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COMMENTARY

Engineering bone organoids: Recent advances and future prospects

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Abstract

Bone organoids have emerged as powerful three-dimensional (3D) culture systems that recapitulate key aspects of bone physiology and pathology, offering superior translational relevance compared with traditional two-dimensional cultures and animal models. By integrating stem cell-derived lineages with biomimetic matrices that mimic the native extracellular matrix, bone organoids faithfully reproduce cellular heterogeneity, structural organization, and dynamic remodeling. Recent advances in natural and synthetic hydrogels, bioactive signaling, and diverse cell sources—including osteogenic, osteoclastic, hematopoietic, and adipogenic populations—have further enhanced organoid fidelity. Biofabrication strategies, such as scaffold-assisted assembly, 3D bioprinting, and organ-on-a-chip platforms enable spatial control and vascularization, while CRISPR-based gene editing and artificial intelligence-driven optimization offer unprecedented precision and scalability. The development of vascularized, innervated, and multi-system–integrated bone organoids holds great promise for disease modeling, drug screening, and regenerative therapies. This review outlines present strategies, technological advances, and future directions in bone organoid engineering.

Keywords: Bone organoids; Biofabrication strategies; Prospects

1. Introduction

Bone-related disorders, such as critical-sized bone defects, bone tumors, osteoporosis, and osteoarthritis have long been major global health concerns, imposing substantial socioeconomic and clinical burdens. A comprehensive understanding of bone development, remodeling, and disease pathogenesis is therefore essential for improving therapeutic strategies. Conventionally, pre-clinical bone research has relied heavily on two-dimensional cell cultures and animal models. While these approaches have provided valuable insights, their poor physiological relevance, lack of tissue heterogeneity, and species-specific differences frequently result in translational failure in clinical settings.[1]

Organoids, derived from stem cells and supported by bioactive matrices, are self-organizing and self-renewing three-dimensional (3D) culture systems that recapitulate the architectural complexity and functional characteristics of native tissues. Bone organoids, in particular, hold great promise for faithfully mimicking the *in vivo* bone microenvironment, capturing cellular heterogeneity, and

preserving genomic stability. These properties enable modeling of physiological processes, such as ossification, bone remodeling, and hematopoiesis, as well as pathological changes associated with bone diseases. By offering a more accurate and human-relevant platform, bone organoids are advancing our understanding of skeletal development and disease mechanisms, with significant potential for drug screening, precision medicine, and regenerative therapies.[2]

2. Biomimetic materials for bone organoid engineering

The extracellular matrix (ECM) of bone tissue is a hierarchically organized microenvironment composed predominantly of type I collagen (>95% of dry weight), together with elastin, glycosaminoglycans, and proteoglycans. These components collectively provide structural integrity, biochemical signaling, and mechanical support critical for bone development, remodeling, and regeneration. Designing biomimetic materials for bone organoid engineering thus requires recapitulating the native ECM's stiffness, geometry, fiber diameter, and biochemical milieu to guide cell adhesion, proliferation, differentiation, and tissue-specific organization.[3]

2.1. Natural biomaterials

Natural hydrogels, such as gelatin, collagen, alginate, chitosan, hyaluronic acid, and fibrin closely resemble human ECM in both structure and function. These materials are biocompatible and biodegradable, supporting cell adhesion, migration, and signal transduction. They also serve as reservoirs for soluble cytokines and growth factors, facilitating sustained release and gradient formation within the organoid microenvironment. However, natural materials often exhibit batch variability, limited mechanical strength, and rapid degradation, restricting their utility in long-term cultures. Some natural materials, such as alginate and chitosan, lack inherent cell-adhesive domains and require chemical modification (e.g., RGD conjugation). Crosslinking and composite strategies (e.g., gelatin-methacrylate, collagen–polyethylene glycol [PEG] blends) can improve mechanical properties, but precise tuning remains challenging. In addition, their immunogenicity and degradation byproducts may interfere with bone matrix deposition.

