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REVIEW

Recent advances in chemically defined and tunable hydrogel platforms for organoid culture

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Abstract

Recent developments in organoid culture technologies have made it possible to closely recapitulate intrinsic characteristics of different tissues under in vitro conditions. These organoids act as a translational bridge between the traditional 2D/3D cultures and the in vivo models for studying the tissue development processes, disease modeling, and drug screening. Matrigel and tissue-specific extracellular matrix have been shown to support organoid development, efficiently; however, their chemically undefined nature, non-tunable properties, and associated batch-to-batch variations often limit reproducibility of the assembly process. In this regard, chemically defined platforms offer wider opportunities to optimize and recreate tissue-specific microenvironment. The present review delineates the current research trends in this sphere, focusing on material perspective and the target tissues (e.g., neural, liver, pancreatic, renal, and intestinal). The review winds up with a discussion on the current limitations and future perspective to provide a basis for future research.

Keywords Organoid · 3D culture · Biomaterials · Chemically defined hydrogels · Cell-instructive microenvironment

Abbreviations

2D	Two dimensional
3D	Three dimensional
SD	Standard deviation
ECM	Extracellular matrix
EHS	Engelbreth–Holm–Swarm
LMN	Laminin
COLIV	Collagen IV
HA	Hyaluronic acid
PEG	Polyethylene glycol
PIC	Polyisocyanopeptide
MMPs	Matrix metalloproteinases
GLDH	Glutamate dehydrogenase
ESCs	Embryonic stem cells
HSPCs	Hematopoietic stem and progenitor cells
BMSCs	Bone marrow stromal cells
PEGDA	Poly(ethylene glycol) diacrylate
MSC	Mesenchymal stem cells
FN	Fibronectin
DV	Dorsal–ventral
iPSCs	Induced pluripotent stem cells
ALB	Albumin
MDR1	Multidrug resistance protein 1
CNF	Cellulose nanofibril
ALAT	Alanine aminotransferase
ASAT	Aspartate transaminase
PEGDE	Poly(ethylene glycol) diglycidyl ether
LGR5	Leucine-rich repeat-containing G-protein-coupled receptor 5
BME2	Basement Membrane Extract Type 2
dPMP	Degradable PEG-MMP-PEG
ndPH	Non-degradable PEG–heparin
dPMH	Degradable PEG-MMP-Heparin
LEC	LMN–entactin complex
HNF4α	Hepatocyte nuclear factor 4α
E-cad	E-cadherin
KRT19	Cytokeratin 19
NHE3	Sodium–hydrogen exchanger 3
Muc2	Mucin 2
Lyz	Lysozyme

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Introduction

Consistent efforts toward developing biomimetic complex tissue structures have unfolded strategies for organoids' growth and maintenance under in vitro conditions [1–3]. Organoids could be generated from stem cells, progenitor cells, or even tissue fragments, which undergo self-organization to form tissue-like structures, exhibiting key architectural and functional features along with multicellular complexity, similar to native ones [3, 4]. To date, organoids equivalents of different organs including (but not limited to) gastrointestinal [5–7], pancreatic [8, 9], hepatic [10, 11], renal [12, 13], endometrial [14, 15], cardiac [16, 17], retinal [18, 19], lungs [20, 21], immune [22, 23], neural [24, 25], and skin [26, 27] have been generated. These organoids are believed to have endless translational applicability in R&D, healthcare, diagnostics, and pharmaceutical sectors as they address many limitations of current in vitro and in vivo models [3, 4]. Besides, organoids' potential implementation as a cell-based therapy for tissue repair and regeneration cannot be overlooked [28].

The traditional method of generating organoids relies on the use of complex animal-derived extracellular matrix (ECM) hydrogels, primarily Matrigel, with a recent inclination toward tissue-specific ECM [28–35]. Matrigel is a solubilized basement membrane preparation derived from the Engelbreth–Holm–Swarm (EHS) mouse sarcoma. It majorly consists of laminin (LMN), collagen IV (COLIV), entactin, and heparin sulfate proteoglycan, along with several growth factors [28, 29]. On the other hand, tissue-specific ECM is derived from tissues/organs using decellularization procedures; the obtained ECM is further solubilized to form a pre-gel solution [31, 36, 37]. The stem cells or tissue fragments encapsulated in these hydrogels undergo morphogenesis to form tissue organoids via interaction with various biochemical/biophysical cues offered by the hydrogel [28–30]. Although these gold standard animal-derived materials have been shown to sustain the growth and long-term culture of almost all the known organoids, to date, they have their limitations [28, 29]. Compositionally, both these matrices are still ill-defined, contain xenogenic constituents and growth factors, and suffer batch-to-batch variability, thereby introducing variability in organoid assembly and developmental processes [28, 29]. Moreover, the lack of control over different tunable properties, including biochemical, biomechanical, and biodegradation, further impedes their advancements [28–30]. Cancerous origin of Matrigel also limits its translational aspects [28].

In this regard, organoid culture technology has evidenced increasing efforts to explore chemically defined alternatives [28–30]. Such platforms offer to tune intricate components of the tissue microenvironment and guide the formation, maintenance, and differentiation of organoids in a controlled and reproducible manner [28–30, 35]. The current review highlights developments in organoid technology employing chemically defined hydrogel platforms.

Chemically defined hydrogels: cell-instructive behavior

The organoid formation is a dynamic process; hence, the matrix that encapsulates the organoid should also be dynamic. Cell–matrix interaction is one of the dominant determinants in translating single cells into organized and structured 3D forms. During organoid formation, the matrix characteristics significantly impact cell viability, proliferation, cell–cell crosstalk, cytoskeletal changes, and homeostasis, controlled in a spatiotemporal manner (Fig. 1) [38].

Early organoid studies have used solubilized decellularized ECM, thanks to its complex tissue-specific composition, favorable mechanical properties, and biocompatibility. Nevertheless, batch-to-batch differences in ECM composition and mechanical properties, unfortunately, resulted in a lack of reproducibility of these results [35]. Therefore, various natural and synthetic biomaterials were adapted into organoid research to improve the standardization of the process. The presence of adhesion motifs, mechanical properties, biodegradability, cross-linking conditions, and the biocompatibility of these natural and synthetic biomaterials are remarked as the distinctive selection criteria. They play a substantial role in creating a dynamic microenvironment for the organoid cultures. For instance, while the presence or density of the adhesion motifs on the polymeric backbone intermediates and affects cytoskeletal dynamics and morphogenesis, the bulk/localized matrix mechanical properties control mechanotransduction-associated cell signaling cascades [39]. Even the variability in the motif type or density could also modulate cellular phenotype. RGDS motif, but not RGES, better supported the human hepatocytes' functional characteristics [40]. In another study, the presence of RGD motifs at high density was shown to inhibit osteogenesis while promoting adipogenic differentiation of mesenchymal stem cells (MSCs) [41].

Chemically defined hydrogels: material perspective

Up to date, the proposed chemically defined natural and synthetic biomaterials cannot yet recapitulate the natural ECM composition complexity, structure, and bioactivity. However, the precise and consistent production of these materials ensures high reproducibility and effective customization.

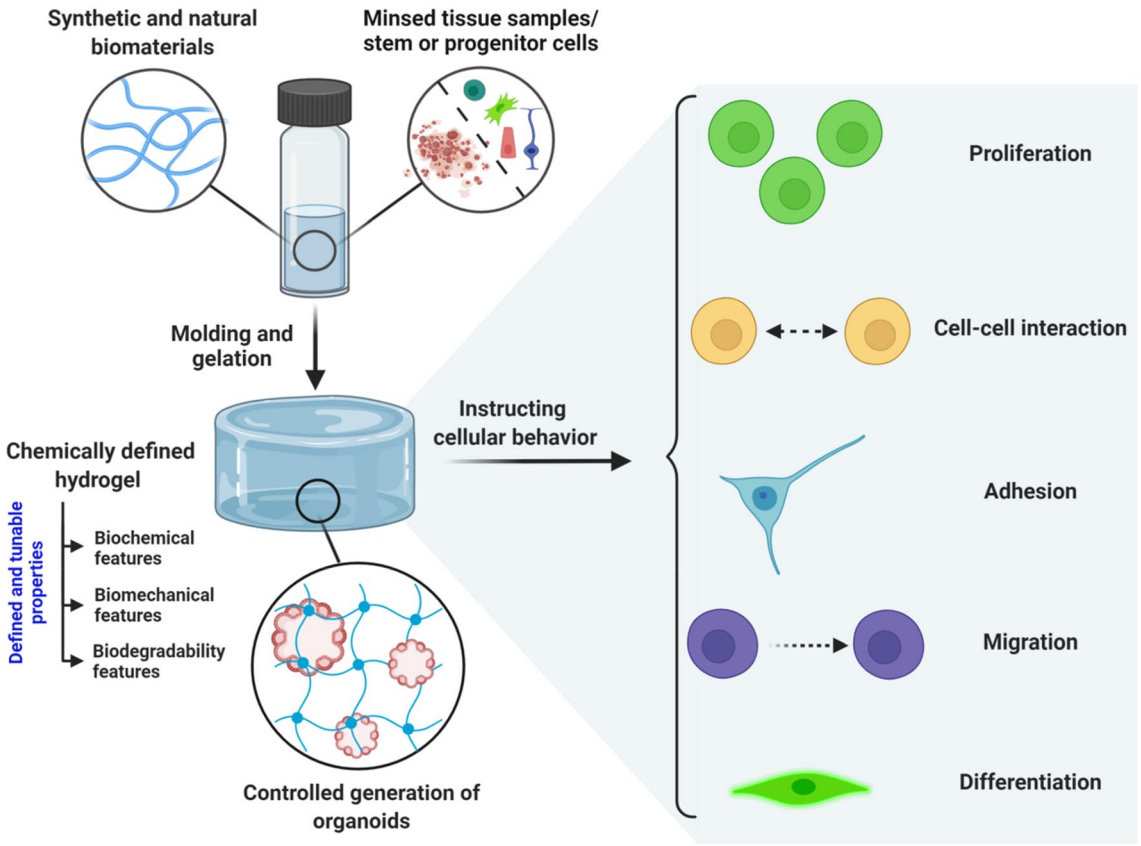

Fig. 1 Schematic of defined hydrogels with tunable physicochemical properties to instruct cellular behaviors in building 3D organoid models

Aiming to reduce the complexity and increase the experiment reproducibility, natural single biopolymer components such as collagen type I, gelatin, hyaluronic acid (HA), alginate, fibrin or mixtures thereof have been investigated [35]. However, small differences in matrix composition or biopolymer properties are still present that could negatively affect the results' reproducibility. Therefore, matrices entirely based on synthetic polymers have also been considered [29]. Polyethylene glycol (PEG) and polyisocyanopeptide (PIC) are the two pioneer synthetic polymers used closely in organoid research. Table 1 presents a comparative evaluation of natural and synthetic materials in different aspects.

Natural biomaterials and their derivatives

Single biopolymer components based on natural protein, glycosaminoglycans, and polysaccharide are used in organoid research [35]. Among protein-based biopolymers, collagen type I, gelatin, and fibrin are the most popular ones. Collagen is the most abundant ECM protein in the vertebrates, while gelatin is a collagen derivative [42, 43]. The primary structure of collagen is a triple-stranded helix that is responsible for collagen's thermo-responsive characteristic. Depending on the chemical composition, pH, and temperature, collagen can self-assemble into strong, long cross-linked fibrils. Thanks to its significant mechanical properties, collagen type I is resistant to tensile forces, thus typically used in connective tissue organoids [44–47]. Despite collagen's desirable biochemical characteristics, its limited solubility in cell-friendly conditions hinders its processability.

