Potential of organ-on-a-chip in advancing synthetic extracellular matrix technology for bone tissue engineering in dentistry (Review)

MUHAMMAD HIDAYAT SYAHRUDDIN[1,2], IKA DEWI ANA[2-4], DMITRY BELYAEV[5], DYAH IRNAWATI[6], HEVI WIHADMADYATAMI[4,7], NATALIA BESHCHASNA[5] and RAHMI ANGGRAENI[4,8]

[1]Doctoral Program in Dental Sciences, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; [2]Center of Excellence for Carbonate Apatite-based Extracellular Matrix and Adjuvant, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; [3]Department of Dental Biomedical Sciences, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; [4]Research Collaboration Center for Biomedical Scaffolds, National Research and Innovation Agency-Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; [5]Department of Bio- and Nanotechnology, Group of Biodegradation and Nanofunctionalization, Fraunhofer Institute for Ceramic Technologies and Systems, D-01109 Dresden, Germany; [6]Department of Dental Biomaterials Sciences, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; [7]Department of Anatomy, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; [8]Research Center for Preclinical and Clinical Medicine, National Research and Innovation Agency of The Republic of Indonesia, Cibinong Science Center, Bogor 16915, Indonesia

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Abstract. Bone tissue engineering (BTE) is a cutting-edge approach within biomedical sciences, especially in regenerative medicine, addressing key challenges such as organ transplantation and complex tissue repair. At the core of BTE is the development of biomimetic scaffolds to replicate native tissue environments. However, conventional models often fall short in accurately mimicking the complexity of human tissue microenvironments. Organ-on-a-chip (OOAC) technology offers a transformative alternative. These microscale systems combine microfluidics, biomaterials and cell cultures to emulate the structural and functional characteristics of human tissue. OOAC platforms facilitate dynamic, real-time evaluation of scaffold biocompatibility, cellular interactions and mechanical properties under physiological conditions. By overcoming the limitations of traditional preclinical models, OOAC systems minimize the need for animal testing, improve predictive accuracy for *in vivo* outcomes and accelerate the path to clinical translation. The present study aimed to summarize scaffold development for BTE, with a focus on dental applications, and highlights the integration of OOAC technology. These innovations possess the potential to revolutionize scaffold design and advance broader biomedical research applications.

Correspondence to: Professor Ika Dewi Ana, Department of Dental Biomedical Sciences, Faculty of Dentistry, Universitas Gadjah Mada, Jalan Denta 1, Sekip Utara, Yogyakarta 55281, Indonesia
E-mail: ikadewiana@ugm.ac.id

Professor Natalia Beshchasna, Department of Bio- and Nanotechnology, Group of Biodegradation and Nanofunctionalization, Fraunhofer Institute for Ceramic Technologies and Systems, Winterbergstr 28, D-01109 Dresden, Germany
E-mail: natalia.beshchasna@ikts.fraunhofer.de

Abbreviations: 3R, reduction, refinement and replacement; BBB, blood-brain barrier; BTE, bone tissue engineering; ECM, extracellular matrix; FDA, Food and Drug Administration; FSS, flow shear stress; GBD, global burden of disease; MSC, mesenchymal stem cell; NGF, nerve growth factor; OOAC, organ-on-a-chip; OS, osteosarcoma; RM, regenerative medicine; SLA, stereolithography

Key words: organ-on-a-chip, bioengineering, biomedicine, microfluidics, scaffold, dentistry, sustainable development goal

Contents

1. Introduction

Certain animal species exhibit regenerative abilities, such as amphibians and zebrafish, which can restore lost or damaged body parts (1). As scientific understanding of this phenomenon

has deepened, focus has shifted toward overcoming the limitations of human healing (2-4). Unlike regenerative species, humans lack the capacity to fully regenerate complex tissue after injury or disease. This biological limitation has spurred the advancement of regenerative medicine (RM), an interdisciplinary field that integrates biology, engineering, and clinical sciences to promote tissue repair or replacement, stimulate organ regeneration, restore lost function, and reduce reliance on donor transplants in cases of disease, trauma, or congenital conditions (2-4).

Traditional medical treatments frequently encounter two major challenges: The scarcity of suitable tissue or organ donors and the potential for immune rejection in recipients (5,6). These issues have driven the evolution of tissue engineering (TE), now a pivotal component of RM. TE employs biomaterials, biological signals and engineering techniques to develop structures that facilitate healing or replace damaged tissue (7). This approach offers therapeutic solutions that are adaptable, scalable, and more compatible with natural repair mechanisms of the body (7-9).

Bone tissue, although dynamic and naturally capable of self-repair, can be critically impaired by traumatic injury or congenital conditions such as osteogenesis imperfecta and fibrous dysplasia (10,11) In such cases, its regenerative capacity may prove inadequate (12-16). Surgical interventions remain a standard treatment, yet they are not always effective, particularly in cases involving complex defects (17,18). TE provides an alternative pathway to the creation of constructs that replicate the native structure and function of bone, aiming to improve biological integration and expedite recovery.

Progress in bone TE (BTE) has introduced a systematic approach involving the application of porous scaffolds, bioactive signals and cell-based approaches (19-22). These scaffolds serve as three-dimensional (3D) frameworks that simulate the extracellular matrix, offering physical support and directing cell activity. Crucial parameters such as porosity, mechanical strength and surface chemistry determine the effectiveness of these constructs *in vivo* (23). To fulfil these criteria, advanced fabrication techniques, such as microfluidics-assisted manufacturing, have been used to produce biomaterials with precise microscale architectural control (24,25).

Organ-on-a-chip (OOAC) technology is a micro-engineered platform capable of mimicking organ functions on a chip-scale device (26). By integrating principles from materials science, fluid dynamics and cellular biology, OOAC enables the modeling of tissue interactions under controlled physiologically relevant conditions (27). Compared with static cultures and animal studies, OOAC offers more accurate insight into cellular responses and biomaterial compatibility, while also addressing ethical concerns and translational challenges (28-30). Although the applications of OOAC in BTE remain limited due to the novelty of the technology and the predominance of conventional *in vitro* and *in vivo* platforms in preclinical research, initial studies indicate potential for preclinical scaffold evaluation (31-33).

In dental and craniofacial research, OOAC systems have demonstrated promise in replicating the bone and periodontal microenvironments. These models facilitate real-time observation of cell behavior under mechanical forces, responses to biomaterials and contributions to mineral formation. Such platforms provide innovative methods for assessing the performance of scaffolds, drug delivery carriers and regenerative therapies, thereby decreasing reliance on animal testing (31,34). As personalized medicine continues to advance, OOAC may become a key interface between laboratory innovation and clinical application, particularly in the development of customized regenerative solutions (34). The present review aimed to summarize the role of OOAC in BTE and its challenges and prospects in comparison with conventional approaches.

2. Methods

The literature search was performed using PubMed (pubmed.ncbi.nlm.nih.gov/), ScienceDirect (sciencedirect.com/), and Scopus (https://www.scopus.com/) databases, employing a combination of controlled vocabulary and free-text terms. The search string applied was: ('organ-on-a-chip' OR 'organ chip' OR 'organ-on-chip') AND ('scaffold' OR 'bone tissue engineering' OR 'biomaterial'). The search was further refined using the following criteria: i) Full text availability, ii) published between January 2014 and January 2024 and iii) written in English language (Fig. 1). These criteria were chosen to ensure the inclusion of the most recent, relevant and high-impact advancements in the rapidly evolving field of OOAC technology and its incorporation into BTE applications. Titles and abstracts were screened by two independent reviewers (MHS and IDA), and the full texts of eligible articles were subsequently reviewed for inclusion. Any discrepancies were resolved through discussion. The initial PubMed and ScienceDirect searches yielded 2,311 and 2,945 studies related to OOAC, respectively. Of these, 118 articles were associated with scaffold fabrication, TE or BTE. Scopus database yielded 43 additional articles; six of these overlapped with those identified in PubMed and were therefore excluded. In total, 155 articles were identified. Inclusion criteria were applied to exclude manuals, notes, or guidelines, retaining only review articles, systematic reviews or original research papers.

3. Bone overview: Biology, common problems and the remodeling process

Bones are complex, dynamic and highly organized structures (35). They possess a unique combination of stiffness and the ability to transmit compressive, bending and torsional loads (36). In TE, a deep understanding of the fundamental structure of bone tissue is essential for effectively replicating its structure and function. The bone framework is composed of two layers of osseous tissue. The cortical bone, which forms the external surface, is characterized by low porosity and high mechanical strength. This dense outer layer provides structural support and protection. By contrast, cancellous (trabecular) bone exhibits a porosity ranging from 80 to 90%. This spongy inner layer is metabolically active and plays a critical role in facilitating joint function and limb movement (36-39).