2.2. Synthetic polymers and composite materials

PEG, polyvinyl alcohol, polylactic acid, and polyacrylamide offer superior control over chemical composition, stiffness, porosity, and degradation kinetics. Being free of animal-derived components, they enhance reproducibility and clinical compatibility. Advanced modifications allow the incorporation of matrix metalloproteinase-cleavable motifs and integrin-binding domains to better recapitulate ECM remodeling and cell–matrix interactions. Across rational design, synthetic hydrogels can mimic the matrix stiffness (10–30 kPa), fiber diameter (~100 nm), and topography of bone tissue, thereby promoting osteogenic lineage commitment and spatial organization. However, purely synthetic systems lack intrinsic signaling capacity and often require external supplementation with exogenous growth factors. Furthermore, the degradation of certain polymers can produce acidic byproducts that compromise long-term cell viability. Compared with natural hydrogels, synthetic polymers are more predictable and scalable but biologically inert, making them more suitable for translational applications where standardization is prioritized over microenvironmental complexity.[4]

3. Cell source selection for bone organoid construction

Effective bone organoid engineering relies not only on the scaffold but also on the selection and integration of appropriate cell types that recapitulate the dynamic cellular ecosystem of bone. Bone homeostasis involves a triad of coordinated biological processes: Osteogenesis, osteoclastogenesis, and hematopoiesis.

3.1. Osteogenic cells

Human induced pluripotent stem cells (hiPSCs) are particularly attractive due to their patient specificity and genetic stability, which enable personalized disease modeling. However, hiPSC-derived osteoblasts often display immature phenotypes, requiring extended differentiation protocols or additional cues, such as mechanical stimulation to achieve full functionality. By contrast, mesenchymal stromal cells (MSCs) remain the most widely used due to their robust osteogenic potential and immunomodulatory properties. However, MSCs exhibit donor-to-donor variability and limited long-term expansion, complicating reproducibility across laboratories. Embryonic stem cells demonstrate strong osteogenic capacity but are associated with ethical concerns and stringent regulatory barriers.[5]

3.2. Osteoclast pre-cursors

The incorporation of monocyte/macrophage-derived pre-cursors is essential to recapitulate bone resorption dynamics. While these cells add physiological relevance, they present challenges, such as short lifespan in culture and high sensitivity to cytokine concentrations. Studies have shown that co-culturing osteoclast pre-cursors with MSC-derived osteoblasts enhances remodeling fidelity; however, maintaining the balance between bone formation and resorption remains difficult in long-term culture.[6]

3.3. Adipocytes

Adipocytes are increasingly recognized as key modulators of bone homeostasis through their interaction with

osteoblasts and osteoclasts. They contribute to establishing a physiologically relevant marrow-like environment but, if not tightly regulated, may skew organoids toward adipogenesis. Adipose-derived stem cells provide a convenient, accessible source for generating both osteogenic and vascularized constructs; however, their osteogenic capacity is generally weaker than that of bone marrow-derived MSCs.[7]

3.4. Hematopoietic and supportive niche cells

Hematopoietic stem and progenitor cells play a pivotal role in coupling bone formation with immune and vascular networks. Their integration into bone organoids enhances physiological relevance and supports the construction of multifunctional hematopoietic niches that more closely recapitulate *in vivo* bone marrow environments.[8]

4. Cytokines and small-molecule inducers

Biochemical cues are indispensable for directing lineage specification, tissue organization, and functional maturation in bone organoid systems. A well-orchestrated combination of cytokines and small-molecule inducers can recapitulate the *in vivo* signaling cascades that regulate osteogenesis, chondrogenesis, and hematopoietic niche formation. However, their application requires nuanced control over dose, timing, and context to avoid off-target effects or aberrant differentiation.

4.1. Bone morphogenetic proteins (BMPs)

BMPs, particularly BMP-2 and BMP-7, are potent osteoinductive factors that activate Smad1/5/8 signaling to promote Runx2-mediated osteoblast differentiation. In bone organoids, BMPs facilitate mineralized matrix deposition; however, supraphysiological doses can cause ectopic ossification and fibrosis. Sustained release via hydrogel encapsulation or microfluidic perfusion systems helps mitigate these risks by enabling spatiotemporal control.[9]

