi-channel extrusion printing—in which these materials are printed from separate cartridges than those containing growth factors—can be employed.^{12,32,44,45} 3D printing may also require exposure of components to potentially denaturing organic solvents.⁴⁶ Alternatively, using water-based rather than organic components to print hydrogel-growth factor combinations can permit the 3D printing of functionally bioactive growth factor-laden tissue engineering constructs.^{6,14,41,47}

Preservation of growth factor bioactivity must occur not only during printing, but also in the preceding and subsequent processing methods. For example, while growth factors may withstand elevated temperatures for the duration of printing itself, caution must be applied when the time of heat exposure is lengthened for the technique of heating extrusion mixtures or stereolithography resins in order to reduce their viscosity before printing.^{48–50} Similarly, the amount of ultraviolet light employed for crosslinking of scaffold materials after printing must be tolerable to the incorporated biomolecules.^{32,51} Subjecting the printed construct to heated or cooled air for the purpose of solvent evaporation must also be performed at a temperature compatible with retained growth factor bioactivity.^{46,52,53}

Preservation Strategy 2: Encapsulation in Protective Delivery Vehicles—Some scaffold biomaterials are not conducive to adaptation for 3D printing conditions within bioactivity-compatible ranges, such as synthetic polymers which require hightemperature printing conditions.⁵⁴ Therefore, growth factors printed into scaffolds made of these materials must be protected from direct exposure to this harsh environment. One viable approach consists of enclosing growth factors within a delivery vehicle which can then be included in an extrusion printing cartridge or stereolithography resin.⁵⁵ For example, poly(D,L-lactic-*co*-glycolic acid) (PLGA), a biodegradable polymer commonly used for drug delivery applications, can be used to encapsulate growth factors for subsequent incorporation into scaffold materials.⁵⁶

Nevertheless, the process of growth factor encapsulation in microparticles or nanoparticles may itself threaten the bioactivity of the protein. For strategies requiring sonication or high-temperature incubation, heat-sensitive growth factors may denature; the same consequence may occur in emulsion microparticle synthesis processes upon the proteins' interaction with a water-oil interface.⁵⁷

(II) Spatial Localization

One of the leading justifications for pursuing 3D printing as a means of producing growth factor-loaded tissue engineering scaffolds is the substantial capacity to define the incorporated growth factors' location within the construct architecture. When growth factors

are successfully printed with preserved bioactivity, they can induce spatially definitive responses from cells.¹⁴ Samorezov *et al.* compiled an extensive survey on methods of spatially-specific delivery of growth factors and other bioactive molecules.⁶ Demonstrated approaches for localization of growth factors by 3D printing involve including growth factors in bulk scaffold material which is directed to print at certain sites, or using delivery vehicles to define growth factor location.

Localization Strategy 1: Printing Growth Factor-Loaded Material in Distinct

Construct Regions—The 3D printing technique of stereolithography can be used to incorporate growth factors into a confined layer of a tissue engineering scaffold.⁵⁸ However, for a given horizontal plane, the growth factors and their delivery vehicles are homogeneously distributed in the scaffold after stirring into the resin, without further localization capacity in the dimensions of the plane, unless intervening steps of resin removal and switching as well as scaffold rinsing are implemented.^{49,59}

3D printing localization of one or multiple growth factors can also be accomplished in multi-channel extrusion printing by regionally segregating the growth factor-loaded material in the CAD file. For example, material optimized for contact with host tissue can be printed in the periphery, while cells would be directed to migrate towards the source of growth factors printed in the scaffold's interior.^{13,43} This approach is especially appealing for localization of VEGF, which can be chosen to match central regions of hypoxic cell staining, in order to direct vascularization to where it is most needed.¹³ Constructs can also be designed to have a gradient composition, such that increasingly higher proportions of growth factor-loaded scaffold strands exist in successive layers.⁴³

Localization Strategy 2: Distributing Growth Factor-Loaded Delivery Vehicles

—In addition to preserving growth factor bioactivity, delivery vehicles can dictate the location of incorporated growth factors by way of their distribution within printed scaffolds.^{30,60,61} For example, multiple extrusion printing cartridges can be alternately loaded with delivery vehicles containing different growth factors to yield distinct and interfacing tissue types.³⁰ This controlled localization can be used to replicate the complex architecture of anatomical sites with varied tissue compositions and orientations.⁶¹ Scaffold materials co-printed with cells can also be guided to exhibit selective tissue formation only in regions with printed microparticles or nanoparticles enclosing growth factors.⁶²

