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Tissue Engineered Platforms for Studying Primary and Metastatic Neoplasm Behavior in Bone

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Abstract

Cancer is the second leading cause of death in the United States, claiming more than 560,000 lives each year. Osteosarcoma (OS) is the most common primary malignant tumor of bone in children and young adults, while bone is a common site of metastasis for tumors initiating from other tissues. The heterogeneity, continual evolution, and complexity of this disease at different stages of tumor progression drives a critical need for physiologically relevant models that capture the dynamic cancer microenvironment and advance chemotherapy techniques. Monolayer cultures have been favored for cell-based research for decades due to their simplicity and scalability. However, the nature of these models makes it impossible to fully describe the biomechanical and biochemical cues present in 3-dimensional (3D) microenvironments, such as ECM stiffness, degradability, surface topography, and adhesivity. Biomaterials have emerged as valuable tools to model the behavior of various cancers by creating highly tunable 3D systems for studying neoplasm behavior, screening chemotherapeutic drugs, and developing novel treatment delivery techniques. This review highlights the recent application of biomaterials toward the development of tumor models, details methods for their tunability, and discusses the clinical and therapeutic applications of these systems.

Keywords

biomaterials; 3D tumor model; mechanical properties; cancer therapy; tumor microenvironment

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Conflict of Interest

The authors have nothing to report.

Introduction

Cancer is the second leading cause of death in the United States, claiming more than 600,000 lives in 2019 (Siegel et al., 2019, 2020). Most fatalities are attributed not to the primary tumor itself but to metastasis from the primary tumor to other tissue sites. Bone is a common metastatic site, and bone metastasis is considered incurable with a poor patient survival prognosis of 6 to 48 months (Cortini et al., 2019; Macedo et al., 2017). Primary bone cancers, or sarcomas, are relatively rare, but most are quite aggressive, requiring multi-agent cytotoxic chemotherapy, surgery and/or radiation therapy. While these two entities, metastatic cancer to bone and bone sarcomas, are biologically and categorically quite distinct, understanding the bone microenvironment and its ability to facilitate neoplastic progression is of critical importance.

Patient-derived xenografts (PDX) are prominent cancer model systems increasingly used in translational cancer research (Hidalgo et al., 2014; Siolas and Hannon, 2013). However, this approach is costly, time-intensive, and fails to accurately recapitulate human disease due to differences between organisms, rate of tumor growth, and genomic stability throughout propagation (Aparicio et al., 2015; Ben-David et al., 2017). For decades, two-dimensional (2D) *in vitro* models have been the cornerstone of cell-based research due to their reduced cost, reproducibility, and ease of analysis (Cortini et al., 2019). Yet, the nature of 2D models makes it impossible to fully describe the biochemical and biomechanical cues present in three-dimensional (3D) cell microenvironments for the study of cancer. To address this shortcoming, 3D cultures including spheroids and polymeric scaffolds have been developed to model and interrogate the cellular interactions within a tumor and the effect of biomechanical properties of the ECM on neoplasm behavior (Fig. 1).

Dramatic advances have emerged from the study of tumor cells on 3D substrates. The development of novel materials, as well as the tunability of these materials to mimic the dynamic nature of tumor growth, provides an exciting opportunity to describe cell behavior or identify druggable targets to combat cancer. This review will describe recent developments in biomaterial systems for studying cancer. We will describe how neoplasm behavior of primary bone cancer and metastatic bone cancer, specifically breast and lung, are influenced by microenvironmental properties of the engineered constructs (Fig. 2). We will also discuss the many potential applications of these models in therapeutic applications.

1. Scaffold composition to tune biophysical properties

Cancer is characterized by a dysregulation in critical signaling pathways that elicits changes in gene expression, cell behavior, and tissue architecture. The interplay between cells and the surrounding microenvironment is attributed to dynamic reciprocity – a model describing the bidirectional interaction between the cell and its surrounding extracellular matrix (ECM) (Bissell et al., 1982; Jorgens et al., 2017). Taken together, the communication between cancer cells and their ECM is a critical regulator for tumor progression (Fig. 2A). Thus, it is imperative to apply our evolving understanding of the biological nature of cancer to develop improved models to understand and combat tumorigenesis.

Biomaterials play an integral role in the development of engineered microenvironments for the study of cancer. Natural biomaterials can be derived from proteins (e.g., collagen, fibrin, silk, gelatin, Matrigel), polysaccharides (e.g., hyaluronic acid (HA), chitin/chitosan, alginate), and decellularized tissues (Aravamudhan et al., 2014; Chaudhuri et al., 2014). These materials are advantageous for their biocompatibility and morphological, mechanical, and adhesive properties similar to native ECM. Synthetic biomaterials can be constructed from metals, ceramics, and polymers, both nonbiodegradable (e.g., polyethylene glycol (PEG), polydimethylsiloxane (PDMS)) and biodegradable (e.g., polyacrylamide (PAM), poly ε-caprolactone (PCL), poly(lactide-*co*-glycolide) (PLG)) (Tian et al., 2012). Unlike natural materials, these are chemically inert and mechanically durable. Hydrogels, which are highly water-absorbent polymeric scaffolds, are common due to their biocompatibility and efficient transportation of oxygen and nutrients. These platforms may be developed from a single polymer or combination of natural and/or synthetic materials.

Physical properties of the biomaterial scaffold including stiffness, porosity, and adhesivity can be tuned to explore cell response by adjusting the type and concentration of the polymer and crosslinker (Fig. 2B) (Duval et al., 2017). Furthermore, biomaterials can be tailored to mimic specific physiological microenvironments to interrogate certain cell behaviors and diseases. For example, PLG scaffolds or type 1 collagen gels loaded with hydroxyapatite (HAp) were used to model the bone microenvironment and investigate the metastatic behavior of breast cancer (BC) cells (Choi et al., 2019b; He et al., 2019). Cells in collagen gels containing HAp exhibited morphological changes associated with increased invasiveness and motility compared to collagen controls. Bioreactors and microfluidic devices are also useful because they integrate fluid flow into the system – a crucial aspect for cell function (Clay et al., 2016). The tunability and expansive array of biomaterials enable the development of physiologically relevant systems to study cancer, which cannot be captured with traditional culture studies on glass or tissue culture polystyrene (TCP).

2. Biomechanical properties of engineered substrates

Tissue homeostasis is commonly disrupted during tumor progression and is associated with changes in tumor stroma stiffness, ECM degradation, and remodeling (Maller et al., 2020). Thus, interrogation of the role of ECM stiffness and degradability on cancer cell behavior represents an exciting strategy to discover mechanisms that facilitate the development of malignant tumors.

