




# Bio-manufacturing innovation lights up the future

## Report from the Second Bio-Design & Manufacturing Youth Forum

Liang Ma<sup>1,2,3</sup>  · Huayong Yang<sup>1,2,3</sup>

Received: 29 January 2023 / Accepted: 31 January 2023  
© Zhejiang University Press 2023

Bio-manufacturing, a frontier interdisciplinary subject, is a promising field full of creative thinking and innovation [1]. In order to create a platform for young scientists and students in the bio-manufacturing field to have a good chance of communication and discussion, based on the success of *First Bio-Design & Manufacturing Youth Forum* in 2021 [2], the *Second Bio-Design & Manufacturing Youth Forum* chaired by the editor-in-chief Academician Huayong Yang and jointly sponsored by the journal of *Bio-Design and Manufacturing*, School of Mechanical Engineering, Zhejiang University and the State Key Laboratory of Fluid Power and Mechatronic Systems was successfully held in Hangzhou on November 19, 2022. Ten prestigious young scientists were invited to deliver a speech on their own research both onsite and online, and more than 400 attendants in this area from various universities and research institutes were attended. In this forum, they discussed the novel bionic design, innovative three-dimensional (3D) bioprinting process, novel biomaterials and new biomedical applications. In this report, we summarize all the hot topics delivered by the speakers and list them by the research area.

### Innovative 3D bioprinting processes

#### Multimaterial light-based bioprinting

The Laboratory of Engineered Living Systems directed by Prof. Yu Shrike Zhang is focused on generating functional

tissues and their models toward applications in regenerative medicine and personalizable medicine using a collection of medical engineering strategies, including 3D bioprinting, organ-on-a-chip, biomaterials, and bioanalysis. Of particular interest, the laboratory develops numerous bioprinting methodologies to enhance tissue biofabrication, spanning from hardware/software engineering to bioink designs and downstream applications.

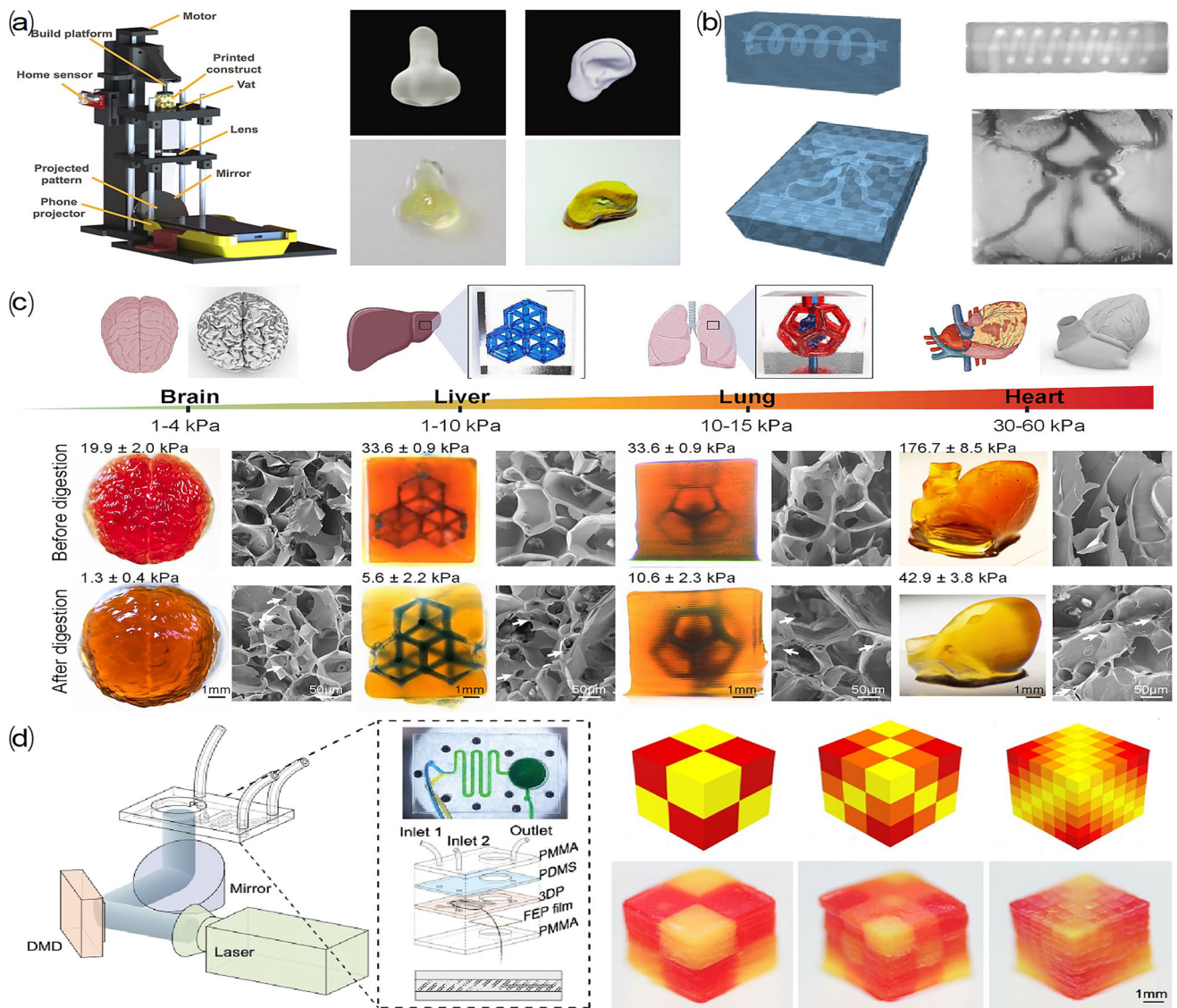
One of the key bioprinting modalities that the laboratory investigates is vat-polymerization bioprinting, specifically digital light processing (DLP) bioprinting. Examples from the hardware/software engineering perspective included the miniaturization of the bioprinter device to a low-cost smartphone projector-enabled configuration that allowed convenient fabrication of complex organ-like structures and in situ tissue creation (Fig. 1a) [3]. Moreover, designing high-performance bioinks for DLP-based bioprinting is of importance. With our collaborators, we demonstrated the adoption of fish-derived gelatin methacryloyl (GelMA) for DLP bioprinting of sophisticated tissue-embedded vascular patterns (Fig. 1b) [4]. We ourselves further invented a molecular cleavage strategy to make possible DLP bioprinting of physiologically relevant tissues with native-matching mechanical properties using a multicomponent bioink that would allow initial high stiffness for printability and selective enzymatic digestion of the desired component to lower the matrix mechanics (Fig. 1c) [5]. Finally, we heavily investigate the multimaterial capacity of DLP bioprinting toward functional tissue engineering—through integration of a microfluidic chip design, we demonstrated the ability to produce discrete multimaterial patterns in an automated, fast manner [6]; more recently, such a system was upgraded to be able to also produce continual and precisely composable multimaterial patterns via additional incorporation of a microfluidic mixer setup (Fig. 1d) [7].

