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Dynamic in vitro models for tumor tissue engineering

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ABSTRACT

Cancer research uses in vitro studies for controllable analysis of tumor behavior and preclinical testing of therapeutics. Shortcomings of basic cell culture systems in recreating in vivo interactions have driven the development of more efficient and biomimetic in vitro environments for cancer research. Assimilation of certain developments in tissue engineering will accelerate and improve the design of these environments. With the continual improvement of the tumor engineering field, the next step is towards macroscopic systems such as scaffold-supported, flow-perfused macroscale tumor bioreactors. Surface modifications of synthetic scaffolds allow for targeted cell adhesion and improved ECM development. Flow perfusion has emerged as means to expose cancerous tissues to critical biomechanical forces for tumor progression while simultaneously improving nutrient and waste transport. Macroscale perfusable systems allow for non-destructive real-time monitoring using biosensors capable of improving understanding of in vitro tumor development at reduced cost and waste. The combination of macroscale perfusable systems, surface-modified synthetic scaffolds, and non-destructive real-time monitoring will provide advanced platforms for in vitro modeling of tumor development, with broad applications in basic tumor research and preclinical drug development.

1. Introduction

In 2018, the American Cancer Society estimated that there will be 609,640 cancer deaths and 1,735,350 new cancer diagnoses within the United States, with cancer being the second most common cause of death [1]. 2D culture systems are the primary means for therapeutic testing due to their cost and reliability, but 3D microenvironments dictate the phenotypic progression of cultured cells and tumors such as complex extracellular matrices, vascularization, and cellular networks [2]. Surface geometry intricacies have been shown to alter cell signaling, gene expression, and protein localization in cardiomyocytes [3–5]. The changes in 3D microenvironments also push differentiation alterations for mesenchymal stem cells to hepatocyte-like cells [6] and affect human pluripotent stem cell differentiation [7]. Tumorigenic cells are also affected by 3D microenvironments with 100 different cancer cell types being classified into four different 3D morphology types [8] coupled with cell type specific conditions like high diffusion barriers [9]. The impact of 3D microenvironments on cancer cells affect not only their phenotypic responses, but also their gene expressions and inhibitions of regulatory responses [10].

To overcome limitations of 2D systems, 3D culturing has become a new frontier for cancer research. The most promising models for in vitro cancer analysis are spheroids, organoids, and scaffold-supported bioreactor models [11,12] (Fig. 1). Spheroid models are commonly used for 3D tumor models and have made advancements in the field such as chemoresistance [13] and gene expression [14], but these models neglect important stressors for tumor progression by culturing them in static systems [15]. Fluid flow and interstitial shear forces have been shown to play a critical role in mimicking in vivo biomechanical forces in vitro not only in several normal cell types [16], but in tumor cells as well [17,18]. Interstitial fluid flow plays a critical role in microenvironment alteration by acting as a transport for multiple soluble factors to lymph nodes [19], applying direct and indirect biomechanical forces to the cells via the matrix [20,21], driving fibroblast differentiation [22], and altering growth factor extracellular gradients [23,24]. Beyond transport and biomechanical force generation, cell stiffening by stagnation has been shown to reduce metastasis and correlate poor prognosis thereby failing to mimic in vivo conditions in breast, skin, liver, lung, and brain cancers [25–27].

Another recent addition to oncological culturing is the use of organoids, which are self-organizing tissue-derived stem cells that form organotypic structures [28] and can even be derived via induced pluripotent stem cells [29]. Multiple studies ranging from breast [30], colon [31], and prostate [32] have shown that tumor-derived organoids

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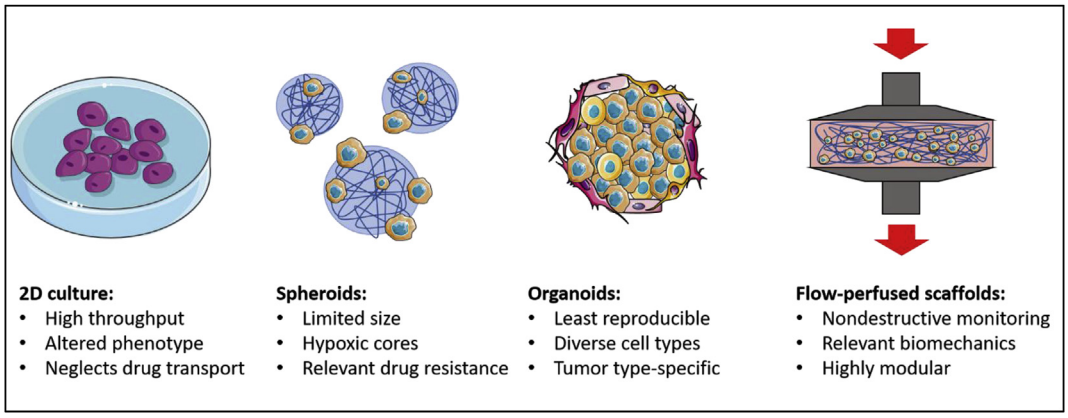