2.1 Stiffness and viscoelasticity of model platforms—

Tumor stroma stiffness is frequently increased during tumor development compared to healthy tissue. For example, cancerous breast tissues may be 20-fold stiffer, while lung carcinomas may be 30 times stiffer than normal tissue (Joyce et al., 2018; Paszek et al., 2005; Shukla et al., 2016; Umemoto et al., 2014). In contrast, osteosarcoma (OS), the most common cancer in adolescents and young adults, does not exhibit an increase in tumor stroma stiffness in canine models compared to healthy bone (Steffey et al., 2017). The mechanical properties of OS in human patients have not yet been reported. For tissues exhibiting increases in stiffness, the cascade of events that lead to malignant transformation are initially triggered by the stiffening of the tumor microenvironment (TME). Cells generate contractile forces on

the stiffening ECM, which increases cytoskeletal tension, drives the assembly of focal adhesions, and promotes the growth of the tumor mass (Domura et al., 2017a; Paszek et al., 2005). Thus, ECM stiffness is a key parameter for study in models of disease.

ECM stiffness influences tumorigenesis by inducing invasive cell morphology, enhancing migratory abilities, and upregulating the expression of epithelial-mesenchymal transition (EMT) markers. For instance, metastatic and non-metastatic breast cancer (BC) and hepatocellular carcinoma (HCC) cells were seeded on stiff PAM and PDMS substrates (>55 kPa), representative of tumor stiffness, and soft substrates (5–10 kPa), representative of healthy tissue. Cells on stiff surfaces were characterized by well-spread, polygonal, flattened morphology and increased cell adhesion compared to cells with rounded morphology on softer surfaces (Ansardamavandi et al., 2018; Azadi et al., 2019; Peng et al., 2019; Zhao et al., 2018). Highly metastatic mammary MDA-MB-231 cells exhibited a 35% increase in proliferation on 36 kPa PEG diacrylate-Gelatin-Methacryloyl (PEGDA-GelMA) gels compared to a 25% increase on 16 kPa gels (Li et al., 2016). OS cells exhibited the greatest migration on PAM and PEGDA hydrogels of 34 kPa (Dai et al., 2019; Jabbari et al., 2015). Interestingly, there was a decrease in migration on substrate stiffnesses greater than these moduli. High matrix stiffness enhanced BC cell migration by upregulating mesenchymal and EMT signaling markers (e.g., N-cadherin, Snail, vimentin, TWIST-1, MMP-2) and downregulating epithelial markers (e.g., E-cadherin) (Wei et al., 2015). Similar trends were observed for OS, a non-epithelial tumor, on rigid substrates, suggesting that these cells were undergoing an “EMT-like” process that facilitated their metastatic ability (Dai et al., 2019; Jiang et al., 2019). However, EMT signaling markers in lung adenocarcinoma cells demonstrated a biphasic relationship with substrate stiffness (Alonso-Nocelo et al., 2018; Shukla et al., 2016). These data suggest that the activation of EMT is dependent on matrix stiffness.

Compared to 2D models, 3D systems facilitate a more extensive exploration of cell activities in a biomimetic environment. While increases in proliferation and spreading were consistent with monolayer culture, BC cells cultured in alginate hydrogels resulted in cell aggregates as occurs *in vivo*, confirming the importance of 3D culture (Cavo et al., 2016). Furthermore, 3D models are amenable to dynamic modulation of substrate stiffness as occurs physiologically through photocrosslinking techniques (Ondeck et al., 2019). These models successfully recapitulate malignant transformation of non-tumorigenic cells demonstrated by enhanced mesenchymal phenotype markers on stiffened ECM (Joyce et al., 2018). Microgels are under investigation for their ability to enhance cell-matrix interactions, cell proliferation, and nutrient and water transport. In addition to the ease of tuning microgel rigidity by varying polymer concentration, microgels have been used to explore the role of the oxygen microenvironment, as tumor hypoxia is a key regulator of cancer progression (Lee and Cha, 2018, 2020). Other models have incorporated the use of decellularized ECM (dECM) to retain the structure, biochemical, and biomechanical cues of the native ECM. Cell invasion and upregulation of EMT signaling markers were increased in stiffer tumor niches modeled by porcine liver dECM-GelMA-based scaffolds (Ma et al., 2018). Additionally, 3D platforms are useful to study the contribution of ECM stiffness on the effectiveness of chemotherapeutic treatments. MDA-MB-231 cells treated with doxorubicin (DOX) were 3-fold more chemoresistant in stiff alginate-Matrigel hydrogels (2 kPa)

compared to their softer counterparts (200 Pa) (Joyce et al., 2018). These data emphasize the importance of stiffness on cell proliferation, migration, and chemoresistance, and thus, this property must be considered in the development of model systems to study cancer.

Soft tissues throughout the body are comprised of a network of viscoelastic proteins and biopolymers. Cancer cells demonstrate changes in cellular viscoelasticity compared to noncancerous cells, motivating the need to explore this characteristic (Chaudhuri, 2017; Xie et al., 2019). Polymer film fluidity, which is inversely related to viscosity, can be tuned via the molecular weight of the polymer. MCF-7 breast cancer cells exhibited more proliferation and higher metabolic activity on poly(ε-caprolactone-*co*-D,L-lactide) (PCL-*co*-DLLA) films with high fluidity compared to films with low fluidity (Najmina et al., 2020). Upon treatment with doxorubicin, BC cells on high fluidity surfaces formed 3D aggregates and were highly chemoresistant versus cells on low fluidity surfaces (Najmina et al., 2020). U2OS human OS cells exhibited amplified cell spreading and stress fiber formation on stress-relaxing alginate hydrogels compared to elastic gels (Chaudhuri et al., 2015). Viscoelasticity has also been tuned in noncancerous cell studies using oxidized alginate or mixtures of agarose and acrylamide, which could be further applied to study cancer cell behavior (Cacopardo et al., 2019; Hafeez et al., 2018; Hung et al., 2020). While several studies confirm that viscoelasticity affects cell proliferation and chemoresistance, the effect of substrate viscoelasticity on cell invasiveness is poorly described, representing an important area for future study.

2.2 Degradability—Cells must degrade the surrounding ECM and basement membrane to facilitate tumor growth, which is commonly achieved via matrix metalloproteinases (MMPs) secreted at increased levels by tumor cells (Kessenbrock et al., 2015). Continual degradation and remodeling of the ECM influences the microenvironmental stiffness and resultant neoplasm behavior. Hence, ECM degradability is a key aspect to consider when designing new models to study cancer cell behavior.

