EXPERT OPINION

Composite polymer-bioceramic scaffolds with drug delivery capability for bone tissue engineering

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Introduction: Next-generation scaffolds for bone tissue engineering (BTE) should exhibit the appropriate combination of mechanical support and morphological guidance for cell proliferation and attachment while at the same time serving as matrices for sustained delivery of therapeutic drugs and/or biomolecular signals, such as growth factors. Drug delivery from BTE scaffolds to induce the formation of functional tissues, which may need to vary temporally and spatially, represents a versatile approach to manipulating the local environment for directing cell function and/or to treat common bone diseases or local infection. In addition, drug delivery from BTE is proposed to either increase the expression of tissue inductive factors or to block the expression of others factors that could inhibit bone tissue formation. Composite scaffolds which combine biopolymers and bioactive ceramics in mechanically competent 3D structures, including also organic–inorganic hybrids, are being widely developed for BTE, where the affinity and interaction between biomaterials and therapeutic drugs or biomolecular signals play a decisive role in controlling the release rate.

Areas covered: This review covers current developments and applications of 3D composite scaffolds for BTE which exhibit the added capability of controlled delivery of therapeutic drugs or growth factors. A summary of drugs and biomolecules incorporated in composite scaffolds and approaches developed to combine biopolymers and bioceramics in composites for drug delivery systems for BTE is presented. Special attention is given to identify the main challenges and unmet needs of current designs and technologies for developing such multifunctional 3D composite scaffolds for BTE.

Expert opinion: One of the major challenges for developing composite scaffolds for BTE is the incorporation of a drug delivery function of sufficient complexity to be able to induce the release patterns that may be necessary for effective osseointegration, vascularization and bone regeneration. Loading 3D scaffolds with different biomolecular agents should produce a codelivery system with different, predetermined release profiles. It is also envisaged that the number of relevant bioactive agents that can be loaded onto scaffolds will be increased, whilst the composite scaffold design should exploit synergistically the different degradation profiles of the organic and inorganic components.

Keywords: bone tissue engineering, composite scaffolds, drug delivery

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Article highlights.

- The use of bioactive glass or hydroxyapatite nanoparticles incorporated into a polymer matrix and the application of bioinspired approaches are being increasingly considered to closely mimic the nanosized features of natural bone.
- Biomolecular agents can be loaded in a scaffold either by attachment or immobilization to/on the scaffold surface or by entrapment within the scaffold, where the affinity of the molecular agent to the biomaterial needs to be modulated to release the agent at the desired rate.
- Most delivery systems applied in bone tissue engineering depend on the diffusion of the molecular agent through the scaffold during delivery, which does not always provide adequate (sustained) release behavior.
- Entrapment of the molecular agent through multilayered loaded polymer coatings onto the preformed scaffold surface or the use of drug loaded nano/microspheres incorporated in the scaffold are useful to control the release rate of the molecular agents or therapeutic drugs.
- The possibility of loading different (two or even more) biomolecular agents in nano/microspheres with different degradation profiles in order to be released at different time frames is being envisaged.

This box summarizes key points contained in the article.

1. Introduction

Tissue engineering and regenerative medicine can potentially extend and improve life of patients who are suffering from tissue loss by providing functional tissue replacements, typically by creating a controlled environment that promotes and directs cell proliferation and new tissue growth [1-10]. The transformation of newly formed tissue into a functioning organ follows a stepwise process with cells responding to different biological signals and stimuli for the different stages of development, such as growth factors, extracellular matrix (ECM) proteins, cell–cell interactions or mechanical stresses. In the particular case of bone tissue engineering (BTE), biocompatible, biodegradable and highly porous scaffolds in a three-dimensional (3D) geometry should create in the first instance sufficient space for new tissue formation. Additionally, the scaffold needs to provide a substrate for cell attachment, proliferation and differentiation, and for supporting new tissue growth. Further, the degradation rate of the scaffold into nontoxic products should ideally match the time required for tissue regeneration [9,11-15]. Thus, the selection of the most suitable scaffold biomaterial should be done considering chemical composition as well as physical structure, mechanical properties, biocompatibility and absence of adverse immune response [5,16,17]. In addition, scaffolds can be useful as matrices for controlled delivery of therapeutic drugs and tissue inductive molecules such as growth factors [18-27]. A growth factor is any intracellular signaling protein (e.g., a cytokine) or steroid hormone which plays a significant role for enhanced cell function and tissue regeneration (e.g., they are involved in cellular growth, proliferation and cellular differentiation processes) [10,12,15,28,29]. For example, bone morphogenic proteins (BMPs) stimulate bone cell differentiation, while fibroblast growth factors and vascular endothelial growth factor (VEGF) stimulate blood vessel formation (angiogenesis) [30-32]. While endogenous signaling molecules are useful to provide signals to trigger the healing process at smaller local injury sites, for larger size defects the delivery of exogenous growth factors is required [15,23-25]. Cells are sensitive to the concentration of growth factors and since growth factors usually have short half-lives, their successful application depends on the efficacy of the delivery technologies [12,15,21,23-25,33].

