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Mini-review

# Designer hydrogels: Shedding light on the physical chemistry of the pancreatic cancer microenvironment

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is currently the third leading cause of cancer mortality in the United States, with a 5-year survival of ~8%. PDAC is characterized by a dense and hypo-vascularized stroma consisting of proliferating cancer cells, cancer-associated fibroblasts, macrophages and immune cells, as well as excess matrices including collagens, fibronectin, and hyaluronic acid. In addition, PDAC has increased interstitial pressures and a hypoxic/acidic tumor microenvironment (TME) that impedes drug delivery and blocks cancer-directed immune mechanisms. In spite of increasing options in targeted therapy, PDAC has mostly remained treatment recalcitrant. Owing to its critical roles on governing PDAC progression and treatment outcome, TME and its interplay with the cancer cells are increasingly studied. In particular, three-dimensional (3D) hydrogels derived from or inspired by components in the TME are progressively developed. When properly designed, these hydrogels (e.g., Matrigel, collagen gel, hyaluronic acid-based, and semi-synthetic hydrogels) can provide pathophysiologically relevant compositions, conditions, and contexts for supporting PDAC cell fate processes. This review summarizes recent efforts in using 3D hydrogels for fundamental studies on cell-matrix or cell-cell interactions in PDAC.

## 1. Tumor microenvironment in PDAC

Many solid cancers have cancer cells that exhibit self-sufficiency in growth signals, unlimited cell growth, sustained ability to obtain nutrients, apoptosis resistance, insensitivity to growth inhibitory pathways, and the capacity to invade and metastasize [1]. Pancreatic ductal adenocarcinoma (PDAC) has similar characteristics. PDAC is currently the third leading cause of cancer mortality in the United States, with a 5-year survival of ~8% [2]. These slight increases in survival statistics in patients with PDAC are the consequence of improved imaging strategies and advances in chemotherapy but minimal improvement in cancer-directed immune activation strategies [3–5]. Thus, therapies with gemcitabine in combination with nab-paclitaxel or the combination of leucovorin (folinic acid), fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX) have led to improvements in overall survival rates [6,7]. However, in spite of expanding options in targeted therapy [8], PDAC has mostly remained-treatment recalcitrant. Moreover, the incidence of PDAC continues to increase slightly due to the aging of the United States population and the high prevalence of obesity and type 2 diabetes [9]. Moreover, it is anticipated that PDAC will become the second leading cause of cancer death in the United States during the 2020's [10].

PDAC is generally resistant to chemotherapy or radiotherapy, and only 15%–20% of patients with PDAC have resectable disease at the time of diagnosis [11]. PDAC's biological aggressiveness is caused, in part, by the high frequency of major driver mutations that include KRAS, TP53, SMAD4, and CDKN2A, co-existing with numerous low-frequency driver mutations and enhanced cancer cell survival pathways including STAT3, NFkB, and epigenetic alterations [12–15]. This aberrant genomic landscape leads to multiple dysfunctions that are compounded by the overexpression of tyrosine kinase receptors (TKRs) such as epidermal growth factor (EGF) receptor (EGFR), human EGFR 2 (HER2) and HER3, fibroblast growth factor (FGF) receptors (FGFRs), hepatocyte growth factor (HGF) receptor (MET), the AXL receptor, and insulin-like growth factor 1 (IGF-1) receptor [16]. In addition, serine-threonine kinases, such as the type 1 transforming growth factor beta (TβRI) and bone morphogenetic protein receptors (BMPR) can be overexpressed [17]. Often, the corresponding ligands such as

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transforming growth factor alpha (TGF-α), fibroblast growth factors (FGFs), insulin-like growth factor 1 (IGF-1), and hepatocyte growth factor (HGF), are also abundant, leading to multiple cross-talk pathways that promote mitogenic signaling and chemoresistance [18].

PDAC is characterized by a dense stroma with limited vascularization. PDAC stroma also contains proliferating cancer associated fibroblasts (CAFs), collagens, fibronectin, and hyaluronic acid, leading to increased interstitial pressures, compression of scant vascular beds, impaired drug delivery, and a hypoxic and acidic tumor microenvironment (TME) [19–21]. The TME is also infiltrated with inflammatory cells and macrophages that produce immune suppressive cytokines to suppress cancer directed immune mechanisms [22]. Moreover, CAFs release large quantities of HGF that acts in a paracrine manner on the adjacent pancreatic cancer cells (PCCs) [23]. These cells then produce CXCL12 to attenuate cytotoxic T cell penetration into the tumor mass [22].

