Biomaterials Translational

Segmental long bone regeneration guided by degradable synthetic polymeric scaffolds

*Xiaowen Xu, Jie Song**

Key Words:
3D printing; bone grafting; critical-size defect; hydrogels; long bone segmental defect; synthetic degradable polymers

From the Contents

ABSTRACT

Recent developments in synthetic bone grafting materials and adjuvant therapeutic agents have opened the door to the regenerative reconstruction of critical-size long bone segmental defects resulting from trauma, osteoporotic fractures or tumour resections. Polymeric scaffolds with controlled macroporosities, degradability, useful surgical handling characteristics, and the ability to deliver biotherapeutics to promote new bone ingrowth have been developed for this challenging orthopaedic application. This review highlights major classes of degradable synthetic polymers and their biomineral composites, including conventional and amphiphilic polyesters, polyanhydrides, polycarbonates, and polyethylene glycol-based hydrogels, that have been explored for the regenerative reconstruction of critical-size long bone segmental defects over the past two decades. The pros and cons of these synthetic scaffold materials are presented in the context of enabling or impeding the functional (mechanical and radiographic) repair of a long bone segmental defect, with the long bone regeneration outcomes compared with healthy long bone controls or results achieved with current grafting standards.

Department of Orthopaedics & Physical Rehabilitation, University of Massachusetts Medical School, Worcester, MA, USA.

***Corresponding author:**
Jie Song,
Jie.Song@umassmed.edu.

http://doi.org/10.3877/cma.j.issn.2096-112X.2020.01.004

How to cite this article:
Xu, X.; Song, J. Segmental long bone regeneration guided by degradable synthetic polymeric scaffolds. *Biomater Transl.* **2020**, *1*(1), 33-45.

Introduction

Despite the inherent healing capacity of bone, regenerative reconstruction of critical-size long bone segmental defects (LBSDs) resulting from traumatic injuries, osteoporotic fractures among the elderly, or tumour resections remains a formidable clinical challenge due to inadequacies of existing bone grafting technologies.[1-6] Gold standard cancellous autografts retrieved from the non-weight-bearing region of the patient's own skeleton (e.g. iliac crest, ribs) are known for rapid resorptions *in vivo* and often result in poor unions in LBSD reconstruction.[7] Although autologous cortical bone grafts (e.g. fibular segments) can achieve higher union rates and superior mechanical strength restoration in LBSD reconstruction,[8] their limited availability and associated donor site morbidity present major hurdles for widespread clinical use.[9] Meanwhile, devitalized allogenic long bone grafts harvested from donor cadavers, although less limited by supplies, are known for notoriously high failure rates when used for LBSD reconstruction due to their structural instability and poor vascularization.[10, 11] For instance, 18% and 46% of traumatic long bone injury patients in a retrospective study experienced structural allograft failures in the first 3 years and the longer term, respectively.[12] These limitations associated with autografting and allografting have driven the demands for viable synthetic bone graft alternatives.[13]

Current clinically-used synthetic bone graft substitutes such as bioceramics, collagen sponges/hydrogels, demineralized bone matrix paste/putty, or their coarse combinations[14, 15] are known for their tendency to break and their inadequate/inconsistent *in vivo* performances. For LBSD repair, auxiliary metallic mesh cages are often used in addition to conventional fixations to help locally retain these mechanically-inferior formulations within the defect.[16, 17] In addition, the slow resorption of

calcium apatite-based ceramic grafts, known to persist for years *in vivo*,[18] presents a hurdle to the timely restoration of the mechanical integrity of the long bone.[19] Although human recombinant bone morphogenetic proteins 2 and 7 (rhBMP-2 and rhBMP-7) have been clinically used to improve bone graft osteointegration and are approved by the United States Food and Drug Administration for certain indications,[20-22] the supraphysiological clinical doses (e.g. milligrams scale) required are associated with local or systemic complications ranging from ectopic bone formation to death.[23-25] Overall, synthetic bone grafts combining attractive surgical handling characteristics, structural stability, suitable degradability and safe doses of biotherapeutics expediting osteointegration are still lacking for limb salvage applications.

Recent progress in understanding the cellular and molecular processes governing long bone regeneration has offered new clues for the design of next-generation synthetic bone grafts. LBSDs are known to disrupt multiple tissue compartments including the bone, bone marrow, periosteum, endosteum, vasculature and surrounding muscles and nerves. Accordingly, its regenerative healing is governed by tightly-orchestrated signalling pathways involving a large number of cells (e.g. immune cells, blood cells, mesenchymal and hematopoietic stem cells, musculoskeletal cells), starting with acute inflammation and ending with the remodelling of the regenerated bone.[6] Synthetic bone grafts implanted into LBSDs directly interact with the myriad of endogenous cells recruited to the defect site, impacting their cross-talk during the dynamic competition between the processes of tissue regeneration *vs.* degeneration. Strategies for modulating immune responses, osteogenesis, vascularization, and bone remodelling through the manipulation of biomaterial hydrophilicity, surface charge, microroughness, porosities[26] and/or temporally-controlled delivery of biotherapeutics[6, 26] have been actively explored. Varied successes of these approaches, although not always generalizable across a broad spectrum of biomaterials, point to a broad range of means to augment the performance of synthetic bone grafts for LBSD reconstruction.

The past two decades have also witnessed rapid developments and popularization of a range of rapid prototyping/three-dimensional (3D) printing technologies, particularly fused deposition modelling and bioprinting, for the fabrication of biomaterial scaffolds to guide tissue regenerations.[27, 28] Compared to more conventional porous scaffold fabrication techniques such as gas foaming, particulate leaching, thermally-induced phase separation, freeze drying and freeze casting,[27, 28] these 3D-printing techniques have the distinct advantage of precise spatial and geometrical controls over scaffold micro/macro-porosities and, in the case of bioprinting, co-delivery of biotherapeutics/cells. Combined with electrospinning[29] and 3D weaving,[30, 31] these enabling tools have made it possible to recapitulate complex mesoscale structural features of skeletal tissues in biomimetic synthetic bone grafts to promote vascularization, osteointegration and effective bone remodelling.

In this review, we highlight recent synthetic bone grafts fabricated from degradable synthetic polymer/mineral composites, particularly polyesters, polyanhydrides, and polycarbonates, as well as degradable poly(ethylene glycol) (PEG)-based hydrogels for the regenerative repair of critical-size LBSDs (**Table 1**). An electronic search of Google Scholar, PubMed, Medline, EMBASE, and Cochrane Library for literature describing "critical-size long bone defect regeneration", published in English between 2000 and 2020, was performed. The results were then screened by title and abstract to only include those involving degradable synthetic polymers. Finally, we further narrowed down the list by excluding animal studies that employed neither proper controls nor quantitative outcome measures. Some of these grafts were fabricated by 3D printing and will be pointed out accordingly. Whenever possible, the guided bone regeneration outcomes including the radiographic union, degree of new bone formation, synthetic graft degradation/resorption, and the functional properties of regenerated bone are compared to those achieved with current grafting standards or healthy controls. Although angiogenesis is known to be tightly coupled to osteogenesis and an important parameter of functional bone regeneration,[32] the highly varied (or lack of) quantitative assessments of angiogenesis in most studies makes head-to-head comparisons difficult. Accordingly, we choose to focus on restoration of the mechanical properties of the regenerated long bone as a key indicator of functional long bone regeneration as its success requires sufficient vascularization and integration with the host bone. The varied successes and limitations of these synthetic bone grafts will be appreciated from their physiochemical properties, degradation characteristics and the dose of their integrated biotherapeutics. Most examples highlighted involve the delivery of osteogenic proteins and peptides rather than exogenous therapeutic cells that might represent a higher translational barrier in terms of regulatory approvals.

Biodegradable Synthetic Polymer-Based Composites for Critical-Size Long Bone Segmental Defect Regeneration

According to the definition of the American Society for Testing and Materials, biodegradability refers to the susceptibility of a material to be decomposed into carbon dioxide, methane, water, and/or inorganic compounds as well as biomass.[33] Here we focus on synthetic biodegradable polymers capable of undergoing decomposition in humans and vertebrate animals into fragments that can be further metabolized and readily removed from the body through natural pathways (e.g., excretion or metabolism).[34] The most commonly-used biodegradable polymers for orthopaedic applications include polyesters (conventional and amphiphilic polylactides, poly(propylene fumarate) (PPF)), polyanhydrides, and polycarbonates. Here we discuss recent applications of synthetic bone grafts composed of these degradable polymers and other structural additives such as the osteoconductive biominerals hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP) for the regenerative repair of LBSDs.

Polyester-based composite bone grafts

Conventional polylactide-based composites

Polyesters remain the most popular and widely used biodegradable polymers for medical uses.[35] Of them, polylactides are the most extensively investigated, with poly(lactic acid) (PLA), polyglycolic acid, poly(D,L-lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL) and their copolymers cleared by the United States Food and Drug Administration for various medical applications

Table 1. Degradable synthetic polymeric scaffolds for long bone segmental defects

Graft composition	Animal	Segmental defect	Therapeutics	Regeneration outcomes	Limitations
3D printed PCL/β-TCP composite	Sheep[44]	3 cm tibial	3.5 mg rhBMP-7	Radiographic union; mechanical restoration	Slow graft resorption
PLGA microparticles	Sheep[38]	2.5 cm femoral	4-mg rhBMP-2	Radiographic union (no mechanical testing)	Tendency of PLGA breakage
PLGA-coated gelatine sponge	Dog[39]	2.5 cm tibial	0.4 mg/mL rhBMP-2	Radiographic union; mechanical restoration	Small sample size; Bone resorption
Porous PLA-PEG/HAp	Rabbit[46]	1.5 cm radial	5–20 μg rhBMP-2	Radiographic union (no mechanical testing)	Slow graft resorption
PLA-DX-PEG/b-TCP	Rabbit[47]	1.5 cm femoral	50 mg rhBMP-2	Radiographic union; mechanical restoration; full graft resorption	Graft distortion within defect
3D-printed PELGA/HAp	Rat[54]	5 mm femoral	400 ng rhBMP-2/7	Facile & stable graft fixation; rapid union, full graft resorption & mechanical restoration	Larger animal translation unknown
Solid PPF rod/porous sleeve with PLGA microparticle	Rat[60]	5 mm femoral	2–8 μg rhBMP-2	Improved defect fixation by solid rod; improved bone formation	Regeneration impeded by solid rod; no union
Crosslinked PPF/PPF diacrylate with PLGA microparticle	Rabbit[61]	1.5 cm radial	200 μg TP508	Improved osteointegration	No union; slow graft resorption
Salicylic acid-based poly(anhydride-ester)/ PCL membrane	Rat[65]	5 mm femoral	12 μg rhBMP-2	Ectopic bone formation suppressed; long bone regeneration improved (no mechanical testing)	Poor graft mechanical property; long-term remodelling unclear
Tyrosine-derived polycarbonate/CP	Rabbit[99]	1.5 cm radial	17–35 μg rhBMP-2	Improved bone formation (no mechanical testing)	No union
pHEMA-HAp composite	Rat[116]	5 mm femoral	400 ng rhBMP-2/7	Radiographic union; mechanical restoration	Slow graft resorption
MMP-sensitive 4-arm PEG hydrogel with integrin binding GFOGER	Murine[126]	2.5 mm radial	30 ng rhBMP-2	Radiographic union; mechanical restoration; MMP-responsive degradation	Potentially high manufacturing cost