✉ Liang Ma  
liangma@zju.edu.cn

<sup>1</sup> State Key Laboratory of Fluid Power and Mechatronic Systems, Zhejiang University, Hangzhou 310058, China

<sup>2</sup> School of Mechanical Engineering, Zhejiang University, Hangzhou 310058, China

<sup>3</sup> The Editorial Office of Bio-Design and Manufacturing, Zhejiang University, Hangzhou 310058, China



**Fig. 1** Innovations in digital light processing (DLP) bioprinting strategies. **a** A smartphone projector-enabled low-cost DLP bioprinter and bioprinting of solid organ-like structures (reproduced from [3], Copyright 2021, with permission from Wiley-VCH GmbH). **b** DLP bioprinting of tissue-embedded microchannel networks using the fish-GelMA bioink (reproduced from [4], Copyright 2021, with permission from the authors). **c** The molecularly cleavable bioink for DLP bioprinting of tissue constructs with native-matching mechanical properties

(reproduced from [5], Copyright 2022, with permission from the authors). **d** Microfluidic mixer-based multimaterial DLP bioprinting of composable gradients (reproduced from [7], Copyright 2021, with permission from Wiley-VCH GmbH). GelMA: gelatin methacryloyl; DMD: digital micromirror device; PMMA: poly(methyl methacrylate); PDMS: polydimethylsiloxane; 3DP: 3D printing; FEP: fluorinated ethylene propylene

### Laser additive manufacturing

Defect repair and tissue regeneration are always worldwide problems that humanity continues to explore. It is expected that grafts will be developed from short-term functional replacement to permanent regenerative repair. Therefore, Prof. Cijun Shuai’s team (from Central South University and Jiangxi University of Science and Technology) focuses on common challenges in tissue repair, such as difficulties in fine structure preparation and poor biomechanical

adaptation of grafts. They propose a new idea of laser additive manufacturing of tissue regeneration structures, establish theoretic and/or technical systems of “equipment-materials-process-performance,” and achieve many key breakthroughs in personalized tissue repair from 0 to 1. The main achievements and contributions are as follows:

The principle and method of laser additive manufacturing for micronanoscale grain and macroscale tissue regeneration structures are proposed. The interaction mechanism between the laser and material under multifield coupling is revealed.

High-precision laser additive manufacturing equipment that can realize the precise control of macro-, micro- and nanostructures is developed, which fills the domestic gap in this area. Moreover, the evolution mechanism of powder geometry under the coupling of a complex heat flow field is revealed. The spheroidizing and shaping system of special powder for laser additive manufacturing is developed, which breaks through the bottleneck of the preparation of high fluidity biodegradable powders and solves a conundrum that powders cannot be 3D printed due to poor fluidity. Meanwhile, the powder processed by this system has high sphericity ( $\geq 98\%$ ) and good fluidity (angle of repose improved from  $41.3^\circ$  to  $28.5^\circ$ ).

The theory and technology of laser additive manufacturing with a cross-scale microporous structure and individual shape are established. The formation principle and preparation methods of multiscale microporous structures are proposed. Moreover, matching criteria between the forming accuracy of the tissue regeneration structure and the laser additive manufacturing process are established. The mechanism of defect formation in the forming process and its influence on the dimensional accuracy are revealed. Strategies such as zone scanning and multistage preheating are proposed to integrate high-precision forming and high-performance additive manufacturing. The prepared tissue regeneration structure has both a personalized shape and hierarchical porous structure with 10–100 nm, 10–100  $\mu\text{m}$  and 150–800  $\mu\text{m}$  pores, high porosity  $>70\%$  and interconnection rate of 100%, which is similar to the natural bone structure and provides space for the ingrowth of new bone and blood vessels.

The regeneration theory and regulation method of the transformation from artificial structure to biological structure are developed. A sandwich-like codispersion and self-dispersion system constructed by zero-dimensional nanoparticles, one-dimensional nanotubes and two-dimensional nanosheets is innovatively constructed, which realizes the synergistic dispersion and strengthening of the second nanophase in the tissue regeneration structure and solves the agglomeration problems of the nanophase. Furthermore, boron nitride nanotubes and graphene oxide can induce the directional differentiation of stem cells, which further improves tissue regeneration ability. More significantly, the scientific nature of stem cell directional differentiation is revealed, thus overcoming the conundrum of biological and mechanical adaptation of tissue regeneration structures. Figure 2 summarizes the scheme of laser-based additive manufacturing for bone regeneration.

### High-precision bioprinting of micronanoscale fibers/living cells

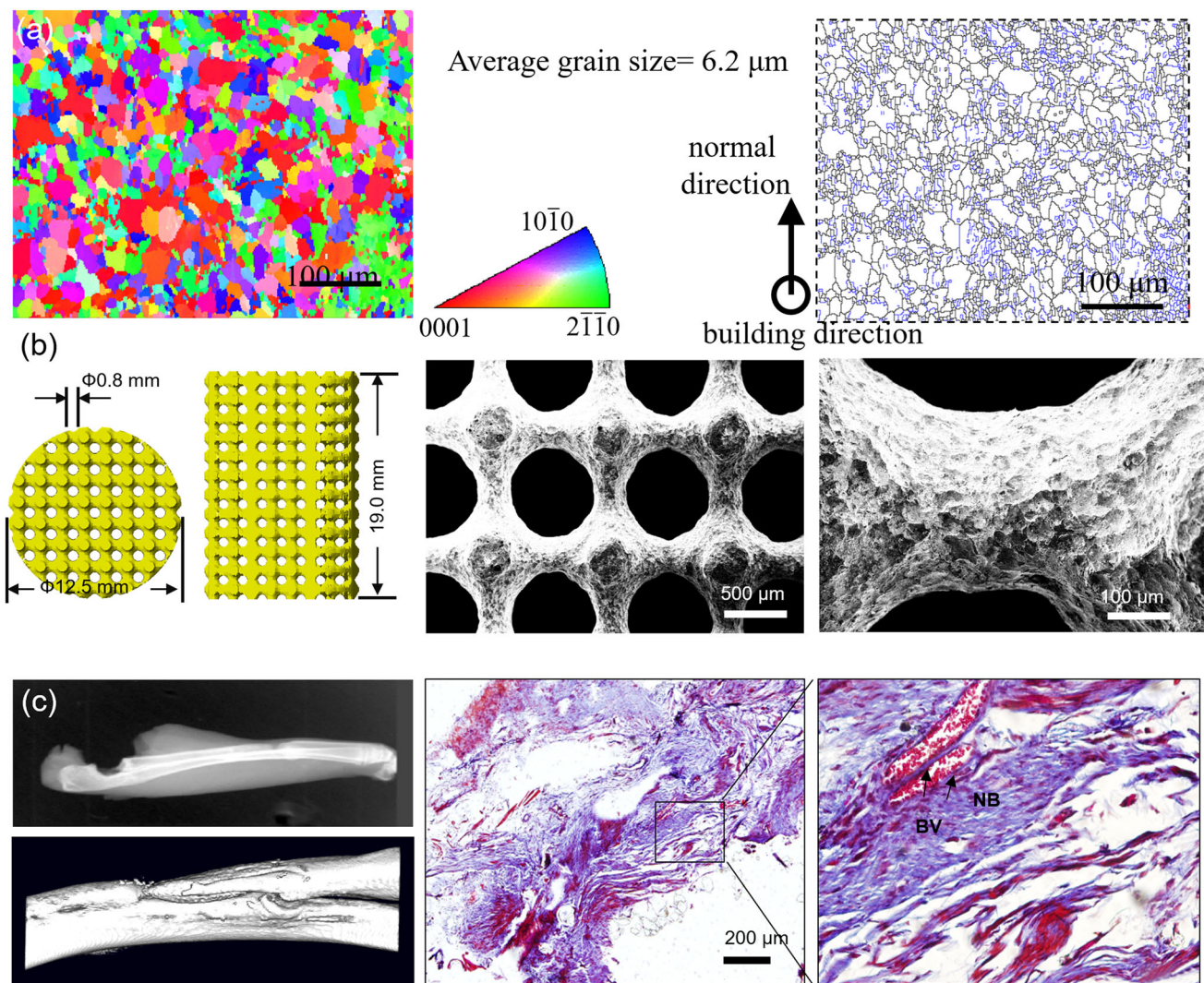
Aiming at the functional regeneration and clinical repair of human soft tissues and organs, the research group from Prof.