4.2. Transforming growth factor beta (TGF-β) family

TGF-β1 and TGF-β3 are crucial for early-stage mesenchymal condensation, chondrogenesis, and ECM remodeling. In 3D cultures, TGF-β signaling supports matrix deposition and progenitor proliferation but may suppress late-stage mineralization if not carefully modulated. Its dual role in promoting osteogenesis and fibrosis underscores the importance of stage-specific application. Crosstalk with BMP, Notch, and Hippo pathways further complicates its utility, necessitating combinatorial and sequential induction strategies.[10]

4.3. Dexamethasone, ascorbic acid, and β-glycerophosphate

This classical osteogenic cocktail remains a cornerstone in bone tissue engineering. Dexamethasone upregulates osteogenic transcription factors (Runx2, Osterix), ascorbic acid stimulates collagen synthesis, and β-glycerophosphate supplies inorganic phosphate for hydroxyapatite formation. However, prolonged dexamethasone exposure can induce senescence and impair stem cell potency. Optimizing dose and duration, or replacing glucocorticoids with small molecules, such as purmorphamine (a Hedgehog pathway activator), has emerged as a strategy to improve physiological relevance.[11]

4.4. Emerging inducers and combinatorial approaches

Recent studies have introduced Wnt agonists (e.g., CHIR99021), vascular endothelial growth factor for angiogenesis, and interleukin-6 family cytokines for hematopoietic support within organoids. Combinatorial gradient systems, often implemented through microfluidics, allow regional specification (e.g., cortical vs. trabecular zones), thereby mimicking native bone compartments. However, the field still lacks consensus on optimal induction protocols, and most approaches remain empirically designed rather than guided by predictive modeling.[12]

5. Advanced biofabrication strategies for bone organoids

5.1. Scaffold-assisted techniques

Scaffold-based strategies mimic the architecture and function of bone by combining cells with biomaterial scaffolds. Key considerations for scaffold selection include mechanical strength, porosity, biocompatibility, and degradation profile. Embedding induced pluripotent stem cell (iPSC)-derived chondrocytes or co-culturing dental pulp stem cells and bone marrow-derived MSCs within 3D scaffolds has been shown to promote vascularization, enhance viability, and facilitate matrix mineralization.

5.2. Cellular self-assembly

Cellular self-assembly leverages the intrinsic ability of cells to organize into complex 3D structures without the use of external scaffolds. By selecting appropriate stem and progenitor cells and optimizing their density, distribution, and exposure to growth factors and matrix cues, cells can self-organize into tissue-like architectures. Mechanical stress, electrical stimulation, and inductive biomolecules further enhance tissue maturation. For example, induced pluripotent stem cells (iPSCs) cultured in Matrigel–collagen hydrogels with sequential growth factor supplementation have been guided to form vascularized bone marrow organoids containing fibroblasts, endothelial cells, and hematopoietic lineages.[13]

5.3. 3D bioprinting

3D bioprinting enables the layer-by-layer deposition of cell-laden bioinks—comprising cells, hydrogels, and

bioactive molecules—to fabricate complex bone-like architectures with controlled spatial resolution. Recent advancements in extrusion-based, inkjet, and light-assisted printing have facilitated the recreation of osteon-mimetic architectures, vascular channels, and stiffness gradients, thereby enhancing the structural and functional fidelity of engineered bone tissues. The integration of bioactive materials with MSCs or iPSC-derived osteoblasts allows precise regulation of both mechanical and biological parameters. Furthermore, bioprinting facilitates the spatial co-localization of multiple cell types, such as osteoblasts and endothelial cells, within defined microdomains.[14]

5.4. Organ-on-a-chip technology

Microfluidic organ-on-a-chip systems integrate ECM scaffolds, dynamic flow, and mechanical cues to simulate physiological conditions. These platforms enable real-time manipulation of microenvironmental factors, such as shear stress, nutrient gradients, and mechanical loading, all of which are critical for bone development and remodeling. Recent studies have established co-culture systems that mimic synovial–cartilage interactions on 3D chip platforms, offering valuable insights into joint pathophysiology and drug response.[15]

5.5. Gene editing

Gene editing has become integral to mechanistic studies and patient-specific modeling within 3D systems. In hiPSC-based models of osteogenesis imperfecta, CRISPR/Cas9-mediated correction of *COL1A1* restored osteogenic differentiation, providing a blueprint for generating isogenic pairs to validate therapeutics in bone organoids.[16] More recently, deletion of non-coding *RUNX1* loci identified through genome-wide association studies in hiPSC-derived mesenchymal progenitors enhanced osteogenic potential and *in vivo* bone regeneration, illustrating how editing regulatory elements can fine-tune lineage output.[17]