Natural polymeric biomaterials are frequently used in tissue engineered platforms for their ability to support cell adhesion and biocompatibility. However, compared to synthetic biomaterials, natural biomaterials are more vulnerable to cell degradability and remodeling. In order to achieve more consistent and predictable results, substrate degradation can be controlled using MMP-degradable crosslinkers, such as GPQG↓IWGQ (PQ, ↓ denotes cleavage site), or non-degradable crosslinkers, such as *N*-vinyl pyrrolidone (NVP). MDA-MB-231 breast cancer cells cultured on HA-based hydrogels crosslinked with PQ invaded twice as far into the substrate compared to cells on non-proteolytic degradable crosslinked HA-based hydrogels (Fisher et al., 2015). In another example, MDA-MB-231 cells were entrapped in acrylate-PEG-succinimidyl valerate (acrylate-PEG-SVA) hydrogels crosslinked with NVP. As NVP concentration decreased, and thus hydrogel degradability increased, BC cells exhibited greater proliferation, formed large cell clusters with filopodial protrusions, and were more metabolically active, each indicative of invasive tumor characteristics (Pradhan and Slater, 2019). Collectively, these studies decoupled substrate degradability from other compounding factors such as stiffness and adhesivity to observe changes in cell invasiveness. Additional studies are necessary to examine the synergistic effect of these potent stimuli on tumor cells.

3. Substrate topography

Substrate topography describes the finely spaced surface properties or fiber alignment that results in changes in contact area, protein adsorption, cell adhesion, and cell alignment (Choudhury and Chinchanikar, 2017). In the vicinity of tumors, primary cancer cells had increased radial alignment, yet during invasion, cells were predominantly oriented along aligned collagen fibers (Conklin et al., 2011; Provenzano et al., 2006). The phenomenon of cell orientation in cancer microenvironments motivates the exploration of how topographical features, specifically surface patterns and pore size, affect neoplasm behavior.

3.1 Surface patterns—Photolithography is a common technique to manufacture patterned substrates, enabling the production of surfaces with defined morphological patterns. Lung carcinoma cell lines exhibited increased migration on PDMS-grated patterns (5 μm ridges with 5 μm spacing) compared to arc and square configurations. Although non-metastatic A549 cells and metastatic H1299 cells possessed different morphologies, migration speeds were faster on grated surfaces compared to flat controls (Zhou et al., 2017). MDA-MB-231 cells cultured on PDMS gratings (widths from 2–4 μm) had increased extension, alignment along the grating length, and spreading area compared to planar controls (Chaudhuri et al., 2016). Furthermore, histone modifications in cancer cells primed to a tumorigenic state occurred on PAM gels of different patterns (i.e., spiral, star, pentagon, square) (Lee et al., 2020). In another study, MDA-MB-231 cells exhibited greater eccentricity, a measurement of protrusion width, on flat polystyrene ribbon controls compared to curved fibers. For curved fibers, eccentricity correlated with fiber diameter (Koons et al., 2017). Similarly, BC cells had a 20% increase in cell spreading on type 1 collagen fibers with an 850 nm diameter compared to 550 nm. Cell invasiveness increased with fiber diameter, yet proliferation was unchanged (Sapudom et al., 2015). These data demonstrate that cell protrusion, cytoskeletal arrangement, and tumor invasiveness are dependent on surface patterns and fiber diameter.

Orientation of fibers within the substrate, whether anisotropic or isotropic, was controlled via electrospinning of PLLA and PCL nanofibers or stretching of type 1 collagen hydrogels. In 2D and 3D culture, MDA-MB-231 cancer cells cultured on aligned fibers formed more focal adhesions, more F-actin bundles, larger nuclear elongation, and fewer but more elongated protrusions (1.5-fold longer) along the fiber orientation. Directional persistence was increased with fiber alignment, allowing for increases in net distance traveled (Domura et al., 2017b; Riching et al., 2014; Saha et al., 2012; Wang et al., 2015). BC cells on anisotropic fibers expressed increased vimentin expression, a marker of EMT signaling, and lower levels of E-cadherin compared to cells on isotropic fibers, demonstrating the potential implications of fiber orientation in EMT activation (Domura et al., 2017b; Saha et al., 2012). While these data confirmed that anisotropic fibers affect tumor behavior, the studies also revealed the role of substrate biomechanical properties (i.e., stiffness) as a confounding factor. Some studies indicated that anisotropic fibers stimulated faster migration speeds compared to isotropic fibers while others reported the opposite (Domura et al., 2017b; Riching et al., 2014; Wang et al., 2015). Fiber alignment is associated with increases in stiffness. The synergistic effects of this relationship have not been effectively decoupled and represent an important area for future studies.

3.2 Pore size—Rapid proliferation of cancer cells will cause local crowding and resultant cell restriction in primary tumors (Nia et al., 2020). During metastasis, tumor cells must extravasate through confining pores of the ECM and circulating capillaries (1–20 μm in diameter) or fiber- and channel-like tracks (3–30 μm in width) (Weigelin et al., 2012). These confined spaces are dictated by the fibrillar network in the matrix and impose morphological changes to the cells, altering their malignancy. Macropores (>75 μm) are also crucial for facilitating oxygen and nutrient passage and driving certain cellular processes, such as differentiation, as demonstrated by noncancerous cells (Vissers et al., 2015). In engineered systems, porosity can be controlled via particle leaching, freeze-drying, electrospinning, chemical crosslinker type and density to form hydrogels, and microchannels (Annabi et al., 2010). While limited studies have investigated the influence of pore diameter on cancer cell behavior, preliminary findings on the success of macropores on promoting BC cell adhesion and growth have established this as a promising area for future exploration (Xiong et al., 2014).

The migratory properties of tumor cells are hindered in constricted environments. When migrating through tight interstitial spaces, cells incur physical stress and undergo extensive deformation of the nucleus and cell membrane (Denais et al., 2016). This was demonstrated by increased nuclear deformation for cancer cells in 3 μm versus 50 μm microchannels, resulting in decreased cell proliferation (Moriarty and Stroka, 2018). As pore size decreased in collagen-PEG mesh networks, BC cells exhibited reduced cell spreading, leading to rounded morphology, increased cell-cell adhesion protein expression, larger cell aggregates, and triggered morphogenesis (Ranamukhaarachchi et al., 2019). Furthermore, BC cells exhibited decreased protrusion formation when encountering smaller pores, impeding cell velocity and invasiveness (Ranamukhaarachchi et al., 2019; Reynolds et al., 2018). Pore size is associated with fiber length. Shorter fibers, and thus smaller pore size, induced morphogenesis in MDA-MB-231 cells, steering the cells away from single cell behaviors to invasive networks of aggressive tumors (Ranamukhaarachchi et al., 2019; Velez et al., 2017). During confinement, nuclear influx, volume expansion, and blebbing are elevated, which could promote uncontrolled rupture events and DNA damage (Mistriotis et al., 2019). These data suggest the implications of pore size in cancer metastasis, which could aid in developing novel therapeutics.