Fig. 1. Comparisons of culturing techniques from 2D to multiple macroscopic methods.

resemble the genetic and phenotypic epithelium of which they were derived [33]. Although organoids present worthwhile benefits, their limitations to full-scale modeling reduce their viability. Organoids lack the development of vessels, immune cells, and stroma [34], and their interaction with serum and serum-derivatives cause unfavorable long-term results [35]. Unreliable growth and heterogeneity between organoid samples [33] yield undesirable conditions for macroscale modeling.

In recent years, macroscopic cultures and 3D perfusable systems have been utilized in the cancer field to better understand the effects of the local environment and structure on tumor progression and therapeutic solutions. These systems create biomechanical stimulation through continuous or semi-continuous fluid flow [36] as well nutrient delivery and waste removal [37]. To withstand the perfusable systems, scaffolds have been used for cell adhesion and to help mimic the tumor microenvironment, as hydrogels [38], synthetic hard scaffolds like polycaprolactone (PCL) and poly(lactic-co-glycolic acid (PLGA) [39] and modified hydrogels [40] have been shown to be suitable scaffolds for specific cancer cell culturing; however, scaffold stiffness, pore and fiber size, and interconnectivity affect cell culturing [41]. To overcome these limitations, 3D printing has emerged as a solution to precisely construct uniform, or even random, scaffold geometries to mimic microenvironments [42]. Although some 3D printing scaffold material can present adhesion or microenvironmental limitations like limited bioactive sites [43], studies have successfully shown the viability of surface modifications on certain hard polymers such as poly-(lactic acid) (PLA) and poly-(ε-caprolactone) (PCL) using peptides and other linkers [43].

The customizability of perfusable systems has been demonstrated the ability to control biomechanical forces as well as niche tumor environments through scaffold modifications. Real-time monitoring has been shown to be a viable technique for perfusable systems using optics and biochemical assays [44]. By using the macroscopic scale and the correlated real-time systems, the combination of all these available tools create a unique environment for tumor culturing and research. This review will cover the status of perfusable 3D models for tumor engineering alongside their customizable parts including scaffolds and real-time monitoring.

2. Scaffolds & surface modifications

The challenge of mimicking tumor microenvironments stems from the complexity from which these environments are produced. In native tumors and in vivo models. 2D morphologies are distinctly different from their 3D counterparts with differences such as the presence of secondary cell types, ECM components, and matrix stiffness leading to increased diffusion limitations and chemoresistance [45]. Heterogeneity exists amongst the environments where different phenotypes and differentiation stages occur simultaneously with varying proliferation rates [46,47]. Subsections of varying cell types within the tumorous growth can remain inactive and add to chemoresistance as well as tumor relapse and metastasis [48–50]. The gap between native morphologies and 2D cultures has been partially bridged using natural and synthetic 3D constructs [45].

Macro-scale 3D models expand on microfluidic applications to more fully replicate tumorigenic environments, extracellular matrices, and tumor physical properties [51–53]. Hydrogels, like collagen and alginate [53,54], are commonly used for macroscopic 3D scaffolds due to their biocompatibility, and they are typically used to explore drug efficacy and tumor development in a microscopic view. Sung et al., and Huang et al., used hydrogels to investigate cancer metastasis into adjacent cell hydrogels and have shown the utility of macroscopic hydrogels to study cell to cell interactions in relation to extracellular matrix environments [55,56].

