Biomaterial-driven kidney organoid maturation
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Abstract
Differentiation of human-induced pluripotent stem cells (hiPSCs) toward kidney organoids is known to suffer from batch-to-batch differences, off-target populations, and skewed cellular compositions. Application of synthetic hydrogels as a tool for hiPSC-differentiation may provide additional control over this variable process. This review discusses important material properties that affect kidney organoid generation. We summarize cellular adhesive cues for synthetic materials, that allow transduction of the mechanical forces to the cell, and how these signals directly affect Hippo-Canonical Wnt signaling and morphogenetic events. In addition, chemical strategies are discussed that allow spatiotemporal presentation of biochemical agents to the cell in a material-dependent approach.

Introduction
The engineering of a human kidney has not yet been achieved. Most endeavors attempt to recapitulate the developmental events observed during kidney organogenesis. Human-induced pluripotent stem cells (hiPSCs) may be primed using similar developmental programs to generate tissue that contains renal microstructures. These hiPSC-derived tissues are known as kidney organoids. A variety of protocols have been developed for differentiation of hiPSCs to produce the different progenitor cells of the kidney (i.e., nephron, ureteric, endothelial, and interstitial progenitors). Depending on which progenitor populations are produced, it is possible to generate nephrons, collecting ducts, vasculature, and/or interstitium, respectively [1-6].

Current protocols still face many hurdles in terms of batch-to-batch differences, off-target populations, skewed cellular compositions, and incorrect organization of functional microstructures [7]. These limitations need to be resolved in order for kidney organoids to realize their full potential as regenerative therapy. The root of many of these problems appears to be the manner in which hiPSCs are differentiated toward intermediate mesodermal lineages. Biochemical signals (e.g. fibroblast growth factor 9 (FGF9), bone morphogenetic protein 4 (BMP4), glial cell-derived neurotrophic factor (GDNF), activin A, or CHIR99021) used to differentiate hiPSCs are dissolved in media, which is then exposed to the cells in a bulk-approach manner [1,3,4,8]. This approach neglects the spatiotemporal accuracy in which these signals need to be presented to cells, which is critical for proper lineage patterning. The micro-niches in which renal progenitor cell populations reside, play a major role in modulating timing and location of these signals. This micro-niche is a complex environment consisting of extracellular matrix (ECM) proteins, proteoglycans, and carbohydrates that together serve as: (1) a reservoir for soluble factors and mediators, forming concentration gradients and being presented to cells, (2) compartmentalization of different cellular populations and microstructures, and (3) mechanotransductive signals to modulate cytoskeletal and chromatin remodeling [9]. In contrast, differentiation of hiPSCs toward mesodermal lineages occurs on materials that appear the polar opposite of this native environment. These cells are cultured in non-adherent plates or on rigid polystyrene plates coated with recombinant proteins or basement membrane extract, derived from Engelbreth-Holm-Swarm tumor (i.e., Matrigel). [1,3,4,8,10] Matrigel is rich in biological signals and allows for culturing and differentiation of hiPSCs in both two and three dimensions. Nonetheless, this biomaterial is extremely complex in terms of biochemical agents, and its composition is undefined and variable, which could affect differentiation in negative ways [11]. Furthermore, this matrix lacks tunability of material properties such as stiffness, degradability, anisotropic structure, and biochemical composition. It is precisely...
these material properties that may be harnessed to influence differentiation of hiPSCs and induced-renal progenitor cells within developing kidney organoids.

Synthetic hydrogels (i.e., cross-linked polymeric networks that contain water as dispersion medium) are good alternatives to Matrigel, due to their decreased complexity and defined composition, resulting in more controlled biomaterial–cell interactions. Here, we argue for improving and fine-tuning hiPSC differentiation toward kidney organoids with additional tools in the form of synthetic hydrogels. The different aspects of hydrogels are shortly discussed from a material science point-of-view. Then it is evaluated how these aspects may be harnessed to tap into biological processes and influence lineage commitment and morphological events.