4. Substrate adhesivity

Cell adhesion proteins such as integrins and cadherins play a vital role in tumor cell proliferation, migration, and invasion by facilitating adhesion to the ECM (Desgrosellier and Cheresh, 2010). The contribution of cell adhesion to the malignant potential of tumor progression can be investigated by modulating the type and density of adhesive ligands within an engineered platform (Fig. 2B).

The endogenous ECM is comprised of numerous adhesive ligands, which can be broadly categorized into Arginine-Glycine-Aspartic Acid (RGD), laminin, and collagen receptors. The overexpression of certain integrins in primary tumors enables and enhances metastasis. For instance, several integrins have been implicated in bone metastasis including $\alpha v\beta 3$ (vitronectin receptor), $\alpha 2\beta 1$ (collagen receptor), and $\alpha 4\beta 1$ (fibronectin receptor) (Esposito

and Kang, 2014). For bone metastatic BC cells, αvβ3 increased cell adhesion to vitronectin but not to other matricellular proteins such as collagen or fibronectin and exhibited strong migration towards osteopontin, an ECM protein found in the bone matrix (Sloan et al., 2006). These studies established the role of αvβ3 on spontaneous metastasis of breast tumors to bone. Similarly, MDA-MB-231 cells exhibited enhanced invasion into fibronectin- or RGD-modified collagen matrices that triggered α5β1 integrin engagement compared to unmodified collagen gels (Mierke et al., 2011). However, no dependency on ligand type was observed for non-metastatic BC cells cultured in PEG-heparin hydrogels functionalized with RGD (a binding motif of fibronectin), IKVAV (an adhesion peptide derived from laminin), and GFOGER (a binding motif found in collagen type 1). MCF-7 cells formed spheroids in the hydrogels irrespective of ligand type (Taubenberger et al., 2016). Collectively, these data establish that the type of adhesive ligand can influence cell adhesion, migration, and metastasis depending on the cell's metastatic potential.

Ligand density also influences neoplasm behavior and can be tuned in engineered platforms by modulating ligand concentration and spacing. BC cells cultured in acrylate-PEG-succinimidyl valerate (SVA) and HA-based hydrogels modified with RGD exhibited increased proliferation and cluster formation compared to unmodified gels (Fisher et al., 2015; Pradhan and Slater, 2019). Furthermore, αvβ6 is an integrin that is significantly upregulated in many epithelial-derived cancers and drives invasion and metastasis (Ganguly et al., 2020). Interestingly, osteosarcoma cells exhibited no change in cell proliferation or tumorigenic markers with various adhesion ligand densities (Jiang et al., 2019). Ligand density modulated via ligand spacing would be interesting to further explore in cancer studies, as ligands with a critical separation length between 58–73 nm are speculated to be a universal length scale for the formation of stable focal adhesions (Arnold et al., 2004; Deng et al., 2017). The implications of ligand density on cancer extravasation and the interplay between stiffness and ligand spacing should be investigated to better understand the mechanisms associated with tumor progression.

Ligand density and type impacted chemotherapy drug sensitivity in cancer cells, demonstrating the potential for preferentially targeting specific ligands for therapeutic applications. BC cells on gold nanoparticles (AuNPs) with larger ligand spacing and αvβ3-coating were more sensitive to paclitaxel treatment than those on surfaces with smaller ligand spacing and α5β1-coating (Young et al., 2020). Currently, AuNPs and liposomes are used for nanoscale drug delivery for cancer therapy (Zhong et al., 2014). Ligands are anchored and presented on the surfaces of these drug-encapsulated nanostructures to be taken up by cancer cells. By modulating ligand properties on drug-loaded nanoparticles, such treatments may offer improved therapeutic benefit in patients while reducing the necessary dosage of chemotherapeutic agents.

5. Mechanical stresses experienced by cancer cells

Cancer cells are exposed to a variety of mechanical stresses including tensile, compressive, and shear forces imposed by neighboring cells or surrounding ECM (Nia et al., 2020). Tensile stress is a consequence of increasing ECM stiffness where assembled actin stress fibers increase actomyosin contractions and subsequent intracellular tension. Compressive

stress arises when cells migrate through narrow constrictions or are subjected to confined spaces by enhanced tumor cell proliferation. Shear stress occurs from blood and interstitial fluid flow experienced by cancer cells. These biomechanical forces shape the tumor microenvironment, influence cellular behaviors, and can drive malignancy, making it imperative to better understand the effects of these forces in tumor progression and the implications of these stresses in cancer treatment.

Biomaterials have been used to effectively establish the role of tensile and compressive stresses in driving tumorigenic behaviors. For instance, BC cells were cultured on a PDMS cell stretching device and stretched cyclically over 4 hours to observe the effects of tensile stress. Initially, there was an increase in cell length, filopodia and actin formation, cell alignment, cell area, and cell-cell interactions until around 2 hours, after which prolonged stretching induced cell necrosis (Yadav et al., 2019). Furthermore, physiologically relevant compressive forces were modeled by compressing BC cells between a membrane and agarose gel. Human and murine BC cells showed no change in proliferation, yet cells exhibited a 1.3–2-fold increase in migration rate, more elongated actin filaments, and more microtubule rearrangement compared to noncancerous MCF10A cells (Tse et al., 2012). These data emphasize the dependence of malignant cell morphology and metastatic behavior development on tensile and compressive forces.