Natural scaffolds, such as collagen or alginate hydrogels, remain commonplace in tumor engineering due to their physiological relevance [57–60]; however, their limitations become more significant when transitioning to more complex and niche tumor microenvironments. Limitations for natural scaffolds range from low modularity and batch variability to inconsistent ligand bioactivity as well as a failure to promote cell-cell interactions amongst a dynamic biomechanical environment [61]. Control of natural scaffolds' biomechanical properties, compositions, and geometries remains a challenge [62]. Synthetic polymer scaffolds which include PCL, poly-(glycolic acid) (PGA), PLA and their derivatives [63] have been developed as an alternative solution to certain natural scaffold limitations. Precise control over synthetic polymer architectures and geometries, as well as a high degree of chemical composition precision [64], alleviate variability and provide a starting point to attain desired ECM characteristics [43]. These polymers demonstrate in vivo biocompatibility, biodegradability, and critical cell encapsulation properties [61]. Although synthetic polymerics overcome some of the limitations of other common scaffolds, limited cell adhesion and delayed ECM development due to the hydrophobicity of these materials requires further consideration [65,66].

Cell-matrix and cell-cell interactions control cell morphology and motility [67], affecting tumor progression and proliferation [68]. Interestingly, cellular survival and proliferation have been shown to depend heavily on cell adhesion through integrin-related interactions [69]. Cell adhesion molecules are typically surface glycoproteins with variable effects on different cell types while E-cadherins [69], R-cadherins [70], and peptide chains can become linkers for cell adhesion [43]. It has been shown that cancer cell lines, especially metastatic cancer cell lines, exhibit weaker cell adhesion strength [71] and when coupled with the lower bioactive site count of synthetic hard polymers [72] necessitate the surface functionalization of the scaffolds with biomolecules that promote the adhesion of these cells. By modifying the

surfaces of synthetic polymer scaffolds to present adhesion proteins and peptides, the limitations of cell-surface interactions can be reduced while simultaneously retaining the fine control of production characteristic of synthetic polymers.

Synthetic scaffolds for tumor engineering have emerged as an investigative means to facilitate 3D cancer research by creating controlled and uniform platforms with improved cell seeding density and homogeneity [61]. Human oral cancer using oral squamous cell carcinoma was modeled using synthetic PLGA scaffolds, showing that chemosensitivity, cellular penetration, and angiogenic ability increased compared to 2D cultures and 3D natural scaffolds [73]. The incorporation of hydroxyapatite within PLGA scaffolds has improved breast cancer metastases [74], and electrospun PCL scaffolds have shown improved chemoresistance and gene expressions compared to 2D cultures [75]. In an effort to increase cell adhesion, Girard et al. compared PLGA and PLA scaffolds modified with polyethylene glycol (PEG). When seeded with several cancer cell lines including BG1 ovarian cancer cells, MCF-7 breast cancer cells, and B16 melanoma cancer cells, modified synthetic scaffolds demonstrated increased adhesion compared to non-modified PLGA and PLA scaffolds enabling the culture of tumor biopsies [76].

The use of scaffolds in tumor engineering improve the ability to mimic tumor microenvironments as well as provides stability and adhesion for cancer cell culturing. Despite the increasingly diverse applications of scaffold surface modifications in tissue engineering, few surface modifications have been applied that directly focus on cancer cell interactions. Select complete scaffold-fabrication-to-culture studies are summarized in Table 1. Synthetic biomaterial scaffolds allow for precise control over architectures, and the addition of surface modification improves their poor cell adhesion and proliferation. Surface-modified scaffolds make perfusion bioreactor systems viable by reducing cell detachment due to fluid flow induced shear stress.

3. 3D flow perfusion

The oncological community has taken notice of microfluidic systems from its modeling potentials of tumorigenesis factored by spatial development such as metastasis, drug resistance, and intercellular communication. Controlled microscale conditions, such as laminar flow, allow for a reproduction of niche concentration gradients for tumors [77,78], as well as better defined single cell drug transport and biological process actions [79]. Cell sorting [80] and pharmacodynamic studies [81] have been using microfluidics as an effective analytical tool for tumor biology. Jang et al. developed a microfluidic platform for chemo resistant patients that tested up to 100 differing drug combinations [82]. Microfabrication techniques elicit better control over topology and surface chemistry [83], as shown by Dickinson et al. and Shin et al., where microcontact-printed scaffolds were used to test extravasation and metastasis on tumor cells and endothelial cells using hyaluronic acid and fibronectin [84,85].

