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REVIEW ARTICLE

Biomaterials and biosensors in intestinal organoid culture, a progress review

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Abstract

As an emerging technology, intestinal organoids are promising new tools for basic and translational research in gastroenterology. Currently, culture of intestinal organoids relies mostly on a type of tumor-derived scaffolds, namely Matrigel, which may pose tumorigenic risks to organoid implantation. Apart from the traditional detection methods, such as tissue slicing and fluorescence staining, the monitoring of intestinal organoids requires real-time biosensors that can adapt to their three-dimensional dynamic growth patterns. In this review, we summarized the recent advances in developing definite hydrogel scaffolds for intestinal organoid culture and identified key parameters for scaffold design. In addition, classified by different substrate compositions like pH, electrolytes, and functional proteins, we concluded the existing live-imaging biosensors and elucidated their underlying mechanisms. We hope this review enhances the understanding of intestinal organoid culture and provides more practical approaches to investigate them.

KEYWORDS

biosensor, intestinal stem cell, organoid, regenerative medicine, scaffold design

1 | INTRODUCTION

In 2009, Sato et al. (2009) firstly established long-term culture conditions to generate intestinal organoids from small intestinal crypts. Subsequently, increasing studies began to describe the intestinal organoids, which are three-dimensional (3D) tissues that develop from specific stem cells in vitro and bear physiological resemblance to the in vivo intestine (Ohsaka & Sonoyama, 2018; Watson et al., 2014; Workman et al., 2017). Hence, it has been regarded as an advantageous platform to conduct studies such as molecular signaling,

pathogen–host interactions, preclinical drug screening, toxin testing, and tissue engineering (Gunasekara et al., 2018; Nakamura & Sato, 2018; Rahmani, Breyner, Su, Verdu, & Didar, 2019; Shin, Hinojosa, Ingber, & Kim, 2019). Routinely, Matrigel is used to culture intestinal organoids under different manipulations of growth factors determined by the stem cell ages and sources. However, the Matrigel is poorly defined basement membrane derivatives extracted from Engelbreth–Holm–Swarm mouse sarcoma cells. As a result, the application of intestinal organoids is limited especially in the field of regenerative medicine (Huang, Ren, Wu, Li, & Ren, 2019). Additionally, morphological and functional evaluations of intestinal organoids are mainly dependent on the 3D imaging that usually involves the organoid fixation (Dekkers et al., 2019). Unfortunately, this procedure causes the death of intestinal organoids and hinders observation of organoid development.

New insights have been put forward regarding the two concerns above. First, natural and synthetic hydrogel scaffolds are potential alternatives to Matrigel, since they exhibit a 3D network structure similar to the environment of stem cell niches (Kratochvil et al., 2019). Most of these scaffolds have definite composition and clear description of their biocompatibilities (Brown & Anseth, 2017; Huang, Li, et al., 2018). They can control the development of intestinal organoids by creating different biophysical signals. These signals can stimulate specific cellular receptors to regulate cell growth. For example, yes-associated protein 1 (YAP) is a nuclear effector that has been implicated in cellular mechanosensing and mechanotransduction (Aragona et al., 2013). This protein can sense alternation of biomaterial stiffness to regulate self-renewal of intestinal stem cells (ISCs; Gjorevski et al., 2016) and determine symmetry-breaking events such as crypts and villi (Serra et al., 2019). Therefore, as potential candidates, selection of appropriate scaffolds requires overall assessment on their physical (e.g., stiffness) as well as biological (e.g., molecule–cell interactions and intracellular signaling alternation) properties.

In addition, dynamic physiological changes such as electrolytes and functional proteins may distinguish disease states from the normal states (Rao, 2019). Like the native intestine, homeostasis of intestinal organoids relies on the balance of electrolyte exchange and integrity of intestinal barriers. Electrolyte disturbance can cause diarrhea or constipation, while injury to the intestinal barriers can lead to bacterial translocation and excessive inflammation. By targeting specific electrolytes or functional proteins, live-imaging biosensors can continuously monitor them in a real-time manner, while traditional assays such as western blot and fluorescence staining can only detect transient information.