The quantity and quality of bone are key determinants of its strength. Bone quantity is influenced by factors such as bone mineral density and porosity, whereas bone quality is associated with material composition and characteristics, including microstructures, geometry and mechanical properties (Table I) (39-49). Bone tissue features a highly

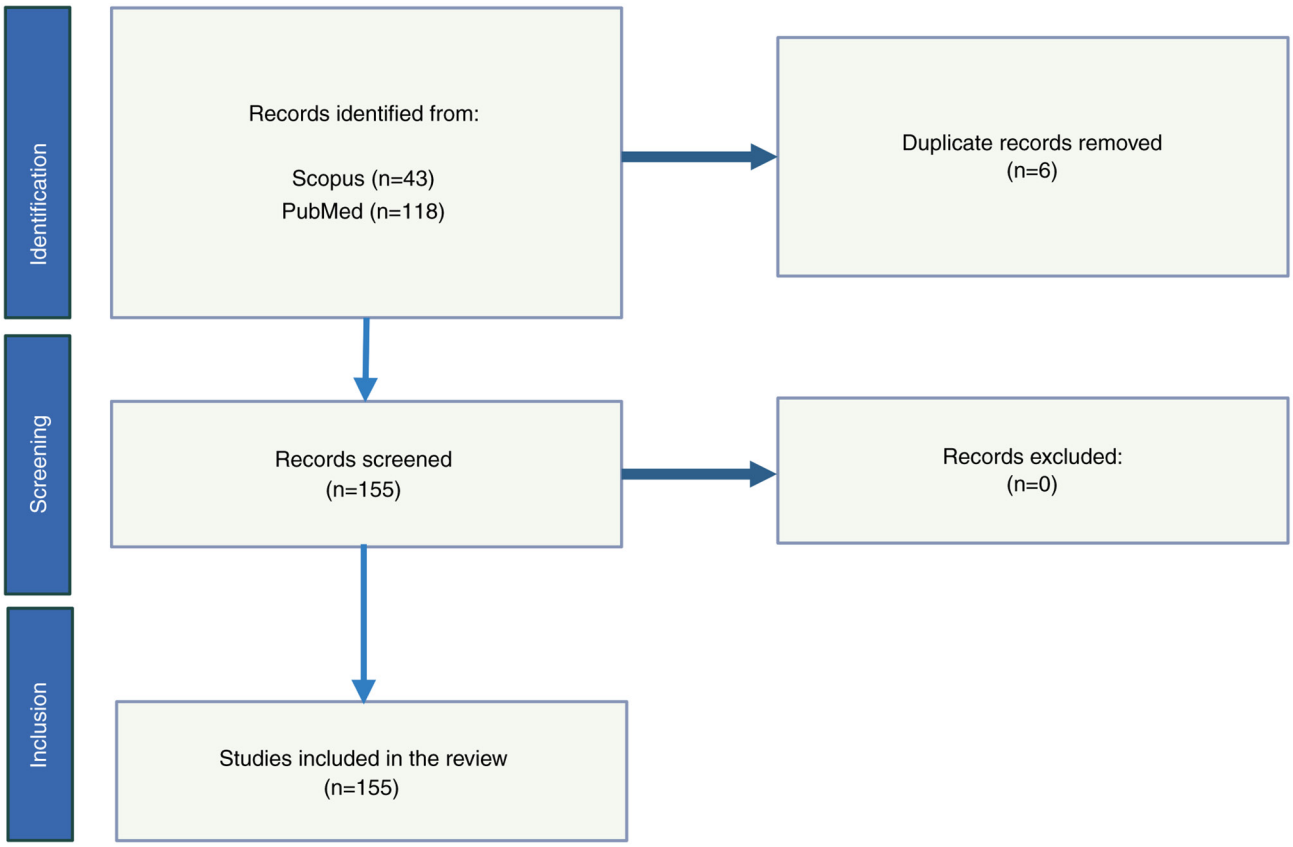

Figure 1. Identification, screening and inclusion of the articles for the study.

developed vascular network and comprises multiple components, including the osteoid matrix, primarily made of type I collagen fibers, along with the mineral bone salt hydroxyapatite, calcium phosphate (CaP), water, non-collagenous protein, proteoglycans, lipids, growth factors, cytokines and bone cells (Fig. 2) (50-54). The skeletal system includes four distinct types of bone cells (osteoblasts, osteoclasts, osteocytes and bone lining cells), which maintain an integrated skeletal structure (55,56). Osteoblasts are responsible for bone formation (57-60), while osteoclasts mediate bone resorption (61,62). Bone lining cells contribute to osteoclast differentiation and inhibit direct contact between osteoclasts and the bone matrix (56,57). Furthermore, osteocytes possess mechanosensitive capabilities that enable intercellular communication between bone cells and serve a regulatory role in the activity of osteoblasts and osteoclasts (63-70).

While bone tissue is key for providing structural support and serving as a reservoir for vital ions in the body (71), it is susceptible to various conditions that compromise its function and integrity, including osteoporosis, osteonecrosis, infection and inflammation, cancer, metastases and autoimmune conditions, all of which impair this tissue (72). These conditions contribute to decreased quality of life and/or increased mortality. For example, osteoporosis notably diminishes bone mass and alters bone structure, thereby increasing the risk of fractures (73). Recognized as a serious public health concern, osteoporosis affects ~200 million people worldwide (74), with a global prevalence of ~18%, according to a meta-analysis (75). Data from the US and the United Kingdom further indicate that fractures are highly prevalent, with >500.000 (UK) and 1.5 million (US) fragility fractures each year impacting millions of patients and placing a considerable financial strain (76,77). In addition to osteoporosis, evidence, including findings from the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD), consistently highlights the high global incidence and prevalence of osteoarthritis (78,79). This condition is especially widespread among the elderly, female patients, and correlated with factors such as age, sex, race/ethnicity, education level, comorbidities, and BMI (80-84).

In addition to degenerative conditions such as osteoporosis and osteoarthritis, malignant conditions such as osteosarcoma (OS) pose significant challenges in tumor therapy. These challenges stem from difficulties in establishing reliable biomarkers, understanding the mechanisms behind recurrence and accurately identifying the involved cell type. OS is the most common malignant primary bone tumor affecting both children and adults. Identifying the specific cell type is key, as conventional OS can be subdivided into distinct histological subtypes, including osteoblastic, fibroblastic, chondroblastic, epithelioid, giant cell-rich, small cell and telangiectatic forms (85-88). The estimated global incidence of OS is 2-4 cases per million individuals per year. The disease shows two distinct peaks in age distribution: The first, more pronounced, occurs between 15 and 19 years, and the second, less prominent, is observed in individuals aged >60 years (89,90). Additionally, bone tissue is susceptible to metastasis, and disorders such as OS can invade adjacent tissue (91,92).

Table I. Mechanical properties of human bone.

Bone type	Porosity, %	Modulus, GPa		Strength, MPa		Poisson's Ratio	(Refs.)
Compact	3-5	Longitudinal	17.90±3.90	Tension	135.00±15.60	0.40±0.16	(41-49)
				Compression	205±17.30		
		Transverse	10.10±2.49	Tension	53.00±10.70	0.62±0.26	
				Compression	131.00±20.70		
		Shear	3.30±0.40	Shear	65.00±4.00		
Trabecular	>90	Vertebra	0.07±0.05	2.40±1.60			(41-49)
		Tibia	0.45±0.26	5.30±2.90			
		Femur	0.44±0.27	6.80±4.80			

MPa, megapascals; GPa, Gigapascals.

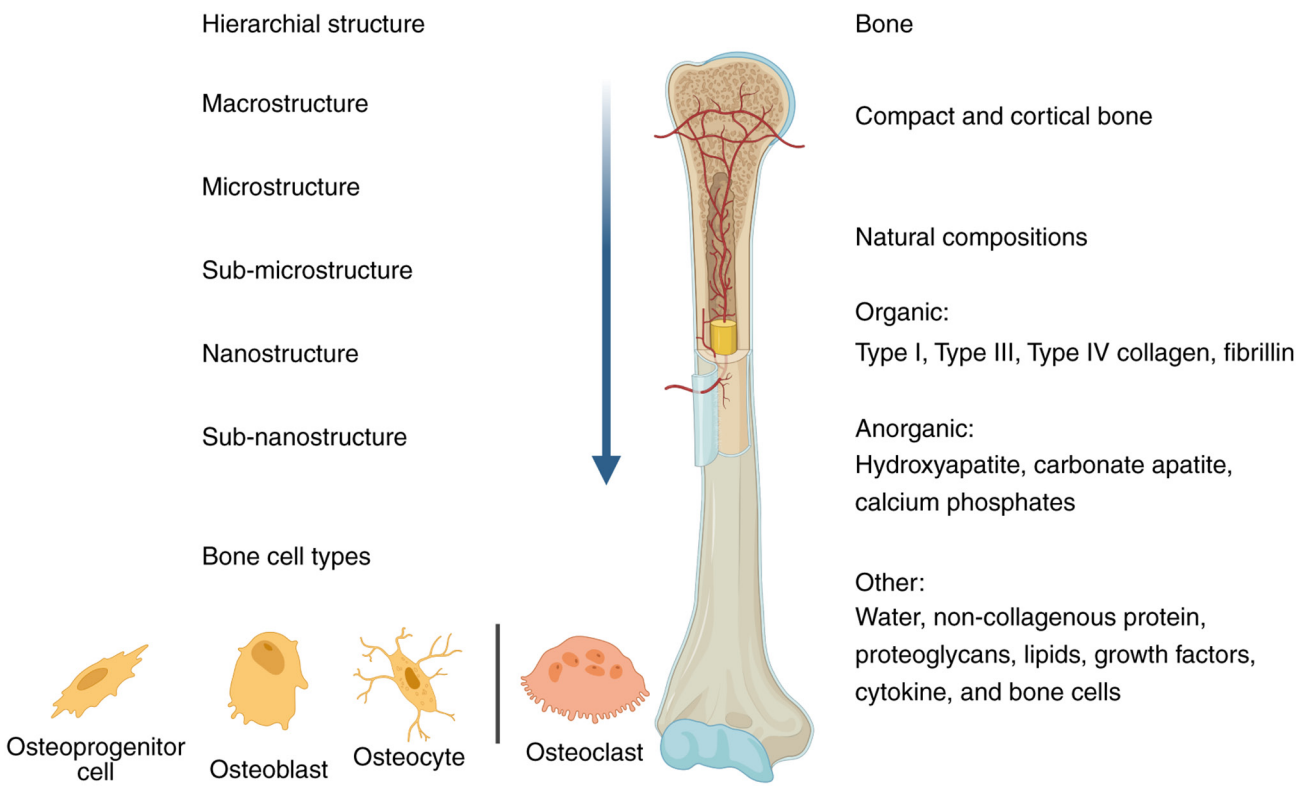

Figure 2. Biology of the bone. Bone is composed of hybrid organic and inorganic materials. Created with BioRender.com.