**Introducing cell-adhesive cues**

The mechanical environment surrounding cells is known to prime lineage commitment of stem cells. In their native environment, cells adhere to ECM components via integrins. Mechanical information of these ECM components is propagated to the cell via a series of protein–protein interactions, which transmit this information by generating tension in cytoskeletal actin filaments [9]. The transmission of mechanical forces along the cytoskeleton (i.e., mechanotransduction) directly affects cell shape, opening/closing of nuclear pore complexes, signaling pathways, and gene expression. In this manner, changes in the mechanical environment impact cellular differentiation programs [12].

Synthetic biomaterials are also capable of inducing mechanotransduction. However, the often hydrophilic nature of the polymeric network in synthetic hydrogels makes these unsuitable for adhesion of cells. As such, these networks require functionalization with biochemical cues that allow adhesion of cells to provide a physical cell–material interaction [13]. Recombinant basement membrane proteins (e.g., vitronectin, laminin, collagen IV, and fibronectin) are used to coat polystyrene plates for culturing and differentiating of hiPSCs toward mesoderm in 2D [14]. Incorporating these recombinant proteins in a synthetic hydrogel is one way to induce cellular adhesion of organoids [15]. An alternative is the incorporation of integrin-binding peptide sequences derived from these proteins [16]. The various cell types found in kidney organoids all contain a different array of integrins. Owing to this integrin heterogeneity, it is possible to introduce phenotype specific cell–material interaction depending on which peptide sequence is incorporated (Table 1).

Most hiPSC-derived kidney organoids are generated from 2D cultures, in which hiPSCs are cultured on polystyrene plates coated with ECM proteins such as Matrigel. Replacing these ECM coatings with a synthetic substrate not only results in a more defined culture, it also provides the opportunity to control the exact mechanical and biochemical environment of the cells. 2D substrates, however, do not comprise the geometry and mechanical environment of the native ECM, therefore constraining the cells to behave and differentiate naturally. 2.5D matrices may therefore aid to improve the representation of the natural ECM (Figure 1a). Here, the cells are cultured in between two layers of ECM, also referred to as an ECM sandwich. While the cells normally only receive signals from the ventral side, the cells are now also able to interact dorsally, affecting focal adhesion formation, integrin expression and the formation of the actin cytoskeleton. This subsequently results in altered cell signaling. These 2.5D matrices have been shown to mimic a 3D environment, while preserving the ease of 2D cultures [17,18]. Freedman et al. generated tubular organoids when hiPSCs were cultured underneath a layer of Matrigel, showing the potential of 2.5D matrices [3]. Synthetic 2.5D matrices can be created by using a diluted synthetic material, offering sufficient nutrient exchange while sustaining the cell–matrix interaction. In addition to the aforementioned cell–matrix outcomes, these matrices can also be used to retain or release bioactive molecules and growth factors to modulate stem cell behavior, which will be discussed in more detail later. Similarly, 3D matrices can be created for improved cell–matrix interaction as well, supporting the geometry of the native ECM and offering the possibility for growth factor functionalization (Figure 1a). For kidney research this is an opportunity, as currently embryoid bodies, renal progenitor cell aggregates, and kidney organoids are grown in unnatural conditions of lacking an ECM (i.e., on ultra-low attachment plates) or being placed on top of a transwell insert.

**Mechanotransduction biases lineage commitment**

Stiffness is a critical material property that primes stem cells toward a certain lineage [29]. The stiffness of a hydrogel with a linear-elastic regime is often defined by an elastic or Young’s modulus, which describes the amount of force that is required to deform an area. If the hydrogel network demonstrates more viscoelastic behavior, then it is described by a storage modulus (G’ — the elastic component) and a loss modulus (G” — the viscous component). These moduli are tunable by changing factors such as polymer weight content and the number of cross-links. An increase in either leads to a stiffer material [30].

