The Translational Application of Hydrogel for Organoid Technology: Challenges and Future Perspectives

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Abstract

Human organoids mimic the physiology and tissue architecture of organs and is of great significance for promoting the study of human diseases. Traditionally, organoid cultures rely predominantly on animal or tumor-derived extracellular matrix (ECM), resulting in poor reproducibility. This limits their utility in for large-scale drug screening and application for regenerative medicine. Recently, synthetic polymeric hydrogels, with high biocompatibility and biodegradability, stability, uniformity of compositions, and high throughput properties, have emerged as potential materials for achieving three-dimensional (3D) architectures for organoid cultures. Compared to conventional animal or tumour-derived organoids, these newly engineered hydrogel-based organoids more closely resemble human organs, as they are able to mimic native structural and functional properties observed in-situ. In this review, we will summarise recent developments in hydrogel-based organoid culture, highlight emergent hydrogel technology and discuss future challenges in applying them to organoid culture.

1. Introduction

Two-dimensional (2D) cell culture has been commonly used in biomedical research due to the convenience it offers as an in-vitro model and its associated cost effectiveness. However, they poorly imitate the *in vivo* three-dimensional (3D) environment, thereby significantly limit their applicability. On the other hand, animal models have been widely used to investigate physiological processes for translational medicine because of these "avatar" correlation between animal and human.[1] However, intrinsic limitations of animal models include higher costs involved in animal husbandry, longer time-line to develop genetic disease models, and poor genotype-phenotype correlation to human diseases. [2, 3] Organoids overcome these limitations as they utilize human instead of animal cells, and at the same time are able to mimic in-vivo 3D environment more closely than 2D in-vitro cultures. This has driven stem cell researchers to further investigate organ-level biology in organoid models with the aim to closely mimic physiological of human in a cost-affordable manner.[4, 5]

Organoids are able to recapitulate the organ developmental processes to achieve 3D miniature versions of organs.[6-9] Organoid is an intermediate model encompassing the advantages of both in-vivo animal models and 2D cell culture, and yet offers a sophisticated model to study various clinically relevant scientific questions.[5, 10-12] Clevers *et al.* presented that organoid technology have already opened up a novel pathway between organ development and human pathologies.[13] In this case, organoids provide new possibilities for more realistic simulation of biological processes related to human physiology, and can be widely used in many fields, including "biobanking" of patient derived organoids, disease modeling, drug testing, host-microbe interactions, transcriptome analysis, or targeted gene editing.[14-20]

A myriad of culture systems been used for producing organoids. These includes magnetic suspension culture system, crypt isolation method, air-liquid interface method, embryoid model, bioreactor, or 2D monolayer culture method.[5, 21] The success of organoid culture rely on various physical and chemical characteristics of the culture microenvironment, which in turn affects spatial-temporal specification, biochemical

signaling and tissue architecture.[5, 22] Amongst them, extracellular matrix or ECM, which is made of functional and structural molecules including proteins, glycosaminoglycans, and glycoconjugates, plays a defining role by forming complex networks to support cells in all tissues or organs.[12, 13, 23-28] Therefore, the type of ECM may be a crucial consideration for directing human organoid cultures.

Decellularized tissues (DT)-derived ECM hydrogels have tissue-specific biochemical characteristics and potential to provide an environment for cell growth directly, which is important for translational medicine.[29] Matrigel is the most widely used animal tissue-derived ECM, and is derived from Engelbreth-Holm-Swarm (EHS) murine sarcoma basement membrane. It has been widely regarded as the "gold standard" scaffold for cell growth *in vitro* and first generation ECM for organoids culture systems.[30] Matrigel has been used in myriad of cell culture applications for more than four decades. It consists of four basement membrane ECM proteins including collagen IV, entactin (usually acts as a crosslinker), laminin, or heparin sulfate proteoglycan perlecan, and has been successfully applied to the 3D culture of many cell types (*i.e.*, cardiomyocytes, human pluripotent stem cells, or neurons).[21] However, due to various undefined components of matrigel, most of the organoids may have the tendency to undergo irregular development processes to form tissues of uncontrollable sizes, shapes, or cell types resulting in batch to batch variability.[31-33] This poor reproducibility has in turn impacted the translatability of organoid research.

With the rise of tissue engineering, natural and synthetic polymer-based hydrogels have become popular components of organoid scaffold due to their inherent advantages. Key features includes biocompatibility, biodegradability and the ability to mimic native ECM due to their highly tunable physical and chemical properties.[34] Current reported hydrogels with potential for organoid application, include naturally-derived alginate, chitosan, hyaluronic acids and collagen, as well as some synthetic polymer-based hydrogel (e.g., polyethylene glycol, poly acrylamide and polyvinyl alcohol). They have been experimentally proven useful for translational medicine including wound repair or cell transplantation. This review aims to summarize recent developments in the use

of de-cellularized tissue derived, natural and synthetic polymer-based hydrogels for organoid culture, with a focus on polymeric characteristics and material compositions (**Figure 1**).[4, 27, 35-40] Lastly, future perspectives and challenges of organoid hydrogel for translational research will be discussed.

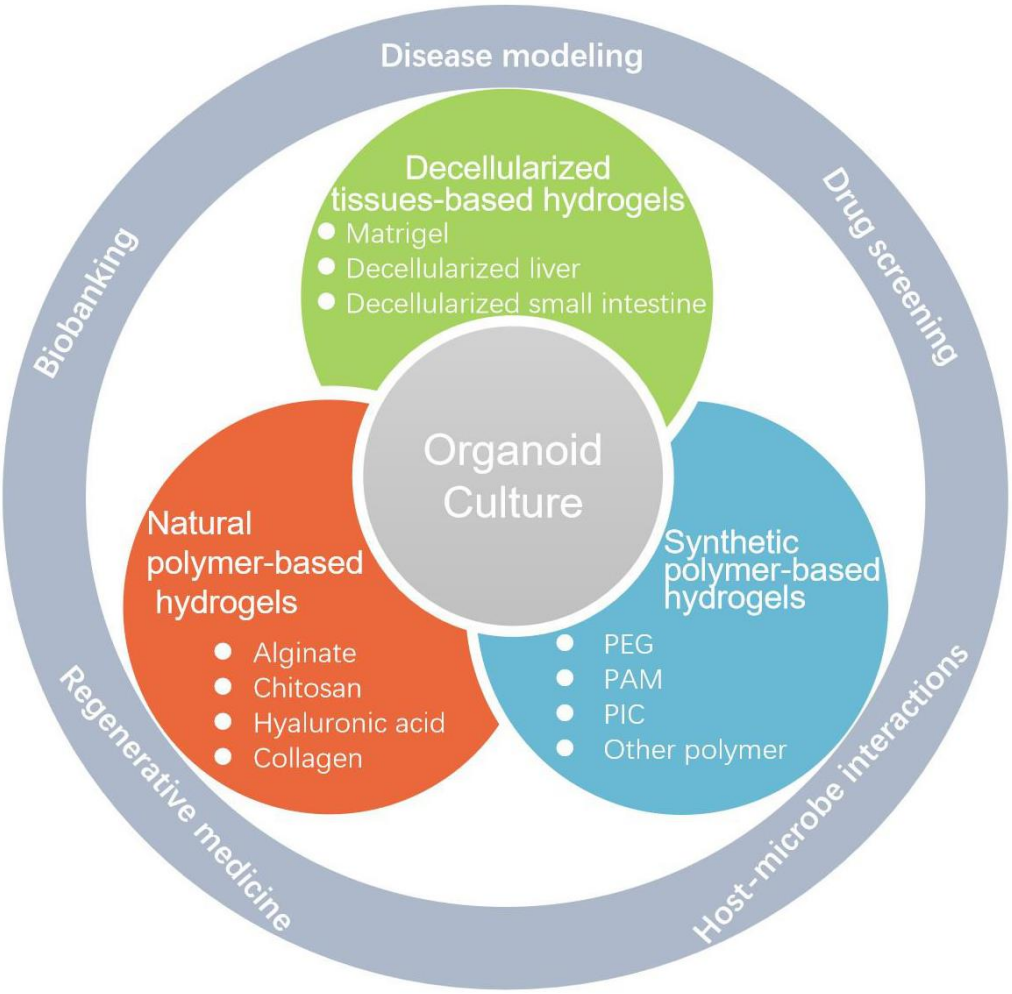

Figure 1. Schematic diagram showing the different types of hydrogel used in organoid culture and related biomedical applications.