In general, bone diseases associated with infection, such as osteomyelitis, pose significant concerns, with healthcare costs for fracture-associated infections ~6.5 times higher than those for non-infected cases (93,94). Osteomyelitis can develop by three primary mechanisms: Trauma or contamination, vascular insufficiency or neuropathy and acute hematogenous spread. The condition is most commonly caused by the invasion of *Staphylococcus aureus*, a Gram-positive bacterium that induces pathological skeletal remodeling (94-96). Moreover, epidemiological data from Germany, the most populous country in the European Union, reveal that the prevalence of osteomyelitis has increased by 10.44% over the past decade, with an incidence rate of 16.7 cases per 100,000 people reported in 2018 (97,98).

In dentistry, periodontitis is a common inflammatory oral disease that affected >1 billion individuals globally in 2021, with numbers continuing to rise, according to the GBD study (99,100). Periodontitis can cause the degradation of connective tissue attachments, leading to progressive bone loss (101,102). This condition is triggered by lipopolysaccharide and other virulence factors produced by specific Gram-negative anaerobic bacteria, commonly known as the red complex, including *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* (103-105). Periodontitis adversely impacts quality of life due to clinical manifestations such as gingival inflammation, clinical attachment loss, radiographic

signs of alveolar bone loss, deep periodontal pockets, tooth mobility, bleeding on probing and pathological tooth migration (106).

Bone damage or associated conditions severely impair function and compromise overall wellbeing (107-109). Bone regeneration is a dynamically regulated process involving metabolic activity and complex interactions between tissue cells, immune cells and the musculoskeletal system (110-112). Despite rapid progress in TE, achieving effective tissue repair by leveraging natural healing mechanisms remains a substantial challenge for clinicians (110,113,114).

A key aspect of this challenge is the role of niche cells in the bone repair process. Niche cells are essential in establishing a specific microenvironment and transmitting signals that regulate stem cell responses. Cells within this niche (such as inflammatory, endothelial and Schwann cells) influence molecular regulation and thereby enhance osteoblast function following bone injury (115). Following an acute bone injury, inflammatory cells become activated, initiating a cascade triggered by hematoma formation (116,117). The hypoxia and low pH conditions within the hematoma create a transient environment that supports the active infiltration of tissue macrophages and polymorphonuclear neutrophils into the affected area. In fracture healing, resident macrophages from the endosteal and periosteal surfaces contribute to intramembranous ossification. By contrast, inflammatory macrophages recruited to the injury site are involved in endochondral ossification (117). Molecules such as IL-6, chemokine ligand 2, bone morphogenic protein 4 and vascular endothelial growth factor (VEGF) promote osteogenic differentiation by interacting with nascent osteoprogenitor cells and directing their development toward the osteoblast lineage, thereby enhancing the bone repair process (117-123).

In bone repair, revascularization serves a vital role. Endothelial cells interact with osteoprogenitor cells to establish a microenvironment conducive to osteogenesis. The osteogenesis process is associated with the presence of endothelial cells in the metaphysis and endosteum of long bone (124-126). These cells secrete surface markers such as platelet and endothelial cell adhesion molecule-1 and endomucin, which promote the differentiation of osteoprogenitor cells into osteoblasts through the Notch signaling pathway (127). Additionally, the maintenance of skeletal homeostasis and the bone repair process are influenced by the vital roles of sensory and sympathetic nerves, particularly secretion of nerve growth factor (NGF) (128,129). Both NGF, produced by nerve cells, and VEGF, secreted by Schwann cells, enhance the osteogenic differentiation of osteoprogenitor cells, thereby facilitating new bone formation (130). The fate of the osteoblast lineage is determined by the microenvironment, which delivers essential signals that stimulate vascularization and subsequent bone matrix formation, leading to new bone development (115).

To support these complex biological processes, biomaterials in the form of scaffolds are essential in bone regeneration, serving a central role in reestablishing the bone microenvironment. These biomaterials exhibit key properties, including variable surface characteristics, such as topographies (size and shape), roughness and patterning, as well as mechanical traits such as stiffness (131). 3D bioengineered environments, including scaffolds, hydrogels and mono- or multicellular aggregates (spheroids and organoids), have been developed from various biomaterials to recreate a microenvironment that supports bone cell function (131-134). Moreover, multiple factors can be modified in 3D scaffolds, such as overall geometry, porous structure quality and mechanical behavior, all of which are influenced by the scaffold materials and the fabrication method (135).

Another key feature of biomaterials used in bone regeneration is the incorporation of bioactive CaP, which supports osteoconductive, osteoinductive and osseointegration processes as potential regenerative mechanisms (136-139). Both osteoconductive and osteoinductive properties are 3D phenomena (140,141). Osteoconduction involves the ingrowth of capillaries, perivascular tissue and osteoprogenitor cells from the surrounding bone into the 3D porous structure of the implant. It serves both as a guide for osteogenic growth and as a structural bridge across bone defects, supporting the regeneration of bone tissue (142,143). By contrast, osteoinduction refers to the stimulation of undifferentiated mesenchymal stem cells (MSCs) to differentiate into osteoprogenitor cells, with the goal of generating bone in heterotopic sites (144). Osteointegration, however, is primarily considered a two-dimensional (2D) or surface-level phenomenon, defined by the formation and long-term maintenance of direct contact between the biomaterial surface and bone under functional loading conditions (145-147).

Additionally, advancements in biomaterials research have focused on the application of mechanical stimuli, such as compressive, tensile and flow-induced stresses, using microfluidic devices. This method is being developed to investigate cellular responses and tissue organization involved in bone remodeling (148,149). The present review will further explore the integration of microfluidic-based technology with BTE scaffolds as a key framework for OOAC design.

4. Scaffolds for BTE applications

TE is an interdisciplinary field that integrates cellular biology, synthetic materials and biochemical components to develop a synthetic extracellular matrix (ECM) capable of preserving or regenerating tissues that are diseased, damaged or congenitally impaired (143,144,150,151). The success of a TE approach depends on interaction among these key components, collectively referred to as the TE triad (Fig. 3). First introduced in late 1980s, TE has experienced advancements, contributing to personalized therapy, organ transplants, implants and drug delivery systems, all supported by promising research outcomes (37,152,153). Scaffolds are key elements in TE, designed to mimic the function of native tissue and create an optimal microenvironment that supports the proliferation and differentiation of host cells, ultimately enabling the regeneration of new, functional tissue (154).

Scaffolds are impermanent matrices made from biodegradable biomaterials, serving as 3D frameworks for tissue reconstruction or as carriers for cells, signaling molecules or therapeutic agents (21,155-157). For effective regeneration, cell-free scaffolds must facilitate the recruitment and migration of host cells to the site of injury following implantation. By contrast, cell-combined scaffolds, incorporating various