Early approaches for perfusable culture systems usually included natural scaffolds like immobilized 3D hydrogel scaffolds that use diffusion for nutrient and solute transport despite their drawbacks [86], which included limited transport and biophysical stresses through their use of flow around rather than flow though the scaffold [87]. Toh et al. developed a microfabricated pillar system of immobilized cells using a polyelectrolyte coacervation reaction to support their 3D matrix to enhance the diffusion transport. Three days of cultured perfusion using this system formed multicellular aggregates with MCF-7 and HEP G2 carcinoma cell lines [88]. To investigate microscopic interstitial flow to study tumor migration, Polacheck et al. used 3D collagen I constructs in a microfluidic channel using hydrostatic pressure gradients to demonstrate applicable tumor flow [89]. Along similar lines, Haessler et al. has shown that interstitial flow affects cell migration, as well as generates differential responses from cell populations of the tumor exposed to interstitial flow [90]. Despite natural scaffold successes, diffusive transport limitations can be better controlled with porous scaffolds

Table 1
Surface modifications for cancer model scaffolds.

Reference	Cancer type	Cell line	Scaffold	Modification	*Results*
Pathi et al. [151]	Breast	MDA-MB231	PLGA	Hydroxyapatite incorporation	Hydroxyapatite promoted metastatic behavior, enhanced IL-8 secretion.
Ma et al. [152]	Osteosarcoma	MG-63	Tricalcium phosphate (TCP)	Graphene oxide (GO) coating	GO-coated TCP scaffolds facilitated photothermal therapy to lyse cancerous cells with subsequent functionality as an osteoconductive scaffold for bone regeneration.
Szot et al. [153]	Prostate, renal, breast	PC-3, RENCA, MDA-MD-231	PCL/collagen	Nanofibrous topography	PCL/collagen scaffolds promoted cancer cell adhesion and proliferation.
Hartman et al. [154]	Prostate Cancer	C4-2B	PCL and PCl/gelatin	Immobilization of perlecan domain IV peptide (PlnDIV)	PlnDIV increased C4-2B cell focal adhesion activity, promoting cell adhesion, proliferation, and migration across the scaffold.

using synthetic hard polymers, but few studies have explored the use of hard porous scaffolds for tumor studies. Ma et al. used PLA scaffolds to house gliomastoma brain cancer cells and liver cells in a microscale two-chamber perfusion system and has shown the potential for these types of scaffolds and perfusion systems to study toxicity of therapeutic drugs [91].

Macroscale flow-perfusion bioreactors may be an appropriate next step to more accurately model tumor progression since tumorigenesis is a delicate balance of both macroenvironmental and microenvironmental aspects [92] wherein the functional connections between cancer cells and their neighboring microenvironments can result in tumorigenesis [93] and phenotypic alterations [94]. Differences in cellular responses are observed when comparing microfluidic systems to biological assays and their macroscopic counterparts [95,96]. Nutrient availability, cell viability, and volume densities changed between micro and macroscopic testing of mammary fibroblasts. Increased cell viability and nutrient availability was observed for macroscopic systems, and the volume densities varied as much as 5 times between scales [96]. The literature in the oncological field for perfusion systems is limited but growing due to increasing appreciation for these systems' ability to overcome limitations of current microscopic systems [97]. Mishra et al. developed an ex vivo lung model by utilizing decellularization techniques to form a scaffold. They then used perfusable systems for cell seeding and culture, showing characteristics similar to the original cancer lung sample [98]. In another study, the same group cultured their 3D ex vivo decellularized cancer lung for 15 days and showed that their 3D model produced matrix metalloproteinases that were not present in 2D cultures [99].

The translation of perfusable macroscopic systems from normal tissue to tumor engineering is in its infancy. Cardiac tissue [100] and musculoskeletal tumors [101–103] have successfully incorporated macroscopic perfusable systems using cellular contractions, electrical stimuli, and shear stress to guide tissue and tumor development. Using mathematical models available to macroscopic systems [100,104], 3D biomimetic tissue and tumor systems have been modeled [73,105] using porous polymeric scaffolds [106,107]. Macroscopic perfusable systems have been shown to be viable means of testing drug responses to cultured tumors [36,37,108]. In a seminal study, Fischbach et al. developed a 3D tumor engineered model that has shown in vivo-like tumor responses to therapeutics and angiogenesis [73]. To overcome large scale limitations for pre-clinical testing, Marshall et al. modified existing microfluidic devices to support extended tumor culturing for preclinical trials of drug responses [37].