2. Decellularized tissues-based hydrogels in organoid culture

Recent reports indicate that the ECM hydrogels derived from de-cellularized tissue (DT) facilitates proper cell growth , reproducibility, and differentiation (**Figure 2**).[29, 41] Compared to synthetic hydrogels, ECM hydrogels provide more physiologically relevant chemical composition, which will in turn help to support biologically relevant functions (*i.e.*, enhancing cell attachment or regulating cellular proliferation).[42]

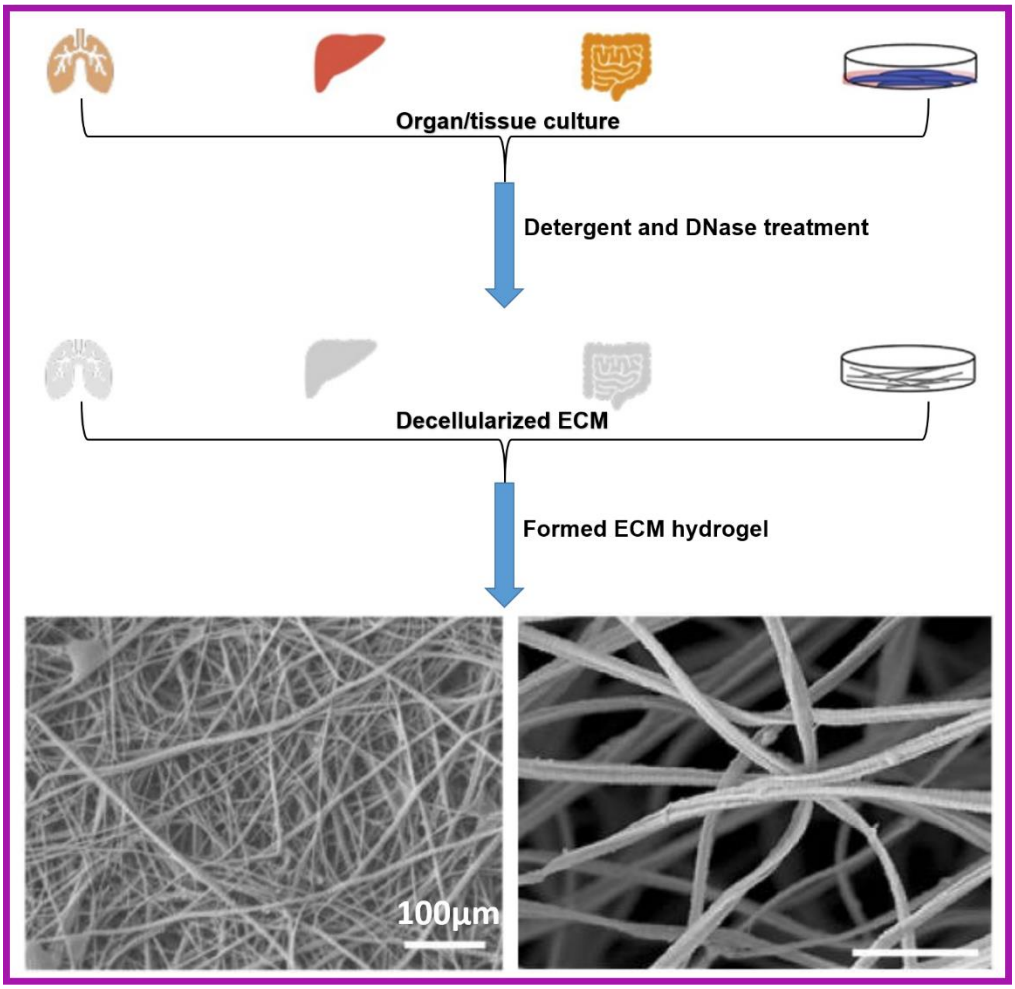

Figure 2. The process of generating decellularized extracellular matrix (ECM) hydrogels and imaging of surface topography using scanning electron microscopy (SEM).[29] Copyright 2019, Nature communication.

Amongst the ECM hydrogels, Matrigel, a gelatinuous protein containing substance secreted from Engelbreth-Holm-Swarm (EHS) murine sarcoma basement membrane, has played a vital role in development of organoid culture. [21] Chen *et al.* utilized human induced pluripotent stem cells (hiPSCs) for lung bud organs (LBOs) generation after xenotransplantation and Matrigel 3D culture. This represents an effective pathway for lung development and lung disease research.[43] In another report, Matrigel was used successfully as a scaffold for neural stem cells (NSCs) survival and differentiation in a spinal cord injury model.[44] Furthermore, Matrigel has also been successfully used to generate gastruloids with somites produced in the correct rostral-caudal patterning *in vitro*.[45] However, Matrigel's biomedical application is restricted by its complex, undefined, highly variable constituents and undesirable xenogenic by-

products. Difficulty in ensuring precise control over Matrigel's composition and its inherent spatial heterogeneity renders it difficult to ensure reliable and reproducible tissue culture .[21, 29, 46]

In recent years, de-cellularized ECM hydrogels from different animal sources have been used as an alternative to Matrigel in tissue engineering or regenerative medicine. For instance, a de-cellularized ECM from porcine liver tissue by detergent and enzyme treatments was successfully incorporated with induced hepatic cells (iHep) to produce artificial liver organoids that can mimic liver functions in-vitro by Jin *et al.*[47] (**Figure 3**). De-cellularized porcine testicular tissue (DTT) has also been used by Vermeulen *et.al* to generate testicular organoids (TOs) and has been shown to be superior to collagen hydrogel in terms of organization function or cell numbers due to preserved growth factors.[48] Furthermore, proteomic analysis conducted by Giobbe *et al.* showed that de-cellularized ECM-based hydrogels from small intestine (SI) contained the main components of porcine SI tissue and had similar physical behavior to commercial hydrogels.[29] More interestingly, this SI ECM gel is capable of facilitating organoids formation from endoderm (*i.e.*, hepatic, pancreatic, or gastric sources), thus indicates its potential to direct *in vitro* or *in vivo* human organoids growth and differentiation.[4, 49, 50] Lastly, Xu *et al.* compared two kinds of de-cellularized matrix hydrogels, one from the spinal cord and the other from peripheral brain tissue.[51] They showed that only de-cellularized matrix hydrogel from spinal cord could enhance the vitality, reproduction, and migration of neural stem and progenitor cells (NSPCs) in early 3D culture, indicating the importance of de-cellularized ECM hydrogel designs.

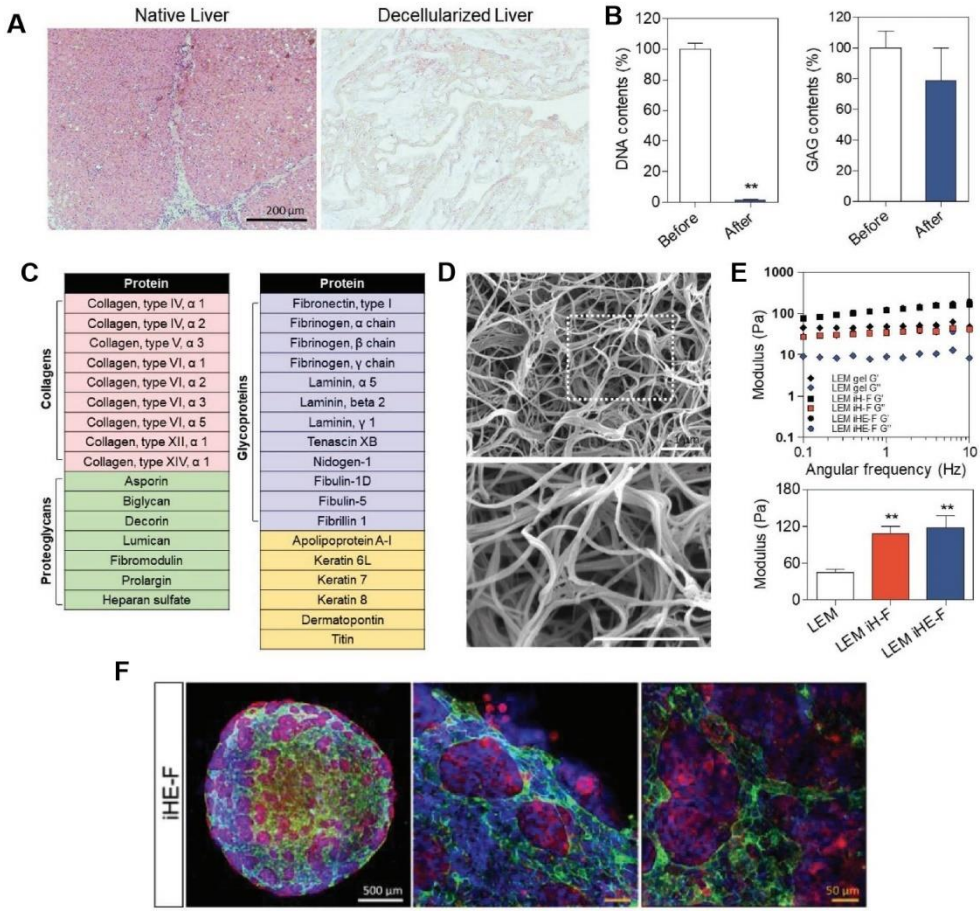

Figure 3. A de-cellularized ECM-based hydrogel from liver tissue. (A) Comparison of native tissue and de-cellularized tissue by hematoxylin & eosin (HE) staining. (B) Biochemical analysis of different tissues in terms of deoxyribonucleic acid (DNA) or glycosaminoglycans (GAG) contents. (C) Proteomic evaluations of different tissues. (D) Scanning electron microscopy analysis of ECM hydrogel. (E) Physical property analysis of ECM hydrogel. (F) Immunofluorescence analysis of ECM hydrogel-based organoids with co-culture of iHep and epithelial cells.[47] Copyright 2019, Advanced Functional Materials.