Dichen Li and Prof. Jiankang He at Xi'an Jiaotong University has conducted three aspects of scientific explorations on the design and fabrication of flexible biodegradable scaffolds, high-precision bioprinting of micronanoscale fiber/living cell composite constructs and the construction of vascularized organoid models with capillary scale. Critical technical challenges such as the flexible structural design of degradable materials and high-precision additive manufacturing have been overcome [11] (Fig. 3a). The dynamic mechanical evolution behavior during the degradation of the flexible scaffold in vivo and the tissue regeneration process has been clarified. In addition, clinical trials including postbreast cancer filling and tracheal softening repair by using flexible degradable/renewable scaffolds have been realized, which provides a new strategy for the accurate and personalized repair of some soft tissue defects. In addition, we developed an innovative method for high-precision electrohydrodynamic printing of micro/nanobiological fibers and living cells [12] (Fig. 3b). Commercialized multinozzle bioprinting equipment was finally developed with micronanoscale printing resolution. It was found that micro/nanofibers and hydrogels can guide/restrain the 3D directional growth of myocardial cells, muscle cells and nerve cells, which can significantly enhance the functionalities of engineered constructs in vitro. This new bioprinting capability provides a promising method for 3D controllable manufacturing and functional regulation of complex living tissues and organs. Furthermore, inspired by natural leaf venation, we presented a microfabrication method to produce a microfluidic network with capillary scale inside biologically relevant biomaterials for the first time, and the fluid transport mechanism inside the artificial microchannels was clarified (Fig. 3c) [13]. The vascularization of microchannel networks and the construction of multiorgan models in vitro were realized, which provides a new strategy for the precise manufacturing of vascularized organoid tumor/tissue models in vitro. In the future, the applications of high-precision 3D-printed living soft tissues and organoids in the fields of clinical repair and in vitro drug modeling will be further explored.

## Novel bionic design

### Bionic structure design and precision biofabrication for artificial bone scaffolds

The performance of bioceramic bionic bones depends on the biomaterials, their porous structure, and the forming process used. To understand the mutual effects of bionic structure, biomaterials, process and performance requirements of artificial bones, Prof. Yanen Wang's team (from Northwestern Polytechnical University) believes that the



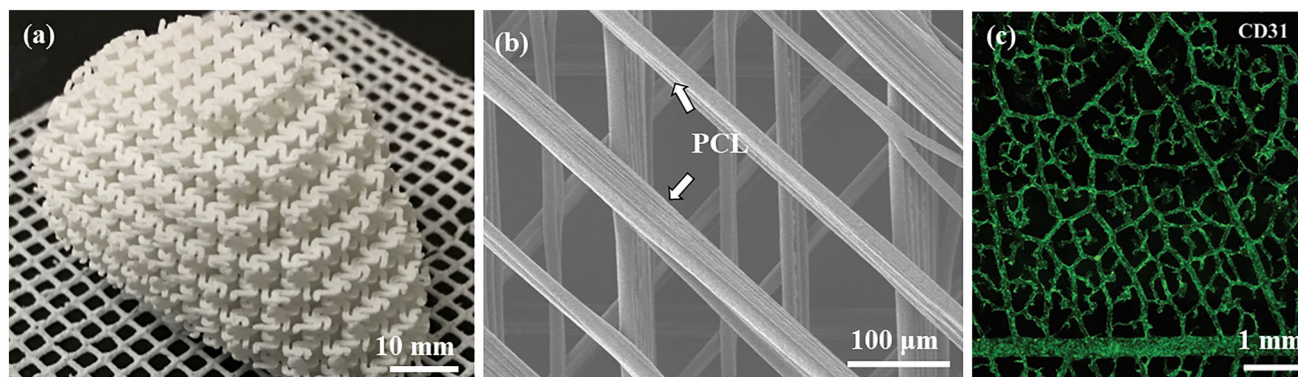
**Fig. 2** Additive manufactured artificial structure for tissue repair. **a** Microscale grain (reproduced from [8], Copyright 2022, with permission from Zhejiang University Press). **b** Porous structure (reproduced

from [9], Copyright 2021, with permission from Elsevier Ltd.). **c** Bone tissue formation after implantation for 8 weeks (reproduced from [10], Copyright 2021, with permission from Zhejiang University Press)

following considerations need to be developed: (1) improving the bionic design from the perspectives of nutrition supply and mechanical optimization, (2) achieving optimal manufacturing using an improved 3D printing process with parametric optimization, and (3) investigating performance testing and verification considering the demands of product applications. To fulfill these criteria, the following investigations were conducted.

(1) Based on molecular dynamics, an interaction model between polymer adhesives and bioceramics was established to determine the toughening effects of different adhesives on ceramic bone scaffolds. A research method was proposed to study the toughening modification of

bioceramic bone scaffolds. The effects of different component concentrations on the structure and properties of bone scaffold matrix materials and the aggregation behavior of nanoparticles were studied using molecular dynamics and dissipative particle dynamics theories. The process parameters of blending materials with nanoparticle dispersion characteristics were optimized, which made up for the defects of mechanical properties of the matrix materials and provided the theoretical and parameter basis for the selection of bonding agents for strength toughening of hydroxyapatite (HA) bone scaffolds prepared by 3D printing technology (Fig. 4a). (2) Simulation modeling of the spreading system of bioceramic fine powder was established, and the flow characteristics of fine ceramic particles during the powder



**Fig. 3** Fabrication of microstructural features to regulate cellular growth. **a** Additive manufacturing of flexible biodegradable scaffolds for soft tissue engineering (reproduced from [11], Copyright 2020, with permission from the authors). **b** Electrohydrodynamic printing of microfibrillar architectures for cellular alignment (reproduced from [12],

Copyright 2019, with permission from The Royal Society of Chemistry). **c** Leaf-venation-inspired fabrication of a microfluidic network for endothelialization at the capillary scale (reproduced from [13], Copyright 2022, with permission from Wiley-VCH GmbH). PCL: polycaprolactone

spreading process were studied and analyzed. The vibration powder spreading method was found to improve the powder spreading quality of bioceramic particles that are easy to agglomerate and harden. The problem that the wet sticky sample, with weak mechanical strength, cannot be directly detected was solved by modeling the microstrength wet sticky particle stacking system (Fig. 4b).