Incorporation of particulate delivery vehicles can also modify scaffold surface topography in a manner which guides the morphology of adhered cells.⁶³ This advantage must be balanced with the potential alterations of scaffold mechanical properties resulting from inclusion of delivery vehicles.³⁰

(III) Controlled Release

After growth factors are incorporated at a specific location in the biomaterial scaffold, they must be allowed to come into contact with the membrane receptors of either exogenously introduced or native cells. Upon release from the printed scaffold material or delivery vehicle, the original growth factor location constitutes the peak of a concentration gradient,

from which the released growth factors diffuse to other sites as permitted by scaffold architecture, particularly pore structure.^{52,53,64–66}

The timing of growth factor release should aim to mimic the biomolecule's pattern of appearance during a particular physiologic process, while without excessive burst release nor dosage-sequestering retention. This objective becomes increasingly complex for growth factors that must be present at early timepoints, yet should also recur in later phases of tissue formation, such as for BMP and TGF- β .^{67,68} Proper timing of growth factor exposure can determine whether release profiles from 3D printed scaffolds are effective for influencing cell behavior. For example, predominant burst release of loaded growth factor may fail to provoke cell differentiation, possibly due to an insufficient duration of exposure for triggering desired cellular responses.³²

An additional consideration is the method of growth factor release, which depends on the nature of its incorporation. For growth factors directly integrated into the scaffold material, their release rate will be determined initially by release from the surface and ultimately by degradation of the scaffold biomaterial.⁵⁶ For growth factors encapsulated in microparticles or nanoparticles which are embedded in scaffold material, the degradative capacity of the delivery vehicle material and subsequent biomolecule diffusion will also determine the rate of growth factor release.^{69,70} Additional reviews on the controlled release of growth factors have been conducted by Vo et al., Lee et al., and Porter et al.^{10,71,72}

Release Strategy 1: Degradation of Construct Material—For growth factors directly loaded into biomaterials for 3D printing, the known degradation rates of these materials can guide the design of constructs with one or multiple growth factors releasing at different rates, to coincide most advantageously with signals needed for the tissue formation process.^{13,47,73,74} The rate and degree of growth factor release may depend on processing techniques conducted after printing that determine scaffold degradation rate, such as material crosslinking.³² Moreover, after growth factor release by the mechanism of degradation, attention must be given towards possible threats to bioactivity of the resulting environment. For example, the acidic nature of degradation products of PLGA may denature growth factors whose bioactivity was initially preserved during 3D printing.^{44,46,75}

To prevent excessive burst release of growth factors from the 3D printed structures, tissue engineering scaffolds can feature an affinity to growth factors which allows their more gradual release.^{66,76,77} Genetic recombination or preliminary complexation can produce growth factor proteins with enhanced scaffold binding and resulting control over initial burst release.^{5,46} Scaffold biomaterials can thus be chosen or modified to dictate their growth factor affinities and subsequent release profiles.^{14,43,76} However, if the affinity of growth factors for the scaffold is excessive, the proteins may be undesirably delayed in releasing from the material and diffusing away to access their cellular targets.^{32,58}

Release Strategy 2: Degradation of Delivery Vehicles—For growth factors printed with scaffold biomaterials in intermediary delivery vehicles, the degradation rate of the vehicles' material will influence the timing of growth factor release.⁷⁸ Various works have employed microparticles or nanoparticles to enable controlled growth factor release by using

different compositions of encapsulating material such as PLGA, gelatin, and chitosan-dextran.^{30,41,58,60–64,79,80}