Given the above environmental alterations, PCC gain a growth advantage through the utilization of aberrant metabolic pathways, glutamine, alanine and lipids, and acquisition of additional nutrients through macropinocytosis and autophagy [24,25]. It has therefore been suggested that it is important to reprogram the TME in a manner that decreases the high interstitial pressures, enhances drug delivery into the tumor mass, and interferes with the metabolic advantages created by that above pro-survival processes [26–29]. To achieve these goals, conventional two dimensional (2D) cell culture devices are insufficient, while animal studies may impose additional challenges regarding mechanistic understanding of the complex cell-matrix and cell-cell interactions, as well as the physical alterations that occur in the PDAC TME (Fig. 1). As such, extracellular matrix (ECM)-derived and synthetic polymer-based three-dimensional (3D) hydrogels (Table 1) are increasingly developed and used for understanding the influences of microenvironment cues on cancer progression [30–35]. While the use of 3D matrices for PDAC research is in early stage, great strides have been made in recent years (Table 2). The remaining sections highlight recent advances in the use of 3D matrices for studying PDAC cell-matrix and cell-cell interactions.

## 2. 3D culture of PDAC cells with ECM-derived hydrogels

### 2.1. Matrigel®

Matrigel is derived from basement membrane of Engelbreth-Holm-Swarm (EHS) mouse sarcoma and is rich in collagen IV, laminin, heparin sulfate proteoglycans (HSPG), as well as a variety of growth factors. Matrigel solidified/gelled when the temperature is above 10 °C. Because of its tumor origin, Matrigel has been used for culturing a variety of cancer cells in 3D [36,37], including PCCs [38]. For example, Reddy and colleagues showed that pancreatic ductal epithelial cells organized into spheroids with apical-basal polarity in Matrigel, whereas PCCs (e.g., MIA Paca-2, PANC-1) exhibited irregular cell shapes [38]. Using Matrigel overlaid on top of noble agar, Korc and colleagues examined morphological changes and cellular response of PCCs in 3D monocultures (ASPC1, BxPC3, COLO-357, T3M4, PK-1, PK-2, Rlnk-2) to EGF/TGF-β1, pharmacological inhibitors, and chemotherapeutics [39]. The authors concluded that 3D culture and co-incubation with SB431542 and erlotinib enhanced the efficacy of gemcitabine and cisplatin in PCCs and in primary cells derived from genetically-engineered mouse models of PDAC. The same group later developed a 3D co-culture system to show that growth of murine PCCs was enhanced in the presence of murine SVEC4 endothelial cells (ECs) and the enhanced growth was suppressed by ruxolitinib [40]. Interestingly, ruxolitinib failed to inhibit growth of these PCCs or ECs in monoculture, which was attributed to an angiocrine mechanism whereby ECs produce factors that promote PCC proliferation that could be suppressed by targeting JAK1–2 with ruxolitinib [30]. By contrast, when human PCCs were co-cultured with human vascular endothelial cells (HUVECs) growth suppression was only obtained through concomitant targeting of TβRI kinase with SB505124 and JAK1 with ruxolitinib [41].