One of the first examples demonstrating the importance of stiffness were naive mesenchymal stem cells that specified their lineage commitment based on the underlying substrate stiffness [29]. Pluripotent stem cells (PSCs) are also sensitive to their mechanical environment, as changes in substrate stiffness have shown to
bias their specification toward ectoderm, mesoderm, or endoderm [6, 31, 32]. The influence of the mechanical environment on germ layer specification is caused by the sensitivity of certain developmental signaling pathways to mechanotransduction. Among these mechanosensitive pathways is Hippo signaling, which directly affects the efficiency of mesodermal differentiation more canonical Wnt signaling compared to those differentiated on rigid substrates, allowing for improved mesodermal differentiation efficiency. Upon culturing of intermediate mesodermal cells derived from soft substrates in a 3D setting, more renal vesicles were generated, which also appeared one day earlier compared to those derived from stiff substrates. The resulting kidney organoids also contained more nephron-like structures and showed higher expression for genes related to nephrons [6].

A number of studies have investigated the extent to which material stiffness affects the efficiency of mesoderm differentiation. PSCs cultured on soft (0.4–1 kPa) and rigid (60 kPa) polyacrylamide hydrogels showed different colony morphology, which originates from a change in preference between cell–cell contacts or cell–ECM contacts. On the rigid substrates, the cell-ECM interactions dominate in the PSC colonies. This leads to an elaborate filamented actin network that inactivates the Hippo pathway, allowing YAP nuclear translocation where it forms the YAP-TEAD transcription factor complex [6,32,34]. This complex also functions as a gene repressor in the nucleus, as it binds and blocks promoter sites, preventing transcription of a number of genes critical for mesoderm specification, such as Wnt3a, T/Brauchy, EOMES, NODAL, and MIXL1 (Figure 1b) [33,35,36]. On soft substrates, PSC colonies have a preference for cell–cell contacts based on E-cadherin. This results in a predominant cortical actin network. A decreased amount of cytoplasmic filamented actin leads to active core Hippo kinases, which phosphorylates YAP leading to its cytoplasmic degradation [6,32]. The effect of absent mechanotransduction in PSC colonies on soft-substrates is two-fold. (1) The increased amount of E-cadherin binds more β-catenin, functioning as a reservoir, and preventing its cytoplasmic degradation. This means that at the start of mesodermal differentiation more canonical Wnt signaling is possible [32]. (2) YAP remains in the cytoplasm and no longer blocks promoter/enhancer sites of genes important for mesoderm specification (Figure 1b). As a result, hiPSCs differentiated on soft substrates have enhanced canonical Wnt signaling compared to those differentiated on rigid substrates, allowing for improved mesodermal differentiation efficiency. Upon culturing of intermediate mesodermal cells derived from soft substrates in a 3D setting, more renal vesicles were generated, which also appeared one day earlier compared to those derived from stiff substrates. The resulting kidney organoids also contained more nephron-like structures and showed higher expression for genes related to nephrons [6].

Currently, PSCs are differentiated in 2D on polystyrene culture plates (~3 GPa) to induce renal progenitor-like cells [37]. These studies suggest that this results in a lower differentiation efficiency. This may very well result in off-target populations which are often detected in kidney organoids, as an increased differentiation efficiency would yield a more homogeneous cellular population. Off-target populations arise in a later stage of kidney organoid development, indicating that these cells initially follow a similar differentiation trajectory as renal cells [38]. Minor transcriptional differences caused by a sub-optimal mechanical environment at earlier stages may skewer differentiation to different phenotypes. Single-cell transcriptomics from multiple studies highlighted glial-, neuronal-, muscle-, cartilage-, and melanoma-like phenotypes as off-target populations that arise at later stages [38–41]. The aggressive rise of cartilage-like tissue within kidney organoids after long-term culture or transplantation appears to be the main driving force behind degradation of renal structures. Interestingly, the amount of cartilage was reduced in transplanted kidney organoids that were encapsulated in decellularized kidney extracellular matrix hydrogels [41]. This indicates that encapsulation by hydrogels that resemble the native mechanical environment and contain cell-adhesive cues may reduce off-target populations or halt their rapid expansion at a
later differentiation stage. Furthermore, each pheno-
typical population secretes a different array of bioactive
agents, which influences differentiation of neighboring
cells. A reduction in off-target populations would yield a
more homogeneous secretome of the organoid, which
results in less noise during commitment toward renal
lineages. This potentially reduces batch-to-batch dif-
f erences observed during kidney organoid generation.

