REVIEW

Recent Advances in Graphene Oxide-Based on Organoid Culture as Disease Model and Cell Behavior – A Systematic Literature Review

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Abstract: Due to their ability to replicate the in vivo microenvironment through cell interaction and induce cells to stimulate cell function, three-dimensional cell culture models can overcome the limitations of two-dimensional models. Organoids are 3D models that demonstrate the ability to replicate the natural structure of an organ. In most organoid tissue cultures, matrigel made of a mouse tumor extracellular matrix protein mixture is an essential ingredient. However, its tumor-derived origin, batch-to-batch variation, high cost, and safety concerns have limited the usefulness of organoid drug development and regenerative medicine. Its clinical application has also been hindered by the fact that organoid generation is dependent on the use of poorly defined matrices. Therefore, matrix optimization is a crucial step in developing organoid culture that introduces alternatives as different materials. Recently, a variety of substitute materials has reportedly replaced matrigel. The purpose of this study is to review the significance of the latest advances in materials for cell culture applications and how they enhance build network systems by generating proper cell behavior. Excellence in cell behavior is evaluated from their cell characteristics, cell proliferation, cell differentiation, and even gene expression. As a result, graphene oxide as a matrix optimization demonstrated high potency in developing organoid models. Graphene oxide can promote good cell behavior and is well known for having good biocompatibility. Hence, advances in matrix optimization of graphene oxide provide opportunities for the future development of advanced organoid models.

Keywords: organoid, cell culture, graphene, matrigel, alternative matrigel, cell behavior, host-pathogen

Introduction

Many researchers focus on developing 3D cell culture models for organoid production since they provide advantages as valuable tools for studying disease mechanisms and testing potential treatments. The 2D cell culture does not replicate the in vivo microenvironment, as cell–cell interactions are limited and a tissue-specific architecture is lacking.[1] Moreover, study with references revealed that the 2D cell culture models have some disadvantages, such as the inability to control cell shape or loss of cell regulatory abilities, limitation to single-cell types, and inability to reflect the complexity of physiology in tissues, leading to bias in predicting specific tissue response.[2–6] Therefore, 3D culture is introduced as an alternative solution to address these disadvantages. Recent studies demonstrated that the 3D cell culture model promoted an environment that mimics an in vivo situation, stimulated cells by enhancing cell proliferation, migration, matrix creation, stem cell differentiation, and even replicated an environment of a particular tissue's physiology and pathophysiology.[5–8] An organoid is a complex three-dimensional structure that can simulate various interactions, including those between cells and the matrix, the physiological function of a particular tissue that can imitate

Graphical Abstract
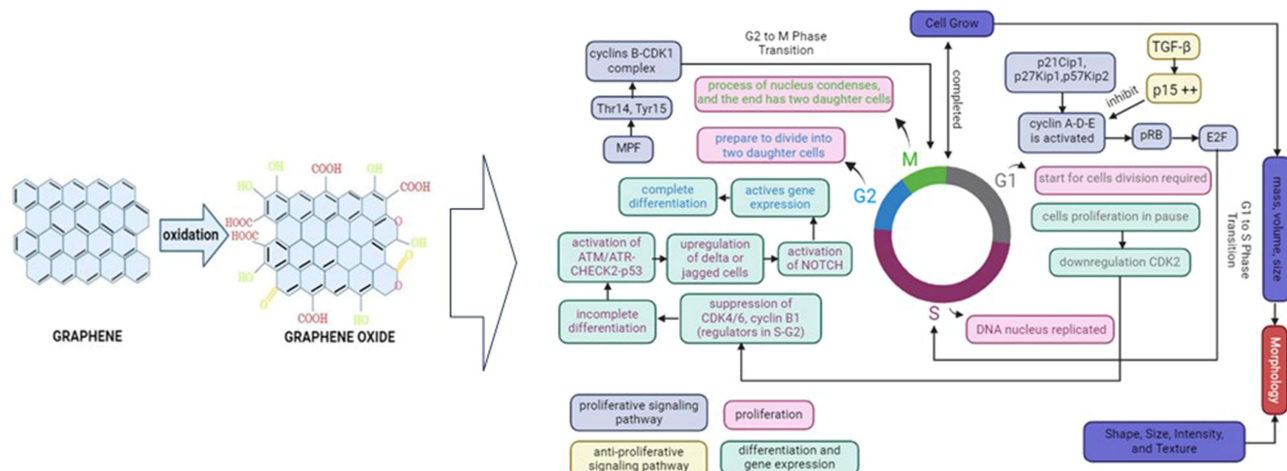
the physiology of an organ, and the composition of different cell types that can sustain cell function.[9–11] Organoids have demonstrated promising outcomes in recreating the native organ through metabolic activity, gene and protein expression, and tissue microfabrication.[12] Organoid models have been constructed for disease models that have explored pathologic agents, such as viruses, bacteria, and parasites, that cause the disease.[13–29] Nevertheless, there are currently certain obstacles preventing the growth of organoid needs. The drawback of organoid development is due to high cell diversity, survival in the culture system, as well as maturity and function limitations.[12]

One of the significant elements in promoting cell differentiation and potentially reducing the populations of dying cells is the physics and chemistry of matrix optimization's characteristics.[30,31] The results of organoid culture are affected by matrix optimization.[32,33] The primary function of the cell's matrix is to sustain and promote cell development. Most organoid cultures have recently been cultivated in a matrix using matrigel.[34–37] Matrigel is a commercially available matrix generated from a mouse tumor that contains high levels of laminin, collagen, and heparan sulfate proteoglycans mixed with other small amount of ECM proteins and growth factor.[38,39] Nevertheless, matrigel is limited in its ability to selectively develop organoid culture due to its varied composition. The complex composition of matrigel and thousands of identical peptides in matrigel can cause high abundances of undissolved matrigel proteins, resulting in less identification of organoid proteins; organoid proteins may be misidentified due to identical peptides from matrigel contaminants; and the abundances of organoid proteins uniquely may be estimated with bias in proteomic analysis, even the complex composition can influence cell culture in unexpected ways.[40,41] Moreover, the complex composition of matrigel may interfere to cell behaviour, making it difficult to distinguish biological effect, and even xenogenic contaminants were detected in matrigel may restrict therapeutic potential of cell or tissue culture.[42] Therefore, it is crucial to develop materials as alternative materials of matrigel for organoid cultivation. However, organoid research is still in its infancy, with preliminary exploration of construction strategies, assessment, and application methods.

This review aims to investigate the significance of the latest advances in developing alternative materials for matrigel in cell culture applications and how they enhance build network systems by generating proper cell behavior. This review will focus on recent advances in using materials as an alternative for matrigel in organoid culture applications, such as tissue engineering and even disease therapy. We highlight critical studies for each application in which the materials used as matrix were directly compared to matrigel to observe cell characteristics as one of the factors to see the effects of matrix on a suitable microenvironment. Aside from that, we examine cell behavior and other factors such as cell proliferation, differentiation, and gene expression. Also, we study how they can be utilized to develop tissue systems that resemble the structure of native tissue. In the present review, we focused on graphene oxide or combined graphene oxide,

as one of the materials that have succeeded in developing in the biomedical field, such as potential drug delivery[43–45] and even tissue engineering.[46–49] Finally, we discuss the current situation of alternative materials to matrigel and give the author's perspective on the graphene oxide potency for developing organoids with additional model applications such as investigating and studying disease, drug discovery, and even vaccine development.

Materials and Methods

Based on the purpose of this review, we have three research questions that we will explain in this review: (1) Does alternative matrigel, matrigel or combined graphene oxide in organoid culture create a more suitable microenvironment, (2) How do effects of cell proliferation, cell differentiation, and gene expressions as further parameters of behavior cell sign on organoid culture, (3) How many doses of graphene oxide have shown non-toxicity in organoid culture.

Study Design and Literature Search

A systematic literature review based on The Preferred Reporting Items for Systematic Review (PRISMA).[50] The searched electronic databases used SpringerLink, ScienceDirect, and Google Scholar with English language restrictions. The following keywords were used: "organoid AND cell culture AND graphene oxide OR graphene AND matrigel OR alternative matrigel AND cell behavior AND host pathogen". We use Boolean operators OR and AND within our search.

Study Selection and Eligibility Criteria

The search was restricted to experimental studies in English published in 2012–2024, article types, area subjects, or knowledge sciences. The search results was screened by looking at article titles, abstract records, and duplicates within Mendeley software. After screening articles based on article titles and abstract records, the full text of these articles was screened based on a reference list of inclusion and exclusion criteria, as summarized in Table 1.

Results

Search Results

For the first search strategy, 1.319 articles were received based on years of publication in 2012–2024. Of which, 1.319 were screened based on article types, years of publication, subject area or knowledge sciences, and language. Of the remaining 301 articles, titles, abstracts, article types, and duplicates were screened, and then 193 articles were screened for full text. After reviewing the full text, 142 articles were screened based on inclusion and exclusion criteria. The remaining 51 articles were included in the analysis. The flow diagram of the results of the screening process is illustrated in Figure 1.