Unlike collagen, gelatin's high solubility in the water at physiological temperature allows its easy processability while maintaining a proper cell environment. As gelatin is water-soluble at physiological temperatures, permanent cross-linking of gelatin matrices is ensured through chemical or enzymatic polymerization using Genipin or transglutaminase [48, 49]. Additionally, photoinitiated radical polymerization is also prevalently used for cross-linking of gelatin matrices; nevertheless, for such applications, gelatin should be functionalized with acrylic/methacrylic moieties [50]. Fibrinogen is another abundant protein in vertebrates, although to a lesser extent compared to collagen. Fibrinogen can naturally self-assemble into fibrin in the presence of thrombin to form a gel [51]. Like gelatin and collagen, fibrin also possesses different cell adhesion motifs, including RGD, allowing it to support cell viability, proliferation, hemostasis, differentiation, and function without any further

Table 1 Summary of the distinctive hallmarks of biomaterials suited for developing organoids

Biomaterials	Distinctive hallmarks	Cross-linking mechanism	Source	Cost	References
Natural hydrogels					
Collagen	Intermolecular cross-links among the triple-helical structures pH- and temperature-dependent self-assembling fibril-forming properties Amino acid repeating sequence $[Gly–X–Y]_n$, X positions by proline and Y positions by hydroxyproline	**Physical gelation**: Thermal self-assembly **Chemical gelation**: Glutaraldehyde, EDC/NHS, photopolymerization (methacrylate, thiol derivatives)	Bovine skin and tendons, porcine skin, rat tail, sponges, fish, shark, and jellyfish	$$$	[77, 78]
Gelatin	Derived through hydrolytic processes of collagen Molecular weight can be controlled through the hydrolytic processing parameters such as pH, temperature, and time Amino acid repeating sequence $[Gly–X–Y]_n$, X positions by proline and Y positions by hydroxyproline	**Physical gelation**: Thermal self-assembly **Chemical gelation**: Glutaraldehyde, EDC/NHS, photopolymerization (methacrylate, thiol derivatives) **Enzymatic gelation**: Transglutaminase	Bovine skin and tendons, porcine skin, rat tail, sponges, fish, shark, and jellyfish	$	[79, 80]
Fibrinogen	A complex triglobular, elongated protein Capable of conveying matrix proteins such as fibronectin and growth factors Possess both strong and weak binding sites for calcium ions Thermal and enzymatic clotting	**Enzymatic gelation**: Thrombin, Factor XIII **Chemical gelation**: Glutaraldehyde, EDC/NHS	Human plasma, bovine plasma, rat plasma	$$$	[81]
Heparin	Linear polysaccharide Made up of repeating disaccharides, primarily uronic acid and glucosamine with varying degrees of sulfation and *N*-acetylation High affinity for growth factors	**Physical gelation**: Electrostatic interaction **Chemical gelation**: Photopolymerization (thiol, maleimide derivatives)	Porcine intestinal mucosa, intestinal tissue of Turkey, bovine lung	$	[57, 82]
Hyaluronic acid	Non-sulfated anionic polysaccharide from glycosamine glycan family Composed of repeating units of disaccharides D-glucuronic acid and *N*-acetyl-D-glucosamine Specific binding site for CD44	**Chemical gelation**: Glutaraldehyde, EDC/NHS, Photopolymerization (methacrylate, thiol derivatives)	Bacterial *Streptococcus equi*, bacterial *Streptococcus pyrogenes*, bovine vitreous humor, rooster comb	$$$	[83, 84]
Alginate	A natural anionic polysaccharide It is composed of *repeating units* of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues Low protein adsorption	**Physical gelation**: Ionic cross-linking, electrostatic interaction **Chemical gelation**: Glutaraldehyde, EDC/NHS, photopolymerization (methacrylate, thiol derivatives)	Brown algae, including *Laminaria hyperborea*, by treatment with aqueous alkali solutions	$	[85–87]
Chitosan	A linear polysaccharide consisting of *N*-acetylglucosamine and *N*-glucosamine repeating units Cationic molecules possessing abundantly functional amine and hydroxyl groups on the molecular chain An excellent protein binding Antimicrobial characteristics	**Physical gelation**: Ionic cross-linking, Electrostatic interaction **Chemical gelation**: Glutaraldehyde, EDC/NHS, photopolymerization (methacrylate, thiol derivatives)	Exoskeleton of crustaceans	$	[88, 89]

Table 1 (continued)

Biomaterials	Distinctive hallmarks	Cross-linking mechanism	Source	Cost	References
Synthetic hydrogels					
PEG	Non-ionic synthetic hydrophilic polymer Available in different molecular weights (300 Da–40 kDa) Efficient cell binding to the once functionalized with conjunction of cell adhesion peptides	**Chemical gelation**: Photopolymerization, click chemistry, radical polymerization	Synthetic (N/A)	$$	[90–92]
PIC	Exhibit reversible thermo-responsive self-assembly Strain-stiffening property Efficient cell binding to the once functionalized with conjunction of cell adhesion peptides	**Physical gelation**: Thermal self-assembly **Chemical gelation**: Click chemistry	Synthetic (N/A)	–	[69, 75, 76]

$: 1$ < price per gram polymer < 10$, $$: 10$ < price per gram polymer < 100$, $$$: 100$ < price per gram polymer

functionalization. Hence, fibrin can enable long-term expansion of organoids [52, 53].

Besides protein-based biopolymers, glycosaminoglycans also hold great potential for designing dynamic organoid microenvironment. For example, HA matrices can activate cascade signals which affect cell attachment, migration, proliferation, and morphogenesis thanks to their capacity of interaction with several cell transmembrane receptors such as CD44, CD54, and CD168 [54]. However, the resilience of HA hydrogels and their ability to resist degradation by hyaluronidases, reactive oxygen and nitrogen species, highly hinder their applicability. In order to improve HA degradation, protease-sensitive motifs such as that of matrix metalloproteinases (MMPs) have been exploited for preparing HA-based platforms [55, 56]. Heparin is another natural glycosaminoglycan that has witnessed broad applicability for tissue engineering, mainly due to its anticoagulant and antithrombotic properties [57]. Owing to its negative charge, it also has a high affinity for various growth factors [57, 58]. Moreover, sulfonic acid, hydroxyl, and carboxyl groups on the heparin backbone enable functionalization with light-sensitive moieties and conjugation with synthetic and natural polymers to create multifunctional 3D matrices for organoid cultures [59, 60].

Additionally, polysaccharides have also been abundantly employed in organoid research, alginate being the most favorable one due to its biocompatibility and ease of manipulation [61, 62]. The cross-linking of alginate could occur in the presence of di-/trivalent cations under physiological conditions, averting a need for its further modification [63]. However, due to the lack of cell adhesive moiety, alginate often requires either functionalization with cell-binding cues or mixed with more instructive biopolymers to support organoid formation [45, 64]. Chitosan is another natural polysaccharide, derived from the partial deacetylation of chitin; its chemical structure constitutes D-glucosamine and *N*-acetyl-D-glucosamine [65, 66]. Unlike alginate, chitosan is protonated in acidic aqueous solutions and acts as a polycationic polymer. Thanks to its polycationic behavior, chitosan can form polyionic complexes with different anionic polymers (such as HA, gelatin, chondroitin sulfate) and thus could be utilized for organoid culture [67]. Besides, the amine groups on its backbone provide an opportunity to conjugate biologically active molecules (fibronectin (FN), LMN, and other peptides/proteins) to support cell growth and differentiation [68].

Synthetic biomaterials and their derivatives

Biocompatible synthetic polymers have also been studied extensively in the last decades, with the most common materials being polyvinyl alcohols, PEG, PIC, and polyacrylamide and their derivatives. PEG and PIC derivatives

come forward in organoid research as they closely mimic the fibrous and porous architecture and mechanical properties of structural ECM proteins (Fig. 2) [69, 70]. PEG-based hydrogels are prevalently used in this research area as they are commercially available in a wide range of structures (for example, linear or multi-arm star versions) and molecular weights, enabling different matrix designs, mechanical and rheological properties [71, 72]. Moreover, they can easily be functionalized, using thiol-ene or azide-alkyne chemical strategies, to incorporate different biological ligands, signaling molecules, and cross-linking/degradation sites, allowing the precise tuning of physical and biochemical niche properties [73, 74]. These reactions are rapid and quantitative in yield, making them attractive tools for the fabrication of functionalized PEG hydrogels.

On the other hand, PICs are a relatively newer class of synthetic hydrogels employed for organoid research due to their thermo-responsive nature [69]. Rapid gelation of PIC solutions can thermally be induced by heating them beyond the gelation temperature ($T_{gel} \approx 18$ °C). Moreover, PIC gelation is fully reversible, particularly advantageous for cell and organoid extraction [69, 75]. Regarding the mechanical properties, PIC hydrogels can be formed at low polymer concentrations (0.1–1 wt%), and they display low shear modulus $G' = 0.1$–4 kPa that overlaps with the stiffness of various soft tissues [69]. Despite these attractive properties, PIC hydrogels lack cell-binding sites; however, a rapid functionalization with cell adhesion cues such as RGD can be achieved through strain-promoted azide-alkyne cycloaddition reaction [76].

Organoid culture in defined hydrogels

To date, numerous studies have already explored the defined and tunable hydrogels for establishment and growth of organoid cultures, via recreating cell-instructive microenvironment. This section elaborates over such representative studies, segregated based on the different tissue types, including, neural, hepatic, pancreatic, renal, intestinal, and others.

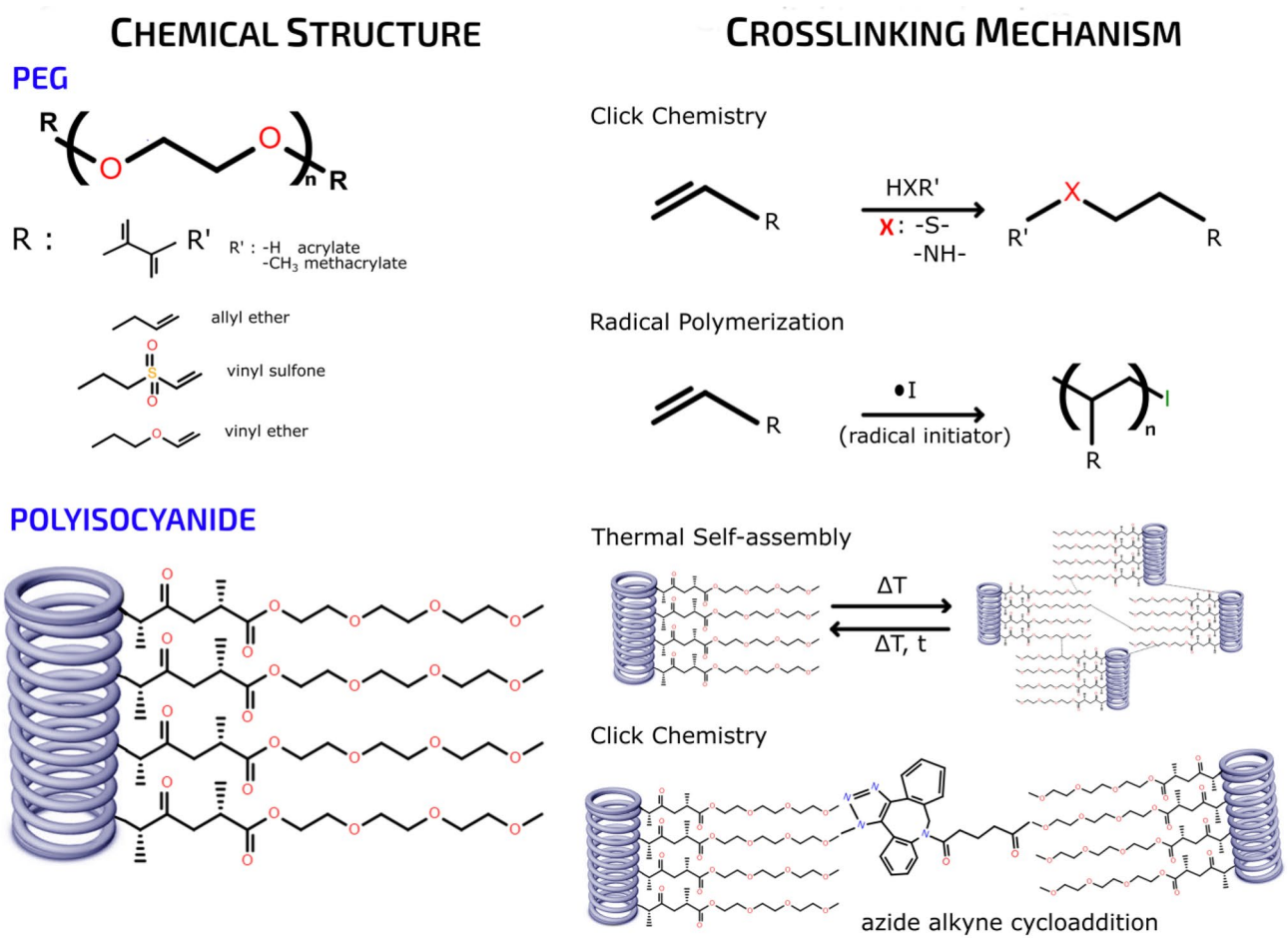

Fig. 2 Chemical structure and cross-linking mechanism of various PEG derivatives and PIC polymer

Neural organoids

The neural development is one of the most complex processes, making it challenging to model and study in the absence of an animal model; however, the recent advancements in cerebral and neural organoid technologies have made it possible, to an extent [93].