**Influence of network dynamics on morphogenesis**

Morphogenesis is one of the main phenomenological
driving forces during kidney development, and relies on
reorganization of cells into novel tissue shapes and
structures [42,43]. During this process, cells are
required to actively degrade their surrounding micro-
environment, and reform it in order to migrate and form
novel shapes [43]. Morphogenesis occurs in every stage
of kidney development and is critical during ureteric
bud (UB) branching, nephrogenesis, and vascularization
of the fetal kidney [42]. This indicates that kidney
organoids, which also undergo these stages, cannot be
cultured in all types of synthetic microenvironments.
Instead, a synthetic microenvironment needs to be
spatially compliant to permit expansion and morpho-
gensis of kidney organoids (Figure 2a). Stress-
relaxation is a critical material property underlying
spatial compliance of a hydrogel and is defined as a decrease in stress in the polymeric network in response to strain generated in the material [30]. It is dependent on the type of degradation and re-organization that may occur based on chemical composition and type of cross-links (Figure 2b). Here, hydrogels are divided in three classes based on the degree of stress-relaxation behavior they exhibit: (1) static hydrogels, (2) controlled degradable hydrogels, and (3) dynamic hydrogels. Each of these classes shows stress-relaxation to a certain extent, but they differ in terms of timescale.

Static hydrogels contain a network that only contain cross-links based on covalent bonds. These types of chemical bonds are high in energy and are therefore difficult to break using cellular force. Common chemistries used for covalent cross-linked hydrogels are methacrylate chemistry and bio-orthogonal click-reactions [44]. These types of polymeric networks are degradable via hydrolysis if they contain ester bonds [45]. However, degradation through this mechanism longer than the morphogenesis occurring during development. This makes hydrogels based on covalent networks relatively static and unsuitable for culturing kidney organoids.

Controlled degradable hydrogels are similar to static hydrogels in the sense that their network is solely based on covalent bonds. However, these types of hydrogels contain chemical moieties in their network that are degraded by an external input (Figure 2a). Incorporating matrix metalloproteinases (MMP)-cleavable peptide sequences into the network leads to local network degradation by cell-secreted MMPs. The degradation kinetics of the hydrogel may be tuned by altering the MMP-cleavable peptide sequence to moderate MMP affinity [46]. MMP-2 and MMP-9 are both critical metalloproteinases in ureteric branching and nephrogenesis [47]. To this end, a polymeric network containing a ‘GPQGIWGQ’ (pan-MMP), ‘GDFIPVSLRSGGK’ (MMP-2 specific), and/or ‘KGPRQFT’ (MMP-9 specific) is degradable by a wide range of cells present at different

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**Figure 2**

Effect of stress-relaxation on morphogenesis. (a) Schematic representation of the effect of hydrogel network dynamics on budding of organoid structures. Organoids in static hydrogels are physically ‘trapped’ and do not undergo morphogenetic changes. Secretion of MMPs by organoids degrades MMP-cleavable networks, allowing local migration out of the organoid. Dynamic hydrogels based on supramolecular hydrogels actively reorganize in response to physical stress applied by the organoid, allowing omni-directional migration. (b) Stress-relaxation behavior of different type of hydrogels depending on their network degradability.
stages during the development of kidney organoids [46, 48–50]. This allows UB cells and nephron progenitor cells (NPCs) to actively degrade their synthetic micro-environment, and subsequently engage in morphological events. Several studies already demonstrated that MMP-regulated network degradation induces tubulogenesis of proximal renal epithelial cells in 3D-culture in vitro, whereas non-degradable (i.e., laser) counterparts remained spheroids [51,52]. Whether induced-NPCs would also undergo tubulogenesis is still unknown. A more spatially controlled method for hydrogel degradation is the introduction of photocleavable bonds. These polymeric networks contain moieties that react to specific wavelengths, which allows controlled degradation in three-dimensions using two-photon laser scanning microscopy. Photodegradable hydrogels have only been used for intestinal epithelial organoids, which demonstrated spatially controlled villi budding upon photodegradation [53]. This type of chemistry may be valuable in modulating ureteric branching in vitro, which is a morphological process that also depends on budding events from epithelial structures.