Matrigel-based matrices have also been used to study the influence of physical cues on PDAC cell fate. Rowat and colleagues used a modified scratch wound invasion assay where PCCs were overlaid with Matrigel [42]. PCCs exhibited significantly lower wound confluence and invasion through the Matrigel layer than a non-transformed control cell line. The study also found that cells with higher vimentin levels are more compliant (i.e., softer) but less invasive, which was in contrast to the established phenomenon that cells often express higher levels of vimentin and are more motile/invasive during epithelial-to-mesenchymal transition (EMT). Fisher and colleagues examined the effect of hypoxia on enhanced aggressive phenotype, metastatic potential, and impaired therapeutic efficacy of PCCs [43]. In addition to studying the effect of hypoxia on APE1/Ref-1 redox signaling activity, the authors performed 3D co-culture of PCCs with CAFs in reduced growth factor Matrigel. Results showed that APE1/Ref-1 signaling was dramatically enhanced in *ex vivo* 3D CAF/PCC co-culture. Dual blockade of APE1/Ref-1 and CA9 (carbonic anhydrase IX) using APX3330 and SLC-0111 induced effective tumor cell killing. del Rio Hernandez and colleagues showed that culturing pancreatic stellate cells (PSCs) on top of Matrigel for 6 days reverted activated PSCs to a quiescent-like phenotype as cells lost their spindle morphology and regain cytoplasmic lipid droplets [44]. The authors also cultured cells on top of fibronectin-coated polyacrylamide hydrogels with different stiffness (1–25 kPa). It appeared that increased matrix stiffness alone was sufficient to induce activation of PSCs. Furthermore, matrix with a stiffness gradient caused durotaxis of PSCs. Although these studies were conducted on 2D surface, the combination of Matrigel and synthetic hydrogels provide important insights into PSC activation and quiescence.

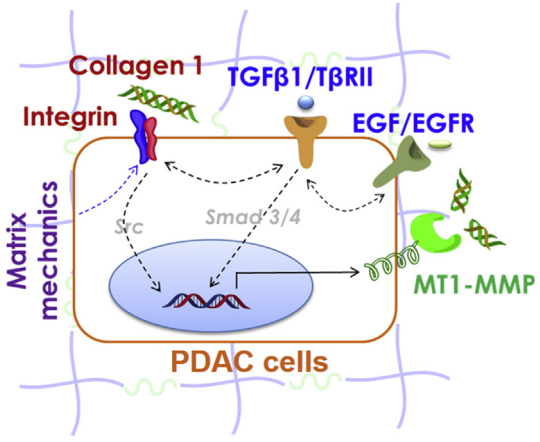


**Fig. 1.** Schematic of PDAC tumor microenvironment. Major matrix factors regulating PDAC cell fate include, but not limited to, matrix mechanics, matrix ligand (e.g., collagen) and cytokine (e.g., TGFβ1 and EGF) signaling, and matrix metalloproteinase (e.g., MT1-MMP) activation. Src is downstream of many signaling cascades, including integrins and non-canonical TGF-β signaling, whereas Smad3/4 mediated canonical TGF-β signaling.

### 2.2. Collagen gels

Collagen 1 is one of the most highly secreted ECM proteins in PDAC stroma, or desmoplastic reaction. Depending on the source, the properties of collagen gels vary significantly [45]. For example, bovine collagen exhibits slow gelation and hence can be processed at room temperature. Bovine collagen also forms more regular fibrils that does not resemble collagen structure *in vivo*. On the other hand, rat tail-derived collagen gels faster and must be placed on ice to prevent premature gelation. Rat tail collagen forms irregular fibril structure that may be more *in vivo*-like. Regardless of the source, all collagen fibrils are soluble and stable in acidic solutions at low temperatures. At above

**Table 1**
Advantages and disadvantages of commonly used cell-laden 3D hydrogels.

| Materials | Advantages | Disadvantages |
|---|---|---|
| Matrigel | • Derived from basement membrane of animal tissue<br>• Contains bioactive motifs for cell recognition | • Difficult to tune matrix mechanics<br>• Contains undefined compositions and residual growth factors<br>• Pre-chilled tips/vessels are required as gelation occurs when temperature is above 10 °C |
| Collagen gel | • Forms fibrous gel to closely mimic collagen-rich tumor ECM<br>• Contains bioactive motifs for cell recognition | • Acidic solution is required for dissolving collagen |
| Hyaluronic acid | • Provide HA ligands for receptor recognition<br>• Mimic HA-rich tumor microenvironment | • Purely HA gel does not provide integrin signaling |
| Purely synthetic polymers | • Well defined and tunable physical properties | • Lack bioactive motifs for cell recognition and degradation |