Included Studies

All 51 articles were studied in biomedical applications. Of the 32 articles related to tissue engineering or regeneration,[51–80] five articles refer to therapeutic agents,[81–85] two articles related to cancer therapy,[86,87] 1 article refers to skin therapy,[70] 1 article refers to biosensing system,[88] and 10 articles refer to organoid models.[89–98] Matrix optimization with matrigel or alternative matrigel or combined with graphene was assessed in 51 articles. The quantity of matrigel was depicted in 13 studies,[53–58,80,88,94–98] alternative matrigel in 17 studies,[51,52,63,74–79,81–83,89–91,93] and combined with graphene in 21 studies.[56,58–62,64–73,84–87]

Table 1 Inclusion–Exclusion Criteria

| Inclusion Criteria	Exclusion Criteria
Cell culture or organoid experimental studies	Abstract only and review article
Studies written in English	Off-topic studies
Cell culture or organoid intervention-related medium optimization with matrigel or alternative matrigel or graphene	Nothing medium optimization used matrigel or alternative matrigel or graphene
Measurement of cell behavior such as cell characteristics, cell proliferation, cell differentiation, and gene expression of combined graphene effects	
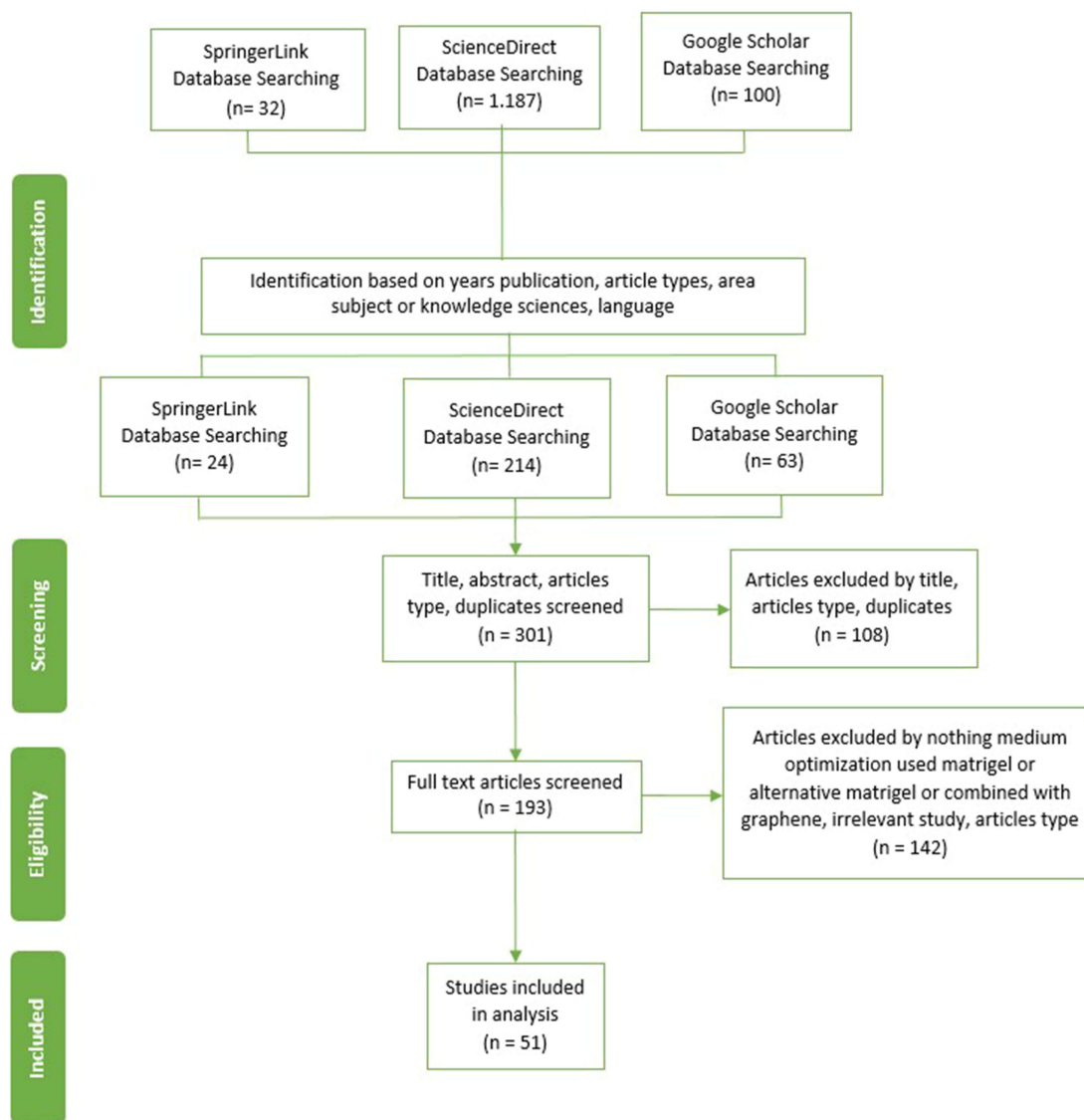
Figure I Article Screening Process based on PRISMA Guidelines.[50]

Generally, all 51 articles will describe matrix optimization that affects the microenvironment in vitro, such as cell characteristics, proliferation, differentiation, and gene expression. In 18 studies, it is referred to graphene combinations, which will be presented more in detail in this review based on in vitro cell culture.[56,58,60–62,64,66–73,84–87]

Graphene Oxide as Nanomaterial for Biomedical Application

Graphene is a single layer made from sp2-hybridized carbon atoms arranged in honeycomb lattice structures.[99,100] The sp2-hybridized carbon atoms are garnering attention in the field of biomedicine, including biosensing, drug delivery, bioimaging, tissue engineering, antimicrobial agents, and even cancer therapy.[101,102] However, two-dimensional (2D) graphene lattice structures have some drawbacks, such as unstable chemical formations and a limited ability to interact with other molecules or materials due to graphene's limited active component, resulting in incompatibility.[103] Graphene oxide (GO), a member of the graphene family, is produced via oxidation using strong oxidizing agents.[100] Oxidation methods for generating GO include Staudenmaier, Hofmann, Tour, and Hummers.[104] The Hummers method is the most often employed to produce GO by giving graphite a chemical treatment through oxidation, then dispersing and

exfoliating in water or organic solubility.[105] Furthermore, the Hummers method is the most widely used and developed because it produces high-quality GO quickly.[106,107]

GO possesses physicochemical properties and biocompatibility due to its unique structure, which is a graphene basal plane attached with functional groups like hydroxyl (OH) and carboxylic (COOH), which leads to further functionalization and conjugation or immobilization of other nanoparticles on its surface.[108–112] In addition, the GO surface's morphology, shape, size, and functional groups stimulate biological activity and may even improve cell–cell interactions.[113] Because GO has excellent surface functionality and is rich in oxygen-containing groups, it can be easily modified with other materials, improving solubility, selectivity, and biocompatibility.[100,102,114–116] Also, GO possesses amphiphilicity properties, which means it can be hydrophobic or hydrophilic, as well as a high affinity, which makes it helpful in interacting with biomolecules such as enzymes, peptides, DNA, and proteins in the construction of 3D models.[103,113] Interestingly, because GO is photoluminescent and permeable to cell membranes, it can be used in drug delivery and gene delivery.[117] As a result, GO has been predominantly used in biomedical applications such as tissue engineering, cancer therapy, drug delivery, gene delivery, and biosensing,[108,118–120] as illustrated in Figure 2.

Graphene and graphene derivatives have been used to create a variety of biosensors due to their outstanding sensing performance (high specific surface area, extraordinary electronic properties, electron transport capabilities, and ultrahigh flexibility).[121] Also, due to hydrophilic oxygen-containing functional groups, GO has good water dispersibility, biocompatibility, and high affinity for certain biomolecules.[122] Graphene-based biosensors provide quantitative detection of cancer-related biomarkers such as DNA, miRNA, small molecules, and proteins.[121] Thus, GO as biosensors have been applied in surface plasmon resonance (SPR),[123] fluorescence resonance energy transfer (FRET)[124,] and electrochemical-based techniques.[125] On the other hand, GO typically contains both hydrophobic and hydrophilic regions. In the hydrophobic region, the π–π conjugated system on the surface makes it capable of connecting multiple molecules through the non-covalent bond interactions.[126,127] Moreover, GO has better water solubility than graphene due to its abundant hydrophilic groups, such as –O–, –COOH, –OH. These groups could form the hydrophilic region, which allows further functionalization by attaching to various molecules including protein, DNA, and RNA.[128,129] Thus, GO met for developing future anticancer graphene drug delivery since an efficient accumulation of anticancer drugs in tumor targets/tissues, controlled cellular uptake properties, tumor-targeted drug release behavior, and selective toxicity toward the cells.

GO modified with a non-toxic cationic material and a tumor-specific monoclonal antibody (anti-EpCAM) for the delivery of survivin-siRNA (GCE/siRNA) had a strong antitumor effect in vitro, which was attributed to GCE/siRNA's efficient antiproliferation, migration, and invasion inhibition effect.[85] The presence of several hybrid layers of carbon atoms (sp2) of graphene oxide has a large surface for highly efficient drug loading.[127] Besides that, the high degree of
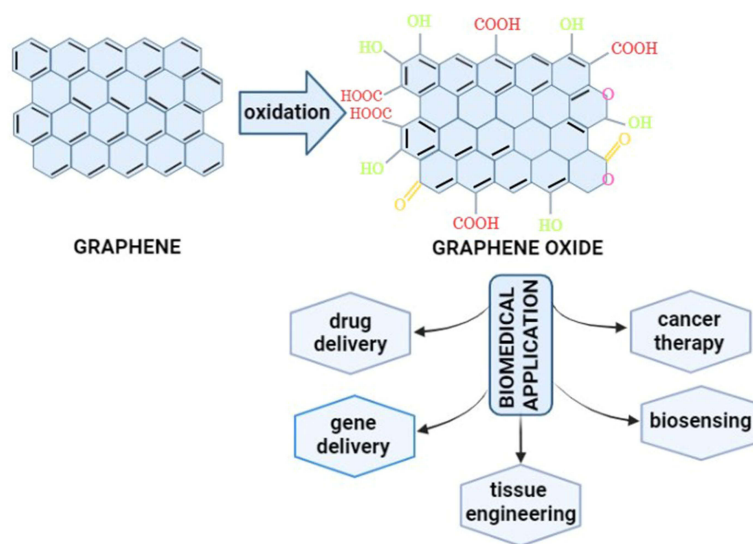

Figure 2 Graphene Oxide as Nanomaterial's for Biomedicine Application.

functionalized graphene oxide (GO) nanoparticles improves intelligent controlled release and gene silencing capability.[130] Moreover, GO is an effective nanocarrier that allows for the targeted delivery of small drug molecules, antibodies, nucleic acids, and peptides to the liquid or solid tumor sites.[130] Therefore, graphene oxide can play a significant role as a drug delivery system in gene delivery, and cancer therapy, especially as a drug carrier.[127,130,131]