3. Natural polymer-based hydrogels in organoid culture

Natural polymer-based hydrogels are composed of naturally-derived components and include protein-based (*i.e.*, gelatin, fibronectin, or collagen) or polysaccharide-based (*i.e.*, chitosan, alginate, or hyaluronic acid) materials.[4, 34, 52] As a Good manufacturing practice (GMP)-standard system for organoid expansion is critical for clinical applications, single component polymer-based hydrogel would be ideal for promoting 3D organoids culture. Previous endeavors have showed that organoids can also be cultured in single-component and biopolymer-based hydrogels (*i.e.*, alginate) to

generate intricate structures (**Figure 4**).[4, 53, 54] These natural hydrogels are readily abundant, demonstrate good biocompatibility and can be tuned to respond to external stimulus such as temperature, pH and ions etc. However, their limitations include; low stability, poor mechanical properties and rapid degradation.[5, 55-57]

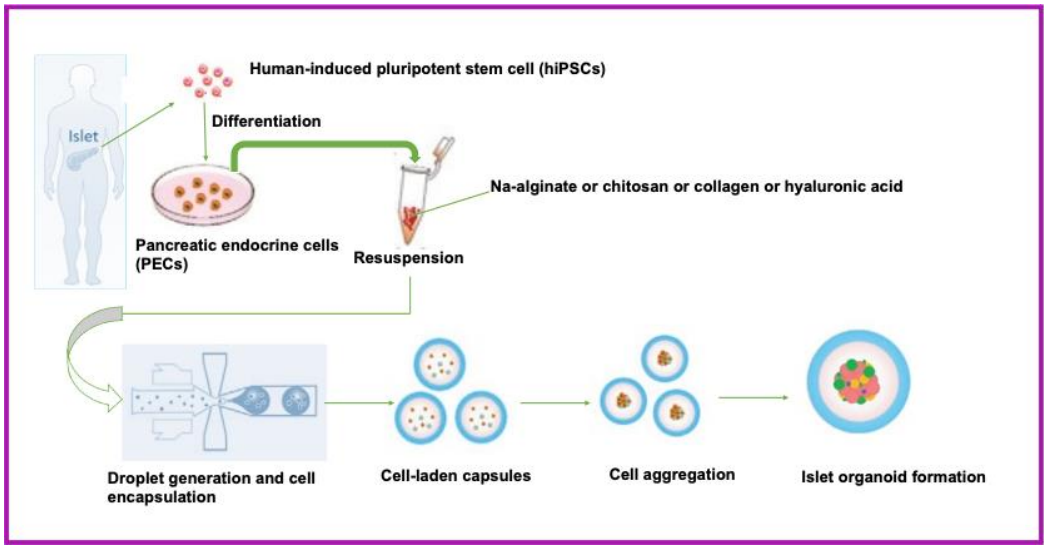

Figure 4. Natural polymer-based hydrogels (such as Na-alginate, chitosan, collagen and hyaluronic acid) in the production of islet organoids. Reproduced with permission.[58] Copyright 2020, Advanced Science.

3.1 Alginate

Alginate is a natural polymer derived from algae. The gelation process of alginate occurs spontaneously when alginate interacts with divalent ions such as barium, magnesium and calcium ions (**Figure 5**).[34, 59, 60] Due to its biodegradability, biocompatibility, and simple gelation process, alginate has been widely used in regenerative medicine, drug screening, and other biomedical fields.[61] For instance, Capeling *et al.* demonstrated that unmodified alginate can support *in vitro* Human intestinal organoids (HIOs) growth, comparable to Matrigel-grown HIOs.[59] By comparing the cell surface marker changes of hESC-derived or hiPSC-derived embryoid bodies in different medium culture (*i.e.*, hyaluronic acid (HA), HA/gelatin hydrogels, 0.5% and 1% peptide arginine-glycine-aspartate (RGD)-alginate), Hunt *et al.* found that RGD-alginate hydrogel was more suitable for transporting the retinal cells into the compromised retina than HA-based hydrogel.[62]

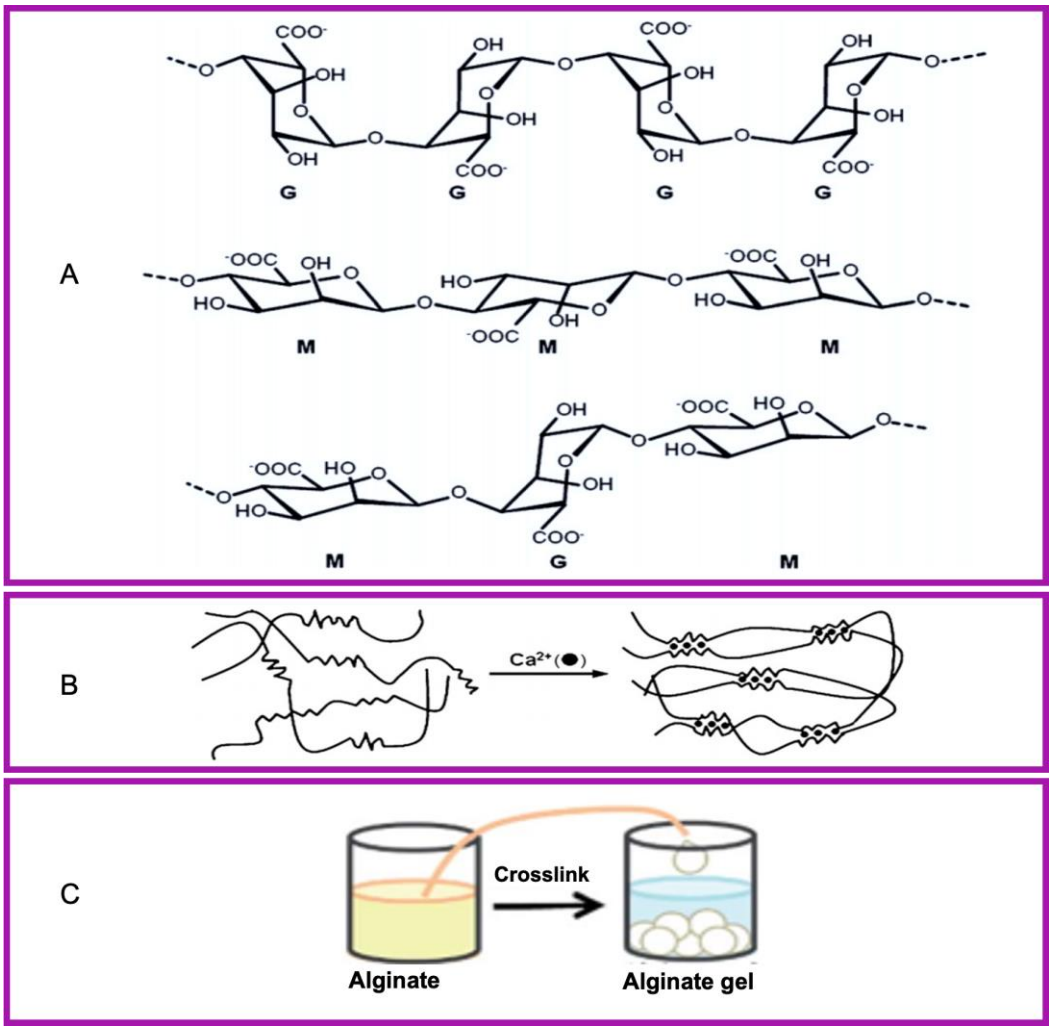

Figure 5. Based on basic chemical structures of alginate (A) and alginate hydrogels prepared by ionic cross-linking (B). Reproduced with permission.[60] Copyright 2012, Progress in Polymer Science. Egg-box model (C). Reproduced with permission.[63] Copyright 2016, Journal of Materials Chemistry.

3.2 Chitosan

The structure of chitosan is similar to glycosaminoglycan which is a key component of ECM is required for intercellular adhesion.[64, 65] Although chitosan is not widely found in nature, it is easily obtained from another biopolymer - chitin, via an alkaline N-deacetylation reaction on chitin that is ubiquitously present in the cell walls of fungi and the shells of marine crustaceans.[66] For chitosan's biomedical applications, environmental pH value is a very important factor. Chitosan's solubility and gelation can be adjusted by pH changes.[34, 67, 68] In addition, chitosan has additional unique characteristics such as natural antibacterial properties, biocompatibility, bio-

absorbability with controllable biodegradation, as well as non-toxic degradation by-products. (**Figure 6**).[65] As a typical example, Patel *et al.* successfully prepared a bioengineered complete ventricle by using a bioengineered trilobular valve (BETV) mold and a chitosan scaffold to simulate the geometric shape of human newborn aortic valve.[69]