Finally, dynamic hydrogels contain a supramolecular polymeric network with cross-links based on directed non-covalent interactions (for detailed reviews on supramolecular biomaterials, see references [54–56]). Supramolecular moieties that facilitate network formation via hydrogen-bonding are ureido-pyrimidinones, benzenetricarboxamides, and bisurea. Other well-known supramolecular cross-linking strategies are based on metal–ligand interactions (e.g., catechols– and imidazole groups) and host-guest chemistry (e.g., cycloexodextrin and cucurbit[n]uril) [54]. These types of bonds are typically low in energy, meaning they are easily broken by low forces. As such, stress induced onto the network by spatial cellular processes (i.e., cell migration, proliferation, or matrix deposition) lead to breaking of these non-covalent bonds, thereby accommodating space for morphogenesis of the organoid (Figure 2a). The reversible nature of supramolecular interactions allows the reformation of the network in a different state after breaking, resulting in reciprocal mechanical behavior.

**Biofunctionalization of synthetic matrices**

Instead of presenting required molecules and growth factors to the cells via the culture medium, bioactive cues can also be tethered to a synthetic matrix, resulting in prolonged exposure and increased signaling of the specific differentiation pathways. Particularly growth factors important during the differentiation, such as BMP4 and FGF9, could be of interest to attach to the synthetic ECM. The proteins can be loaded into the hydrogels directly, covalently bound to the hydrogel, or bound via a carrier or via interactions with other proteins, due to electrostatic interactions [57]. Especially for this application, the use of heparin or heparan sulfate to bind growth factors such as FGF could be useful.

Also, synthetic alternatives for growth factors can be employed. This was demonstrated, for instance, with the development of FGF2-mimicking peptide amphiphiles to activate the FGF2 signaling pathway in human umbilical vein endothelial cells [58]. Designing synthetic ligands also allows for the development of more sensitive and efficient ligands, such as multivalent ligands. Multivalent ligands are composed of a scaffold with multiple copies of the recognition element of a ligand. The high density of these copies induces the assembly of multiple receptors on the cell membrane, resulting in receptor clustering. This clustering of integrins facilitates cell adhesion and intracellular signaling more efficiently than when activated with monovalent ligands. This was demonstrated with star polymers with a high density of YGRGD moieties to bind multiple integrins simultaneously [59].

Spatial and temporal control over the bioactivity creates anisotropy in biomaterials. Several techniques are available for spatial and temporal patterning of hydrogels, of which photolithography is the most common technique. Hydrogels can be functionalized with molecules, peptides and proteins after gelation to pattern specific regions of a material (local patterning) or to introduce certain cues at a specific timing (timed patterning). Most of these reactions occur via click reactions, such as thiolene reactions, strain-promoted azide–alkyne cycloaddition, Diels–Alder ligation and oxime/hydrazone [60]. This offers the possibility to introduce growth factors and molecules to the cells at specific points during the differentiation, e.g., via thiol-norbornene photopolymerization [61]. Proteins such as Activin A, BMP4, GDNF, FGF9 may be introduced at certain timepoints to induce signaling in their corresponding pathways. For example, FGF9 could be bound to the materials to obtain prolonged and increased signaling for improving the maturation of the kidney organoid.

While the introduction of certain bioactive cues can be important to induce signaling at a specific timepoint, it might also be necessary to release these cues in order to obtain correct differentiation. CHIR99021, for instance, a non-endogenous small molecule used during differentiation from PSC toward primitive streak, activates canonical Wnt signaling. However, this molecule needs to be removed after a few days. Biochemical release from materials can happen when photocleavable groups are attached to the molecule, peptide or protein. Hydrogels with nitrobenzyl based linkers are widely applied to release proteins upon exposure to light [45]. While the introduction and release of biochemicals opens up opportunities for spatial and temporal patterning, mimicking the dynamic ECM requires more reversible
patterning chemistries. The incorporation of two bioorthogonal photochemistries introduces coupling and subsequently releasing bioactive cues. The reversible patterning of vitronectin, for example, resulted in reversible differentiation of hMSCs into osteoblasts [62]. Other stimuli-labile moieties can be coupled to the bioactive cue as well. This was shown by Gawade et al. who developed a hydrogel with various types of proteins; all released by different stimuli involving light, enzymes and reductants [63]. This confirms that the introduction and release of multiple bioactive cues can be regulated.