Parallel to the concern of excessive growth factor affinity to the bulk scaffold material, delivery vehicles may not release all of their contents within timeframes relevant for guidance of tissue formation.⁷⁹ Microparticles printed into scaffolds demonstrate slower release patterns relative to free microparticles, given that scaffold material degradation might be necessary before microparticles and their growth factor contents become accessible, and the scaffold material also presents an additional diffusional barrier for released growth factors.^{33,41,49,63,70,81,82} Post-printing scaffold treatments can be included to encourage more rapid and complete growth factor release; however, techniques involving immersion of the scaffold in a processing solution should be ensured not to result in excessive release of loaded growth factor before the scaffold is used for its intended application.^{30,64,74} Therefore, amid attempts to control growth factor release through methods such as encapsulation in microparticles or nanoparticles, other characteristics of biomaterials and processing methods chosen for tissue engineering scaffolds may either enhance or interfere with desirable release.⁸³

Greater initial loading dosages of growth factors within these delivery vehicles might also be attempted to ensure sufficient quantities of release, yet this approach may be dissatisfactory for avoiding the supraphysiologic dosing of existing products.⁶⁴ Alternatively, a greater number of delivery vehicles can be incorporated, as they may also benefit the scaffold degradation process by serving as porogens in the scaffold material, which may then facilitate microparticle or nanoparticle release.⁶⁴ The safety of these workarounds may be undesirable in the *in vivo* setting where conditions antagonistic to delivery vehicle integrity may suddenly introduce the retained dose. Nevertheless, delivery vehicles' ability to release growth factor at a slower rate than that for direct incorporation into a scaffold can enable prolonged exposure of local cells to growth factor presence with resulting superior guidance of tissue formation.^{58,62,79}

Experimental Considerations

As researchers continue to invest time and resources into developing techniques to preserve bioactivity, localize spatially, and release controllably while 3D printing with growth factors, a wide view of experimental setups for previous work can guide the design of future efforts.

A necessary decision in these studies is the quantity of loaded growth factor. An optimized growth factor dosage can be determined before conducting 3D printing with the growth factors. The desire to increase the growth factor dose for greater tissue responses must be tempered by the intent to avoid adverse effects and unnecessary costs resulting from use of excess growth factor quantities.³²

After choosing appropriate loading dosages, assessing the effects of growth factor incorporation warrants a thoughtful selection of included controls. Ideally, preserved growth factor bioactivity ought to be verified by comparing evidence of cellular responses among three experimental groups—printed scaffolds with and without growth factor, as well as free

unprinted growth factor.^{49,62} Retained growth factor functionality can also be demonstrated by dosage-dependent effects of delivered growth factors.⁶¹ Multiple measures of cellular responses can be included to ensure comprehensive yet selective detection of growth factor effects.⁴⁹ In studies with scaffolds delivering different growth factors, preserved growth factor bioactivity is further emphasized by specificity of tissue responses both to the growth factor protein's family—such as BMP—and to its specific isoform, BMP-2 or BMP-7.⁸⁰

When planning *in vitro* measures of released 3D printed growth factor bioactivity, careful consideration must also be given to the frequency of cell media exchange. For constructs exhibiting burst release, an early change of media may remove a majority of the growth factor, which could exacerbate the insufficient duration of growth factor exposure to the cells.³² Additionally, when the strategy of growth factor encapsulation in delivery vehicles is chosen because scaffold materials require printing conditions inhospitable to growth factor activity, the earliest fraction of growth factors collected may be unprotected biomolecules undergoing burst release from the surface of the scaffold or delivery vehicle.⁸⁴

To investigate the goal of spatial localization by 3D printing, fluorescent labeling and microscopy would allow determination of the growth factors' positions within the scaffold. Growth factors can be incorporated in a form preconjugated to fluorescent molecules, or printed scaffolds with incorporated growth factors can be stained with fluorescently labeling solutions.^{85,86} Alternatively, delivery vehicles may be designed to include fluorescent probes.³⁰

Furthermore, crucial questions for choosing how and when to measure growth factor bioactivity, localization, and release include whether the method is non-destructive, quantitative, and dynamic with time.⁸⁷ These criteria are especially important for monitoring of controlled growth factor release; researchers can employ radiolabeling, or the tagging of growth factors with elemental isotopes, to monitor the release kinetics quantitatively and non-invasively *in vivo* without disrupting the growth factors' bioactivity.^{88,89}