Note: 3D: three-dimensional; CP: calcium phosphate; DX: p-dioxanone; GFOGER: α2β1 integrin-specific hexapeptide sequence Gly-Phe-Hyp-Gly-Glu-Arg; HAp: hydroxyapatite; MMP: matrix metalloproteinase; PCL: polycaprolactone; PEG: poly(ethylene glycol); PELGA: poly(lactic-co-glycolic acid)-b-poly(ethylene glycol)-b-poly(lactic-co-glycolic acid); pHEMA: poly(2-hydroxyethyl methacrylate); PLA: poly(lactic acid); PLGA: poly(D,L-lactic-co-glycolic acid); PPF: poly(propylene fumarate); rhBMP: recombinant human bone morphogenetic protein; TP508: Chrysalin, a 23-amino acid peptide representing amino acids 508–530 of human prothrombin; β-TCP: β-tricalcium phosphate.

ranging from resorbable sutures, drug delivery formulations to orthopaedic applications.[36] These polymers can be prepared by ring-opening polymerization or copolymerization of glycolide, lactide, and/or ε-caprolactone. They undergo biodegradation via nonspecific hydrolytic scissions of the ester linkages at varied rates. End degradation products such as lactic acid, a natural metabolite, can be transported to the liver for metabolic conversions.[37]

The most common use of polylactides for LBSD reconstruction is to exploit their degradability for the *in vivo* delivery of osteogenic therapeutic factors. An earlier study delivering rhBMP-2 and autologous blood via PLGA microparticles to 25-mm, critical-size femoral segmental defects in sheep[38] demonstrated the efficacy of 2- and 4-mg rhBMP-2 or autologous blood in improving new bone formation within the LBSD compared to the empty PLGA microparticle carriers. With the delivery of therapeutics, new bone mineral content within the LBSD was found to reach that of the intact femur by 4 months while the recanalization of the intramedullary canal approached completion by 12 months. However, the tendency of the weak PLGA to break *in situ* was noted and the biomechanical restoration of the regenerated bone was not evaluated. PLGA was also used to coat gelatine sponge grafts for the delivery of 0.4 mg/mL rhBMP-2 into 2.5-cm tibial segmental defects in dogs.[39] Unlike the defect treated with the polymer-coated gelatine sponge scaffold alone that failed to bridge by 4 months, the defects treated with the polymer-coated gelatine sponge grafts impregnated with rhBMP-2 achieved radiographic union by 4 months (**Figure 1A–E**). Upon removal of the fixation plate, the regenerated bone continued to be remodelled over 2 years (**Figure 1F–H**), with the torsional stiffness exceeding that of the intact tibiae at 8 months and returning to the level of intact tibiae at 2 years.

The osteoconductive minerals β-TCP and HAp, known for their varying *in vivo* resorption rates,[40] abilities to absorb a wide range of protein factors[41] and to buffer acidic degradation products of polylactides, have long been used to fabricate degradable polymer-mineral composite bone graft substitutes.[42, 43] A successful use of

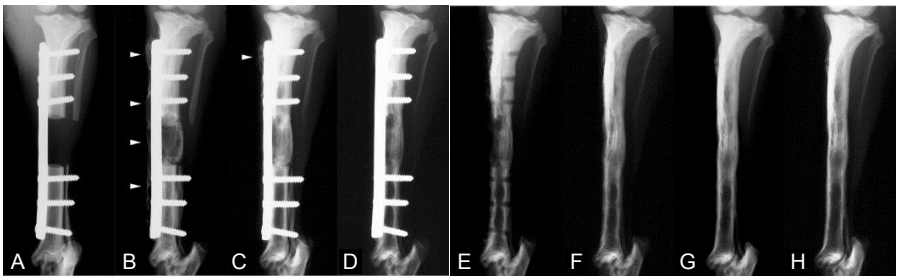

Figure 1. Radiographs of a defect treated with polymer-coated gelatin sponge impregnated with recombinant bone morphogenetic protein 2 (0.4 mg/cm^3) (anteroposterior view). (A–H) Radiographs were taken at 0 (A), 4 (B), 8 (C), 16 (D and E; before and after plate removal, respectively), 32 (F), 52 (G) and 104 (H) weeks postoperatively. Arrowheads in B and C indicate the hypertrophic bone beyond the metal plate. Reproduced from Kokubo et al.[39] with permission from Elsevier.

3D-printed macroporous PCL/β-TCP composite scaffold for the repair of 3 cm, critical-size tibial segmental defects in sheep was demonstrated with the incorporation/delivery of 1.75 or 3.5 mg rhBMP-7.[44] Bony callus bridged over the critical-size LBSDs by 3 months, with the torsional strength of the regenerated bone in both groups reaching the level achieved by the autologous cancellous bone grafting control. By 1 year, the mechanical restoration resulting from the PCL/β-TCP/3.5-mg rhBMP-7 treatment transcended those treated with the autologous cancellous bone graft control, although the synthetic bone graft was not fully resorbed, likely due to the slow degradation of PCL.

It should also be noted that the milligram scale of recombinant bone morphogenetic protein (BMP) protein therapeutics used in combination with the polylactide-based scaffolds in these large animal LBSD repair studies resembled the high human clinical doses known for adverse local and systemic health risks. More recently, 3D-printed elastic composites of HAp/PCL or HAp/PLGA with mineral contents as high as 90 wt% were developed as BMP delivery carriers for spinal fusion and calvarial bone repair applcations.[45] It remains to be seen whether these high-mineral content polylactide composites may facilitate the functional regeneration of LBSDs, especially with substantially reduced effective loading doses of BMP therapeutics.

Amphiphilic polylactide-based composites

To improve the aqueous wettability, enhance the interfacial bonding with hydrophilic biominerals, and expedite the hydrolytic degradation of polylactide-based composite grafts, amphiphilic block copolymers containing both hydrophilic PEG block and hydrophobic PLA, PGLA or PCL block have been developed. A number of studies have explored the use of these amphiphilic polylactide-mineral composites for the repair of critical-size LBSDs. Porous amphiphilic copolymer PLA-PEG/HAp composites in combination with 5 or 20 μg rhBMP-2 were shown to enable bony callus formation bridging over 15-mm radial segmental defects in rabbits within 2 months.[46] The composite grafts, however, were not fully resorbed by 2 months, and it was unclear how the mechanical integrity of the repaired defect compared to that of intact controls. Similarly, composite grafts composed of amphiphilic random copolymers consisting of PLA, p-dioxanone and PEG (PLA-DX-PEG) and β-TCP were used to deliver 50 μg of rhBMP-2 for the repair of 15-mm femoral segmental defects in rabbits.[47] Bony callus bridged over the defects at 2 months (**Figure 2**), restoring the bending stiffness to 40% of the intact controls. The implant was completely resorbed by 6 months, accompanied by the restoration of mechanical integrity and natural anatomical structure of the regenerated femur through continued remodelling of the new bone.

We have developed multi-functional shape memory composite bone grafts based on the amphiphilic copolymers PLA-PEG-PLA or PLGA-PEG-PLGA with HAp, and elucidated how useful physical handling characteristics and biological performances

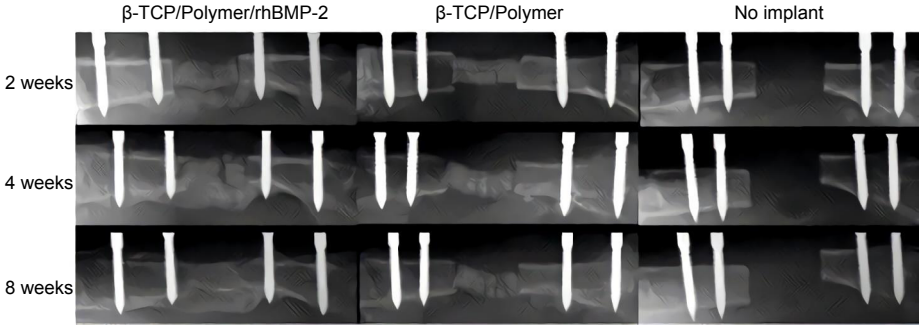

Figure 2. Representative femoral radiographs. From left, implanted with β-TCP with PLA-DX-PEG and rhBMP-2, β-TCP with PLA-DX-PEG without rhBMP-2, and critical size bone defect without implantation (sham surgery). Sequential radiographs show bone repair at 2, 4, and 8 weeks after implantation in the experimental group. Reproduced from Yoneda et al.[47] with permission from Elsevier. DX: p-dioxanone; PEG: poly(ethylene glycol); PLA: poly(lactic acid); rhBMP-2: recombinant bone morphogenetic protein 2; β-TCP: β-tricalcium phosphate.