Shear stress (SS) has been more extensively studied compared to tensile and compressive stress. During metastasis, tumor cells primarily encounter interstitial SS and blood SS. Bioreactors are effective for modeling and investigating dynamic cancer metastasis because they can mimic the natural forces experienced in the tumor microenvironment. For instance, aggressive human MDA-MB-231 BC cells cultured in alginate-Matrigel hydrogels in a multi-organ bioreactor migrated from the gels and attached to a porous electrospun PCL-gelatin membrane in the bioreactor that mimicked vascular walls (Cavo et al., 2018). The cells formed invadopodia to anchor to the membrane and exhibited cytoskeletal irregularities and cell elongation characteristic of their malignancy. To investigate the role of interstitial SS, human BC cells were seeded in 3D collagen-agarose IPN hydrogels and cultured in a bioreactor applying 5.4 dyn/cm^2 SS (Novak et al., 2019). SS increased cell proliferation, cell area, and chemoresistance to paclitaxel by 2-fold compared to controls. The flow rate through perfusion bioreactors can be controlled to support *ex vivo* culture of breast cancer tissue to evaluate the efficacy of various cancer therapies. *Ex vivo* triple negative breast cancer tissue treated with anti-estrogen and checkpoint-inhibitors, such as anti-programmed death ligand (PDL-1), demonstrated an anti-proliferative effect and significant cancer cell death, respectively (Muraro et al., 2017). Lung cancer cells cultured on PDMS with applied hydrostatic pressures (HPs) ranging from 0–20 mmHg had increased cell volume, filopodia number, migration, and EMT marker expression with elevated HPs (Kao et al., 2017). Contrary to modeling interstitial SS, blood SS was studied using microfluidic devices to mimic the circulatory microenvironment experienced by cancer cells during metastasis. Oscillatory shear forces of 5 dyn/cm^2 promoted proliferation of MDA-MB-231 suspension cells and increases in stemness markers (e.g., Nanog, Oct4B, Sox2) (Choi et al., 2019a). Interestingly, SS (20 dyn/cm^2) sustained for 2 hours impeded BC cell adhesion, and cell viability decreased by up to 50% after 12 hours of shear treatment (Xin et al., 2019). This suggests the dependence of tumor cell suspensions on SS magnitude and duration.

Furthermore, hemodynamic shear flow triggered EMT as demonstrated by increased vimentin and TWIST gene expression under 20 dyn/cm^2 and greater transendothelial extravasation of BC cells under 15 dyn/cm^2 (Ma et al., 2017; Xin et al., 2019). Patient-derived, doxorubicin-treated primary epithelial tumor cells exposed to 20 dyn/cm^2 stress exhibited greater cell suspension growth and increases in stemness and EMT-promoting gene expression (Choi et al., 2019a). This establishes the crucial role of hemodynamic SS in promoting MSC-like phenotype, which stimulates EMT and metastasis to distant organs. Overall, mechanical stresses influence tumor progression and have important implications in cancer treatment, representing a promising focus to identify new therapeutic targets for inhibiting neoplasm advancement.

6. Spheroids as a platform to study internal and external mechanical properties of tumors

Tumor spheroids, also known as tumorspheres, are models that improve *in vitro* mimicry of the native tumor and represent a method to understand the crosstalk between cancer cells, the tumor mass, and the TME (Fig. 3) (Bregenzer et al., 2019; Weiswald et al., 2015). This model is especially advantageous for understanding the mechanical ramifications of heterogeneous niches within tumors that typically arise from variations in oxygen, nutrient, chemical, and physical exposures (Bregenzer et al., 2019).

6.1 Tumor spheroid mechanics—

Understanding the intratumoral biomechanical properties and their influence on whole tumor behavior is integral to an improved understanding of cancer pathobiology and identification of therapeutic targets (Stylianopoulos, 2017; Weiswald et al., 2015). Tumor spheroids are compact cell aggregates defined only by cell-cell and cell-endogenous ECM interactions that are typically studied as either homotypic spheroids made only with cancer cell lines or heterotypic spheroids containing cancer and stromal cells (*e.g.* fibroblasts, endothelial cells, immune cells, etc.) (Weiswald et al., 2015). Experiments using tumor spheroids composed solely of cancer cell lines enable exclusive observation of cancer cell behavior, which is particularly important in elucidating cancer-derived changes in mechanical properties. Force-sensing microtweezers were used to characterize the initial storage modulus of BC tumor spheroids to understand inherent changes in tumor stiffness compared to noncancerous tissue (Jaiswal et al., 2017). Additionally, internal tumor propagation and responses to external stresses, such as those induced by surrounding tissue and fluid stress, can be directly measured within spheroids using mechanical stress microsensors (Dolega et al., 2017). Furthermore, stresses on the tumor induced by rapidly proliferating cancer cells (growth-induced stress), can also be modeled with tumor spheroids. HCT116 colon carcinoma spheroids formed with more cells and cultured for a shorter time had increased susceptibility to chemotherapeutics compared to spheroids formed with fewer cells and cultured longer (Guillaume et al., 2019). These studies demonstrate the importance of tumor spheroids in understanding inherent behaviors of tumor masses and characterizing the intratumoral responses to mechanical stimuli.

6.2 Tumor spheroid-biomaterial interactions—

Tumor spheroids, when entrapped in biomaterials, can model tumor invasion into surrounding tissue and associated tumor-driven alterations in mechanical properties (Thakuri et al., 2018). The incorporation of spheroids into biomaterials reflects distinct cell behaviors compared to monodisperse cells due to the

dense cell-cell and cell-ECM interactions within spheroids and limited cell-biomaterial interactions on their periphery (Gionet-Gonzales and Leach, 2018; Guillaume et al., 2019; Weiswald et al., 2015). These interactions are most readily characterized in studies interrogating the effects of substrate stiffness on tumor spheroid behavior. MCF-7 breast cancer cell spheroids in stiff (1.5 kPa) MMP-degradable PEG hydrogels had higher surface stiffness and spheroid compaction but lower metabolic activity and proliferation compared to spheroids in compliant (0.75 kPa) gels. Furthermore, increased tumor spheroid growth was observed upon pharmacological disruption of cytoskeletal rearrangement and spheroid interaction with the hydrogels (Taubenberger et al., 2019). Primary breast cancer tumoroids and isolated breast cancer mesenchymal cell spheroids embedded in collagen gels exhibited collective contractile forces of at least 200 μN against the gel after integrin engagement along the periphery (Mark et al., 2020). The characterization of substrate stiffness and its influence on tumor behavior is important for understanding primary tumor growth and development. Moreover, the mechanical characteristics of the microenvironment are key for studying metastasis to tissues of different stiffnesses compared to the primary tumor, such as metastasis of carcinomas to the bone (e.g., breast and lung cancer) or metastasis of osteosarcoma from bone to the lung.