Therapeutic drugs for the treatment of bone diseases with local drug delivery have several advantages compared to systemic administration to minimize side effects and risk of overdose, as well as to improve the bioavailability of the drug with the appropriate therapeutic concentration effectively reaching the target site [21,22,34]. Further, the competition between the integration of the scaffold with the surrounding tissue and adhesion of bacterial with subsequent biofilm formation, onto its surface upon implantation might be prevented with the local release of antimicrobial agents [22,35-37]. The major challenge of the next generation of scaffolds for BTE thus lies in presenting the appropriate combination of mechanical support and morphological guidance for cell proliferation and attachment while at the same time serving as matrices for sustained delivery of therapeutic drugs and/or biomolecular signals, which may need to vary temporally and spatially (**Figure 1**) [12,21,38-40]. In addition, scaffolds have to be suitable for easy sterilization without either loss of mechanical function or denaturation of the incorporated drug or biomolecules.

3D composite scaffolds for BTE have been fabricated from a range of biodegradable natural, synthetic and biotechnology-based materials where cell infiltration is usually supported by a network of pores to enable the initial transport of oxygen and nutrients, removal of metabolic waste and degradation products, as well as cell migration and cell–cell interactions [1-10,41,42]. In addition, porosity and pore structure determine the scaffold mechanical properties [5-9]. Indeed 3D scaffolds should be a bridge between standard two-dimensional (2D) *in vitro* culture systems and the native 3D *in vivo* environment [43-46]. The desired characteristics of traditional scaffolds for BTE and their fabrication technologies have been described in several review articles (e.g., in Refs. [5,8,10,15,29,47]). While the design aspects of the scaffold can influence tissue formation, the addition of biomolecular agents such as growth factors or therapeutic drugs can promote the desired cellular response needed to accelerate the formation of functional tissue, as stated above, and/or to treat a disease or infection locally. Moreover direct delivery of a biomolecular agent from the scaffold should enable protection against extracellular

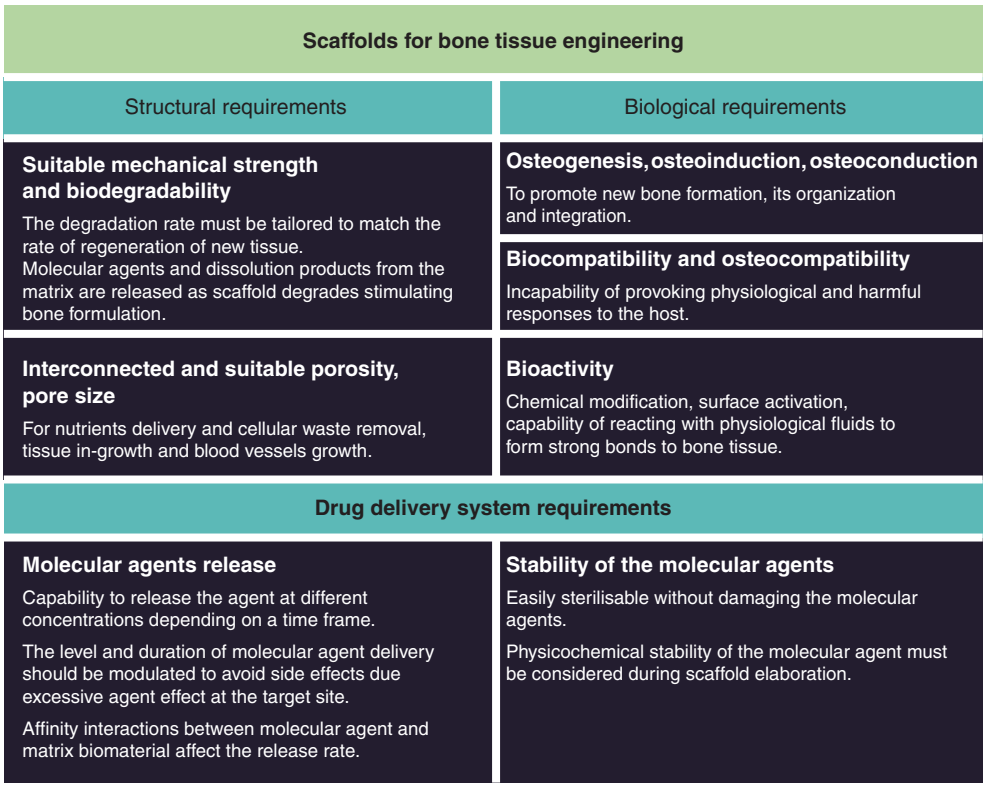

Figure 1. Structural and biological requirements for bone tissue engineering scaffolds incorporating a drug delivery function.

barriers that could reduce their therapeutic efficacy by shielding them from being attacked by immune responses [48]. Additionally, scaffold-based delivery systems should have the capability to release sustained effective levels of the biomolecular agent for prolonged periods of time at the local site, which would compensate drug lost due to clearance or degradation.