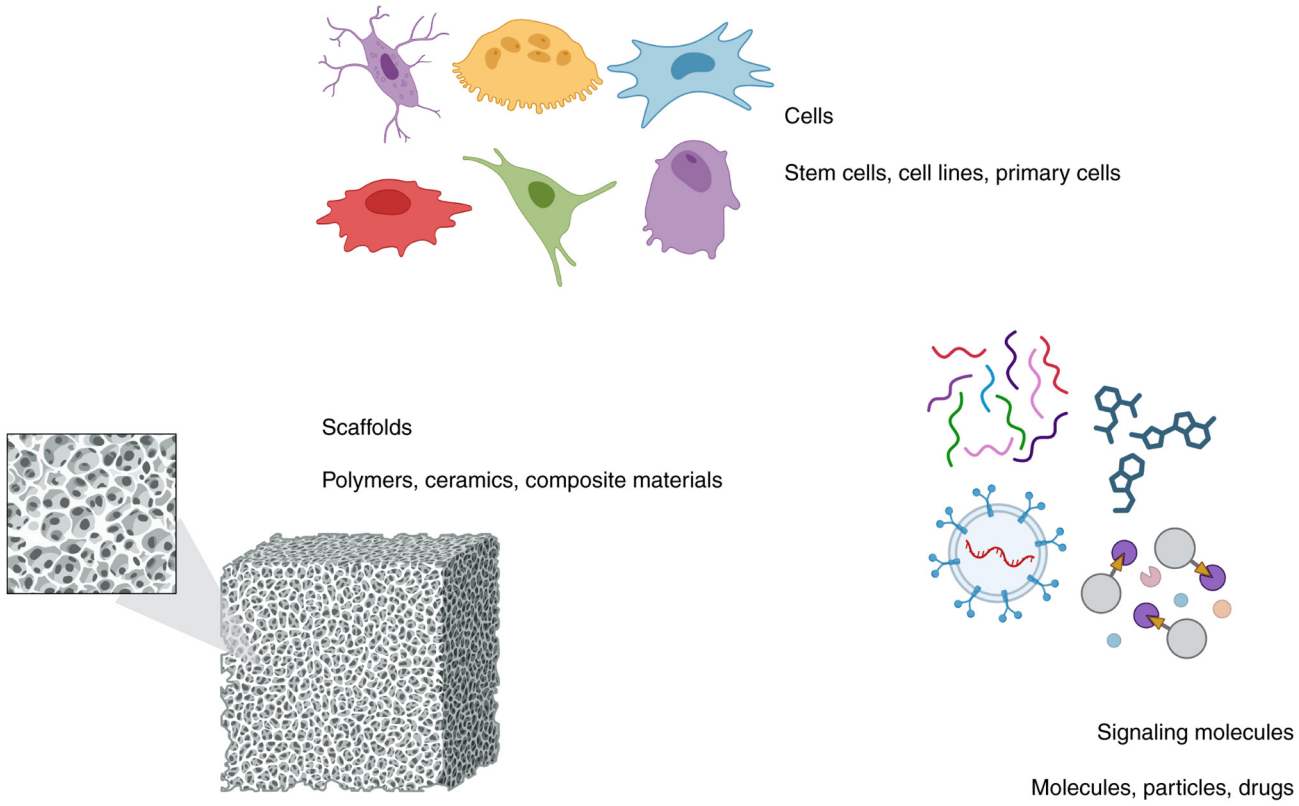

Figure 3. Tissue engineering triad consists of three essential components, scaffolds, signaling molecules, and cells, which function either independently or synergistically to promote tissue regeneration. Created with BioRender.com.

cell types, including stem cells, are engineered to enhance tissue formation through cell proliferation and differentiation. Additionally, substance-combined scaffolds interact with the body by releasing specific molecules, particles or drugs during the degradation process (154,156,157).

Ideal TE scaffolds exhibit key attributes essential for successful tissue regeneration and organ engineering applications, including 3D structure, biocompatibility, controlled and reproducible microarchitectures, surface chemistry conducive to cellular activity, mechanical strength appropriate for the regenerated tissue, tissue-specific biodegradability, porosity and interconnected network for perfusion of beneficial factors and sterilizability (Fig. 4) (158-164). The 3D structure of scaffolds is largely determined by the properties of the biomaterial used and its intended applications. For example, dense tissue such as bone requires a rigid polymeric framework, whereas soft tissue such as nerves demand a more pliable scaffold, and flexible tissue such as skin or blood vessels benefits from a highly adaptable polymeric structure (164). Therefore, adjusting scaffold characteristics is key to meet specific biological, clinical, manufacturing, economic and regulatory demands. In the context of BTE, scaffolds must address key factors such as load-bearing capacity during the repair phase, biological properties, vascularization, integration with host tissue, ease of handling, minimally invasive implantation and the ability to support osteoconduction and osteoinduction (13,154,165).

TE scaffolds must also demonstrate a range of biological characteristics to effectively support the development of new tissue. Non-toxicity and biocompatibility are fundamental biological properties that ensure scaffolds mimic and serve as a temporary ECM without disrupting cell processes during degradation (13,166). The progression of scaffolds into smart materials capable of responding dynamically to environmental stimuli underscores the importance not only of biocompatibility (167,168) but also of immunomodulatory capability (167-169) and bioactivity (43,166). Additionally, indicators such as cell vitality, attachment, proliferation, differentiation and surrounding cell signaling, are key parameters in evaluating scaffold performance in both preclinical and clinical settings (170-173).

Scaffolds used in TE must accelerate bone healing while conforming to specific architectural frameworks and functional requirements tailored to the target tissue and site of the defect (174-177). Mechanical properties, including tensile strength, elastic modulus, pore size, surface topology, load-bearing capacity, stiffness, fracture toughness, fatigue resistance and elongation percentage, are critical considerations. These mechanical parameters influence other properties; for example, stiffness can direct specific cellular differentiation, while pore size impacts the scaffold environment, affecting nutrient distribution and cell migration. Furthermore, surface topography serves a crucial role in regulating cellular behavior, by influencing cell adhesion, morphology, proliferation and differentiation (164,178-181). Thus, an optimally designed TE scaffold with appropriate biomechanical characteristics should closely resemble the bone or the tissue intended for replacement.

Equally vital to scaffold performance are its pore and porosity attributes, which are directly associated with structural functionality. The effectiveness of a scaffold is influenced

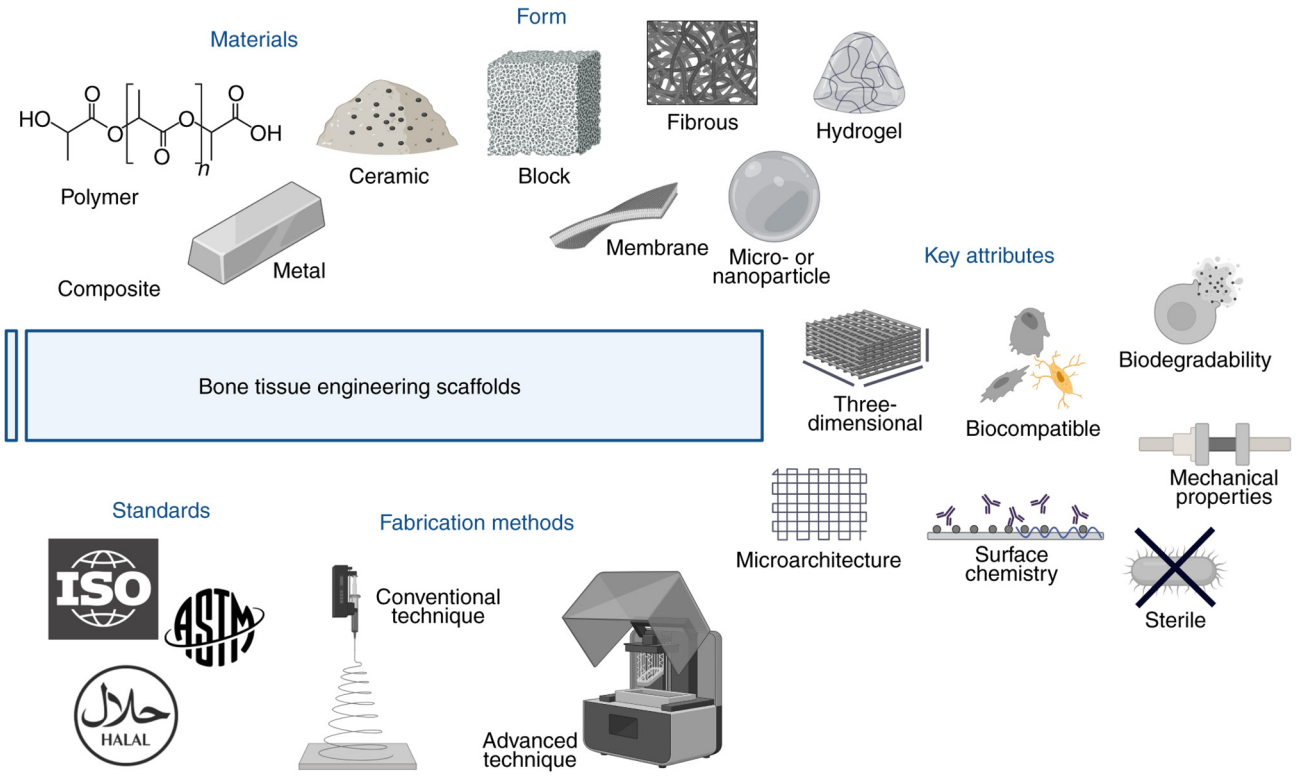

Figure 4. Aspects in developing bone tissue engineering scaffolds for biomedicine include materials, methods, key attributes, and standards. Created with BioRender.com.

by parameters such as pore size, volume and interconnectivity (42,182,183). Porosity is a key factor in enabling oxygen and nutrient penetration, waste elimination and the development of new blood vessels within the ECM (184). Furthermore, surface topography affects how host cells adhere to the scaffold, directing their responses and facilitating communication between cell types (181,185). Host cells interact with the scaffold surface through adhesion receptors such as integrins, which mediate signal transmission between the scaffold and cells. This interaction prompts integrins to associate with other proteins to form focal adhesion complexes, activating intercellular signaling pathways that guide cellular responses to the implant (178,186-188).

Moreover, sterilizability is a crucial characteristic of TE scaffolds, ensuring their safe application within the body. Polymer scaffolds can be fabricated aseptically or sterilized post-production (189,190). The Food and Drug Administration (FDA) of the United States of America has approved several sterilization techniques for medical devices, including ethylene oxide gas, autoclaving and γ irradiation, all of which can be applied to TE scaffolds (191). Additional methods, such as ethanol treatment, plasma sterilization, UV irradiation, antibiotic exposure and bleaching with sodium dichloroisocyanurate dihydrate, are also used. It is essential that these methods maintain sterility while preserving the material properties of the scaffold. As most TE scaffolds are composed of polymers, the sterilization technique must not compromise polymer integrity, which could result in hydrolysis, melting or depolymerization (192).