may be engineered to enhance LBSD regeneration outcomes through a series of studies.[48-54] With the hydrophobic PLA or PLGA blocks providing tunable degradability while the hydrophilic central PEG block enables strong bonding with HAp, the amphiphilic composites exhibited enhanced elasticity and aqueous wettability.[48, 50, 51] Compared to conventional polylactide-HAp composites, the well-integrated HAp in the amphiphilic composites more effectively promoted osteochondral lineage commitment of bone marrow-derived stromal cells in unstimulated culture and supported far more potent osteogenesis upon *in vitro* osteogenic induction.[51] The well-dispersed HAp distribution within the amphiphilic composites, maximizing mineral surface area for protein absorption, also enabled the sustained release of BMP protein therapeutics from both electrospun fibrous meshes[53] and 3D-printed macroporous scaffolds[54] for guided long bone regeneration. By controlling the block length and the ratio of hydrophobic PLA/PLGA *vs.* hydrophilic PEG, we also programmed hydration-induced stiffening behaviour, driven by microphase separation and PEG crystallization, to enable stable self-fixation of the amphiphilic composites within confined defects.[49, 50] Finally, taking advantage of the respective thermal transitions of the amphiphilic blocks, we programmed shape memory behaviours that enable facile temporary shape programming at ambient temperature and shape recovery at safe physiological temperatures.[48-50, 52]

The successful translation of these multifunctional amphiphilic degradable shape memory composites for safer and more effective regenerative LBSD repair was recently demonstrated in rodents.[54] Specifically, the macroporous 25% Hap-PLGA-PEG-PLGA(8/1) grafts incorporating PLGA-PEG-PLGA triblock copolymers, with a lactide to gylcolide ratio of 8:1, and 25 wt% Hap, were 3D printed to fit 5-mm, critical-size femoral segmental defects in rats. The graft could be compressed into a short cylinder at the time of surgery for convenient placement within the LBSD, and then underwent shape recovery at body temperature and spontaneous swelling and stiffening upon contact with bodily fluid. These unique graft characteristics translated into much shorter surgery time compared to the placement of the weak collagen sponge controls, and resulted in superior graft fixation as demonstrated by the substantially-higher graft fixation force measured (> 2 orders of magnitude higher than that of collagen sponge) and the 100% graft stability achieved *in vivo*. Importantly, when the graft was loaded with 400 ng of a rhBMP-2/7 heterodimer, (equivalent to ~13 μg in a 60-kg human), it led to the formation of bridging bony callus as early as 1 month (**Figure 3A**). Continuous remodelling led to steady increases in bone volume and bone mineral density (**Figure 3B**), the recanalization of regenerated bone, and the full resorption of the bone graft by 3 months (**Figures 3A** and **C**), culminating in the restoration of torsional strength to the level of intact controls by 4 months (**Figure 3D**). It should be noted that such a functional LBSD regeneration was achieved with a recombinant BMP protein therapeutic dose > 2 orders of magnitude lower than those typically required with collagen sponge carriers. Indeed, the significantly lower effective BMP loading dose on the amphiphilic composite graft, along with the excellent graft fixation stability, translated into the complete elimination of ectopic bone formation that was consistently observed in LBSDs treated with collagen/BMP controls. It remains to be seen whether this exciting shape memory bone graft technology may translate into safer and more effective limb salvage in larger animals and humans.

Poly(propylene fumarate)-based composites

Poly(propylene fumarate) (PPF), an unsaturated linear polyester used for orthopaedic applications,[55, 56] can be prepared by the transesterification of di-(2-hydroxypropyl) fumarate. Fumaric acid, the main degradation product of PPF, is one of the essential Kreb's cycle acid intermediates and is widely used in the food industry. The fumarate double bonds in PPF allow the polymer to be further crosslinked *in situ* for applications ranging from injectable biodegradable bone cements[57, 58] to fabricating macroporous scaffolds crosslinked in 3D-printed negative moulds.[59] By altering the composition and crosslinking of the polymers, PPF with compressive strength ranging from tens to hundreds of megapascals can be prepared. Solid PPF intramedullary rods, with or without a porous PPF sleeve for encouraging osteointegration, and with rhBMP-2 delivery via embedded PLGA microparticles, were examined for the stabilization and repair of 5-mm, critical-size femoral segmental defects in rats.[60] The solid PPF intramedullary rod, applied in addition to plate fixation, improved defect fixation. Unfortunately, although the porous coating incorporating 2 or 8 μg of rhBMP-2 promoted new bone formation, complete union was not achieved in any treatment groups examined, suggesting that the solid PPF rod impeded long bone regeneration.

Porous, thermally-crosslinked PPF/PPF diacrylate composite scaffolds containing PLGA microparticles loaded with the osteogenic peptide TP508 were also examined for guided regeneration of 15 mm critical-size radial segmental defects in rabbits.[61] Whereas the composite scaffold containing 100 or 0 μg of TP508 led to minimal bone formation (< 10% bridging), the scaffolds bearing 200 μg of TP508 via the PLGA microparticles improved the osteointegration (up to 80% bridging). Unfortunately, the PPF scaffold remained largely undegraded and the defect failed to be fully bridged by 12 weeks. These studies point to the limitation of slowly degrading PPF scaffolds for functional long bone regeneration.

Polyanhydride-based composites

Polyanhydrides, another class of biodegradable polymers frequently used for drug delivery,[62, 63] can be prepared by ring-opening polymerization, melt polycondensation, dehydrochlorination and dehydrative coupling. The carboxylic acid degradation products resulting from the hydrolytic cleavage of the anhydride linkages should be carefully chosen for *in vivo* applications, to minimize mutagenicity or cytotoxicity.[64] Of note, polyanhydrides that can be hydrolysed into therapeutic acids such as salicylic acid, a nonsteroidal anti-inflammatory drug, have been explored for limiting undesired ectopic bone formation associated with LBSD repair when high doses of rhBMP-2 are delivered via collagen sponge carriers.[65] The idea was to utilize the ability of nonsteroidal anti-inflammatory drugs to suppress bone formation via the inhibition of cyclooxygenase-2[66-68] to counter the excessive release of rhBMP-2 into the tissues surrounding the LBSD. Specifically, salicylic acid-based poly(anhydride-ester) (SAPAE) was electrospun with PCL into thin membranes capable of fast

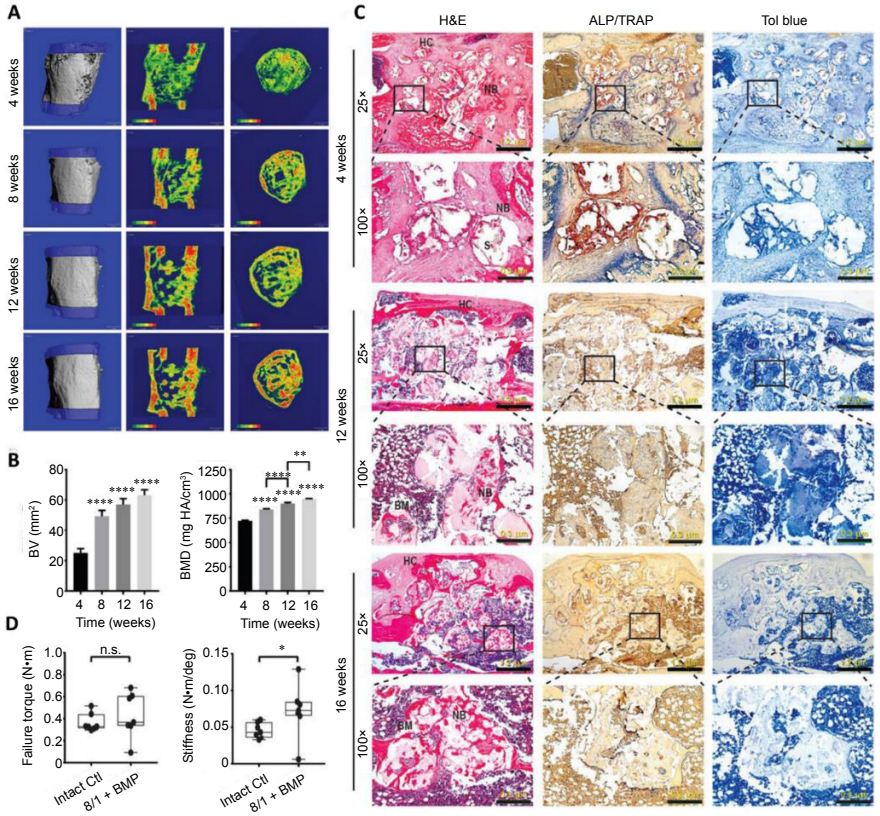

Figure 3. Accelerated healing of 5-mm rat femoral segmental defects by 25% HAp-PELGA(8/1) grafts preabsorbed with 400-ng rhBMP-2/7. (A) 3D μCT images and BMD colour maps (centre sagittal and axial slices) of the ROI showing maturing regenerated bone within the defect over time. Global thresholding was applied to exclude bone densities below 518.2 mg HAp/cm^3 (HAp-PELGA graft invisible at this threshold). (B) Longitudinal μCT quantification of BV and BMD ($n \geq 12$) within the ROI over time. Data are presented as means ± SEM. $^{**}P < 0.01$, $^{****}P < 0.0001$ (one-way analysis of variance with Tukey's *post-hoc* test). The global lower threshold of 518.2 mg HAp/cm^3 was applied for all quantifications. (C) Histological micrographs of H&E-, ALP/TRAP-, and Tol blue–stained sections of explanted graft-filled femurs over time. Scale bars: 1.2 mm (25× magnification) and 300 μm (100× magnification). Boxed regions shown at higher magnification in bottom rows. (D) Boxplots of failure torque and stiffness of intact (control) *versus* regenerated femur (8/1 + BMP) 16 weeks after being treated with HAp-PELGA(8/1) grafts preloaded with 400-ng rhBMP-2/7 ($n = 7$). $^{*}P < 0.05$ (Wilcoxon–Mann–Whitney rank sum test). Reprinted from Zhang et al.[54] with permission from AAAS. 3D: three-dimensional; ALP: alkaline phosphatase; BM: bone marrow; BMD: bone mineral density; BMP: bone morphogenetic protein; BV: bone volume; Ctl: control; H&E: haematoxylin and eosin; HAp: hydroxyapatite; HC: healing callus; n.s.: $P > 0.05$; NB: new bone; PELGA: poly(lactic-co-glycolic acid)-b-poly(ethylene glycol)-b-poly(lactic-co-glycolic acid); rhBMP: human recombinant bone morphogenetic protein; ROI: region of interest; S: scaffold; TRAP: tartrate-resistant acid phosphatase; μCT: micro-computed tomography.

degradation (FD-SAPAE) or slow degradation (SD-SAPAE). Collagen sponges loaded with 12-μg BMP-2 were placed within 5-mm rat femoral segmental defects with or without FD-SAPAE, SD-SAPAE or PCL control membranes.[65] Whereas massive ectopic bone formation was observed in the groups without any membrane wrapping or those wrapped with PCL control or SD-SAPAE by 4 weeks, the treatment with FD-SAPAE membrane improved bone formation within the LBSD while suppressing ectopic bone formation. The study, however, did not investigate the longer-term bone remodelling outcome or the mechanical integrity of regenerated bone as a function of salicylic acid release kinetics and membrane degradations. It should be noted that the poor mechanical properties of the polyanhydride precludes its use as a standalone bone graft for LBSD repair, and the complex dynamics and interplay of the biological actions of BMP-2 and salicylic acid could present barriers to the clinical translation of this strategy.