Tissue engineering involves the development of biotechnologically produced and functional tissues and organs for repair or replacement. Graphene oxides have excellent physical, physicochemical, and biological properties for biomedical applications due to their large surface area and ability to interact with proteins and peptides.[132] Therefore, GO has been successfully used to optimize scaffold architectures for a variety of organs, from skin to heart tissue.[113] The protein-graphene oxide structures formed either as nanocomposites or as biocomplexes. It is emphasized that the effects of carbon-containing nanostructures on protein conformation and structural stability are being investigated for applications in tissue engineering and regenerative medicine.[133,134] The biological activity of GO nanocomposite bioconjugates also plays an important role in terms of cell viability and proliferation, as well as the ability of these constructs to sustain the formation of new and functional tissue.[133]

Cell Behavior

For many years, in vivo animal experiments or animal models have been used in biomedical research to improve the quality of life of humans and animals through toxicity tests, diagnostic tools, and the development of new drugs or vaccines.[135] Furthermore, in vivo experiments were used for the characterization and analysis of molecules and biological systems, such as molecular markers for neurodegenerative diseases, models for cancer treatment, and clinical tests of drugs.[136–140] However, in vivo testing or animal models concern ethics, require more expenses, and involve multiple cells and agents, thereby impeding the evaluation of the intended effect,[141] whereas in vitro experiments do not require the technical skills needed when handling animals and are generally less expensive, quicker, and easier to perform and quantify.[142] Recent studies have shown that animal organoid models (eg, prostate cancer, bladder cancer) can represent tumors in vivo.[143,144] In addition, organoid models can be used in the modeling of diseases and in transplantation or replacement studies.[145] Therefore, 3D culture or organoid models provide some aspects of the tissue microenvironment that could potentially be used for in vitro phenotyping to mimic in vivo situations and reduce in vivo animal experiments or animal models.

In vitro cell cultures are required for various biomedical applications, including cellular and organismic biology, drug discovery, and regenerative medicine.[146] 3D cell culture enhances physiological formation and allows cells to recapitulate many tissue features such as shape, proliferation rate, differentiation, and even gene expression.[147–150] Previous studies on disease models have shown that organoids as 3D models are more physiology-relevant and pathologic. Cell number, viability, morphology, proliferation, differentiation, gene expression, and even in vivo relevance were improved by the 3D model.[151] Therefore, organoids as 3D models are considered capable of being developed in such a way that the unique microenvironment that occurs in vivo can be imitated the existing in vitro. Figure 3 is an illustration of the concepts of morphology, proliferation, differentiation, and gene expression as biological mechanisms for optimizing cell behavior in cell culture.

Cell morphology is an important aspect corresponding to the cell-cultured to understand cell behavior, such as the cell phenotype,[152] and it is even associated with cell functions.[153] The morphological characteristics of cells are assessed based on their form, size, intensity, and texture of cellular compartments.[154] Animal cell culture, particularly mammalian cell culture, has been classified into three fundamental types based on shape and appearance: fibroblastic, epithelial, and lymphoblastic.[155] Fibroblastic cells grow connected to substrates and have elongated forms. Epithelial cells have polygonal shapes with more uniform dimensions and grow in discrete patches adhering to the substrate. Lymphoblast cells are spherical form and grow in suspension, unattached to a surface. Cell shape and growth are classified according to the type of cells developed in cell culture. Cell morphology is also linked to cell proliferation since it regulates the expansion of their mass, volume, and size.[156] Therefore, growth speed is the time derivative of mass or volume [dV/dt], and mass or volume-specific growth rate is the growth speed divided by mass or volume [1/V dV/(dt)].[156]

Cell growth requires proliferation. Proliferation is a process in which cells must duplicate the new cell by growing and then dividing into two equal copies by passing through the cell cycle, which includes the G0 phase, G1 phase,
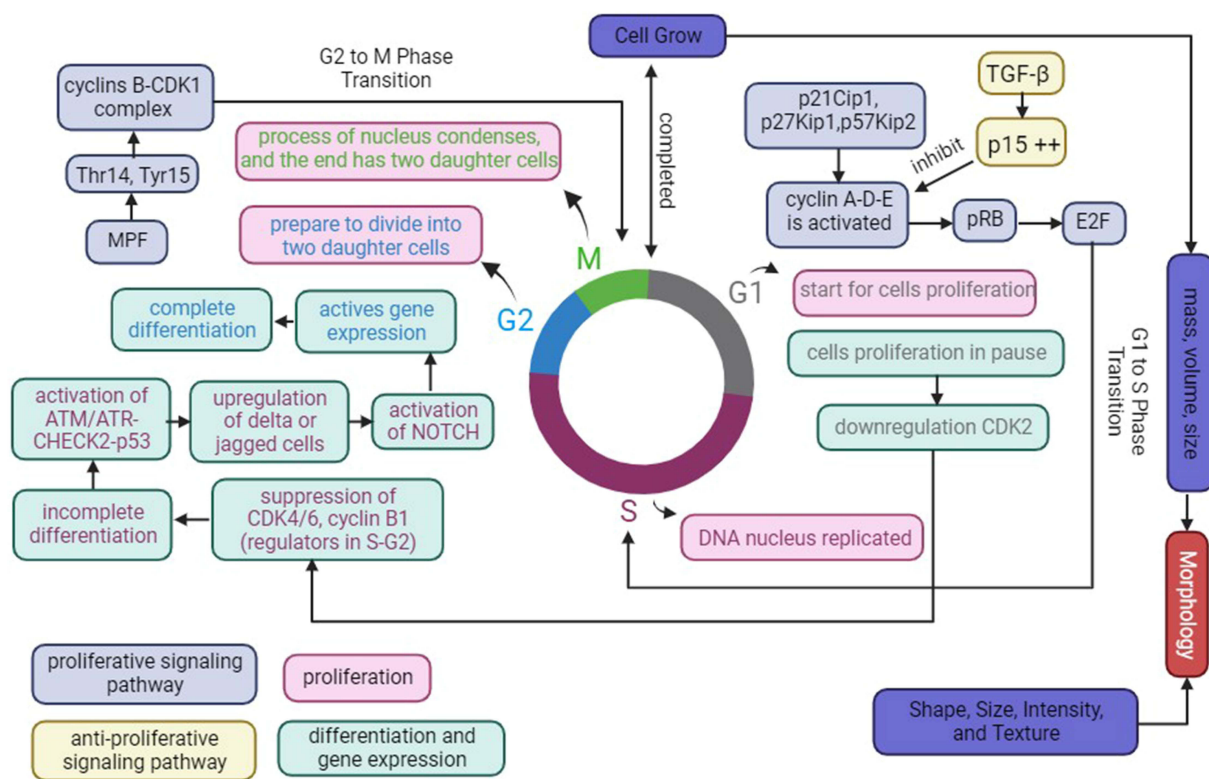
Figure 3 Principle of Morphology, Proliferation, Differentiation, and Gene Expression in Cells Grow.

S phase, G2 phase, and M phase.[157,158] Most cells in the first are stable and are in the gap (G0) phase of the cell cycle, also known as the resting cell period.[157,158] When cell division is required, the cell will come into the G1 phase of the cell cycle.[157] The S (synthesis) phase occurs when DNA in the nucleus is reproduced for a limited portion of the cell cycle. The cell will then enter the second gap (G2) phase and then will prepare to divide into two daughter cells during the M (mitosis) phase. The cell will enter the M phase, in which the contents of the nucleus condense to form a visible chromosome that is pulled apart into two equal sets via an elaborately organized series of movements. In the end, the cell divides into two daughter cells. Furthermore, the M phase is divided into five stages: prophase, prometaphase, metaphase, anaphase, and telophase.[158]

Prophase is the stage during which the paired chromosomes that were duplicated during S phase initiate condensing to form sister chromatids. Then, condensing complexes and histone phosphorylation are employed to condense chromatin, while the nuclear membrane begins to break down, and these steps are driven by the cyclin B/cyclin-dependent kinase 1 (CDK1) complex working in tandem with the Polo-like kinases (Plks). Prometaphase is the next stage in which the nuclear membrane disappears, and the spindle begins to assemble. The chromatids then align in one plane at the metaphase plate, a process known as metaphase steps. The chromatids are suitably positioned to promote the anaphase-driven separation process. As the chromosomes separate and migrate to the spindle poles, the process of cell cleavage, known as telophase, begins.

Cell proliferation is controlled by the proliferative signaling system, which propels cells into the cell cycle by activating processes early in G1, with cyclin D activation controlling the G1 phase.[158] The obligation to divide occurs during the G1 phase, which is regulated by cyclin-D-CDK4/6 and cyclin-E-CDK2.[159] Cyclin D-CDK4 and cyclin D-CDK6 have functions in the phosphorylation of retinoblastoma protein (pRB), while cyclin E-CDK2 contributes to pRB phosphorylation in late G1, which promotes cell entry into the S phase.[160] Furthermore, cyclin D, E, and A are regulated by the CDKI family, which includes p21Cip1, p27Kip1, and p57Kip2, whereas p21Cip1 expression is regulated by p53.[161] pRB regulates the G1 phase, which suppresses the activity of E2F transcription factors in mitotic cells.[159] As a result, the pRB-E2F pathway regulates the transition from G1 to S. The G2 to M phase transition is then mediated by

the cyclin B-CDK1 complex, previously known as maturation (mitosis) promoting factor (MPF), which is dephosphorylated at Thr14 and Tyr15 by the phosphatase CDC25.[159,160,162] Furthermore, CDK1-cyclin B phosphorylates activate Wee1/Myt1.[163] Wee1 and Myt1 hinder mitotic progression by monitoring the G2/M phase checkpoint and providing inhibitory phosphorylation to CDK1.[160,164] These inhibitors role significant functions in cancer cells and can even be used as cancer therapy targets.[164] While there are anti-proliferative signaling pathways, transforming growth factor-β (TGF-β) inhibits cell cycle entry and even encourages cells to differentiate.[158] TGF-β, smad signaling pathway, suppresses cell proliferation by up-regulating the CDK inhibitor p15, which inhibits cyclin E and cyclin A and even prevents the formation of transcription factor Myc, which is a key regulator of cell proliferation.[158]