In an effort to study early neurogenesis pattern, tunable synthetic hydrogel system was developed and further used for culturing and differentiating mouse embryonic stem cells (ESCs) (Fig. 3) [94]. Hydrogels were formed by 8-arm PEG precursor molecules conjugated with lysine-donor (with/without MMP-sensitive motif) and glutamine-acceptor factor XIIIa substrate peptides in the presence of thrombin-activated FXIIIa. The hydrogels' mechanical properties could be tuned via alteration in precursor polymer density, while a change in the ratio of MMP-sensitive and insensitive lysine-donor peptides affected the degradability of gels. The optimal synthetic hydrogel compositions supported the formation of more homogeneous neuroepithelial colonies, a higher proportion of cysts having apicobasal polarity, and neural tubelike patterning along the dorsal–ventral (DV) axis. An in-depth evaluation of tunable characteristics revealed that non-degradable PEG hydrogel with stiffness range between

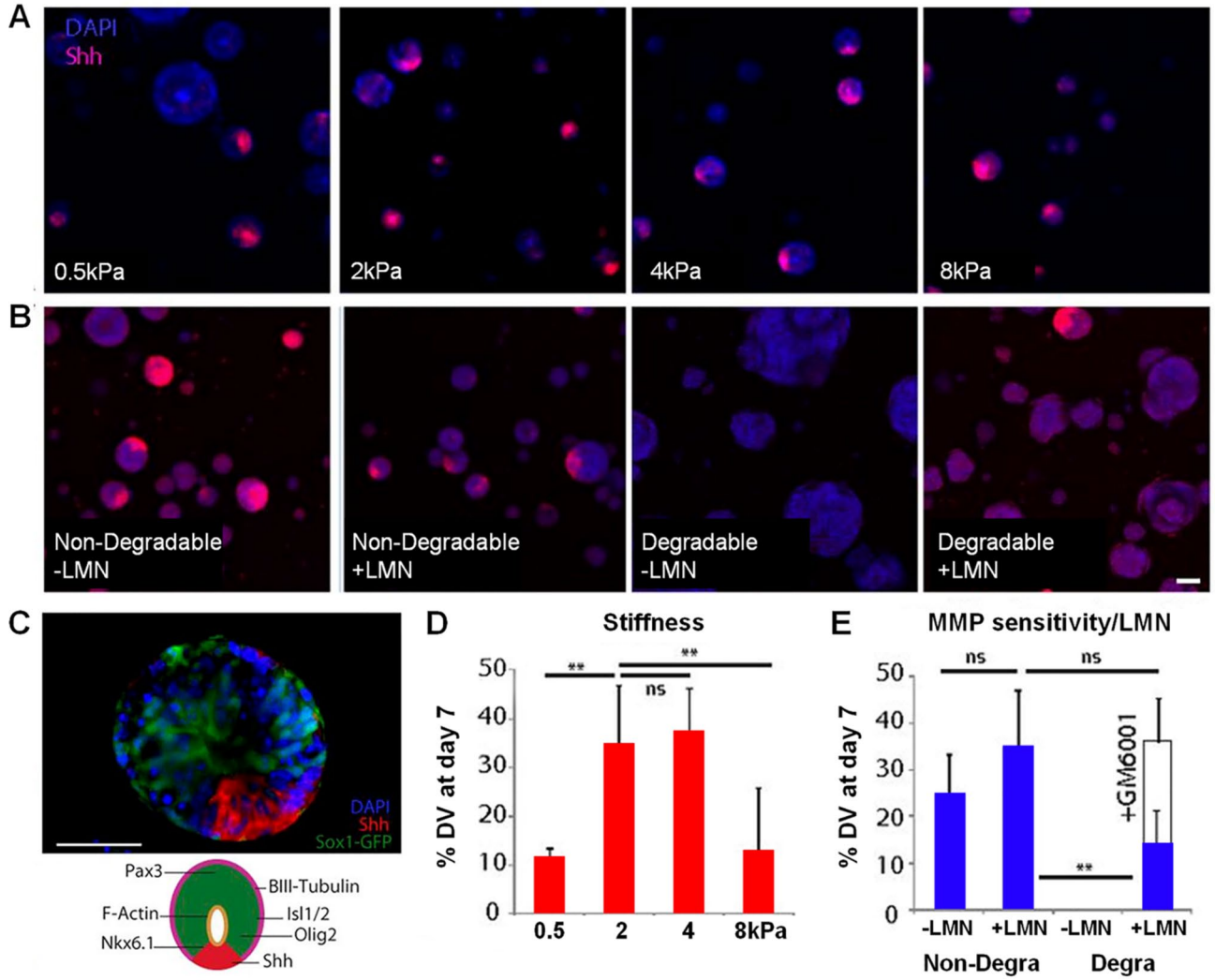

Fig. 3 Representative images of Shh-immunostained neural cysts with DV patterning, cultured in non-degradable PEG matrices with **a** variable stiffness and **b** variable degradability and LMN composition (stiffness ~ 2 kPa). Nucleus was stained with DAPI. Scale bar: 100 µm. **c** Representative fluorescent micrographs and schematics demonstrating DV patterning with key features of neural tube architecture, Shh+ floor plate, Olig2+ progenitor motor neuron, Isl1/2+ motor neuron, βIII tubulin+ post-mitotic neuron, as well as Nkx6.1+ ventral and Pax3+ dorsal positional identities. Scale bar: 50 µm. Quantitative evaluation of DV patterning efficiency of neural organoids in non-degradable PEG hydrogels with **d** variable stiffness and **e** variable degradability and LMN composition, at day 7 of culture. Error bars: standard error (SE). "**" represents significant difference with p value < 0.01. Reproduced with permission from Ref. [94]. Copyright © 2016, Ranga et al.

2 and 4 kPa and supplemented with LMN supported a better progression of neurogenic events. Another two variants of PEG-based synthetic hydrogel with tunable biochemical features, first consisting of PEG-conjugated with collagen-like peptide (PEG-CLP) and the other with collagen-like peptide attached to RGD motif (PEG-CLP-RGD), were reported to support the self-organization of cerebellar cells into functional organoids [95]. PEG-CLP-RGD hydrogels demonstrated a higher neurite outgrowth, though they were not organized as compactly as in PEG-RGD.

Recently, carboxylated HA and protonated chitosan hydrogel (together, termed as Cell-Mate3D matrix) were employed for robust generation of cerebral organoids from human-induced pluripotent stem cells (iPSCs) [67]. In a short culture duration (~ 10–14 days), in the defined hydrogels, early corticogenesis was observed in the cerebral organoids characterized by the formation of neural rosettes and neural tubelike structures and expression of neural differentiation markers (β-III tubulin, SOX2, nestin). At this stage, the organoid was spherical with a maximum dimension of ~ 1 mm; which further increased to 2.5–3 mm by 28th day of culture with an increase in their complexity. The cerebral organoids demonstrated an enhanced expression of the forebrain, midbrain, and hindbrain markers; however, the expression levels were greatly influenced by the hiPSC line and neural differentiation time. Besides, physiologically relevant glutamate responsiveness and cellular depolarization were also evident in the developed organoids.

Hepatic organoids

The liver is the largest internal organ, consisting of hepatocytes (major population ~ 70%), biliary epithelial cells, hepatic stellate cells, liver sinusoidal endothelial cells, Kupffer cells, and hepatic stem/progenitor cells, which are organized in unique lobular architecture and carries out over 500 different functions [96, 97]. The emergence of liver organoid technology has provided a newer dimension to the pharmaceutical sector, particularly those linked with the assessment of hepatotoxic drug responses [98–100]. They may also have potential applicability for tissue engineering and disease modeling domain [101].

A recent study demonstrated that synthetic MMP-sensitive, RGD-conjugated PEG hydrogel matrices (PEG-RGD) efficiently supported the formation and differentiation of liver organoids, comparable either to Matrigel or PEG hydrogels conjugated with either COLIV (PEG-COLIV) or LMN-111 (PEG-LAM-1) or fibronectin (PEG-FN) (Fig. 4) [101]. Besides, mechanical aspects also play a critical role in determining the phenotypic characteristics of the liver organoids. To further ascertain its applicability in this domain, PEG-RGD hydrogels of variable stiffness were prepared by altering the polymeric density. The hydrogels mimicking the physiological liver stiffness ranges (between 1.3 and 1.7 kPa) were optimal for generating liver organoids, while those with lower or higher stiffness limited the process. Interestingly, organoids in PEG-RGD hydrogels (stiffness ~ 4 kPa) displayed characteristics of fibrotic liver tissue with downregulation of hepatic progenitor cells markers and upregulation of hepatic injury-associated genes. These optimized PEG-RGD hydrogels also offer immense avenues for establishing patient-specific human liver organoids directly from human biopsies, a step toward developing personalized therapies.

Another synthetic hydrogel matrix based on PIC, supplemented with either LMN–entactin complex (PIC-LEC) or recombinant human LMN-111 (PIC-LMN-1), supported the expansion and differentiation of liver organoids; unconjugated PIC (PIC-plain) or RGD-conjugated PIC (PIC-RGD) gels, instead, were insufficient for the same (Fig. 5) [75]. The phenotypic characteristics of organoids were greatly influenced by the molecular weight of PIC (1 kDa or 5 kDa), polymer density (2.5, 1.75, 1 mg/mL), and concentration of LEC/LMN-111 (1, 2, 3 mg/mL). Organoids differentiated in PIC hydrogels exhibited albumin (ALB) secretion, glutamate dehydrogenase (GLDH) production, ammonia elimination, multidrug resistance protein 1 (MDR1) rhodamine 123 transporter activity, and the expression of mature hepatocyte markers, comparable or better than Matrigel. Moreover, the thermo-responsive nature of these PIC hydrogels adds an advantage for organoid subculturing, while maintaining organoid functional characters even after 10 passages.

Alternatively, hydrogels composing a single or composition of multiple biomacromolecules could provide a defined microenvironment for culturing liver organoids. For instance, cellulose nanofibril (CNF) hydrogels supported differentiation of liver organoids with the expression of hepatocyte-specific markers, ALB secretion, glycogen accumulation, GLDH production, alanine aminotransferase (ALAT), and aspartate transaminase (ASAT) activity, comparable or better than those cultured in Matrigel [102]. In another study, composite hydrogel capsules consisting of fibrin core and alginate–chitosan shell were fabricated using droplet microfluidic technique for generating liver organoids from human iPSCs-derived hepatic progenitor cells [53]. Organoids in hydrogel capsules expressed hepatocyte and cholangiocyte markers and showed ALB and urea synthesis. The presence/absence of fibrin hydrogels in the capsule core significantly affected phenotype of the liver organoids.

Pancreatic organoids

The pancreas is a compound gland that exhibits both exocrine (via acinar and ductal cells) and endocrine (alpha, beta, gamma, epsilon, and PP cells) functions, arranged in a specialized manner, forming islets of Langerhans. Pancreatic organoids have great importance for understanding

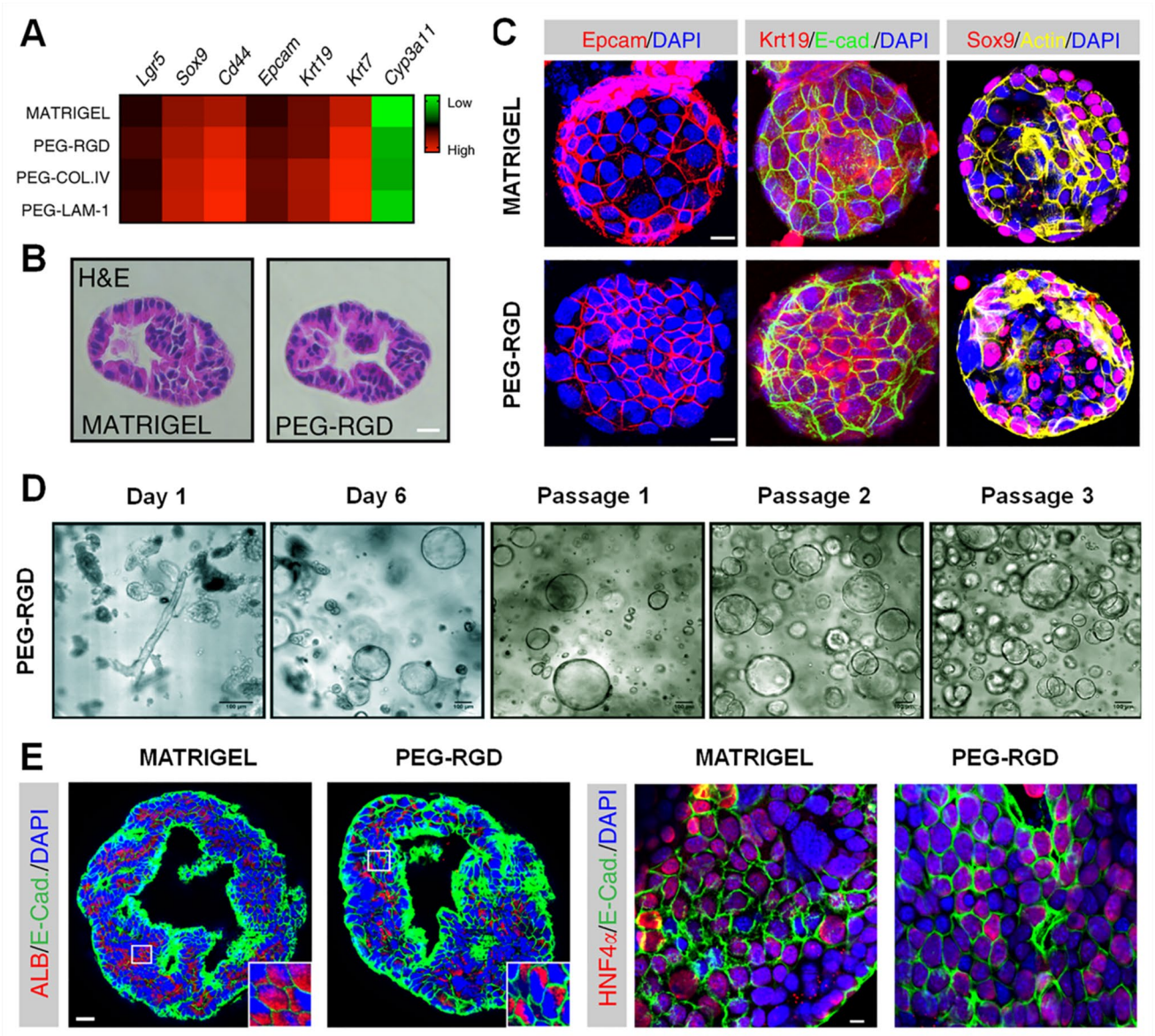

Fig. 4 **a** Heat map representation of gene expression of different markers in liver organoids cultured in Matrigel, PEG-RGD, PEG-COLIV, and PEG-LAM-1. **b** Representative images of H&E stained section of liver organoids cultured in Matrigel and PEG-RGD hydrogels. Scale bar 25 µm. **c** Representative images of immunostained sections of liver organoids in Matrigel and PEG-RGD hydrogels. **d** Bright-field micrographs of liver organoids at different days of seeding and different passages. Scale bar 100 µm. **e** Representative immunofluorescence confocal micrographs of ALB, hepatocyte nuclear factor 4α (HNF4α), and E-cadherin (E-cad)-stained liver organoids in Matrigel and PEG-RGD hydrogels. Scale bars: 25 µm (left), 10 µm (right). Reproduced with permission from Ref. [101]. Copyright © 2020, Sorrentino et al.

the disease biology and finding therapeutic interventions, mainly associated with common pancreatic diseases, like diabetes (type 1 or type 2) and cancer [103].