Research on the effect of flow-perfusion on tumor progression has emerged recently. When compared to static 3D cultures, flow perfusion has been shown to increase cell proliferation and cell homogeneity within tissue like structures and exhibit morphology and phenotypes like xenografts [108]. Biomechanical stimulation due to shear stresses has been often overlooked as a means of tumor progression. Studies have shown the physiological responses of cancer cell lines to shear stress from fluid flow [36,109]. Munson et al. modeled tumor cell invasion of glioma cells by increasing the pressure differential thereby increasing interstitial flow, which showed that the increased flow enhances glioma cell motility and resistance to drug treatments [110]. Mathematical and computational models of fluid flow through tumorous environments have emerged due to the relevance of macroscopic flow-perfusion in cancer research. The developed models relate fluid flow to tumor permeability [111], drug delivery [112], as well as blood flow [113].

Our lab participated in a recent study investigated tumor cell phenotypic responses based on shear gradients and scaffold architecture with Ewing sarcoma in collaboration with the Mikos group [114]. Previous work in the Mikos group has shown that the tumor microenvironment has an impact on tumor cell drug response and phenotype [115] with secretion of IGF-1 being present during flow-perfusion [36]. The limitations of common scaffolds for tumor engineering are due to a lack of scaffold architecture control [41]. Poly-(propylene fumarate) (PPF) scaffolds were 3D printed since PPF has been a suitable biomaterial for bone-related applications [116–119] while extrusion-based 3D printing allowed fine control over scaffold architecture to create artificial physical gradients within the scaffold [42,120]. It was hypothesized that the tumor phenotype would be affected by shear gradients from 3D printed scaffolds, which would ultimately benefit cancer therapeutic [121] and cancer modeling [122] research.

For this study, 3D printed PPF 3-layered scaffolds were designed with varying gradients of increasing (1 mm–0.6 mm to 0.2 mm), decreasing (0.2 mm–0.6 mm to 1 mm), and uniform (0.6 mm) pore sizes with computational models utilized to estimate shear stresses. Scaffolds were seeded and cultured for 10 days in both static and perfusion cultures with a custom designed 3D printed bioreactor. After 10 days both cultures were harvested. Increased cell proliferation was observed in flow-perfusion compared to static cultures. Smaller pore sizes led to higher proliferation, likely from the increased surface area for cell adhesion. Although there were no statistically significant changes within scaffold layers, the orientation of the gradients to the shear stresses affected cell proliferation, protein and ligand expression. Interestingly, IGF-1 was affected by not only the shear, but also the direction of the gradient, where the decreasing pore size scaffold showed increased IGF-1 secretion compared to the uniform and increasing pore size scaffold. Further scaffold and bioreactor optimization could exploit scaffold complexity by changing pore size gradients and adjusting flow-perfusion to better mimic biomechanical cues similar to in vivo conditions.

With the cancer research community developments towards the promising advances of flow perfusion, macroscopic culture has the potential to tremendously enhance our understanding of the interplay of biomechanical, biochemical, and architectural cues. The degree to which each aspect of a macroscopic system can be altered in vitro provides tremendous opportunities to explore scaffold designs, surface attachment, perfusion rates and styles, as well as the ability to perfuse components of interest such as chemokines, therapeutic drugs, and other soluble components. The ultimate goal of tumor engineering is to completely and fully recreate the tumor environment in vitro, such that macroscopic cultures should translate as well as adapt bioreactor systems to model the tumor environment.

4. Non-destructive real-time monitoring

One of the predominant limitations of the current state of the tissue engineering field is the need to harvest samples to monitor tissue development. Literature addressing non-destructive methods for determining construct quality is limited [123]. Due to the easily sampled media reservoir and flow-based gradients across the scaffold, perfusion bioreactor systems are uniquely situated to real-time monitor the viability of seeded cells. Fig. 2 illustrates the flow perfusion bioreactor system modified for metabolite monitoring. Tumor microenvironments are complex - and their development shifts depending on biomechanical forces and ECM changes [124,125]. Macroscopic bioreactor systems trade fine system control, compared to their microfluidic counterparts, for increased complexity, size, and tissue-like behavior. Due to their scale, macroscopic perfusable systems ideally should incorporate analytical methods of in-line testing to determine key analyte concentrations without biopsy sampling [126]. Biosensors offer in-line detection ranging from optical, chemical, and biological sensors [127]. Common in-line detections are mainly optical, with mid and near infrared spectroscopy for bioprocesses [128,129]. Studies have measured particle trajectories through perfusable systems for microcarrier particles [130,131]. Manley et al. used high-precision lasers to detect intracellularly fluorescently labelled cancerous cells and their aggregation patterns over several hours in a high aspect ratio vessel rotating wall vessel (RWV) bioreactor containing PC12 rat pheochromocytoma cells and HepG2 human hepatocellular carcinoma cells [132].