Differentiation leads to different specialized cell types, commencement through the progressive difference of developmental pathways, and completion through the consecutive programming and final elaboration of each functional cell type.[165] Differentiation is regulated by the cell cycle through cell cycle regulators, which directly affect the expression of differentiation genes.[166] The cell cycle initiates as daughter cells enter the G1 phase, which has been particularly permissive to differentiation signals and even commits to specific lineages.[166] While cell proliferation is paused in G1, cells remain in G1, committing to terminal differentiation into a functional cell type.[166,167] Several cycle regulators, such as CDK2, CDK4, and CDK6 proteins, remain relatively constant at steady levels during the normal cell cycle and in quiescent, aging, and even terminally differentiated cells.[159] Down-regulation of CDK2 lengthens the G1 phase, resulting in differentiation, while during G1 cell cycle progression, it is mediated by the activation of CD4/6.[168–170] Whereas suppression of CDK4/6, cyclin B1, a regulator in the S and G2 phases, led to a rapid decrease in expression and also activated the ATM/ATR-CHECK2-p53 by incomplete differentiation to prevent abnormal cell generation.[171,172] Additionally, DNA methylation and histone marks are inherited in S and G2 phases, indicating regulation of differentiated phenotype.[172] Then, cells commit to differentiation by activating notch signaling by upregulating delta or jagged cell surface ligand, which then transits to the nucleus, activating gene expression.[166] Therefore, cell differentiation is completed in G2 phase through cell cycle dependent.

ECM roles include providing structural support cells, which have an impact on morphology, promoting cell proliferation; organizing to facilitate tissue function, which has an impact on cell differentiation, and regulating gene expression.[8,173–175] The ECM surrounding the cells influences the topology of cell-cell, cell-matrix contact, and distribution of the signaling biomolecules.[176–178] The cell–matrix interaction begins cell adhesion by integrin, and then the ECM can exert physical effects to regulate cell topology by anchoring, signal transmission, and cell migration.[179] In addition, cell migration during environmental cues can be biochemical or physical, which can regulate differentiation.[180–182] Physical properties trigger mechanical cues so the ECM can regulate morphology.[179] Cell morphology is maintained by the regulation of gene expression via cell interactions and transcriptional regulation.[183,184] Therefore, the structure of the ECM supports biomolecule signaling, which is integrated by the intracellular signaling pathway and provides for cell growth, proliferation, differentiation, morphology, and gene expression,[185] as illustrated in Figure 4.

Organoid models were developed from stem cells and are grown in in vitro culture systems with extracellular matrix (ECM).[186] In addition, matrigel has been employed in a variety of applications including tissue engineering, assays, and organoid assembly.[187,188] Matrigel is a gelatinous ECM protein mixture obtained from the murine Engelbreth–Holm–Swarm sarcoma.[189,190] However, matrigel derived from the Engelbreth-Holm-Swarm mouse sarcoma exhibits significant batch-to-batch variation in grown organoids.[191] Furthermore, tumor matrisomes differ significantly from normal tissue, suggesting that ECM-based matrigel may not provide a tissue-specific microenvironment for organoids.[192] Therefore, this work has shown an alternative to matrigel, as summarized in Table 2.

Study Outcomes and Discussion
Alternative Matrigel in Cell Culture or Organoid

Alternative matrigel can be used as a matrix to have effects on the microenvironment in vitro. The previous studies showed alternative materials such as gelatin, GelMa, collagen, Se-EX-Ch-COL/TA, Col-Ch, chitosan-pectin, PAMB-C-OHA, PLA-HA, PEG, GG, IGF1c-PHM, PUCL@CNT, CPSN, and RSF/Mxene,[51,52,63,74–79,81–83,89–91,93] as summarized in Table 2. The use of gelatin showed that cell aggregation is characterized by spindle-round or spheroid structures.[81,89] These characteristics characterize this type of three-dimensional cell culture, thereby promoting an
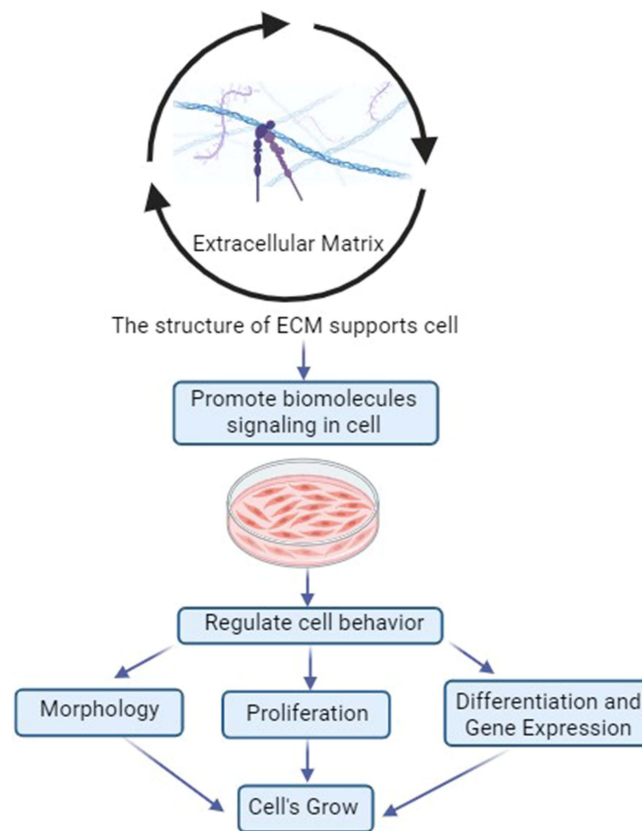
Figure 4 Principle of ECM for Cell Growth.

in vivo-like environment. Besides that, GelMa showed that colonoid formation is new when it is applied to organoid models.[90] However, the number of organoid cells in GelMa is less than that in gelatin, which is applied to organoid models. The number of organoid cells in GelMa is around 30,[90] while the number of organoid cells in gelatin is 39.[89] Similar results for collagen showed that it has characteristics of three-dimensional cell culture,[51,82,83] and even it is applied to organoid models, which shows that it can maintain cell phenotype,[91] and even assisted cECS-like structure formation.[52] Furthermore, interaction of collagen combination has an effect on tissue regeneration, which is implicated by increased cell migration.[52] Furthermore, cell migration is fundamental to establishing and maintaining the proper organization of multicellular organism.[193] In multicellular organisms, cell migration, and collective cell behaviour are essential mechanisms in morphogenesis for development. Therefore, 3D organoid culture requires collective behaviours to emerge with proper three-dimensional (3D) tissue organisation and cellular composition.[194]

Similar results showed that chitosan combinations have effects on cells that mimic the structures of native cells[63] and even cells formed spheroid as characterized by 3D models.[92] Additionally, it was shown that chitosan-based hydrogel formulations loaded with secretomes due to hydrophilic and hydrophobic interaction, which promotes cell migration, so it supported tubule-like blood vessel network formation for HUVECs and even elongated shaped for H9C2 cell.[63] Despite the fact that the chitosan combination has been successfully used for developing 3D models, the high hydrogel formulation showed that the morphology of the spheroids grown was irregular and highly heterogeneous.[92] Other studies showed that polycaprolactone (PCL) combination improving flexibility, so it has better physical properties and is supported for tissue manipulation and adaptation.[74] Additionally, silk fibroin combined with PCL improved hydrophilicity and flexibility.[75,76] Therefore, PCL combination assistance for neuron-like structures formation,[74] myocardium-like structures,[75] and tube formation produces more honeycomb-like structures of HUVECs.[76] In addition to PLA-HA, PEG, GG, and also IGF1c-PHM have shown previous similar results such as forming HUVEC-like structures,[77] cells formed fibroblast-like,[78] neurospheres formation,[79] and even maintaining phenotype cells on organoid models.[93] However, the

Table 2 The Effects on Cell Characteristics for Cells or Organoid Culture

Class Materials	Materials	Cell Line/Primary Cell In Vitro	Applications	Cell Characteristics	Ref.
A. The Positive Effects on Cell Characteristics for Cells or Organoid Culture					
Alternative Matrigel	Col-Ch	HUVECs	Tissue engineering and regeneration	Cells formed "spheric".	[51]
	Se-EX-Ch-COL/TA	cECs	Tissue engineering and regeneration	cECS-like structures formation.	[52]
	PAMB-C-OHA	HUVECs, H9C2 cell	Tissue engineering and regeneration	Tubule-like blood vessel network formation for HUVECs and elongated shaped for H9C2.	[63]
	PUCL@CNT	NSPCs	Tissue engineering and regeneration	Neuron like structures formation.	[74]
	CPSN	BADSCs	Tissue engineering and regeneration	Myocardium-like structures.	[75]
	RSF/Mxene	HUVECs	Tissue engineering and regeneration	Tube formation produces more honeycomb-like structures.	[76]
	PLA- HA	HUVECs, monocyte	Tissue engineering and regeneration	Formed HUVECs- like structures.	[77]
	GG	PDLSCs, DPSCs	Tissue engineering and regeneration	Cells formed fibroblast-like.	[78]
	IGF1c-PHM	NSCs	Tissue engineering and regeneration	Neurospheres formation.	[79]
	Gelatine	Human skeletal muscle tissue	Therapeutic agent	Colony formed "spindle round".	[81]
	Collagen	hPDLSCs, hBMSCs	Therapeutic agent	Cells formed "spindle".	[82]
	Collagen	MDMs, BMDMs	Therapeutic agent	Formed mesenchymal and like structures "rosette".	[83]
	Gelatine	CAFs, PDAC	Organoid models	Cells formed "spheroid".	[89]
	GelMa	Bovine colon crypts	Organoid models	Formed new colonoids and can maintain viable models after 48 hours.	[90]
	Collagen	Fresh primary tumor	Organoid models	Phenotype cell of leader cells derived cancer cells differentiation.	[91]
	Chitosan-Pectin	HCT 116	Organoid models	Cells formed "spheroid".	[92]