In an attempt to recreate artificial 3D pancreatic niche under in vitro conditions, synthetic MMP-sensitive, soft (shear modulus ~ 250 Pa) PEG-LMN hydrogels were developed [104]. These hydrogels were able to maintain and expand pancreatic progenitor cells, forming organoids. The organoids retained the expression of pancreatic progenitor markers (PDX1 and SOX9), epithelial marker (E-cad), internal polarization marker (mucin1), proliferative marker (pHH3), and endocrine differentiation marker (insulin). On the contrary, unconjugated PEG or stiffer PEG-LMN hydrogels (shear modulus > 1 kPa) were insufficient, demonstrating a loss of epithelial and pancreatic features. Notably, the authors did not present any comparative validation of these hydrogels' potency against the gold standard, Matrigel.

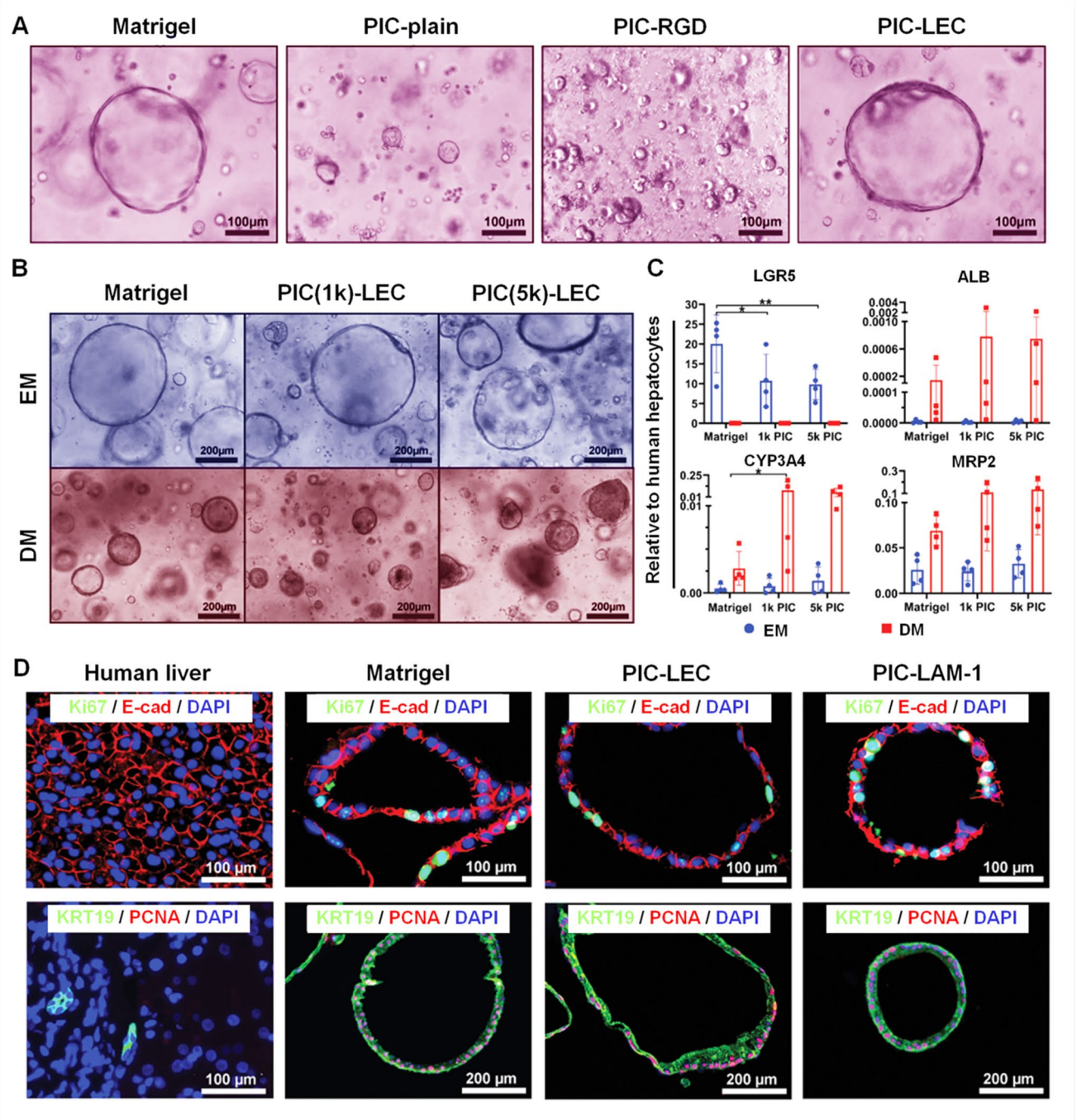

Fig. 5 **a** Representative light microscopy images of hepatic organoids, 7 days post-seeding, in Matrigel, PIC-plain, PIC-RGD, and PIC-LEC hydrogels; cultured in expansion medium. Organoids proliferated in Matrigel and PIC-LEC, but not in PIC-plain and PIC-RGD. Scale bar: 100 μm. **b** Representative images of hepatic organoids in Matrigel and LEC-supplemented PIC hydrogels with different molecular weights—1 kDa (PIC(1 k)-LEC) or 5 kDa (PIC(5 k)-LEC); cultured in expansion and differentiation medium. Scale bar: 200 μm. **c** Gene expression profiles of stem cells (LGR5) and hepatic markers (ALB, CYP3A4, and MRP2) in the organoids expanded or differentiated in Matrigel, PIC(1 k)-LEC, and PIC(5 k)-LEC hydrogels; transcription levels normalized to human hepatocytes. Error bars: standard deviation (SD). "*" represents significant difference with p value $<=0.05$. **d** Representative immunofluorescence micrographs of hepatic organoids for epithelial (E-cad and cytokeratin 19 (KRT19)) and proliferative (proliferating cell nuclear antigen (PCNA) and Ki67) markers, cultured in Matrigel, PIC-LEC, and PIC-LMN-1. Nucleus was stained with DAPI. Native human liver tissue was taken as a control. Scale bars: 100 μm (top), 200 μm (bottom). Reproduced with permission from Ref. [75]. Copyright © 2020, Ye et al.

In a different study, hydrogel composed of Amikacin hydrate and poly(ethylene glycol) diglycidyl ether (PEGDE), formally termed as Amikagel, was employed as a substrate coating material to generate 3D islet organoids [105]. When cocultured with HUVEC on Amikagel, human ESC-derived pancreatic progenitor cells underwent spontaneous maturation and self-organization into functional organoids. The heterogenous organoids, thus formed, showed an enhanced PDX1, insulin 1, and glucagon gene expression compared to those on Matrigel; the organoids also expressed C-peptide protein.

Besides, chemically defined hydrogels formed by RGD-modified SG dextran polymer and cross-linked with thiol-modified HA (together referred as DEX hydrogel) supported the formation and maintenance of human pancreatic organoids (from freshly isolated human pancreatic ducts) (Fig. 6)

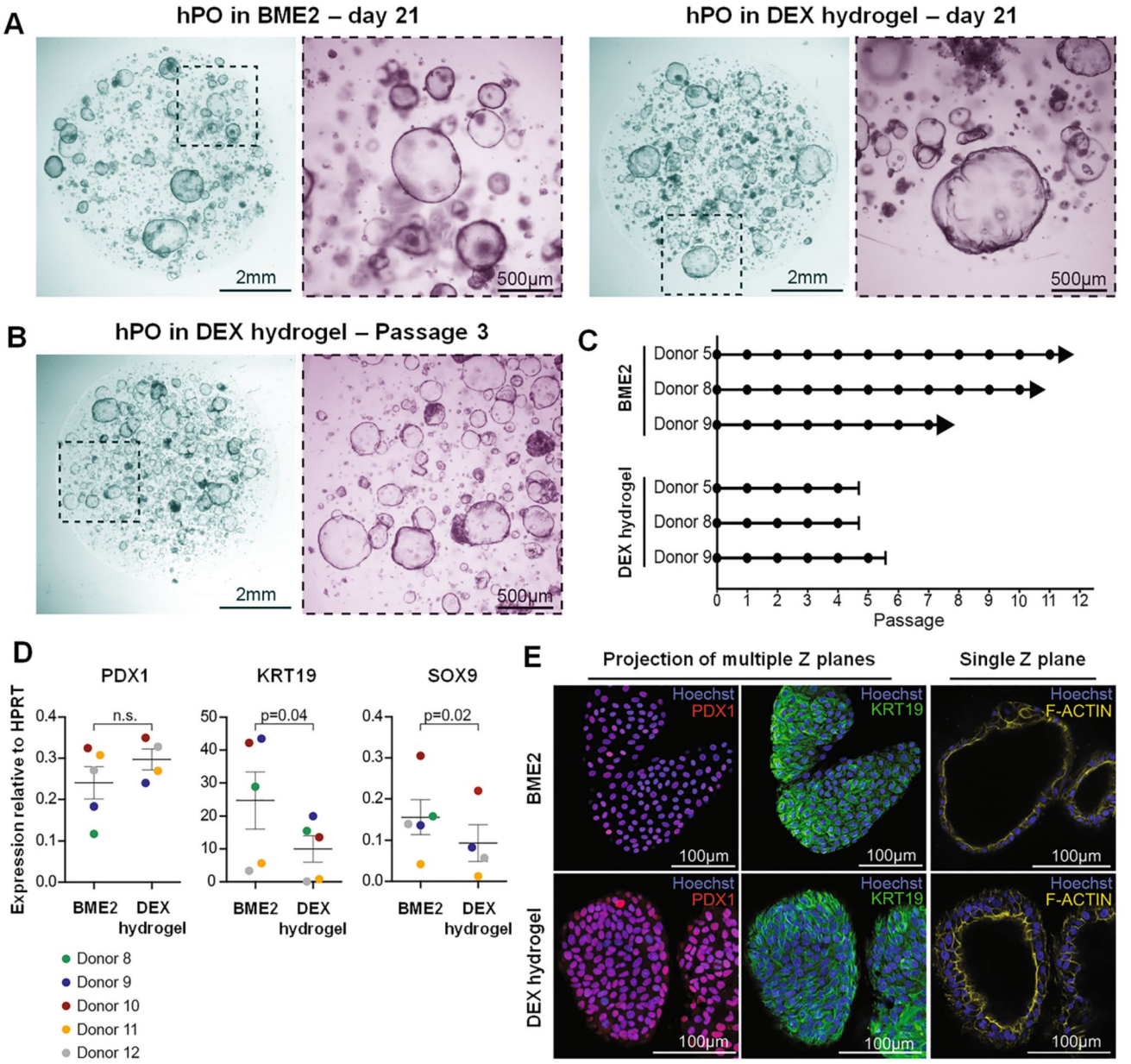

Fig. 6 **a** Bright-field micrographs of human pancreatic organoids in BME2 and DEX hydrogels after 21 days. DEX hydrogels were conjugated with minimal adhesion motif, RGD. Scale bars: 2 mm (left), 500 µm (right). **b** Pancreatic organoids in DEX hydrogels at passage 3. Scale bars: 2 mm (left), 500 µm (right). **c** Graphs demonstrating human pancreatic organoids derived from different donors, expanded via passaging in BME2 and DEX hydrogels. **d** Gene expression profiling of pancreatic organoids derived from different donors in BME2 and DEX hydrogels. **e** Immunofluorescence staining of polarized pancreatic organoids in BME2 and DEX hydrogels. Nucleus was stained with Hoechst. Scale bars: 100 µm. Reproduced with permission from Ref. [56]. Copyright © 2020, Georgakopoulos et al.