Drug delivery from most biomaterial systems likely occurs through a combination of drug interactions with the matrix and its subsequent release, thus the biomaterials employed in the design of the matrix must be rationally selected to regulate these interactions [49-55]. In this context, drug delivery from BTE scaffolds represents a versatile approach to manipulating the local environment for directing cell function and/or to treat common bone diseases, such as osteoporosis, or local infection. In addition, the *in situ* controlled and timely release of antimicrobial agents in conjunction with the degradation of the scaffold should prevent infection or biofilm adhesion to the scaffold surface while simultaneously inducing bone formation [29]. In BTE, the controlled delivery of growth factors is proposed to either increase the expression of tissue inductive factors or to block the expression of others factors that could inhibit bone tissue formation [23-25]. The primary challenge is to achieve sustained and tailored spatial localization of delivery. Research at the interface of biomaterials, drug delivery and pharmacology is focused on the identification of design parameters for the drug delivery system as well as the biomaterial itself. Indeed in selecting the best strategy for delivery i) therapeutic drugs for local treatment of bone diseases and/or ii) growth factors to promote bone formation, multiple aspects must be considered. These include physicochemical requirements and stability of the biomolecular agent (therapeutic drug or growth factor) at the conditions used for scaffold fabrication as well as its desired concentration at the local site and rate of release. The level and duration of the biomolecular agent delivery may need to be modulated to avoid side effects resulting from excessive biomolecular activity at the target site [21-23,38,40,56]. Further, in particular situations, it may be useful for the delivery of the molecular agent to occur in time varying concentrations which should be modulated by the delivery system. Generally, low-affinity interactions between biomolecular agents and matrix biomaterial will increase the release rate, while increasing the affinity can be effective to reduce the delivery rate and to retain the molecular agent within the scaffold.

Popular systems being increasingly investigated for BTE scaffolds are based on suitable combinations of biodegradable polymers and bioactive ceramics and glasses [8,9,47]. These systems include composites as well as organic–inorganic hybrid systems [26,27,42]. Frequently, these composite and hybrid scaffolds are designed to serve also as local drug and/or growth factor delivery devices following the considerations stated above. The following sections address the current capabilities

and identify opportunities and challenges for the application of localized drug delivery using 3D composite scaffolds for BTE and provide an overview of published studies on the development and applications of such scaffolds. A detailed summary of drugs included in composite scaffolds and the several approaches developed to combine the different types of polymers with bioceramics in composites for drug delivery systems in the context of BTE is presented. Sections 2 and 3 discuss several approaches adopted to develop composite BTE scaffolds with drug/biomolecular agent delivery capability. These sections are intended to provide a few relevant examples, but not to exhaustively list all investigated systems belonging to each category. In addition, only 3D prefabricated scaffolds are considered while injectable systems (which are also available, e.g., [57]) are not covered in order to limit the scope of the review. Section 4 summarizes the advances in the field and identify existing challenges while in Section 5 (Expert opinion), the developments that the authors consider likely to be important in the future are discussed as well as avenues of research are suggested to expand as further studies yield more detailed results.

2. Composite scaffolds for bone tissue engineering

2.1 Biomaterials selection and scaffold design

Biomaterials play an essential role in BTE to provide 3D templates and synthetic extracellular-matrix environments for tissue regeneration as outlined above. Although the majority of biomaterials investigated as BTE scaffolds are single component, usually either biodegradable organic polymers or inorganic bioceramics, their main drawback is that one single component usually is not able to meet all the requirements for a successful BTE scaffold [8,9,11-16]. Bone tissue is indeed a natural organic–inorganic composite material consisting of collagen and mineral phase (apatite) which exhibits an excellent balance between strength and toughness, superior to either of its individual components [16]. In this sense, metals such as titanium and its alloys, which are excellent candidate materials for medical implants due to their superior mechanical properties [58], are not generally suitable as BTE scaffolds because of their lack of biodegradability [59]. Bioceramic materials (amorphous or crystalline) such as hydroxyapatite (HAP) and resorbable calcium phosphates, e.g., tricalcium phosphate, and bioactive glasses are all bioactive (surface active) and offer good osteoconductivity however their application as 3D porous scaffolds is usually limited due to their intrinsic brittleness and low fracture strength [60]. These limitations of pure bioceramics have thus led to intense interest in the development of polymer-bioceramic composites which mimic the native bone structure to some degree [8,9,42,61-64]. Composite scaffolds composed of biodegradable polymers (either natural, synthetic or biotechnology designed) and bioactive ceramic materials can benefit from the advantages of both material phases and balance their individual disadvantages considering that the composite composition and microstructure can be tailored to the specific needs [8,9,16,59,65-68]. The inorganic component of BTE scaffolds, such as HAP, calcium phosphate or bioactive glass, is responsible for the enhanced osteoconductivity, while the biodegradable polymer matrix provides the continuous and tough structure incorporating high porosity and high surface area [59]. In addition, biomimetic approaches for example involving self-assembled HA–collagen composites [42] and the application of responsive matrices, e.g., employing magnetic phases [41] and stimuli-responsive (e.g., pH-, thermosensitive) intelligent polymer matrices [68,69] are being continuously explored to add functionality to the scaffolds.