Therefore, in this progress review, we summarize the emerging biomaterials and elucidate how their properties affect the intestinal organoid culture. The design, manufacture, and function of the live-imaging biosensors are also discussed individually based on substrate compositions.

2 | BIOMATERIALS FOR INTESTINAL ORGANOID CULTURE

Hydrogel scaffolds are generated from the crosslinking of polymers (Huang, Deng, et al., 2018; Huang, Ren, et al., 2018). Based on the types polymers extracted, the hydrogels can be classified into natural hydrogels and synthetic hydrogels. Although increasing evidence suggests that the synthetic hydrogels can act as tissue scaffolds with tunable mechanical strength, they lack important bioactive molecules, such as amino acids to support the adhesion, growth, and maturation of the encapsulated cells (Jabbari, 2019). In contrast, natural hydrogels contain the native tissue constituents of mammals, thus offering more appropriate conditions for intestinal organoid culture.

2.1 | Natural scaffolds

It is important to explore the principles of scaffold design for intestinal organoid culture. A recent study has identified three key conditions for natural protein-based hydrogels in the culture of intestinal organoids (Broguiere et al., 2018). First, the hydrogel scaffolds need to provide anchoring molecules such as the arg-gly-asp (RGD) sequence for adhesion of ISCs. Broguiere and his colleagues developed a definite fibrin-based hydrogel that supported intestinal organoid growth. The intrinsic RGD sites in fibrin (35 μM RGD in 3 mg/ml fibrin) are so sturdy that additional coupled RGD peptides do not increase the proliferation of intestinal organoids. Moreover, the stiffness of the fibrin-based hydrogel can affect intestinal organoid differentiation. Usually, scaffold stiffness is regulated by altering polymer density (Jee et al., 2019). The fibrin at 3–4.5 mg/ml has a storage modulus of 80–140 Pa, which is the optimal mechanical strength for intestinal organoid culture (Broguiere et al., 2018). Third, laminin-111, one of the main components of Matrigel, can independently provide biological signals for organoid growth. Supplementation of laminin-111 in fibrin-based hydrogels can achieve analogous colony formation efficiency to that of the Matrigel in organoid culture. Although other hydrogels like alginate hydrogels can support the survival of intestinal organoids (Capeling et al., 2019), their abilities to generate well-differentiated organoids are poorer possibly due to the lack of coupled RGD and additive laminin-111.

2.2 | Synthetic scaffolds

As a representative synthetic hydrogel, the polyethylene glycol (PEG)-based scaffolds can also be used in the intestinal organoid culture. PEG is a type of synthetic polymer that is nontoxic to cells and minimally irritating to tissues (Zhu, 2010). Similar to natural hydrogels, Gjorevski and Lutolf (2017) modified PEG using RGD and laminin-111 for ISC expansion and organoid formation (Figure 1a). At the stage of ISC expansion, a specific PEG reagent with eight thiol reactive vinylsulfone (VS) groups, namely eight-arm PEG-VS, was applied. The VS groups can selectively react with thiol groups for site specific PEGylation of peptides (e.g., Gln and Lys). RGD peptides can conjugate to Gln of PEG-VS-Gln through formation of ε-(α-glutamyl)lysine isopeptide triggered by the activated transglutaminase factor XIII. This mechanism is also responsible for gelation through the crosslinking of PEG-VS-Gln and PEG-VS-Lys. Such PEG-based scaffolds have shear