BTE scaffolds are fabricated from a range of materials, typically categorized into four primary groups: Ceramics, polymers, metal and composites. Each material group offers specific applications and advantages suited to particular tissue repair requirements (193,194). To develop an effective scaffold tailored to a specific tissue defect, several strategies can be employed. These include the use of pre-fabricated porous scaffolds made from natural or synthetic biomaterials, decellularized ECM derived from allografts or xenografts, confluent cells with secreted ECM and cell-encapsulated hydrogels (195). Based on their geometric structures, BTE scaffolds may take the form of porous, fibrous, microspherical or solid freeform constructs (194).

Porous scaffolds with interconnected structures (directional or random) support regeneration, vascularization and ECM deposition by enhancing the transport of gases and nutrients through their internal channels. Optimal pore size varies depending on the target tissue: 200-400 for bone, 50-200 for muscle and 10-75 μm for fibrous tissues (196-200). Fibrous scaffolds, synthesized from biodegradable polymers such as polycaprolactone, polylactic acid, gelatin, cellulose and silk fibroin, are beneficial for application in skin, cartilage, ligament, muscle and blood vessels (201-208). Micro- or nanoparticle scaffolds are extensively utilized in gene therapy, drug delivery and the release of bioactive compounds, with effectiveness often depending on their responsiveness to specific stimuli (209-211). Solid freeform scaffolds, created using 3D printing or additive manufacturing technologies, have attracted interest due to their precision and customization capabilities (212-214). The 3D printing process involves fabricating a product that conforms to a pre-designed digital model, which undergoes multiple stages, including model creation,

stereolithography (SLA), slicing, material preparation, printing and post-printing refinement (215-218).

Scaffold manufacturing techniques are classified into two categories: Conventional techniques and rapid prototyping methods. Each category encompasses processes that result in distinct scaffold properties (13). Although conventional methods remain widely used, they face limitations in producing intricate geometries and maintaining controlled reproducibility. By contrast, rapid prototyping allows greater design complexity and customization (173). Conventional techniques include freeze-drying (219-222), gas foaming (16,223), electrospinning (134,224,225), solvent casting (16,226) and phase separation (16). Rapid prototyping methods include selective laser sintering (227-229), SLA (229,230), fused deposition modeling (229,231), solvent-based extrusion (232,233) and bioprinting (14,233-235). Establishing standardized protocols for BTE scaffolds is key to ensuring the quality, safety and efficacy of TE medical products. These standards are governed by the American Society for Testing and Materials International (236,237), International Organization for Standardization (238), national standardization agencies and emerging halal certification systems.

5. Scaffold assessment using OOAC

OOAC technology has made notable advancements, revolutionizing drug testing and pathology research. This innovative approach integrates microfluidic systems to replicate the complex physiological conditions of human organs, offering improvement over traditional 2D cell cultures. By mimicking intricate organ functions, OOAC technology provides a more precise and comprehensive platform for investigating cell interactions, drug response and disease modeling (239,240). It effectively bridges the gap between *in vitro* and *in vivo* research by establishing a controlled microenvironment for more accurate cell culture studies and system analysis (241). Moreover, the development of OOAC is guided by the replace, reduce and refine (3R) animal testing principle and is supported by regulatory bodies such as the European Medicines Agency and US FDA, both of which actively promote preclinical models aligned with this ethical framework (242,243).

To replicate tissue structure and functions, OOAC technology combines microengineering with TE techniques. Essentially, it constitutes a small-scale fluidic system comprising human-derived cells and physicochemical microenvironments. OOAC platforms typically include key components such as microarchitecture chips, biological elements, biomaterials, physicochemical stimulation, sensors or monitoring devices, membranes and microfluidic technology (240,244,245). The fabrication of OOAC devices often uses soft lithography, a method employing polydimethylsiloxane (246-249). Commonly used in microelectronics, soft lithography involves two primary steps: Photolithography process to produce a stamp, followed by a molding procedure (250). This process starts with a mold, typically composed of photo-resistant silicon, designed to mirror the inverse of the desired channel layout. The mold is filled with material, shaped into the desired chip form and cross-linked. Soft lithography enables the production of multilayer microfluidic devices with flexible microstructures, facilitating rapid prototyping of micro- and nanostructures with specific channel patterns. Following cooling and separation, the resulting block can be sealed either reversibly or irreversibly (251,252). An alternative method for constructing an OOAC device involves using 3D printing technology, specifically light-assisted additive manufacturing, also known as vat photopolymerization, combined with a dynamic photomask (253,254).

The development of OOAC devices requires consideration of several critical factors, including geometry, dimension, channel shape and the biological context (240). The number and configuration of channels within the microfluidic chip (single, double or multiple channels arranged in parallel or sandwich configurations) are associated with the geometry parameters (240,255). Double-channel designs, typically connected by a porous membrane, are commonly employed in various applications such as modelling interface or biological barrier (256,257), co-culturing (258), disease modeling (259,260) and drug evaluation (261) These chips typically have two inlets and outlets to regulate the flow of working fluids or biological materials, such as protein, cells or drugs, in and out of the system (262,263). Microfluidic chips with this configuration are frequently used to model the blood-brain barrier (BBB), intestine and lungs (26,28,264-265). The chip dimensions are typically on the centimeter scale, with channels ranging from millimeters to submicron sizes. The channels may have circular or rectangular shapes and are separated by a natural or synthetic polymeric membrane. This membrane is produced using conventional methods such as electrospinning and freeze-drying or advanced techniques such as 3D printing and SLA (266-270). The membrane serves a critical role in replicating permeability between two environments, enabling cell attachment, separation and communication between compartments (271-273).

A key parameter in replicating physiological conditions within OOAC systems is the biological context, which directly influences how accurately the microenvironment is reproduced to meet specific research goals. This parameter encompasses several key factors, including cell-ECM interaction, cell-cell interactions, biochemical environment regulation and biophysical environment control, such as flow dynamics, clog prevention and tissue mechanics. Each element is essential in ensuring that the system replicates *in vivo* conditions, thereby enhancing the relevance of experimental outcomes. Cell-ECM interactions are key to tissue development and homeostasis. In addition to providing structural support, ECM proteins play a key role in facilitating cell-matrix communication by modulating cell adhesion, migration and differentiation. These interactions direct cellular behavior and development by activating key intracellular signaling pathways, which are vital for processes such as tissue formation and remodeling. This is particularly beneficial for research investigating the impact of microenvironmental factors on cellular proliferation, differentiation and tissue-specific function, offering insights into how the ECM influences cell fate and performance. Cell-cell interactions are key for cell proliferation, morphogenesis and tissue repair. Achieving a complex, organized and synergistic assembly of diverse cell types is essential for generating structured tissue with functional intercellular interactions (253,271-277). Research on cell migration and cancer metastasis typically utilizes OOAC systems to investigate these parameters

(253,274-277). OOAC systems can regulate the biochemical environment, based on the understanding that cellular behavior, such as proliferation, differentiation, migration and angiogenesis, is influenced by biochemical factors within the tissue microenvironment. These factors include concentration and oxygen gradient (278-280).

As part of biochemical and biophysical environment regulation, fluid flow serves a key role in mass transport, enabling the delivery of nutrients, dispersal of soluble substances and removal of cell waste throughout the human body. In OOAC systems, precise control of fluid flow is key for generating accurate results. The flow rate influences shear stress, polarity, concentration gradients of oxygen and nutrients, factors that affect cell adhesion, proliferation, morphology and protein expression (281,282). Two types of flow (steady and pulsatile) are employed in OOAC systems. Various methods are used to regulate flow, including pressure-driven methods, electroosmotic (electrokinetic flow), surface tension, shear flow, gravity, buoyancy, squeeze-film effects, laser-induced flow and biologically driven flow. The regulation of these flows is typically achieved using micropumps, such as peristaltic micropumps and syringe, electrokinetic and capillary pumps (253). In addition to fluid dynamics, it is vital to consider organ-specific chemical and mechanical stimuli, such as traction, compression, pressure and the exposure to drugs, toxins or radiation (247,283-287). A common challenge in these systems is clogging, which occurs due to the accumulation of particles that obstruct flow, especially in small-scale or highly sensitive systems. Preventive measures such as maintaining optimal pressure and temperature, controlling surface roughness and incorporating bubble traps are essential to prevent clogging. Although clogging can interfere with cell proliferation and compromise the functionality of organ-on-a-chip models, it may be advantageous to reinforce porous or fractured structures, or to facilitate the detection of specific biological components such as cells or protein (288-291).

Currently, there are two main approaches for conducting an OOAC experiment. The first involves constructing a custom-built OOAC system, while the second uses commercially available platforms. Self-built systems allow creation of highly specialized and complex models, making them valuable for investigating human disease mechanisms or other niche applications. However, these systems often lack standardization and may be less reliable (292,293). By contrast, commercially available OOAC platforms are gaining popularity in both academic and industrial settings due to their robustness and user-friendly design, especially in drug discovery and development. These commercial systems offer enhanced stability and operation simplicity, making them attractive to the pharmaceutical industry (292,294). At present, >15 companies manufacture commercially available OOAC devices and associated components (Table II) (292,294-327).