Beyond the selection of the biomaterials (polymer matrix and bioceramic), the development of composite scaffolds involves the optimization of volume fraction, size and shape of the inorganic phase, establishment of a suitable bonding at the polymer–bioceramic interface and design of porosity and pore structure. Nanoscale particulate bioactive glasses and nano-HAP are being increasingly considered in BTE composite scaffolds to closely mimic the nanosized features of natural bone, and promising results in terms of improving the mechanical properties and enhancement of protein adsorption over micronsized bioceramic/polymer scaffolds have been achieved [70,71]. Nanoscale particulate bioceramics exhibit also advantages over their conventional (micronsized) counterparts due to the possibility to induce nanotopographic surface features in composite materials in addition to their larger surface area, enhanced solubility and higher bioreactivity [70-72]. Moreover, mesoporous bioactive glasses are also very promising biomaterial candidates for developing multifunctional scaffolds with drug delivery capability [73-75]. On the other hand, it is well known that a wide range of biodegradable polymers, both of natural and synthetic origin and exhibiting different degradation rates, are available for BTE, such as alginate, chitosan, collagen, poly(lactic acid) (PLA), poly(lactide-*co*-glycolide) (PLGA) among many others [20]. A number of techniques have been developed for the fabrication of porous polymer/bioceramic composite scaffolds which usually need to be adapted if a therapeutic drug or bioactive molecule must be incorporated. The main concern associated with the incorporation of either therapeutic drugs or growth factors into scaffolds is the possible degradation during scaffold manufacture [15,22]. Commonly used processing techniques for 3D scaffolds are blending and phase separation techniques, freeze-drying, foam coating, solvent casting/particulate leaching/evaporation and thermally induced phase-separation. Biomimetic approaches are also being increasingly considered [42]. In addition, computer-aided scaffold design strategies are receiving continuous attention and are additive manufacturing technologies have been shown to be attractive to produce 'designed' porous scaffolds for BTE [76-79]. Microfabrication techniques, such as 3D printing and similar additive methods, are being developed to create 3D scaffolds with controllable feature sizes and

patterned topography [46,78-80]. Merging these scaffold technologies with drug delivery function is attractive to enable these patterning and microfabrication strategies to become methods of choice for fabricating next generation multifunctional BTE scaffolds.

2.2 Drug incorporation

When a biomolecular agent is loaded into a 3D scaffold, both high encapsulation efficiency as well as a sustained release rate allowing a therapeutic dose during a desired time frame are needed. Biomolecular agents can be loaded in a scaffold either by attachment to the scaffold surface or by entrapment within the scaffold. Thus biomolecular agent incorporation into 3D scaffolds for BTE generally occurs according to three basic approaches (**Figure 2**):

1) Pre-encapsulation of the agent (e.g., using micro- or nanospheres) followed by loading of the encapsulating system into the scaffold, which has been shown to be relatively effective in retaining the bioactivity of various therapeutic drugs [53].
2) Surface immobilization of the agent by nonspecific mechanisms such as hydrophobic, electrostatic or van der Waals interactions, whereby these nonspecific bindings depend on composition of both the biomolecular agents (e.g., protein, sugar, lipid, polymer) and the biomaterial, as well as on swelling ratio and density of the biomaterial and relative quantity of functional groups present in each component.
3) Specific interaction, which may be introduced through the incorporation of functional groups on the molecular agent or in the biomaterial to achieve a better control of the binding as well as the incorporation of the molecular agent within a micro/nanocarrier [48].