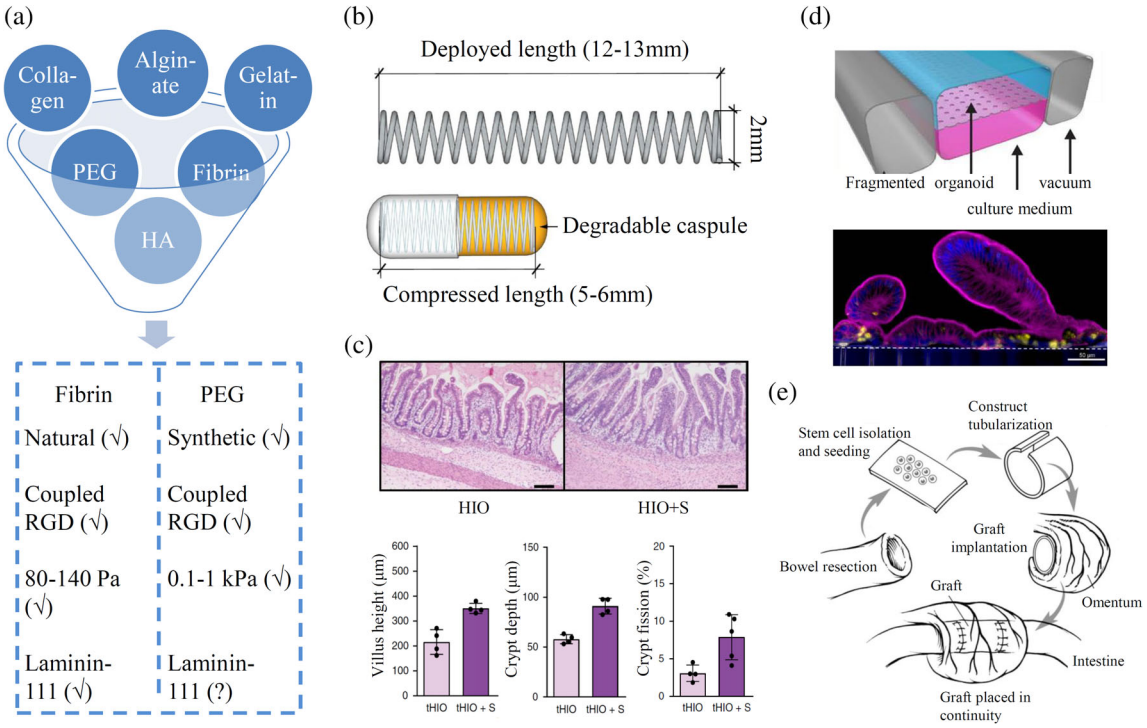

FIGURE 1 Utility of multiple biomaterials in the culture and implantation of intestinal organoids. (a) Among conventional biocompatible polymers, the fibrin and PEG-based bioscaffolds have been identified suitable for intestinal organoid culture under specific conditions including the presence of active biomolecules and proper stiffness. (b, c) The mechanical stimulation by the nitinol spring can promote the morphological maturation of intestinal organoids. Reproduced with the permission from Poling et al. (d) The microfluidic devices enable an easily accessible cell culture system for intestinal villi derived from human organoids. Reproduced with the permission from Kasendra et al. (e) Tubularization of scaffolds seeded with intestinal organoids is more easily anastomosed to native bowel tract because of consistent shape. Reproduced with the permission from Ladd et al. HA, hyaluronic acid; HIO, human intestinal organoid; PEG, polyethylene glycol; S, spring

modulus of 1,000 Pa to support ISC proliferation in the presence of Wnt and Notch agonists. At the stage of organoid formation, laminin-111 was added to a softer PEG-based scaffold (shear modulus ~200 Pa) for ISC differentiation and morphogenesis (Gjorevski et al., 2016). In a separate study, Cruz-Acuna et al. (2017) and Cruz-Acuna et al. (2018) reported another type of PEG-based hydrogels to deliver intestinal organoids in vivo. Their scaffolds were generated from a four-arm PEG bearing maleimide groups at each terminus, which could be conjugated by RGD peptide thiol groups. The gelation of the PEG-based hydrogels also depended on the crosslinking of terminal maleimide groups and thiol groups in GPQ-W (GCRDGPQGIWGQDRCG) peptides. Of note, although lacking laminin-111, this scaffold effectively supported the cell expansion and organoid formation, which implied the laminin-111 was not indispensable. In this case, transplantation of intestinal organoids would be simpler and safer.