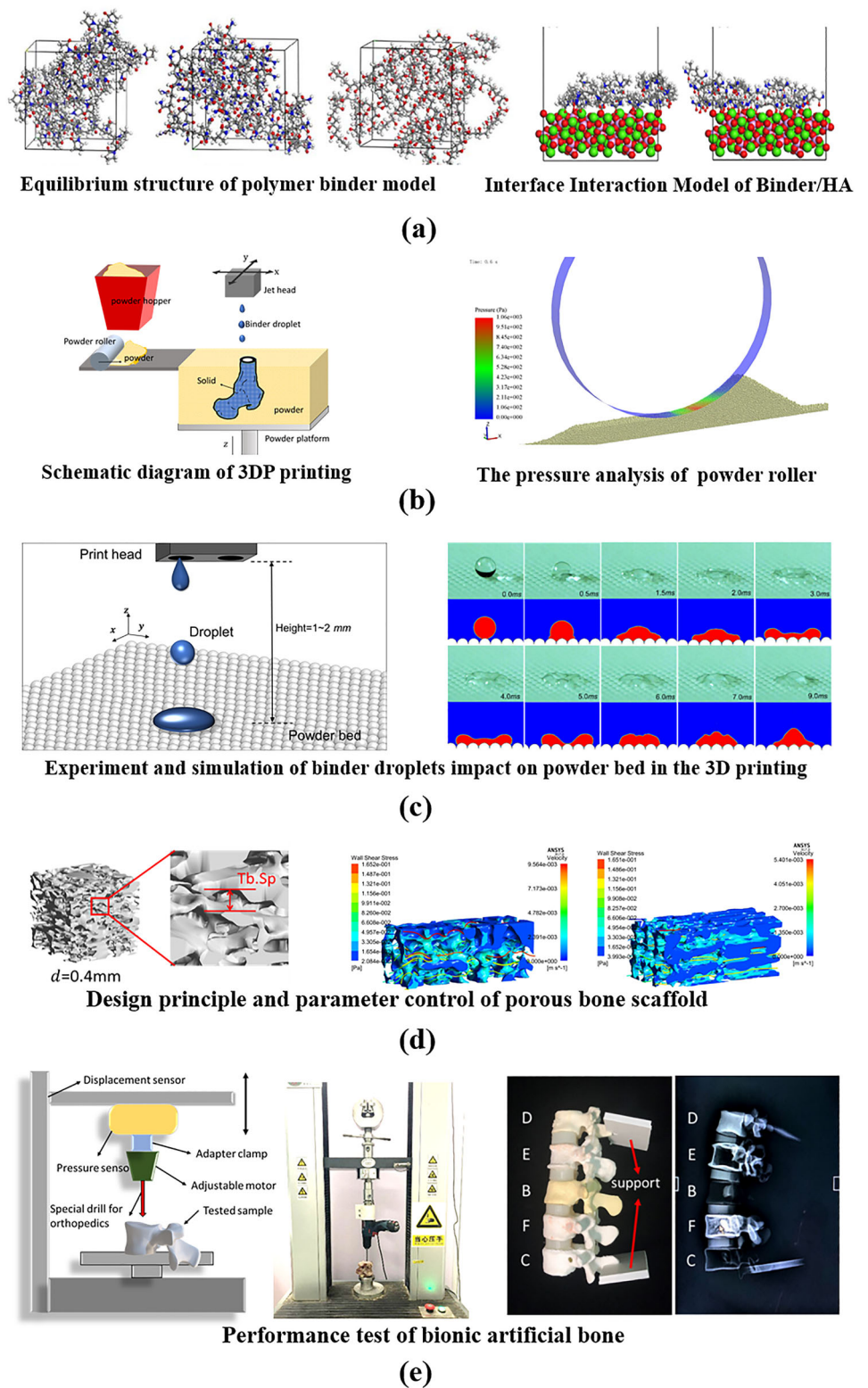
- (3) A model of low-impact bio-binder impacting ceramic particles was established to study the impact behavior of bio-binder on ceramic particles. Understanding the microdroplet particle interaction process helps find the optimal printing parameters of 3D printing bioceramic powder for establishing different binder/ceramic material systems for toughening modification (Fig. 4c) [14].
- (4) Based on the design principle of bionic artificial bone with biological and histological characteristics, a structural design method of complex porous bionic artificial bone with controllable histological parameters was obtained. The topological reconstruction and simulation modeling of the complex gradient large segment human cancellous bone/bionic porous artificial bone structure and the seepage characteristics have solved the problem of structural design evaluation of porous artificial bone from the macronutrient supply and microcellular fluid shear effect (Fig. 4d) [15].
- (5) The proposed method for testing the characteristics of bionic artificial bone based on the clinical applications of complex orthopedic patients has solved the problem that the viscoelastic, anisotropic, uneven preoperative exercise model of bionic artificial bone, as a new medical device, lacks corresponding performance testing methods (Fig. 4e).

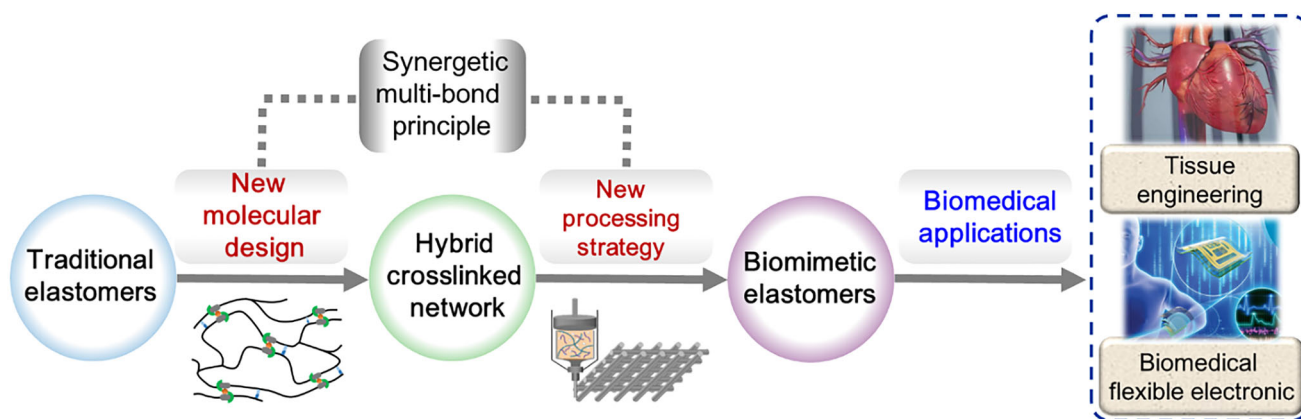
## Novel biomaterials

### Biomimetic elastomers, 3D printing and their biomedical applications

Biocompatible elastomers can resemble the mechanical properties of the extracellular matrix and soft tissues and are compliant with the dynamic mechanical environment in the body; thus, they are very useful for many biomedical applications. However, typical biomedical elastomers, such as the widely used poly(glycerol sebacate) (PGS), still have difficulty in finely mimicking the mechanical features of tissues, lack self-healing properties, and are difficult to process. Accordingly, Prof. Zhengwei You's group at Donghua University has conducted systematic studies to address the aforementioned issues. A multibond interaction molecular strategy of synergistic strong bonds and weak bonds has been proposed. First, three synergistic mechanisms of “mechanically invisible” weak bonds at small strain, forming copper coordination bonds for strengthening and catalyzing the recombination of dynamic covalent bonds, construct a multibond hybrid cross-linking molecular network, which solves the conflict that high mechanical strength, low modulus, and self-healing are difficult to balance. Accordingly, a skin-like strong, tough, and soft PGS-based elastomer with a nonlinear mechanical profile [16] and a room temperature self-healing Cu(II)–dimethylglyoxime–urethane–complex-based poly(oxime-urethane) elastomer with world-recorded tensile strength and toughness have been created [17]. This work provides a universal molecular design for the development of soft, strong, and self-healing bionic elastomers. Second, a general processing strategy of “creating strength from weakness” in synergetic multibond systems is proposed. Utilization of the weak noncovalent interaction between food

**Fig. 4** Bionic structure design and precision biofabrication for artificial bone scaffolds. **a** A molecular dynamics simulation model of the interaction between polymer adhesives and bioceramics. **b** Study the flow characteristics of the spreading system of bioceramic fine powder. **c** Study the deformation process of binder droplet spreading on the layer of powder bed surface (reproduced from [14], Copyright 2022, with permission from the authors). **d** Study the topological reconstruction and simulation modeling of the complex gradient large segment of porous artificial bone structure and its seepage characteristics (reproduced from [15], Copyright 2022, with permission from Elsevier B.V.). **e** Study the characteristics of bionic artificial bone based on the clinical applications of complex orthopedic patients