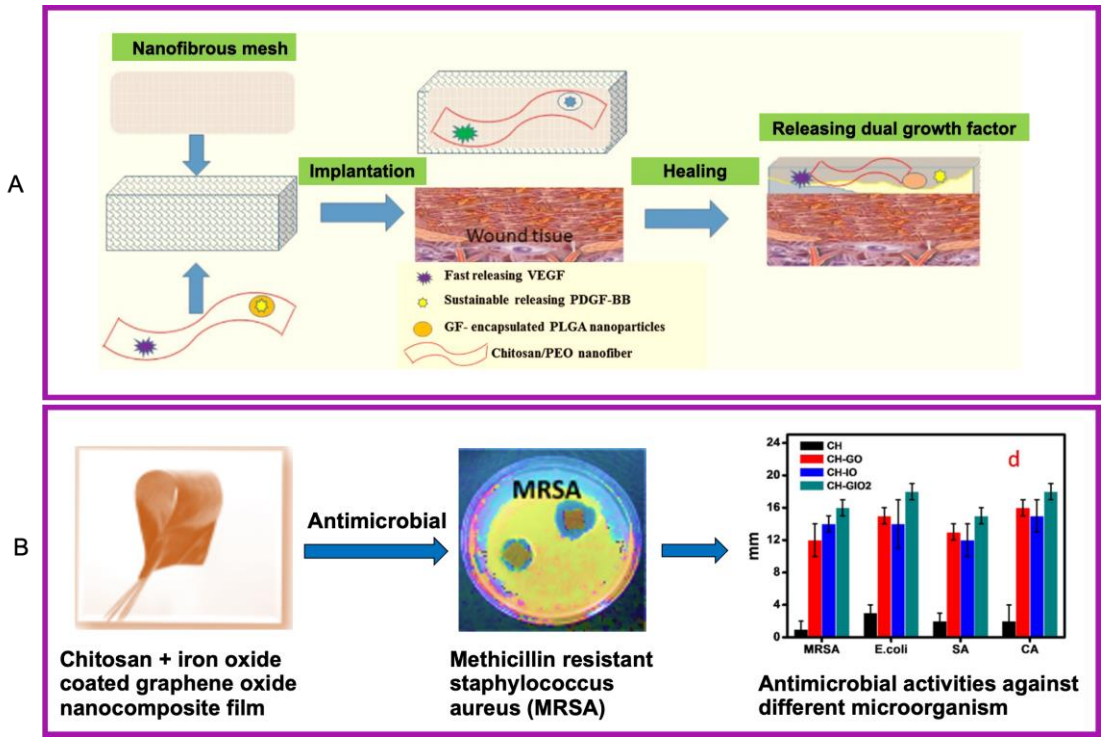

Figure 6. Chitosan and polyethylene oxide formed nanofibers enhancing wound healing by release a dual growth factor (A). Chitosan based composite films with antimicrobial activity (B). Reproduced with permission.[70] Copyright 2016, ACS Applied Materials & Interfaces.

3.3 Hyaluronic acid

Hyaluronic acid (HA), a negatively charged unbranched polysaccharide synthesized in the body,[39, 52, 71-73] modulates angiogenesis, cell signaling, matrix organizations and morphogenesis. HA has wide-spread applications as it is biocompatible and non-immunogenic, with biofunctionality . In addition, it can be easily functionalized due to the presence of numerous reactive groups, However, its weak mechanical properties might prove to be an obstacle for its future use (**Figure 7**).[34, 74-76] For example, Wu *et al.* showed that the methacrylated hyaluronic acid (Me-

HA) hydrogel can be used to effectively promote the differentiation of human induced pluripotent stem cell-derived neural progenitor cells (hiPSC-NPCs) *in vitro*, and they demonstrated the relationship between hydrogel stiffness and morphological characteristics of hiPSC-NPCs.[39]

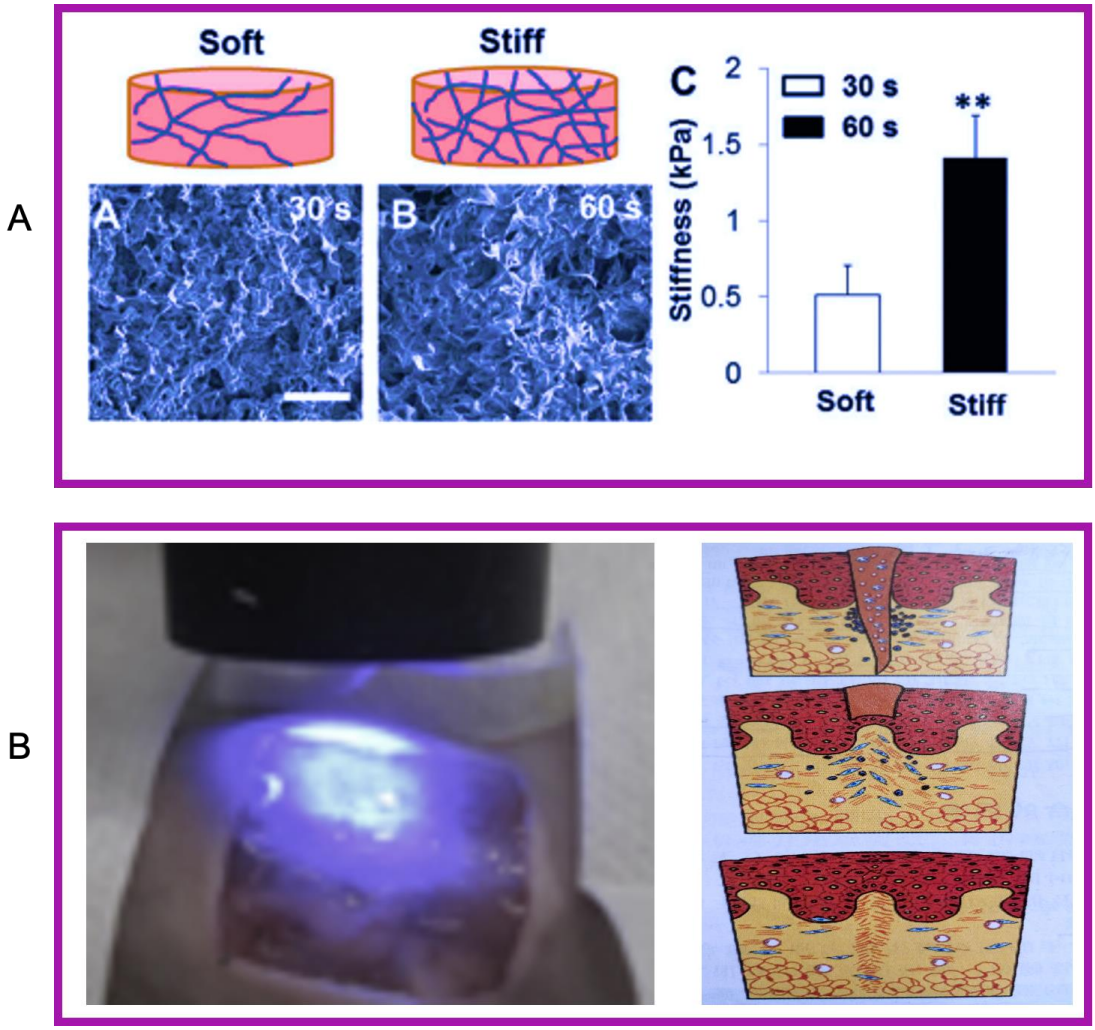

Figure 7. Different crosslinking time of HA-based hydrogels have different physical properties, such as soft and stiff (A). Reproduced with permission.[39] Copyright 2017, Journal of Materials Chemistry B. HA hydrogel used for wound healing *via* photocrosslinking process *in situ* (B).

3.4 Collagen

Collagen is a crucial component of ECM. It has a triple helical structure and is heavily reliant on firm hydrogen bonds.[34, 77] Collagen has significant advantages over other natural polymers, as it has low immunogenicity, is biodegradable and

biocompatible. In addition, it possesses unique self-assembling fibril-forming properties. Yeung *et al.* showed that human osteoarthritis chondrocytes could re-realize their phenotypes *in vivo* in 3D collagen microcapsules, confirming the potential of collagen microcapsules in translational medicine.[78] Nevertheless, its poor mechanical strength, thermal stability, and enzyme tolerance might limit the clinical potentials of natural collagen in biomedical field. [79-81]

4. Synthetic polymer-based hydrogels in organoid technology

Hydrogels play a vital role for the development of 3D cell culture models as they can produce tissue-like matrices that are structurally akin to native ECM.[75, 76, 82, 83] In recent years, many studies have focused on developing bio-inspired synthetic materials to replace tissue derived ECM matrices, to enable consistent, reproducible and clinically relevant organoid culture protocols.[76, 84-86] Herein, the recent novel 3D culture matrices generated using synthetic hydrogels will be summarized.

4.1 Poly(ethylene glycol) (PEG)-based hydrogels

In recent years, many studies have used modified poly(ethylene glycol) (PEG) instead of Matrigel, because it is inexpensive and commercially available. More importantly, modified PEG ligands could be engineered to satisfy the needs of different matrix designs. In addition, PEG can also be easily functionalized to bind signaling molecules or biological ligands, or cross-linking points can be incorporated to allow precise adjustment of biochemical or physical properties (**Table 1**).[87-90] Notably, the stiffness of PEG-based hydrogel has been shown to affect the structure and morphology of 3D cultures. [12] For example, an appropriately tailored stiffness for PEG-gelatin based hydrogel was crucial for successful generation of blood vessels and liver organoid tissue (**Figure 8**).[53] Inspired by this work, subsequent groups have successfully cultured different kinds of organoids (e.g. mouse colon, neural tube and human intestinal) using modified PEG hydrogel instead of ECM.[12, 35, 36, 53, 91-96] Compared to other synthetic polymers, PEG hydrogel replacement matrix has obvious

advantages. This includes optical clarity and ease of reproducible consistency during batch manufacturing.