2.3 | Shear force of scaffolds

Despite components and stiffness, the shear force of materials also affects the development of intestinal organoids (Shyer et al., 2013; Shyer, Huycke, Lee, Mahadevan, & Tabin, 2015). Recently, Poling et al. (2018) utilized a spring coated by a degradable capsule to simulate intrinsic shear stress (Figure 1b). After spring implantation, morphology of the intestinal organoids resembled human jejunum more closely, characterized by increase of villus height, crypt depth, and smooth muscle thickness (Figure 1c). The transcriptome analysis further revealed the upregulation of ErbB receptors and TGF-β signaling in organoids plus spring group compared with organoids alone. This study provides the basis for the use of strain force generators in intestinal organoid culture.

2.4 | Microfluidic chip

Hydrogel-based 3D intestinal organoid culture can largely restore the in vivo environment for gut epithelia. However, the spheroidal structure of organoids prevents the access of intraluminal-polarized epithelia to the external stimuli, thereby restricting their application in high-throughput tests (Liu & Chen, 2018). An updated study conducted by Kasendra et al. (2018) has emerged as the approach to solve the problem. They dissociated patient biopsy-derived intestinal organoids through enzymatic treatment and seeded these fragments on the porous membrane of a microfluidic chip coated by Matrigel (Figure 1d). This technology has two main advantages including genetic similarity to human intestinal cells and externally accessible

polarized villi. Other than Matrigel, collagen-based hydrogels can also support the growth and polarization of intestinal epithelia. Wang et al. (2017) fabricated a collagen scaffold with micropatterned cylindrical protrusions and depressions. The intestinal epithelia seeded on the scaffold can undergo polarization and recreate crypt-villus architecture. Overall, though combination of organ microchips with specific hydrogels, we can successfully achieve monolayer culture of intestinal organoids and further investigate the impacts of exogenous reagents and microbes on crypt-villus structure (Workman et al., 2018).

2.5 | Tubularization of scaffolds

Unlike other applications, in the field of regenerative medicine, transplantation of the intestinal organoid-derived tissues requires tubular, stiff, and elastic scaffolds that are anatomically relevant to native intestine. Polylactic-glycolic acid (PLGA) is a set of synthetic biodegradable polymers with sufficient mechanical strength and biosafety. Shaffiey et al. (2016) coated tubular PLGA scaffolds with Matrigel as carriers of intestinal organoids, which were then implanted into dog rectum. Through transplantation, intestinal organoids improved rectal mucosal regeneration. However, the elasticity of PLGA was so poor that such scaffolds were not able to be implanted in the folded and coiled small intestine, and implantation could have a high risk of postoperative leakage and obstruction. Zakhem, Tamburrini, Orlando, Koch, and Bitar (2017) used tubular chitosan sheet that is biodegradable and biocompatible, and improves material flexibility. Their bioengineered bowel made of the tubular chitosan sheet was confirmed to have vascularization and normal cell phenotype and function after implantation in the small intestine of rats. Moreover, to obtain scaffolds with crypt-villus architecture, Ladd et al. (2018) created a template array of 500 μm deep and ordered holes on a polymethylmethacrylate template. They poured either poly(ethylene-co-vinyl acetate) or poly(glycerol sebacate) onto the template to cast the crypt-villus structure on the surface of scaffolds. After seeding with ISCs, the scaffolds were tubularized, followed by transplantation into piglets' small intestine for treatment of short bowel syndrome (Figure 1e). Although the results from the above studies were exciting, the finding of an ideal hollow tubular scaffold still has a long way to go since it ultimately should be self-driven in order to adapt to intestinal peristalsis (Bitar, Raghavan, & Zakhem, 2014).

