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Synthetic alternatives to Matrigel

Elizabeth A. Aisenbrey[1], **William L. Murphy**[2,3,†]

[1]Department of Surgery, University of Wisconsin–Madison, WI, USA.

[2]Department of Biomedical Engineering, University of Wisconsin–Madison, WI, USA.

[3]Department of Orthopedics and Rehabilitation, University of Wisconsin–Madison, WI, USA.

Abstract

Matrigel, a basement-membrane matrix extracted from Engelbreth–Holm–Swarm mouse sarcomas, has been used for more than four decades for a myriad of cell culture applications. However, Matrigel is limited in its applicability to cellular biology, therapeutic cell manufacturing and drug discovery owing to its complex, ill-defined and variable composition. Variations in the mechanical and biochemical properties within a single batch of Matrigel — and between batches — have led to uncertainty in cell culture experiments and a lack of reproducibility. Moreover, Matrigel is not conducive to physical or biochemical manipulation, making it difficult to fine-tune the matrix to promote intended cell behaviours and achieve specific biological outcomes. Recent advances in synthetic scaffolds have led to the development of xenogenic-free, chemically defined, highly tunable and reproducible alternatives. In this Review, we assess the applications of Matrigel in cell culture, regenerative medicine and organoid assembly, detailing the limitations of Matrigel and highlighting synthetic scaffold alternatives that have shown equivalent or superior results. Additionally, we discuss the hurdles that are limiting a full transition from Matrigel to synthetic scaffolds and provide a brief perspective on the future directions of synthetic scaffolds for cell culture applications.

Toc Blurb

Matrigel is widely used for cell culture; however, its ill-defined composition, batch-to-batch variability, and animal-derived nature lead to experimental uncertainty and a lack of reproducibility. In this Review, we discuss the limitations of Matrigel and highlight synthetic alternatives for stem cell culture, regenerative medicine and organoid assembly.

† wlmurphy@wisc.edu.

Author contributions

All authors contributed equally to the preparation of this manuscript.

Competing interests

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Introduction

The origin of Matrigel dates back more than 40 years to the discovery of a murine tumour that produced large quantities of extracellular matrix (ECM) proteins reminiscent of a basement membrane[1] — a specific ECM that serves as a structural support for cells in most epithelial and endothelial layers[2]. Later named the Engelbreth–Holm–Swarm (EHS) tumour, extracts from this basement-membrane-producing tumour were developed and marketed as Matrigel or EHS matrix[3-5] (herein referred to as Matrigel). The primary components of Matrigel are four major basement membrane ECM proteins: laminin (~60%), collagen IV (~30%), entactin (~8%) and the heparin sulfate proteoglycan perlecan (~2–3%)[6]. Multiple isoforms of laminin have been identified in Matrigel, including β2, α5, α3 and α4, with the most predominate being α1, β1 and γ1, which make up the heterotrimer laminin 1 (also known as laminin 111)[7,8]. Laminin 1 contains multiple adhesion sites for the attachment of various cell types, including stem, epithelial, endothelial and tumour cells[1,8-12]. Moreover, the laminin-1-derived peptides Ile-Lys-Val-Ala-Val (IKVAV) and Try-Ile-Gly-Ser-Arg (YIGSR) promote differentiation[13,14] and angiogenesis[11,15], as well as tumour growth and metastasis[16,17]. Although collagen IV is most abundant, other collagens found in Matrigel include collagen I, XVIII, VI and III[7]. Matrigel also contains tumour-derived proteins, including growth factors, such as transforming growth factor (TGF) family peptides (for example, TGF-β) and fibroblast growth factors (FGFs)[18,19], as well as enzymes, such as matrix metalloproteinases (MMPs)[5,20]. Collectively, these structural and biological proteins contribute to the biological function of Matrigel.

During preparation, the reconstituted form of Matrigel undergoes gelation at temperatures in the range 22–37 °C, during which entactin acts as a crosslinker between the laminin and collagen IV to create a hydrogel — a water-swollen, crosslinked network. Owing to its inherent bioactivity, Matrigel has been used for various applications for different cell types. As a thin gel coating, Matrigel has been used to culture and expand cells, such as human pluripotent stem cells (hPSCs)[21], neurons[22,23] and cardiomyocytes[24]. Thicker Matrigel coatings have been used to develop assays to investigate endothelial tubulogenesis[25,26], and 3D Matrigel constructs allow for cell encapsulation in tissue engineering[27,28] and organoid assembly[29,30]. In these contexts, Matrigel has been a useful, yet perhaps poorly understood, tool for cell culture.

The applicability of Matrigel is, however, severely limited by the variability in its composition and the presence of xenogenic contaminants. Indeed, multiple reports have indicated a need to use caution in interpreting results based on Matrigel-cultured cells[18,31]. However, researchers continue to use Matrigel for cell culture owing to its availability, ease of use and versatility for culturing different types of cells. The ubiquitous use of Matrigel may also in part be due to a historical lack of comparable synthetic alternatives. However, recently developed synthetic materials have shown results equivalent or superior to those of Matrigel. These synthetic alternatives can provide a chemically defined, xenogenic-free environment that can be modified for desired outcomes and provide reproducible results. In particular, synthetic materials used for cell culture, often termed scaffolds, have been designed and developed for stem cell culture, tissue engineering and organoid assembly for toxicant and therapeutic screening (Fig. 1).

In this Review, we begin by briefly discussing the limitations of Matrigel, before assessing the use of Matrigel in three specific areas of research: stem cell culture, regenerative medicine and organoid assembly. For each application, we highlight key studies in which the performance of synthetic scaffolds has been directly compared with that of Matrigel and analyse the suitability of synthetic alternatives (Table 1). Lastly, we discuss the current impediments to replacing Matrigel with synthetic scaffolds and provide our perspective on the future of synthetic scaffolds for cell culture applications.

Limitations of Matrigel

Although Matrigel is commonly used as a cell culture tool[7], it is inherently limited in its applicability for fundamental research, therapeutic cell manufacturing and cell-based assays, owing to its complex, ill-defined and variable composition[5,32,33] (Fig. 1a). Inconsistencies in biochemical properties between batches of Matrigel — and within a single batch — has led to uncertainty and a lack of reproducibility in cell culture experiments[18,19,34,35]. More than 14,000 unique peptides and nearly 2,000 unique proteins have been identified in Matrigel[4-6,8,13,14]. The majority of those identified are structural proteins, but others include growth factors[7,18,19], transcription factors[7] and cytokines[19]. Numerous proteomic analyses on Matrigel have revealed considerable variability, with each new study discovering proteins that have not yet been recorded or not detecting proteins that had been previously reported[7,18,19,36,37]. For example, in one study, growth factors such as insulin-like growth factor 1 and epidermal growth factor, which are important and promiscuous signalling molecules, were expressed at quantifiable levels (on the order of nanograms per millilitre[18]) but were not detected in four independent Matrigel batches investigated in a later study[19]. The reported concentration of growth factors has also been inconsistent, including an order of magnitude difference in FGF2 and platelet-derived growth factor concentrations between batches[19]. Growth-factor-reduced (GFR) Matrigel is an alternative Matrigel product that is similar in structure to standard Matrigel but with lower growth factor concentrations[18]. When compared, 480 unique proteins were identified in standard Matrigel and 424 in GFR Matrigel, with only a ~53% batch-to-batch similarity in proteins between the two products[7]. This difference in protein content was not only attributed to the lower concentration of growth factors in GFR Matrigel, but also to variations in the structural protein content[7].

