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Highlights from the latest articles in advanced biomanufacturing at micro- and nano-scale

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Digital microfluidics for on-demand manufacturing & handling of microscale hydrogel materials

Evaluation of: Eydelnant IA, Betty Li B, Wheeler AR. Microgels on-demand. *Nat. Commun.* 5, 3355 (2014).

The ability to generate and assemble content-varying hydrogel composites can benefit broad fundamental studies and industry applications, including screening stem-cell differentiation niche, engineering complex microphysiological system and evaluating drug safety and efficacy [1]. Despite great efforts in developing such technologies, most current techniques are unable to simultaneously generate multiple types of hydrogel composites. In this work, the authors addressed this challenge by introducing an addressable digital microfluidic method [2]. Digital microfluidics enables individual control over picoliter- to microliter-sized droplets on an open electrode array by digitally addressing wettability of each electrode via electrowetting [3]. Every droplet is addressable by applying an electric potential on the electrode it places on. Multiple droplets can be manipulated in parallel with serial operations by programming wettability of electrodes. Using this technique, the authors dispensed precursor solutions of different hydrogel materials from the reservoirs to the electrode arrays with a volumetric variation less than 3.3% and further generated content-varying droplet arrays by moving, mixing, merging, and splitting the dispensed droplets. These droplets containing hydrogel precursor solution can be gelled by photo-, chemical or thermal crosslinking. Hydrogel composites can be formed layer by layer by dispensing and crosslinking another type of hydrogel precursor solution around the initial crosslinked gel structure. In addition, the geometry of microgels can also be controlled by changing the

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shape of the hydrophilic sites and the thickness of interplate spacing. The authors utilized this approach for 3D cell culture and tissue formation. Kidney cells suspended in Geltrex™ (Gibco®, CA, USA) were encapsulated in the gel *in situ* and cultured over 5 days with daily media exchange. Lumen-like structure was observed after 96-h tissue culture and polarization of kidney spheroids was confirmed by immunostaining with ZO-1 and E-cadherin.

The digital microfluidic technique presented in this study allows generation of multiple hydrogels or hydrogel composites in parallel with individually tailored content; compatible with multiple crosslinking mechanisms; does not require moving parts or robotics. However, current device only allows formation of hydrogels sized from 500 µm to 2 mm, which is much larger than the size of a single cell. Thus, it loses feasibility for applications that require single-cell level control. This issue might be addressed by fabricating a device with smaller electrode and hydrophilic spot size. In addition, throughput of this method is also expected to increase by expanding the number of electrodes and introducing smart control programs. In brief, this work opens a new way for parallel manufacturing of diverse hydrogel materials, which could potentially many fields including regenerative medicine and drug discovery.

Biomanufacturing of tunable multiscale materials with genetically engineered cells

Evaluation of: Chen AY, Deng Z, Billings AN *et al.* Synthesis and patterning of tunable multiscale materials with engineered cells. *Nat. Mater.* 13(5), 515–523 (2014).

In addition to manufacturing biotic materials using nonbiological systems, as shown in the previous article, biological systems have also been exploited to produce and pattern multiscale biotic materials or bioticabiotic composites. As an emerging field, synthetic biology aims at engineering cells with synthetic DNA to accomplish desired function and plays an increasingly significant role for advanced biomanufacturing. In this work, the authors demonstrated production of fibrils and their composite with inorganic materials across multiple length scales using a synthetic biology approach [4]. The authors explored the fact that *Escherichia coli* produces amyloid fibrils via self-assembly of secreted curli subunit CsgA on subunit CsgB. Using synthetic riboregulators, CsgA subunits were conjugated with various peptide tags that can interface with inorganic materials. Production of amyloid fibrils was achieved by either external control or autonomous patterning. In the external control, cellular consortia were engineered with inducible riboregulators acyhomoserine lactone (AHL)_{Receiver}/CsgA and anhydrotetracycline (aTc)_{Receiver}/CsgA_{His} to produce fibrils composed of CsgA and CsgA_{His}. Fibrils can be tuned with different length distribution and relative proportions of the CsgA and CsgA_{His} by changing the relative lengths/amplitudes of AHL pulses versus aTc pulses or by producing simultaneous expression of CsgA variants with different concentrations of aTc and AHL. In the autonomous patterning, engineered *E. coli* autonomously produces fibrils with structure and composition changing over time. Fibril composition could be tuned by varying the initial seeding ratio of two engineered cell types. Fibril can be patterned across multiple-length scales ranging from tens of nanometers to micrometers via spatially varying inducer

concentrations. Furthermore, production of fibrils can also be interfaced with inorganic materials (e.g., gold nanoparticles and quantum dots) to create functional materials for broad applications including colocalization of heterogeneous nanoparticles, modulation of quantum dot fluorescence lifetimes and formation of responsive biofilm-based electrical switch. These functional materials could be further utilized as building blocks or components for engineering more complex materials.