3 | LIVE-IMAGING BIOSENSORS FOR DETECTION OF INTESTINAL ORGANOIDS

Intestinal organoids may experience physiological signal alternation from their own development and aging or responses to external stimuli such as toxins, drugs, and microbial invasion. These signals are usually vital in maintaining gastrointestinal functions including pH regulation, electrolyte balance, and barrier functions. Targeting the specific signal, live-imaging technology can help us learn more about the status of organoids.

3.1 | pH monitoring

As shown in Figure 2, the epithelia in the intestinal organoids can regulate the luminal pH value via important transmembrane channels like DRA, NHE3, and cHKA. Therefore, abnormal fluctuations in luminal pH may imply intestinal epithelial dysfunction. Traditionally, to detect pH value, pH electrodes can be used to capture electric current of hydrogen ions, which are too invasive (Kim, Ginga, & Takayama, 2018). More recently, a set of novel pH indicators, the seminaphtharhodafluor (SNARF) family, has been utilized to monitor luminal and cytosolic pH of intestinal organoids (McCracken et al., 2017;

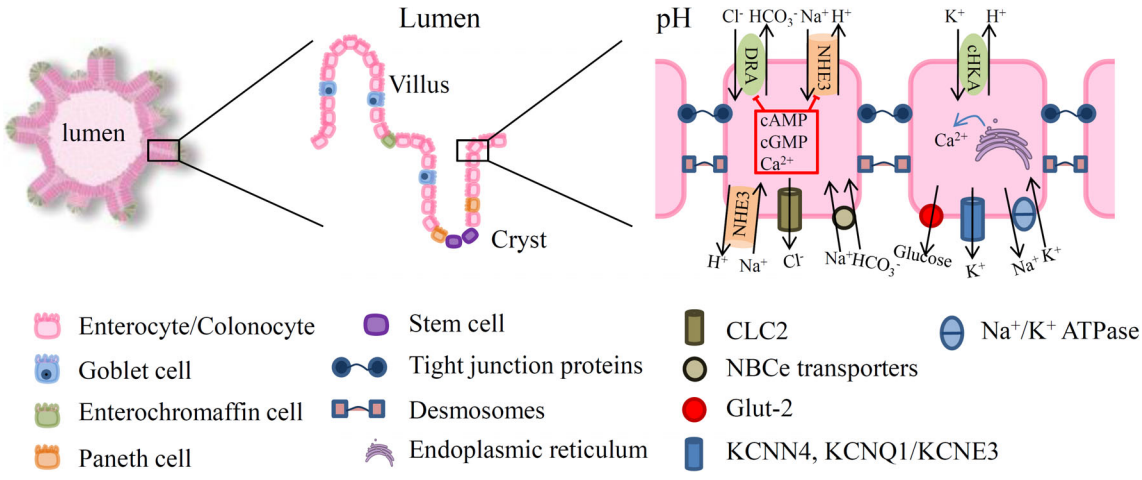

FIGURE 2 Physiology of intestinal organoids in terms of pH, electrolytes, and functional proteins. DRA, Cl^-/HCO_3^- exchanger. NHE3, Na^+/H^+ exchanger. cHKA, colonic H^+/K^+ ATPase. CLC2, chloride channel 2. NBCe transporters, sodium-coupled bicarbonate transporters. Glut-2, glucose transporter-2. KCNN4 and KCNQ1/KCNE3, K^+ channel sorted by conductance, KCNN4 (intermediate conductance), KCNQ1/KCNE3 (small conductance)