room temperature (20–25 °C), collagen fibrils self-assemble into bundled fibers with diameters ranging from 12 to 120 nm [45]. The fibers further crosslink into 3D microporous matrix through physical association. Notably, the pH value of the gel solution must be adjusted to neutral for cell culture. Collagen regulates many aspects of cell fate processes and has been widely used in 3D culture of cancer cells. For example, Munshi and colleagues studied the effect of 3D PCC cultures in collagen gels on tumor suppression, expression of matrix metalloproteinase (MMP), invasion, and chemo-resistance [46–48]. In particular, PCCs grew in 3D collagen gels repressed tumor-suppressive let-7 family of microRNAs, partly via up-regulation of membrane type-1 MMP (e.g., MT1-MMP, also named MMP-14) expression and ERK1/2 activation [47]. These cells also demonstrated enhanced TGF-β1 signaling in collagen gel, whereas blocking TGF-β1 signaling attenuated collagen-induced signaling (e.g., MT1-MMP expression, ERK1/2 activation, and let-7 repression) [47]. Collagen 1 also induced Snail expression and MT1-MMP-dependent invasion [48]. PCCs were more resistant to gemcitabine treatment when culturing in collagen gels via HMGA2-dependent histone acetyltransferase expression [46]. However, the stiffness of these collagen gels was not reported.

Schneider and co-workers correlated the activity of MMP14 with stiffness of collagen gels. Specifically, MMP14 blocking antibodies were used to reveal its role on activating other soluble MMPs (e.g., MMP-2 and 9) under various matrix stiffness conditions [49]. Furthermore, inhibition of MMP-14 diminished invasion of PANC-1 cells into 3D collagen gels. However, this study did not characterize the exact stiffness of collagen gels formed at different concentrations (1 and 5 mg/mL), nor did it decouple the effect of matrix stiffness and collagen content on MMP expression. To address these issues, the authors used glutaraldehyde and transglutaminase to increase chemical crosslinking and stiffness of collagen gels without changing collagen concentration [50]. As such, ECM density and stiffness was decoupled. It was demonstrated that MMP activity was modulated by substrate stiffness (50-2000 Pa).

Voytik-Harbin and colleagues examined EMT in PCCs using 3D collagen oligomer gels mixed with different ratios of Matrigel while maintaining constant matrix stiffness [51]. PCCs exhibited more spindle-shaped and single-celled morphology when cultured in soft (100 Pa) collagen oligomer gels, as opposed to round clusters when grew in Matrigel. Furthermore, exposure of PCCs to fibrillar collagen oligomer gels was sufficient to induce EMT. As fibril collagen oligomer density (0.9, 1.5, 2.1 mg/mL) and gel stiffness increased (100, 500, and 1000 Pa), all PDAC lines (BxPC3 and MIA PaCa-2) growing as tight clusters owing to increased spatial constraints and matrix stiffness. It is worth noting that the stiff hydrogels produced from 2.1 mg/mL collagen oligomer had shear modulus (G′) of 1 kPa (equivalent to Young's

**Table 2**
3D hydrogels for PDAC cell culture.

| Materials | Focus | Stiffness | Cell types used | Reference |
|---|---|---|---|---|
| Matrigel | Cell polarity & organization | N/A | Panc-1, MIA PaCa-2, Su.86.86, BxPC-3 | [38] |
| | Response to cytokines and inhibitors, and chemotherapeutics | N/A | AsPC-1, BxPC3, COLO-357, T3M4, PK-1, PK-2, Rlnk-2 | [39] |
| | Effect of ruxolitinib on co-culture of PCCs and endothelial cells | N/A | Endothelial cells, Panc-1, IUSCC-PC1 | [41] |
| | Invasion assay | N/A | MIA PaCa-2, Panc-1, Hs766T, HPDE | [42] |
| | Effect of hypoxia on enhanced aggressive phenotype | N/A | Pa03C, Pa02C, Panc10.05, CAF19, MIA PaCa2, UH1303-02 | [43] |
| | Quiescence/activation of pancreatic stellate cells | N/A | PSC | [44] |
| Collagen | Gemcitabine resistance | N/A | Panc-1, CD18/HPAF-II | [46] |
| | *let-7* regulation & TGF-β1-mediated MT1-MMP expression | N/A | Panc-1 | [47] |
| | MMP expression, EMT | N/A | HPDE, Panc-1, CD18, AsPC-1 | [48] |
| | MMP14 activation & invasion | N/A | Panc-1 | [49] |
| | Cellular contractility & matrix stiffness | 0.05–2 kPa | Panc-1, BxPC-3, and AsPC-1, HaCat (keratinocyte), MDA-MB-231 (breast cancer) | [50] |
| | Collagen fibrillar microstructure & EMT | 0.1–1 kPa | Panc-1, BxPC3, MIA PaCa-2 | [51] |
| Hyaluronic acid | 3D cell carrier for orthotopic implantation of PCCs | N/A | MIA PaCa-2 | [52] |
| | Effect of 3D matrix stiffness on PCC growth | 1, 2, 5 kPa | COLO-357 | [53] |
| | Effect of dynamic matrix stiffening on EMT | 1–5 kPa | COLO-357, Panc-1 | [66] |
| Fibronectin-coated polyacrylamide hydrogels (2D) | Substrate rigidity on activation & durotaxis of pancreatic stellate cells | 1–25 kPa | PSC | [44] |
| PEG-peptide hydrogels | Effect of integrin ligands & matrix stiffness on cell morphogenesis & EMT | 3–6 kPa | Panc-1 | [56] |
| | Matrix properties and EGF receptor inhibition on the growth of PANC-1 cells | 2–12 kPa | Panc-1 | [55] |
| | Effect of matrix stiffness and collagen 1 on MT1-MMP expression & EMT | 2 kPa | COLO-357 | [54] |
| | Effect of dynamic matrix stiffening on PSC | 1–5 kPa | PSC | [65] |