Alternative Matrigel	PEG	Human jejunal (J2, J3, J11) and duodenal epithelium (D109)	Organoid models	Maintaining phenotype cells of epithelial.	[93]
Matrigel	Matrigel	AT2S	Organoid models	Cell epithelial characteristics.	[94]
	Matrigel	Crypts (ileum), adult jejunum	Organoid models	Maintaining viable models.	[95]
	Matrigel	H9 ES cell	Organoid models	Formed neuroepithelium and rosette-like structures.	[96]
	Matrigel	Ileum biopsy	Organoid models	Cell epithelial characteristics.	[97]
Optimization Alternative Matrigel to Matrigel	Matrigel-Collagen-Manuka Honey	HUVECs, HMSCs	Tissue engineering and regeneration	Endothelial network formation.	[80]
	Matrigel-OEO-BG-PLGA-Gel	HUVECs	Tissue engineering and regeneration	Tubule-like blood vessel network formation.	[53]
	Matrigel-Gel-TCP/SC	HUVECs	Tissue engineering and regeneration	Tubule-like blood vessel network formation.	[54]
	Matrigel-EPO /CNP@FPH	HUVECs	Tissue engineering and regeneration	Tubule-like blood vessel network formation.	[55]
	Matrigel-dL-EG	PC12 cell line	Tissue engineering and regeneration	Neuron-like structures formation.	[56]
	Matrigel-AG-PCL	ADSCs, iSGCs	Tissue engineering and regeneration	Capillary-like network formation.	[57]
	Matrigel-BG-GO	EA-hy9.26 cell line	Tissue engineering and regeneration	Endothelial structure branch formation.	[58]
	Matrigel-MNSs-CNT-COOH	NSCs, C2C12 cells	Biosensing system	Neuromuscular junction's formation.	[88]
	Matrigel - PSMPs@B	ISCs	Organoid models	Colon structure formation.	[98]
B. The Negative Effects on Cell Characteristics for Cells or Organoid Culture					
Alternative Matrigel	Gelatine	CAFs, PDAC	Organoid models	The lack of maintaining phenotype cells.	[89]
	Collagen	Fresh primary tumor	Organoid models	The lack of cells is expressed.	[91]
	Chitosan-Pectin	HCT 116	Organoid models	Spheroid was irregular.	[92]
Matrigel	Matrigel	AT2S	Organoid models	The lack of maintaining recapitulate infection physiology.	[94]
Optimization Alternative Matrigel to Matrigel	Matrigel-Collagen-Manuka Honey	HUVECs, HMSCs	Tissue engineering and regeneration	Inhibited vessel formation.	[80]
	Matrigel - PSMPs@B	ISCs	Organoid models	Enhanced the damage to organoid models.	[98]

materials used as a matrix that have been developed for organoid models are still challenging, such as the fact that only a minority of cells are expressed,[91] histological processing is related to the difficulty of staining cells cultured on the hydrogel,[93] and mutation expression is insufficient to confer phenotype cells on organoid models.[89]

Furthermore, matrigel is currently being used as a matrix for organoid models. AT2s and ileum organoids showed that formed cell epithelial characteristics successfully.[94,97] Crypts and adults jejunum organoid showed that this model can remain viable after added bacteria.[95] Similar results were found for organoid models, as 3D models that improved the in vivo microenvironment showed that they formed neuroepithelium and rosette-like structures.[96] However, the development of organoid models showed that it is unlikely to faithfully recapitulate infection physiology when the virus acts as a pathogen is treated,[94] and only measurement showed viable models after being treated with bacteria after one day.[95] Besides that, matrix optimization with the various added biopolymers to matrigel is developing recently,[53–58,80,88,98] as summarized in Table 2. The addition of biopolymers to matrigel, such as collagen-manuka honey,[80] PSMPs@B,[98] OEO-BG-PLGA/Gel,[53] Gel-TCP/SC,[54] EPO/CNP@FPH,[55] dL-EG,[56] MNSs-CNT-COOH,[88] AG-PCL,[57] and BG-GO[58] that is used for developing matrix for tissue engineering, regeneration, and even organoid models successfully.

Previous studies showed that there are challenges with the various added biopolymers such as the high concentration of manuka honey added to collagen-inhibited vessel formation,[80] the number of damaged organoids was higher after added PSMPs@B due to benzo [a] pyrene (B [a] P) enhancing toxicity,[98] and the high concentration of bioceramics, BG and β-TCP, did not significantly promote angiogenicity due to their toxicity, inhibitory effect on cell chemotaxis, and weak osteoinductive activity.[53,54,58] Additionally, the optimization matrix with EPO/CNP@FPH shows that it can promote the function of HUVECs, as it is signed by tubule-like blood vessel network formation and even it had effects on osteogenesis under inflammatory conditions, but it was absent in the normal condition.[55] A similar result showed that the optimization matrix used AG-PCL promoted the maturation of ISGCs, but further verification of their significance is needed due to complexity interaction.[57] Interestingly, the added graphene to matrigel had good biocompatibility, showing that GO and graphene promoted growth cells and even successfully mimicked native cells such as neuron formation[56] and endothelial structure branch formation.[58] The overall results of the studies showed that matrigel can help microenvironment in vitro related to cell characteristics similar in vivo, especially for developing organoid models.[94–98]

Furthermore, based on this work, there are some alternative matrigel that have positive effects on cell characteristics for cell culture or organoid culture, such as gelatin, chitosan, polycaprolactone, collagen, polyethylene glycol, hyaluronic acid, ceria nanoparticle, graphene oxide, alginate, and carbon nanotubes.[51,52,55–58,63,74–79,81–83,88,90,93,95–97] Besides that, some alternative matrigel showed negative effects on cell characteristics, such as gelatin, collagen, matrigel, optimization manuka honey, and benzo [a] pyrene (B (a) P)-loaded polystyrene microplastics, as summarized in Table 2B.[80,89,91,92,94,98] Interestingly, graphene oxide has no negative effects on cell characteristics at a certain content.[58] In addition, graphene oxide will be explained next section.

Materials used in graphene combinations are currently in development for tissue engineering and regeneration, therapeutic agent, and even cancer therapy.[59–62,64–68,70,84,86] Furthermore, combination graphene showed that cell characteristics promoted cell elongation,[64,66,84] maintained cell phenotype,[64,68,84] formed spheroid[61] and neurospheres,[59] grew like native cells,[60,62,64,65,67,70] and even promoted breast cancer cells metastated.[86] Furthermore, implanted rGO did not induce DNA breakage genotoxicity at the local and systemic levels, so it can maintain cell morphology.[65] Various cell formations are obtained by emulating the native cells as summarized in Table 3. Additionally, the GO combination improves viscoelastic properties due to the anionic interaction of the GO and the cationic interaction of the biopolymer, so it may allow cells to mimic native cells.[64,70] GO combination promotes migration activity of cells, reduces and even inhibits apoptotic activity, so it gives effects on cellular spheroid formation.[61,70] Besides that, the GO combination possessing physical antibacterial function is needed for wound healing dressing application.[70]

Both the GO and rGO combinations showed that they assisted cells grow like native cells.[60,64–67,70,84] The growth cell depends on contact-mediated cues which can be delivered by graphene combinations and diffusible cues in the medium.[67] Both GO and rGO also offer unique topographic properties that influence the cell's biological response creating physicochemical properties in combination with additional biopolymers that allow easy tissue modification which is required in tissue engineering applications.[84] Additional biopolymer-to-graphene combinations also improve their mechanical properties,[132] such as hydrogel may support the formation of scaffolds,[64] and resemble MSU-1.1 cell,

Table 3 The Graphene Oxide and Their Composite to Microenvironment in Cell Culture or Organoid Culture

Materials	Cell Line/ Primary Cell In Vitro	Applications	Cell Characteristics	Ref.
PANI–GO	SH-SY5Y, cortical neuronal cell	Tissue engineering and regeneration	Neurospheres formation.	[59]
CMCs-GelMa-GO-PLGA	HUVECs	Tissue engineering and regeneration	Tubule-like blood vessel network formation.	[60]
Ce-GO	HUVECs, HMSCs	Tissue engineering and regeneration	Spheroid formation.	[61]
EU/GL/TiO2	mESCs	Tissue engineering and regeneration	Embryoid bodies formation and colonies ESCs structures.	[62]
CH-GO	SH-SY5Y	Tissue engineering and regeneration	Cell phenotype like epithelial, cell growth is spread and elongated uniformly on the cell surface.	[64]
rGOM	ARPE-19, HCE-T	Tissue engineering and regeneration	Cells formed "polygonal".	[65]
rGO-PCL	ADSCs	Tissue engineering and regeneration	Elongated spindle – like morphology.	[66]
adECM-PDA-rGO	NE-4C neuronal cell line	Tissue engineering and regeneration	Neuron like structures formation.	[67]
rGO-Mxene	MSU-1.1, HeLa, SH-SY5Y	Tissue engineering and regeneration	Maintaining cell phenotype.	[68]
SPI-SH-GA@PGO	HUVECs	Skin therapy	Tubule-like blood vessel network formation.	[70]
GO/rGO	MSCs	Therapeutic agents	Cells elongated with large nucleus (type of MSCs) and maintaining cell phenotype.	[84]
PEG-GO	MDA-MB-231, MDA-MB-436, MCF-10A	Cancer Therapy	Breast cancer cells metastated.	[86]

HeLa cell, and SH-SY5Y cell to receive the well native extracellular matrix (ECM) environment.[68] Therefore, graphene in combination with proper biopolymer is a potential material enabling development of organoid models.

Cell Behavior on Graphene-Based Organoid Models

Various studies investigate the effects of graphene on cell behavior as an evidence rule on improving a suitable microenvironment, as summarized in Table 4.