Zachos et al., 2016), because it can be ratiometrically measured based on the dual-emission properties (Nakata et al., 2010). In detail, SNARF exists in two switchable forms in which it converts to a phenol form with the λem of 583 nm in an acidic environment or alternatively a phenolate form with the λem of 627 nm in basic environment (Figure 3a). The ratio of the two forms can be used to calculate the pH value. However, the fluorescent intensity-based sensing method is unreliable. This is especially true when making quantitative measurement, because it is easily influenced by optical interaction, optical artifacts, and spatial distribution of fluorescent dyes. Moreover, biological fluorescent proteins can be used to measure the pH with higher biocompatibility to intestinal organoids. O'Donnell et al. (2018) designed a gene sequence that coexpressed a cellulose-binding domain (CBD) and a fluorescent protein (ECFP) with strong pH-sensitivity of fluorescence lifetime in a conjugated form (CBD-ECFP; Figure 3b). Afterward, they linked CBD-ECFP to cellulose-based scaffolds. The entire scaffolds were able to reveal the pH value changes in the lumen of intestinal organoids timely under the fluorescence intensity and lifetime imaging (FLIM) modes. Compared with the intensity-based sensing technology, this FLIM mode enables higher accuracy.

3.2 | Electrolytes monitoring

Intracellular electrolyte balance is important to maintain cell viability and functions. For example, Na^+/K^+ ATPase assists in the transmembrane exchange of Na^+ and K^+ to keep the osmotic pressure within normal ranges; Ca^{2+} released from the endoplasmic reticulum can act as a second messenger to regulate signal transduction (Figure 2). Thus, the biosensors detecting these electrolytes have wide applications. GCaMP2 is one of the most robust Ca^{2+} indicators (Wang, Shui, Kotlikoff, & Sondermann, 2008). It is essentially a fluorescent protein fused by circularly permutated EGFP (cpEGFP), M13, and calmodulin (CaM; Figure 3c). Analysis of the crystal structure reveals the conformational changes of CaM when binding to Ca^{2+}, thus creating a new domain interface between CaM and cpEGFP and blocking solvent