5.6. Artificial intelligence (AI)

Machine-learning pipelines increasingly support the optimization of bioink formulations, printing parameters, and culture regimens, thereby reducing trial-and-error and improving reproducibility across laboratories. Recent reviews and methodological studies describe closed-loop frameworks that predict printability and fidelity from rheology and process inputs. Meanwhile, deep-learning image analysis—using optical coherence tomography, convolutional neural networks, or volumetric networks—automates morphometric analysis, quantification of mineral deposition, and detection of vascular networks in 3D constructs. Although many of these applications are tissue-agnostic, they are technically transferable to bone and are already being used to accelerate optimization cycles and standardize quality control at scale.[18]

6. Future research prospects

Future research should focus on enhancing structural and functional complexity, improving vascularization, integrating multiple physiological systems, and advancing clinical readiness. Present bone organoid models often replicate isolated features, such as mineralization zones, trabeculae, or marrow-like cavities, but fail to recapitulate the fully integrated cortical, cancellous, and marrow architecture. Advanced 3D bioprinting, high-resolution lithography, and self-assembling biomaterials hold promise for producing hierarchical, osteon-mimetic structures with precise spatial organization and biomechanical properties. In parallel, efforts should be directed toward integrating vasculature, lymphatic-like structures, and innervation to more accurately simulate the native bone microenvironment.

Vascularization remains a central bottleneck, as diffusion limits for nutrient and oxygen diffusion restrict organoid size, maturation, and longevity. Strategies, such as co-culturing with endothelial cells, incorporating angiogenic factors, and employing microfluidic organ-on-a-chip systems can provide dynamic perfusion and promote vessel formation. Combining these approaches with hypoxia pre-conditioning or gradient-based signaling may further enhance vessel stability and functionality, enabling long-term culture and physiologically relevant models. In addition, emerging four-dimensional printing enables the use of stimuli-responsive biomaterials to produce structures that change shape over time in response to intrinsic or external cues, such as self-folding tubes formed in culture media.[19] These dynamic scaffolds can adapt to vascularization and cell behaviors in defect-specific microenvironments, potentially overcoming the vascularization barrier in bone tissue engineering without the need for pre-formed vascular networks.

Multi-system integration represents a critical frontier in bone organoid research, as bone physiology is intricately linked with the immune, endocrine, and nervous systems. Future platforms should incorporate immune cell populations, hormonal signaling, and neuro-osteogenic interactions to enable the study of systemic regulation and the modeling of complex disorders, such as osteoporosis, osteoarthritis, bone metastases, and rare skeletal dysplasias. Concurrently, the integration of AI and data-driven optimization is expected to accelerate advances in culture protocols, biomaterial formulations, and differentiation strategies. Machine learning can predict optimal combinations of bioactive factors, mechanical cues, and environmental conditions, thereby enabling reproducible large-scale production. In parallel, AI-assisted imaging and high-content screening will enhance quality control and functional evaluation. Gene-editing technologies,