The study of tumor spheroids within biomaterials is also relevant for the study of early tumor development and therapeutic response (Lam et al., 2014; Li and Kumacheva, 2018). Spheroid formation within biomaterials provides a unique opportunity to decipher key parameters, such as adhesivity, involved in initial tumor formation and growth. Monodisperse LNCaP prostate cancer cells formed spheroids following encapsulation in a HA hydrogel (Hao et al., 2016). Tumor spheroids formed in RGD-modified hydrogels exhibited increased size, metabolic activity, and E-cadherin expression compared to spheroids formed in hydrogels with scrambled RDG peptides. These findings suggest that adhesive ligand presentation and density may play a key role in tumor development and growth rate. Additionally, the dense nature of spheroids allows for more accurate modeling of drug penetration and cell resilience for therapeutic testing. MG-63 osteosarcoma cell spheroids embedded in GelMA and Matrigel showed increased migration and chemotherapeutic resistance compared to monodisperse cells (Monteiro et al., 2020). Substrate stiffness as a correlate to tumor spheroid therapeutic resistance is also reported in the literature. MDA-MB-231 metastatic breast cancer cell spheroids were loaded in 300 Pa, 1200 Pa, and 6000 Pa collagen hydrogels and exposed to chemotherapeutic treatments. Spheroids in 300 Pa gels demonstrated increased cell migration from the spheroid as well as treatment-mediated apoptosis compared to those in stiffer gels (Lam et al., 2014). These data emphasize the importance of accurate tumor modeling in the identification and development of effective cancer therapeutics.

7. Application of computational models

Computational models integrate key biological findings to simultaneously study numerous cellular effects, molecular interactions, and environmental effects. For instance, the contribution of hydrodynamics on circulating tumor cell vascular colonization was studied using an advanced computational flow model to understand the effects of microenvironmental biophysical forces on the tumor cells (Hynes et al., 2020).

Computational models further enable better clinical prediction for breast cancer through the classification of gene expression data based on the analysis of genetic patterns (Nandagopal et al., 2019). Models of individual cancer cells are used to predict cytokine and cellular biomarker profiles found in cancerous tissues and explore how microenvironmental conditions (e.g., hypoxia) drives tumorigenesis and invasion (Fischer et al., 2019). Furthermore, *in silico* models are advantageous for their ability to describe patterns of metastatic spreading and permit high throughput testing of various therapeutic strategies to guide precision cancer medicine while avoiding a trial and error style of approach (Cheng et al., 2020; Munoz and Tello, 2017). Overall, computational models are informative to study primary and metastatic neoplasm behavior and reduce costs associated with determining appropriate treatments. However, their inability to recapitulate native physiology is a major limitation that must be considered.

8. Application of *in vitro* models to the *in vivo* condition

Engineered platforms can create more accurate biomimetic systems that mimic the cancer microenvironment for *in vitro* study. However, *in vivo* models are necessary to observe overall effects on a living subject and facilitate translational studies for diagnosis and treatment. Ductal carcinoma in situ (DCIS) specimens seeded on PLG scaffolds containing HAp had increased IL-8 expression and more than 30% less cell clustering than on PLG controls (He et al., 2019). When murine xenografts were implanted with the scaffolds, the authors reported decreased cellular organization, potentially enhanced IL-8 secretion, and enhanced fibrosis. These findings corresponded with pathological analyses conducted for clinical DCIS specimens, where the presence of microcalcification correlated with increased IL-8 staining and cell proliferation, emphasizing the usefulness of PDXs to model behaviors that occur physiologically. Similarly, OS and BC cells cultured on 3D-printed polydopamine-modified nagelschmidtite (NAGEL) bioceramic scaffolds undergoing irradiation exhibited increased cell death *in vitro*. This was in agreement with impaired tumor growth observed in mice (Ma et al., 2016). Additionally, when B16-F1 melanoma cell spheroids were entrapped within soft (90 Pa) fibrin hydrogels and implanted subcutaneously or injected intravenously into both syngeneic and immunocompromised mice, they exhibited significantly increased tumorigenesis and lung metastasis than cells grown on TCP or soft hydrogels (Liu et al., 2012). This suggests that 3D culture of spheroids selects for functionally aggressive cell and tumor populations that translate to *in vivo* models. These studies indicate the role of engineered platforms in developing relevant culture systems and the physiological relevance of *in vivo* studies to recapitulate human tumors for cancer research.

Future outlook and clinical translation

Targeted treatments identified in preclinical studies have too often failed to translate into successes in randomized controlled trials for bone sarcoma patients (Choy et al., 2014; Kopp et al., 2019). Models that accurately recapitulate human disease are imperative for the development of novel therapeutics, especially for rare cancers like osteosarcoma (OS) for which there have been few advancements in the past 30 years (Wedekind et al., 2018). Both *in vitro* cell monolayer models and PDX *in vivo* models have helped elucidate disease

mechanisms but also have limitations in the development and testing of novel therapeutics. PDX models may not adequately recapitulate disease evolution, as copy number alterations differ between tumor progression in patients and those acquired during PDX passages. Genomic stability of PDXs may be related to responsiveness to chemotherapy (Ben-David et al., 2017). Additionally, the translation of therapeutics that target these aspects of tumorigenesis and metastagenesis to human trials is limited by the replacement of human stromal elements, such as cancer-associated fibroblasts, endothelial cells, and immune and inflammatory cells, with murine constituents in PDX murine models (Aparicio et al., 2015).

The effectiveness of immunotherapy in treating other adult cancers inspires further exploration of its potential role in bone sarcoma. However, for immunotherapeutic strategies to become successful in OS, further detail is required of the OS immune microenvironment and its interaction with the host immune system. It is likely that combination approaches will be required for targeting the tumor's methods of immunosuppression, including downregulation of human leukocyte antigens from tumor cell surface, recruitment of T-regulatory cells, myeloid derived suppressor cells, and tumor-associated M2 macrophages (Wedekind et al., 2018). Unfortunately, current PDX models in immunodeficient hosts are limited in their ability to study and interrogate the immune microenvironment as well as modulate the host immune system (Di Modugno et al., 2019). 3D tumorspheres would also limit the tunability of the stroma, ECM, and immune infiltrate. Bioengineered models that allow more precise tunability of the ECM, substrate stiffness, hypoxia, and content of the local immune system niche are attractive for their potential to recapitulate human tumors while enabling interrogation of the contribution of the stroma and other cell-cell interactions and immune infiltrate. Additionally, these models may provide a more efficient and cost-effective platform with higher throughput testing of new therapeutics and holds promise for patient tumor specific treatments.