[56]. The sensitivity of DEX hydrogels toward dextranase allows easy passaging of pancreatic organoids. However, reduced expansion potential and limited passage numbers (up to passage 4, before cultures start to deteriorate), compared to Basement Membrane Extract Type 2 (BME2) matrix, highlight the need for further mechano-biochemical tuning of DEX hydrogels.

Renal organoids

The kidney is a bean-shaped organ, involved in the filtration of blood to remove wastes and control the body's fluidic and electrolytic balance. It consists of almost 20 different cell types, structured into the glomerulus, and proximal and distal tubules [106, 107]. Kidney organoids have witnessed widespread applicability in developmental biology, disease modeling, and drug screening [12, 108].

With the aim of studying renal tubulogenesis and evaluating drug-mediated nephrotoxicity, PEG and heparin-based hydrogels with tunable biochemical features (via the addition of heparin) and degradability (via incorporation of MMP-sensitive peptide) were reported (Fig. 7) [59]. Three different sets of hydrogels, namely degradable PEG-MMP-PEG (dPMP), non-degradable PEG-heparin (ndPH), and degradable PEG-MMP-heparin (dPMH) were prepared and evaluated parallelly to study the assembly process of human renal proximal tubule epithelial cells. The results revealed that dPMH hydrogels, over 4 weeks of culture, significantly promoted cellular organization into polarized renal tubules with a clear lumen, characterized by the presence of some cilia and microvilli at the luminal face. On the other hand, dPMP gels did not show any sign of polarization, while in ndPH gels, polarized spheroids with lumens filled with cellular debris were evident.

Another PEG-based hydrogel system, providing independent control over its mechanical features, tethered adhesion motif density, and hydrogel degradability, was employed to study epithelial morphogenesis [109]. The cysts formed under different formulation were scored in terms of their apicobasal polarity and lumen formation. Under optimal conditions, i.e., 4–4.5% PEG polymer density, 2 mM RGD peptide adhesion motif, and ~ 90% or more MMP-sensitive peptide threshold density (adjusted by replacing protease-degradable peptide with a slow degrading peptide variant), showed cyst growth, polarization, and lumenogenesis, similar to that observed in control collagen gels; hydrogels with altered properties resulted in abnormal morphogenesis.

Unlike the above studies, recent research revealed that the conjugation of MT1-MMP-sensitive IPES peptide to PEG hydrogels, instead of conventional MMP-sensitive peptide, was crucial for renal tubulogenesis [110]. Similarly, functionalization of RGD adhesion motif, but not collagen I-mimetic triple-helical peptide or LMN β1 chain-derived peptide, to the hydrogels efficiently supported renal tubulogenesis. PEG macromer size (10 or 20 kDa) and their polymeric density (together affecting the hydrogels' mechanical properties) were also shown to affect epithelial morphogenesis, thereby suggesting independent multi-tunability of these hydrogels.

Intestinal organoids

The intestine is the longest organ of the digestive tract and forms a major site of digestion, nutrient absorption, waste removal, and immune system homeostasis [93]. Anatomically, the intestinal epithelium is organized in a defined crypt/villus structure. Crypts contain intestinal stem cell niche that continuously divides to generate differentiated cells (enterocytes, goblet cells, enteroendocrine cells, Tuft cells, and Paneth cells), present on the villus unit [111, 112]. Intestinal organoids are among the first to be generated, and still, substantial research efforts have been focused on generating fully functional intestinal epithelium for investigating developmental processes, disease modelling, drug pharmacokinetics, and toxicity [73, 113–115].

A study demonstrated that defined, degradable PEG hydrogels conjugated with collagen I-mimetic triple-helical cell adhesion peptide, in addition to FN-binder peptide and COLIV-LMN binder peptide, and a cross-linker peptide with MMP- and sortase-sensitive sites, efficiently sustained the formation and differentiation of duodenal enteroids (Fig. 8) [74]. The generated undifferentiated enteroids appeared as cysts, expressing crypt-associated markers and evident proliferative capacity across passages, whereas, upon differentiation, markers of intestinal epithelium were observable. Moreover, alterations in the biomechanical features, caused by the change in polymeric density and size of PEG macromer (20 or 40 kDa or a combination of both) of the hydrogels, affected enteroid formation efficiency.

In the same milieu, to provide evidence toward the need for a dynamic environment for the maintenance of stem cell niche and organogenesis, soft PEG-RGD hydrogels were recently fabricated [115]. These hydrogels initially provided a stiff microenvironment that allowed maintenance and expansion of intestinal stem cells. Over the culture duration, hydrogels slowly underwent degradation, reducing the gel stiffness and promoting intestinal organogenesis. It is essential to mention that RGD adhesion motifs alone could suffice the stem cell niche maintenance; however, for organogenesis, supplementation of full-length LMN-111 protein was necessary. The non-degradable, static variants of the hydrogels could support cystogenesis and maintain leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5) positive intestinal stem cells even after repeated passaging but were incapable of inducing in vitro organogenesis. On

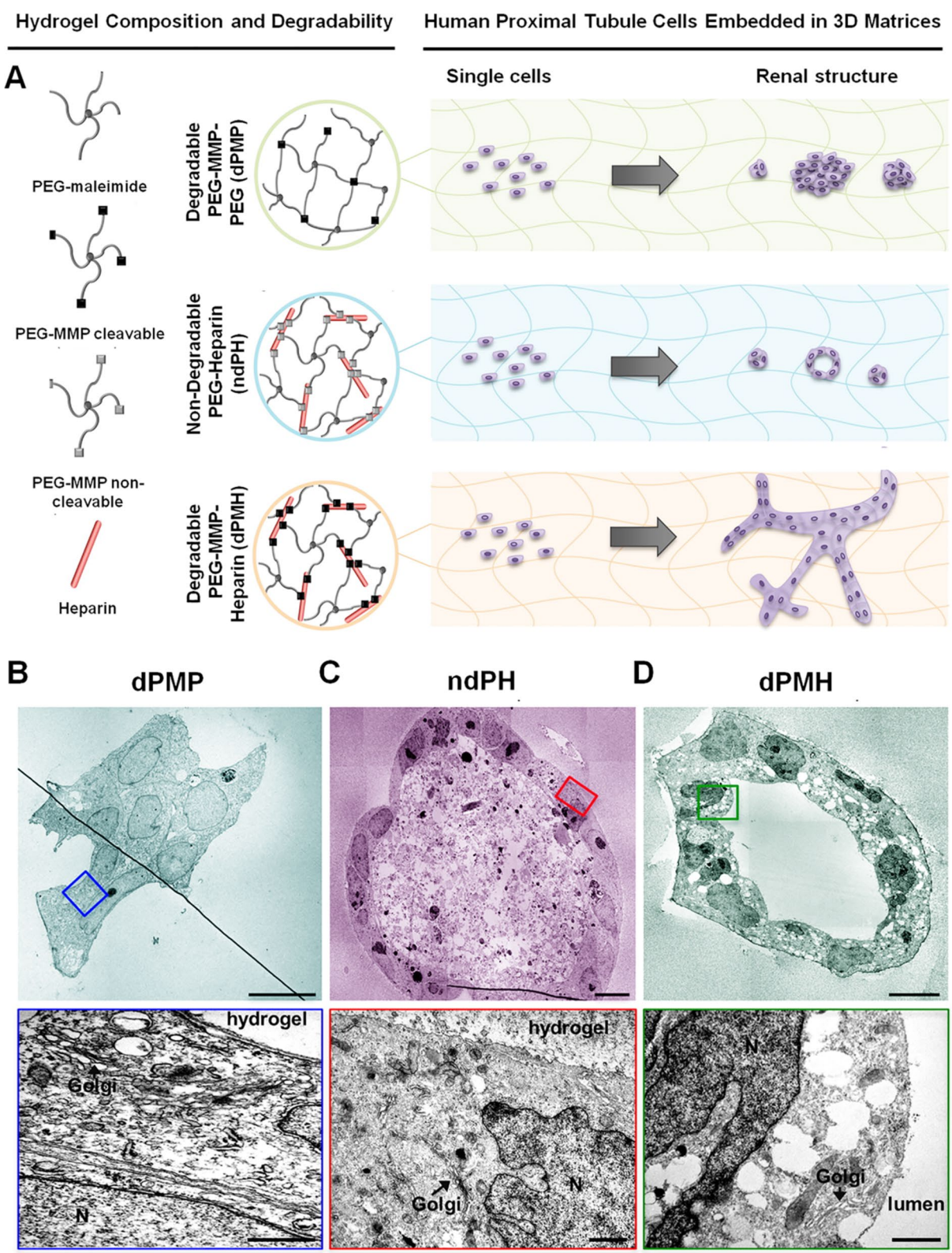

Fig. 7 **a** Schematic representation of chemically defined hydrogels with variable composition (PEG or heparin), degradability (MMP-sensitive or insensitive), and structural morphogenesis of human proximal tubule cells in different hydrogels. Electron micrographs of HK-2 cell-derived renal structures in **b** degradable PEG-MMP-PEG, **c** non-degradable PEG-heparin, and **d** degradable PEG-MMP-heparin hydrogels (scale bar: 10 μm). Zoomed images have been provided below (scale bar: 1 μm). Golgi and N represent the Golgi apparatus and the nucleus, respectively. Reproduced with permission from Ref. [59]. Copyright © 2017, Elsevier B. V.

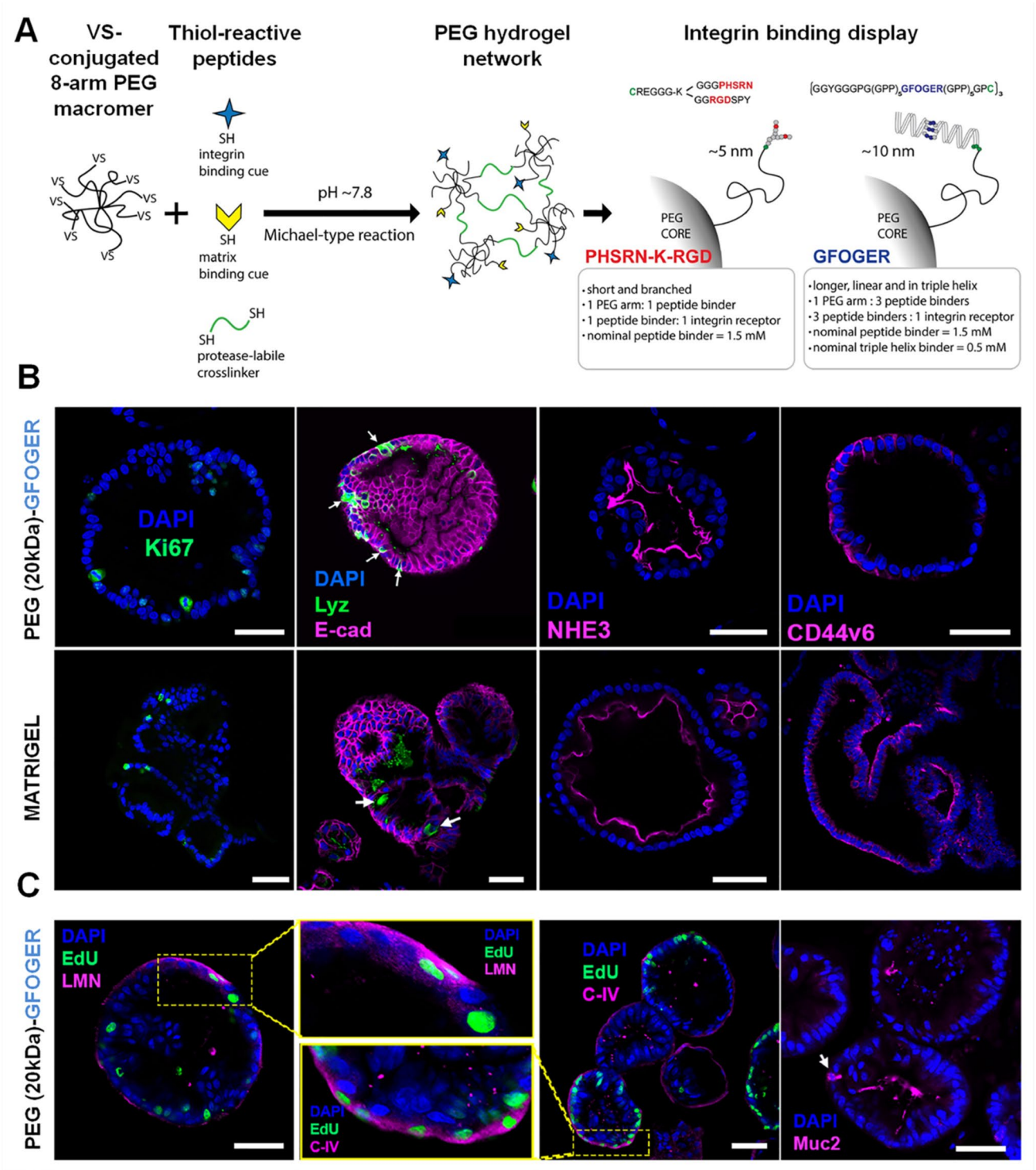

Fig. 8 **a** Schematic representation of assembling tunable, chemically defined, synthetic PEG hydrogel matrix. VS-conjugated 8-arm PEG macromers (20 or 40 kDa), in combination with thiol-reactive peptides, namely integrin-binding cues (PHSRN-K-RGD and GFOGER, with intrinsic affinity toward α5β1 and α2β1, respectively), matrix binding cues (FN-binding and COLIV-LMN biding), and protease-liable cross-linkers (MMP and sortase-sensitive), to form hydrogels via Michael-type reaction. The figure also highlights branched (PHSRN-K-RGD) and triple-helical (GFOGER) conformation of the integrin-binding peptides, bound to the PEG arm. **b**, **c** Representative immunostained polarized 10-day-old intestinal organoids in Matrigel and PEG (20 kDa)-GFOGER hydrogel demonstrating proliferative cells (EdU, Ki67), mature enterocytes (sodium–hydrogen exchanger 3, NHE3), goblet (mucin 2, Muc2), Paneth (lysozyme, Lyz), epithelial (E-cad), and basolateral (LMN, COLIV, and CD44-v6) markers. Nucleus was stained with DAPI. Scale bar 50 μm. Reproduced with permission from Ref. [74]. Copyright © 2020 Elsevier B. V.

the other hand, the fast degrading variants generated lesser cysts with lower LGR5+ stem cell population.