**Fig. 5** Design, fabrication, and applications of bionic biomedical elastomers

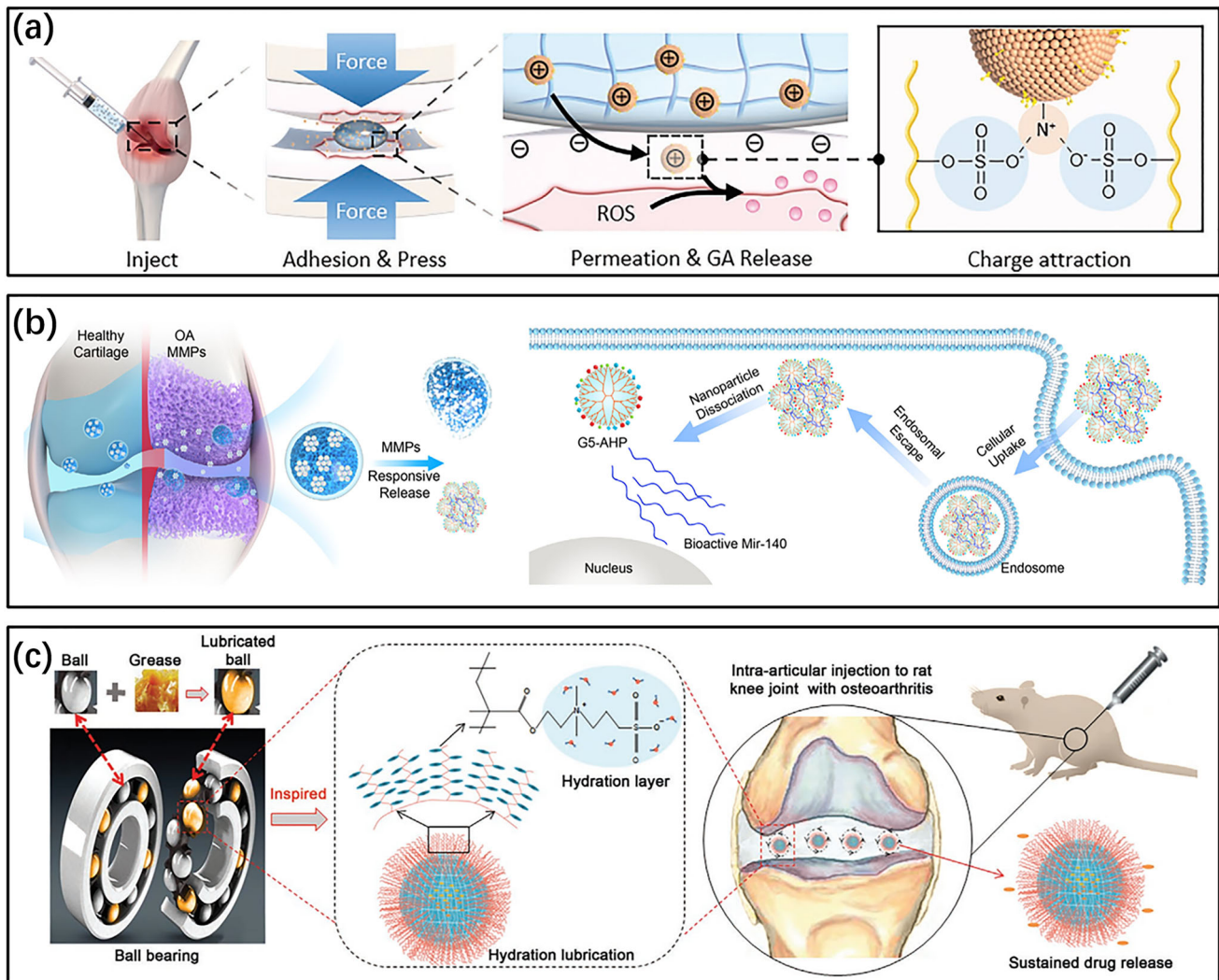
materials and polymers to regulate polymer viscoelasticity facilitates the formation of a strong covalently crosslinked network, which overcomes the difficulty of thermoset elastomer processing [18]. Accordingly, new 3D printing strategies have been established to enable facile personalized customization of thermosetting porous scaffolds and the construction of complex structures such as perfusable and permeable biomimetic vascular networks [19]. Third, we demonstrate a “print-healing” strategy to solve several bottlenecks in fused deposition modeling printing [20]. Inspired by Lego bricks, we rationally split the complex 3D structure into simple modules for printing. Then, complete complex architectures were assembled from these modules and spliced by self-healing. This strategy enables the construction of complex structures and large objects from a compact printer. The multiple dynamic bonds induce strong binding between layers to avoid typical poor layer-to-layer adhesion. This strategy also enables in situ fabrication of devices in vivo. Accordingly, a series of new medical devices with outstanding performance for tissue repair, including myocardial infarction, aortic aneurysm, nerve coaptation, and biomedical flexible electronics, have been developed [21–23]. The molecular design concepts, synthetic methods, molding strategies, and device construction ideas are general and may be used to construct other functional biomaterials for diverse biomedical applications. The scheme of design, fabrication, and applications of bionic biomedical elastomers is summarized in Fig. 5.

### Injectable hydrogel microspheres promote articular cartilage repair

The repair and regeneration of articular cartilage have been an essential clinical concern for medical researchers in recent decades. The scientific research team led by Prof. Wenguo Cui (from Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine and Shanghai Institute

of Traumatology and Orthopaedics) has developed various multifunctional hydrogel microspheres using microfluidic technology for the repair and regeneration of articular cartilage with promising results.

First, to solve the problem of cartilage drugs not being retained in the joint cavity for a long time, Prof. Wenguo Cui’s team constructed microscale hydrogel microspheres with different functional modules to achieve the long-term slow release of drugs and resist the clearance of synovial tissue. Lin et al. [24] presented a charge-guided micro/nano-hydrogel microsphere system that can penetrate deeply into the cartilage matrix and release drugs close to chondrocytes guided by the negative charge of the matrix. This system effectively improves drug utilization and efficacy and significantly promotes cartilage repair and regeneration (Fig. 6a). Moreover, gene therapy is an effective treatment for cartilage injury of interest to Prof. Wenguo Cui’s team. The team innovatively constructed hydrogel microspheres with the function of performing gene therapy to achieve cartilage repair by regulating the gene expression of chondrocytes. For instance, Li et al. [25] successfully constructed gene hydrogel microspheres encapsulated with nanoparticles containing microRNA-140, which enabled gene therapy targeting cartilage to mitigate degradation of articular cartilage (Fig. 6b). Moreover, since the cartilage matrix is constantly being worn and torn by the movement of the knee joint, the duration of the disease can be greatly reduced if the cartilage damage caused by friction is reduced during the process of cartilage repair and regeneration. Inspired by mechanical ball bearings, Yang et al. [26] prepared hydrogel microspheres with surface lubrication functions capable of converting sliding friction into rolling friction based on their unique microscale spherical structure, which was demonstrated to significantly reduce frictional damage to cartilage. This research has pioneered a new application mode of hydrogel microspheres (Fig. 6c).



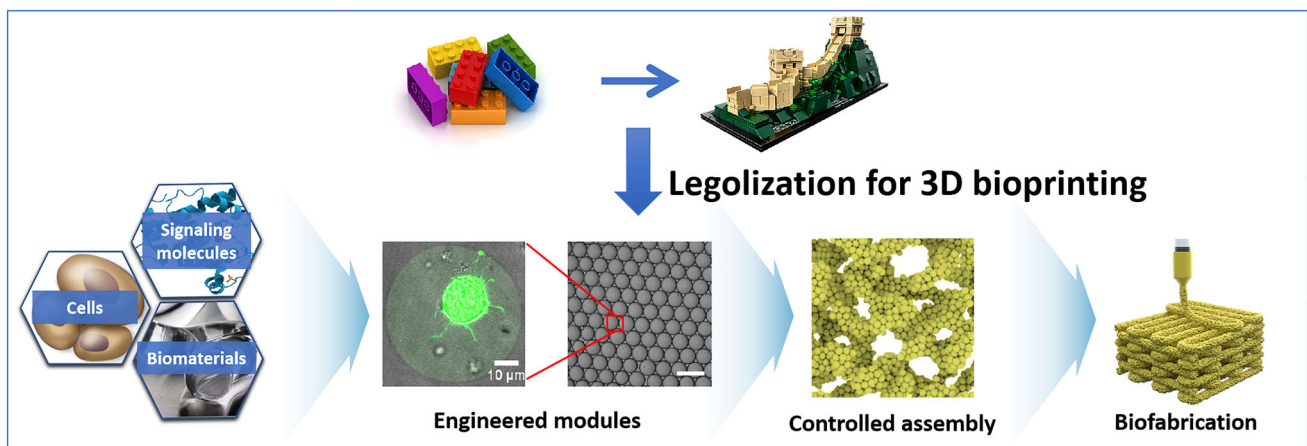
**Fig. 6** Hydrogel microspheres have been used for cartilage repair and regeneration. **a** The charge-guided hydrogel microspheres achieved penetration of the cartilage matrix and long-term slow release of drugs (reproduced from [24], Copyright 2021, with permission from Wiley-VCH GmbH). **b** Gene hydrogel microspheres have enabled gene