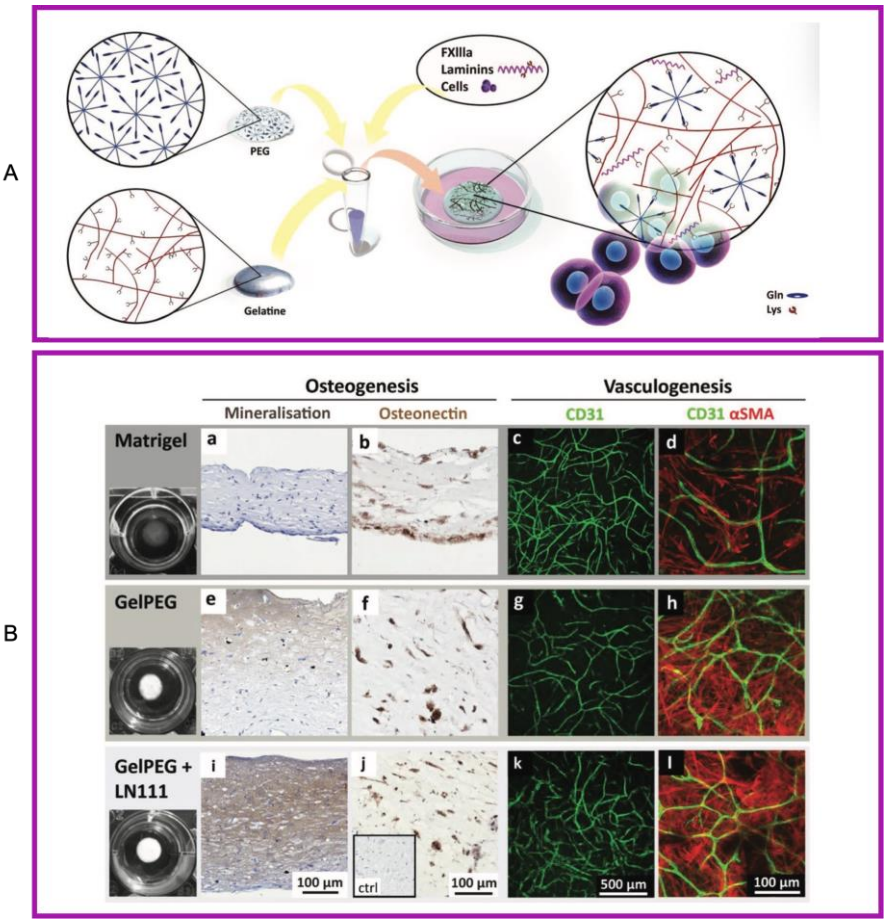

Figure 8. (A) Hydrogel formed by PEG and gelatin *via* enzymatic crosslinking. (B) Comparison of PEG based hydrogel and Matrigel in terms of organoid development. Reproduced with permission.[53] Copyright 2019, Advanced Healthcare Materials.

The key advantage of PEG hydrogels lies in its tunability. This is due to the presence of multiple functional groups, which can be modified to fine-tune the hydrogel's chemistry, biophysical, and biochemical properties. For example, Cruz-Acuña *et al.* developed a multiple-armed PEG core with four maleimide side chain modifications (PEG-4MAL) for organoids development. The maleimide groups on the backbone of the PEG-4MAL hydrogel can react with adhesion ligands and cross-link peptides (*i.e.*, peptide or laminin) to enhance PEG-4MAL's bio-interactions. This improves the adaptability of PEG-4MAL to promote the development of different organoids types.[94] Furthermore, PEG has also been incorporated with natural polymers

(*i.e.*, chitosan) to render it injectable and has been shown to achieve more than 80% intrinsic recovery of nerve development, by ways of dynamic covalent chemistry reaction (**Figure 9**)[34].

More intriguingly, the modified PEG hydrogels have attractive mechanical properties (*i.e.*, stiffness, composition, or modulus), which are important for the development of organoids. For example, Lutolf *et al.* found that stiffness indeed influenced intestinal stem cell (ISC) colony formation. Furthermore, a hybrid hydrogel, composed of a stable poly(ethylene glycol) (sPEG, or mechanically static PEG) as the main chain and degradable or hydrolysable PEG (dPEG, or mechanically dynamic PEG) as the side chain, was created for the purpose of turning the stiffness of this hybrid hydrogel. Although a hard matrix was reported to favorably support the expansion of intestinal stem cells, a compressed and strictly enclosed environment on the other hand, might impair colony growth and delay morphogenesis. The ideal stiffness lies within a narrow mechanical window of about 190Pa (**Figure 10**). Inspired by this work, further studies were performed to explore the link between mechanical properties and organoids growth. Candiello *et al.* described a novel modified PEG hydrogel (named Amikagel), which promoted spontaneous assembly of pancreatic progenitor cells into robust spheroids, with tunable heterogeneous cellular composition or spheroid size.[16] Amikacin was used as one of the monomers to adjust both the gel's physical or chemical properties, by adjusting its molar ratio. This strategy was successful for fine-tuning the gel's protein adsorption ability or surface biochemistry/amine contents, and thereby influence cell-substrate interaction, as well as promote the formation of pancreatic islet organoids. Of interest, only hESC committed to the pancreatic lineage was suitable for reproducible assembly into solid spheroids with Amikagel, and an elastic modulus of around 225 kPa best facilitated the formation of pancreatic islet organoids.

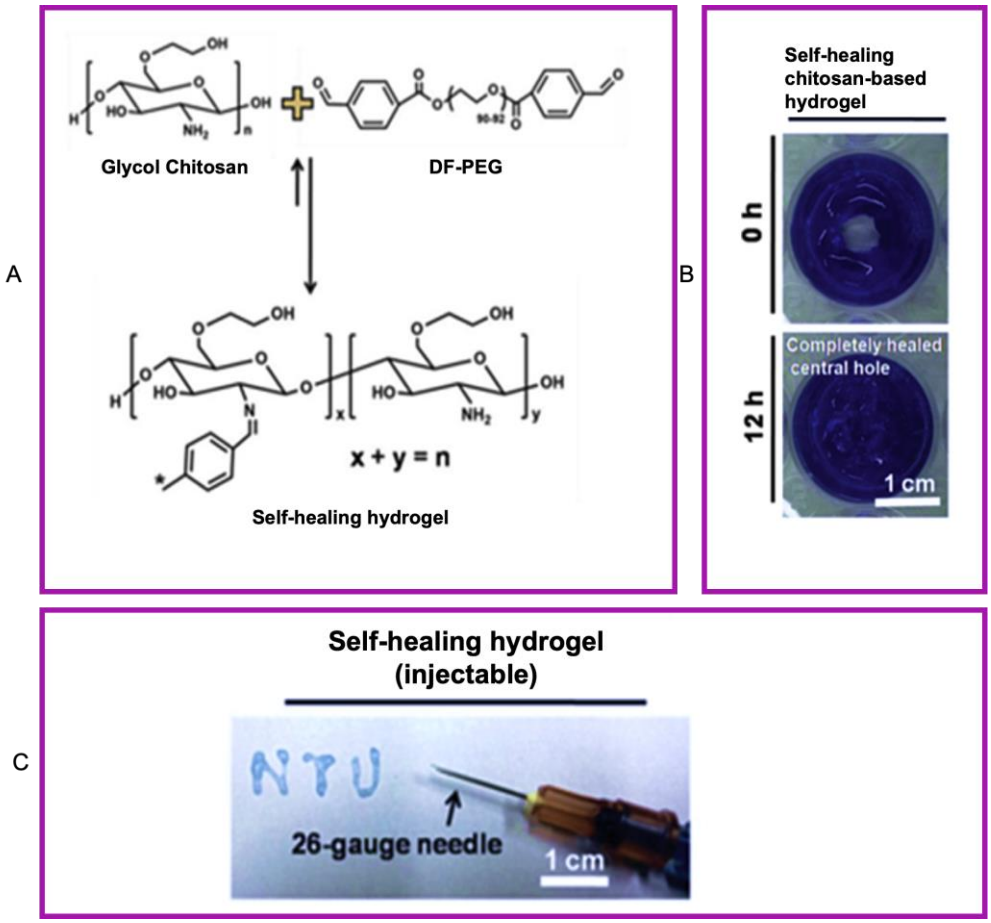

Figure 9. A schematic illustrating the self-healing hydrogel based on chitosan and modified with PEG. Based on basic process of forming self-healing hydrogel (A), chitosan-based hydrogel completely healed by itself after 12 h (B); and it can easily pass through syringe and have injectable property (C). Reproduced with permission.[34] Copyright 2016, Bioactive Materials.