The extent of these molecular interactions as well as the influence of the environmental conditions, such as pH and ionic strength, will dictate whether the biomolecular agent will be bound or released. In addition, for the three cases, the molecular agent can be loaded directly onto the surface of the prefabricated 3D scaffold, thus avoiding degradation of any active substance through for example a high temperature process. In all cases, the affinity of the molecular agent to the biomaterial needs to be modulated to release the agent at the desired rate. Most delivery systems applied in BTE depend on the diffusion of the molecular agent through the scaffold during delivery, which does not always provide adequate (sustained) release behavior. Diffusion-based delivery systems have decreasing rates of drug release with time. Among the basic procedures available to load biomolecular agents into scaffolds the following can be considered: i) the direct addition of the drug into a polymer solution or emulsion used to fabricate scaffolds [81,82], ii) the straightforward immersion of a prefabricated scaffold into a drug-containing solution for drug adsorption onto the surface of the scaffold, iii) a more sophisticated version of ii), which involves soaking the prefabricated scaffold into a drug-containing polymer solution in order to get a polymer coating with better ability to control the drug release [10,83].

Even though these techniques can achieve certain slow release characteristics, the control over the release kinetics may be limited, eluting the greatest percentage of the loaded molecular agent very fast in a characteristic "burst release" manner. This behavior makes difficult to establish a suitable therapeutic release profile for a given drug, leading also to concerns over long-term complications associated with the high dosage of released drug [15,84]. Even though in certain cases a fast release of high doses of a certain drug or biomolecular agent might be needed, the 'correct therapeutic dose' needs to be determined by dedicated clinical studies. It is important to note that there is a need of clinical information regarding the effective level of doses for the majority of the biomolecular agents, particular growth factors, currently investigated for BTE. Some investigations have considered the dissolution of the matrix itself (the multifunctional scaffold, which, in the context of the systems considered in this review, is a biopolymer or hydrogel incorporating inorganic fillers) as an element to control drug release. Of particular interest is the possibility to load charged molecular agents on scaffolds with alternating layers of charged polymers and contra-ion charged agents. In this manner, the duration of drug release can be tuned through changing the degradability or electrochemistry of the polymer layers [16,85]. Further, multilayered scaffolds have the potential capability, with the appropriate design of each layer, to release multiple molecular agents in sequential manner. Methods for surface immobilization have been developed for growth factors as well as modification of growth factors by conjugation to water-soluble carriers [48,86]. In particular, supercritical fluid (SCF) processing [40,87-89] is attractive for drug delivery, since it does not require the use of solvents which could adversely affect a loaded drug or growth factor. Drugs and bioactive molecules can also be directly incorporated into electrospun nanofibers [15,90-95]. Further, it is possible to incorporate the drug into a nanofiber by using coaxial electrospinning wherein a secondary polymer solution containing the drug is electrospun within the core of the forming nanofiber surrounded by a shell polymer [15,96,97]. In addition, electrospraying has emerged as an effective technique for the formation of microparticles for drug delivery [95,98].

It is important to note that in BTE, the scaffold needs to provide structural support to the newly formed tissue which often requires slow degradation of the matrix to warrant mechanical integrity during tissue regeneration [15,99,100]. Conventional bone scaffold materials are, thus, at least at a first view, not optimal for direct loading of biomolecular agents since their release will only be possible through the degradation of the scaffold matrix, which should be relatively slow. In this context, the biomolecular agent entrapped within the scaffold will be released when the scaffold starts to