These chemistries could be integrated into hydrogels to mimic the reciprocal interaction between the metanephric mesenchyme (MM) and the UB and the ECM, e.g. to stimulate UB branching via the functionalization of MM-secreted GNDF (Figure 3a), or to support the differentiation of NPCs via the incorporation of the UB-ECM peptide nephronectin or UB-secreted protein WNT9b.

Gradients of bioactive cues can be created on and within hydrogels with photochemical patterning, providing an alternative to microfluidics. In this way gradients of RGD peptides [64], N-cadherin or fibronectin [65] can be created, spatially influencing the differentiation of the stem cells. Morphogen gradients play an essential role during embryogenesis, e.g., for the formation of the primitive streak (via gradients of Nodal, BMP4, and canonical WNT) and the intermediate mesoderm.

Figure 3

Suggested hypothetical examples of biofunctionalized synthetic matrices that interact with hiPSC-derived cells. (a) Spatial patterning of GDNF can induce ureteric bud branching. (b) Gradients of morphogens within a 3D matrix stimulate both the anterior and posterior intermediate mesoderm formation in primitive streak cells. RA: retinoic acid, FGF9: fibroblast growth factor 9, FGFR: fibroblast growth factor receptor, WNT: Wnt signaling ligand.
Kidney organoids are plagued by off-target populations and deficient maturation. The differentiation protocols are currently being re-invented to solve these issues. However, the role and effect of material properties on lineage commitment of hiPSCs is unrepresented in this process. The extent to which the mechanical environment affects developmental programs is significant and deserves consideration. As such, we propose that biomaterials need to be used as an additional tool to control organoid development. Kidney organoids are now generated on rigid materials that do not change over time. Instead, this process would benefit from a soft and mechanically compliant material— as this enhances induction of mesodermal lineages. Furthermore, static materials are no longer sufficient for complex cellular aggregates that evolve in composition and behavior over time. A dynamic material is required that ‘grows’ with the organoid. Such a living-type material is created by encoding dynamic reciprocal behavior into a material. Organoid-material reciprocal interactions would allow for accurate spatiotemporal modulation of organoid behavior through dynamic adjustment of both presented ligands, and cell surface receptors. Mechanical reciprocity already exists in supramolecular materials that demonstrate stress-relaxation, and adjustable and recyclable ligands. Cellular movement serves as an input by placing stress upon the material network, which leads to reorganization of the network and in turn provides space for morphogenetic behavior. Incorporating reciprocal biochemical elements into a material that modulate developmental signaling pathways would allow accurate spatiotemporal control of cellular differentiation. In this manner, biomaterials gain a biological instructive role that goes beyond their initial role of cellular adhesion. Development of such complex materials would greatly advance both the organoid- and biomaterial field.