Compared with nonbiological systems, this synthetic biology approach enables smart and precise control over material synthesis via hierarchy assembly. Compared to previous synthetic biology methods, this approach enables engineering complex biotic–abiotic composites. In addition, *E. coli* based production can be potentially transformed from bench to large scale through fermentation industry. This work establishes a foundation for biomanufacturing complex functional biotic-abiotic with genetically engineered cells.

Printing 3D tissue constructs with decellularized extracellular matrix

Evaluation of: Pati F, Jang J, Ha DH *et al.* Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nat. Commun.* 5, 3935 (2014).

Bioprinting has emerged as a promising cell and biomaterial patterning strategy that uses an additive manufacturing method to build functional tissue mimicking constructs [5–10]. However, utilizing bioprinting in biomanufacturing industry has been hampered by several limitations, including the inability to reconstitute the intrinsic tissue morphology and function [11]. The authors of this report developed a bioprinting method to construct a cell-laden structure incorporating a bioink composed of decellularized extracellular matrices (dECMs) to create a conducive environment for the growth of 3D structural tissue [12]. The authors explored in their experiments three kinds of tissue: cartilage tissue, heart tissue and adipose tissue. Three separate bioinks were developed, one for each kind of tissue. They were then printed in a repeating lattice formation, which then were stacked one on top of the other and allowed to incubate to achieve gelation. Following 14 days in culture, viability results demonstrated that the dECM bioinks were capable of successfully nurturing cells. In evaluating their approach and negating various possible detractors, the authors examined various aspects of the dECM bioink, printing process and gel product in detail. When the rheological properties on cells within the dECM bioink during printing were measured, the authors concluded that shear stress was of minimal deleterious effect on cells. In addition, they determined that the dECM gel postprinting retained its shape and form, essential to creating a favorable environment for cell growth and proliferation. Tissue-specific gene expression was also tested, with positive mRNA expression for various key genes such as *SOX9* in cartilage dECM, *Myh6* in heart dECM and the *PPAR-γ* receptor in adipogenic dECM. Similarly, tissue formation was measured, with markers indicating positive growth patterns in all three dECMs. By exploiting the versatility of the dECM bioink, the authors have engineered a tissue printing method capable of producing cells better supported by their natural matrix. However, the study did not encompass the evaluation of the heterogeneous tissue formation during this process. Nevertheless, the dECM gels can be used for larger-scale tissue engineering purposes, drug screening and *in vitro* disease modeling.

Induce vascularization in 3D printed bone scaffold

Evaluation of: Wang J, Yang M, Zhu Y, Wang L, Tomsia A, Mao C. Phage nanofibers induce vascularized osteogenesis in 3D printed bone scaffold. *Adv. Mater.* 2014, 26, 4961–4966.