Polycarbonate-based composite bone grafts

Polycarbonates[69] are a class of thermoplastics that have broad-ranging applications from construction materials, digital storage media to containers and automobile parts. Poly(bisphenol A carbonate), prepared from the condensation of bisphenol A with phosgene or diphenyl carbonate, is a leading example due to its high impact resistance, ductility, optical transparency and low production costs.[70] Unfortunately, the oestrogen-like behaviour of bisphenol A[71] causes major concerns for its use in applications such as food containers as well as biomedically *in vivo*.[72] Aliphatic polycarbonates that are degradable into non-xenoestrogenic alkyl alcohols and carbon dioxide under physiological conditions have

thus attracted attention for biomedical uses.[73-79] For guided bone regenerations, porous poly(butylene carbonate) membranes[80] and poly(trimethylene-carbonate) barrier films[81] were examined for treating non-weight-bearing calvarial and mandibular defects, respectively; they were found to perform comparably in terms of bone regeneration outcome to the respective PCL and polytetrafluoroethylene controls. These aliphatic polycarbonates, mechanically inferior to PCL, have not been applied to the regenerative repair of weight-bearing LBSDs. Meanwhile, to enable the introduction of functionalities and hydrophilicity desired for potential therapeutics delivery and osteointegration, carbonate monomers with "clickable" functionalities including alkyne-,[82] azide-[83] and (methyl)acrylate84 have been prepared. For instance, we developed an azido-substituted cyclic trimethylene carbonate monomer that can be used for controlled homopolymerization and copolymerization with lactides,[82] and demonstrated the facile functionalization of the resulting polycarbonates and poly(ester-carbonates) via either copper-catalyzed[85] or copper-free, strain-promoted[86] azido-alkyne cycloaddition "click" chemistries. Whether and how mineral composites prepared with these functional polycarbonates translate into the regenerative LBSD repair remains to be determined.

Tyrosine-derived polycarbonates (Tyr-PCs), with hydrolytically-labile carbonate linkages and ester linkages along the main chain and connecting the side chains, respectively, were first developed by Kohn and Langer in 1987 as structural analogues of conventional poly(amino acids).[87-89] Being mouldable,[90] biocompatible and exhibiting good bone apposition,[91-93] this relatively new class of degradable polycarbonates have been explored for orthopaedic applications ranging from fixation rods[89] to guided craniomaxillofacial bone regeneration.[94-98] A Tyr-PC terpolymer, polymerized from 89 mol% desaminotyrosyl-tyrosine alkyl ester and 10 mol% desaminotyrosyl-tyrosine and incorporating 1 mol% 1000-Da PEG, was explored for the repair of 15-mm, critical-size radial segmental defects in rabbits, with the polymer scaffold coated with calcium phosphate and 0-, 17- or 35-μg rhBMP-2.[99] Whereas the Trp-PC + calcium phosphate scaffold alone induced minimal bone formation (< 2.5%), the loading of 17- or 35-μg rhBMP-2 led to significant increases in new bone formation within the LBSDs at 4 and 8 weeks. However, the defects were not fully restored at 8 weeks and the mechanical integrity of the regenerated bone was not evaluated.

As scaffold degradation kinetics and the immunogenicity of degradation products directly impact immune responses including macrophage polarization and the efficiency of osteogenesis, osteointegration and remodelling, the hydrolytically-degradable carbonate and ester linkages in Tyr-PCs provide a unique opportunity to modulate the osteoimmunological responses. For example, by manipulating side chain chemistry, Tyr-PCs can be engineered to achieve faster hydrolysis of the carbonate linkages, thereby delaying/mitigating the acute inflammatory responses often observed with the acidic degradation products of polyester-based scaffolds.[100] Long-term local and systemic safety profiles, however, will have to be established after any chemical modifications under the context of specific *in vivo* applications.

Synthetic Biodegradable Polyethylene Glycol-Based Hydrogels for Critical-Size Long Bone Segmental Defect Regeneration

Limitations of polysaccharide-based hydrogels

Naturally-occurring polysaccharide-based hydrogels such as hyaluronic acid,[101] alginate,[102-108] and chitosan[109-111] have been explored for LBSD repairs with varying degrees of success, with the polysaccharides often chemically modified with cell-adhesive peptides/proteins and/or loaded with osteogenic/angiogenic factors. Due to the intrinsically weak mechanical properties, these hydrogel formulations were often augmented with other structural components including osteoconductive minerals, or delivered within a secondary containment to the site of LBSDs. The microgram-scale rhBMP-2 delivered via most of these scaffolds for treating rodent critical-size LBSDs, when scaled to human, is unlikely to address the safety concerns associated with the current clinical doses delivered with collagen sponge carriers. Finally, covalent modifications of naturally-occurring polysaccharides in a regioselective manner and with reproducible stoichiometric control can be challenging.

Limitations of non-degradable crosslinked synthetic hydrogel composites

In comparison, wholly synthetic hydrogels crosslinked from well-defined building blocks present unique advantages in addressing some of the challenges associated with naturally occurring polysaccharide-based hydrogels. For instance, poly(2-hydroxyethyl methacrylate)-based hydrogels bearing biomimetic mineral-binding ligands can be readily prepared by copolymerizing functional methacrylate monomers.[112, 113] We demonstrated that high-mineral content (up to 70 wt%) poly(2-hydroxyethyl methacrylate)-HA composite with outstanding structural integrity, interfacial adhesion and compressive elasticity can be prepared for bone tissue engineering applications.[114, 115] When the poly(2-hydroxyethyl methacrylate)-mineral composite grafts containing 50 wt% HA and 400-ng rhBMP-2/7 were press-fit within 5-mm, critical-size femoral segmental defects in rats, they led to robust bridging bony callus formation by 6 weeks, with the torsional integrity of the remodelled new bone comparable to that of healthy controls.[116] However, due to the non-hydrolytically degradable nature of the polymethacrylate network, the composite graft remained sandwiched within the new bone, likely taking a very long time for the graft to be resorbed. This is also a limitation of conventional photo-crosslinked polyethylene glycol di(meth) acrylate-based hydrogels as synthetic bone grafts. Although lower molecular mass PEG oligomers can be excreted through the urine, the slow degradation of the crosslinked system could impede timely tissue integration and graft resorption for LBSD reconstruction.[117] Accordingly, there is a need for PEG-based hydrogels with more controlled degradation and bio-functionalities (e.g. cell-adhesiveness to overcome its bioinert nature[118]) for LBSD reconstructions.

Integrating controlled degradation to crosslinked polyethylene glycol-based hydrogels

To expedite the hydrolytic degradation of crosslinked PEG-based hydrogels, degradable polymer segments (e.g. polylactide,[119]

polycarbonate[120]) could be covalently integrated within the 3D network. Alternatively, we showed that when isolated degradable ester linkages were strategically placed near the strain-promoted azide-alkyne cycloaddition crosslinking site of well-structured PEG hydrogels, a broad range of degradation rates (from days to years) predicted by first-order hydrolytic degradation kinetics could be engineered.[121] Finally, PEG hydrogels crosslinked by matrix metalloproteinase-sensitive peptide crosslinkers[122] have seen numerous applications for bone and cartilage tissue engineering where the neotissue integration benefited from timely, environmentally-responsive scaffold degradation.[123-125] For instance, Shekaran et al.[126] crosslinked four-arm PEG-maleimides bearing the pro-osteogenic α2β1 integrin-specific hexapeptide Gly-Phe-Hyp-Gly-Glu-Ar (GFOGER) with matrix metalloproteinase-sensitive crosslinkers. These hydrogels were loaded with varying doses of rhBMP-2 for the repair of 2.5-mm murine radial segmental defects. The GFOGER-functionalized hydrogel alone was shown to result in substantial new bone formation within the defect, outperforming those tethered with the more commonly used cell adhesive RGD peptides. Furthermore, with the delivery of a low dose of 30-ng rhBMP-2, the GFOGER-modified, matrix metalloproteinase-responsive hydrogel underwent timely *in vivo* degradation and BMP-2 release, effectively repairing the LBSD with bridging new bone that fully restored its torsional integrity by 2 months (**Figure 4**). By contrast, the collagen control absorbed with the same dose of rhBMP-2 was unable to fully bridge the defect within the same timeframe. This well-designed scaffold has the potential to be a more effective and safer BMP therapeutics carrier for a range of orthopaedic applications.