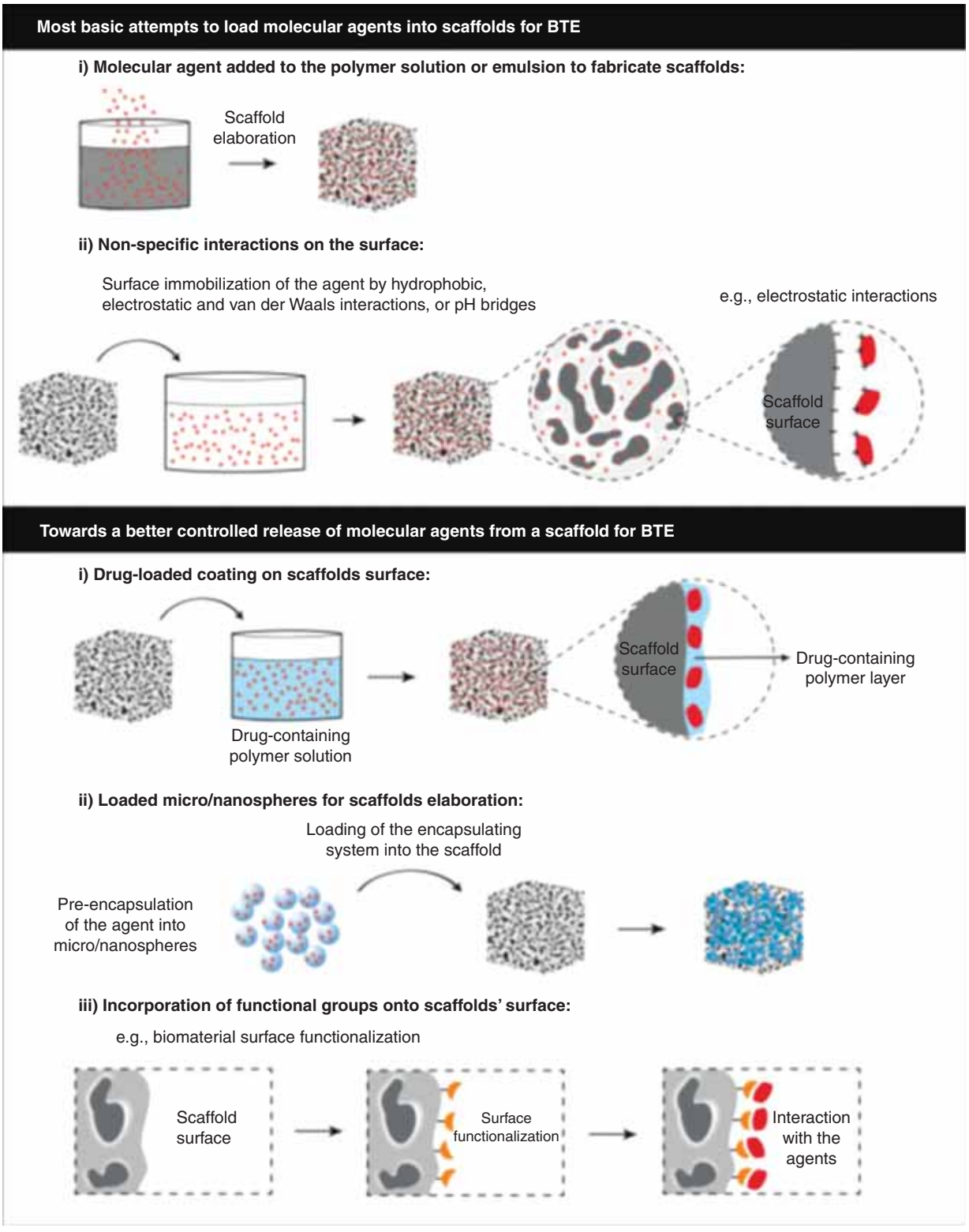

Figure 2. Overview of processing approaches to develop BTE scaffolds with drug delivery ability.

degrade, that is, also losing its mechanical integrity. Thus the drug release mechanism must be decoupled from the intrinsic scaffold degradation kinetics. Some investigations have combined the incorporation of the biomolecular agent within the scaffold with the adsorption of the agent onto the scaffold surface; however, the pattern of release rate obtained (an initial burst release followed by a very slow release rate) may not be necessarily clinically useful for the combination of therapeutic drugs or growth factors studied. Advances in this task can be reached by the entrapment of the molecular agent through multilayered loaded polymer coatings onto the preformed scaffold surface or by the use of loaded nano/microspheres which are incorporated in the scaffold [101,102]. Thus, in order to maximize efficiency of a scaffold for BTE, the loaded biomolecular agents should preferentially be readily and constantly released whilst the scaffold can still provide the needed structural support. A common technique employed to sustain the release of a molecular agent from scaffolds is through encapsulation in nano/microspheres and hydrogels. These approaches, and particularly nano/microspheres to be loaded into a preformed scaffold, provide interesting composite alternatives since the encapsulation system and the scaffold can be made from different biomaterials: i) a biodegradable polymer for the drug encapsulation system to provide faster degradation and sustained release of the entrapped drug, and ii) a composite biomaterial with lower degradation rate and robust mechanical properties for the manufacture of the scaffold to closely fit the requirements for BTE. Moreover encapsulation creates a physical barrier to prevent the ability of a molecular agent to diffuse away until the encapsulation system (generally a degradable biopolymer network) has been sufficiently degraded. Thus, by modifying the composition of the encapsulation system, the degradation rate of the system and the subsequent release rate of the entrapped drug can be tuned to a certain extent in order to maintain a therapeutic dose for the required period of time [103]. Further, drug loading into an encapsulation system allows a better control of drug release, since the system can be studied and optimized independently before incorporation into the scaffold [15,103-106]. Additionally, it can be envisaged the possibility of loading different (two or even more) biomolecular agents in nano/microspheres with different degradation profiles in order to be released at different time frames [22,107].

3. Multifunctional BTE composite scaffolds

3.1 Natural polymer-based composite scaffolds

Natural polymers are obtained from natural sources and possess attractive properties such as biocompatibility and degradability, being generally easily solubilized in physiological fluids. The most common natural polymers being considered in BTE are collagen, gelatin, chitosan, fibrin, silk and hyaluronic acid [108-110]. The combination of natural polymers and inorganic phases is receiving increasing attention for BTE scaffolds with drug delivery capability and representative examples of such composite systems are presented in **Table 1**.