The mechanical properties of Matrigel also show batch-to-batch variability. Although some variability in elastic modulus (or ‘stiffness’) can be attributed to different testing methods and temperatures[38-40], inherent variability between batches and within a single batch have been identified[41,42] (Fig. 1a). For example, using atomic force microscopy, the average elastic modulus of two batches of Matrigel was reported to be 400–420 Pa. However, a third batch had an average elastic modulus twice as high (840 Pa)[35]. Moreover, heterogeneities within the ECM resulted in local areas of the Matrigel with even higher elastic moduli (1–3 kPa)[35]. Using in situ mechanical interferometry to analyse local mechanical properties, the median elastic modulus of Matrigel was found to agree well with that of bulk measurements (~650 Pa)[42]. However, on the microscale, the Matrigel was non-uniform, with regions of higher elastic modulus (1–2 kPa)[42]. Optical thickness images revealed that these stiffer areas corresponded to areas of higher material density. Variations in the stiffness have also been

attributed to the underlying substrate[34] and the gradual changes in Matrigel thickness over time, perhaps caused by ECM remodelling[42].

Another complexity inherent in Matrigel is the potential for antigenicity. The introduction of xenogenic contaminants from an animal-derived ECM such as Matrigel may limit the therapeutic potential of cells or tissues expanded in Matrigel-containing culture. Evidence of viral contamination, specifically lactate dehydrogenase-elevating virus (LDHV), has been found in multiple batches of animal-derived ECM products, including Matrigel[43,44]. LDHV is a natural mouse virus that infects macrophages and can affect the immune system and tumour behaviour[44-46]. Matrigel's complexity and animal origin may also interfere with mechanistic studies of cell behaviour, making it difficult to distinguish biological effects caused by controlled experimental variables from those caused by Matrigel itself. The ambiguity in experimental results and the presence of xenogenic contaminants are often compounded when serum-containing media is used in conjunction with Matrigel (Box 1).

Synthetic alternatives to Matrigel

The limitations of Matrigel have driven the search for synthetic alternatives. Over the past two decades, numerous synthetic scaffolds, both 2D and 3D, have been developed using synthetic polymers. Unlike Matrigel, the physical, mechanical and biological properties of synthetic polymeric scaffolds can often be tuned independently by altering the composition, molecular weight, crosslinker, crosslink density and method of polymerization[47,48] (Fig. 1b). The density and presentation of biofunctional moieties, often in the form of peptides, can also be controlled[48]. Owing to the diversity of scaffolds that have been designed and developed as alternatives to Matrigel, this Review is limited to describing only some of the key properties of the various scaffolds. An in-depth description of scaffold synthesis and characterization is beyond the scope of this Review. However, because many of the scaffolds presented here are derived from polyacrylamide (PAM) and polyethylene glycol (PEG), we provide an overview of these synthetic materials.

PAM is a synthetic polymer that forms a hydrogel upon reaction of an acrylamide monomer and bis-acrylamide crosslinker in the presence of ammonium persulfate and tetramethylethylenediamine. PAM is uncharged and bioinert, and therefore does not react with proteins or bind directly to cells[49]. However, these materials are commonly used for cell culture[50,51] because the stiffness and biofunctionality of PAM hydrogels can be tuned, enabling user-defined control of cell–material interactions. For instance, cell-adhesion peptides and ECM proteins have been crosslinked to PAM hydrogels to engage cell interactions[49]. Owing to the toxicity of the hydrogel precursors and the polymerization reaction, however, PAM hydrogels are limited to 2D cell culture and cannot be used for 3D cell encapsulation[47].

PEG is one of the most studied and widely used synthetic polymers for the construction of synthetic scaffolds[52,53]. This material is advantageous for cell culture as it is hydrophilic, bioinert and highly amenable to chemical modification[54]. PEG can be modified with diverse functional groups and formed into hydrogels using various polymerization techniques[55,56]. PEG hydrogels are often formed through photopolymerization, whereby multiarm PEG

chains are functionalized with reactive groups (such as acrylate, norbornene or thiol), combined with a photoinitiator and then exposed to UV or visible light[57-59]. Other polymerization methods include Michael addition reactions, including the thiol-Michael addition reaction[60], and enzymatic reactions using, for example, the activated transglutaminase enzyme factor XIIIa[61,62]. These polymerization techniques are typically non-toxic, which allows for cell encapsulation within the forming hydrogel[47,63-65]. Additionally, the thiol–ene chemistry permits cysteine-containing peptides, either as pendant peptides or crosslinkers, to be covalently tethered to the polymer, thus introducing biofunctionality into the otherwise inert system[57,66].

Pluripotent stem cell culture

hPSCs, including embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs), proliferate indefinitely and maintain their ability to differentiate into cells from all three germ layers (the endoderm, mesoderm and ectoderm) when cultured in appropriate conditions[67]. The ability to expand and generate large numbers of hPSCs in vitro has great potential to serve as a feedstock for applications in disease modelling, drug screening and cellular therapies[68-71]. When they were first isolated, hESCs needed to be cultured on a feeder layer of mouse embryonic fibroblasts to maintain their pluripotency[67]. However, this method inevitably resulted in complications associated with co-culture, including the need to remove animal-derived contaminants. Matrigel was used in initial efforts to eliminate embryonic fibroblast feeder layers, and a pivotal study showed that it supported proliferation and maintenance of the stem cell phenotype of hESCs, as determined by a normal karyotype and high telomerase activity for up to 130 population doublings[21]. Although the use of Matrigel removed some complications associated with mouse fibroblast co-culture, it did not entirely rid the cultures of xenogeneic components that are undesirable for hPSC clinical applications[72]. Moreover, the ill-defined, animal-derived nature of Matrigel can influence cellular behaviour[5,18], ultimately calling into question conclusions derived from stem cells grown on Matrigel.

Synthetic scaffolds that support hPSC proliferation and maintenance at similar or superior levels to those of Matrigel have been developed. For instance, the zwitterionic polymer, poly(2-(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl)ammonium hydroxide) (PMEDSAH) was the first fully synthetic polymer coating reported to sustain long-term culture of hESCs. The physical properties of the synthetic coating, including the hydrophilicity, thickness and surface charge, can be altered by varying the mode of polymerization and reaction time. Collectively, these physical properties influence the self-renewal of the hESCs[73]. Compared with Matrigel, the hESCs cultured on the PMEDSAH coating had a similar gene expression profile after 20 passages[74,75]. In another study, 90 polymers, varying in chemical composition and molecular weight, were evaluated for their ability to support the pluripotency of hPSCs. Of those screened, 16 polymers performed similarly to Matrigel and supported short-term proliferation and maintenance of hPSC pluripotency. However, poly(methyl vinyl ether-*alt*-maleic anhydride) was the only polymer to sustain long-term hPSC culture while reducing spontaneous differentiation of hESC and hiPSC lines to a similar extent as Matrigel[76]. Although the mechanism by which the polymer coating sustained long-term culture was not investigated, it was postulated that the

anionic nature of the synthetic polymer mimics the structural and functional features of heparin, including its propensity for growth-factor binding, which may have a central role in regulating the self-renewal of hESCs.

Since these initial discoveries, various synthetic scaffolds have been developed to recapitulate the key cell–matrix interactions necessary for maintaining hPSC pluripotency. In addition to the physical properties, such as stiffness, topography and surface charge, the biochemical properties of the cellular microenvironment, including cell adhesivity, biochemical functionality and degradability, also have a key role in stem cell fate. Unlike Matrigel, the biochemical properties of synthetic scaffolds can also be tuned.