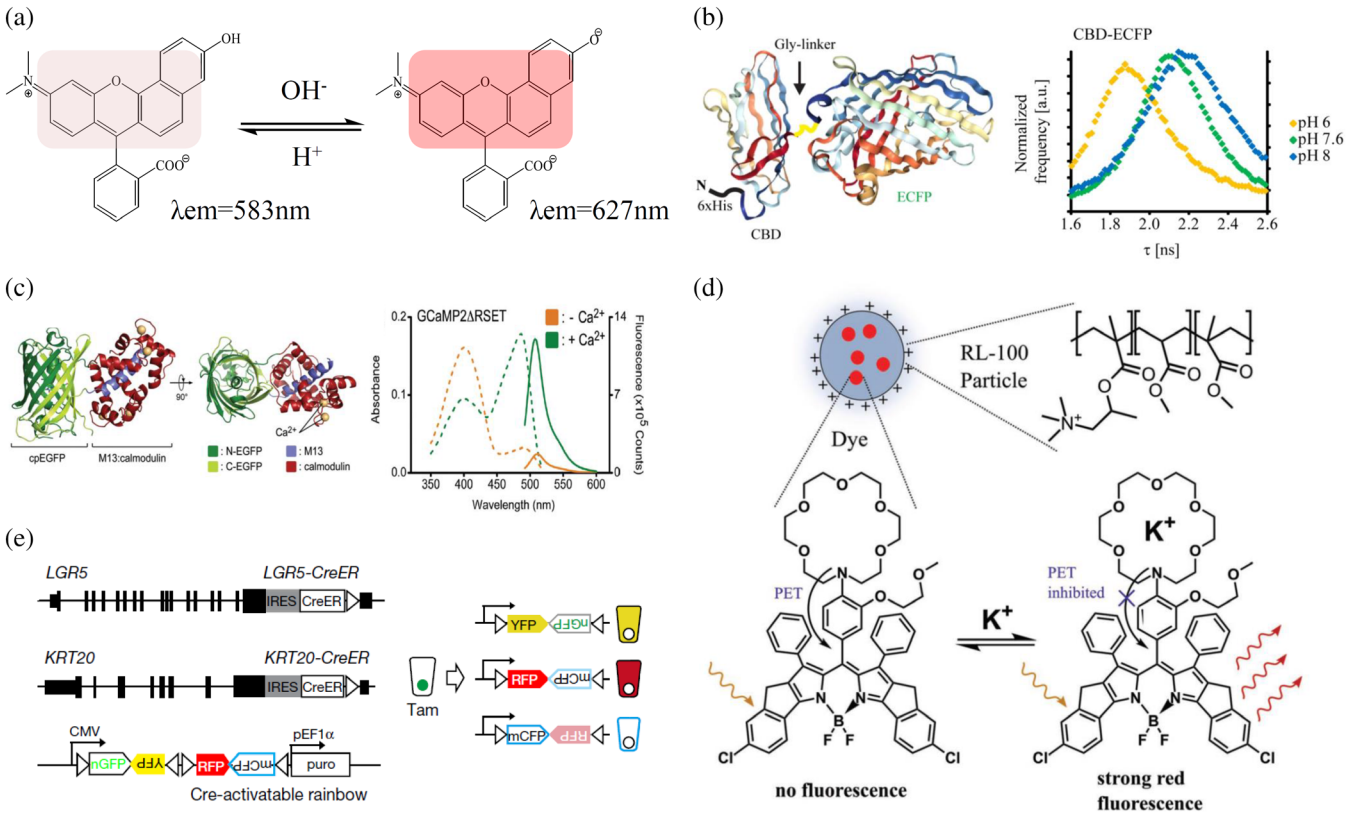

FIGURE 3 Live imaging of intestinal organoids by specific fluorescent dyes and fluorescent gene editing. (a) Phenolic substituent of SNARF regulated by pH accounts for the dual-emission properties. Reproduced with the permission from Nakata et al. (b) CBD-ECFP has disparate fluorescence lifetime distributions when exposed to different pH. Reproduced with the permission from O'Donnell et al. (c) Binding of Ca^{2+} to GCaMP2 can lead to a change of conformation (left) and spectroscopic properties (right). Absorbance, dashed lines. Fluorescence emission, solid lines. Reproduced with the permission from Wang et al. (d) The photostability of K^+-sensitive FI3 dye is improved when encapsulated in RL100 nanoparticles. Reproduced with the permission from Müller et al. (e) The multicolor rainbow reporter gene activated by CreER was fused with LGR5 gene and KRT20 gene using CRISPR-Cas9. upon tamoxifen (Tam) treatment, the $LGR5^+$ or $KRT20^+$ cells can switch their genetic color code to either YFP, RFP, CFP, or GFP at random. LGR5, marker of intestinal stem cells. KRT20, marker of differentiated cells. Reproduced with the permission from Shimokawa et al. CBD, cellulose-binding domain; ECFP, enhanced cyan fluorescent protein; FI3, boron-dipyrromethene (BODIPY)-based K^+-sensitive fluoroionophore; SNARF, seminaphthorhodafluor

access to the chromophore to emit fluorescence (Akerboom et al., 2009). Because fluorescence is highly conformation dependent, additional proteins linked to GCaMP2 may compromise the structure of GCaMP2, which leads to the loss of discriminated fluorescent lifetime when detecting Ca^{2+} in intestinal organoids (O'Donnell et al., 2018). To make the fluorescent dyes more stable, Müller et al. (2018) encapsulated a boron-dipyrromethene-based K^+-sensitive fluoroionophore, FI3 into cationic polymer RL100 nanoparticles to image the real-time fluxes of K^+ in intestinal organoids (Figure 3d). Via a series of experiments, they found that (a) the nano-coated FI3 has a higher efficiency of intracellular transportation than the free one. In addition, (b) the nanocoating helps to reduce interference of the biological environment on the fluorescence lifetime. This study indicates a new direction for nano-based strategy of cell imaging with enhanced photostability of fluorescent dyes.