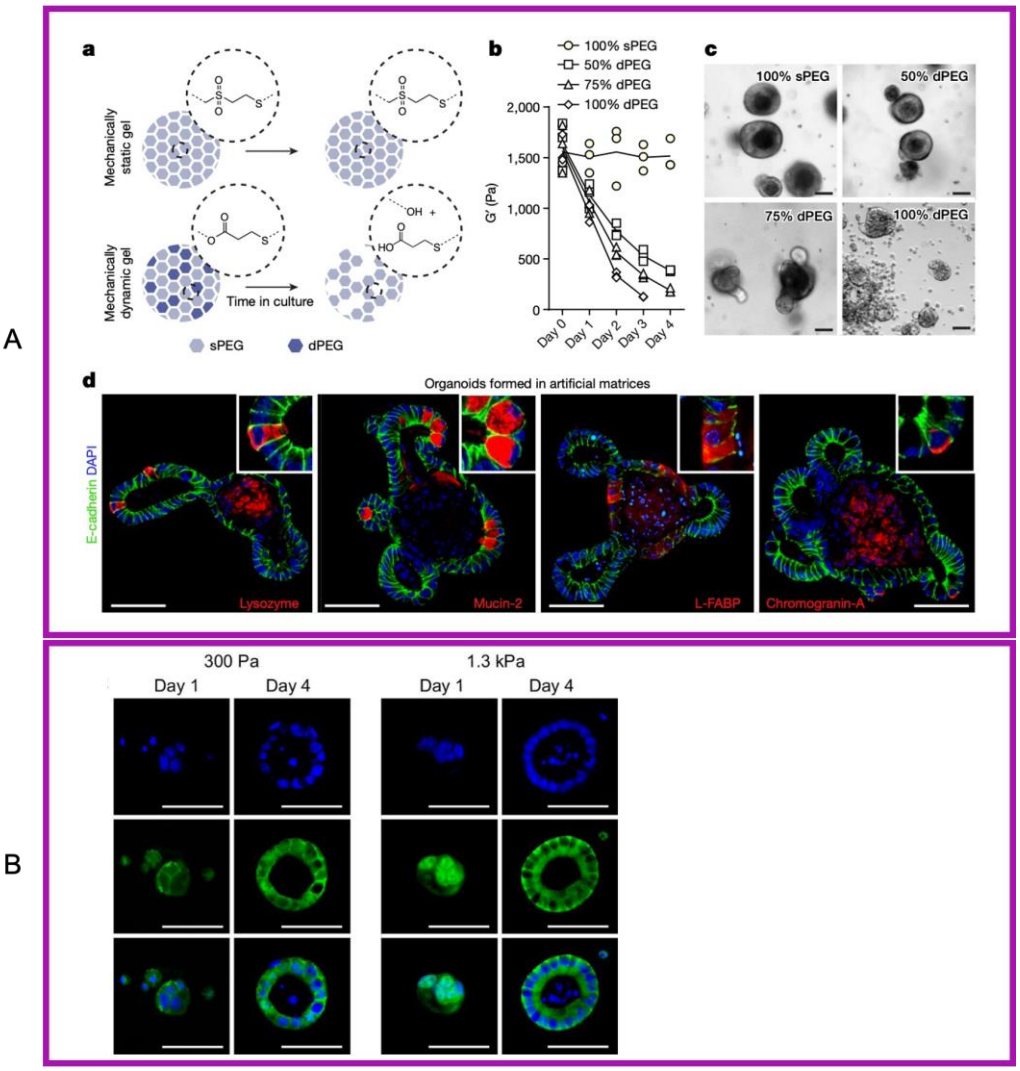

Figure 10. (A) Based on mechanically tunable PEG hydrogel formation (static and dynamic PEG, sPEG, and dPEG) (a), the shear modulus of different PEG hydrogel formulations were controllable in different days (b). The images of different concentrated PEG hydrogel were shown (c); which contributed to the final formation of organoids (d). (B) Localization in colonies formation after 4 d in different stiffness of PEG RGD gels. Reproduced with permission.[12] Copyright 2016, Nature.

The application of modified PEG hydrogels to the cultivation of organoids may be helpful for establishing 3D disease models to interrogate disease pathogenesis but also drug screening. Wilson *et al.* constructed a PEG hydrogel stent as a coating of intestinal villi topography to mimic the human small intestinal epithelium in 2D,[97] and designed a cross-linked collagen hydrogel stent for 3D intestinal crypt-villus architecture.[98] Casey *et al.* develop a modified PEG micro-well array platform to simulate the breast cancer tumor microenvironment. This was further used for anti-cancer drug screening

studies.[99] Tumor behavior is known to be related not only to the interaction between malignant cells and tumor microenvironment, but also to the three-dimensional structure of the tumor. A study by Bufalo *et al.* reported a three-dimensional cancer model based on PEG-fibrin hydrogel, which was able to accurately mimic the tumor microenvironment and allow the study of how the 3D-environment affects tumour behavior.[100] Compared to 2D cultures, tumor cells cultured in PEG-fibrin hydrogel were able to self-assemble into complex structures, mimicked *in vivo* dynamics better, and had stronger tumor proliferation with invasion abilities. Undoubtedly, numerous studies have demonstrated that the modified PEG hydrogel culture model is an effective platform for studying cancer biology and tumor response to biological treatments. This is crucial for cancer drug screening and in turn hasten the drug development process.

4.2 Poly(acrylamide) (PAM)-based hydrogel

Poly(acrylamide) (PAM) or poly (N-isopropylacrylamide) (PIPAAm) based hydrogel have also been used as scaffolds for organoid culture. They are polymers synthesized *via* chemical reaction. PIPAAm, a derivative of PAM, has a critical gelation temperature around 37 °C and is useful in temperature responsive cell culture (*i.e.*, controllable cell detachment upon temperature changes). Generally, PAM hydrogels are bioinert, hydrophilic, and electroneutral, thus they cannot bind directly to cells or react with proteins. However, cell-PAM hydrogel interactions can be modulated by its stiffness or biofunctionality properties. Nonetheless, PAM hydrogel is generally regarded as having poor cell adhesion with minimum cell toxicity. For example, Farrukh *et al.* constructed a methylsulfonyl PAM hydrogel and experimentally proved that this hydrogel has low protein absorption. Its bio-interaction can be tuned by specific covalent coupling with bioligands.[101-106]

4.3 Polyisocyanopeptides (PIC)-based hydrogels

Recently, Ye *et al.* described a new polyisocyanopeptides (PIC) and laminin-based hydrogel, which is able to efficiently support liver organoids differentiation. They

demonstrated ease of handling due to its thermo-sensitive properties (**Figure 11**).[107] As a first step, the structure of PIC hydrogel was optimized to satisfy the need of liver organoids reproduction. Secondly, for a better understanding of the PIC hydrogel's performances, its mechanical properties were evaluated. Softer PIC hydrogel was observed to be more helpful for the growth of liver organoids. The liver organoids were able to efficiently differentiate into functional hepatocyte-like cells, and also experienced long-term expansion. Although PIC hydrogel was a relatively new synthetic hydrogel, these proof-of-concept results showed that it could mimic the mechanical properties and structure of ECM protein (*i.e.*, fibrin or collagen). More interestingly, when the polymer solution is heated to 18 °C, PIC hydrogel could rapidly change from liquid to gel status, which made it attractive for rapid and reversible extraction of cells or organoids from the hydrogel scaffold. Although PIC hydrogels are physically cross-linked, they remain stable for several weeks' in cell culture experiments.[108] Zhang *et al.* further described a well-defined synthetic biomimetic matrix based on PIC hydrogel, used to culture mammary gland organoids from single breast epithelial cells or breast fragments,[109] indicating that PIC platform may serve as an ideal substrate for in-depth study of organoids or disease models.

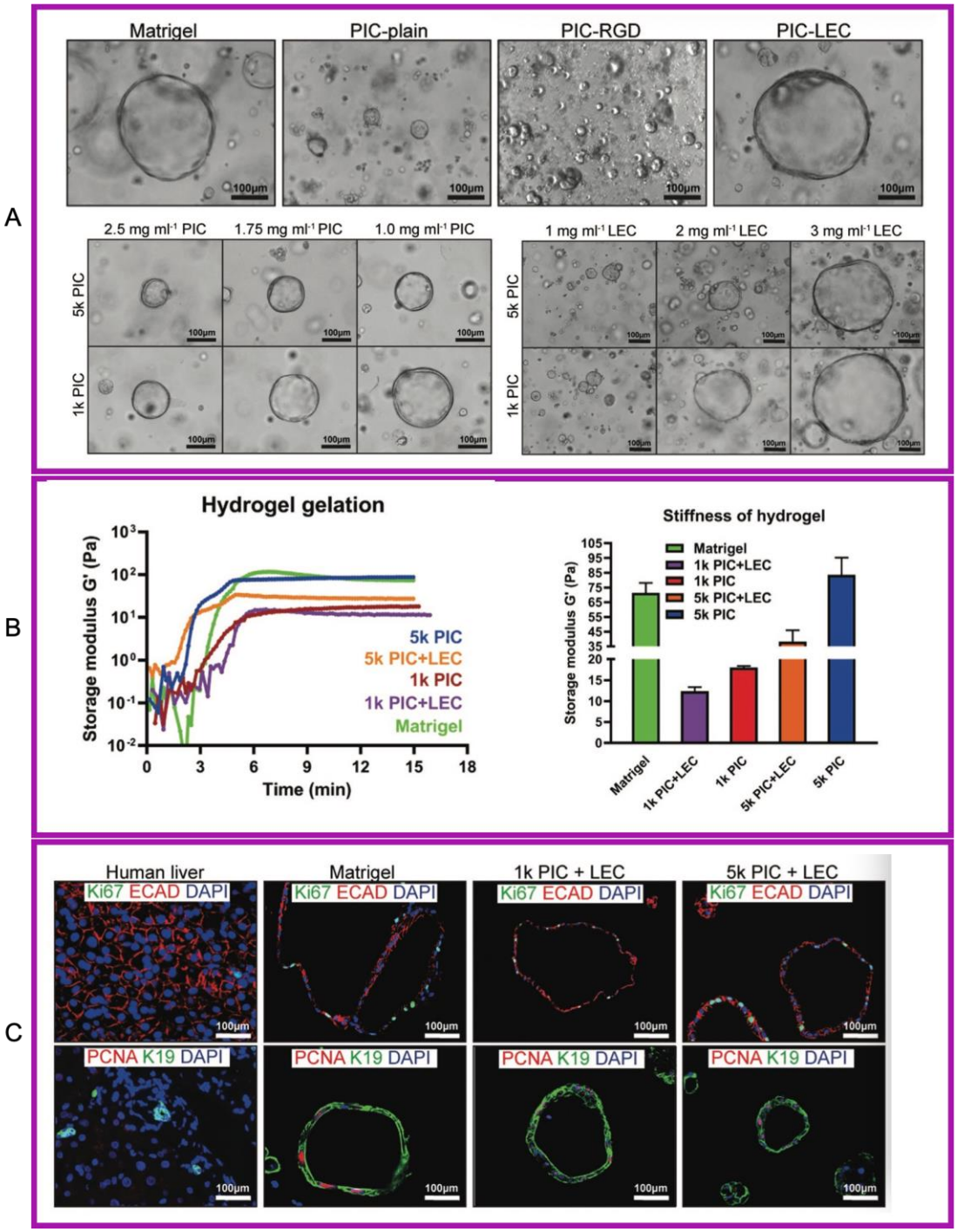

Figure 11. Based on optimized PIC hydrogel, liver organoid expansion (A) as well as its relationship with hydrogel stiffness (B) were observed, which was also evidenced by immunofluorescent marker imaging (C). Reproduced with permission.[107] Copyright 2020, Advanced Functional Materials.