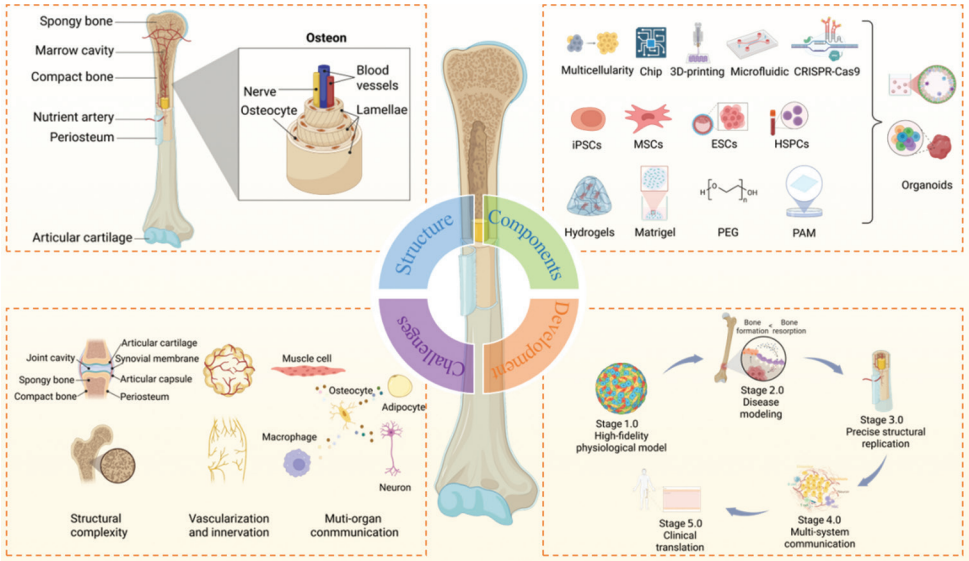

Figure 1. Schematic overview of bone organoid design and developmental roadmap, highlighting anatomical and histological features of native bone, workflows and strategies for engineering bone organoids, present challenges in their development, and predicted future developmental stages. Image created by the authors.

Abbreviations: 3D: Three-dimensional; ESCs: Embryonic stem cells; HSPCs: Hematopoietic stem and progenitor cells; iPSCs: Induced pluripotent stem cells; MSCs: Mesenchymal stromal cells; PAM: Polyacrylamide; PEG: Polyethylene glycol.

particularly CRISPR/Cas9, will further support the generation of patient- and disease-specific models, allowing precise dissection of the genetic determinants of bone development and pathology, validation of therapeutic targets, and testing of personalized interventions. The convergence of patient-derived iPSCs, organoid engineering, AI, and genomic medicine holds the potential to fundamentally transform skeletal disease research and regenerative therapies.

In the long term, the evolution of bone organoids is expected to follow a staged roadmap. Stage 1.0 will involve the development of high-fidelity physiological models that replicate the cellular composition, ECM architecture, and basic functions of native bone. Stage 2.0 will introduce pathological features through gene editing and microenvironment modulation, enabling disease modeling of osteoporosis, osteoarthritis, tumors, and rare dysplasias. Stage 3.0 will focus on precise structural replication to reconstruct cortical–trabecular interfaces and osteon-like units, while achieving vascularization, innervation, and stable long-term multi-cell co-cultures. Stage 4.0 will integrate immune, endocrine, and nervous systems, guided by AI-driven optimization of culture conditions, biomaterials, and quality control processes. Ultimately, Stage 5 will mark clinical translation, with Good Manufacturing Practice-compliant, scalable manufacturing of patient-specific organoids for drug screening and regenerative therapies (Figure 1). Achieving these milestones will require close multidisciplinary collaboration across developmental biology, materials science, mechanical engineering, computational biology, and clinical orthopedics, positioning bone organoids as versatile platforms for research, precision medicine, and tissue engineering.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

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Writing – review & editing: Wenzhao Wang, Haijian Sun

Ethics approval and consent to participate

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References

1. Zujur D, Kanke K, Lichtler AC, Hojo H, Chung UI, Ohba S. Three-dimensional system enabling the maintenance and directed differentiation of pluripotent stem cells under defined conditions. *Sci Adv*. 2017;3(5):e1602875.

   doi: 10.1126/sciadv.1602875

2. Kratochvil MJ, Seymour AJ, Li TL, Pasca SP, Kuo CJ, Heilshorn SC. Engineered materials for organoid systems. *Nat Rev Mater*. 2019;4(9):606-622.

   doi: 10.1038/s41578-019-0129-9

3. Zhao T, Huang Y, Zhu J, *et al*. Extracellular matrix signaling cues: Biological functions, diseases, and therapeutic targets. *MedComm (2020)*. 2025;6(8):e70281.

   doi: 10.1002/mco2.70281

4. Vallmajo-Martin Q, Broguiere N, Millan C, Zenobi-Wong M, Ehrbar M. PEG/HA hybrid hydrogels for biologically and mechanically tailorable bone marrow organoids. *Adv Funct Mater*. 2020;30(48):1910282.