Conclusion

The low-cost, high throughput screening, tunability, and expansive variety of biomaterials have paved the way for new opportunities to improve our understanding of cancer and develop new therapies. The applications of biomaterials range from developing biomimetic models for interrogating the influences of ECM biomechanical properties on neoplasm behavior to screening cancer drug efficacy and establishing novel treatment delivery techniques for therapeutic applications. While 2D *in vitro* models have been the cornerstone of cell-based research for decades, 3D substrates have emerged as robust tools to enable a more comprehensive representation of the biochemical and biomechanical cues present in tumor microenvironments. Bioengineered models are under continual development to recapitulate human tumors in cancer research and have potential for identifying new druggable targets and treatments. These systems can be manipulated to reflect changes in the local tumor microenvironment by regulating their biophysical properties including stiffness, degradation, topographical features, substrate adhesivity, and application of physical forces. Engineered models of cancer represent a more efficient and cost-effective platform for the translation of therapeutics to human trials that may overcome the limitations of current PDX models. While this review highlights the use of engineered model systems to study both primary tumors of the bone and tumors that commonly metastasize to the bone, it is

important to recognize that these are two distinct disease states, and the utility of model systems must be carefully considered.

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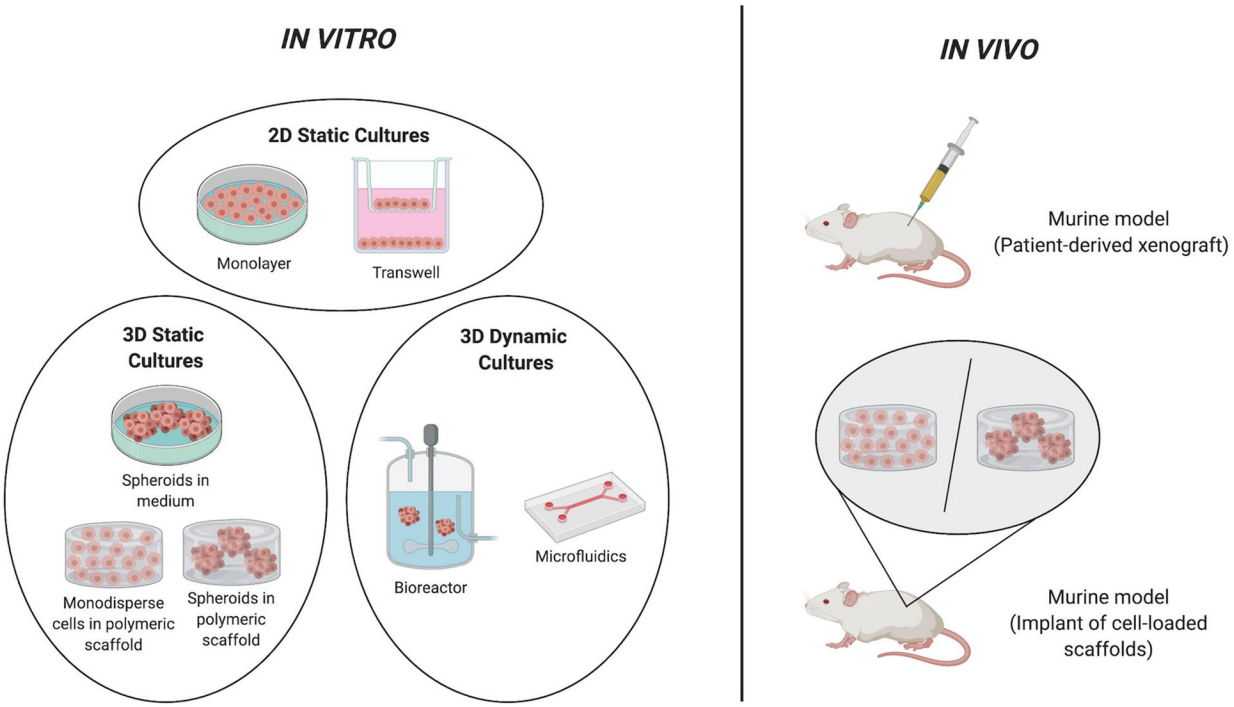

Figure 1. Schematic representation of commonly used 2D and 3D *in vitro* and *in vivo* model systems.

In vitro 2D static models include monolayer and Transwell cultures (top left), *in vitro* 3D static models utilize spheroids and polymeric scaffolds (bottom left), and *in vitro* 3D dynamic models leverage cells cultured in bioreactors and microfluidic systems (bottom middle). Common *in vivo* murine-based models incorporate PDX (top right) or cell-loaded polymeric scaffolds (bottom right).