4.4 Other polymer-based hydrogels

The key advantage of synthetic hydrogel materials lies in their ability to demonstrate stimulus responsive behaviors (*i.e.*, pressure sensitivity, magnetic

sensitivity, temperature sensitivity, and photosensitivity). For example, Yavitt *et al.* designed a allyl sulfide based photodegradable hydrogel, which could be degraded within 15 s upon exposure of 365 nm wavelength light in the presence of photoinitiator.[110] Furthermore, Hushka *et al.* also demonstrated that allyl sulfide based hydrogel could tune the crypt-villi structure formation in the small intestine organoids.[111] Besides photosensitive hydrogels, temperature-sensitive hydrogels could also be useful in organoid research. For example, biofunctionalized poly(vinyl alcohol) (PVA) based thermogels demonstrate superior biocompatibility and can be used as a matrix to regulate cellular differentiation.[112-114]

5. Challenge and opportunities of hydrogel-based organoids culture

5.1 The advantages of hydrogel-based organoids in translation medicine

Hydrogel technology has been utilized to construct 3D scaffold for organoid models to achieve high biocompatibility, uniformity of compositions, stability, and high-throughput properties. For example, a number of rationally designed hydrogels (*i.e.*, one-step synthesis composite hydrogel capsules;[2] allyl sulfide and thiol formed photodegradable hydrogel;[110] soft fibrin matric consisting of laminin supplementation and RGD adhesion domains on the scaffold;[115] polylactic acid and adipic acid dihydrazide hydrogel[116]) have been shown to support long-term culture and expansion of organoid. Furthermore, chemically defined hydrogels (*i.e.*, PEG hydrogels) are able to regulate organoid growth, proliferation, or differentiation by modulating the synthetic microenvironment (*i.e.*, the mechanical environments of organoid with tunable stiffness). For example, liver organoids require the activation of yes-associated protein 1 (YAP) and the Src family of kinases (SFKs) for liver organoid development. As the activation of tyrosine kinase Src might affect the cellular behavior of epithelial cells, a lower stiffness hydrogel might be more optimal for organoid proliferation.[86, 107] For example, a synthetic PEG (composing with maleimide-terminated and four-armed macromer) hydrogel, with injectable property for easy cell encapsulation through protease-degradable peptides cross-linking, has been experimentally proven to

support robust and reproducible organoid growth or expansion (**Figure 12**)[35, 94] It is able to significantly accelerate colonic wound repair via hiPSC-derived and hESC-derived spheroids. As another example, a low-cost and defined matrix plant-based nanocellulose hydrogel, composing of 0.1% nanocellulose fibers, was designed and could provide the microenvironment for SI organoid development or budding.[88]

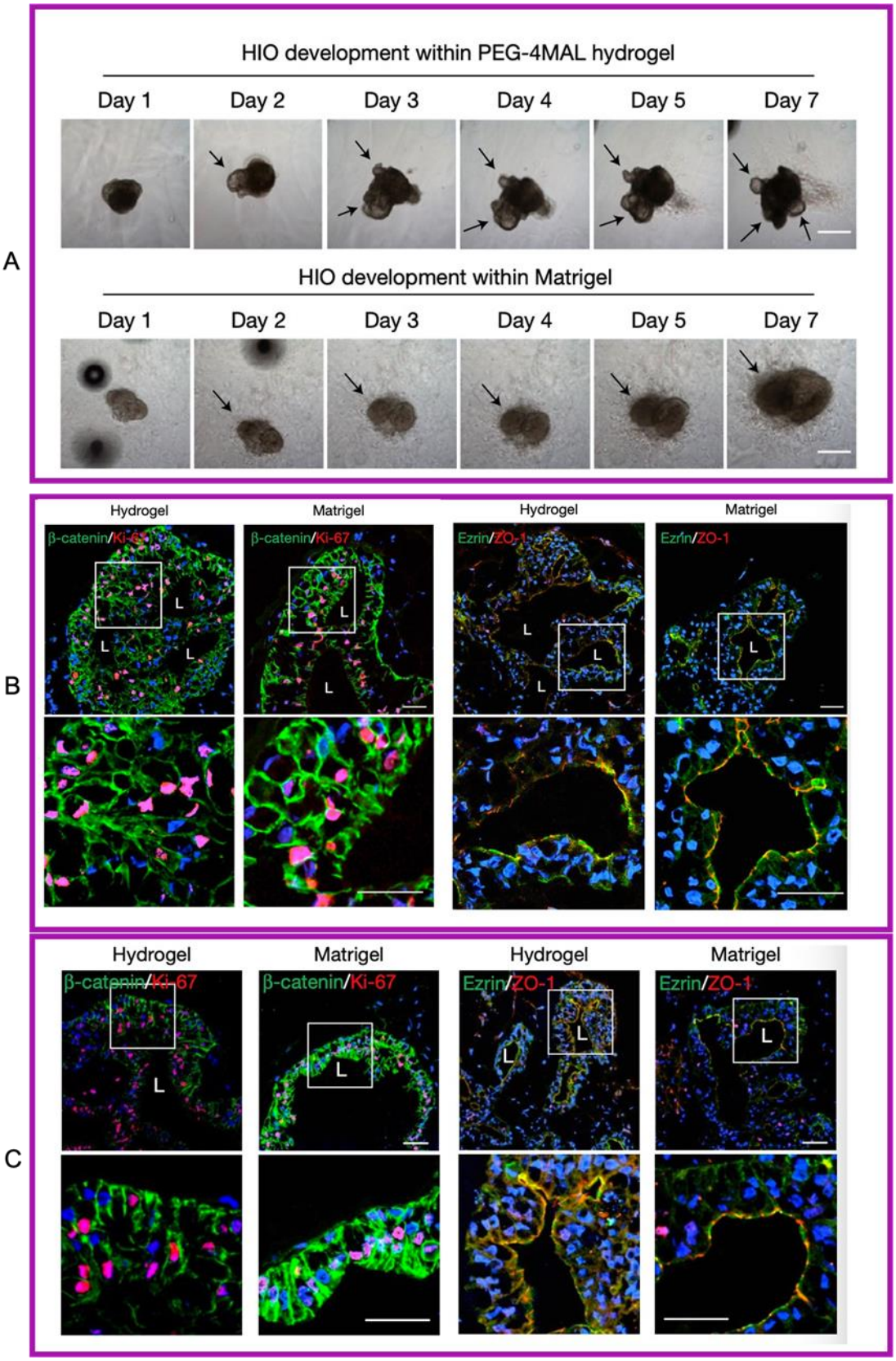

Figure 12. Development of human intestinal organoid (HIO) with PEG-4MAL polymer-based hydrogel in comparison with Matrigel (A) and imaging of fluorescence microscopy were recorded after 7 d (B) or 21 d (C). Reproduced with permission.[35] Copyright 2017, Nature Cell Biology.

To mimic the unique adipose-laden tissue microenvironment of the human breast, researchers grew large mammary organoids in hydrogels. This improved 3D culture systems highlights the importance of signaling components from mammary ECM for sustaining the growth of large organoids structurally.[49] For this purpose, a well-defined, thermosensitive properties or photosensitive biomimetic matrix has been developed.[107, 109, 110] Another example includes the development of a hydrogel-based platform by Candiello *et al.*, to promote spontaneous self-organization and assembly of pancreatic progenitor cells into solid 3D spheroids, with the intention to specificity control the size and heterogeneous cellular compositions.[16]

Last but not least, sacrificial or degradable hydrogels were utilized by Rossen *et al.* to combine cellular clusters with satisfactory reproducibility or scalability,[117] indicating the importance of hydrogel structure designs. Notably, due to the advantage of 3D unique structure, organoid technology might also be useful for immunology research. It allows for an in-depth study of interactions between epithelial and immune cell, and thereby form the basis for developing novel immune therapeutics for cancer.[118, 119]

5.2 The limitations of conventional materials used in organoid culture

A key limitation of conventional materials such as Matrigel is the presence of xenogenic components as they are derived from animals. Animal-derived matrices contain a heterogeneous mixture of ECM proteins, growth factors (GFs), proteoglycans, and other biological proteins (**Figure 13**), and cannot be used for clinical applications.[54, 120, 121] In fact, all commercially available organoid culture matrixes such as Matrigel (Corning), Cultrex BEM (Trevigen), and Geltrex (Gibo), are derived from cancerous murine cells. Similarly, the presence of heterogenous and undefined component might act as antigens and elicit spurious, physiologically irrelevant pleiotropic effects.[122-125]