Cell Proliferation

All ten studies showed that graphene combinations affected cell proliferation. Graphene combinations such as graphene, GO and rGO showed that they had cell proliferation capabilities. Both GO and rGO showed that the cell proliferation ratio is 1 and even greater than.[60,66,73] Nevertheless, other studies showed that GO combinations had cell proliferation ratio less than 1.[58,70,87] Cell proliferation of BG/GO showed that the low GO content promoted cell proliferation[58] and similarly on GO combinations received similar result.[87] Other study showed that cell proliferation ratio less than 1 due to post-irradiation, indicating that radiation severely thwarted cellular growth, though growth cell for irradiated reverted to normality after extended timeframes.[70] However, GO showed a greater cell proliferation ratio than less rGO and graphene,[62,68,69,84] in addition cell proliferation ratio with GO more than 1 is obtained[69] than proliferation ratio of rGO, and graphene is more than 0.8[75,84] and more than 0.5,[62] respectively. Suitable environment for proliferation cells possibly due to the increase in cell's adhesion greater than 1000% in the present of GO because of surface functional

Table 4 Effects of Graphene Combination as Matrix Towards Proliferation, Differentiation, and Gene Expression

Materials	Cell Line/ Primary Cell In Vitro	Applications	Cell Behaviour				Ref.
			Cell Proliferation Ratio	Cell Differentiation	Gene Expression		
					Upregulation	Downregulation	
dL-EG	PC12 cell	Tissue engineering and regeneration	-	Neuron differentiation	-	-	[56]
BG/GO	rBMSCs, Raw264.7 cells	Tissue engineering and regeneration	0.7	Osteogenic and angiogenic differentiation	ALP, OCN, RUNX2, VEGF	-	[58]
CMCs-GelMa-GO-PLGA	MC3T3 cells	Tissue engineering and regeneration	>1	Osteoblastic differentiation	-	-	[60]
Ce-GO		Tissue engineering and regeneration	-	Angiogenic differentiation	HIF1α, VEGF, FGF2	-	[61]
EU/GL/TiO2	ESCs	Tissue engineering and regeneration	>0.5	Cardiomyocytes and neuron differentiation	Mef2c, Map2	Oct-04	[62]
CH-GO	SH-SY5Y	Tissue engineering and regeneration	-	neuroblast cells differentiation	-	-	[64]
rGO-PCL	ADSCs, DRG	Tissue engineering and regeneration	>1	Neuron differentiation	MBP, NGF, Akt, Tuj1	-	[66]
adECM-PDA-rGO	NSCs	Tissue engineering and regeneration	-	Neuron differentiation	Tuj1, MAP	-	[67]
rGO-Mxene	MSU-1.1, HeLa, SH-SY5Y	Tissue engineering and regeneration	>0.8	-	-	-	[68]
RGD/GO	L-929	Tissue engineering and regeneration	>1	-	-	-	[69]
SPI-SH-GA@PGO	HaCat cell	Tissue engineering and regeneration	0.6	-	Nrf2	NLRP3	[70]
rGO-Col	HUVECs, cardiomyocytes	Tissue engineering and regeneration	-	Cardiac differentiation	TrpT-2, Actn4, Cx43	-	[71]
GOG	BMSCs, HSCs, HUVECs	Tissue engineering and regeneration	-	Osteoclast, osteoblast, and angiogenic differentiation	ALP, Runx2, OCN, RANKL, VEGF	OPG	[72]

NGO/GelMa	HUVECs	Tissue engineering and regeneration	I	Angiogenic differentiation	LPAR6, YAP1	Lats1, p-YAP Ser127	[73]
GO/rGO	MSCs	Therapeutic agents	>0.8	Angiogenic and cardiac differentiation	GATA-4, ACTC1, MEF2C, GATA2, ENDOGLIN, VE-CADHERIN	-	[84]
GCE	MCF-7 cell	Therapeutic agents	Anti-proliferation	Osteogenic differentiation	-	-	[85]
PEG/GO-XN	MDA-MB-231, MDA-MB-436, MCF-10A	Cancer Therapy	Anti-proliferation	-	SNAIL, CDH2, VIM, TWIST1	CDH-1	[86]
GO	PC3 cell	Cancer therapy	>0.3	-	-	-	[87]

chemical compare to rGO.[64,69,84,195] Furthermore, enhanced addition properties promote cell–surface interactions, derived from an excellent ultrathin topological substrate with cell contact adhesion ability.[69] Besides that, the GO combination enhanced the ductility, so it displayed mechanical properties, which is beneficial for practical applications that allow cell culture.[70] Hydrophilic properties of graphene oxide consider responsible in cell culture medium preventing cell agglomeration[84] which may limit the nutrient supply and even induced oxidative stress which triggers the apoptotic pathway.[196] In contrast, rGO is hydrophobic which is usually promotes agglomerates in water solutions.[197]

Additionally, graphene oxide with chitosan and a tumor-specific monoclonal antibody (anti-EpCAM) for the delivery of survivin-siRNA give tumor-targeting effects[85] that cause inhibition proliferation cells. Other study reported that xanthohumol (XN) modified with PEGylated (PEG) and GO (PEG-GO@XN) can inhibit breast cancer cells.[86] Therefore, it reduces the production of ATP, blocks the migration and invasion of cancer cells even can suppress the metastasis of cancer cells.[86] Interestingly, the GO combination promoted metastatic breast cancer cells, but after adding xanthohumol (XN) to GO, it provided suppression of metastatic tumors.[86] Furthermore, the graphene oxide-based surfaces may influence the therapeutic agent's applications, such as small flakes and thick graphene layers. Interestingly, small flakes of graphene oxide are 0.2–2 μm can reduce cell proliferation and induce their apoptosis.[84] While the most suitable surfaces for cells of graphene oxide layer thickness are 10 and 15 μg/cm2.[84] Similarly, an average thickness of GO of 2 nm shows biocompatibility indicated by no apparent cytotoxicity.[86]

Cell Differentiation and Gene Expression

The biocompatibility of biomedical materials is the most important requirement. The combination of graphene has great potential for nanomedicine and biomedical applications. Recent studies have shown that graphene combinations have been widely explored for bioimaging, biosensing, and antibacterial applications, as summarized by Bellier et al.[198] Interestingly, there is a new study showing that inhalation of GO in humans is well tolerated and has no adverse effects.[199] Therefore, graphene combinations have been considered as biocompatible materials. In this review, graphene combinations have supported biomedical applications such as tissue engineering and regeneration, cancer therapy, and even therapeutic agents, as summarized in Table 4. Furthermore, graphene combinations provide a microenvironment necessary for cell differentiation and gene expression, which are important for tissue engineering. Appropriately engineered graphene materials are not only biocompatible but often greater when it comes to creating the microenvironment necessary for cell growth, differentiation, and development.[200–202]

Cell differentiation is needed for different functions to develop multicellular cells. Therefore, cell differentiation is a cellular process for developing cell types, with appropriate functions including for developing organoids. The various cell types of differentiated cellular processes for developing mature organoids. Growth factors and chemical agents are frequently used to increase and regulate cell differentiation. Furthermore, organoids, as artificial three-dimensional cell cultures, also require growth factors and chemical agents for tissue construction. However, the use of growth factors and chemical agents has shown drawbacks such as being unstable, inefficient, and even hazardous.[203,204] Therefore, nanomaterials have been developed currently to overcome these limitations due to their physical and chemical properties.[205] Nanomaterial has bioactive effects so it can improve and even control cell differentiation.[206] Graphene is one of the nanomaterials that has shown effects on cell differentiation. Biofunctionalization of graphene in combination with growth factor and ECM proteins may further improve biological properties.[84] Graphene in combination with various biomaterial effects on cell differentiation reported in ref.[56,58,60–62,64,66,67,71–73,84,85]

Graphene combination has shown cell differentiation such as neuronal differentiation,[56,62,64,66,67] osteogenic and osteoblastic differentiation,[58,60,72,85] angiogenic differentiation,[58,61,72,73,84] and cardiac differentiation.[62,71,84] Both graphene combinations, GO and rGO, have shown neuronal differentiation. In contrast, rGO has shown success in developing neuron cells, with the axon length of DRGs up to 738 μm.[66] Other studies of GO in combination with chitosan showed that GO can give neuritic extension from the center neurospheres about 39 μm.[64] Similar results showed that GO in combination with decellularized lotus petioles gives neuritic extension is more than 60 μm.[56] Decellularized lotus petioles in combination with GO improve carriers for neural cells.[56] rGO has shown longer than GO for neuronal differentiation. However, these studies showed that GO affected cell differentiation into neurons and neurospheres. However, remained acceptable for the successful development of GO in 3D cell culture. Furthermore, rGO combined

with gelatin promotes osteogenic differentiation,[72] similarly, results GO combination showed that GO combination improves osteogenic and osteoblastic differentiation[58,60] and even similarly only GO receives similar result.[85] Besides that, angiogenic differentiation has shown the capability of GO and rGO.[58,61,72,73,84] Furthermore, the graphene combination showed that it supported differentiating cells and the formation of functional cardiomyocytes.[62] Similarly, the results of the rGO combination showed that it can induce cardiac differentiation in MSCs.[71] GO and rGO induced hUC-MSCs differentiation into cardiomyocyte-like cells.[84] Moreover, GO and rGO can maintain multipotency capabilities, promote growth cells, and even maintain viability cells.[84] Interestingly, Nano Graphene Oxide (NGO) gave wound healing effects for tissue engineering and even regeneration, which is proved by wound area up to 20%.[73] Furthermore, cell differentiation could regulate the expression of proteins.[85] It means that the process of cellular differentiation is regulated by transcription factors. Therefore, transcription factors that affect whether or not a gene is transcribed play a role in the specialization.[207]