The cell–matrix interactions crucial for hPSC expansion and pluripotency can be reconstructed on synthetic scaffolds by incorporating cell-adhesion motifs. Integrin receptor subunits involved in hPSC adhesion to Matrigel include α5, α6, αv, β1 and β5 (refs[7,34,77,78]). Peptides that bind to these integrin receptors have been developed and presented on synthetic scaffolds in different combinations to promote cell adhesion and proliferation for long-term hPSC culture[32,77]. One of the most ubiquitously used peptides to encourage cell adhesion to synthetic scaffolds is the fibronectin-derived three-amino-acid peptide Arg-Gly-Asp (RGD), which binds to both αvβ3 and αvβ5 integrins[79]. In one study, RGD and a range of other peptides were covalently tethered to poly(acrylamide-*co*-propargyl acrylamide) (PAPA) brushes[80]. The PAPA coatings were prepared through photoinitiator-free photopolymerization using high-intensity UV light. Unlike Matrigel and other naturally derived scaffolds, the PAPA brushes offer a stable surface coating that has a longer shelf-life, are modifiable and can be sterilized using industry standard methodologies. A cyclic form of RGD , cyclo(Arg-Gly-Asp-D-Phe-Lys) (cRGDfK), was identified as the most effective peptide for hPSC culture; the cRGDfK peptide compared favourably with other peptides derived from laminin, fibronectin and vitronectin. The cRGDfk-coupled PAPA-coated scaffold maintained long-term undifferentiated cultures of three independent hPSC lines, similar to what is observed with Geltrex[80] (the GFR Matrigel produced by Gibco), and eliminated karyotypic abnormalities observed in Geltrex-cultured cells. Moreover, cyclic RGD in a different form, cyclo(Arg-Gly-Asp-D-Phe-Cys), has also supported short-term hESC expansion[81]. Through high-throughput screening of an array of 64 PEG thiol–norbornene synthetic hydrogels of varying stiffness and cyclic RGD concentrations, several hydrogel formulations were identified that showed similar or enhanced maintenance of hESC pluripotency, as evaluated by NANOG expression, relative to that of hESCs cultured on Matrigel. One hydrogel formulation, containing 2 mM of cyclic RGD and with a modulus of 10 kPa, supported hESC expansion and pluripotency, even in the absence of a rho-associated protein kinase (ROCK) inhibitor, which is typically needed to maintain hPSC adhesion and expansion[81].

In addition to RGD, other peptides, such as those derived from vitronectin (an ECM glycoprotein abundant in serum[82] and present in Matrigel in only trace amounts[6]), have been tethered to synthetic scaffolds and the resulting materials investigated for their ability to maintain hPSC pluripotency. In one study, peptide sequences derived from natural ECM proteins, including laminin, bone sialoprotein and vitronectin, were conjugated to synthetic peptide–acrylate surfaces and screened for their ability to culture undifferentiated hESCs.

Surfaces conjugated to the vitronectin-derived peptide supported hESC pluripotency to a level comparable to that of Matrigel for more than ten passages[83]. Moreover, a film composed of a copolymer of oligo-(ethylene glycol) methacrylate and 2-hydroxy-ethyl methacrylate (poly(OEGMA-co-HEMA)) functionalized with a vitronectin-derived peptide supported hiPSC self-renewal at a level similar to that of Matrigel for ten passages, but in a xeno-free and chemically defined media[84]. In a separate study, hPSC culture was investigated on poly(vinyl alcohol-*co*-itaconic acid) hydrogels of varying elasticities and grafted with a vitronectin-derived peptide. A hydrogel with an elasticity of 25 kPa and grafted with high concentrations (500–1,500 μg ml^{-1}) of the vitronectin-derived peptide maintained hiPSC and hESC culture at levels similar to those of Matrigel for more than 20 passages under xeno-free conditions[85]. Synthemax, a commercially available, synthetic vitronectin scaffold functionalized with RGD, also supported hiPSC self-renewal to a similar extent as Matrigel[86] but in chemically defined and growth-factor-free conditions[87].

Synthetic scaffolds have also been used to mimic the role of heparin sulfate proteoglycans such as perlecan, a major component of Matrigel[88], to support hPSC culture. Evidence suggests that heparin sulfate proteoglycans have a key role in maintaining the self-renewal of hPSCs, owing to their ability to bind to soluble basic fibroblast growth factor (bFGF), a crucial growth factor required for hPSC maintenance, and to protect bFGF from denaturation and proteolytic degradation[89-91]. In one study, a heparin-mimicking synthetic hydrogel was developed by copolymerizing poly(sodium 4-stryenesulfonate) (PSS) with PAM at different ratios. The resulting heparin-mimetic scaffold, PAM_6-*co*-PSS_2, supported long-term hPSC expansion and maintained pluripotency similar to Matrigel, as defined by NANOG and OCT4 expression, for more than 20 passages in a chemically defined media[92]. In addition, synthetic scaffolds that display proteoglycan-binding peptides, which can interact with glycosaminoglycans found on the surface of cells, are effective for sustained stem cell renewal[93,94]. For instance, PAM hydrogel scaffolds functionalized with a vitronectin-derived, glycosaminoglycan-binding peptide maintained hPSC pluripotency with similar gene expression profiles to those cultured on Matrigel. However, long-term hESC proliferation on these functionalized hydrogels was stiffness dependent: hESCs cultured on stiff hydrogels (10 kPa) proliferated into robust colonies whereas those on softer hydrogels (0.7 kPa and 3 kPa) eventually detached[94].

hPSC culture and expansion using synthetic scaffolds has been extended from 2D surface coatings to 3D systems to further encourage pluripotency and self-renewal[95-97]. In contrast to 2D culture, the 3D environment allows for control over cell morphology and enhanced cell–cell interactions, which are both potent regulators of stem cell growth and phenotype[98,99]. For example, 3D PEG hydrogel scaffolds with customized stiffnesses, degradability and biochemical composition have promoted mouse ESC proliferation and hiPSC generation from somatic cells[97,100]. In one study, MMP-degradable, RGD-functionalized PEG hydrogel scaffolds, developed using factor-XIIa-mediated crosslinking of peptide-functionalized PEG monomers, increased the reprogramming efficiency of human fibroblasts into hiPSCs by 2.5-fold compared with a conventional 2D culture[100]. The 3D synthetic scaffold also supported homogenous hiPSC colony generation, which was not achievable in 3D Matrigel or 3D collagen scaffolds[100]. In a separate study, integrin-binding peptides — inspired by motifs involved in iPSC binding to Matrigel — were presented on a

photopolymerized PEG thiol–ene hydrogel scaffold for 3D hiPSC culture[97]. The presentation of both a laminin-derived peptide, YIGSR, and an αvβ5-binding RGD-containing peptide on the scaffold were key to hiPSC pluripotency and enabled downstream differentiation into neural progenitor cells[97]. In both studies, the cell–matrix interactions that supported hiPSC culture in the 3D systems were different from those that supported culture in the 2D systems, indicating that stem cell–matrix interactions are system dependent[101].