Alternatively, natural biomaterials have also been used for culturing intestinal organoids. For instance, alginate hydrogels (concentration 1%, storage modulus ~ 78 Pa) were shown to sustain the growth of human intestinal organoids derived from human ESC or iPSC lines; however, the yield of organoids was significantly lower than in the case of Matrigel [64]. Interestingly, alginate-grown organoids showed polarized architecture with the expression of different intestinal epithelial differentiation markers, similar to those grown in Matrigel. Upon in vivo implantation organoids underwent maturation, closely resembling the native human intestinal tissue. Another defined hydrogel composition of fibrin (concentration 3.5–4 mg/mL, storage modulus ~ 77–140 Pa) supplemented with LMN (2 mg/mL) promoted the formation, growth, and epithelial morphogenesis of the intestinal organoids, similar to Matrigel (Fig. 9) [52]. Supplementation of other ECM components, namely COLIV and heparin, were insufficient for the purpose, highlighting the critical role of LMN in intestinal morphogenesis.

Other organoids

Apart from the above-discussed ones, chemically defined hydrogels could also sustain organoids' growth associated with other tissues/organs.

The mammary gland is a branched tubular organ that undergoes two distinct developmental phases: (i) embryonic and (ii) postnatal during puberty and pregnancy; to study this, organoid models are often preferred [116]. Besides, their widespread applicability in breast cancer biology cannot be disregarded. Recently, soft, degradable, and chemically defined PEG-heparin hydrogels were shown to promote morphogenesis of non-transformed mammary epithelial cells into polarized multicellular organoids with a clear lumen, resembling terminal ductal lobular units (acini), with growth characteristics similar to those in Matrigel [60]. The cells in non-degradable variants also formed cysts but had lower lumen clearance, while the cells in stiffer variants exhibited higher proliferation, lower polarization, and invasive features.

Endometrium, the inner lining of the uterus, comprising epithelial and mesenchymal cells, is subjected to periodic alterations in growth, differentiation, and degradation under the influence of ovarian hormones [117]. Endometrial organoids have emerged as potential candidates for recapitulating these periodic changes and endometrium-associated pathobiological issues. In this regard, fully synthetic matrices coupled with collagen I-mimetic triple-helical cell adhesion peptide, FN-binder peptide, COLIV-LMN binder peptide, and a cross-linker peptide with MMP- and sortase-sensitive sites could provide necessary cues to generate polarized endometrial organoids with EdU+ and EpCAM+ proliferative and differentiated epithelial cells, similar to that of Matrigel (Fig. 10) [74].

The immune system is highly intricate and is still one of the most challenging systems to study. In order to recapitulate such complex microenvironment, lymph node organoids were developed in MMP-sensitive, defined PEG hydrogels using Naïve B cells and 40LB stromal cells (genetically modified to express CD40 ligand and produce B cell-activating factor) [118]. The study revealed that maleimide group, but not vinyl sulfone and acrylate, when used as end-group functionality in PEG hydrogels, sustained B cells' survival and germinal center-like induction. Moreover, incorporating collagen I-mimetic peptide, similar to that present in the niche, promoted germinal center-like dynamics and epigenetics; peptides mimicking FN/vitronectin and VCAM1 were insufficient for the purpose.

Bone marrow is a soft, spongy tissue inside the bones, wherein hematopoietic stem and progenitor cells (HSPCs) and bone marrow stromal cells (BMSCs) reside and contribute in sustained repair and regeneration of the body. Developing a highly controlled microenvironment for studying the cellular dynamics in the bone marrow is challenging. In this regard, bone marrow organoids were formed in a tunable and defined PEG-HA composite hydrogels [92]. Hydrogels were hydrolytically sensitive to MMP peptide and were conjugated with RGD motif. The study revealed that the hydrogels could maintain, expand, and differentiate human HSPCs and BMSCs in vitro. Additionally, PEG hydrogels coupled with 20 different cell-instructive peptide moieties (including a set of integrin-binding and MMP-sensitive domains of marrow-specific ECM proteins) and native bone marrow-mimetic mechanical properties can also be employed for generating bone marrow organoids [119].

Tables 2 and 3 summarize current developments in chemically defined hydrogels from natural and synthetic materials for organoid culture, respectively.

Conclusion and outlook

The capacity of organoids to recapitulate and reflect the intricacies of native tissue and organs has led to their potential applications in biomedical and pharmaceutical industries. Over the years, Matrigel continues to be the most suitable platform for a myriad of organoid-based applications. Alternatively, recent interventions have highlighted a similar potency of decellularized tissue-derived ECM for organoid generation. However, the lack of spatiotemporal control over their tunable characteristics limits their translational aspects, calling upon the need to explore advanced platforms.

Chemically defined matrices, involving the use of natural, synthetic materials or their biohybrids, offer tailoring

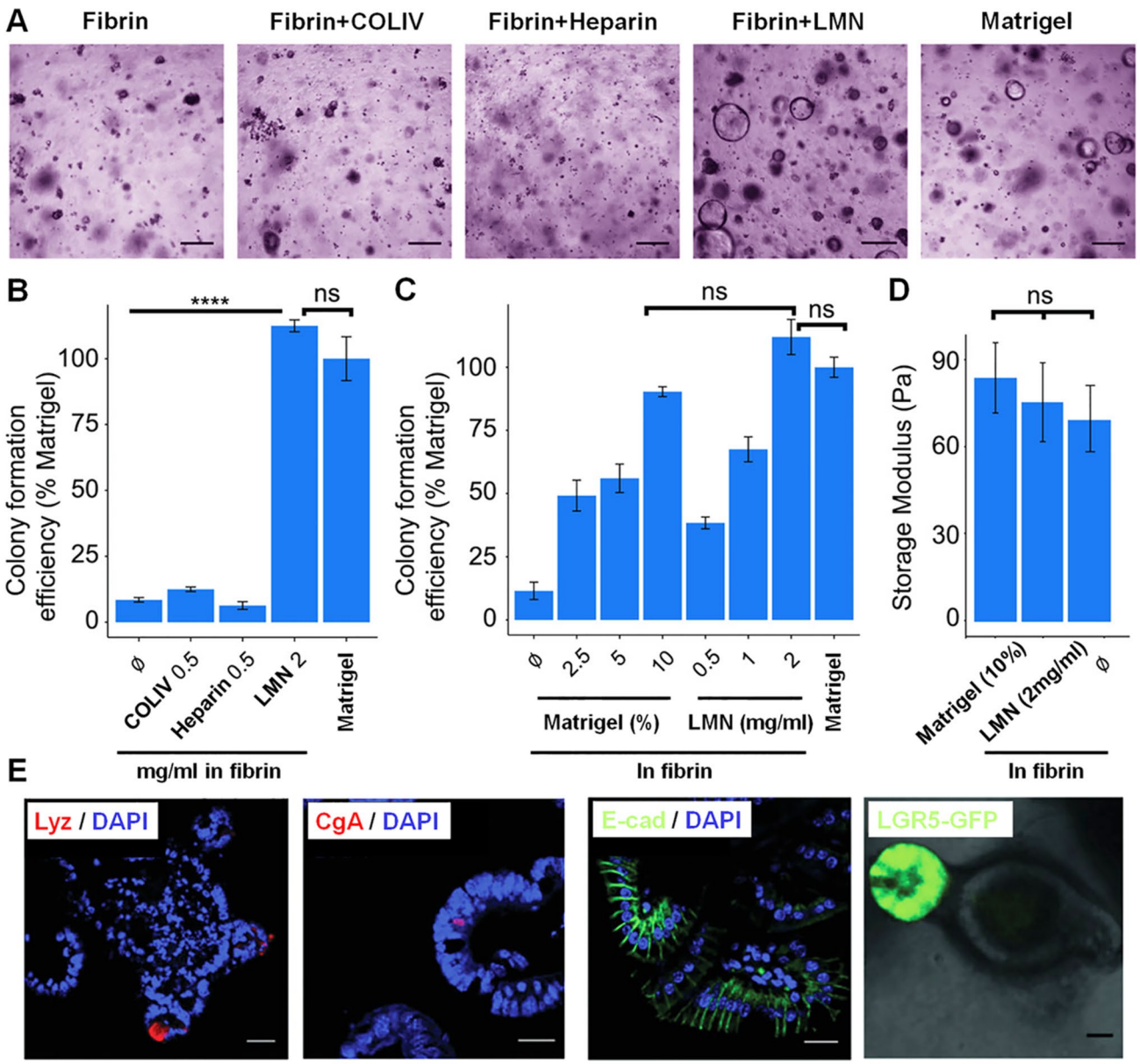

Fig. 9 **a** Bright-field micrographs, showing morphogenesis of mouse small intestinal stem cells in pure Matrigel or fibrin hydrogels (pure or supplemented with COLIV, heparin, and LMN). Scale bar 200 µm. **b** Quantitative analysis of colony formation efficiency of mouse small intestinal stem cells under different supplementation of fibrin hydrogels normalized to pure Matrigel. Error bars: SD. **c** Quantitative analysis of colony formation efficiency of mouse small intestinal stem cells in fibrin hydrogel supplemented with varying concentration of Matrigel or LMN, normalized to Matrigel. Error bars: SD. **d** Quantification of mechanical properties of pure and supplemented fibrin hydrogels. Error bars: SD. **e** Representative images of intestinal organoid sections stained for Lyz (Paneth cells), chromogranin A (CgA, enteroendocrine cells), E-cad (epithelial cells), and LGR5-GFP (intestinal stem cells). Paneth and intestinal stem cells were exclusively present in the crypt-like domains. Nucleus was stained with DAPI. Scale bar: 20 µm. Reproduced with permission from Ref. [52]. Copyright © 2018, Broguiere et al. Published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

advantages over biochemical, mechanical, and degradation-related features, thereby guiding the generation, expansion, differentiation, and maturation of organoids in a reproducible manner, as highlighted in the present work (Fig. 11). Further expansion in the organoid technology needs a critical upgradation from the archaic cell culture regimes and a better understanding of cellular responses upon interaction with different biointerfacial cues. The contact printing technology or combinatorial approaches, wherein different compositions of ECM proteins or bioactive peptides could be spotted on a hydrogel platform (maybe of variable stiffness), can turn out to be handy, ascertaining their influence