Finally, a noticeable trend in 3D printing literature is that printing parameters such as pressure, speed, and temperature are often listed in the methodology section without an explanation of how these values were selected and optimized, nor how the printed structure would change if these values were increased or decreased.⁹⁰ Therefore, engineers employing 3D printing should systematically isolate and analyze each variable of the printing conditions and its effectiveness towards addressing the challenges discussed.⁹¹ For example, printing at lower temperatures may enable preserved bioactivity, yet prevent sufficient reductions in scaffold material viscosity to allow uniform distribution of growth factors and their delivery vehicles within the scaffold material.⁹² The outcomes of these investigations can be disseminated both through traditional means of written journal publications and through online communities which offer open source sharing of CAD files for 3D printing software programs.^{93,94}

Outlook

Each of the reviewed studies contributes progress towards the spatially precise 3D printing of biologically active growth factor molecules within tissue engineering scaffolds. Additional strategies for 3D printing with growth factors can also be conceived. For the goal of ensuring growth factor bioactivity after printing, a novel approach might involve printing growth factors in an initially inactive then subsequently activable form, such as by association with a protective component which is later removed, as a biomimetic design inspired by the ECM-directed activation of TGF- β .⁹⁵ Alternatively, researchers could synthesize and print with smaller peptide sequences which act on the same cellular growth factor receptors.^{6,96–98}

Moreover, a particular advantage of natural polymer compositions for scaffold biomaterials or delivery vehicles is their susceptibility to enzymatic degradation, which dictates the release of their growth factor contents. Cell-directed tunability of growth factor release could be imparted such that after initial stimulation of cells by growth factor released from the construct, cells would differentiate and release enzymes characteristic of their tissue development stage. These enzymes would then encourage release of additional growth factor, so tissues would dictate their own timeline for growth, similar to the natural phenomenon of transient sequestration of bioactive molecules in the ECM.⁹⁹

Once retained bioactivity during the 3D printing process, precise positioning of growth factors, and efficacious temporal release profiles can be accomplished using these different strategies, engineers can then expand these techniques from printing single growth factors in a simple homogeneous pattern to developing 3D printed gradients of multiple growth factors.¹⁰⁰ Such gradient patterns will be essential to optimize the scaffold's interaction with surrounding tissues of potentially different types via an intertissue interface.¹⁰¹ This strategy will be particularly important for applications such as the craniofacial complex, in which multiple tissue types exist and high standards of integration are required to meet aesthetic goals; as well as osteochondral locations like the knee, for which mechanical functionality depends on the transition from subchondral bone to cartilage.^{102,103}

In vivo studies represent an essential demonstration of the proposed techniques and their efficacy in such applications for human health. Multiple studies have featured animal models with notable tissue formation after implantation of 3D printed growth factor-laden scaffolds into tissue defects.^{12,13,30,32,42,44,46,49,62,79} Meanwhile, computational materiomics approaches can assist with prediction of the biological response to the combination of various components for these biomolecule-incorporating constructs.¹⁰⁴

The motivation to pursue this technology is the capacity for eventual assimilation into medical practice. Development of these systems should aim to illustrate the ultimate clinical paradigm, with 3D imaging of target tissue sites directing the outer shapes and dimensions of printed scaffolds.³⁰ For instance, in the field of craniofacial surgery, computed tomography data of a patient's opposite, unilateral facial feature could provide a template for the synthesis of a symmetrical replacement of absent bone tissue.^{105,106}

Another benefit of the therapeutic integration of 3D printing with growth factors is the speed and non-invasiveness of planning and producing an individualized tissue construct for a patient. The process of manufacturing a construct can be completed within days of acquiring non-invasive imaging studies that are already included in standard medical practice.^{107,108} This aspect represents an advantage over autologous cellular or gene therapy approaches, which can require invasive surgical extraction procedures and weeks of preparation.^{109–111} Additionally, both localization and controlled release of growth factors using 3D printing technology are promising for avoidance of the therapeutic complications of ectopic tissue growth.¹¹² 3D printing with growth factors can also be used for extra-therapeutic applications of tissue engineering. For example, localization of aberrantly expressed growth factors in tumor modeling can represent the complex phenomena of vascularization due to hypoxic cells in the center of a tumor.¹¹³