Recently, real-time monitoring of perfusion bioreactors has been

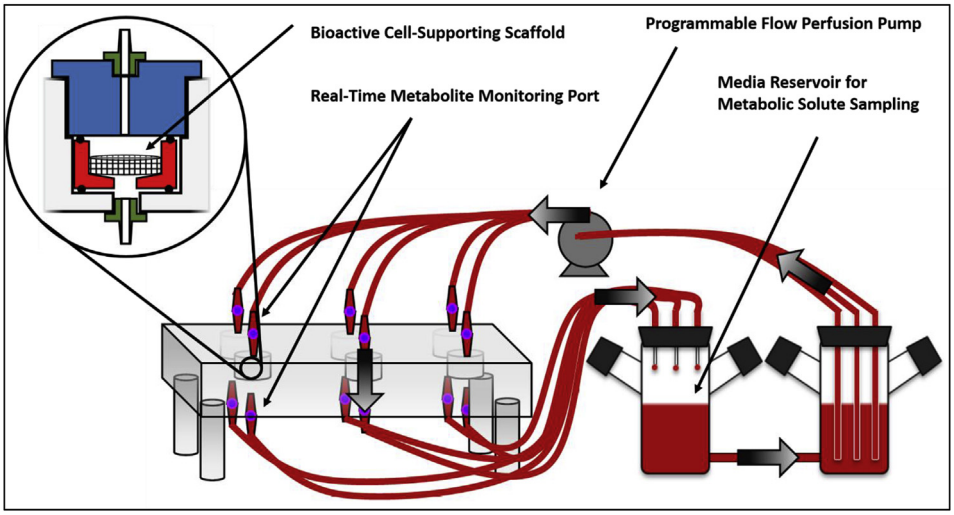

Fig. 2. Flow perfusion bioreactor with metabolite monitoring modifications [155].

achieved using metabolite monitoring approaches. Construct monitoring is in early stages with studies that have shown oxygen consumption drop through chondrocyte samples [133] and microfluidic 3D cultures assays [134,135]. In recent years, metabolite monitoring has been used to compare therapeutic drug responses and gradient modeling [134,136,137]. Our lab has investigated real-time non-destructive metabolite monitoring on macroscopic perfusable bioreactor systems currently undeveloped in literature. The goal of our experiments was to demonstrate the feasible correlation between construct cellularity over time [138] and monitoring for bone differentiation over time [139]. Although studies have been done in 2D cultures for metabolite monitoring [134,135], most of these studies luck the ability to monitor cellularity, thereby providing an opportunity to expand metabolite monitoring for 3D environments with the incorporation of real-time cellularity monitoring. We explored the utilization of glucose consumption, oxygen uptake, and accumulation of key metabolites to accurately and non-destructively monitor construct cellularity [138] and osteogenic differentiation [139] over time. Mesenchymal stem cells were chosen for their wide use in tissue engineering [140] and their high metabolic rates [141]. Non-differentiating rat mesenchymal stem cells (rMSCs) were seeded onto non-woven fiber mesh spunbonded PLLA scaffolds and cultured in a flow-perfusion bioreactor system up to 21 days [138] in conjunction with a sample group with osteo-inducing medium [139].

The oxygen measurements were taken with a ruthenium complexed coated fiber optic probe emitting blue fluorescent light. The ruthenium became excited when in the contact with molecular oxygen and emitted a red light which was correlated between the fluorescent and oxygen partial pressure. The signal was sent to a computer software that returned oxygen concentrations. By calibrating the probe with 0% oxygen and approximately 21% oxygen in air, we inserted the probe at both bioreactor chamber inlets and outlets and then related the cell-specific oxygen uptake rate with the known values of flow rate, number of cells, and inlet and outlet oxygen concentration. The glucose measurements were performed two ways: using a colorimetric glucose assay [138] and with glucose test strips [139]. Other key components tested were lactate, osteoprotegerin, and osteocalcin [142], with the last two tested due to their prevalence in the differentiation of mesenchymal stem cells into osteoblastic regions.