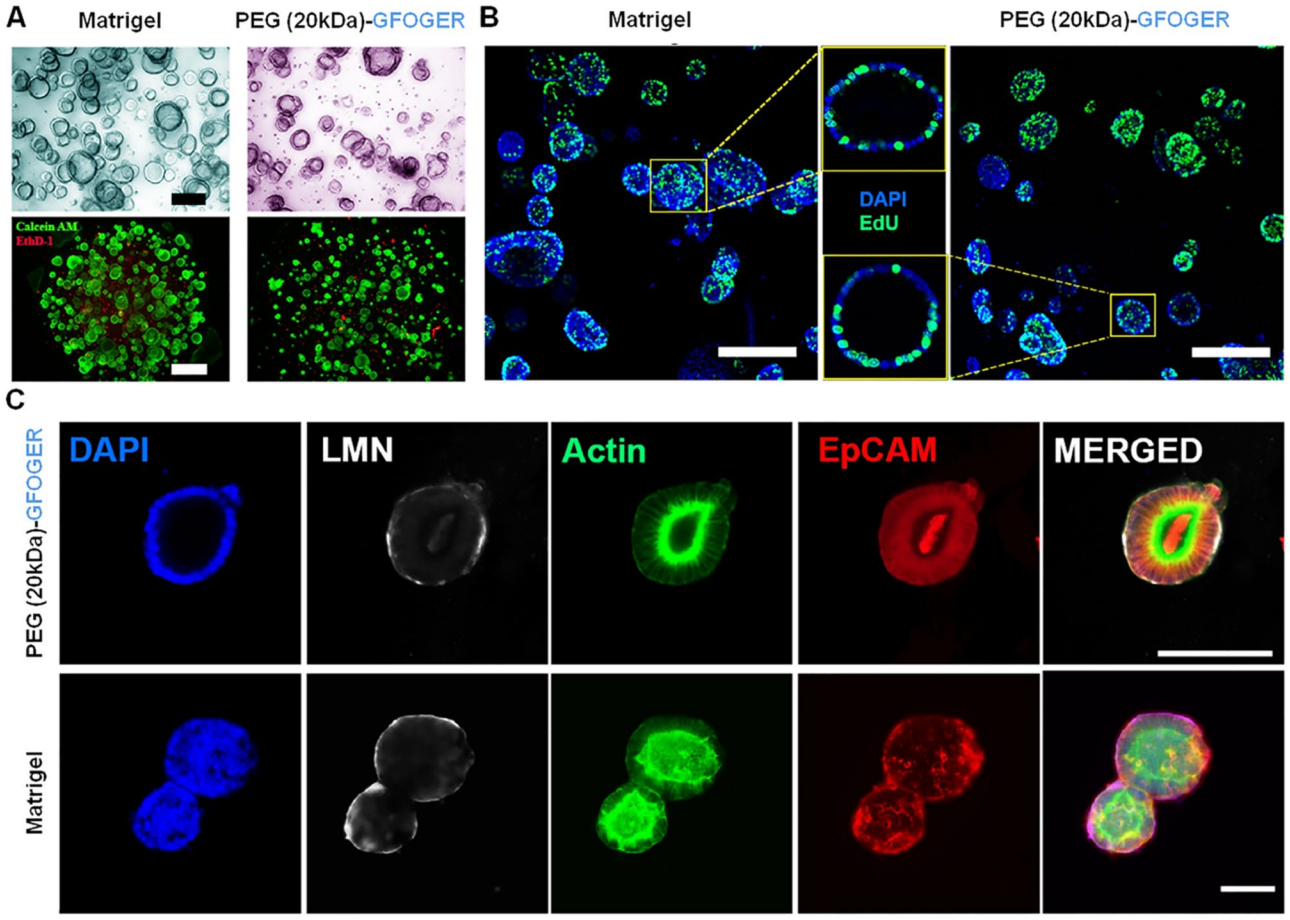

Fig. 10 **a** Representative bright-field and corresponding live/dead images of endometrial organoids in Matrigel and PEG (20 kDa)-GFOGER hydrogels. Scale bars: 200 µm (top), 500 µm (bottom). **b**, **c** Immunostained endometrial organoids in Matrigel and PEG (20 kDa)-GFOGER demonstrating proliferative cells (EdU+), apical (actin+), basolateral (LMN+), and epithelial (EpCAM+) markers. Scale bar 200 µm (**b**) and 100 µm (**c**). Nucleus was stained with DAPI. Reproduced with permission from Ref. [74]. Copyright © 2020, Elsevier B. V.

on phenotypic characteristics of either stem cells or progenitor cell population [130, 131].

Further impactful development in this domain can be attributed to (i) synthetic hydrogels that are fully or partially cross-linked by physical interactions, or (ii) photomutatable-synthetic hydrogels [29, 132–135]. In contrast to chemically cross-linked hydrogels, physically cross-linked ones are more dynamic and exhibit a broader sensitivity to the surrounding environment. The use of photomutatable-synthetic hydrogels also offers targeted dynamic tuning of matrix mechanics or generating a local display of growth factors via light and photo-cleavable components attached to the matrix.

However, for research progress, a fruitful collaboration between material scientists, biologists, engineers, and clinicians across academia and industry, aiming toward popularization and improvement in these defined platforms, is necessary. It is also essential to ensure the availability and finding strategies for easy, user-friendly usage of these platforms among the practitioners. At the commercial level, QGel, a start-up company, is making an effort to meet the demand of chemically defined synthetic matrices for a wide range of organoid-related applications (https://www.qgel.com/).

Besides, organoids related to many tissues, including cardiac and retinal, have still not been reported in chemically defined hydrogels, presenting another scope for future development in this sphere.

On a concluding note, we upbeat that dedicated effort in this direction can open up new vistas in generating clinically compatible organoid tissue models in the near future.

Table 2 Chemically defined hydrogels from natural biomaterials and their derivatives for organoid culture

S. No.	Organoid type	Polymer	Peptide sequence (function)	Tunable features (if reported)	Mechanical properties	Cells used	Control matrices	References
1	Cerebral	HA and protonated chitosan	NA	NA	Young's modulus: 9.8 kPa	Human iPSCs	NA	[67]
2	Neural	Thiolated heparin and HA methacrylate	NA	Biochemical features	Storage modulus: 0.3 (soft) and 1.17 kPa (rigid)	hiPSK3 cells	Geltrex	[120]
3	Pancreatic	SG dextran (thiol-reactive) and thiol-modified HA	RGD	NA	NA	Pancreatic duct/pre-established organoid fragments	BME-2	[56]
4	PDAC	Collagen	NA	NA	NA	PDAC cancer PDX	NA	[44]
5	Liver	Cellulose	NA	Mechanical features	Young's modulus: 0.255 kPa	Pre-established human liver organoid	Matrigel	[102]
6	Liver	Fibrin, alginate, chitosan	NA	Biochemical features	NA	iHeps	NA	[53]
7	Colorectal	Gelatin–phenol and HA–phenol	NA	Mechanical and biochemical features	Young's modulus: 14.6 kPa	Colorectal cancer PDX	Geltrex	[121]
8	Intestinal	Alginate	NA	NA	NA	Pre-established human intestinal organoid	Matrigel	[45]
9	Intestinal	Alginate	NA	Mechanical features	Storage modulus: < 0.1 kPa	Human ESC/iPSC lines	Matrigel	[64]
10	Intestinal	Collagen	NA	NA	NA	Pre-established human intestinal organoid	Matrigel	[46]
11	Intestinal	Collagen	NA	NA	NA	Human crypt fragments and intestinal subepithelial myofibroblasts	Matrigel	[47]
12	Intestinal	Collagen	NA	Mechanical features	Storage modulus: 0.029 kPa	Mouse crypt fragments	NA	[122]
13	Intestinal	Fibrin and LMN	NA	Mechanical and biochemical features	Storage modulus: 0.077 kPa	Mouse small intestinal stem cells	Matrigel	[52]

BME-2, Basement Membrane Extract Type 2; PDAC, pancreatic ductal adenocarcinoma; PDX, patient-derived xenograft; hiPSCs, human-induced pluripotent stem cells; hESCs, human embryonic stem cells; iHeps, hiPSCs-derived hepatocyte-like cells; NA, not available

Table 3 Chemically defined hydrogels from synthetic biomaterials and their derivatives or biohybrids for organoid culture

S. No.	Organoid type	Polymer	Conjugated peptide sequence	Conjugated peptide source/function	Peptide conjugation strategy	Tunable features (if reported)	Mechanical properties	Cells	Control matrices	References
1	Cerebellar	PEG-8MAL	$CG(PKG)_4(POG)_4(DOG)_4$	Collagen-like peptide	Thiol–maleimide "click" chemistry	Biochemical features	Young's modulus: 80.1 kPa	Cerebellar neuronal–glial cell	–	[95]
			$CG(PKG)_4(POG)_4(DOG)_4$-RGDSPG	Collagen-like peptide with cell adhesion motif			Young's modulus: 61.2 kPa			
2	Neural	PEG-8NB	CRGDS	Cell adhesion motif	Thiol–Norbornene "click" chemistry	Mechanical and biochemical features	NA	Human ESC-NPCs, MSCs, pericytes, endothelial cells, and microglia precursors	–	[123]
			KCGG-PQGIWG-QGCK	MMP-sensitive peptides						
3	Neural	PEG-8VS	FKGG-GDQGIAGF-ERCG	MMP-insensitive, lysine-donor transglutaminase factor XIIIa substrate peptide	Thiol–vinyl sulfone "click" chemistry	–	–	mESCs	Matrigel	[124]
			NQEQVSPL-ERCG	Glutamine-acceptor transglutaminase factor XIIIa substrate peptide						

Table 3 (continued)

S. No.	Organoid type	Polymer	Conjugated peptide sequence	Conjugated peptide source/function	Peptide conjugation strategy	Tunable features (if reported)	Mechanical properties	Cells	Control matrices	References
4	Neural	PEG-8VS	FKGG-VPMSMRGG-ERCG	MMP-sensitive, lysine-donor transglutaminase factor XIIIa substrate peptide	Thiol–vinyl sulfone "click" chemistry	Mechanical features, degradability, and biochemical features	Young's modulus: 2–4 kPa	Mouse ESCs	Matrigel	[94]
			FKGG-GDQGIAGF-ERCG	MMP-insensitive, lysine-donor transglutaminase factor XIIIa substrate peptide						
			NQEQVSPL-ERCG	Glutamine-acceptor transglutaminase factor XIIIa substrate peptide						
5	Pancreatic	Amikacin hydrate and PEGDE	–	–	–	Mechanical features	Young's modulus: 300 kPa	Human ESC-PP with HUVEC	Matrigel	[105]
6	Pancreatic	PEG-VS (with LMN)	FKGG-GPQGIWGQ-ERCG	MMP-sensitive, lysine-donor transglutaminase factor XIIIa substrate peptide	Thiol–vinyl sulfone "click" chemistry	Mechanical and biochemical features	Shear modulus: 0.25 kPa	Embryonic pancreas progenitor cells	Matrigel	[104]
			NQEQVSPL-ERCG	Glutamine-acceptor transglutaminase factor XIIIa substrate peptide						

Table 3 (continued)

S. No.	Organoid type	Polymer	Conjugated peptide sequence	Conjugated peptide source/function	Peptide conjugation strategy	Tunable features (if reported)	Mechanical properties	Cells	Control matrices	References
7	Intestine	PEG-4MAL	GRGDSPC	Cell adhesion motif (FN or vitronectin mimic)	Thiol–maleimide "click" chemistry	Polymer density and biochemical features	Storage modulus: < 0.1 kPa	Human iPSCs	Matrigel	[73]
			CGGRKRLQVQLSIRT	LMN α1 chain-derived (AG73 peptide)						
			GYGGGP(GPP)$_5$GFOGER(GPP)$_5$GPC	Collagen I-mimetic triple-helical peptide						
			CGGAASIKVAVSADR	LMN α1 chain-derived						
			GCRD-GPQGIWGQ-DRCG	Protease-degradable peptide (collagen-derived)						

Table 3 (continued)

S. No.	Organoid type	Polymer	Conjugated peptide sequence	Conjugated peptide source/function	Peptide conjugation strategy	Tunable features (if reported)	Mechanical properties	Cells	Control matrices	References
8	Intestinal	PEG-8VS and PEG-8Acr	FKGG-GPQGIWGQ-ERCG	MMP-sensitive, lysine-donor transglutaminase factor XIIIa substrate peptide	Thiol–vinyl sulfone "click" chemistry	Mechanical features, tethered adhesion motif density, hydrogel degradability, and biochemical features	Shear modulus: 1.3 kPa	Mouse crypt fragments or mouse intestinal stem cells	Matrigel	[91, 115]
			FKGG-GDQGIAGF-ERCG	MMP-insensitive, lysine-donor transglutaminase factor XIIIa substrate peptide						
			NQEQVSPL-ERCG	Glutamine-acceptor transglutaminase factor XIIIa substrate peptide						
			NQEQVSPL-RGDSPG	Glutamine-acceptor transglutaminase factor XIIIa substrate peptide with RGD motif						
9	Intestine	Dibenzylcyclooctyne-functionalized 4-arm PEG with azide-functionalized cross-linkers	Azide-functionalized RGD	Cell adhesion motif	Strain-promoted azide-alkyne cycloaddition (SPAAC) reaction	Polymer density and hydrogel photodegradability	Storage modulus: < 1.5 kPa	Single cells from pre-established small intestinal organoids	–	[125]

Table 3 (continued)

S. No.	Organoid type	Polymer	Conjugated peptide sequence	Conjugated peptide source/function	Peptide conjugation strategy	Tunable features (if reported)	Mechanical properties	Cells	Control matrices	References
10	Intestinal	QGel CN99 (Proprietary formulation)	–	–	–	–	Shear modulus: 0.45 kPa (tends to reduce to 0.01–0.05 Pa upon culture)	Colonic crypt-derived single cells	Matrigel	[126]
11	Intestinal	Polypeptide chain-based hydrogels	–	–	–	Mechanical and biochemical features	Storage modulus: 0.18 (soft) and 1.22 kPa (rigid)	Mouse intestinal tissue fragments	Collagen I	[114]
12	Intestinal	PEG-4MAL	GRGDSPC	Cell adhesion motif (FN or vitronectin mimic)	Thiol–maleimide "click" chemistry	Mechanical and biochemical features	Storage modulus: 0.1 kPa	Human iPSCs	Matrigel	[90]
			GCRDGPQGIWGQDRCG	Protease-degradable peptide (collagen-derived)						
13	Intestinal	PEG-8VS	GGYGGG $PG(GPP)_5GFOGER(GPP)_5GPC$	Collagen I-mimetic triple-helical peptide	Thiol–vinyl sulfone "click" chemistry	Mechanical features, tethered adhesion motif density, and biochemical features	Storage modulus: 0.81–0.93 kPa	Pre-established organoid fragments	Matrigel	[74]
			PHSRNGGGK-(GGG-ERCG)-GGRGDSPY	FN-derived cell adhesion peptide						
			GCRD-VPMSMRGG-DRCG	MMP-sensitive peptide						
			GCRE-TLQPVYEYMVGV	FN-binding peptide						
			GCRE-ISAFLGIPFAEPPMG-PRRFLPPEPKKP	COLIV and LMN binding peptide						