Conclusion and future directions

Kidney organoids are plagued by off-target populations and deficient maturation. The differentiation protocols are currently being re-invented to solve these issues. However, the role and effect of material properties on lineage commitment of hiPSCs is unrepresented in this process. The extent to which the mechanical environment affects developmental programs is significant and deserves consideration. As such, we propose that biomaterials need to be used as an additional tool to control organoid development. Kidney organoids are now generated on rigid materials that do not change over time. Instead, this process would benefit from a soft and mechanically compliant material— as this enhances induction of mesodermal lineages. Furthermore, static materials are no longer sufficient for complex cellular aggregates that evolve in composition and behavior over time. A dynamic material is required that ‘grows’ with the organoid. Such a living-type material is created by encoding dynamic reciprocal behavior into a material. Organoid-material reciprocal interactions would allow for accurate spatiotemporal modulation of organoid behavior through dynamic adjustment of both presented ligands, and cell surface receptors. Mechanical reciprocity already exists in supramolecular materials that demonstrate stress-relaxation, and adjustable and recyclable ligands. Cellular movement serves as an input by placing stress upon the material network, which leads to reorganization of the network and in turn provides space for morphogenetic behavior. Incorporating reciprocal biochemical elements into a material that modulate developmental signaling pathways would allow accurate spatiotemporal control of cellular differentiation. In this manner, biomaterials gain a biological instructive role that goes beyond their initial role of cellular adhesion. Development of such complex materials would greatly advance both the organoid- and biomaterial field.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Takasato M, et al.: Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. Nature 2015, 526:564–568.
2. Taguchi A, Nishinakamura R: Higher-order kidney organogenesis from pluripotent stem cells. Cell Stem Cell 2017, 21:730–746. e6.
3. Freedman BS, et al.: Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. Nat Commun 2015, 6.
4. Morizane R, et al.: Nephron organoids derived from human pluripotent stem cells model kidney development and injury. Nat Biotechnol 2015, 33:1193–1200.
5. Low JH, et al.: Generation of human PSC-derived kidney organoids with patterned nephron segments and a de novo vascular network. Cell Stem Cell 2019, 25:373–387. e9.
6. Garreta E, et al.: Fine tuning the extracellular environment accelerates the derivation of kidney organoids from human pluripotent stem cells. Nat Mater 2019. https://doi.org/10.1038/s41563-019-0287-6.
7. Nishinakamura R: Human kidney organoids: progress and remaining challenges. Nat Rev Nephrol 2019, 9.
8. Taguchi A, Nishinakamura R: Higher-order kidney organogenesis from pluripotent stem cells. Cell Stem Cell 2017, 21:730–746. e6.
9. Maurer M, Lammerding J: The driving force: nuclear mechatransduction in cellular function, fate, and disease. Annu Rev Biomed Eng 2019, 21:443–468.
10. van den Berg CW, et al.: Renal subcapsular transplantation of * PSC-derived kidney organoids induces neo-vasculogenesis and significant glomerular and tubular maturation in vivo. Stem Cell Rep 2018, 10:751–765.
11. Kratochvil MJ, et al.: Engineered materials for organoid systems. Nat Rev Mater 2019. https://doi.org/10.1038/s41578-019-0129-9.
12. Eroshkin FM, Zaraisky AG: Mechano-sensitive regulation of gene expression during the embryonic development. Genesis 2017, 55:1–8.
13. Deforest CA, Anseth KS: Advances in bioactive hydrogels to probe and direct cell fate. Ann Rev Chem Biomol Eng 2012, 3:421–444.
14. Ireland RG, et al.: Combinatorial extracellular matrix micro-array identifies novel bioengineered substrates for xenofree culture of human pluripotent stem cells. Biomaterials 2020, 248:120017.
15. Gjorevski N, et al.: Designer matrices for intestinal stem cell and organoid culture. Nature 2016, 539:560–564.
16. Huettner N, Dargaville TR, Forget A: Discovering cell-adhesion peptides in tissue engineering: beyond RGD. Trends Biotechnol 2018, 36:372–383.
17. Beningo KA, Dembo M, Wang YL: Responses of fibroblasts to anchorage of dorsal extracellular matrix receptors. Proc Natl Acad Sci U S A 2004, 101:18024–18029.
18. Ballester-Beltrán J, Moratal D, Lebourd M, Salmerón-Sánchez M: Fibronectin-matrix sandwich-like microenvironments to manipulate cell fate. Biomater Sci 2014, 2:381–389.
Rowland TJ, et al.: Roles of integrins in human induced pluripotent stem cell growth on Matrigel and vitronectin. Stem Cell Dev 2010, 19:1231–1240.

Jiang C, et al.: Screening of pure synthetic coating substrates for induced pluripotent stem cells and iPSC-derived neuro-epithelial progenitors with short peptide based integrin array. Exp Cell Res 2019, 380:90–99.

Kreiberg JA, et al.: Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. Development 1996, 122: 3537–3547.