Regenerative medicine

Stem cell differentiation

Interest in stem cells has increased owing to their tremendous potential for developing treatments in regenerative medicine[102-104]. However, before stem-cell-based therapies can be taken from ‘bench to bedside’, challenges associated with stem cell culture, such as directing lineage-specific stem cell differentiation, producing homogenous cell populations and ensuring localized in vivo delivery, must be addressed[105]. Various strategies have been developed to overcome these issues, including the development of cell culture environments that instruct stem cell behaviour. It is widely accepted that stem cell fate is directly affected by the interaction of the cells with their surrounding ECM[98], whereby factors such as the composition, mechanics and architecture of the ECM act in concert to give rise to a series of spatially and temporally coordinated events that regulate cell differentiation and function. To unlock the full potential of stem cells in vitro, it has been posited that aspects of their in vivo native 3D environment must be reconstructed to provide the necessary cues[106,107]. Owing to the ill-defined composition of Matrigel, it is difficult to match the properties of Matrigel to the specific ECM requirements for different tissue types, and its spatially heterogeneous properties do not provide the tightly governed, spatio-temporal cues found during stem cell differentiation in vivo[108,109] (Fig. 2). Together, these drawbacks limit the ability to control stem cell differentiation in Matrigel-based cultures. As an alternative to Matrigel, synthetic scaffolds have been used to identify appropriate environments to differentiate stem cells, maintain differentiated cell phenotypes and produce homogenous cell populations (Fig. 3).

The advent of highly tunable synthetic scaffolds has made it possible for researchers to probe the role of mechanical and biochemical factors on stem cell fate. Notably, parameters such as scaffold stiffness and degradability, as well as the presence of tethered cell-adhesion peptides and growth factors, can be systematically varied to customize materials to encourage stem cell differentiation[100,110,111]. For example, self-assembled peptide nanofibre hydrogels, consisting of a peptide sequence derived from brain ECM that is known to inhibit neuronal apoptosis, supported stem cell differentiation into neurons, astrocytes and oligodendrocytes[112]. The synthetic hydrogel scaffolds also stimulated neuronal cell attachment, neurite outgrowth and the formation of active and functional synapses, overall showing superior cell survival and differentiation properties than those of Matrigel or collagen scaffolds[112]. Moreover, photopolymerizable PEG thiol–ene hydrogel scaffolds with cysteine-flanked MMP-sensitive crosslinks to encourage endothelial differentiation and vascular morphogenesis demonstrated similar gene expression profiles to those of cells cultured on Matrigel[113]. Several other synthetic hydrogel scaffolds have been found to

support stem cell differentiation[114-116]; however, as they were not directly compared with Matrigel, they are not discussed in this Review.

In addition to biochemical cues, lineage-specific differentiation of stem cells is highly sensitive to mechanical and physical stimuli, such as scaffold stiffness[117,118]. Cell culture methods that recapitulate the stiffness of the natural tissue environment can direct stem cell differentiation. Soft scaffolds that mimic the elastic modulus of the brain (0.1–1 kPa) can be neurogenic, scaffolds of intermediate stiffness that mimic skeletal muscle (8–17 kPa) can be myogenic, and rigid scaffolds that mimic bone (25–40 kPa) can be osteogenic[118]. In comparison, Matrigel stiffness is relatively low (with an elastic modulus of ~400 Pa)[35] and differs from that of most tissue-specific ECMs[35]. Although Matrigel stiffness can be increased slightly by increasing the overall protein concentration, this alters the biochemical composition and, thus, alters the biological functionality[119]. By contrast, the stiffness of synthetic hydrogel scaffolds can be varied over a wider range while maintaining their biochemical functionality[110,120-122]. For instance, in one study, the biochemical composition of a PEG hydrogel scaffold used to support adipogenic differentiation of human mesenchymal stem cells was identical to what was needed to support osteogenic differentiation, but the substrate stiffness requirements were drastically different[120]. The modularity of the PEG hydrogel scaffolds meant that the biochemical composition could be maintained while the stiffness of the scaffold was varied, enabling the physical and biological cues to be decoupled and the development of tissue-specific synthetic scaffolds.

Complex, yet defined, architectures that mimic cell morphologies and cell–matrix interactions in native tissues can also be achieved using synthetic scaffolds. Techniques such as electrospinning[123,124], micropatterning[125,126] and 3D printing[127] have been developed to produce synthetic scaffolds that mimic the ECM down to the nanometre scale. These techniques have been used in several studies to control stem cell differentiation and/or maintain cell phenotype for a wide range of applications, although studies that report a direct comparison with Matrigel are limited. However, in one example, electrospun synthetic polyamide nanofibres, consisting of two polyamide polymers ($(C_{28}O_4N_4H_{47})_n$ and $(C_{28}O_{4.4}N_4H_{47})_n$), promoted murine and human ESC and iPSC differentiation into functional hepatocytes. With these synthetic materials, the expression of hepatocyte-specific genes and albumin secretion was higher than on Matrigel or collagen, owing to manipulation of the cellular morphology[128].

Emerging applications in regenerative medicine require pure populations of defined cells to be manufactured, but achieving this using Matrigel has been difficult[100,129,130] (Fig. 4). Owing to heterogeneities between batches of Matrigel and within a single batch, the cells can experience different microenvironments, which can lead to different cell fates. In a 2017 protocol for the directed differentiation of iPSCs into functional cholangiocytes, variability in the differentiation efficiency between Matrigel batches was observed[131]. Heterogeneities have also been reported in neuroepithelial differentiation; colonies cultured on Matrigel were highly dissimilar in morphology and size, and exhibited both epithelial and mesenchymal phenotypes[132]. By contrast, colonies developed on PEG hydrogel scaffolds, generated using factor-XIIIa-mediated crosslinking, were homogenous and led to a pure population[132]. The mixed population present in Matrigel-cultured cells is postulated to be due to conflicting

signals present in Matrigel that are not found in the chemically defined PEG. Furthermore, through manipulation of various properties, such as biofunctionalization with specific cell-binding peptides or enzymatically degradable crosslinks, synthetic scaffolds have been used to select for, or against, a certain cell type to achieve a more homogenous final population[109,132].

In vivo tissue regeneration

Scaffolds for tissue regeneration must provide a stable and supportive vehicle to deliver cells to the desired location in vivo. Materials that can be injected directly into the desired location (that is, the defect site), form a scaffold in situ and achieve a seamless transition from a cell-laden scaffold to neotissue are desirable, but require precise control of the formation and degradation of the material[133,134] (Fig. 3). Matrigel gelation cannot be precisely controlled as it occurs over a wide temperature range (22–37 °C) and on timescales ranging from minutes to hours[7,42]. Protocols suggest gelling at physiological temperature, but gelation can occur at room temperature, making Matrigel difficult to prepare and handle in clinical settings[40]. Moreover, Matrigel degradation is not controllable. Matrigel degrades by exposure to MMPs, such as MMP2 and MMP9, but heterogeneities in Matrigel composition and crosslink density can result in unpredictable and non-uniform degradation[135-137]. This heterogeneous degradation jeopardizes the bulk material properties of Matrigel and limits its mechanical integrity[138]. Additionally, Matrigel contains growth factors and cytokines that can induce cell migration and angiogenesis, resulting in undesirable degradation and blood vessel formation when implanted in vivo[139,140].

Synthetic scaffolds can substantially reduce complications associated with the in vivo administration of Matrigel. Some synthetic polymer precursors can be injected directly into a defect site, polymerized in situ and provide encapsulated cells with a space-filling scaffold that enables cells to produce their own ECM while simultaneously degrading the surrounding synthetic scaffold[141-143]. For instance, materials have been designed to photopolymerize on timescales on the order of seconds to ensure controlled cell delivery, and their ease of use has made them popular for tissue engineering applications[59,64,144]. Synthetic scaffolds can be designed to undergo multiple modes of degradation, including hydrolytic, enzymatic, physical (for example, thermal or pH) or a combination thereof[145]. Unlike Matrigel, the rate of degradation of these synthetic scaffolds can be tuned to match the rate of ECM deposition by manipulating the polymer concentration, crosslink density and peptide lability to ensure mechanical stability[133,134].