While utilizing bioprinting in conjunction with novel bioinks to fabricate 3D tissue constructs, as shown in the previous report, the present paper investigates the ability to design a safe virus-activated matrix (VAM) by filling a 3D printed biomimetic bone scaffold with Arg-Gly-Asp (RGD) phage nanofiber to promote the generation of vascularized bone [13]. Although blood vessels regeneration (angiogenesis) has been shown to promote new bone formation (osteogenesis) [14], current strategies of bone tissue engineering achieved a limited success in producing vascularized new bones [13]. The authors first used 3D bioprinting to construct a biomimetic bone scaffold comprising biphasic calcium phosphate with hydroxyapatite and B-tricalcium phosphate. The RGD-phage and chitosan are then integrated into 3D printed scaffolds and the scaffolds were then lyophilized to prepare VAM pores and column that would support cell adhesion. The authors utilized RGD-phage due to its ability to differentiate mesenchymal stem cells (MSCs) into osteoblast without any osteogenic supplements. Rat MSCs are subsequently seeded into the constructed scaffold and the scaffold is then implanted into a rat radial bone defect. Following 8 weeks *in vivo*, authors evaluated the newly formed bone at the site of defect using histological analysis and micro-computed tomography. The authors have shown that the formation of a new bone is enhanced and oriented along the channels of bone scaffold when the scaffold is filled with RGD-phage to form VAM. Based on microcomputed tomography results, the authors also demonstrated that the VAM successfully achieved bone repair *in situ* and the level of new bone matched the control. Following these observations, the authors then evaluated the new blood vessel formation by using immunofluorescence and histological staining. The newly formed blood vessels were identified using CD31. The authors concluded that the endothelialization and subsequent vascularization result by recognition of the integrins, which are highly expressed on activated EC cells, by RGD-phage. This recognition process promotes the recruitment of activated ECs from surrounding tissue. Upon hematoxylin and eosin staining of the regenerated bone, the authors further observed a newly formed blood vessel, which includes some red blood cells. In addition, authors demonstrated that vascular regeneration (angiogenesis) and bone repair (osteogenesis) were promoted more when RGD-phage was used as compared to control (i.e., 3D printed scaffold filled with wild-type phage). The regeneration of vascularized bone was further promoted when VEGF was added to VAM. Despite that the phage does not demonstrate any toxicity *in vivo*, the present study did not investigate the fate of phage in bone. The presented approach, where the RGD-phage nanofiber integrated with 3D printed biomimetic bone scaffold, can further create new directions to regenerate vascularized bone for regenerative medicine applications.

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References

1. Chen P, Luo Z, Güven S, et al. Microscale assembly directed by liquid-based template. *Adv. Mater.* 2014; 26:5936–5941. [PubMed: 24956442]
2. Eydelnant IA, Betty Li B, Wheeler AR. Microgels on demand. *Nat. Commun.* 2014; 5:3355. [PubMed: 24566526]
3. Choi K, Ng AH, Fobel R, Wheeler AR. Digital microfluidics. *Annu. Rev. Anal. Chem.* 2012; 5:413–440.
4. Chen AY, Deng Z, Billings AN, et al. Synthesis and patterning of tunable multiscale materials with engineered cells. *Nat. Mater.* 2014; 13:515–523. [PubMed: 24658114]
5. Durmus NG, Tasoglu S, Demirci U. Bioprinting: functional droplet networks. *Nat. Mater.* 2013; 12:478–479. [PubMed: 23695742]
6. Gurkan UA, El Assal R, Yildiz SE, et al. Engineering anisotropic biomimetic fibrocartilage microenvironment by bioprinting mesenchymal stem cells in nanoliter gel droplets. *Mol. Pharm.* 2014; 11(7):2151–2159. [PubMed: 24495169]
7. Mironov V, Boland T, Trusk T, Forgacs G, Markwald RR. Organ printing: computer-aided jet-based 3D tissue engineering. *Trends Biotechnol.* 2003; 21:157–161. [PubMed: 12679063]
8. Demirci U, Montesano G. Single cell epitaxy by acoustic picolitre droplets. *Lab Chip.* 2007; 7:1139–1145. [PubMed: 17713612]
9. Moon S, Hasan SK, Song YS, et al. Layer by layer three-dimensional tissue epitaxy by cell-laden hydrogel droplets. *Tissue Eng. Part C Methods.* 2009; 16:157–166. [PubMed: 19586367]
10. El Assal R, Guven S, Gurkan UA, et al. Bio-inspired cryo-ink preserves red blood cell phenotype and function during nanoliter vitrification. *Adv. Mater.* 2014; 26:5815–5822. [PubMed: 25047246]
11. Tasoglu S, Demirci U. Bioprinting for stem cell research. *Trends Biotechnol.* 2013; 31:10–19. [PubMed: 23260439]
12. Pati F, Jang J, Ha DH, et al. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nat. Commun.* 2014; 5:3935. [PubMed: 24887553]
13. Wang J, Yang M, Zhu Y, Wang L, Tomsia AP, Mao C. Phage nanofibers induce vascularized osteogenesis in 3D printed bone scaffolds. *Adv. Mater.* 2014; 26(29):4961–4966. [PubMed: 24711251]
14. Street J, Bao M, deGuzman L, et al. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. *Proc. Natl Acad. Sci. USA.* 2002; 99:9656–9661. [PubMed: 12118119]