therapy targeting cartilage to mitigate degradation of articular cartilage (reproduced from [25], Copyright 2021, with permission from the authors). **c** Ball-bearing inspired hydrogel microspheres as bio-lubricants attenuate osteoarthritis (reproduced from [26], Copyright 2020, with permission from Wiley-VCH GmbH)

Finally, the development of biomedical materials has brought a new idea for the repair and regeneration of articular cartilage. However, due to the unclear pathophysiological mechanism and the complex pathological environment of cartilage, promoting articular cartilage repair and regeneration in a more efficient and targeted way is also a major challenge. Therefore, researchers need to continuously explore the unknown mechanism of cartilage injury and the various factors that affect the efficacy of cartilage repair and regeneration. Only from the clear pathophysiological mechanism can the new generation of hydrogel microspheres be more efficient and targeted to promote articular cartilage repair and regeneration.

### Micro- and nanomodules for the legalization of organ fabrication

Living organisms can be considered to be assembled by a variety of building blocks of different length scales, such as small molecules on the order of angstroms, proteins on the order of nanometers, or individual cells or tens of microns. To replicate and engineer these building blocks and enable further assembly of these components into macroscale constructs, similar to Lego games, can enable major advances in the design and fabrication of engineered tissues/organs, which opens a new avenue for the design of multifunctional biomaterials and the engineering of tissue mimics. Aiming at this, Huanan Wang's group at Dalian University of



**Fig. 7** Scheme showing the design rationale of micro- and nanomodules for the legalization of organ fabrication

Technology endeavored to develop innovative material systems and microfabrication techniques toward the design of high-performance biomaterials and engineered tissue mimics (Fig. 7). Specifically, they focus on two scales, (1) to generate functionalized nanomaterials as Lego building blocks to assemble into hierarchical constructs with fascinating viscoelastic behaviors, including self-healing and shape memory, and (2) to fabricate microtissue modules as building blocks to form granular gels of desirable printability and fidelity. The former nanostructured system is represented by so-called colloidal gels, which are typically composed of a continuous network of assembled particles dispersed in a liquid. These colloidal gels with special viscoelastic behavior have recently attracted increasing attention for biomedical applications such as tissue engineering, controlled delivery of biomolecules, bone substitution, and additive manufacturing [27–30]. Unlike monolithic polymeric gels, colloidal gels exhibit a heterogeneous structure characterized by the assembly of colloidal particles into strands that form a mechanically stable particulate network due to attractive interparticle forces. Specifically, by exploiting the reversibility of noncovalent interparticle interactions, colloidal gels can be rendered shear-thinning and self-healing, making them ideal candidates as printable ink materials for customized biofabrication of tissues/organs. At the micrometer scale, on the other hand, Wang's group took advantage of microfluidic droplet-based techniques to generate cellularized tissue modules in a high-throughput and controllable manner [31–34]. This strategy allows encapsulation of single cells in microgels to significantly improve nutrient/waste exchange, precisely control microenvironmental cues at the microscale, and enable on-target delivery of bioactive cargoes; all these can enable major advances in applications such as tissue engineering, cell biology, and cell-based therapies. More importantly, these cell-laden microgels can also serve as building blocks to form granular gels with shear-thinning and self-healing behavior, thereby functioning as injectable

or printable scaffolds toward the generation of highly cellularized tissues/organs [35]. Generally, recent progress from Wang's group as well as other studies has demonstrated the proof of concept to design biomaterials or engineered tissues via a legolization approach, in which we expect to witness the translation of these techniques into clinical trials for regenerative medicine in the near future.

## Novel biomedical applications

### Construction and maturation of vascularized functional myocardial tissue

Reconstruction of functional tissues/organs with complex structures in vivo and in vitro has been a dream of human beings. Three-dimensional bioprinting technology can realize the precise assembly of cells and materials in 3D complex structures, so it has attracted wide attentions. It has become a hot spot in the field of regenerative medicine and biological manufacturing and has great application prospects in the fields of damaged tissue/organ regeneration and in vitro pathology/pharmacology/toxicology model construction.

Among them, cardiac tissue is highly complex in structure and function, and its in vitro regeneration has been faced with major challenges such as vascularization, large-scale function realization and tissue maturation. Prof. Ting Zhang's team (from Tsinghua University) focuses on the key and difficult issues of the construction and maturation of vascularized functional myocardial tissue, and reviews the progress, main research strategies and development trends of relevant research at home and abroad. At the same time, they focus on the research and development of microgel dual-phase bioink, the maturation of multi-cell structure promoted by electrical stimulation, and the research on the construction of cardiac chamber structure by embedded printing.

## Biomaterials for male reproductive repair

In recent years, there has been a significant decline in male fertility; however, this issue has not been widely appreciated. Through surveys of male reproductive and urological surgeons, Prof. Xuetao Shi's team (from South China University of Technology) found that the problems of male patients can be divided into the following three categories: (1) normal testicular function but impaired penile function, making it difficult to complete normal sexual intercourse; (2) normal penile function and normal sexual intercourse, but no sperm in the semen, or even unable to obtain sperm through microscopic sperm extraction surgery; (3) abnormal endocrine function of the testes, resulting in decreased libido, erectile dysfunction and even general physical decline. To address these clinical challenges, the following three issues are considered:

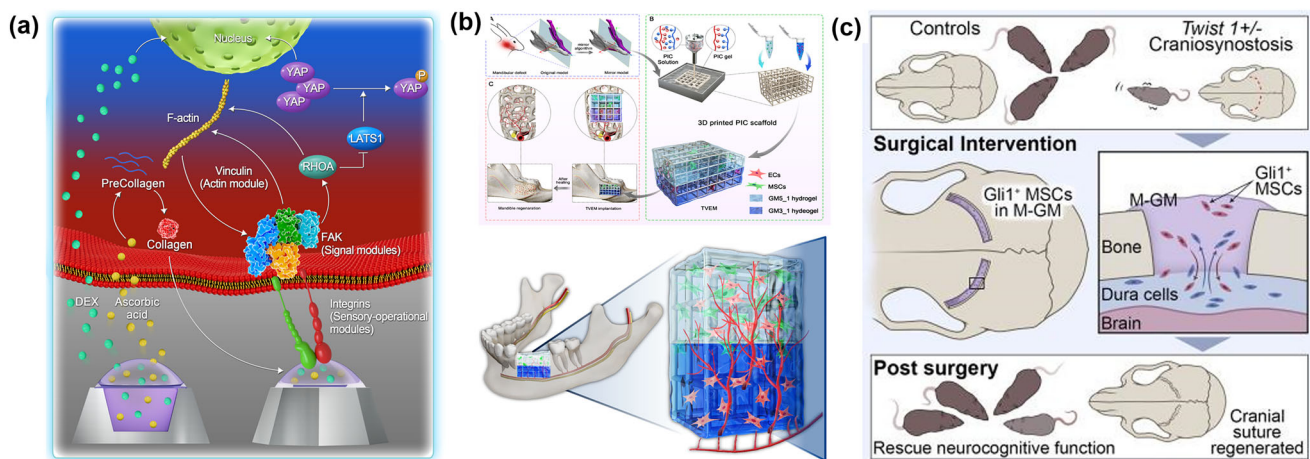
- (1) Injury of the corpus cavernosa results in erectile dysfunction, but its treatment has been very difficult. We constructed heparin-coated 3D-printed hydrogel scaffolds seeded with hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ )-mutated muscle-derived stem cells (MDSCs) to develop bioengineered vascularized corpora. HIF-1 $\alpha$ -mutated MDSCs significantly secrete various angiogenic factors in MDSCs regardless of hypoxia or normoxia. The biodegradable scaffolds, along with MDSCs, are implanted into corpus cavernosa defects in a rabbit model to show good histocompatibility with no immunological rejection, support vascularized tissue ingrowth, and promote neovascularization to repair the defects. Evaluation of the morphology, intracavernosal pressure, elasticity and shrinkage of repaired cavernous tissue proved that the bioengineered corpora scaffolds repaired the defects and successfully recovered penile erectile and ejaculation function. Functional recovery restores the reproductive capability of injured male rabbits.
- (2) Erectile dysfunction (ED) therapy has been extensively explored in preclinical and clinical studies via intracavernous injection of mesenchymal stem cells (MSCs). However, the low tissue retention, poor survival rates and nonuniform distribution of MSCs limit the therapeutic potential and clinical application of MSCs in the ED. We developed a novel adaptive magnetorobot with a reactive oxygen species (ROS) scavenging-mediated MSC delivery system (MSC-R) to enhance the therapeutic effect of MSCs. The magnetorobots improve the retention (>14 days) and survival capacities of MSCs through assistive wearable magnetic-control equipment and ROS scavengers, respectively. In both beagle and rat cavernous injury models, MSC-R accelerates the repair of cavernous tissue and the recovery of erectile and

fertility function. Mechanically, single-cell sequencing revealed that MSC-R has an increased ability to remodel tissue homeostasis via multiple targets.

- (3) As testicular resident mesenchymal stromal cells, stem Leydig cells (SLCs) play a crucial role in tissue homeostasis and regeneration. However, the mechanism by which SLCs sense and respond to damage from the native environment has not been fully illustrated. We discovered the intercellular connections between SLCs and resident macrophages. Upon their activation, SLCs establish a cellular network with macrophages primarily by tunneling nanotubes (TNTs), which transfer mitochondria from SLCs to neighboring activated macrophages. This process enhances the therapeutic efficacy of SLCs following their engraftment in acute inflammation mouse models of testicular torsion. Mitochondrial propagation attenuated the inflammatory profile of macrophages and promoted their ability to adopt a proregenerative M2-like phenotype. By reshaping the interstitial microenvironment, SLCs promoted endogenous Leydig cells (LCs) regeneration and restored spermatogenesis and fertility. Mechanistically, suppression of TRPM7 by short interfering RNA reduced intercellular mitochondrial transfer and worsened therapeutic efficacy.

## Repair and regeneration of craniofacial tissue defects based on biomaterials and stem cells

Aiming at the mechanism resolution and bionic functional regeneration of craniomaxillofacial diseases, the research group from Prof. Mengfei Yu at Zhejiang University has conducted three scientific explorations on the mechanosensitivity of stem cells, the bionic design and fabrication of vascularized engineered mandibles and the construction of MSC-based cranial suture regeneration. The combined effect of chemical and mechanical cues on the osteogenic differentiation of human mesenchymal stem cells was examined (Fig. 8a) [36]. High-resolution, highly ordered hollow microfrustum arrays using double-layer lithography combined with modified methacrylate gelatin loaded with pre-defined soluble chemicals to provide both chemical and mechanical cues to cells. This approach ultimately facilitates the achievement of cellular osteodifferentiation and enhances bone repair efficiency in a model of femoral fracture in vivo in mice. In addition, we fabricated a hierarchical vascularized engineered bone inspired by intramembranous ossification for mandibular regeneration (Fig. 8b) [37]. The hierarchical vascular network and bone structure generated by hierarchical vascularized engineered bone match the particular anatomical structure of the mandible. Bone regenerated using hierarchical vascularized engineered bone is



**Fig. 8** Mechanistic analysis and functional reconstruction of craniomaxillofacial lesions. **a** Schematic illustration of the mechanism by which chemical cues induce mechanical sensitivity in stem cells (reproduced from [36], Copyright 2022, with permission from the authors). **b** Schematic depicting the hierarchical vascularized engineered bone

for mandibular reconstruction (reproduced from [37], Copyright 2022, with permission from the authors). **c** Suture regeneration offers a biological solution for craniosynostosis patients (reproduced from [38], Copyright 2020, with permission from Elsevier Inc.)

similar to natural mandibular bone in terms of morphology and genomics. The concept based on developmental processes and bionic structures provides an effective strategy for tissue regeneration. Furthermore, using a biodegradable material combined with MSCs, we successfully regenerated a functional cranial suture that corrects skull deformity, normalizes intracranial pressure, and rescues neurocognitive behavior deficits (Fig. 8c) [38]. We show that *Twist1*<sup>±</sup> mice with craniosynostosis have increased intracranial pressure and neurocognitive behavioral abnormalities, recapitulating features of human Saethre-Chotzen syndrome. We demonstrate that MSCs combined with M-GM can regenerate a cranial suture, restore normal increased intracranial pressure, and rescue neurocognitive function in a highly clinically relevant craniosynostosis model.

**Acknowledgements** This forum was sponsored by the National Key Research and Development Program of China (No. 2018YFA0703000), the National Natural Science Foundation of China (Nos. 52275294 and 51875518), and the Zhejiang University Global Partnership Fund.

## References

- Santoni S, Gugliandolo SG, Sponchioni M et al (2022) 3D bioprinting: current status and trends—a guide to the literature and industrial practice. *Bio-Des Manuf* 5(1):14–42. <https://doi.org/10.1007/s42242-021-00165-0>
- He Y (2021) Biomanufacturing: from biomedicine to biomedicine. Report from the First Bio-Design & Manufacturing Youth Forum. *Bio-Des Manuf* 4(4):912–913. <https://doi.org/10.1007/s42242-021-00161-4>
- Li W, Wang M, Mille LS et al (2021) A smartphone-enabled portable digital light processing 3D printer. *Adv Mater* 33(35):2102153. <https://doi.org/10.1002/adma.202102153>
- Levato R, Lim KS, Li W et al (2021) High-resolution lithographic biofabrication of hydrogels with complex microchannels from low-temperature-soluble gelatin bioresins. *Mater Today Bio* 12:100162. <https://doi.org/10.1016/j.mtbio.2021.100162>
- Wang M, Li W, Hao J et al (2022) Molecularly cleavable bioinks facilitate high-performance digital light processing-based bioprinting of functional volumetric soft tissues. *Nat Commun* 13(1):3317. <https://doi.org/10.1038/s41467-022-31002-2>
- Miri AK, Nieto D, Iglesias L et al (2018) Microfluidics-enabled multimaterial maskless stereolithographic bioprinting. *Adv Mater* 30(27):1800242. <https://doi.org/10.1002/adma.201800242>
- Wang M, Li W, Mille LS et al (2022) Digital light processing based bioprinting with composable gradients. *Adv Mater* 34(1):2107038. <https://doi.org/10.1002/adma.202107038>
- Yang M, Yang L, Peng S et al (2022) Laser additive manufacturing of zinc: formation quality, texture, and cell behavior. *Bio-Des Manuf* (Early Access). <https://doi.org/10.1007/s42242-022-00216-0>
- Yang Y, Cheng Y, Yang M et al (2022) Semicohere strengthens graphene/zinc scaffolds. *Mater Today Nano* 17:100163. <https://doi.org/10.1016/j.mtnano.2021.100163>
- Yang YW, Cheng Y, Deng F et al (2021) A bifunctional bone scaffold combines osteogenesis and antibacterial activity via in situ grown hydroxyapatite and silver nanoparticles. *Bio-Des Manuf* 4(3):452–468. <https://doi.org/10.1007/s42242-021-00130-x>
- Meng Z, He J, Cai Z et al (2020) Design and additive manufacturing of flexible polycaprolactone scaffolds with highly-tunable mechanical properties for soft tissue engineering. *Mater Des* 189:108508. <https://doi.org/10.1016/j.matdes.2020.108508>
- Lei Q, He J, Li D (2019) Electrohydrodynamic 3D printing of layer-specific oriented, multiscale conductive scaffolds for cardiac tissue engineering. *Nanoscale* 11(32):15195–15205. <https://doi.org/10.1039/C9NR04989D>
- Mao M, Chen P, He J et al (2022) Deciphering fluid transport within leaf-inspired capillary networks based on a 3D computational model. *Small* 18(16):2108102. <https://doi.org/10.1002/sml.202108102>
- Li XP, Wang YE, Ahmed A et al (2022) The study of biological glue droplet impact behavior of bioceramic powders applied in 3D