In multiple in vivo studies, injectable synthetic scaffolds have shown similar, and in some instances better, tissue regeneration than Matrigel, demonstrating enhanced cell viability, engraftment and neotissue formation. For example, an enzymatically degradable, PEG–maleimide hydrogel functionalized with RGD was established as a cell-delivery system for treating muscle trauma in dystrophic mice. Specifically, mouse muscle satellite cells were encapsulated in the PEG hydrogel and delivered directly into the injured muscle. Compared with cells encapsulated in Matrigel or collagen, the hydrogel-delivered cells showed superior in vivo survival, proliferation and engraftment[146]. In another comparative study, six synthetic scaffolds derived from maltodextrin and of varying polymer molecular weight,

crosslink density and RGD concentration were evaluated for their ability to serve as a vehicle and niche to transport mouse myoblasts in vivo[147]. After injection, a synthetic scaffold that supported skeletal myotubule formation similar to that in Matrigel-treated mice was identified. Injectable synthetic scaffolds can also be combined with other synthetic materials, such as microparticles or nanoparticles, to further direct cellular behaviour. For instance, mouse myoblasts encapsulated within a nanocomposite hydrogel scaffold comprising the biodegradable copolymer poly(lactide-*co*-glycolide)-*b*-poly(ethylene glycol)-*b*-poly(lactide-*co*-glycolide) (PLGA–PEG–PLGA) and synthetic clay nanoparticles (Laponite) were used to treat skeletal muscle injuries, in vivo, in a mouse model[148]. In comparison to those treated with Matrigel or the PLGA–PEG–PLGA scaffold without the nanoparticles, the mice treated with the nanocomposite hydrogel scaffold exhibited considerably greater muscle tissue regeneration and functional recovery[148]. Although it was not investigated, it was postulated that the Laponite nanoparticles provide a large surface area and a highly anisotropic charged surface to facilitate strong adsorption of bioactive proteins and polysaccharides in situ, which can regenerate the native microenvironment and provide necessary cues to initiate tissue regeneration.

Organoid assembly

Organoids are stem cell or progenitor-derived tissues that exhibit key features found in organs in vivo, including characteristic tissue architecture, gene expression, cell function and multicellular complexity[29,149-151]. Within the past decade, notable progress has been made in developing various human organoids, including brain[30,132], kidney[152,153], retina[154], lung[155,156], prostate[157], liver[153,158,159] and gastrointestinal tissues[156,160-163]. These organoids have the potential to model embryonic development and disease, provide an in vitro platform for drug discovery and toxicity testing and serve as an implantable, cell-based therapy for tissue regeneration. Many of the organoid assembly protocols developed to date rely on the spontaneous differentiation and self-organization of cells, cell aggregates or embryoid bodies encapsulated in 3D Matrigel scaffolds[160,164]. However, owing to the inherent heterogeneity of Matrigel, this technique often results in batch-to-batch variability and organoids that are developmentally immature. The use of Matrigel in organoid culture also makes it difficult to decouple toxic or therapeutic effects from effects induced by the matrix itself[81] (Fig. 4). Although the tremendous potential of organoids as a scientific and therapeutic tool remains, the lack of control over organoid formation, owing to the poorly defined Matrigel scaffold in which they are grown, impedes their advancement.

Scaffolds for organoid assembly

Synthetic scaffolds can be used to guide differentiation and influence organoid formation in a reproducible and controlled manner by recapitulating key cell–matrix interactions (Figs 2, 4). For example, a PEG hydrogel scaffold, crosslinked using factor XIIIa, was developed for the formation of neuroepithelial tubule organoids[132], which required a scaffold of intermediate stiffness, non-degradable crosslinks and the presentation of laminin-derived peptides. A PEG–maleimide hydrogel scaffold, generated through Michael addition, was used to develop Madin–Darby canine kidney (MDCK) cyst organoids. Although the scaffold stiffness required for MDCK cyst organoid formation was the same as that for the formation

of neuroepithelial tubule organoids (~4 kPa), the formation of MDCK cyst organoids required RGD in the place of laminin and degradable crosslinks to enable dynamic, cell-mediated remodelling of the microenvironment[109]. Similarly, a highly tunable biohybrid PEG hydrogel scaffold has been modified for a wide range of organotypic culture studies, including renal tubulogenesis, mammary epithelial morphogenesis, Alzheimer disease and acute myeloid leukemia[165-168]. Unlike the other scaffolds described in this Review, this biohybrid PEG scaffold contains the naturally derived glycosaminoglycan heparin and, thus, is not entirely synthetic. However, owing to its highly amenable nature, the scaffold was tuned for each application and in every case outperformed Matrigel[166-168]. These examples support the assertion that Matrigel's 'one-size-fits-all' approach may not be appropriate for diverse organoid formation processes and that alternative synthetic scaffolds may provide superior tools.

One area of organoid research for which there are multiple studies that directly compare synthetic scaffolds with Matrigel is the formation of intestinal organoids from intestinal stem cells (ISCs). For example, the stiffness of a hydrolytically degradable, RGD-containing PEG hydrogel scaffold, generated through factor-XIIIa-mediated crosslinking, could be modulated to encourage ISC maturation. The mechanically dynamic hydrogel scaffold softened as it degraded, permitting the formation of organoids similar to those formed in Matrigel, with a similar gene expression profile, but only in the presence of the animal-derived protein laminin 1 (ref.[163]). A subsequent study reported a fully synthetic maleimide-terminated PEG hydrogel scaffold, polymerized through Michael-type addition, to eliminate the need for laminin 1 (refs[156,161]). In this case, the RGD-functionalized PEG hydrogel scaffold was tailored with protease-degradable crosslinkers to encourage cell-mediated degradation. Similar to intestinal organoids formed in Matrigel, the organoids formed in the PEG hydrogel scaffold remained viable and produced intestinal epithelium that resembled that of mature human intestine. The modular nature of the fully synthetic hydrogel allowed for further adaptation, and the same approach was used to generate other human organoids, such as lung[156]. These modifications of the scaffold are crucial for reproducible and controlled organoid assembly, and are not possible when using Matrigel to support organoids.

Organoid applications

Organoids offer an in vitro platform to evaluate drug efficacy and toxicity, and thereby aid drug discovery[152]. Through the use of patient-derived cells, organoids also offer the potential to accurately predict therapeutic response and guide personalized treatment strategies. However, although multiple types of organoids have been established as preclinical human tissue models, there is notable concern regarding the accuracy and reproducibility of Matrigel-cultured organoids in their response to chemical compounds (Fig. 4). In a study evaluating the effects of known toxicants on vascular tissue assembly by human umbilical vein endothelial cells (HUVECs), a customized PEG-based hydrogel scaffold combined with human endothelial cells was superior to the commonly used Matrigel-based assay in its ability to detect putative vascular disrupting compounds[81]. More than 500 hydrogel scaffolds were screened to identify the customized hydrogel that best supported human vascular tissue assembly by HUVECs, and the same screening approach

also identified custom hydrogel scaffolds for hiPSC-derived endothelial cell assembly, hPSC expansion and human mesenchymal stromal cell expansion[110]. Additional drug-screening studies revealed that Matrigel can strongly influence cell-based assays owing to scaffold-induced effects, such as the introduction of xenogenic contaminants and growth factors into the culture environment[169]. In one study, prostate cancer cells cultured on synthetic polystyrene scaffolds were less responsive to drug treatment than those cultured on Matrigel[170]. By contrast, Matrigel and other naturally derived scaffolds have also been associated with enhanced tumorigenicity and chemotherapeutic drug resistance[171]. For organoids to be used in drug discovery and other cell-based assays, there is an imperative need for reproducible, standardized cell-based assays that are devoid of complicating components such as Matrigel.