Zent R, et al.: Involvement of laminin binding integrins and laminin-5 in branching morphogenesis of the ureteric bud during kidney development. Dev Biol 2001, 238:289–302.

Chen D, et al.: Differential expression of collagen- and laminin-binding integrins mediates ureteric bud and inner medullary collecting duct cell tubulogenesis. Am J Physiol - Ren Physiol 2004, 287:602–611.

Hamerksi DA, Santoro SA: Integrons and the kidney: biology and pathobiology. Curr Opin Nephrol Hypertens 1999, 8:9–14.

Wada J, et al.: Cloning of mouse integrin alpha v cDNA and role of the alpha v-related matrix receptors in metastatic development. J Cell Biol 1996, 132:1111–1176.

O’brien LL, et al.: Wnt11 directs nephron progenitor polarity and motile behavior ultimately determining nephron endowment. Elife 2018, 7:1–25.

Sato Y, et al.: Molecular basis of the recognition of nephronectin by integrin alpha6beta1. J Biol Chem 2009, 284: 14524–14536.

Caiado F, Dias S: Endothelial progenitor cells and integrins: adhesive needs. Fibrogenesis Tissue Repair 2012, 5:4.

Engler AJ, Sen S, Sweeney HL, Discher DE: Matrix elasticity directs stem cell lineage specification. Cell 2006, 126: 677–689.

Madi CM, Heilshorn SC: Engineering hydrogel microenvironments to recapitulate the stem cell niche. Annu Rev Biomed Eng 2018, 20:21–47.

Chen YF, et al.: Control of matrix stiffness promotes endothelial lineage specification by regulating SMAD2/3 via IncRNA LINC00458. Sci Adv 2020, 6:

Przybyla L, Lakins JN, Weaver VM: Tissue mechanics orchestrate wnt-dependent human embryonic stem cell differentiation. Cell Stem Cell 2016, 19:462–475.

Estarás C, Hsu HT, Huang L, Jones KA: YAP repression of the WNT3 gene controls hESC differentiation along the cardiac mesoderm lineage. Genes Dev 2017, 31:2250–2263.

Das A, Fischer RS, Pan D, Waterman CM: YAP nuclear localization in the absence of cell-cell contact is mediated by a filamentous actin-dependent, Myosin Ilband Phospho-YAP-independent pathway during extracellular matrix mechanosensing. J Biol Chem 2016, 291:6096–6110.

Estarás C, Benner C, Jones KA: SMADs and YAP compete to control elongation of β-catenin; LEF1-Recruited RAPN1 during hESC differentiation. Mol Cell 2014, 58:780–793.

Beyer TA, et al.: Switch enhancers interpret TGF-β and Wnt11 directs nephron progenitor polarity and motile behavior ultimately determining nephron endowment. Elife 2018, 7:1–25.

Yang C, Tibbitt MW, Basta L, Anseth KS: Mechanical memory and dosing influence stem cell fate. Nat Mater 2014, 13: 645–652.

Wu H, et al.: Comparative analysis and refinement of human PSC-derived kidney organoid differentiation with single-cell transcriptomics. Cell Stem Cell 2018.1–13. https://doi.org/10.1016/j.stem.2018.10.010.

Subramanian A, et al.: Single cell census of human kidney organoids shows reproducibility and diminished off-target cells after transplantation. Nat Commun 2019, 10.
62. DeForest CA, Tirrell DA: A photoreversible protein-patterning approach for guiding stem cell fate in three-dimensional gels. Nat Mater 2015, 14:523–531.

63. Gawade PM, Shadish JA, Badeau BA, DeForest CA: Logic-based delivery of site-specifically modified proteins from environmentally responsive hydrogel biomaterials. Adv Mater 2019, 31:1–6.

64. Khetan S, Burdick JA: Patterning hydrogels in three dimensions towards controlling cellular interactions. Soft Matter 2011, 7:830–838.

65. Vega SL, et al.: Combinatorial hydrogels with biochemical gradients for screening 3D cellular microenvironments. Nat Commun 2018, 9:1–10.