- printing of bone scaffolds. *Appl Sci* 12(4):1898. <https://doi.org/10.3390/app12041898>
15. Li XP, Wang YN, Zhang B et al (2022) The design and evaluation of bionic porous bone scaffolds in fluid flow characteristics and mechanical properties. *Comput Methods Progr Biomed* 225:107059. <https://doi.org/10.1016/j.cmpb.2022.107059>
  16. Chen S, Sun L, Zhou X et al (2020) Mechanically and biologically skin-like elastomers for bio-integrated electronics. *Nat Commun* 11(1):1107. <https://doi.org/10.1038/s41467-020-14446-2>
  17. Zhang L, Liu Z, Wu X et al (2019) A highly efficient self-healing elastomer with unprecedented mechanical properties. *Adv Mater* 31(23):1901402. <https://doi.org/10.1002/adma.201901402>
  18. Song J, Chen S, Sun L et al (2020) Mechanically and electronically robust transparent organohydrogel fibers. *Adv Mater* 32(8):1906994. <https://doi.org/10.1002/adma.201906994>
  19. Chen S, Huang T, Zuo H et al (2018) A single integrated 3D-printing process customizes elastic and sustainable triboelectric nanogenerators for wearable electronics. *Adv Funct Mater* 28(46):1805108. <https://doi.org/10.1002/adfm.201805108>
  20. Zuo H, Liu Z, Zhang L et al (2021) Self-healing materials enable free-standing seamless large-scale 3D printing. *Sci China Mater* 64(7):1791–1800. <https://doi.org/10.1007/s40843-020-1603-y>
  21. Huang S, Lei D, Yang Q et al (2021) A perfusable, multifunctional epicardial device improves cardiac function and tissue repair. *Nat Med* 27(3):480–490. <https://doi.org/10.1038/s41591-021-01279-9>
  22. Jiang C, Zhang L, Yang Q et al (2021) Self-healing polyurethane-elastomer with mechanical tunability for multiple biomedical applications in vivo. *Nat Commun* 12(1):4395. <https://doi.org/10.1038/s41467-021-24680-x>
  23. Zhang L, Liang J, Jiang C et al (2021) Peptidoglycan-inspired autonomous ultrafast self-healing bio-friendly elastomers for bio-integrated electronics. *Natl Sci Rev* 8(5):nwaa154. <https://doi.org/10.1093/nsr/nwaa154>
  24. Lin F, Wang Z, Xiang L et al (2021) Charge-guided micro/nano-hydrogel microsphere for penetrating cartilage matrix. *Adv Funct Mater* 31(49):2107678. <https://doi.org/10.1002/adfm.202107678>
  25. Li B, Wang F, Hu F et al (2022) Injectable “nano-micron” combined gene-hydrogel microspheres for local treatment of osteoarthritis. *NPG Asia Mater* 14(1):1. <https://doi.org/10.1038/s41427-021-00351-7>
  26. Yang J, Han Y, Lin J et al (2020) Ball-bearing-inspired polyampholyte-modified microspheres as bio-lubricants attenuate osteoarthritis. *Small* 16(44):2004519. <https://doi.org/10.1002/smll.202004519>
  27. Diba M, Wang H, Kodger TE et al (2017) Highly elastic and self-healing composite colloidal gels. *Adv Mater* 29(11):1604672. <https://doi.org/10.1002/adma.201604672>
  28. Li D, Chen K, Tang H et al (2022) A logic-based diagnostic and therapeutic hydrogel with multistimuli responsiveness to orchestrate diabetic bone regeneration. *Adv Mater* 34(11):2108430. <https://doi.org/10.1002/adma.202108430>
  29. Wang H, Boerman OC, Sariibrahimoglu K et al (2012) Comparison of micro- vs. nanostructured colloidal gelatin gels for sustained delivery of osteogenic proteins: bone morphogenetic protein-2 and alkaline phosphatase. *Biomaterials* 33(33):8695–8703. <https://doi.org/10.1016/j.biomaterials.2012.08.024>
  30. Wang Y, Zhang Y, Chen K et al (2021) Injectable nanostructured colloidal gels resembling native nucleus pulposus as carriers of mesenchymal stem cells for the repair of degenerated intervertebral discs. *Mater Sci Eng C* 128:112343. <https://doi.org/10.1016/j.msec.2021.112343>
  31. Zhang H, Zhang L, An C et al (2022) Large-scale single-cell encapsulation in microgels through metastable droplet-templating combined with microfluidic-integration. *Biofabrication* 14(3):035015. <https://doi.org/10.1088/1758-5090/ac7168>
  32. An C, Liu W, Zhang Y et al (2020) Continuous microfluidic encapsulation of single mesenchymal stem cells using alginate microgels as injectable fillers for bone regeneration. *Acta Biomater* 111:181–196. <https://doi.org/10.1016/j.actbio.2020.05.024>
  33. An C, Zhang Y, Li H et al (2021) Thermo-responsive fluorinated surfactant for on-demand demulsification of microfluidic droplets. *Lab Chip* 21(18):3412–3419. <https://doi.org/10.1039/d1lc00450f>
  34. Zhang L, Chen K, Zhang H et al (2018) Microfluidic templated multicompartiment microgels for 3D encapsulation and pairing of single cells. *Small* 14(9):1702955. <https://doi.org/10.1002/smll.201702955>
  35. An C, Zhou R, Zhang H et al (2023) Microfluidic-templated cell-laden microgels fabricated using phototriggered imine-crosslinking as injectable and adaptable granular gels for bone regeneration. *Acta Biomater* 157:91–107. <https://doi.org/10.1016/j.actbio.2022.11.034>
  36. He J, You D, Li Q et al (2022) Osteogenesis-inducing chemical cues enhance the mechanosensitivity of human mesenchymal stem cells for osteogenic differentiation on a microtopographically patterned surface. *Adv Sci* 9(16):2200053. <https://doi.org/10.1002/advs.202200053>
  37. Ye X, He J, Wang S et al (2022) A hierarchical vascularized engineered bone inspired by intramembranous ossification for mandibular regeneration. *Int J Oral Sci* 14(1):31. <https://doi.org/10.1038/s41368-022-00179-z>
  38. Yu M, Ma L, Yuan Y et al (2021) Cranial suture regeneration mitigates skull and neurocognitive defects in craniosynostosis. *Cell* 184(1):243–256.e18. <https://doi.org/10.1016/j.cell.2020.11.037>