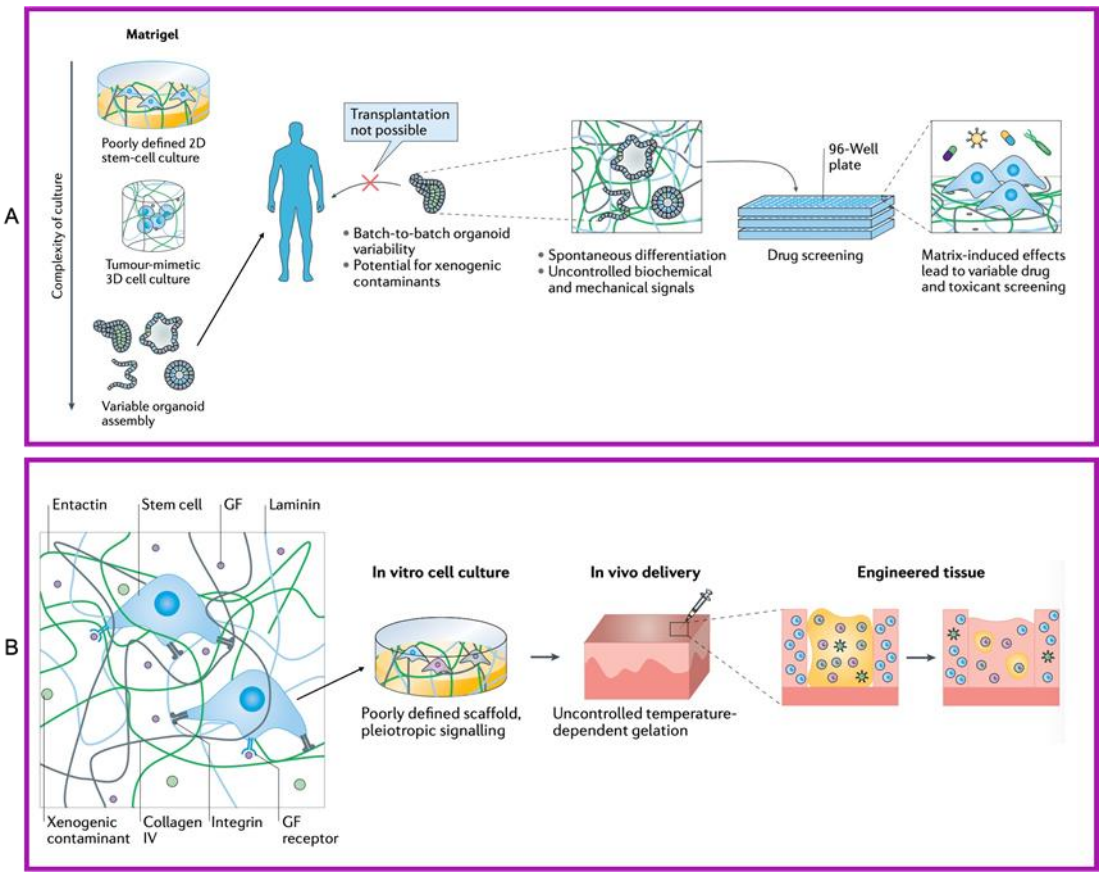

Figure 13. The comparison between scaffolds and Matrigel for cell culture. Reproduced with permission.[21] Copyright 2020, Natural Reviews Materials.

Furthermore, organoids cultured in conventional materials have been shown to exhibit highly heterogenous cell composition due to large (*i.e.*, more than 50%) variability between batches. This prevents the development of reliable, and high quality organoids.[107] Meanwhile, there is lack of suitable materials that are able to achieve the ideal range of matrix stiffness for optimal organoid culture, not to mention tedious gel synthesis and processing methods, which inevitably contributes to high costs and limits the scaling-up of organoid culture for clinical applications. All of which limits their application in clinically relevant, translational medicine.

5.3 The limitations of hydrogel-based organoids

Precise engineering is required to obtain reproducible organoid size and cellular composition, particularly for use in robust bioengineered platforms. [107, 120, 126-128] As it might not be easy to control organization, cellular type, or cell-cell/cell-matrix

interactions by traditional organoid culture manner, there is an urgent need to develop an alternative well-defined organoid culture matrix. Fortunately, the emergence of hydrogel-based scaffolds embedded with artificial ECM provides new hopes. They have the advantage of forming organized architecture, to direct specific cell behavior, and influence cellular differentiation as well as promote intrinsic self-organization ability.[81, 120]

Although synthetic hydrogels possess many ideal characteristics and with potentially low production cost,[88] intrinsic challenges still remain. For example, synthetic hydrogels (*i.e.*, PEG and fibrin-based hydrogels) require an additional step for gelation, either via chemically or enzymatic cross-linking. As this cross-linking step cannot be easily reversed, it might result in poor organoid retrieval from the hydrogel. Moreover, as the cancer micro-environment is highly complex, it remains a challenge for any hydrogel-based organoid to mimic the endogenous situation with high-fidelity and reproducibility. [58, 129, 130] Lastly, potential toxicity arising from biodegradation by-product is an important factor that has to be overcome One such example, is the nanofibril (CNF) hydrogel, which was shown to demonstrate undesirable kidney toxicity in clinical trials.[2, 110, 116]

6. Conclusion and future perspectives

In summary, the successful establishment of hydrogel-based organoids culture has been demonstrated to be a convenient tool for clinically relevant biomedical applications. It is clear that organoids grown in both naturally and synthetic derived hydrogels are able to recapitulate structural and functional characteristics of many organs. However, natural and tissue-derived polymers (such as Matrigel and HA) are limited by their intrinsic variability in manufacturing, resulting in poor reproducibility in organoid culture. Furthermore, they are not amenable for targeting engineering, unlike synthetic hydrogels. Synthetic hydrogels hold the promise as the next generation material for organoid technology for the following key reasons: (i) Reproducibility in manufacturing (due to its synthetic and chemically defined nature), (ii) Scalability

(through the use of bioreactors and newer biofabrication technologies), and (iii) Tunability, which includes stimuli responsiveness, to suit the specific needs of different organoid types. Indeed, synthetic hydrogels have demonstrated that their ease of tenability, enable them to better mimic human organs in health or disease states. This in turn allows biologist to more accurately understand organ-based developmental biology and better predict drug response in cancer organoid models.

However, several of experimental hurdles remain for engineering the ideal synthetic hydrogel for organoid technology. This includes the need for a robust 3D scaffold, that is able to recapitulate the complexity of endogenous tissue micro-environment, wherein an interplay between different ECM proteins and differing hydrogel stiffness range is important to ensure high organoid reproducibility. Consequently, further studies are required, focusing on improving our understanding of the interactions between hydrogel-based organoids and the cellular microenvironment, to enable us to design future ready hydrogel organoids materials.

Future perspective includes harnessing the potential of combining next generation 3D bio-printing with synthetic hydrogel technology, to create next generation hydrogel scaffolds, with remarkable self-organization or bio-functional properties. This is made possible with the development of injectable synthetic hydrogels to create complex 3D micro-scaffolds that are able to mimic endogenous niches known to be embedded within native organs. The organoid based on these hydrogels can reflect the main functional and structural characteristics of organs such as brain, lung, kidney and retina. Moreover, these organoids structural and functional properties can be close resemblance to human organs in health and disease (*i.e.*, better understand the developmental biology of organs, accurately mimics of a patient's cancer property and to predict drug response in a personalized fashion), and easily for translational research. Indeed, successful examples have already been achieved in vascular grafts, heart tissues and bone engraftments. Lastly, the combination of synthetic hydrogels with micro-fluidics technology designed to deliver nutrients, will further contribute to our goal to achieve complex multi-cellular organoids in our bid to best mimic native biology.

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Table 1 PEG based synthetic hydrogels and materials characteristics in organoid culture

PEG-Hydrogels	Advantages over Commercial Matrigel-Like Materials	Use Case Examples	Ref
PEG-CLP-RGD / PEG-CLP	• Well-defined, tissue-like electromechanical environment, their biochemical composition can be tailored by choosing appropriate synthetic peptides, higher level of in vitro organization	• Maturation of self-assembled cerebellar organoids	[96]
Gelatin-PEG	• Similar 3D vasculogenesis and outperformed in liver-like tissue analogues	• Vasculogenesis, prevascularized bone-like Tissue Analogues and Liver-Like Tissue Analogues	[53]
ICC (PEG, PEGDA)	• Can be functionalized with select ECM proteins, highly uniform architecture, provide an easy means to observe cells and intra-cellular fluorescence	• Generate iPSC liver organoids, which are functionally closer to human liver, suitable for disease modelling and form vascularised tissue following transplantation	[91]
PEG-4MAL / cysteine / peptides	• Tenability, lower cost and overcoming the limitations of Matrigel	• Promoting HIO engraftment and accelerated intestinal injury wound healing, generation, culture, and passage of hPSC-derived spheroids and organoids	[94]
PEG / RGD / laminin-111	• Overcome the multiple limitations of Matrigel, higher-purity ISC cultures, modularity	• Expansion of ISCs or the formation of organoids	[36]
8-arm PEG-norbornene / MMP	• Produce model neural tissues for replicate experiments, well-defined, cryopreserved	• Improve assays aimed towards toxicity screening and potency testing, produced 3D neuronal and glial organization	[95]
PEG-RGD	• Well-defined, expands organoids' applicability in basic and clinical research, can change composition to explore ISC extensions	• ISC expansion and organoid formation, control mechanical properties for exploring ISC proliferation	[12]
PEG / Fibrin	• Biocompatible, easy to handle, long-term stablility, transparent and intrinsic limitations of xenograft.	• Enables in vivo exploration of tumor-stromal interactions, simulates the growth and behavior of human lung adenocarcinoma, provide a means for testing biological therapies	[100]