3.3 | Fluorescence imaging of functional proteins

Multiple intestinal organoid bioactivities were performed by specific functional proteins. For instance, tight junction proteins such as ZO-1 and occludin can maintain the integrity of intestinal barriers (Figure 2), and histone 2B participates in the process of mitosis. To visualize them, these protein markers can be fused with a fluorescent protein. Bolhaqueiro et al. (2018) listed the concrete steps of lentiviral infections to mark histone 2B. After intestinal organoids were infected, they were able to stably express the green fluorescence on histone 2B. Through time-lapse microscopy, it was observed that a fluorescent chromophore was gradually separated before entering into two cells, indicating the completion of cell division. Compared with lentiviral transduction, Fujii, Matano, Nanki, and Sato (2015) introduced a more efficient gene delivery method using electroporation. It directly transferred genes into intestinal organoids through plasmids that were prepared without further producing lentivirus or transgenic rodents. This team successfully delivered a GFP-expressing piggyBac vector into the dissociated single cells from organoids and re-expanded these cells into organoids. This method based on the electroporation enabled 10/3 times higher transduction efficiency than that of electrotransfer, allowing real-time monitoring of organoids through green fluorescence. Moreover, a novel genome editing technology based on CRISPR-Cas9 nucleases has been applied to knockouts or knockins of specific genes in intestinal organoids (Chen & Goncalves, 2018). By specific introduction of fluorescence-expressing genes, targeted functional proteins are labeled with fluorescence. To investigate the exact role of cancer stem cells ($LGR5^+$) in colorectal cancer development, Shimokawa et al. (2017) fused the genes of LGR5-$CreER^+$ (stem cell marker) and KRT20-$CreER^+$ (differentiated cell marker) cell lines with a multicolor rainbow reporter that could be activated by Cre. When the Cre stimulant tamoxifen was administered, the fluorescence switched randomly to either YFP, RFP, CFP, or GFP (Figure 3e). Through fluorescent colocalization of the above cell markers and rainbow reporters, it implemented the lineage tracing of $LGR5^+$ cells and investigated their roles in tumor progression. Collectively, this study demonstrated the flexibility, specificity, and stability of fluorescent protein gene fusions using CRISPR-Cas9 as well as showing its potential roles in identifying the function of cell subsets.

4 | FUTURE DIRECTIONS

Concerning the potentials of intestinal organoids in gut repair, preparations of organoid-laden scaffolds that accord with intestinal tract anatomy are of great basic and clinical importance. To realize this purpose, development of printable and organoid-compatible hydrogels is promising because they can be used to produce personalized scaffolds by 3D-printing technology (Huang et al., 2017; Madden et al., 2018; Xu, Ren, Huang, Li, & Ren, 2019). Moreover, compared with chemical fluorescent compounds, fusion of fluorescent protein genes using CRISPR-Cas9 causes the least influence to intestinal organoids. Designing plasmids to edit diverse functional proteins with fluorescence is significant and useful for basic science research.

5 | CONCLUSION

In this review, we summarized the recent applications of hydrogel scaffolds for intestinal organoid culture. Several key modifications to the scaffolds may help to gain appropriateness including (a) proper RGD conjugations; (b) suitable stiffness; and (c) supplement of laminin-111. These three conditions ought to be considered when designing and optimizing new scaffolds. Furthermore, we comprehensively listed the methods of fluorescent dyes and gene manipulations that could be used in live imaging of intestinal organoids. Through these methods, we detected the pH, electrolytes, and functional proteins in real time, allowing us to inspect the intestinal organoids in a consecutive manner.

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DECLARATIONS OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Jinjian Huang performed the literature search and wrote the manuscript; Jinjian Huang and Yungang Jiang drew the schematic diagrams and obtained the copyright permission of the figures; Yanhan Ren edited and commented this manuscript. Jinjian Huang, Xiuwen Wu, Zongan Li, and Jianan Ren conceived this review. Jinjian Huang and

Ye Liu revised this manuscript and got the reproduction permissions from the publishers.

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