3.2 Synthetic polymer-based composite scaffolds

Synthetic polymer-based composite scaffolds usually incorporate inorganic bioactive fillers such as HAP or bioactive glasses in biodegradable synthetic matrices [8,47] and some representative examples are included in **Table 1**.

3.3 Biotechnology-based polymer composite scaffolds

Bacteria-derived polymers are gaining importance in tissue engineering and drug delivery because of their biodegradability and biocompatibility [111-113], in particular, polyhydroxyalkanoates (PHAs), which are accumulated as metabolites in a wide variety of bacterial species like *Pseudomonas* sp., *Bacillus* sp., *Ralstonia* sp., *Aeromonas* sp., *and Rhodobacter* sp. PHA are produced when bacteria are grown under nutrient limiting conditions [114] and range in properties from being stiff and brittle to elastic and stretchable [115]. One of the most common and well characterized, stiff and brittle PHA is poly (3-hydroxybutyrate) P(3HB) [116]. Despite the great promise these polymers offer for tissue engineering applications and for development of composites [113], very little has been published regarding their use in the context of multifunctional BTE composite scaffolds with drug delivery capability, typical examples are shown in **Table 1**.

4. Conclusions

The application of BTE composite scaffolds (and organic–inorganic hybrids) with added drug delivery function, combining biodegradable polymers (natural, synthetic and biotechnology based) with bioceramics has been emerging as an attractive approach in recent years. The combination of controlled release technology and composite scaffolds is likely to produce new delivery methods for various biomolecular agents (and their combinations) that are essential for tissue engineering. The potential of drug delivery using composite scaffolds spans further than bone tissue engineering to other regenerative medicine applications. Nevertheless, it is important to highlight that the clinical effectiveness of most of the approaches presented in literature remains to be determined and further studies are required such as long-term assessment of scaffold behavior in relevant tissue environments involving validated and standardized *in vivo* experiments. Further, several of the novel composites used to fabricate BTE scaffold prototypes must be also investigated in long-term *in vivo* studies or using bioreactors. Thus, the ultimate clinical impact of composite scaffolds for BTE might depend not only on the fine tuning of variables that determine the controlled biomolecule or drug delivery process, but also on the ability to develop bioactive composite scaffolds that fulfill all BTE requirements. The development

Table 1. Examples of three-dimensional composite scaffolds for bone tissue engineering with drug delivery capability.

Scaffold composition	Type of study	Agent delivered	Type of agent delivered	Refs.
Natural polymers				
Bioactive glass porous scaffold coated with alginate	*In vitro*	Gallium	Antimicrobial	[117]
Porous matrix of β-TCP/CP/chitosan	*In vitro*	Gentamicin	Antimicrobial	[121]
Porous scaffold of chitosan/HA	*In vitro*	Tetracycline	Antimicrobial	[122]
β-TCP/agarose porous scaffold	*In vitro*	Vancomycin	Antimicrobial	[123]
Gelatin/β-TCP porous scaffold	*In vivo*	Vancomycin	Antimicrobial	[124]
Starch/PLA porous scaffold	*In vitro*	Dexamethasone	Inductive effect in osteogenic culture	[125]
Synthetic polymers				
PLA–dx–PEG copolymer matrix	*In vivo* (repair of 2-mm bone defects)	rhBMP2	Growth factor	[126]
PLGA/HA composite fibrous scaffolds	*In vitro/in vivo*	rhBMP2	Growth factor	[127,128]
Nano zeolite/PEG/poly acrylic acid/polyacrylamid	*In vitro*	Amoxicillin	Antimicrobial	[129]
EC microspheres in a porous matrix of HA/PU	*In vitro*	Ceftazidime	Antimicrobial	[130]
A porous matrix of HA/β-TCP/PLA	*In vitro/in vivo*	Ciproflozacin	Antimicrobial	[131]
PLGA microspheres in a porous construct of PMMA/CMC	*In vitro*	Colistin	Antimicrobial	[132]
Porous matrix of β-TCP/PCL	*In vitro/in vivo*	Gatifloxacin	Antimicrobial	[133]
PLGA microspheres in a porous matrix of HMS-HA	*In vitro*	Gentamicin	Antimicrobial	[132]
HA/PCL porous matrix	*In vitro*	Tetracycline	Antimicrobial	[83]
HA/PCL porous matrix	*In vitro*	Vancomycin	Antimicrobial	[101]
PDLLA/BCP/alginate porous matrix	*In vitro*	Vancomycin	Antimicrobial	[134]
Porous matrix of PCL/chitosan/nanoclay/β-TCP	*In vitro*	Doxorubicin	Antibiotic/antitumoral	[135]
PLGA nanoparticles in a porous matrix of HA	*In vitro/in vivo*	Dexamethasone	Inductive effect in osteogenic culture	[136]
Porous matrix of bioactive glass/MCM-41	*In vitro*	Ibuprofen	Anti-inflammatory	[137]
Porous matrix of PCL/HA	*In vitro*	Clodronate	Inhibition of the osteoclastic resorption	[138]
Microspheric scaffold of PLGA/HA	*In vitro*	Alendronate	Inhibition of the osteoclastic resorption	[139]
Microspheric scaffold of poly (L-lactide-*co*-epsilon caprolactone)/bioactive glass	*In vitro*	Alendronate	Inhibition of the osteoclastic resorption	[140]
Bacteria derived polymers				
Porous calcium phosphate immersed in a drug containing aqueous solution and a P(3HB) coating afterwards	*In vitro*	Tetracycline	Antibiotic	[141]
P(3HB) microspheres in a Bioglass® scaffold	*In vitro*	Gentamicin	Antibiotic	[102]