modulus (E) of about 3 kPa), which is suboptimal, as recent work has shown that PDAC stroma has a much higher stiffness (i.e., E ~ 12 kPa).

### 2.3. Hyaluronic acid (HA) hydrogels

Hyaluronic acid (HA) is a major glycosaminoglycan (GAG) excessively expressed and accumulated in PDAC stroma. HA binds to and activates CD44 and receptor for hyaluronan-mediated motility (RHAMM), leading to cancer cell proliferation, invasion, and drug resistance. Accumulation of HA also causes elevated fluid stress that not only induces abnormal mechanosensing in the cancer cells but also limits penetration of anti-cancer drugs to tumor cells. Chemically crosslinked HA hydrogels have been used to provide stable network for long-term cell study. Scaife and colleagues used thiolated HA, thiolated gelatin, and PEG-diacrylate (PEGDA) to form chemically crosslinked 'HA-G' hydrogels via Michael-type addition reaction [52]. MiaPaCa-2 cells were homogeneously encapsulated in the injectable HA-G hydrogels for orthotopic implantation in nude mouse model of PDAC. HA-G gels with PCCs appeared to exhibit more consistent tumor growth and higher rate of metastasis at 8 weeks of tumor growth. While this study showed HA as an effective 3D cell carrier for orthotopic implantation of PCCs, the influence of HA on PDAC cell fate processes was not explored. The use of HA hydrogels as scaffold for 3D culture of PCCs did not receive significant attention until recently. We have developed HA-gelatin hydrogels via visible light based thiol-norbornene photopolymerization [53]. Specifically, thiolated HA (THA) and norbornene-modified gelatin (GelNB) were modularly crosslinked into hydrogels within minutes by means of visible light irradiation. The orthogonal reactivity between thiol and norbornene permits modular control over biochemical and biophysical properties without affecting other critical parameters capable of guiding PDAC cell fate. In particular, THA/GelNB hydrogels were formed at identical chemical compositions but varied matrix stiffness (G' = 1, 2, and 5 kPa), which was achieved via supplementing PEG-tetra-thiol (PEG4SH). These modular hydrogels were used to examine the effect of stiffness on growth and expression in COLO-357 cells, a PCC line with wild type *KRAS*. Results showed that gels formed with higher crosslinking density and stiffness (G' ~5 kPa) led to cell cultures with smaller diameters. High stiffness down-regulated CTGF mRNA levels, suggesting potential inactivation of the HIPPO pathway that may contribute to tumor progression and metastasis. On the other hand, the expression of sonic hedgehog (SHH) and MMP14 mRNA were significantly upregulated in cells growing in stiffer gels. SHH has been implicated in enhanced drug resistance, whereas upregulation of MMP14 is indicative of higher matrix cleavage and metastatic potential in the encapsulated cells.