According to previous studies (28,241,264,328-339), OOAC technology is primarily employed in fundamental research to replicate the physiological structure and functions of microenvironments. It has been used in disease and cancer modeling, drug development, toxicity evaluation and in studies exploring its manufacturing and assembly processes (241,328). To date, this bioengineering tool has been applied to study the functions of various organs, including the lung (329,330), brain (331), BBB (264,332), endometrium (333), liver (334), heart (335), kidney (336), bone marrow (337), intestine (28,338) and bone (339). Despite the potential of BTE to address bone-associated challenges, there remains a significant gap in OOAC research focusing on BTE scaffolds in medical and dental contexts, highlighting the need for further investigation. To address this gap, the present review provides a comprehensive overview of studies on scaffold fabrication and evaluation using microfluidic-based technology or OOAC devices, with a focus on their potential applications in BTE and bone repair and regeneration.

Several studies (340,341) have been conducted to evaluate scaffolds using OOAC or microfluidic-based devices. Ng *et al* (342) developed a 3D-printed micro-perfused culture device using SLA, embedded with a 3D fibrous scaffold to enhance biomimicry. This system supports the proliferation of Huh7.5 hepatocellular carcinoma cells within a perfused microenvironment and demonstrates adaptability for various cell lines or tissue types (340). Similarly, Han *et al* (341) developed a 3D-printed film for bone regeneration, evaluated within a scaffold-OAC system fabricated via negative replica molding. The device included a central circular area for scaffold placement, surrounded by a microfluidic channel, allowing observation of osteoblast MC3T3-E1 activity, such as migration, morphology and osteogenesis at the blood-scaffold interface. The scaffold-OAC platform has proven to be an effective tool for monitoring cellular responses and evaluating scaffold performance, with the potential to replace conventional *in vivo* and *in vitro* models (341).

Bahmaee *et al* (342) introduced an innovative microfluidic device designed to replicate bone conditions, incorporating a 3D scaffold within a silicone-based microfluidic unit. This configuration supports the proliferation and differentiation of human embryonic stem cell-derived mesenchymal progenitor cells under dynamic mechanical conditions. The aforementioned study demonstrated enhanced osteogenic differentiation and ECM formation compared with both static and continuous flow environments. This bone-OAC device presents an affordable, reproducible and scalable platform for studying bone biology and evaluating BTE scaffolds, with the potential to decrease dependence on animal models for preclinical testing (342). These findings illustrate diverse approaches to scaffold evaluation using OOAC systems, each tailored to specific research purposes, and highlight the importance of this technology in advancing BTE (Table III) (343-351).

In addition to Bahmaee *et al* (342), several other studies have emphasized the use of microfluidic technology for fabricating scaffolds intended for BTE applications: Parhizkar *et al* (352) explored the creation of complex multidimensional scaffolds by integrating biocompatible particles and fibers through T-junction microfluidics and electrohydrodynamic techniques. This device produced multifunctional bovine serum albumin scaffolds with controlled porosity, incorporating poly(lactic-co-glycolic acid (PLGA), polymethysilsesquioxane (PMSQ) and collagen particles/fibers. The developed scaffolds exhibit potential applications across advanced fields, including biomedical applications such as bone regeneration, as well as

Table II. Comercially available OOACs.

Company	Year of establish-ment	OOAC-associated organ/tissue model	Provision of OOAC-related experimental instruments	Customized model services	(Refs.)
4Dcell	2017	Neuron, heart, muscle	Yes	No	(295)
Aim Biotech	2012	BBB, islet, cartilage, microvasculature	Yes	No	(296)
Altis BioSystems	2015	Gut	Yes	Yes	(297)
ANANDA Devices	2015	Neuron	No	Yes	(298)
Alveolix	2019	Lung	Yes	Yes	(299)
Aracari Bio	2019	Brain/neurons, microvasculature	Yes	Yes	(300)
AxoSim	2014	Brain, nerve	No	Yes	(301)
Beonchip	2016	BBB, gut, epithelium	Yes	Yes	(302)
BiomimX	2017	Knee, lung, gut, heart, cartilage, tooth	Yes	Yes	(303)
Bi/ond	2017	Muscle, tumor	No	Yes	(304)
Cherry Biotech	2014	Endothelium, skin, BBB, liver	Yes	Yes	(305)
Chiron	2022	Cartilage, synovial membrane	No	Yes	(306)
CN-Bio	2009	Brain, liver, lung gut, kidney, heart, skin, multi-organ	Yes	Yes	(307)
DiNABIOS	2023	Heart	Yes	No	(308)
Dynamic42	2018	Lung, liver, gut, vasculature	Yes	Yes	(309)
Draper Laboratory	2019	Upper and lower airway, liver, gastrointestinal, BBB, vasculature, glomerulus, reproductive tract, skin	Yes	No	(310)
Elvesys	2011	Universal organ-chip (brain, kidney, gut, or skin)	Yes	Yes	(311)
Emulate	2014	Brain, colon, duodenum, kidney, liver, lung, bone marrow	Yes	Yes	(312)
Hesperos Inc.	2015	Liver, muscle, heart, brain gut, kidney, neuron, BBB, muscle, skin, bone marrow and multi-organ	Yes	No	(313)
Ibidi GmbH	2001	Brain/neurons, lung, liver, gut, kidney, islet, muscles, heart, skin, cartilage, bone marrow	Yes	No	(314)
InSphero	2009	Liver, islet, tumor	No	No	(315)
Jiksak Bioengineering	2017	Nerve	Yes	No	(316)
Kirkstall Ltd.	2006	Brain, liver, brain, heart, gut, lung	Yes	No	(317)
MesoBioTech SAS	2016	Brain, lung	Yes	No	(318)
MicroBrain BT	2014	Neuron	No	No	(319)
Mimetas	2013	BBB, gut, blood vessel, angiogenesis	Yes	No	(320)
NETRI	2018	Peripheral and central nervous system, skin	Yes	Yes	(321)
Numa Bioscience	2012	Liver, islet, microvasculature	Yes	No	(322)
Quris	2019	Patient-on-a-chip	No	No	(323)
REVIVO Biosystem	2019	Skin	Yes	No	(324)
SynVIVO	2015	BBB, lung, liver, tumor, microvasculature	Yes	No	(325)
TissUse GmbH	2010	BBB, bone marrow, hair follicle,lung, liver, testis, thyroid, bone, heart, endometrium, pancreas, vasculature, muscle, kidney, skin, gingiva, multi-organ	Yes	Yes	(326)
Xona Microfluidics	2008	Neuron	Yes	No	(327)

BBB, blood-brain barrier.

Table III. Scaffold-associated microfluidic-based and OOAC devices.

First author/s, year	Type of BTE scaffold	Practical implication	(Refs.)
Ng *et al*, 2024	3D-printed micro-perfused culture	The study used a 3D fibrous scaffold integrated within a unified micro-perfused cell culture system to create a physiologically relevant microenvironment that supports the viability of Huh7.5 hepatocellular carcinoma cells. The device can be tailored to meet the microenvironmental needs of different types of tissue, including bone	(340)
Han *et al*, 2023	Scaffold-OAC	The platform has been developed to evaluate MCTT3-E1 cellular activity at the blood-scaffold interphase. This may serve as a relevant *in vitro* alternative to traditional *in vivo* animal models, offering a valuable tool for the fundamental study of bone regeneration	(341)
Killinger *et al*, 2023	Bone-OAC	This platform was created, refined and characterized specifically to improve the dynamic cultivation and maturation of MC3T3-E1pre-osteoblasts *in vitro*. This system serves as a potent tool for investigating the pathophysiology of human bone *in vitro*, employing microfluidic technology and multi-spheroid array techniques to create more realistic and dynamic culture conditions	(343)
Scheinpflug *et al*, 2023	Bone formation MPS	The goal of this study was to create MPS for bone biology that allows for perfusion, more precise quantification and control over physicochemical parameters. This platform used primary human osteoblasts seeded onto type I collagen scaffolds and is a beneficial and promising alternative to animal testing and disease modeling	(344)
Nguyen *et al*, 2023	Sample analysis assay chip	Development of a prototype for non-disruptive fluid manipulation in gold standard testing of 3D printed TE scaffold *in vivo*. This enables more feasible real-time monitoring of biomaterials implanted *in vivo* and serves as a proof of concept for a versatile BTE platform that reduces animal use, lowers experimental costs, and provides real-time data	(345)
Salerno *et al*,2022	FSS bioreactor	This study investigated the biological effects of FSS on a 3D B-HA scaffold and its impact on osteoblast cells. The evaluation of the scaffold in this bioreactor demonstrated activity of MC3T3 cells may be affected by both the structure of the scaffold and the applied FSS within the bioreactor	(346)
López-Canosa *et al*, 2022	Microfluidic-based 3D model	Study assesses the impact of bioactive PLA + CaP electrospun composite on the recruitment of rat endothelial progenitor and bone marrow-derived rat mesenchymal stromal cells using a microfluidic-based 3D platform. This novel technique can assess the ability of bioactive scaffolds to stimulate vascularization and expedite the preclinical evaluation of biomaterials	(347)
Bahmaee *et al*, 2020	Osteogenesis-OAC	This study developed a microfluidic chip made of silicon and integrated with a 3D scaffold from polymerized high internal phase emulsion. The cellular activity and ECM production of hES-MPs is better than static condition, this platform has the potential to investigate bone, especially BTE scaffolds, and reduce animal requirements	(342)

Table III. Continued.