Perspective

The importance of cautiously interpreting results from cell cultures that include Matrigel was first acknowledged in 1992 (ref.[18]). However, nearly 30 years later, Matrigel continues to be used for a myriad of applications. Other natural scaffolds that comprise purified proteins (for example, collagen type I, laminin and vitronectin) have been developed and found to be suitable for cell culture studies. However, these naturally derived products are also limited by batch-to-batch variability in composition and structure, as well as the inability to decouple biochemical and mechanical properties[47,56]. There are several potential reasons why Matrigel and other naturally derived products continue to be widely used. Historically, the primary reason has been the lack of synthetic alternatives that support the wide range of cell behaviours thought to be supported by Matrigel. However, the ongoing use of these naturally derived scaffolds can no longer be attributed to a lack of synthetic alternatives, as demonstrated by the range of studies described in this Review and the synthetic scaffolds emerging in the cell culture tools market. Synthetic scaffolds now have highly tunable biological, mechanical and degradation properties, and biofunctionalization can create a unique, fully defined microenvironment to guide stem cell expansion, differentiation or tissue formation. These synthetic scaffolds provide favourable alternatives to Matrigel, and the approaches recently used to customize synthetic scaffolds could result in materials that outperform naturally derived scaffolds.

The cost of a fully defined and synthetic cell culture environment, encompassing the synthetic scaffold and the chemically defined media, remains prohibitive. Although the cost of the raw materials to make PEG hydrogels is about half that of Matrigel[161], the need for one or more synthetic peptides to provide the necessary biochemical cues to drive cellular behaviour can be prohibitively expensive for large-scale production[171]. However, recent advances in synthetic peptide synthesis and purification are generating more cost-effective options[171,172]. Another cost consideration when moving towards chemically defined cell culture environments is the requirement for recombinant growth factors, which are often found in Matrigel. Recent synthetic, xeno-free strategies to increase growth factor stability and availability can be applied to cell culture methods and may considerably reduce the costs associated with chemically defined conditions[173]. For example, an assortment of synthetic materials have been developed to sustain growth factor delivery over time to reduce the dosage needed compared with that for bulk administration[174-177]. For instance,

long-term stabilization of bFGFs was achieved by electrostatically binding them to customized mineral-coated microparticles, reducing the required bFGF dosage for hPSC expansion by more than 80%[178]. Binding to the nanoparticles stabilizes bFGF and enables localized and sustained delivery [179]. This approach could be generalized to other growth factors used in stem cell culture. Chemical compounds have also been used as analogues of recombinant growth factors to reduce costs and can prolong hPSC culture[87]. Ongoing developments in synthetic scaffolds that sequester growth factors and promote long-term growth factor stability[180-183] could notably reduce the costs of chemically defined cell culture and make it economically viable for broad use.

Although synthetic scaffolds have proved to be promising alternatives to Matrigel, challenges remain in using them for cell culture, regenerative medicine and organoid growth. Similar to Matrigel, synthetic scaffolds do not provide a one-size-fits-all approach and can require considerable tuning to achieve a distinct set of physical and biochemical parameters to direct cellular behaviour. The process of screening multiple scaffolds of varying interdependent parameters can be time consuming, cost prohibitive and challenging, and those with little experience with synthetic materials may revert to the familiarity of Matrigel. Additionally, matching the fibre-like architecture to recapitulate the complexity of native tissues is difficult to achieve using synthetic scaffolds. As an alternative, optimized synthetic materials that provide a minimal initial set of conditions conducive to cell function, but then rely on cell-mediated processes to define the extracellular milieu, may produce scaffolds that are suitable for not just one purpose, but for several different cell types and applications.

Creating scaffolds in a form that are easy for an end user to employ is another major challenge. One approach involves providing precursor materials in the form of a kit, which requires the end user to form the scaffold themselves. This approach can be effective but also introduces the potential for user error and may require the end user to have specialized equipment for scaffold formation and quality control analysis. Another approach is to generate devices that are pre-coated with scaffold materials, such as pre-coated multi-well plates, which would require no additional modification or characterization by the end user. However, this approach requires coatings that are robust and reproducible, and the shelf life of the coated device would be an important additional parameter to consider. Although these challenges are not unique to synthetic scaffolds, and indeed are also among the limitations of naturally derived ECMs, they must be addressed in a manner that allows for widespread adoption.

There are several ways in which these challenges are being addressed in academia and industry. For example, in 2017 the US National Science Foundation established the Engineering Research Center for Cell Manufacturing Technologies (CMaT) to develop scalable and low-cost manufacturing of high-quality cells, with one focus being synthetic scaffolds. The demand for alternatives to Matrigel has also led to new product development at existing life science companies, including Corning’s Synthemax, as well as the formation of start-up companies such as Mosaic Biosciences, QGel and Stem Pharm, which provide synthetic scaffolds for the range of applications described in this Review. As research and development progress, it is important to maintain a collaborative dialogue between biologists, material scientists, engineers and clinicians across academia and industry, to not

only improve synthetic scaffolds, but also to ensure their availability and ease of use to practitioners in stem cell therapy, regenerative medicine and drug discovery.

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Box 1 |

Chemically defined, xeno-free cell culture

Fully defined, xeno-free cell culture requires chemically defined, xenogenic-free media as well as a chemically defined scaffold[184]. For routine cell expansion, the media has traditionally included serum of human or animal origin, which is associated with now well-studied risks, including the potential for the transmission of prion, zoonotic or viral infections, and the potential for xenogeneic compounds to trigger undesirable immune responses[185]. Similar to Matrigel, serum is susceptible to batch-to-batch variability, raising concerns regarding the quality and concentration of proteins, and the potential effects on the reproducibility of experimental results[186,187]. Numerous serum-free, chemically defined media have been developed and shown to support the successful expansion of stem cells[87,111,188-192]. Synthetic scaffolds have been combined with chemically defined, xenogeneic media to develop a fully defined, xeno-free environment for cell culture for both fundamental research and cell manufacturing for therapeutic applications[80,84-86,193]. The proliferation and pluripotency maintenance of the cells cultured on synthetic scaffolds was similar to those cultured on Matrigel, while eliminating the possibility of xenogeneic contaminants[32,80,84,156].

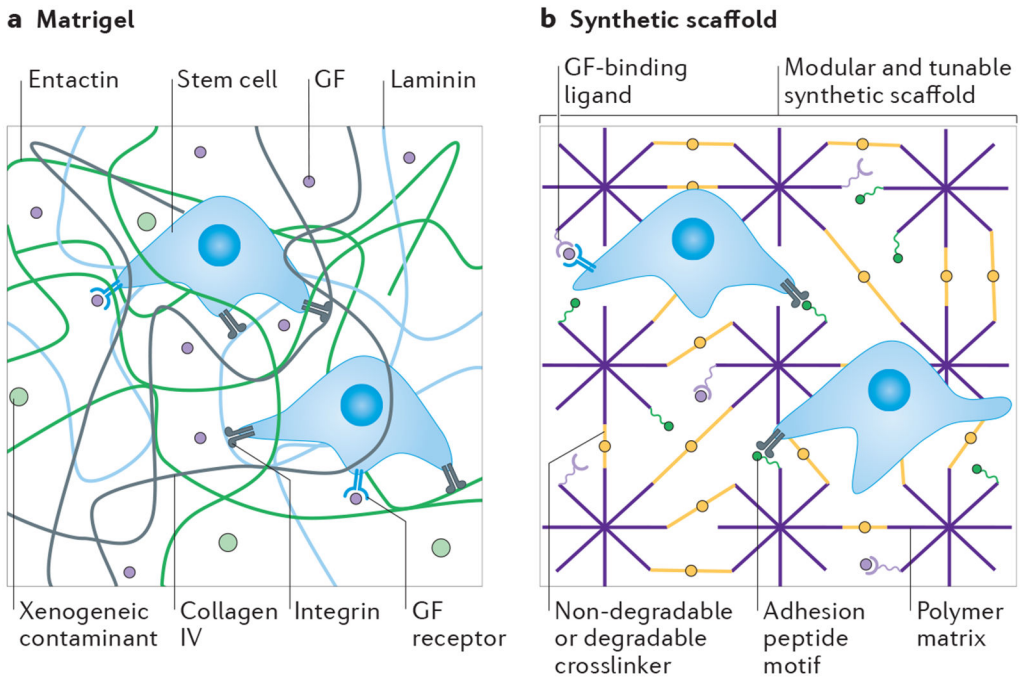

Fig. 1|. Comparison of Matrigel and synthetic scaffolds.