## 3. 3D culture of pancreatic cancer cells with semi-synthetic hydrogels

Hydrogels prepared from synthetic polymers, such as derivatives of poly(ethylene glycol) (PEG), have been used to support the survival of PCCs in 3D [54–56] and to deliver apoptotic/anti-cancer agents to treat PDAC [57–59]. To mimic a cancer cell niche using synthetic hydrogels, it is common to include biomimetic peptides for gel crosslinking and for receptor activation [56,60]. In one example, multi-arm PEG-based macromers (e.g., PEG-norbornene, PEG-maleimide, PEG-vinylsulfone, etc.) are crosslinked by MMP cleavable peptides flanked with bis-cysteines via step-growth photopolymerizations [60]. The resulting 'thiol-ene' networks are more homogeneous and have superior cyto-compatibility when comparing with similar hydrogels formed by random chain-growth polymerization [61].

PEG-peptide hydrogels formed by thiol-norbornene click reaction has been used to evaluate the effect of matrix compositions on PANC-1 cell growth and morphogenesis in 3D [56]. Specifically, PANC-1 cells formed small multicellular clusters in thiol-ene hydrogels within 4 days of *in vitro* culture. After 10-day, the growth and structures of these clusters were significantly impacted by gel matrix properties, including gel degradability, stiffness, and immobilized peptide ligands. The use of matrix metalloproteinase (MMP) sensitive linker or the immobilization of fibronectin-derived RGDS ligand in the matrix promoted PANC-1 cell growth and encouraged them to adopt ductal cyst-like structures. On the other hand, the encapsulated cells formed smaller and more compact aggregates in non-MMP responsive gels. The incorporation of laminin-derived YIGSR peptide did not enhance cell growth and caused the cells to form compact aggregates. Immobilized YIGSR also enhanced the expression of epithelial cell markers including β-catenin and E-cadherin. Our group used a similar PEG-based thiol-norbornene hydrogel to show that the presence of collagen 1 enhanced cell proliferation and Yes-associated protein (YAP) translocation to nuclei of COLO-357 cells [54]. Furthermore, cytokines and collagen 1 synergistically up-regulated MT1-MMP (i.e., MMP-14) expression and induced cell spreading, which could be attributed to EMT. COLO-357 cells grew in 3D developed chemo-resistance even in the absence of collagen 1 and cytokines, as well as expressed high levels of CD24, SHH, and VEGF. In another example, we studied the influence of matrix properties and EGF receptor inhibition on the growth of PANC-1 cells [55]. Unsurprisingly, cells retained high viability and formed clusters in softer hydrogels (G' ~ 2 kPa). On the other hand, more cell death and smaller cell clusters were observed whereas cells encapsulated in stiff hydrogels (G' ~ 12 kPa). Furthermore, the immobilization of an EGFR peptide inhibitor (Asn-Tyr-Gln-Gln-Asn or NYQQN) only caused Akt-dependent cell apoptosis in stiff hydrogels but not in soft hydrogels, highlighting the importance of matrix physical properties on drug sensitivity in PCCs.

## 4. Dynamic hydrogels to probe PDAC cell fate

The biochemical compositions and biophysical properties in TME vary greatly depending on the stages of tumor development. The dynamic evolution of stromal tissue stiffness could lead to mechanosensing to both cancer cells and stromal cells. As such, hydrogels capable of recapitulating the dynamic landscape of extracellular microenvironment are of great importance for fundamental understanding of matrix-induced aberrant cell-matrix interactions [62,63]. Recent work has shown that the stiffness of malignant PDAC tissues ranges from 2 to 6 kPa in shear modulus (equivalent to ~6–18 kPa in Young's Modulus), whereas that of the healthy tissue is around 1 kPa [64]. Synthetic approaches commonly used to mimic a stiffening tissue often rely on performing secondary crosslinking within the primary cell-laden hydrogels [62]. For example, we have reported a cytocompatible enzyme-responsive matrix stiffening strategy [65,66]. The initial gelation and cell encapsulation were achieved by thiol-norbornene crosslinking of 8-arm PEG-norbornene (PEG8NB) and bis-cysteine peptides (i.e., KCYG-PQGIWGQYCK or YGKCYGPQGIWGQYCKGY). This simple peptide linker contains sequence sensitive to matrix metalloproteinase (MMP) induced cleavage, as well as additional tyrosine residues for tyrosinase-triggered di-tyrosine crosslinking [65]. Following thiol-norbornene gelation, the tyrosine residues in the primary network served as substrates for exogenously added tyrosinase (TYR). As TYR diffused in hydrogels, it catalyzed dimerization of tyrosines and led to higher gel crosslinking density and stiffness. We demonstrated that enzyme-triggered and on-demand stiffened hydrogels altered morphology of PSCs cultured in 3D. PSCs also expressed higher level of α-smooth muscle actin (αSMA), a signature marker of myofibroblastic activation.