First author/s, year	Type of BTE scaffold	Practical implication	(Refs.)
Peticone *et al*, 2020	Microfluidic perfusion platform	Study investigates the efficacy of doped microspheres (TiO_2- and CoO-doped PG) in enhancing bone tissue engineering by using a microfluidic perfusion platform involving hMSCs. Proven, controlled perfusion conditions, minimal manual intervention and parameter optimization during cell culture could potentially scale up the device for BTE scaffold assessment	(348)
Lyu *et al*, 2020	Microfluidic chip	This study developed a three-layer structure scaffold from tendon and bone marrow-derived SCs cultured in a decellularized tendon scaffold. A tree-like flow pattern microfluidic chip was used to establish this gradient, guiding stem cell differentiation to promote tissue regeneration. The study presents a potentially effective TE method that could lead to improved tendon-to-bone healing	(349)
Goldman *et al*,.2016	Microfluidic osteochondral graft	The study developed tissue-engineered osteochondral units that closely replicate native cartilage and bone, using bovine MSCs embedded in an agarose scaffold. Key functional parameters can be quantitatively assessed through a microfluidic approach. Furthermore, it holds promise for BTE particularly in regenerative applications.	(350)
Jusoh *et al*, 2015	Microfluidic vascularized bone tissue model	Development of a microfluidic system that accurately mimics the physiological circumstances of bone tissue to study the process of bone angiogenesis. The incorporation o fhydroxyapatite nanoparticles enhance angiogenesis. This platform provides a methodology for studying intricate biological events and evaluating medication reactions and toxicity in bone tissue	(351)

OOAC, organ-on-a-chip; BTE, bone tissue engineering; MPS, microphysiological system; FSS, flow-induced shear stress; B-HA, Hydroxyapatite with B position of carbonation; PLA, polylactic acid; hES-MP, human embryonic stem cell-derived mesenchymal progenitor; MSC, Mesenchymal stem cells.

in pharmaceutical and cosmetic industry (352). Further studies reinforce the ability of microfluidic systems to support the development of diverse scaffold types from various biomaterials (Table IV) (353-363).

6. Future directions and challenges

OOAC platforms are transformative tools in biomedical research, offering precision in replicating human tissue and organ microenvironments (Figs. 5 and 6). These microfluidic systems enable the development of dynamic *in vitro* models that more accurately reflect *in vivo* physiology compared with traditional cell cultures or static assays. OOAC technology has potential across various applications, including disease modeling, cancer metastasis studies, drug toxicity screening and preclinical testing of BTE scaffolds. The integration of fluidic flow, multicellular architecture and biomimetic cues provides a foundation for evaluating regenerative strategies in both research and clinical contexts (Table V).

A strength of OOAC is its flexibility. The platform can accommodate a range of cell types, including primary human cells, immortalized cell lines and SCs. Oral-derived MSCs are particularly advantageous due to their ease of isolation and osteogenic potential, making them ideal for modeling dental and craniofacial bone tissue. Combining MSCs with OOAC platforms offers a promising strategy for testing scaffold performance, analyzing biomaterial behavior and simulating healing processes within complex tissue environments. These systems also facilitate comparative evaluation of biomaterials and BTE constructs under conditions that more closely mimic physiological conditions than conventional 2D or static cultures.

Furthermore, OOAC technology has the potential to streamline the drug and biomaterial development pipeline by decreasing reliance on animal models and accelerating proof-of-concept validation. By bridging the gap between preclinical studies and human trials, OOAC systems shorten therapeutic discovery timelines and reduce development cost. Their alignment with the 3R principle improves the ethical

Table IV. BTE scaffold fabrication using microfluidics technology.

First author/s, year	Type of device	Overview	(Refs.)
Rashidi *et al,* 2024	Calcium-free alginate-encapsulated MSC	Microfluidic system opens up the opportunity to precisely encapsulate cells onto particles while favoring cell retention on substrates, viability and proliferation upon transplantation, with good manufacturing practice requirements	(353)
An *et al*, 2023	Injectable and moldable hMSC-laden microgels	Microfluidic technique via phototriggered imine-crosslinking chemistry is a powerful tool for BTE design strategy that allows enhanced fabrication accuracy, accelerated gelation rate and improved network strength of the scaffold	(354)
Yang *et al*,2021	BMSC-laden collagen microbeads	Microfluidics technology through modular TE is a promising bio-fabrication technique for constructing engineered bone grafts. This strategy enables the creation of complex and patient-specific grafts by integrating tailored bioinks that contain living cells and biomaterials.	(355)
Rajabnejadkeleshteri *et al,* 2021	BMP-2-CD-loaded pectin microparticles	Encapsulating non-invasive bioimaging agents such as BMP-2-conjugated CDs onto a scaffold for bone regeneration made from natural polymer using microfluidic approach. This platform possesses potential in BTE	(356)
Qasim *et al*, 2020	Three-dimensional PLGA-TGF-β3 MPs/PCL nanohybrid scaffold	Microfluidic technology could be used to incorporate synthetic polymers and growth factors. This approach demonstrates a sustained release, improved loading efficacy and enhanced conjugation of growth factor within BTE scaffold for cartilage regeneration	(357)
Moradikhah *et al,* 2020	ALN-loaded chitosan NPs	Microfluidics devices serve as powerful tools for manipulation of liquid solutions in rage of nano-pico volumes to prepare monodispersed and homogenous NPs loaded with bisphosphonate as a promising treatment for osteoporosis	(358)
Hou *et al*, 2018	Injectable PVA microgels co-loaded with hMSCs and BMP-2	Cells and growth factor are co-loaded into polymer-based scaffolds via high-throughput microfluidic devices. This technique protects the stem cells from severe environmental stress and regulates cell differentiation inside precisely specified environment to facilitate bone regeneration	(359)
Lin *et al*, 2018	Sponge-like monodispersed PLGA/nano-MgO-alginate core-shell microsphere	Customized microfluidics can be engineered specifically to control delivery of ions for enhancing bone regeneration such as magnesium to local microenvironment. This approach influences bone cell activity, mineralization and osteogenic differentiation	(360)
Li *et al*, 2017	Biodegradable GelNB hydrogel	Microencapsulation of stem cells into microgel can be engineered through low-cost biocompatible microfluidic-processing conditions. Microfluidic devices can be made into highly cost-effective and user-friendly devices that enable a successful microencapsulation of TE scaffold	(361)
Angelozzi *et al*, 2015	Alginate/gelatin or alginate/urinary bladder matrix composite hydrogel	Microfluidic chip has the potential to be used for cellular embedding and biomineralization. This approach allows production of a complex microfiber made from cells and biomaterial with accurate manipulation of the size and shape of the microfibers	(362)

Table IV. Continued.

First author/s, year	Type of device	Overview	(Refs.)
Ding *et al*, 2015	PCL/Na-alginate/ rBMSC/BMP-2 nano-micro alternating multilayer scaffold	Microfluidic approach is beneficial to engineered complex microbeads encapsulated with cells and growth factors. Combined with continuous electrospinning process, this approach can produce controlled-multi layer BTE scaffold with osteogenic effect *in vitro* and bone formation capability *in vivo*	(363)

BTE, bonetissueengineering; hMSC, Human mesenchymal stem cells; rBMSC, rat bone marrow mesenchymal stem cells; BMP, Bone morphogenic protein; CD, Carbon dots; PLGA, Poly(lactic-co-glycolic) acid; MP, Macroparticles;ALN, Alendronate; NP, Nanoparticle; PVA, Polyvinyl alcohol; PCL, Polycaprolactone.

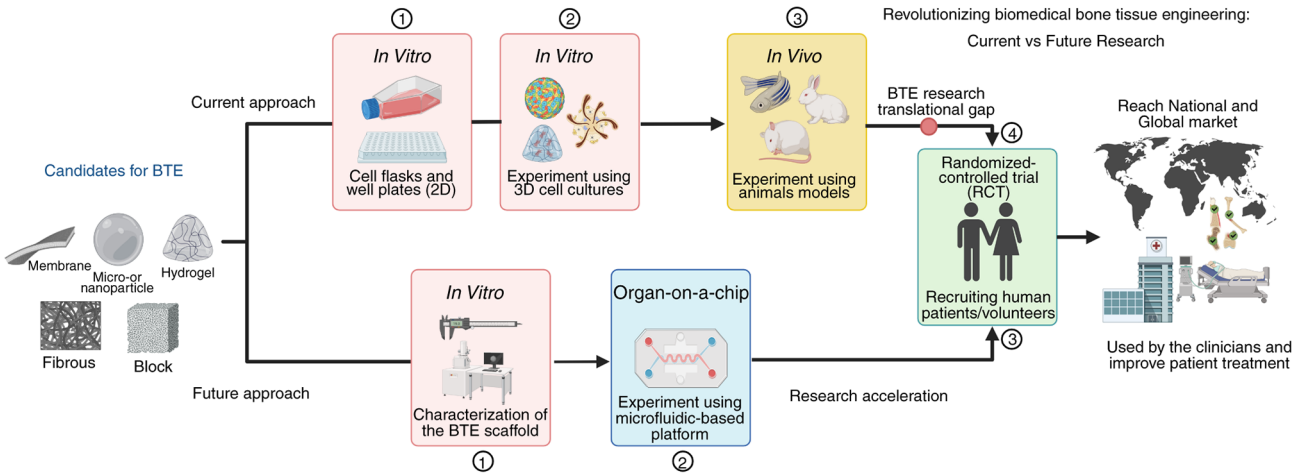

Figure 5. The implementation of Organ-on-a-Chip technology revolutionizes scaffold development in tissue engineering by significantly accelerating the screening and evaluation processes. BTE, bone tissue engineering; RCT, randomized controlled trial. Created with BioRender.com.

profile of research, establishing OOAC as a favorable alternative in translational science. OOAC may overcome the limitations of conventional models-due to its ability to emulate complex human physiology with high fidelity (31,328,364-370).