In addition, studies have shown how the effects of combination graphene on cell behavior are mediated by gene expression.[58,61,62,66,67,70–73,84,86] Some genes, such as SNAI1, CDH2, VIM, and TWIST1, showed that gene upregulated on PEG-GO-XN indicated the formation of endothelial-type cells into mesenchymal-type cells.[86] However, there is a gene downregulated, such as CDH-1 caused, by PEG-GO-XN abolishing TGF-β1. This study showed that gene upregulation and gene downregulation caused epithelial-like maintenance and even decreased the motility potential of breast cancer cells.[86] Therefore, XN loaded PEG-GO can suppress the metastasis of breast cancer. The other study showed genes upregulated, such as LPAR6 and YAP1, while genes downregulated, such as Lats1 and p-YAP Ser127 in HUVEC cells.[73] LPAR6 plays role in suggested angiogenesis, while YAP1 plays a key role in NGO-induced endothelial tip cell angiogenesis.[73,208] In addition, LPAR6 activated the downstream Rho signaling pathway, leading to cell proliferation and migration,[73] whereas Lats1 and p-YAP Ser127 are genes downregulated induced the nuclear translocation of YAP as mediator of activity and cell migration.[73,209,210] Therefore, the formation, growth, and integrity of the blood vessel network are related to angiogenesis. Furthermore, other studies showed that genes upregulated related to angiogenic, such as GATA2, ENDOGLIN, VE-CADHERIN,[84] VEGF,[58] HIF1α, VEGF, and FGF2.[61] The expression levels of GATA2, ENDOGLIN, and VE-CADHERIN are 2.5-fold, 4.5-fold, and 3-fold, respectively.[84] Furthermore, this study compared with rGO and showed that the expression levels of ENDOGLIN and VE-CADHERIN are less than 5-fold and 2-fold, while the expression level of GATA2 is 4-fold.[84] These genes upregulated indicate that they promote the formation of long-length capillaries. Besides that, the upregulation of VEGF indicating effects to pro-angiogenic.[58,61,72] Furthermore, upregulation of VEGF and FGF2, which is considered by HIF1α as a key event that signals promoted secretion of angiogenic factors.[61] The expression levels of VEGF in GO combination and rGO combination are 1.5-fold and 1.75-fold, respectively.[61,72] These results of studies showed that the expression levels of genes promoted to angiogenic of rGO are higher than GO.[61,72,84] However, other study showed that VEGF expression is upregulated, indicating that graphene combination had pro-angiogenic effects through interaction between the osteoblastic and osteoclastic progenitors, promoting angiogenesis to further favor recruitment of osteoblastic and osteoclastic.[58,72] Therefore, these results showed a rapid wound healing area of around 50% and 80% in migration distance in combination with rGO and GO, respectively.[58,72]

Other genes upregulated such as ALP, RUNX2, and OCN indicate osteogenic differentiation due to the hydrophilic of GO significantly promoting early osteogenic differentiation.[58] However, these studies showed that the expression levels of ALP, RUNX2, and OCN decreased due to the high content of GO. A similar study showed that genes upregulated such as ALP, RUNX2, OCN in rGO combinations.[72] Furthermore, this study showed that other genes were upregulated and downregulated, such as RANKL and OPG, respectively, indicating the rGO combination has potential promotive effects on osteoclastogenesis.[72] Besides that, there are genes upregulated and downregulated related to cardiac gene expression that have shown the effects of graphene combination on cardiac differentiation.[62,71,84] GATA2, ACTC1, and MEF2C are genes upregulated in GO combinations with the expression level of them being 2-fold, almost 2-fold, and 1-fold, respectively.[84] Additionally, this study compared with rGO and showed that the level expression of GATA2, ACTC1, and MEF2C in rGO is lower than GO. These genes upregulated indicated that GO improved cardiomyogenic differentiation.[84] Other study showed that genes upregulated such as TrpT-2, Actn4, and Cx43 in rGO

combinations, with the expression level of them is almost 5-fold, 2-fold, and 2.5-fold, respectively.[71] These genes indicated that Actn4 is a gene involved in the cytoskeleton, as is Cx43, a gene involved in cardiac electrical.[71] Besides that, genes upregulated and downregulated, such as Mef2c and oct4, respectively, in graphene combinations.[62] These genes upregulated related to cardiac differentiation, while genes downregulated, indicating a decrease in undifferentiated ESCs.[62]

Besides that, other studies showed that genes regulated in neuronal differentiation.[66,67] MBP, NGF, and Akt are genes upregulated in rGO combinations.[66] These genes upregulated indicated that NGF is related to neurotropic phenotype, while MBP associated with the formation of myelin sheath and Akt related to nerve regeneration.[66] However, this study showed that these expression levels of MBP, NGF, Akt decrease around 2.425-fold, 3.885-fold, and 2.436-fold, respectively, in rGO combinations after treatment LY294002, which is blocked activation of Akt signaling and decreased MBP and NGF.[66] Similar study showed that genes upregulated in rGO combinations such as Tuj1 and MAP with an increase in their levels by 1.26-fold and 1.72-fold, respectively.[67] Additionally, the ratio of Tuj1 in rGO combinations showed that it is higher than without rGO combinations, 35.18% and 21.33%, respectively.[66] Upregulation of Tuj1 and MAP indicating neuronal differentiation alongside the increase in neuritic length and even associated with the maturation of dendrites.[66,67] Interestingly, in GO combinations with loaded XN, there are genes upregulated such as SNAIL, CDH2, VIM, and TWIST1, while genes down-regulated such as CDH-1 cause the maintenance of epithelial-like features and decrease the motility potential of breast cancer.[86] In addition, GO combinations showed that upregulation of Nrf2 roles thwarts oxidative stress, so it effects downregulation of NLRP3 related to prevent inflammation triggering and even DNA injury, so it can reduce radiation-induced skin injury.[70]

The overall results showed that combination graphene improved for cell behavior such as cell proliferation, cell differentiation, and gene expression.[56,58,60–62,64,66–73,84–87] Interestingly, combination GO has the potential for developing in 3D cell culture models. The surface functional chemicals, an excellent ultrathin topological substrate with cell contact adhesion ability, hydrophilic properties, mechanical properties, and suitable size of GO, are considered for development in organoid models as 3D model cell culture.[64,69,70,73,84,195] Additionally, the doses of GO are also considered for development in 3D cell culture models because they influence their cytotoxic effects.[58,87] Therefore, doses of graphene oxide will be described below, along with their effects on cell viability and growth.

Doses of Graphene

Non-toxicity properties of biomaterials are considered for developing cell culture and are signed by cell viability so that they ensure good biocompatibility. Some studies have shown that doses of graphene oxide are related to cell viability and even stimulate cell growth, as summarized in Table 5.[60,70,73,85–87]

Viability ratio cells around 1 as shown by the doses of GO have been shown in Table 5.[85,86] Interestingly, the various concentrations of GO (10–150 μg/mL) showed that the result of cell viability was not significantly different, namely that viability ratio cells around 1.[85] Similar results showed that 10 μg/mL, which is a dose of GO, received a similar ratio of cell viability as the previous study.[86] Therefore, these studies showed that the various concentration doses of GO showed no apparent cytotoxicity.[85,86] Interestingly, these doses of GO tested with added inhibitor cancer cells, XN, inhibited that metastatic breast cancer was vulnerable to XN exposure due to XN inhibiting the cell viability at higher concentrations.[86] A similar study showed that using the various doses of GO tested to suppress cancer cells and adding a tumor-specific monoclonal antibody (anti-EpCAM) for the delivery of surviving siRNA had anti-proliferative effects.[85] The further study using the various doses of GO showed that the cell viability ratio around 1 and even more than 1.[60,73] Interestingly, the doses of GO up to 400 μg/mL did not effect the viability of cells.[73] Additionally, this study assessed that the doses of GO are 0–50 μg/mL for wound healing and showed that doses of GO with 5 μg/mL accelerates migration and enhances endothelial cells.[73] However, the various doses up to 400 μg/mL showed that GO promotes the formation of capillary tubes and networks.[73] Other study used the doses of GO is 0.1 mg/mL showed that cell viability ratio more than 1 and even promoted cell migration.[60] Nevertheless, there are studies showed that cell viability ratio is less than 1.[70,87] The dose of GO is 10 mg/mL used in GO combination tested with radiation treatment showed that it does not influence irradiated culture expansion.[70]

Nevertheless, the cell viability ratio is less than 1 due to post-irradiation, so it influences thwarted cellular growth after 4 days, but growth for irradiated cells reverts to normality after extended timeframes.[70] Additionally, this study showed that GO combinations give angiogenic effects and reduced apoptotic activity, so it promotes cell migration around 50%.[70] Similar results showed that the cell viability ratio is less than 1 with 0–50 µg/mL, though the low doses of GO nearly by 10 µg/mL promote tumor cell proliferation.[87] Furthermore, the previous study showed that the doses of GO around 5–20 µg/mL of no apparent cytotoxicity in vitro assessment and even maintained cell viability at more than 80%, while the doses of GO are more than 50 and 100 µg/mL induced significant decrease in the cell viability.[68,211]

Besides that, the size of GO supported maintaining cell viability and even affected cell growth.[73,87] The thickness of GO layers of 10 and 15 µg/cm2 increased proliferation rates.[84] Furthermore, several studies showed the size of GO, such as the average diameter of 100 nm and thickness of 2 nm,[86] diameter of 80–350 nm,[85] diameter of 0.5–3 µm and thickness of 0.55–1.2 nm.[59] In addition, GO with a size less than 100 nm showed no toxicity.[212] The size of GO provides for biological application because GO, especially, is more hydrophilic and has a large edge-to-area ratio, so it is enriched in carbonyl and carboxyl groups.[213]

The overall studies showed that the doses of GO combination do not show cytotoxic cells. The previous studies have shown that the concentration of graphene oxide shown in Table 5. Besides that, some studies showed that the size, such as diameter and thickness of GO is safe for developing or growing cells. Therefore, the doses and the size of GO appropriately are considered for developing organoid models currently.