Our results showed cells distributed homogeneously throughout the scaffold constructs [138,139] with the osteo-inductive medium group having higher cellularity compared to our standard medium [139]. Inlet oxygen measurements remained constant which assured that the medium was fully reoxygenated when recirculated, and the outlet oxygen measurements saw increasing oxygen uptake rates for the first 7 days, a plateau in consumption until 14 days, and a decrease which is accounted for by the cells growing into the scaffold and acclimating to their environment before covering the construct thereby restricting flow and oxygen transfer at 21 days. Lactate production mirrored the glucose consumption with slightly elevated rates until day 7 and constant lactate production after the cells had grown into the construct [138]. Osteoprotegrin and osteocalcin were measured to assess the time-dependent differentiation of MSCs toward the osteoblastic lineage. Ultimately, it was found that the ratio of macroscopic rates matched the ratio of microscopic rates within the perfusable systems. To non-destructively predict cellularity during metabolic shifts from differentiation we combined the construct-specific rates with their respective cell-specific oxygen uptake and glucose consumption [139].

In relation to tumor engineering, we suggest that these technologies can be used on cancer cell 3D cultures and produce a non-destructive real-time monitoring of cancer construct cellularity and metastatic potential. Cancer metabolism is unique such that mutations in cancer cells can cause increased metabolic rates [143] as well as altering the aerobic glycolysis metabolic pathway to uptake glucose and produce lactate even in the absence of oxygen [144]. High rate of proliferation that exceeds angiogenesis in tumors often subject cancer cells to hypoxic conditions [145]. Alongside metabolic rates, glucose consumption, and lactate production, recent discoveries in cancer research have shown dramatic increases in oncometabolites [146] in tumorous environments [147]. A notable oncometabolite is 2-hydroxyglutarate (D2HG) which is heavily concentrated in tumors with the isocitrate dehydrogenase mutation such as gliomas [148,149]. Jain et al. has modeled the consumption and release profiles of over 200 metabolites across NCI-60 cancer cell lines, showing that glycine uptake has a strong correlation with proliferation rates across multiple cancer cell lines [150]. Tumor engineering is a perfect fit for non-destructive real-time monitoring systems since the recently identified metabolic relations for glucose consumption and lactate production align with cancer metabolism. Cancer cells metabolic rates can be monitored in flow perfusion systems, and testing for differing metabolites has already been shown to be a viable approach.

Furthermore, quantitative oncometabolite monitoring could facilitate analytical in vitro co-culture models with healthy representative tissue. Similarly, the independent monitoring of osteogenic metabolites to determine differentiation progress in the healthy bone tissue model described above [139], oncometabolite concentration over time could be measured independently of cellularity markers, such as oxygen consumption and lactate production, to assess tumor progression under

drug treatment conditions. In a co-culture with healthy cells representative of the tumor's native environment, independent oncometabolite and cellularity monitoring could be used to assess the response of both the cancerous and healthy cells to drug treatment in an advanced preclinical model.

Non-destructive real-time monitoring is a new development within the field of tissue engineering and has the potential to benefit cancer research. Bioreactor systems in conjunction with biosensors result in an efficient way to monitor constructs in real-time and can accurately correlate construct cellularity [138,139]. By combining current macroscale detection with emerging tumor engineering cultures, our understanding of tumor microenvironments can be expanded with real-time culture monitoring.

5. Conclusion

3D perfusable systems are able to overcome the limitations of 2D systems to better recreate the tumor microenvironment thereby enhancing research abilities to study tumors. Flow perfusion systems can better culture tumor cells in environments that more closely mimic the in vivo conditions of tumor progression and allow for the interplay between the complexities of the tumor microenvironments. The flexibility of flow-perfusion bioreactor systems to accommodate different cancer cell lines by utilizing surface modified biomimetic scaffolds implies that these systems can be used for many types of cancer cells.

These technologies, adopted from tissue engineering, will improve cancer treatment development with improved fidelity over 2D systems. The new macroscopic approaches will advance alongside current tissue engineering technologies and can converge towards an ultimate development of in vivo-like in vitro tumor cultures which will advance the field of cancer biology and therapeutics.

Conflicts of interest

None of the authors has a conflict of interest.

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