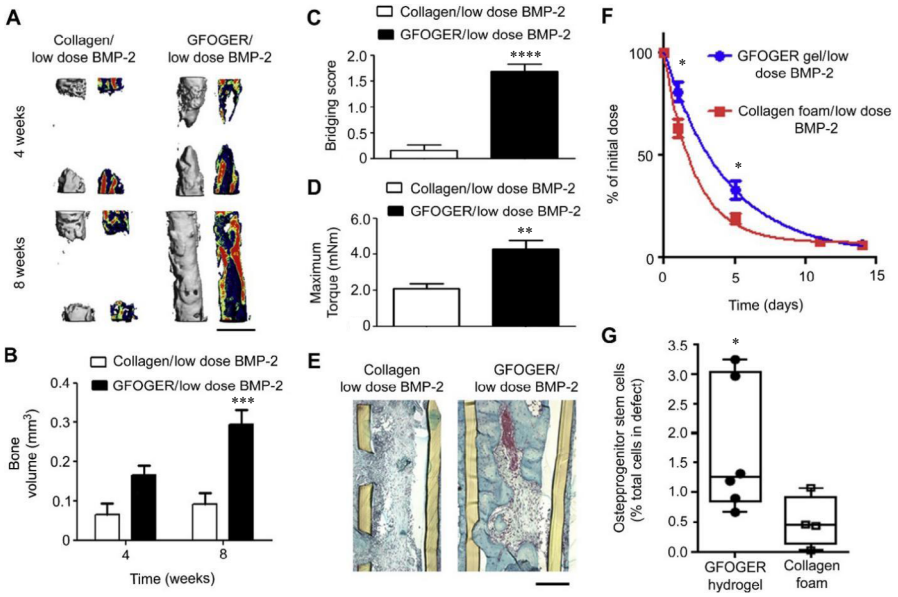

Figure 4. BMP-2 delivery from GFOGER-functionalized gels improves bone regeneration compared to collagen foams. (A) 3D μCT reconstructions of radii (left) and mineral density sagittal sections (right). Scale bar: 1 mm. (B) μCT measures of bone volume in radial defects. (C) Bridging score at 8 weeks post-implantation (n = 13). (D) Maximum torque values for 8 weeks radial samples subjected to torsion mechanical testing to failure (n = 5–9). (E) Sections of 8 weeks radial samples stained with Safranin-O/Fast Green. Scale bar: 200 μm. (F) Retention of infrared dye-labelled BMP-2 at implanted defect sites *in vivo* (n = 6). (G) Quantification of $CD45^-/CD90^+$ osteoprogenitor cells present in the defects 7 days post-implantation (n = 4–6). *P < 0.05, ***P < 0.001, ****P < 0.0001, *vs.* collagen foam/low dose BMP-2. Reproduced from Shekaran et al.[126] with permission from Elsevier. 3D: three-dimensional; BMP-2: bone morphogenetic protein 2; GFOGER: α2β1 integrin-specific hexapeptide sequence Gly-Phe-Hyp-Gly-Glu-Ar; μCT: micro-computed tomography.

Outlook

In the past two decades, a wide range of degradable synthetic polymeric bone grafts have been developed to promote the regenerative repair of LBSDs, some already yielding exciting outcomes in preclinical animal models. For eventual successful clinical translations, further enhancements of the efficacy and safety while ensuring reproducible, scalable and affordable manufacturing of these grafts will be necessary. It is also critical that preclinical studies are more rigorously designed to include functional outcome evaluations such as mechanical property assessment of regenerated long bone against current grafting standards and healthy controls. The assessment of longer-term local tissue responses and systemic safety of the synthetic bone grafts and their degradation products should also be encouraged within the bone tissue engineering community.

Emerging strategies for modulating osteoimmune responses during scaffold-guided bone regeneration such as macrophage polarization and osteoclast-mediated bone remodelling through the manipulation of physiochemical properties of biomaterial scaffolds[26] could lead to more effective regenerative repair of LBSDs. For instance, pro- *vs.* anti-inflammatory cytokines[6, 26] may be tethered to the synthetic bone graft to provide environmentally-responsive, temporally-controlled release to promote regenerative rather than degenerative repair processes.

Meanwhile, recent advances in designing viscoelastic synthetic biomaterials[127-129] that better recapitulate the dynamic tissue mechanics including stress relaxation properties[130] may enable better control over the fate and function of exogenously-delivered cell therapeutics or the myriad of endogenous cells recruited to the site of LBSDs. Furthermore, the engineering of multifunctional synthetic bone graft properties such as shape memory and *in situ* stiffening has the potential to improve the efficiency and precision of the surgical delivery and fixation of personalized bone grafts. Finally, the continued innovation of materials fabrication techniques such as intravital bioprinting[131,132] may open the door for longitudinal delivery of therapeutics to the surface of autogenic, allogenic and synthetic bone grafts in a spatially-defined manner.

Author contributions

XX and JS searched the literature and wrote the review. Both authors approved the final version of this manuscript.

Financial support

This work is supported by an Alex Lemonade Stand Foundation Innovation Grant and a BRIDGE Award from the University of Massachusetts Medical School.

Acknowledgement

None.

Conflicts of interest statement

The authors declare no competing financial interest.

1. Bauer, T. W.; Muschler, G. F. Bone graft materials. An overview of the basic science. *Clin Orthop Relat Res.* **2000**, 10-27.
2. Beekley, A. C.; Starnes, B. W.; Sebesta, J. A. Lessons learned from modern military surgery. *Surg Clin North Am.* **2007**, *87*, 157-184, vii.
3. Brown, K. V.; Guthrie, H. C.; Ramasamy, A.; Kendrew, J. M.; Clasper, J. Modern military surgery: lessons from Iraq and Afghanistan. *J Bone Joint Surg Br.* **2012**, *94*, 536-543.
4. DeCoster, T. A.; Gehlert, R. J.; Mikola, E. A.; Pirela-Cruz, M. A. Management of posttraumatic segmental bone defects. *J Am Acad Orthop Surg.* **2004**, *12*, 28-38.
5. Mauffrey, C.; Barlow, B. T.; Smith, W. Management of segmental bone defects. *J Am Acad Orthop Surg.* **2015**, *23*, 143-153.
6. Winkler, T.; Sass, F. A.; Duda, G. N.; Schmidt-Bleek, K. A review of biomaterials in bone defect healing, remaining shortcomings and future opportunities for bone tissue engineering: The unsolved challenge. *Bone Joint Res.* **2018**, *7*, 232-243.
7. Lauthe, O.; Soubeyrand, M.; Babinet, A.; Dumaine, V.; Anract, P.; Biau, D. J. The indications and donor-site morbidity of tibial cortical strut autografts in the management of defects in long bones. *Bone Joint J.* **2018**, 100-B, 667-674.
8. Hopp, S. G.; Dahners, L. E.; Gilbert, J. A. A study of the mechanical strength of long bone defects treated with various bone autograft substitutes: an experimental investigation in the rabbit. *J Orthop Res.* **1989**, *7*, 579-584.
9. Chiarello, E.; Cadossi, M.; Tedesco, G.; Capra, P.; Calamelli, C.; Shehu, A.; Giannini, S. Autograft, allograft and bone substitutes in reconstructive orthopedic surgery. *Aging Clin Exp Res.* **2013**, *25 Suppl 1*, S101-103.
10. Bostrom, M. P.; Seigerman, D. A. The clinical use of allografts, demineralized bone matrices, synthetic bone graft substitutes and osteoinductive growth factors: a survey study. *HSS J.* **2005**, *1*, 9-18.
11. Betz, R. R. Limitations of autograft and allograft: new synthetic solutions. *Orthopedics.* **2002**, *25*, s561-570.
12. Sorger, J. I.; Hornicek, F. J.; Zavatta, M.; Menzner, J. P.; Gebhardt, M. C.; Tomford, W. W.; Mankin, H. J. Allograft fractures revisited. *Clin Orthop Relat Res.* **2001**, 66-74.
13. Finkemeier, C. G. Bone-grafting and bone-graft substitutes. *J Bone Joint Surg Am.* **2002**, *84*, 454-464.
14. Boyce, T.; Edwards, J.; Scarborough, N. Allograft bone. The influence of processing on safety and performance. *Orthop Clin North Am.* **1999**, *30*, 571-581.
15. Kuttappan, S.; Mathew, D.; Nair, M. B. Biomimetic composite scaffolds containing bioceramics and collagen/gelatin for bone tissue engineering - A mini review. *Int J Biol Macromol.* **2016**, *93*, 1390-1401.
16. Cobos, J. A.; Lindsey, R. W.; Gugala, Z. The cylindrical titanium mesh cage for treatment of a long bone segmental defect: description of a new technique and report of two cases. *J Orthop Trauma.* **2000**, *14*, 54-59.
17. Attias, N.; Lindsey, R. W. Case reports: management of large segmental tibial defects using a cylindrical mesh cage. *Clin Orthop Relat Res.* **2006**, *450*, 259-266.
18. Marcacci, M.; Kon, E.; Moukhachev, V.; Lavroukov, A.; Kutepov, S.; Quarto, R.; Mastrogiacomo, M.; Cancedda, R. Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. *Tissue Eng.* **2007**, *13*, 947-955.
19. Kasten, P.; Vogel, J.; Geiger, F.; Niemeyer, P.; Luginbühl, R.; Szalay, K. The effect of platelet-rich plasma on healing in critical-size long-bone defects. *Biomaterials.* **2008**, *29*, 3983-3992.
20. Geiger, M.; Li, R. H.; Friess, W. Collagen sponges for bone regeneration with rhBMP-2. *Adv Drug Deliv Rev.* **2003**, *55*, 1613-1629.
21. Raina, D. B.; Qayoom, I.; Larsson, D.; Zheng, M. H.; Kumar, A.; Isaksson, H.; Lidgren, L.; Tägil, M. Guided tissue engineering for healing of cancellous and cortical bone using a combination of biomaterial based scaffolding and local bone active molecule delivery. *Biomaterials.* **2019**, *188*, 38-49.
22. Lissenberg-Thunnissen, S. N.; de Gorter, D. J.; Sier, C. F.; Schipper, I. B. Use and efficacy of bone morphogenetic proteins in fracture healing. *Int Orthop.* **2011**, *35*, 1271-1280.
23. Zara, J. N.; Siu, R. K.; Zhang, X.; Shen, J.; Ngo, R.; Lee, M.; Li, W.; Chiang, M.; Chung, J.; Kwak, J.; Wu, B. M.; Ting, K.; Soo, C. High doses of bone morphogenetic protein 2 induce structurally abnormal bone and inflammation in vivo. *Tissue Eng Part A.* **2011**, *17*, 1389-1399.
24. Tazaki, J.; Murata, M.; Akazawa, T.; Yamamoto, M.; Ito, K.; Arisue, M.; Shibata, T.; Tabata, Y. BMP-2 release and dose-response studies in hydroxyapatite and beta-tricalcium phosphate. *Biomed Mater Eng.* **2009**, *19*, 141-146.
25. Mines, D.; Gu, Y.; Kou, T. D.; Cooper, G. S. Recombinant human bone morphogenetic protein-2 and pancreatic cancer: a retrospective cohort study. *Pharmacoepidemiol Drug Saf.* **2011**, *20*, 111-118.
26. Lee, J.; Byun, H.; Madhurakkat Perikamana, S. K.; Lee, S.; Shin, H. Current advances in immunomodulatory biomaterials for bone regeneration. *Adv Healthc Mater.* **2019**, *8*, e1801106.
27. Chocholata, P.; Kulda, V.; Babuska, V. Fabrication of scaffolds for bone-tissue regeneration. *Materials (Basel).* **2019**, *12*, 568.
28. Zhang, B.; Song, J. 3D-printed biomaterials for guided tissue regeneration. *Small Methods.* **2018**, *2*, 1700306.