After fabrication by 3D printing or other means, tissue engineering scaffolds are meant to be surgically introduced into tissue defects of patients. This implantation procedure elicits inflammatory processes that can be manipulated for the benefit of tissue construct integration.¹¹⁴ Such concepts also apply to the body's inflammatory response to introduced growth factors, particularly when excessive burst release occurs. At early timepoints, inflammatory activity may suppress tissue formation, but after resolution of the inflammatory state, an observable increase in tissue formation rate can compensate for the initial delay to yield notable tissue formation.¹² Mechanistically, inflammatory cytokines have been found to stimulate tissue-forming behavior, such as osteogenic differentiation.¹¹⁵ The role of inflammation in the setting of tissue engineering with growth factors is notable given that patients' responses may vary according to their immune systems and any coinciding autoimmune conditions.¹¹⁶

Most importantly, once tissue engineering researchers have harnessed 3D printing for incorporation of growth factors, ethical obligations will require that the amount of bioactive growth factor influencing the local tissue environment falls within a therapeutic window to avoid potentially serious adverse events. Special attention and comprehensive reporting should be provided for the incidence of exorbitant pain, ectopic tissue growth which encroaches on surrounding tissues, inflammatory sequelae, or tumorigenesis in preclinical animal studies and early human trials.¹¹⁷

Ultimately, developing the technique of 3D printing with growth factors will provide a valuable tool that can be combined with other tissue engineering approaches to yield individualized constructs capable of regenerating a variety of tissues for human patients.

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Highlights

- Three-dimensional printing with growth factors occurs directly or by encapsulation
- Printing conditions or delivery vehicles preserve growth factor bioactivity
- Printing in distinct construct regions enables growth factor localization
- Construct or vehicle degradation controls the release of printed growth factors

Fig. 1. Research articles published per year on the topic of three-dimensional (3D) printing with growth factors. As this manuscript was submitted in October 2018, any studies published in 2018 which are discussed in the article and included in Tables 2 and 3 are not included in this figure.

Table 1.

Main goals and strategies for three-dimensional printing with growth factors.

MAIN GOALS		(I) Bioactivity Preservation	(II) Spatial Localization	(III) Controlled Release
STRATEGIES	Direct Inclusion with Biomaterial	Non-denaturing printing conditions	Material printing in distinct regions	Degradation of construct material
	Encapsulation in Delivery Vehicles	Protection by delivery vehicles	Distribution of delivery vehicles	Degradation of delivery vehicles

Primary applications and material systems for published studies on three-dimensional printing with growth factors as of October 2018. * References indicated with an asterisk involve multiple growth factors and/or formation of multiple tissue types.

Table 2.