Although the TYR-stiffened PEG-peptide hydrogels have been useful in studying the effect of dynamic matrix stiffening on cancer stromal cell fate, these gels represented minimal tumor-related matrix components. In a separate study, we designed a pathophysiologically relevant dynamic biomimetic hydrogel system where the gels were crosslinked by thiolated HA (THA) and norbornene/hydroxyphenylacetic acid dually-functionalized gelatin (i.e., GelNB-HPA) [66]. The initial gel network was formed by orthogonal thiol-norbornene photopolymerization of GelNB, GelNB-HPA, THA, and inert macromer PEG-tetra-thiol

(PEG4SH) [53]. With this hybrid dynamic hydrogel system, the effects of matrix biochemical and biophysical cues were easily decoupled for gaining new insights into the effects of matrix compositions on PDAC cell fate processes.

## 5. Conclusion and outlook

Cell-laden hydrogels are increasingly used in cancer cell studies. Overall, animal derived matrices are advantageous owing to their inherent biological motifs for cell attachment and invasion. However, the batch-dependent material compositions and properties, as well as residual growth factors could confound the interpretation of the experimental results (Table 1). While gelation of Matrigel is easily achieved through controlling temperature, pre-cooled pipet tips, microtubes, and cell culture vessels are needed to prevent pre-mature gelation. On the other hand, significant acidic solution is required for preparing collagen gels. Compared with Matrigel and collagen gels, hydrogels crosslinked by derivatives of HA have not been widely used for PDAC cell research. Although HA alone does not provide necessary ligands for integrin signaling, it does activate cell surface receptors such as CD44 and Receptor for hyaluronan-mediated motility (RHAMM). Our work has shown that HA and matrix stiffness synergistically induce EMT in PCCs [66]. Hence, future work should explore the effects of HA and its synergistic signaling with integrin ligands or with other physical cues on PDAC cells fate in 3D.

Conventional hydrogels are often composed of polymers crosslinked by covalent bonds that exhibit purely elastic properties. These elastic hydrogels are excellent artificial tissue mimics for recapitulating aspects of native ECM, including elasticity, permeability, and presentations of bioactive motifs. However, purely elastic hydrogels do not capture the viscoelastic and stress-relaxation properties of native tissues, which may play a significant role in cell fate processes and tissue development. Recent efforts have addressed this through developing advanced hydrogels with reversible crosslinks that can be reformed after breaking up by local cellular processes [67–69]. This new class of reversible/adaptable hydrogels is highly desirable for studies concerning the influence of matrix viscoelastic properties on cell behavior and gene expression. In addition to immobilizing pendant ligand in the presence of cells, one may wish to introduce different integrin ligands at different state of tumor development. In this regard, an addition-fragmentation-chain transfer reaction was developed to allow controlled and reversible exchange of biochemical ligands within an allyl sulfide functionalized PEG hydrogel [70]. Ligand ‘exchange’ could also be achieved via Sortase A mediated peptide ligation or protein labeling [71]. These approaches allows user-defined introduction of immobilized ligands during cell culture, which may be highly useful in understanding the influence of temporal presentation of selective ligands on cancer cell fate. Moreover, bioengineered hydrogels capable of inducing hypoxia could be used to study the synergistic influence of low oxygen tension and other matrix properties on PDAC cell fate [72,73]. Finally, HA-based hydrogels can be designed/fabricated to enable *in vitro* evaluation of hydrostatic pressures on drug delivery to PCCs. Collectively, these approaches will not only increase our understanding of the complex PDAC TME, but may lead to the discovery of novel therapeutic options for this deadly cancer.

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