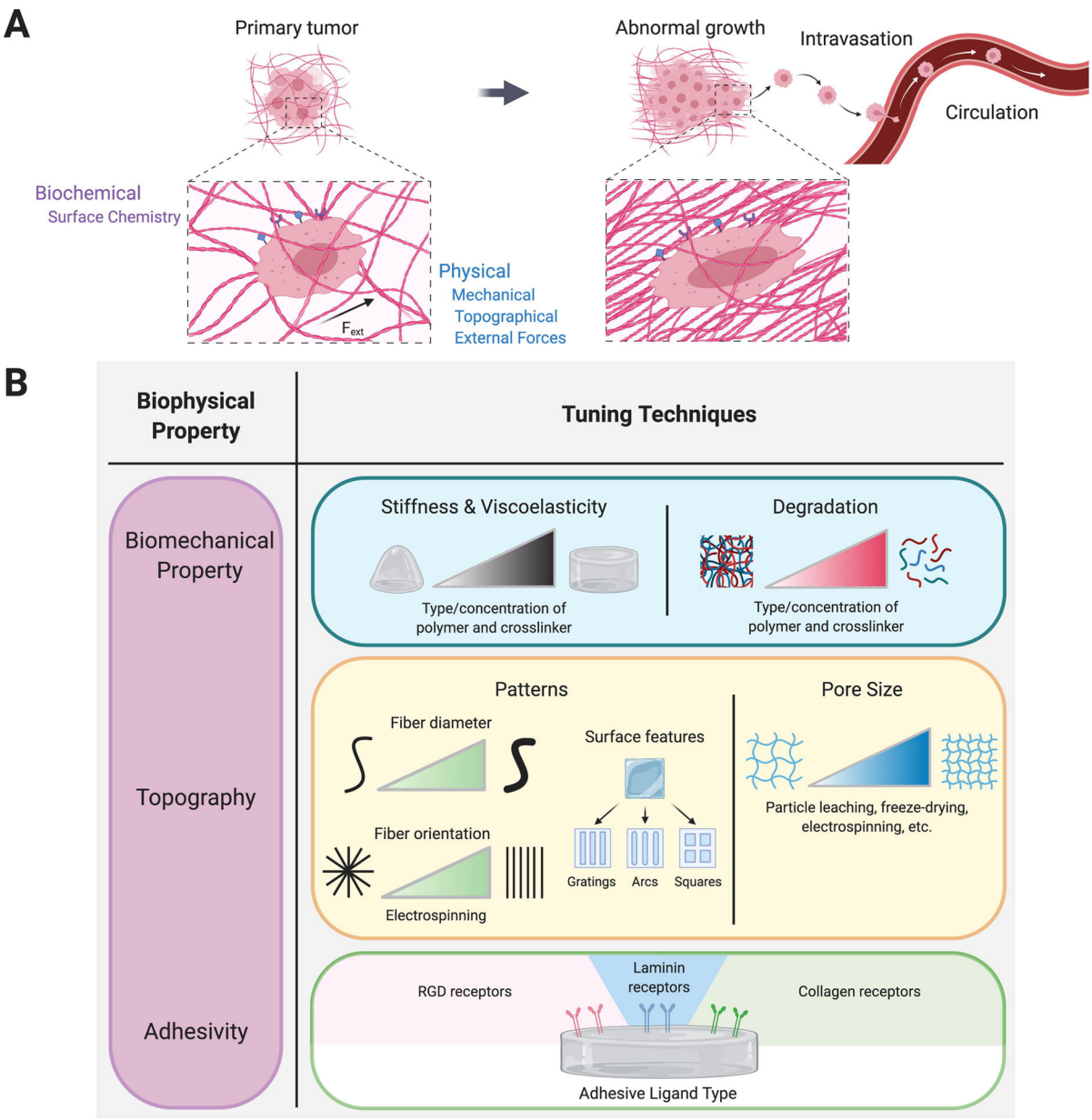

Figure 2. Biochemical and physical properties of the tumor microenvironment and methods to tune them in model systems.

(A) Environmental cues influence neoplasm growth and metastasis. Biochemical cues are impacted by cell surface chemistries. Physical cues are imposed by ECM mechanical properties, ECM topography, and external forces. (B) Techniques to control biophysical characteristics of model systems.

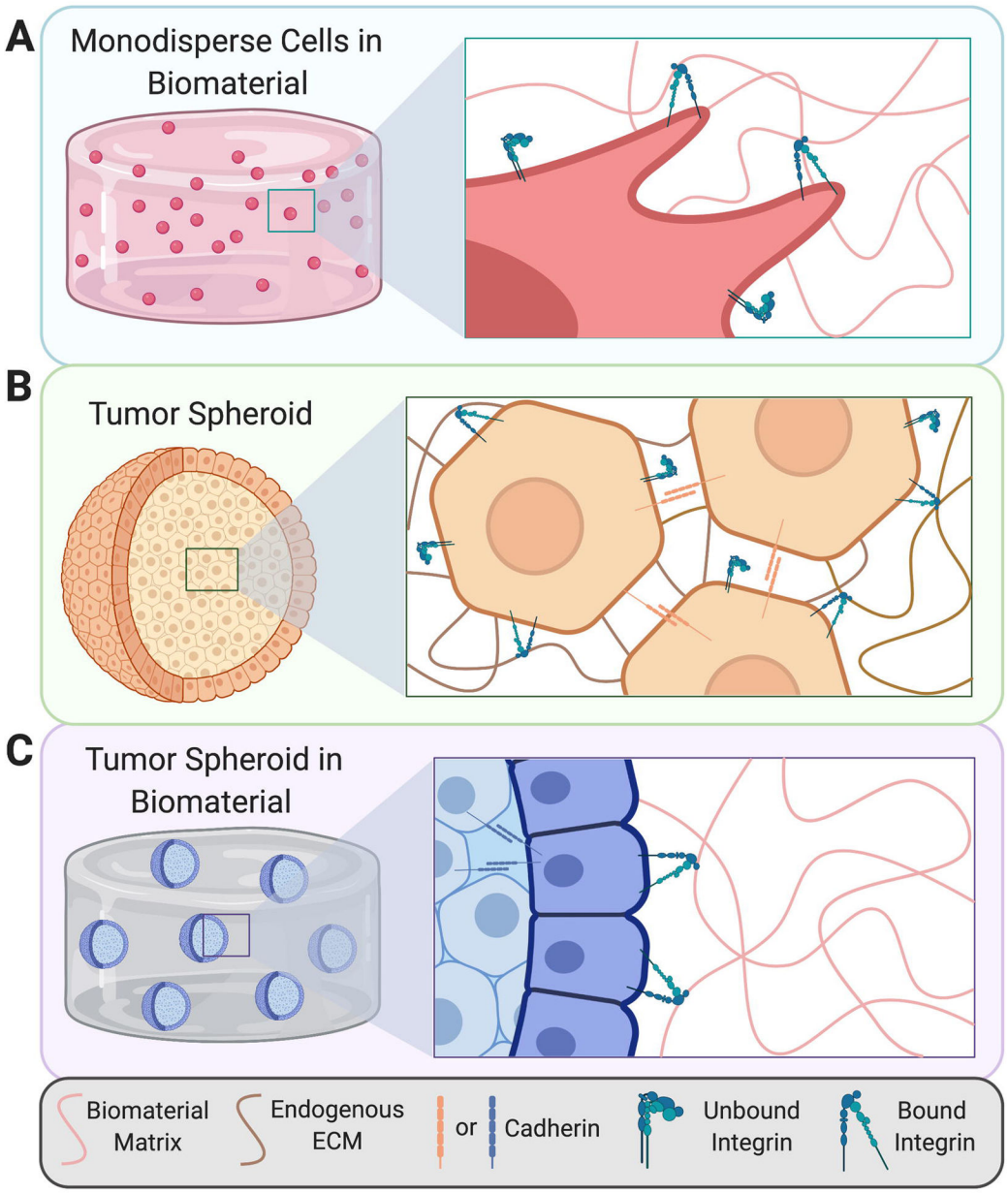

Figure 3. Mechanical interactions of spheroids.

(A) Monodisperse cells within biomaterials engage adhesive ligands *via* integrin binding. (B) Tumor spheroids are dense cell aggregates that internally process mechanical forces through both endogenous ECM ligands and cell-cell cadherin junctions. (C) Within biomaterials, cells within spheroids physically engage with external mechanical stimuli through peripheral cell integrin binding, although these forces may be transmitted throughout the aggregate through multiple mechanisms.