29. Jang, J. H.; Castano, O.; Kim, H. W. Electrospun materials as potential platforms for bone tissue engineering. *Adv Drug Deliv Rev.* **2009**, *61*, 1065-1083.
30. Moutos, F. T.; Freed, L. E.; Guilak, F. A biomimetic three-dimensional woven composite scaffold for functional tissue engineering of cartilage. *Nat Mater.* **2007**, *6*, 162-167.
31. Moutos, F. T.; Glass, K. A.; Compton, S. A.; Ross, A. K.; Gersbach, C. A.; Guilak, F.; Estes, B. T. Anatomically shaped tissue-engineered cartilage with tunable and inducible anticytokine delivery for biological joint resurfacing. *Proc Natl Acad Sci U S A.* **2016**, *113*, E4513-4522.
32. Kanczler, J. M.; Oreffo, R. O. Osteogenesis and angiogenesis: the potential for engineering bone. *Eur Cell Mater.* **2008**, *15*, 100-114.
33. Smith, R. Biodegradable Polymers for Industrial Applications. Woodhead Publishing: **2005**.
34. Cortizo, M. S.; Belluzo, M. S. Biodegradable polymers for bone tissue engineering. In *Industrial applications of renewable biomass products: past, present and future*, Goyanes, S. N.; D'Accorso, N. B., eds.; Springer International Publishing: Cham, **2017**; pp 47-74.
35. Webb, A. R.; Yang, J.; Ameer, G. A. Biodegradable polyester elastomers in tissue engineering. *Expert Opin Biol Ther.* **2004**, *4*, 801-812.
36. Narayanan, G.; Vernekar, V. N.; Kuyinu, E. L.; Laurencin, C. T. Poly (lactic acid)-based biomaterials for orthopaedic regenerative engineering. *Adv Drug Deliv Rev.* **2016**, *107*, 247-276.
37. Temenoff, J. S.; Mikos, A. G. Injectable biodegradable materials for orthopedic tissue engineering. *Biomaterials.* **2000**, *21*, 2405-2412.
38. Kirker-Head, C. A.; Gerhart, T. N.; Armstrong, R.; Schelling, S. H.; Carmel, L. A. Healing bone using recombinant human bone morphogenetic protein 2 and copolymer. *Clin Orthop Relat Res.* **1998**, 205-217.
39. Kokubo, S.; Mochizuki, M.; Fukushima, S.; Ito, T.; Nozaki, K.; Iwai, T.; Takahashi, K.; Yokota, S.; Miyata, K.; Sasaki, N. Long-term stability of bone tissues induced by an osteoinductive biomaterial, recombinant human bone morphogenetic protein-2 and a biodegradable carrier. *Biomaterials.* **2004**, *25*, 1795-1803.
40. Nancollas, G. H.; Henneman, Z. J. Calcium oxalate: calcium phosphate transformations. *Urol Res.* **2010**, *38*, 277-280.
41. Chung, W. J.; Kwon, K. Y.; Song, J.; Lee, S. W. Evolutionary screening of collagen-like peptides that nucleate hydroxyapatite crystals. *Langmuir.* **2011**, *27*, 7620-7628.
42. Godavitarne, C.; Robertson, A.; Peters, J.; Rogers, B. Biodegradable materials. *Orthop Trauma.* **2017**, *31*, 316-320.
43. Cancedda, R.; Giannoni, P.; Mastrogiacomo, M. A tissue engineering approach to bone repair in large animal models and in clinical practice. *Biomaterials.* **2007**, *28*, 4240-4250.
44. Reichert, J. C.; Cipitria, A.; Epari, D. R.; Saifzadeh, S.; Krishnakanth, P.; Berner, A.; Woodruff, M. A.; Schell, H.; Mehta, M.; Schuetz, M. A.; Duda, G. N.; Hutmacher, D. W. A tissue engineering solution for segmental defect regeneration in load-bearing long bones. *Sci Transl Med.* **2012**, *4*, 141ra193.
45. Jakus, A. E.; Rutz, A. L.; Jordan, S. W.; Kannan, A.; Mitchell, S. M.; Yun, C.; Koube, K. D.; Yoo, S. C.; Whiteley, H. E.; Richter, C. P.; Galiano, R. D.; Hsu, W. K.; Stock, S. R.; Hsu, E. L.; Shah, R. N. Hyperelastic "bone": A highly versatile, growth factor-free, osteoregenerative, scalable, and surgically friendly biomaterial. *Sci Transl Med.* **2016**, *8*, 358ra127.
46. Kaito, T.; Myoui, A.; Takaoka, K.; Saito, N.; Nishikawa, M.; Tamai, N.; Ohgushi, H.; Yoshikawa, H. Potentiation of the activity of bone morphogenetic protein-2 in bone regeneration by a PLA-PEG/hydroxyapatite composite. *Biomaterials.* **2005**, *26*, 73-79.
47. Yoneda, M.; Terai, H.; Imai, Y.; Okada, T.; Nozaki, K.; Inoue, H.; Miyamoto, S.; Takaoka, K. Repair of an intercalated long bone defect with a synthetic biodegradable bone-inducing implant. *Biomaterials.* **2005**, *26*, 5145-5152.
48. Zhang, B.; Filion, T. M.; Kutikov, A. B.; Song, J. Facile stem cell delivery to bone grafts enabled by smart shape recovery and stiffening of degradable synthetic periosteal membranes. *Adv Funct Mater.* **2017**, *27*, 1604784.
49. Zhang, B.; DeBartolo, J. E.; Song, J. Shape recovery with concomitant mechanical strengthening of amphiphilic shape memory polymers in warm water. *ACS Appl Mater Interfaces.* **2017**, *9*, 4450-4456.
50. Kutikov, A. B.; Gurijala, A.; Song, J. Rapid prototyping amphiphilic polymer/hydroxyapatite composite scaffolds with hydration-induced self-fixation behavior. *Tissue Eng Part C Methods.* **2015**, *21*, 229-241.
51. Kutikov, A. B.; Song, J. An amphiphilic degradable polymer/hydroxyapatite composite with enhanced handling characteristics promotes osteogenic gene expression in bone marrow stromal cells. *Acta Biomater.* **2013**, *9*, 8354-8364.
52. Kutikov, A. B.; Reyer, K. A.; Song, J. Shape memory performance of thermoplastic amphiphilic triblock copolymer poly(D,L-lactic acid-co-ethylene glycol-co-D,L-lactic acid) (PELA)/hydroxyapatite composites. *Macromol Chem Phys.* **2014**, *215*, 2482-2490.
53. Kutikov, A. B.; Skelly, J. D.; Ayers, D. C.; Song, J. Templated repair of long bone defects in rats with bioactive spiral-wrapped electrospun amphiphilic polymer/hydroxyapatite scaffolds. *ACS Appl Mater Interfaces.* **2015**, *7*, 4890-4901.
54. Zhang, B.; Skelly, J. D.; Maalouf, J. R.; Ayers, D. C.; Song, J. Multifunctional scaffolds for facile implantation, spontaneous fixation, and accelerated long bone regeneration in rodents. *Sci Transl Med.* **2019**, *11*, eaau7411.
55. Peter, S. J.; Yaszemski, M. J.; Suggs, L. J.; Payne, R. G.; Langer, R.; Hayes, W. C.; Unroe, M. R.; Alemany, L. B.; Engel, P. S.; Mikos, A. G. Characterization of partially saturated poly(propylene fumarate) for orthopaedic application. *J Biomater Sci Polym Ed.* **1997**, *8*, 893-904.
56. Temenoff, J. S.; Mikos, A. G. Review: tissue engineering for regeneration of articular cartilage. *Biomaterials.* **2000**, *21*, 431-440.
57. Domb, A. J.; Manor, N.; Elmalak, O. Biodegradable bone cement compositions based on acrylate and epoxide terminated poly(propylene fumarate) oligomers and calcium salt compositions. *Biomaterials.* **1996**, *17*, 411-417.
58. Peter, S. J.; Kim, P.; Yasko, A. W.; Yaszemski, M. J.; Mikos, A. G. Crosslinking characteristics of an injectable poly(propylene fumarate)/beta-tricalcium phosphate paste and mechanical properties of the crosslinked composite for use as a biodegradable bone cement. *J Biomed Mater Res.* **1999**, *44*, 314-321.
59. Lee, K. W.; Wang, S.; Lu, L.; Jabbari, E.; Currier, B. L.; Yaszemski, M. J. Fabrication and characterization of poly(propylene fumarate) scaffolds with controlled pore structures using 3-dimensional printing and injection molding. *Tissue Eng.* **2006**, *12*, 2801-2811.
60. Henslee, A. M.; Spicer, P. P.; Yoon, D. M.; Nair, M. B.; Meretoja, V. V.; Witherel, K. E.; Jansen, J. A.; Mikos, A. G.; Kasper, F. K. Biodegradable composite scaffolds incorporating an intramedullary rod and delivering bone morphogenetic protein-2 for stabilization and bone regeneration in segmental long bone defects. *Acta Biomater.* **2011**, *7*, 3627-3637.
61. Hedberg, E. L.; Kroese-Deutman, H. C.; Shih, C. K.; Crowther, R. S.; Carney, D. H.; Mikos, A. G.; Jansen, J. A. Effect of varied release kinetics of the osteogenic thrombin peptide TP508 from biodegradable, polymeric scaffolds on bone formation in vivo. *J Biomed Mater Res A.* **2005**, *72*, 343-