   doi: 10.1002/adfm.201910282

5. Jones GL, Walton R, Czernuszka J, Griffiths SL, El Haj AJ, Cartmell SH. Primary human osteoblast culture on 3D porous collagen-hydroxyapatite scaffolds. *J Biomed Mater Res A*. 2010;94(4):1244-1250.

   doi: 10.1002/jbm.a.32805

6. Siddiqui JA, Partridge NC. Physiological bone remodeling: Systemic regulation and growth factor involvement. *Physiology (Bethesda)*. 2016;31(3):233-245.

   doi: 10.1152/physiol.00061.2014

7. Yu W, Zhong L, Yao L, *et al*. Bone marrow adipogenic lineage precursors promote osteoclastogenesis in bone remodeling and pathologic bone loss. *J Clin Invest*. 2021;131(2):e140214.

   doi: 10.1172/JCI140214

8. Torisawa YS, Spina CS, Mammoto T, *et al*. Bone marrow-on-a-chip replicates hematopoietic niche physiology *in vitro*. *Nat Methods*. 2014;11(6):663-669.

   doi: 10.1038/nmeth.2938

9. Yan CP, Wang XK, Jiang K, *et al*. β-ecdysterone enhanced bone regeneration through the BMP-2/SMAD/RUNX2/osterix signaling pathway. *Front Cell Dev Biol*. 2022;10:883228.

   doi: 10.3389/fcell.2022.883228

10. Kwon H, Paschos NK, Hu JC, Athanasiou K. Articular cartilage tissue engineering: The role of signaling molecules. *Cell Mol Life Sci*. 2016;73(6):1173-1194.

    doi: 10.1007/s00018-015-2115-8

11. Chen J, He Y, Shan C, Pan Q, Li M, Xia D. Topical combined application of dexamethasone, vitamin C, and β-sodium glycerophosphate for healing the extraction socket in rabbits. *Int J Oral Maxillofac Surg*. 2015;44(10):1317-1323.

    doi: 10.1016/j.ijom.2015.06.011

12. Guo Q, Chen J, Bu Q, *et al*. Establishing stable and highly osteogenic hiPSC-derived MSCs for 3D-printed bone graft through microenvironment modulation by CHIR99021-treated osteocytes. *Mater Today Bio*. 2024;26:101111.

    doi: 10.1016/j.mtbio.2024.101111

13. Ryu NE, Lee SH, Park H. Spheroid culture system methods and applications for mesenchymal stem cells. *Cells*. 2019;8(12):1620.

    doi: 10.3390/cells8121620

14. Zhao X, Li N, Zhang Z, *et al*. Beyond hype: Unveiling the real challenges in clinical translation of 3D printed bone scaffolds and the fresh prospects of bioprinted organoids. *J Nanobiotechnology*. 2024;22(1):500.

    doi: 10.1186/s12951-024-02759-z

15. Rothbauer M, Byrne RA, Schobesberger S, *et al*. Establishment of a human three-dimensional chip-based chondro-synovial coculture joint model for reciprocal cross talk studies in arthritis research. *Lab Chip*. 2021;21(21):4128-4143.

    doi: 10.1039/d1lc00130b

16. Cao Y, Li L, Ren X, *et al*. CRISPR/Cas9 correction of a dominant cis-double-variant in COL1A1 isolated from a patient with osteogenesis imperfecta increases the osteogenic capacity of induced pluripotent stem cells. *J Bone Miner Res*. 2023;38(5):719-732.

    doi: 10.1002/jbmr.4783

17. Khan NM, Wilderman A, Kaiser JM, *et al*. Enhanced osteogenic potential of iPSC-derived mesenchymal progenitor cells following genome editing of GWAS variants in the *RUNX1* gene. *Bone Res*. 2024;12(1):70.

    doi: 10.1038/s41413-024-00369-x

18. Bai L, Wu Y, Li G, Zhang W, Zhang H, Su J. AI-enabled organoids: Construction, analysis, and application. *Bioact Mater*. 2024;31:525-548.

    doi: 10.1016/j.bioactmat.2023.09.005

19. Kirillova A, Maxson R, Stoychev G, Gomillion CT, Ionov L. 4D biofabrication using shape-morphing hydrogels. *Adv Mater*. 2017;29(46):1703443.

    doi: 10.1002/adma.201703443