a | The composition of Matrigel is unamenable to modifications, ill-defined, complex and highly variable, resulting in heterogeneities in both biological and mechanical properties. As it is animal-derived, Matrigel may also contain xenogenic contaminants, and the presence of growth factors (GFs) and other biological proteins can lead to undesirable cellular effects. **b** | Synthetic scaffolds are highly tunable and chemically defined. The mechanical, physical and biological properties of these scaffolds can be modified to direct cellular response while eliminating undesirable matrix-induced effects.

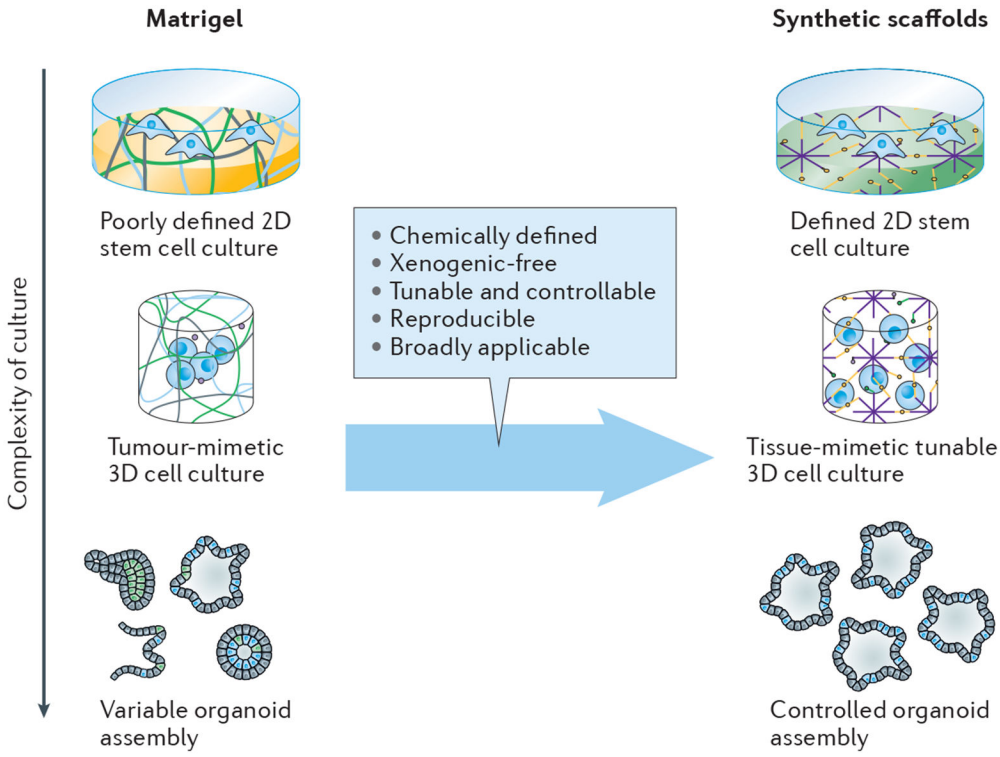

Fig. 2l. Advantages of synthetic scaffolds over Matrigel for cell culture, tissue engineering and organoid formation.

Synthetic alternatives to Matrigel provide a xenogenic-free, chemically defined and reproducible scaffold that can be tuned to guide cellular behaviour for a myriad of applications, including differentiation and organoid formation. The chemically defined nature of synthetic scaffolds also eliminates matrix-induced effects, providing a superior scaffold for toxicant and therapeutic screening assays.

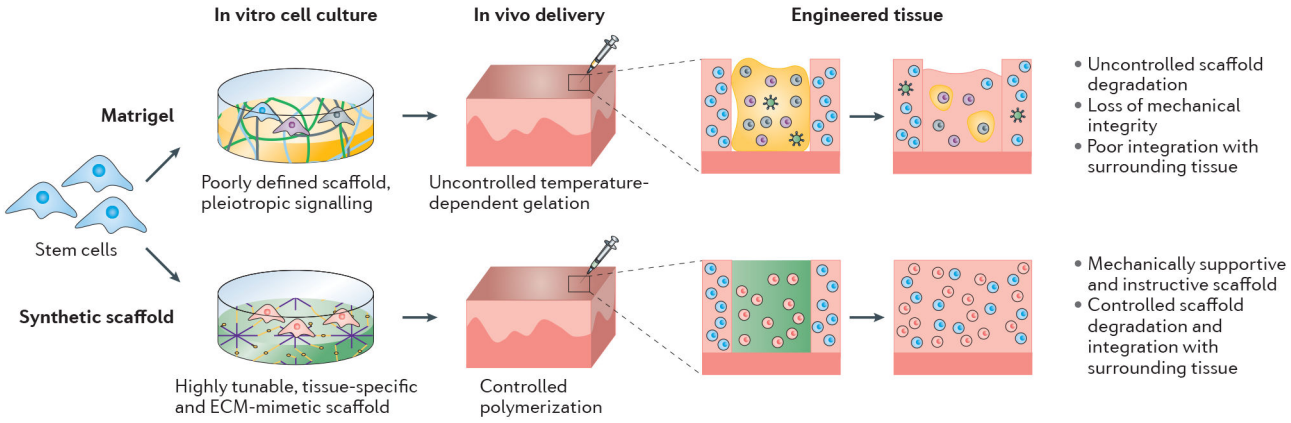

Fig. 3|. Comparison of Matrigel and synthetic scaffolds for stem cell differentiation and tissue engineering.

Unlike Matrigel, which is not tissue specific, synthetic scaffolds can be tuned (often through the addition of peptides) to provide specific biofunctionality to direct cell differentiation. The growth factors and other biologically active proteins in Matrigel lead to the generation of heterogeneous cell populations, whereas synthetic scaffolds generate pure populations of differentiated cells. In the context of in vivo delivery for tissue engineering applications, synthetic scaffolds can be delivered locally to the target site and be tuned to provide sustained mechanical support and biochemical instruction to transition from a cell-laden synthetic scaffold to neotissue. Conversely, the degradation of Matrigel is uncontrolled and its biofunctionality often leads to the formation of blood vessels. The potential for xenogenic contaminants in Matrigel or Matrigel-cultured cells prevents clinical application. Moreover, the handling of Matrigel in clinical settings is difficult owing to its gelation over a wide range of temperatures.