353.
62. Kumar, N.; Langer, R. S.; Domb, A. J. Polyanhydrides: an overview. *Adv Drug Deliv Rev.* **2002**, *54*, 889-910.
63. Basu, A.; Domb, A. J. Recent advances in polyanhydride based biomaterials. *Adv Mater.* **2018**, *30*, e1706815.
64. Katti, D. S.; Lakshmi, S.; Langer, R.; Laurencin, C. T. Toxicity, biodegradation and elimination of polyanhydrides. *Adv Drug Deliv Rev.* **2002**, *54*, 933-961.
65. Subramanian, S.; Mitchell, A.; Yu, W.; Snyder, S.; Uhrich, K.; O'Connor, J. P. Salicylic acid-based polymers for guided bone regeneration using bone morphogenetic protein-2. *Tissue Eng Part A.* **2015**, *21*, 2013-2024.
66. Giannoudis, P. V.; MacDonald, D. A.; Matthews, S. J.; Smith, R. M.; Furlong, A. J.; De Boer, P. Nonunion of the femoral diaphysis. The influence of reaming and non-steroidal anti-inflammatory drugs. *J Bone Joint Surg Br.* **2000**, *82*, 655-658.
67. Goodman, S.; Ma, T.; Trindade, M.; Ikenoue, T.; Matsuura, I.; Wong, N.; Fox, N.; Genovese, M.; Regula, D.; Smith, R. L. COX-2 selective NSAID decreases bone ingrowth in vivo. *J Orthop Res.* **2002**, *20*, 1164-1169.
68. Simon, A. M.; O'Connor, J. P. Dose and time-dependent effects of cyclooxygenase-2 inhibition on fracture-healing. *J Bone Joint Surg Am.* **2007**, *89*, 500-511.
69. Hauenstein, O.; Agarwal, S.; Greiner, A. Bio-based polycarbonate as synthetic toolbox. *Nat Commun.* **2016**, *7*, 11862.
70. Legrand, D. G.; Bendler, J. T. Handbook of polycarbonate science and technology. CRC Press: Boca Raton, **2000**.
71. Calafat, A. M.; Weuve, J.; Ye, X.; Jia, L. T.; Hu, H.; Ringer, S.; Huttner, K.; Hauser, R. Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. *Environ Health Perspect.* **2009**, *117*, 639-644.
72. Kim, J. G. Chemical recycling of poly(bisphenol A carbonate). *Polym Chem.* **2020**, *11*, 4830-4849.
73. Pêgo, A. P.; Poot, A. A.; Grijpma, D. W.; Feijen, J. Copolymers of trimethylene carbonate and epsilon-caprolactone for porous nerve guides: synthesis and properties. *J Biomater Sci Polym Ed.* **2001**, *12*, 35-53.
74. Pêgo, A. P.; Poot, A. A.; Grijpma, D. W.; Feijen, J. In vitro degradation of trimethylene carbonate based (co)polymers. *Macromol Biosci.* **2002**, *2*, 411-419.
75. Rokicki, G. Aliphatic cyclic carbonates and spiroorthocarbonates as monomers. *Prog Polym Sci.* **2000**, *25*, 259-342.
76. Bat, E.; Kothman, B. H.; Higuera, G. A.; van Blitterswijk, C. A.; Feijen, J.; Grijpma, D. W. Ultraviolet light crosslinking of poly(trimethylene carbonate) for elastomeric tissue engineering scaffolds. *Biomaterials.* **2010**, *31*, 8696-8705.
77. Dankers, P. Y. W.; Zhang, Z.; Wisse, E.; Grijpma, D. W.; Sijbesma, R. P.; Feijen, J.; Meijer, E. W. Oligo(trimethylene carbonate)-based supramolecular biomaterials. *Macromolecules.* **2006**, *39*, 8763-8771.
78. Zhu, K. J.; Hendren, R. W.; Jensen, K.; Pitt, C. G. Synthesis, properties, and biodegradation of poly(1,3-trimethylene carbonate). *Macromolecules.* **1991**, *24*, 1736-1740.
79. Feng, J.; Zhuo, R.-X.; Zhang, X.-Z. Construction of functional aliphatic polycarbonates for biomedical applications. *Prog Polym Sci.* **2012**, *37*, 211-236.
80. Xia, Y.; Yao, J.; Shao, C. H.; Shen, X. Y.; Xie, L. Z.; Chen, G.; Peng, S. S.; Zhang, F. M.; Gu, N. Biodegradable poly(butylene-carbonate) porous membranes for guided bone regeneration: In vitro and in vivo studies. *J Bioact Compatible Polym.* **2013**, *28*, 621-636.
81. van Leeuwen, A. C.; Yuan, H.; Passanisi, G.; van der Meer, J. W.; de Bruijn, J. D.; van Kooten, T. G.; Grijpma, D. W.; Bos, R. R. Poly(trimethylene carbonate) and biphasic calcium phosphate composites for orbital floor reconstruction: a feasibility study in sheep. *Eur Cell Mater.* **2014**, *27*, 81-96; discussion 96-97.
82. Han, Y.; Shi, Q.; Hu, J.; Du, Q.; Chen, X.; Jing, X. Grafting BSA onto poly[(L-lactide)-co-carbonate] microspheres by click chemistry. *Macromol Biosci.* **2008**, *8*, 638-644.
83. Xu, J.; Prifti, F.; Song, J. A versatile monomer for preparing well-defined functional polycarbonates and poly(ester-carbonates). *Macromolecules.* **2011**, *44*, 2660-2667.
84. Chen, W.; Yang, H.; Wang, R.; Cheng, R.; Meng, F.; Wei, W.; Zhong, Z. Versatile synthesis of functional biodegradable polymers by combining ring-opening polymerization and postpolymerization modification via michael-type addition reaction. *Macromolecules.* **2010**, *43*, 201-207.
85. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. Click chemistry: diverse chemical function from a few good reactions. A*ngew Chem Int Ed Engl.* **2001**, *40*, 2004-2021.
86. Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. A strain-promoted [3 + 2] azide-alkyne cycloaddition for covalent modification of biomolecules in living systems. *J Am Chem Soc.* **2004**, *126*, 15046-15047.
87. Tangpasuthadol, V.; Pendharkar, S. M.; Peterson, R. C.; Kohn, J. Hydrolytic degradation of tyrosine-derived polycarbonates, a class of new biomaterials. Part II: 3-yr study of polymeric devices. *Biomaterials.* **2000**, *21*, 2379-2387.
88. Kohn, J.; Langer, R. Polymerization reactions involving the side chains of .alpha.-L-amino acids. *J Am Chem Soc.* **1987**, *109*, 817-820.
89. Pyhältö, T.; Lapinsuo, M.; Pätiälä, H.; Pelto, M.; Törmälä, P.; Rokkanen, P. Fixation of distal femoral osteotomies with self-reinforced poly(desamino tyrosyl-tyrosine ethyl ester carbonate) rods: an experimental study on rats. *J Orthop Sci.* **2002**, *7*, 549-556.
90. Abramson, S. D.; Seyda, A.; Sit, P. S.; Kohn, J. Characterization of degradable polymers for orthopedic application. In Polymer based systems on tissue engineering, replacement and regeneration, Reis, R. L.; Cohn, D., Eds.; Springer Netherlands: Dordrecht, **2002**; pp 125-138.
91. Hooper, K. A.; Macon, N. D.; Kohn, J. Comparative histological evaluation of new tyrosine-derived polymers and poly (L-lactic acid) as a function of polymer degradation. *J Biomed Mater Res.* **1998**, *41*, 443-454.
92. Choueka, J.; Charvet, J. L.; Koval, K. J.; Alexander, H.; James, K. S.; Hooper, K. A.; Kohn, J. Canine bone response to tyrosine-derived polycarbonates and poly(L-lactic acid). *J Biomed Mater Res.* **1996**, *31*, 35-41.
93. Magno, M. H. R.; Kim, J.; Srinivasan, A.; McBride, S.; Bolikal, D.; Darr, A.; Hollinger, J. O.; Kohn, J. Synthesis, degradation and biocompatibility of tyrosine-derived polycarbonate scaffolds. *J Mater Chem.* **2010**, *20*, 8885-8893.
94. Saxena, S.; Chang, W.; Fakhrzadeh, A.; Murthy, N. S.; Zhang, W.; Kohn, J.; Yelick, P. C. Calcium phosphate enriched synthetic tyrosine-derived polycarbonate - dicalcium phosphate dihydrate polymer scaffolds for enhanced bone regeneration. *Materialia.* **2020**, *9*, 100616.
95. Asikainen, A. J.; Noponen, J.; Mesimäki, K.; Laitinen, O.; Peltola, J.; Pelto, M.; Kellomäki, M.; Ashammakhi, N.; Lindqvist, C.; Suuronen, R. Tyrosine derived polycarbonate membrane is useful for guided bone regeneration in rabbit mandibular defects. *J Mater Sci Mater Med.* **2005**, *16*, 753-758.
96. Asikainen, A. J.; Noponen, J.; Lindqvist, C.; Pelto, M.; Kellomäki, M.; Juuti, H.; Pihlajamäki, H.; Suuronen, R. Tyrosine-derived polycarbonate membrane in treating mandibular bone defects. An experimental study. *J R Soc Interface.* **2006**, *3*, 629-635.
97. Kim, J.; Magno, M. H.; Waters, H.; Doll, B. A.; McBride, S.; Alvarez, P.;

Darr, A.; Vasanji, A.; Kohn, J.; Hollinger, J. O. Bone regeneration in a rabbit critical-sized calvarial model using tyrosine-derived polycarbonate scaffolds. *Tissue Eng Part A.* **2012**, *18*, 1132-1139.