Primary Application	Growth Factor	Construct Material	Co-Printed Cells	Evaluation Method	Reference Number
Bone formation	Bone morphogenetic protein-2	Collagen, methacrylamide gelatin	Bone mesenchymal stem cells	<i>In vitro</i>	5
		Alginate, collagen, gelatin, poly(e-caprolactone)	Dental pulp stem cells	<i>In vitro, in vivo</i>	13*
		Collagen, gelatin, poly(e-caprolactone), poly(D,L-lactic-co-glycolic acid)	None	<i>In vitro, in vivo</i>	32
		Collagen, poly(e-caprolactone), poly(D,L-lactic-co-glycolic acid) with β -tricalcium phosphate	None	<i>In vitro</i>	44
		Bone extracellular matrix, poly(e-caprolactone) with β -tricalcium phosphate	None	<i>In vitro, in vivo</i>	45
		Hyaluronic acid, poly(ethylene glycol), poly(D,L-lactic-co-glycolic acid)	None	<i>In vitro, in vivo</i>	46
		Poly(D,L-lactic-co-glycolic acid) microparticles in poly(propylene fumarate), diethyl fumarate	None	<i>In vitro, in vivo</i>	49
		Calcium silicate, poly(e-caprolactone)	None	<i>In vitro</i>	50
		Calcium phosphate cement, poly(L-lactic acid)	None	<i>In vitro</i>	53
		Alginate, alginate sulfate	Osteoblasts	<i>In vitro</i>	76
Cartilage formation	Transforming growth factor- β	Gelatin microparticles in alginate	Mesenchymal stem cells	<i>In vitro, in vivo</i>	79
		Alginate, poly(e-caprolactone)	Chondrocytes	<i>In vitro, in vivo</i>	12
		Poly(D,L-lactic-co-glycolic acid) microparticles in poly(e-caprolactone)	None	<i>In vitro, in vivo</i>	30*
		Hyaluronic acid, polyurethane	None	<i>In vitro</i>	47
		Poly(D,L-lactic-co-glycolic acid) nanoparticles in poly(ethylene glycol) diacrylate with nanohydroxyapatite	None	<i>In vitro</i>	58*
		Poly(D,L-lactic-co-glycolic acid) nanoparticles in gelatin methacrylamide, poly(ethylene glycol) diacrylate	Mesenchymal stem cells	<i>In vitro</i>	60
		Poly(D,L-lactic-co-glycolic acid) microparticles in poly(e-caprolactone)	None	<i>In vitro, in vivo</i>	61*
		Poly(D,L-lactic-co-glycolic acid) microparticles in poly(e-caprolactone)	None	<i>In vitro</i>	80*
		Poly(D,L-lactic-co-glycolic acid) microparticles in poly(ethylene glycol) diacrylate	None	<i>In vitro</i>	63
		Collagen, fibrin	Neural stem cells	<i>In vitro</i>	14
Dental tissue formation	Nerve growth factor	Alginate, dermal homogenate, gelatin	Epithelial progenitors	<i>In vitro, in vivo</i>	42
		Poly(D,L-lactic-co-glycolic acid) microparticles in alginate, gelatin with β -tricalcium phosphate	None	<i>In vitro</i>	41
		Alginate, alginate gellan gum, calcium phosphate cement	None	<i>In vitro</i>	43
		Gelatin microparticles in alginate, Matrigel®	Endothelial progenitors	<i>In vitro, in vivo</i>	62
		Chitosan/dextran sulfate microparticles in calcium phosphate cement	None	<i>In vitro</i>	64
		Alginate, Laponite®, methylcellulose	None	<i>In vitro</i>	66
		Poly(D,L-lactic acid-co-trimethylene carbonate)	None	None	52
		Alginate	None	None	74
Vascularization	Vascular endothelial growth factor				
Demonstration of growth factor release	Bone morphogenetic protein-2				
Demonstration of growth factor release	Vascular endothelial growth factor				

Table 3.

Printing methods used in published studies on three-dimensional printing with growth factors as of October 2018.

Printing Technique	Printer	Approximate Printed Strand Diameter (μm)	Approximate Scaffold Pore Size (μm)	Post-printing Processing	Reference Number
Extrusion: single channel	3D-Bioplotter® (EnvisionTec)	100	100	Surface treatment	30
		Unmeasured	500	Crosslinking	41
		300	300	None	61
		Unmeasured	500	None	80
	3DDiscover™ (regenHU)	Unmeasured	Unmeasured	None	74
		Unmeasured	Unmeasured	None	50
	BioScaffolder (GeSiM)	680	1240	Incubation	64
		420	Unmeasured	Crosslinking	66
	Custom-made	Unmeasured	Unmeasured	None	79
		Unmeasured	280, 365	Crosslinking	5
		Unmeasured	Unmeasured	Crosslinking	13
		250	300	Solvent evaporation	46
	Regenovo Bio-Printer™ (Regenovo)	300	700	Freeze-drying	47
		550	965	Crosslinking	76
		Unmeasured	Unmeasured	Crosslinking, incubation	42
		Unmeasured	10	Cryogenic solvent evaporation, incubation	52
	BioScaffolder (GeSiM)	200	300	Crosslinking, incubation	43
		Unmeasured	Unmeasured	None	62
Extrusion: multi-channel	Custom-made	200	400	Crosslinking	12
		Unmeasured	Unmeasured	Incubation	14
		150	250	Incubation	32
		250	250	Incubation	44
	Replicator® 2X (Makerbot)	Unmeasured	Unmeasured	Freeze-drying, incubation	45
		Unmeasured	Unmeasured	Cryogenic solvent evaporation	53
	Custom-made	Unmeasured	Unmeasured	None	49
		215	790	None	58
Stereolithography	Printrbot® (Printrbot)	400	Unmeasured	None	60
		Unmeasured	Unmeasured	None	63