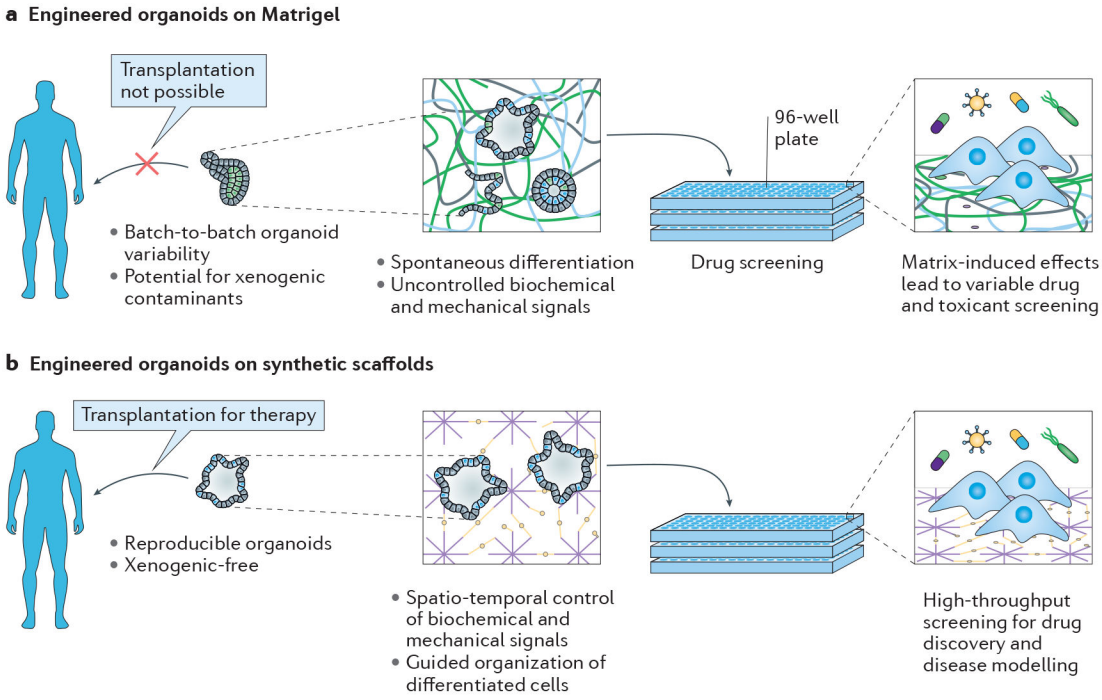

Fig. 4|. Comparison of Matrigel and synthetic scaffolds for organoid assembly and preclinical tissue models.

a | Matrigel scaffolds provide non-specific biochemical and mechanical signals for the spontaneous differentiation and self-assembly of cells into an organotypic model. The biological complexity of Matrigel leads to scaffold-induced effects, which affect the accuracy and reproducibility of preclinical models that rely on Matrigel-cultured cells. **b** | Synthetic scaffolds have a chemically defined structure and the biological, mechanical and physical parameters can be tuned to guide organoid formation.

Table 1

Synthetic scaffolds that have been directly compared with Matrigel

Synthetic scaffold material	Cells and application	Refs.
Pluripotent stem cell culture and maintenance		
PMEDSAH	Long-term 2D hESC and hiPSC culture and maintenance	73-75
PMVE-*alt*-MA	Long-term 2D hESC and hiPSC culture and maintenance	76
PAPA brushes tethered with cRGDfK	Long-term 2D hESC and hiPSC culture and maintenance	80
PEG thiol–ene hydrogels with cyclic RGD	Short-term 2D hESC culture and expansion	81
A peptide–acrylate surface generated from 2-hydroxyethyl methacrylate, 2-carboxyethyl acrylate and tetra(ethylene glycol) dimethacrylate and functionalized with a vitronectin-derived peptide	Long-term 2D hESC culture and maintenance	83
A poly(OEGMA-*co*-HEMA) film decorated with a vitronectin-derived peptide and developed through surface-initiated polymerization	Long-term 2D hiPSC culture and maintenance	84
PVA–IA hydrogels functionalized with a vitronectin-derived peptide	Long-term 2D hiPSC and hESC culture and maintenance	85
PSS and PAM copolymerized hydrogel PAM_6-*co*-PSS_2	Long-term 2D hESC and hiPSC culture and maintenance	92
PAM hydrogels functionalized with a vitronectin-derived glycosaminoglycan-binding peptide	Long-term 2D hESC and hiPSC culture and maintenance	94
RGD-functionalized PEG hydrogel crosslinked using factor XIIIa	3D Human fibroblast reprogramming to hiPSCs and 3D hiPSC culture	100
Stem cell differentiation		
Self-assembled peptide nanofibre hydrogels functionalized with a peptide derived from brain ECM	Mouse neural stem cell differentiation into neurons, astrocytes and oligodendrocytes	112
RGD-functionalized and MMP-sensitive PEG thiol–ene hydrogel	hiPSC-derived endothelial cell and vascular morphogenesis	113
Electrospun synthetic polyamide nanofibres: $(C_{28}O_4N_4H_{47})_n$ and $(C_{28}O_{4.4}N_4H_{47})_n$	Mouse ESC, hESC and iPSC differentiation into functional hepatocytes	126
MMP-sensitive PEG hydrogel crosslinked using factor XIIIa	Mouse ESC neuroepithelial differentiation	128
In vivo tissue regeneration		
RGD-functionalized PEG–MAL protease-degradable hydrogels	Mouse muscle satellite cell engraftment in dystrophic aged skeletal muscle	146
RGD-functionalized maltodextrin-derived scaffolds	Myotubule formation from mouse myoblasts	147
Nanocomposite copolymer PLGA–PEG–PLGA hydrogel with Laponite	Mouse myoblast treatment of muscle injuries	148
Organoid assembly		
Non-degradable PEG hydrogel functionalized with laminin-derived peptides and crosslinked using factor XIIIa	Mouse neuroepithelial tubule organoids	132
RGD-functionalized protease-degradable PEG–MAL hydrogel	Madin–Darby canine kidney cyst organoids	109
MMP-sensitive, heparin-functionalized biohybrid PEG hydrogel	Renal tubulogenesis, mammary epithelial morphogenesis and Alzheimer disease	165-168
Hydrolytically degradable PEG hydrogel functionalized with RGD and laminin and crosslinked using XIIIa	Mouse intestinal organoids	162,163
Protease-degradable, RGD-functionalized PEG–MAL hydrogel	Human intestinal organoids and lung organoids	156,161
Cell-based assays for preclinical tissue models, toxicant screening and drug discovery		
MMP-degradable RGD-functionalized PEG thiol–ene hydrogel	Vascular toxicity screening	81
MMP-degradable, RGD-functionalized PEG thiol–ene hydrogel	Oestrogen-receptor-positive breast cancer assay	169

cRGDfK, cyclo(Arg-Gly-Asp-D-Phe-Lys); ECM, extracellular matrix; ESC, embryonic stem cell; GLN, glutamine; hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; iPSC, induced pluripotent stem cell; LYS, lysine; MAL, maleimide; MMP, metalloproteinase; PAM, polyacrylamide; PAPA, poly(acrylamide-*co*-propargyl acrylamide); PEG, polyethylene glycol; PLGA–PEG–PLGA, poly(lactide-*co*-glycolide)-*b*-poly(ethylene glycol)-*b*-poly(lactide-co-glycolide); PMEDSAH, poly(2-(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl)ammonium hydroxide); PMVE-*alt*-MA, poly(methyl vinyl ether-*alt*-maleic anhydride); poly(OEGMA-*co*-HEMA), poly(oligo(ethylene glycol) methacrylate-*co*-2-hydroxy-ethyl methacrylate); PVA–IA, poly(vinyl alcohol-*co*-itaconic acid); PSS, poly(sodium 4-stryenesulfonate); RGD, Arg-Gly-Asp.