β-TCP: β-tricalcium phosphate; BCP: Biphasic calcium phosphate; BMP: Bone morphogenetic protein; CMC: Carboxymethylcellulose; CP: Calcium phosphate invert glasses; CPA: Calcium phosphate-deficient apatite; ES: Ethyl cellulose; HA: Hydroxyapatite; HMS: Mesoporous silica; MCM-41: Type of mesoporous sílica; P(3HB): Poly(3-hydroxybutyrate); PCL: Poly(ε-caprolactone); PDLLA: Poly(D,L-lactic acid); PEG: Poly(ethylene glycol); PLA: Poly(L-lactic acid); PLGA: Poly(lactide-*co*-glycolide); PMMA: Polymethylmethacrylate; PU: Polyurethane.

of bioinspired concepts for scaffold manufacturing and the application of functional (e.g., stimuli-responsive) matrices are promising approaches in this regard. This multidisciplinary field will further expand with more intensive cooperation among material scientists, pharmaceutical technologists, cell biologists and clinicians in order to identify clinical needs while developing feasible solutions for improving patients' healthcare and quality of live [117]. It is clear that these dual function composite scaffold systems have substantial advantages when compared to traditional scaffolds, as reviewed in this paper, and future progress in BTE approaches will benefit from the further optimization of the functionality and properties of these systems.

5. Expert opinion

The next major challenge for drug releasing scaffolds for BTE is achieving localized drug release in the rate required for tissue regeneration. There is a significant difference between administration of single biomolecular agents and the coordinated delivery of them, temporally and spatially, that is required during tissue regeneration. Indeed the synthesis of

a multidrug releasing scaffold for BTE is challenging. The regeneration of a functional tissue occurs in a series of steps with each step potentially involving different biological signals. Thus a next major challenge for developing composite scaffolds for BTE is the incorporation of a drug delivery function of sufficient complexity to be able to recreate the patterns that may be necessary for an effective osseointegration and bone regeneration. There is still a lack of substantial progress in the clinical application of BTE scaffolds mainly due to insufficient vascularization, which limits the survivability of cells particularly when dealing with large bone defects. Other issues still pending solution are the lack of time-dependant adequate mechanical integrity in the long-term and the susceptibility to infection over long term implantation of the scaffold [21,22,29,117]. The mechanical properties of scaffolds can be improved by tailored combination if biopolymers and inorganic fillers in optimized composite scaffolds while issues like insufficient vascularization and susceptibility to infection should be addressed by the implementation of controlled drug delivery systems within the scaffold, as discussed in this article. Further, the possibility of processing such dual purpose 3D bioactive scaffolds will also enable their usage in more sophisticated concepts such as the loading with different biomolecular agents to produce a co-delivery with different release profiles. In this context, bioinspired approaches and the incorporation of extra functionalities and responsiveness, e.g., magnetic properties, pH/thermo sensitivity, to develop "smart" scaffolds will be relevant. In addition, another interesting possibility is to exploit metallic ions (bioinorganics) release from the inorganic component of the composite scaffold to achieve specific cellular responses in synergy with the added molecular agent or drug [118-120]. Overall, the efficiency of the loading/encapsulation technique, the release rate of the molecular agent/s from the encapsulation system and the rate of clearance are key variables to be considered when designing encapsulation systems within the composite scaffold. With improved understanding of controlled-release mechanisms and further development of technologies to fabricate multifunctional composite scaffolds for BTE, it may be possible to increase the number of relevant bioactive agents that can be loaded onto them, some of them probably not even developed yet as full drug products.

Declaration of interest

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