98. Zhang, W.; Zhang, Z.; Chen, S.; Macri, L.; Kohn, J.; Yelick, P. C. Mandibular jaw bone regeneration using human dental cell-seeded tyrosine-derived polycarbonate scaffolds. *Tissue Eng Part A.* **2016**, *22*, 985-993.
99. Kim, J.; McBride, S.; Donovan, A.; Darr, A.; Magno, M. H.; Hollinger, J. O. Tyrosine-derived polycarbonate scaffolds for bone regeneration in a rabbit radius critical-size defect model. *Biomed Mater.* **2015**, *10*, 035001.
100. Tangpasuthadol, V.; Pendharkar, S. M.; Kohn, J. Hydrolytic degradation of tyrosine-derived polycarbonates, a class of new biomaterials. Part I: study of model compounds. *Biomaterials.* **2000**, *21*, 2371-2378.
101. Han, S. H.; Jung, S. H.; Lee, J. H. Preparation of beta-tricalcium phosphate microsphere-hyaluronic acid-based powder gel composite as a carrier for rhBMP-2 injection and evaluation using long bone segmental defect model. *J Biomater Sci Polym Ed.* **2019**, *30*, 679-693.
102. Kolambkar, Y. M.; Dupont, K. M.; Boerckel, J. D.; Huebsch, N.; Mooney, D. J.; Hutmacher, D. W.; Guldberg, R. E. An alginate-based hybrid system for growth factor delivery in the functional repair of large bone defects. *Biomaterials.* **2011**, *32*, 65-74.
103. Boerckel, J. D.; Kolambkar, Y. M.; Dupont, K. M.; Uhrig, B. A.; Phelps, E. A.; Stevens, H. Y.; García, A. J.; Guldberg, R. E. Effects of protein dose and delivery system on BMP-mediated bone regeneration. *Biomaterials.* **2011**, *32*, 5241-5251.
104. Krebs, M. D.; Salter, E.; Chen, E.; Sutter, K. A.; Alsberg, E. Calcium phosphate-DNA nanoparticle gene delivery from alginate hydrogels induces in vivo osteogenesis. *J Biomed Mater Res A.* **2010**, *92*, 1131-1138.
105. Oest, M. E.; Dupont, K. M.; Kong, H. J.; Mooney, D. J.; Guldberg, R. E. Quantitative assessment of scaffold and growth factor-mediated repair of critically sized bone defects. *J Orthop Res.* **2007**, *25*, 941-950.
106. Kanczler, J. M.; Ginty, P. J.; White, L.; Clarke, N. M.; Howdle, S. M.; Shakesheff, K. M.; Oreffo, R. O. The effect of the delivery of vascular endothelial growth factor and bone morphogenic protein-2 to osteoprogenitor cell populations on bone formation. *Biomaterials.* **2010**, *31*, 1242-1250.
107. Boerckel, J. D.; Dupont, K. M.; Kolambkar, Y. M.; Lin, A. S.; Guldberg, R. E. In vivo model for evaluating the effects of mechanical stimulation on tissue-engineered bone repair. *J Biomech Eng.* **2009**, *131*, 084502.
108. Priddy, L. B.; Chaudhuri, O.; Stevens, H. Y.; Krishnan, L.; Uhrig, B. A.; Willett, N. J.; Guldberg, R. E. Oxidized alginate hydrogels for bone morphogenetic protein-2 delivery in long bone defects. *Acta Biomater.* **2014**, *10*, 4390-4399.
109. Shuang, F.; Hou, S. X.; Zhao, Y. T.; Zhong, H. B.; Xue, C.; Zhu, J. L.; Bu, G. Y.; Cao, Z. Characterization of an injectable chitosan-demineralized bone matrix hybrid for healing critical-size long-bone defects in a rabbit model. *Eur Rev Med Pharmacol Sci.* **2014**, *18*, 740-752.
110. Kim, S.; Bedigrew, K.; Guda, T.; Maloney, W. J.; Park, S.; Wenke, J. C.; Yang, Y. P. Novel osteoinductive photo-cross-linkable chitosan-lactide-fibrinogen hydrogels enhance bone regeneration in critical size segmental bone defects. *Acta Biomater.* **2014**, *10*, 5021-5033.
111. Luca, L.; Rougemont, A. L.; Walpoth, B. H.; Boure, L.; Tami, A.; Anderson, J. M.; Jordan, O.; Gurny, R. Injectable rhBMP-2-loaded chitosan hydrogel composite: osteoinduction at ectopic site and in segmental long bone defect. *J Biomed Mater Res A.* **2011**, *96*, 66-74.
112. Song, J.; Saiz, E.; Bertozzi, C. R. A new approach to mineralization of biocompatible hydrogel scaffolds: an efficient process toward 3-dimensional bonelike composites. *J Am Chem Soc.* **2003**, *125*, 1236-1243.
113. Song, J.; Malathong, V.; Bertozzi, C. R. Mineralization of synthetic polymer scaffolds: a bottom-up approach for the development of artificial bone. *J Am Chem Soc.* **2005**, *127*, 3366-3372.
114. Song, J.; Xu, J.; Filion, T.; Saiz, E.; Tomsia, A. P.; Lian, J. B.; Stein, G. S.; Ayers, D. C.; Bertozzi, C. R. Elastomeric high-mineral content hydrogel-hydroxyapatite composites for orthopedic applications. *J Biomed Mater Res A.* **2009**, *89*, 1098-1107.
115. Xu, J.; Li, X.; Lian, J. B.; Ayers, D. C.; Song, J. Sustained and localized in vitro release of BMP-2/7, RANKL, and tetracycline from FlexBone, an elastomeric osteoconductive bone substitute. *J Orthop Res.* **2009**, *27*, 1306-1311.
116. Filion, T. M.; Li, X.; Mason-Savas, A.; Kreider, J. M.; Goldstein, S. A.; Ayers, D. C.; Song, J. Elastomeric osteoconductive synthetic scaffolds with acquired osteoinductivity expedite the repair of critical femoral defects in rats. *Tissue Eng Part A.* **2011**, *17*, 503-511.
117. Sonnet, C.; Simpson, C. L.; Olabisi, R. M.; Sullivan, K.; Lazard, Z.; Gugala, Z.; Peroni, J. F.; Weh, J. M.; Davis, A. R.; West, J. L.; Olmsted-Davis, E. A. Rapid healing of femoral defects in rats with low dose sustained BMP2 expression from PEGDA hydrogel microspheres. *J Orthop Res.* **2013**, *31*, 1597-1604.
118. Almany, L.; Seliktar, D. Biosynthetic hydrogel scaffolds made from fibrinogen and polyethylene glycol for 3D cell cultures. *Biomaterials.* **2005**, *26*, 2467-2477.
119. Zhang, Z.; Feng, S. S. The drug encapsulation efficiency, in vitro drug release, cellular uptake and cytotoxicity of paclitaxel-loaded poly(lactide)-tocopheryl polyethylene glycol succinate nanoparticles. *Biomaterials.* **2006**, *27*, 4025-4033.
120. Xu, J.; Filion, T. M.; Prifti, F.; Song, J. Cytocompatible poly(ethylene glycol)-co-polycarbonate hydrogels cross-linked by copper-free, strain-promoted click chemistry. *Chem Asian J.* **2011**, *6*, 2730-2737.
121. Xu, J.; Feng, E.; Song, J. Bioorthogonally cross-linked hydrogel network with precisely controlled disintegration time over a broad range. *J Am Chem Soc.* ***2014***, *136*, 4105-4108.
122. Hubbell, J. A. Materials as morphogenetic guides in tissue engineering. *Curr Opin Biotechnol.* **2003**, *14*, 551-558.
123. Culver, J. C.; Hoffmann, J. C.; Poché, R. A.; Slater, J. H.; West, J. L.; Dickinson, M. E. Three-dimensional biomimetic patterning in hydrogels to guide cellular organization. *Adv Mater.* **2012**, *24*, 2344-2348.
124. Lei, Y.; Segura, T. DNA delivery from matrix metalloproteinase degradable poly(ethylene glycol) hydrogels to mouse cloned mesenchymal stem cells. *Biomaterials.* **2009**, *30*, 254-265.
125. Sridhar, B. V.; Brock, J. L.; Silver, J. S.; Leight, J. L.; Randolph, M. A.; Anseth, K. S. Development of a cellularly degradable PEG hydrogel to promote articular cartilage extracellular matrix deposition. *Adv Healthc Mater.* **2015**, *4*, 702-713.
126. Shekaran, A.; García, J. R.; Clark, A. Y.; Kavanaugh, T. E.; Lin, A. S.; Guldberg, R. E.; García, A. J. Bone regeneration using an alpha 2 beta 1 integrin-specific hydrogel as a BMP-2 delivery vehicle. *Biomaterials.* **2014**, *35*, 5453-5461.
127. Rosales, A. M.; Anseth, K. S. The design of reversible hydrogels to capture extracellular matrix dynamics. *Nature reviews Materials.* **2016**, *1*, 15012.
128. Chaudhuri, O.; Gu, L.; Klumpers, D.; Darnell, M.; Bencherif, S. A.; Weaver, J. C.; Huebsch, N.; Lee, H. P.; Lippens, E.; Duda, G. N.; Mooney, D. J. Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nat Mater.* **2016**, *15*, 326-334.
129. Tan, Y.; Huang, H.; Ayers, D. C.; Song, J. Modulating viscoelasticity,

stiffness, and degradation of synthetic cellular niches via stoichiometric tuning of covalent versus dynamic noncovalent cross-linking. *ACS Cent Sci.* **2018**, *4*, 971-981.

130. Fonseca, K. B.; Granja, P. L.; Barrias, C. C. Engineering proteolytically-degradable artificial extracellular matrices. *Prog Polym Sci.* **2014**, *39*, 2010-2029.
131. Chen, Y.; Zhang, J.; Liu, X.; Wang, S.; Tao, J.; Huang, Y.; Wu, W.; Li, Y.; Zhou, K.; Wei, X.; Chen, S.; Li, X.; Xu, X.; Cardon, L.; Qian, Z.; Gou, M. Noninvasive in vivo 3D bioprinting. *Sci Adv.* **2020**, *6*, eaba7406.
132. Urciuolo, A.; Poli, I.; Brandolino, L.; Raffa, P.; Scattolini, V.; Laterza, C.; Giobbe, G. G.; Zambaiti, E.; Selmin, G.; Magnussen, M.; Brigo, L.; De Coppi, P.; Salmaso, S.; Giomo, M.; Elvassore, N. Intravital three-dimensional bioprinting. *Nat Biomed Eng.* **2020**, *4*, 901-915.

Received: August 31, 2020
Revised: October 26, 2020
Accepted: October 29, 2020
Available online: December 28, 2020