From a fabrication perspective, advances such as 3D printing and digital microfabrication have made it more feasible to develop OOAC devices in-house or acquire them from commercial sources (371-373). While self-assembled systems offer customization and design flexibility, commercial models include expert consultation and specialized configurations, making them more accessible to a wider research audience. The global OOAC market has experienced growth, surpassing $100 million in value as of 2020 and continuing to expand (374). As the field progresses, the synergy between OOAC platforms and emerging technologies, such as robotics, artificial intelligence and automated testing, will be vital for scaling applications.

Despite these advances, barriers remain. High setup costs, fabrication and automation complexity, validation, parallelization and lack of standardization hinder widespread adoption (375). Overcoming these challenges requires coordinated efforts across the research community. To promote broader acceptance and reproducibility of OOAC systems in BTE, regulatory protocols must evolve general guidelines to include well-defined standards. This includes developing standardized fabrication protocols, such as defining acceptable ranges for material properties (stiffness, porosity, fluid flow), and establishing preclinical evaluation standards that address metrics such as biocompatibility, barrier function, mechanical stress response and osteogenic performance. Collaboration with national regulatory authorities-including the FDA, European Medicines Agency (EMA), and Pharmaceuticals and Medical Devices Agency (PMDA)-alongside engagement with international standard-setting organizations such as the International Organization for Standardization (ISO) and ASTM International, is essential to establish benchmark testing protocols, validation frameworks, and harmonized reporting standards that can accelerate the acceptance and integration of OOAC technology. Additionally, interlaboratory validation studies and shared databases may facilitate cross-platform comparability, enhancing reproducibility and trust in OOAC outcomes. These efforts are essential for integrating OOAC devices into clinical translation pipelines and positioning them as credible tools for regulatory approval and routine biomedical use. Addressing cost concerns through the development of reusable and sustainable materials for OOAC

Table V. Comparison of study platforms.

Comparison	Conventional approaches *in vitro* and *in vivo*	Organ-on-a-chip	
		Commercially available	Self-assembled
Advantages	Commonly used techniques include standard 2D monolayer cell cultures, 3D cell culture (spheroids and organoids) and animal model ; well-established; standardized, reliable, cost-efficient	Available in various organ models andas multiorgan-on-chip; provided by various companies; includes flow system services and customer support	Customized system; replicable and reliable; cost-efficient; suitable for the research aim
Disadvantages	Conducted mostly with animal-derived cells; potential discrepancies between preclinical findings and clinical outcomes; limited translatability to clinical settings; high cost; ethical issues for animal studies	High cost; difficult to customize; need more time for system delivery	Low validity; long development time; resource-intensive

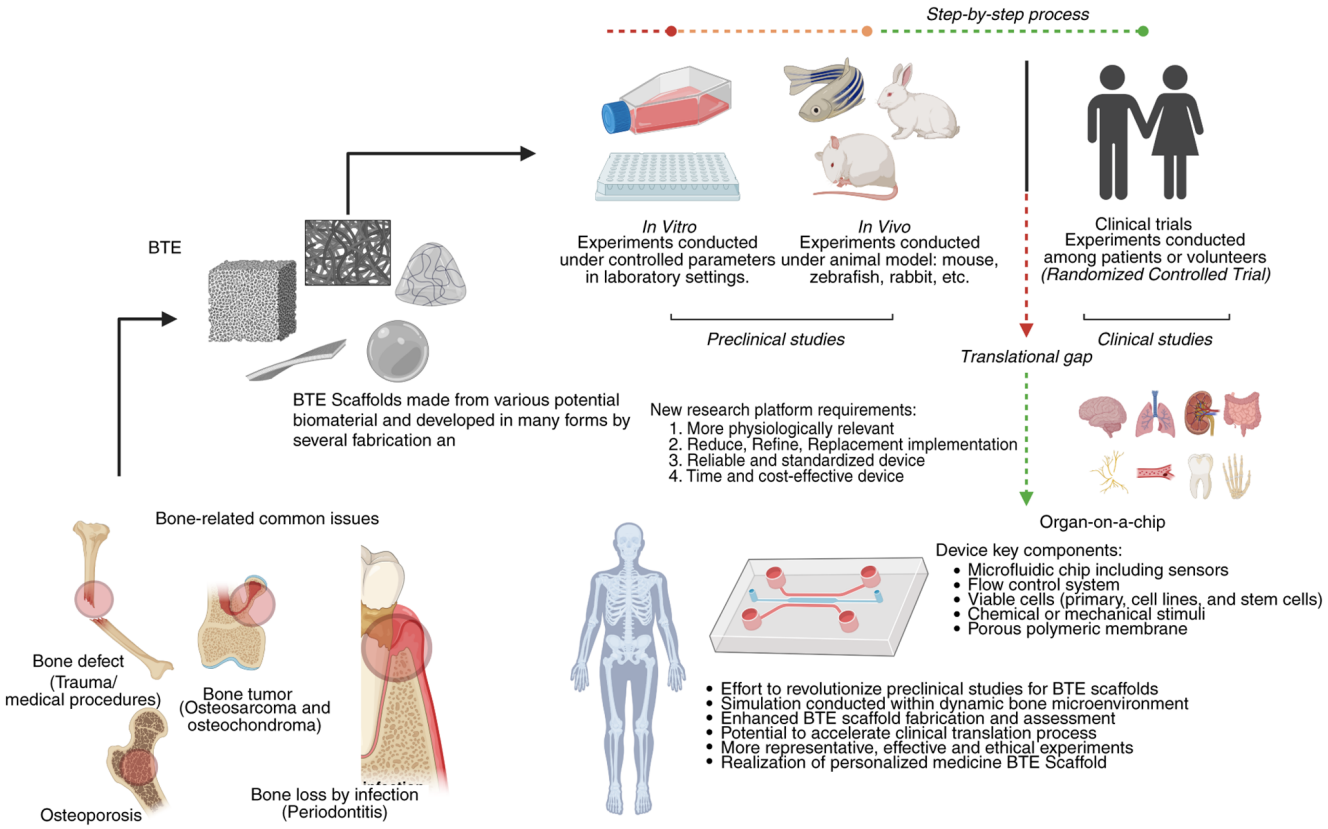

Figure 6. Potential of organ-on-a-chip to advance synthetic extracellular matrix technology in BTE. BTE, bone tissue engineering. Created with BioRender.com.

systems may enhance accessibility. Collectively, these steps will enhance the reproducibility, accessibility and translational capacity of this promising technology in BTE and beyond.

Personalized medicine, which emphasizes the importance of addressing individual variability in genetics, physiology, environment and lifestyle, recognizes that standardized treatments may not be universally effective and that personalized strategies can improve therapeutic outcomes (376). A key component in developing functional OOAC systems is the integration of sensing technology. These sensors monitor specific biomarkers, molecular signals or pathogens, making OOAC platforms well-suited to support precision medicine initiatives (377-379). Moreover, the incorporation of patient-derived cells into OOAC systems introduces a high degree of customization, accounting for genetic heterogeneity and individual biological responses (380). Assessing patient-specific variables, such as immune sensitivity to biomaterials, comorbidities or genetic disorders affecting

scaffold performance, can be effectively achieved using OOAC models. These systems also enable the investigation of rare diseases, cancer progression or individual immune responses in the context of regenerative outcomes (381). As such, OOAC technology may become integral to personalized treatment development and clinical decision-making.

7. Conclusion

OOAC technology represents an innovative biomedical approach with potential to enhance BTE research, particularly in the evaluation of newly developed scaffolds, offering a more physiologically relevant, cost-effective, efficient and ethically acceptable alternative to conventional *in vitro* and animal models. By integrating microfluidics, advanced biomaterials and dynamic cell culture, OOAC systems replicate complex tissue environments, supporting more accurate preclinical testing in dentistry and regenerative medicine. The advancement of OOAC may accelerate clinical translation, enable personalized scaffold development and reduce reliance on animal testing in biomedical research.

The dual availability of self-assembled and commercially available OOAC systems further strengthens their applicability. However, widespread adoption requires addressing key challenges such as standardization, validation, the use of diverse cell types and the integration of multiple organ systems. Overcoming these obstacles may unlock the full potential of this transformative technology, facilitating a gradual shift away from animal studies and paving new paths for bone-related therapies in human healthcare.

Although the present review focused on English-language studies published within a defined time window, relevant insights may also exist outside this scope. Future research would benefit from broader inclusion criteria encompassing earlier and non-English publications to provide a more comprehensive and globally representative understanding of OOAC applications in BTE.

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Availability of data and materials

Not applicable.

Authors' contributions

MHS, IDA and NB conceived the study. MHS and IDA designed the methodology. MHS and DB performed the literature review. MHS and IDA analyzed data, wrote the manuscript and constructed figures. IDA, DI, RA and NB edited the manuscript. IDA, RA, DI and NB supervised the study. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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