Table 3 (continued)

S. No.	Organoid type	Polymer	Conjugated peptide sequence	Conjugated peptide source/function	Peptide conjugation strategy	Tunable features (if reported)	Mechanical properties	Cells	Control matrices	References
14	Endometrial	PEG-8VS	GGYGGG $PG(GPP)_5GFOGER(GPP)_5GPC$	Collagen I-mimetic triple-helical peptide	Thiol–vinyl sulfone "click" chemistry	Biochemical features	Storage modulus: 0.81–0.93 kPa	Pre-established organoid fragments	Matrigel	[74]
			PHSRNGGGK-(GGG-ERCG)-GGRGDSPY	FN-derived cell adhesion peptide						
			GCRD-LPRTG-GPQGIAGQ-DRCG	MMP-sensitive peptide with sortase-sensitive recognition site						
			GCRE-TLQPVYEYMVGV	FN-binding peptide						
			GCRE-ISAFLGIPFAEPPMG-PRRFLPPEPKKP	COLIV and LMN binding peptide						

Table 3 (continued)

S. No.	Organoid type	Polymer	Conjugated peptide sequence	Conjugated peptide source/function	Peptide conjugation strategy	Tunable features (if reported)	Mechanical properties	Cells	Control matrices	References
15	Liver	PEG-8VS	FKGG-GPQGIWGQ-ERCG	MMP-sensitive, lysine-donor transglutaminase factor XIIIa substrate peptide	Thiol–vinyl sulfone "click" chemistry	Mechanical features, degradability, and biochemical features	Storage modulus: 0.3 (soft) and 1.3 kPa (rigid)	Mouse biliary duct fragments	Collagen I and Matrigel	[101]
			FKGG-GDQGIAGF-ERCG	MMP-insensitive, lysine-donor transglutaminase factor XIIIa substrate peptide						
			NQEQVSPL-ERCG	Glutamine-acceptor transglutaminase factor XIIIa substrate peptide						
			NQEQVSPL-RGDSPG	Glutamine-acceptor transglutaminase factor XIIIa substrate peptide with RGD motif						
16	Liver	PEG-8VS (with gelatin); supplemented with either LMN-111 or LMN-521	NQEQVSPL-ERCG	Glutamine-acceptor transglutaminase factor XIIIa substrate peptide	Thiol–vinyl sulfone "click" chemistry	Mechanical and biochemical features	Compressive modulus: 2.6 kPa	Pre-established liver organoid fragments	Matrigel	[127]

Table 3 (continued)

S. No.	Organoid type	Polymer	Conjugated peptide sequence	Conjugated peptide source/function	Peptide conjugation strategy	Tunable features (if reported)	Mechanical properties	Cells	Control matrices	References
17	Liver	PIC azide supplemented with either LEC or human recombinant LMN-111	Dibenzocyclooctyne-conjugated GRGDS	Cell adhesion motif	CuAAC "click" chemistry	Mechanical and biochemical features	Storage modulus: 0.012 (1 kDa weight) and 0.038 kPa (5 kDa weight)	Single cells from pre-established liver organoids	Matrigel	[75]
18	Renal	PEG-4MAL, Heparin-MAL	GCGGPQGWGQGGCG	MMP-sensitive peptide	Thiol–maleimide "click" chemistry	Hydrogel degradability and biochemical features	Storage modulus: 0.5 kPa	Human renal proximal tubule cells	Matrigel	[59]
19	Renal	PEG-4MAL, Heparin-MAL	GRGDSPC	Cell adhesion motif (FN or vitronectin mimic)	Thiol–maleimide "click" chemistry	Mechanical features, tethered adhesion motif density, hydrogel degradability, and Biochemical features	Storage modulus: 0.2 kPa	Immortalized mouse inner medullary collecting duct and mouse renal proximal tubule cells	Matrigel	[110]
			GYGGGP$(GPP)_5$GFOGER $(GPP)_5$GPC	Collagen I-mimetic triple-helical peptide						
			CGGEGYGEGYIGSR	LMN β1 chain-derived						
			GCRD-IPESLRAG-DRCG	MT1-MMP-sensitive cross-linking peptide IPES						
			GCRD-GPQGIWGQ-DRCG	Protease-degradable peptide (collagen-derived)						
20	Renal	PEGDA, Thiolated HA	NA	NA	NA	NA	NA	Mouse proximal tubules	NA	[128]
21	Mammary gland	PEG-4MAL, Heparin-MAL	GCGGPQGIWGQGGCG	MMP-sensitive peptide	Thiol–maleimide "click" chemistry	Mechanical features, hydrogel degradability, and biochemical features	Storage modulus: 0.2–0.35 kPa	MCF10A cell line	Matrigel	[60]
			GCGIGQGQGPWGGCG	MMP-insensitive peptide						

Table 3 (continued)

S. No.	Organoid type	Polymer	Conjugated peptide sequence	Conjugated peptide source/function	Peptide conjugation strategy	Tunable features (if reported)	Mechanical properties	Cells	Control matrices	References
22	Mammary gland	PIC azide	Dibenzocyclooctyne-conjugated GRGDS	Cell adhesion motif	CuAAC "click" chemistry	Mechanical and biochemical features	Storage modulus: 0.3 kPa	Mouse mammary gland fragments or epithelial cells	Matrigel	[69]
23	Prostrate	PIC azide	Dibenzocyclooctyne-conjugated GRGDS	Cell adhesion motif	CuAAC "click" chemistry	Mechanical and biochemical features	Storage modulus: 0.3 kPa	Mouse prostate epithelial cells	Matrigel	[69]
24	Bone marrow	PEG-8VS and vinyl sulfonated HA	FKGG-RGDSPG	Cell adhesion motifs	Thiol–vinyl sulfone "click" chemistry	Biochemical features and hydrogel degradability	Storage modulus: 0.25–0.32 kPa	Human BMSCs and CD34+ hHSPC	NA	[92]
			FKGG-GPQGIWGQ-ERCG	MMP-sensitive, lysine-donor transglutaminase factor XIIIa substrate peptide						
			NQEQVSPL-ERCG	Glutamine-acceptor transglutaminase factor XIIIa substrate peptide						
25	Lymphoma	PEG-4MAL	GRGDSPC	Cell adhesion motif (FN or vitronectin mimic)	Thiol–maleimide "click" chemistry	Biochemical features	NA	Human tonsil-derived FDCs HK, HBL-1, OCI-Ly10, OCI-Ly12, and HuT-78 cells	NA	[129]
			GREDVGC	Cell adhesion motifs (VCAM1 mimic)						
			GCRD-VPMSMRGG-DRCG	MMP-sensitive peptide						

Table 3 (continued)

S. No.	Organoid type	Polymer	Conjugated peptide sequence	Conjugated peptide source/function	Peptide conjugation strategy	Tunable features (if reported)	Mechanical properties	Cells	Control matrices	References
26	Immune	PEG-4MAL or PEG-4VS or PEG-4Acr	GRGDSPC	Cell adhesion motif (FN or vitronectin mimic)	Thiol–ene "click" chemistry (depending upon the reacting groups)	Hydrogel degradability and biochemical features	NA	40LB stromal cells (genetically modified from NIH/3T3 fibroblasts to express CD40 ligand and produce B cell-activating factor) and mouse B cells	NA	[118]
			GREDVGC	Cell adhesion motifs (VCAM1 mimic)						
			GYGGGP$(GPP)_5$GFOGER$(GPP)_5$GPC	Collagen I-mimetic triple-helical peptide						
			GCRD-VPMSMRGG-DRCG	MMP-sensitive peptide						

CuAAC, copper-assisted azide-alkyne cycloaddition; PEG-4MAL, 4-arm PEG macromer with terminal maleimide groups; PEG-8MAL, 8-arm PEG macromer with terminal maleimide groups; PEG-8NB, 8-arm PEG macromer with terminal norbornene groups; PEG-8VS, 8-arm PEG macromer with terminal vinyl sulfone groups; PEG-4VS, 4-arm PEG macromer with terminal vinyl sulfone groups; PEG-4Acr, 4-arm PEG macromer with terminal acrylate groups; PEG-8Acr, 8-arm PEG macromer with terminal acrylate groups; ESC-DE, ESCs-derived definite endoderm; ESC-PP, ESCs-derived pancreatic progenitor cells; ESC-NPCs, ESC-derived neural progenitor cells; Heparin-MAL, heparin maleimide; hTERT, human telomerase reverse transcriptase; BMSCs, bone marrow-derived stromal/stem cells; HSPC, hematopoietic stem and progenitor cells; NA, not available

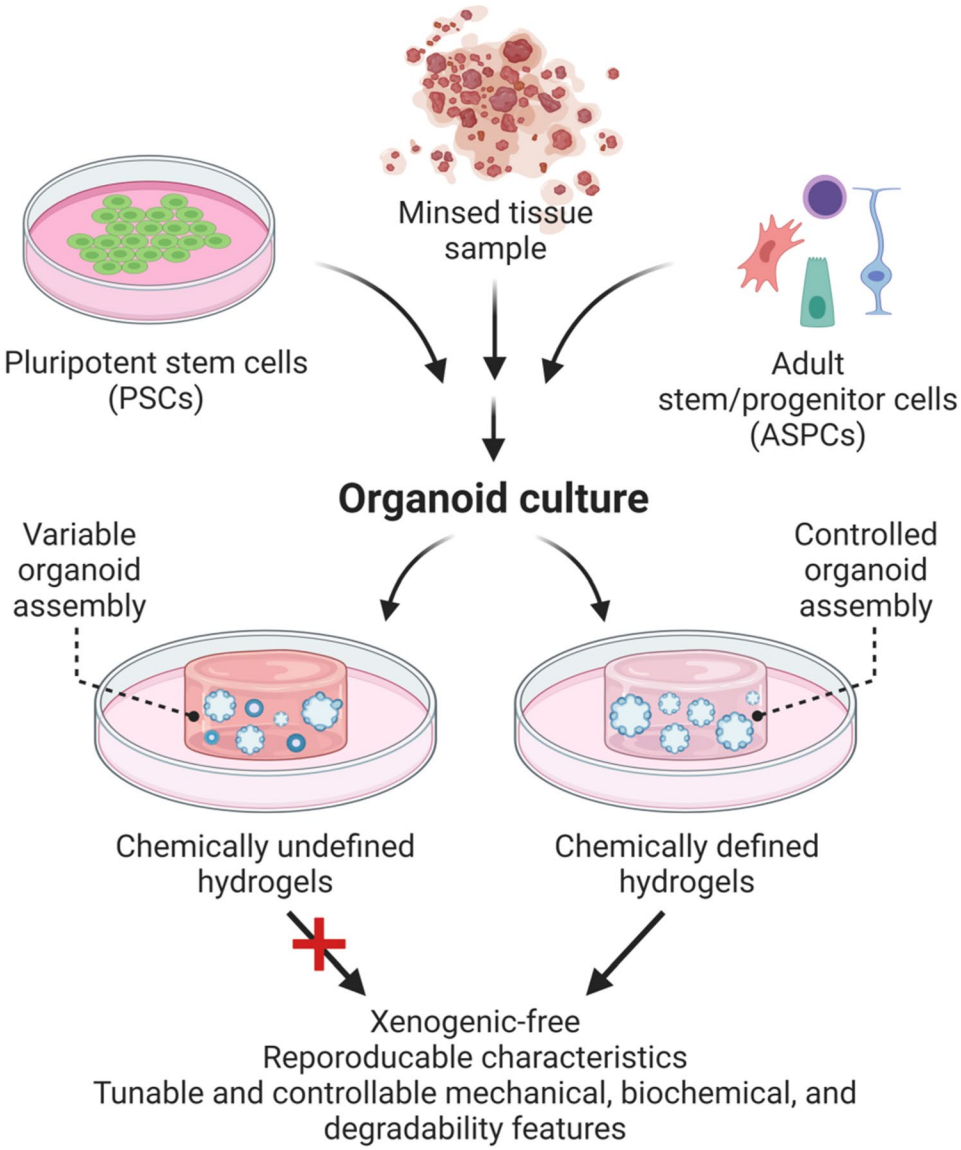

Fig. 11 Schematics highlighting the differences in the organoid formation in chemically defined and undefined hydrogel platforms

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Compliance with ethical standards

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