Author's Perspective

Previous studies showed that the development of a matrix has been used for organoid models. Matrigel has been used for organoid models.[94,95] Those studies showed that matrigel can regenerate epithelial and maintain the viability of models. Matrigel is a matrix that can support cell adhesion so suggest a cell attachment for developing the organoid model.[41] The composition of matrigel consists of laminin, collagen type IV, and nidogen and has been identified in 1800 peptides or proteins, so that it has various compositions.[40,41] However, matrigel has limited manipulation so it is difficult for improving cell behavior and cannot achieve specific biological properties.[214] Therefore, alternative matrigel is considered for substituting matrigel. The alternative matrigel as a matrix has been used for organoid models currently. It is such as gelatin,[89] GelMa,[90] collagen,[91] chitosan-pectin,[92] and PEG.[93] Hydrogel as an alternative matrigel can be more flexible than matrigel for controlling cell behavior, such as cell adhesion and cell rigidity.[215]

Gelatine, as one of the hydrogel systems, has been developed as an ECM.[53,54,81,89,90] Hydrogel can be used for recapitulating the ECM for several advantages, such as being highly hydrated, giving an environment to cells, and being flexible, so it is very similar to what cells experience in vivo.[216] The flexibility of hydrogel means that it can be combined with other biomaterials that are amenable to chemical modification and process engineering techniques that allow for control of their properties.[217,218] The mechanical properties of hydrogel vary across the spectrum that is typical of soft tissue in the body and even much more closely match the microenvironment condition.[216] Additionally, the softest hydrogel influences the mechanical properties with an increase in elastic modulus, which enhances regulated gene expression, while the rate of proliferation decreases.[219] Besides that, some previous studies

Table 5 Doses of Graphene Oxide Effects on Viability Cells

Doses of GO	Viability Ratio	Effect on Cell Growth	Ref.
0.1 mg/mL	> 1	Promoted cell migration	[60]
10 mg/mL	0.6	Promoted cell migration	[70]
0–400 µg/mL	1	Induced cell assembly and new blood vessel formation	[73]
10–150 µg/mL	1	Inhibit MCF-7 cell growth	[85]
10 µg/mL	1	Inhibited the migration and invasion of breast cancer cells	[86]
0–50 µg/mL	> 0.3	Promoted tumor cell proliferation	[87]

explained that gelatin for development related to cell culture in vitro can improve good cell behavior due to mimicking in vivo microenvironments.[72,81,220–222] Gelatine can provide viability for organoid models, supported growth cells, and even cell characteristics that can mimic cell structure types of organoids without changing phenotype cells.[89,90]

Interestingly, graphene oxide as an artificial extracellular matrix due to its honeycomb structures has been developing recently.[223] It is considered biocompatible and mechanically stable for cell growth, differentiation, and even combinations of GO in the cells to promote binding and proliferation.[224,225] Furthermore, GO has good biocompatibility and even effects on cell growth,[69] cell migration,[64,69,73,84,86] and cell adhesion.[64,69,84] Based on this review, we found that GO has the potential to develop organoid models. Previous studies showed that GO has been used a lot in biomedical applications such as for developing tissue engineering and regeneration, as a therapeutic agent, and even for cancer therapy.[56,58–61,64,69,70,73,84–87] Furthermore, GO encourages good cell behavior, such as suitable cell characteristic,[58–61,64,70,84,86] cell proliferation,[58,60,69,70,73,84,87] cell differentiation,[58,60,61,64,73,84,85] and gene expression regulation.[58,61,70,73,84,86] Cell proliferation plays an important role in growth, repair, reproduction as well as renewing damaged cells. Cell differentiation and gene expression are both related to each other for developing cell types with appropriate functions, which are regulated by transcription factors that affect whether or not gene expression relates to the function. Besides that, the structure of GO had unique properties that were preferable for cells that promote protein in culture media or cellular secretions, which improved overall cell–hydrogel interactions.[134,226] In addition, the size of GO provides more hydrophilic properties, so it can be readily combined with other materials,[213] while the doses of GO suitable do not show cytotoxic cells, so it can maintain cell viability and even influence cell growth.[73] Therefore, GO combined with gelatin as hydrogel can be considered for developing organoids as disease models, as summarized in Figure 5.
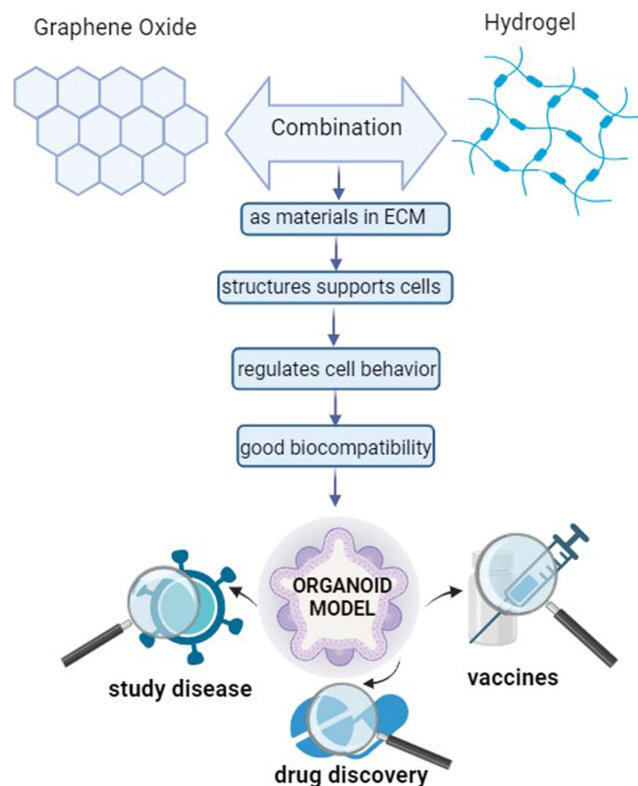

Figure 5 Potentials of graphene oxide for developing organoid Models.

Conclusion

The development of various materials has been used a lot for biomedical applications such as tissue engineering and regeneration, as a therapeutic agent, skin therapy, biosensing system, and even cancer therapy. The development of hydrogel as an alternative to matrigel has been used a lot for developing organoid models. Gelatine is currently one of the hydrogel systems shown in the organoid model as a matrix. Their properties give them flexibility, so they can be readily combined with other materials to mimic the microenvironment in vivo. Besides that, graphene is one of the various materials that has been used a lot for tissue engineering and even drug delivery. Graphene is one of the materials that can be combined with other materials as a matrix, as has been shown in various previous studies. The overall studies related to the use of graphene, especially graphene oxide, showed that it has been successful as a material for matrix in 3D cell culture due to its good biocompatibility. Graphene oxide showed that it has good cell proliferation and differentiation, and even gene expression regulation has shown a specialization role in tissue engineering and regeneration applications. Moreover, the concentration and size of graphene oxide certainly show no cytotoxicity for developing cells. Therefore, optimizing with graphene oxide can be considered to advance the development of organoid models.

Abbreviations

ADSCs, adipose-derived mesenchymal stem cells; adECM-PDA-rGO, adipose decellularized Extracellular Matrix-polydopamine-functionalized reduced graphene oxide; AG-PCL, Alginate Gelatine-poly(ε-caprolactone); BADSCs, brown adipose-derived stem cells; BG/GO, Bioactive Glass/Graphene Oxide; cECs, Cardiac endothelial cells; CMCs-GelMa-GO-PLGA, carboxymethyl chitosan-gelatin methacryloyl-graphene oxide-Poly lactic-co-glycolic acid; CPSN, carbon nanotubes (CNTs)-containing electrospun polycaprolactone/silk fibroin nanofibers; CH-GO, Chitosan-Graphene Oxide; Col-Ch, Collagen-Chitosan; dL-EG, decellularized lotus petiole graphene oxide; EPO/CNP@FPH, erythropoietin (EPO)/ceria nanoparticle (CNP)@Fmoc-Phe3 hydrogel; EU/GL/TiO$_2$, hybrid eumelanin/graphene-like/titanium dioxide; GCE, GO was modified with a non-toxicity cationic material (chitosan) and a tumor-specific monoclonal antibody (anti-EpCAM); GelMa, Gelatin Methacryloyl; Gel-TCP/SC, Gelatin-beta-tricalcium phosphate (β-TCP)/SrCuSi$_4$O$_{10}$ (SC); GG, Gellan gum; GO/rGO, Graphene oxide/reduced Graphene oxide; GOG, gelatin-reduced graphene oxide; HUVECs, human umbilical vein endothelial cells; H9C2, Rat cardiomyoblast cells; IGF1c-PHM, insulin-like growth factor 1 mimetic peptide supramolecular hydrogel microspheres; ISCs, Intestinal Stem Cells; iSGCs, induced sweat gland cell; MESCs, mouse embryonic stem cells; MNSs-CNT-COOH, motor neuron spheroids-carboxylated carbon nanotubes; NGO, Nano Graphene Oxide; NSCs, neural stem cells; NSPCs, neural stem/progenitor cells; OEO-BG-PLGA/Gel, oregano essential oil-Bioactive Glass-poly(L-lactide-co-glycolide)/Gelatin; PANI–GO, Polyaniline-Graphene Oxide; PAMB-C-OHA, Poly-3- amino-4-methoxybenzoic acid carboxymethyl chitosan-oxidized hyaluronic acid; PEG, poly-ethylene glycol; PEG-GO-XN, PEGylated-Graphene Oxide-Xanthohumol; PLA–HA, polylactic acid–hyaluronic acid; PSMPs@B, benzo [a] pyrene (B (a) P)-loaded polystyrene microplastics; PUCL@CNT, polyurethane-polycaprolactone (PUCL)@carbon nanotubes; rBMSCs, rat bone marrow stromal cells; rGO-Col, reduced Graphene Oxide-Collagen; rGOM, reduced Graphene Oxide Membrane; rGO-Mxene, reduced Graphene Oxide - Ti$_3$C$_2$T$_x$; rGO-PCL, reduced Graphene Oxide – Polycaprolactone; RGD/GO, arginine-glycine-aspartic acid peptide and Graphene Oxide; RSF/Mxene, regenerated silk fibroin/Mxene; Se-EX-Ch-COL/TA, Selenium-Exosomes-Chitosan-Collagen/Tannic Acid; SPI-SH-GA@PGO, Soy Protein Isolate-Gallic Acid-phenol and thiol cohorts@Graphene Oxide.

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Disclosure

The authors declare no conflicts of interest in this work.

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