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Engineering cancer microenvironments for *in vitro* 3-D tumor models

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The natural microenvironment of tumors is composed of extracellular matrix (ECM), blood vasculature, and supporting stromal cells. The physical characteristics of ECM as well as the cellular components play a vital role in controlling cancer cell proliferation, apoptosis, metabolism, and differentiation. To mimic the tumor microenvironment outside the human body for drug testing, two-dimensional (2-D) and murine tumor models are routinely used. Although these conventional approaches are employed in preclinical studies, they still present challenges. For example, murine tumor models are expensive and difficult to adopt for routine drug screening. On the other hand, 2-D *in vitro* models are simple to perform, but they do not recapitulate natural tumor microenvironment, because they do not capture important three-dimensional (3-D) cell–cell, cell–matrix signaling pathways, and multi-cellular heterogeneous components of the tumor microenvironment such as stromal and immune cells. The three-dimensional (3-D) *in vitro* tumor models aim to closely mimic cancer microenvironments and have emerged as an alternative to routinely used methods for drug screening. Herein, we review recent advances in 3-D tumor model generation and highlight directions for future applications in drug testing.

Introduction

Tumor growth and aggressiveness are influenced by the microenvironment surrounding the tumor mass [1–5]. The native tumor microenvironment is composed of extracellular matrix (ECM), cell–cell contact, and cell–matrix interactions [6–8]. The ECM consists of a nanofibrous mesh of proteins (i.e., elastin, collagen, fibronectin, and laminin), which fill the extracellular spaces around the cells to help them stay connected with each other by adhesion proteins [9]. In addition, the ECM components are involved in various cell signaling pathways [10,11]. These cell–cell and cell–matrix interactions regulate tumor growth, angiogenesis, aggression, invasion, and metastasis (Fig. 1) [12,13]. In the early stages of cancer, tumor cells undergo certain alterations (a process called immunoediting) to initiate signaling pathways that inactivate the immune system to prevent their elimination from the body [14,15]. Such alterations allow cancer cells to avoid the body's immune response and grow abnormally to form a large tumor mass.

During immunotherapy, the native immune system is reactivated by administration of peripheral blood lymphocytes or immune modulatory drugs [16]. Various immunotherapy drugs have been introduced in the past to treat cancer patients, but many of them have not exhibited a good response during phase I/II clinical trials [17]. For example, the first immunotherapy (Sipuleucel-T, Provenge) for castration-resistant prostate cancer patients approved by United States Food and Drug Administration (FDA) prolonged the survival of cancer patients by only a couple of

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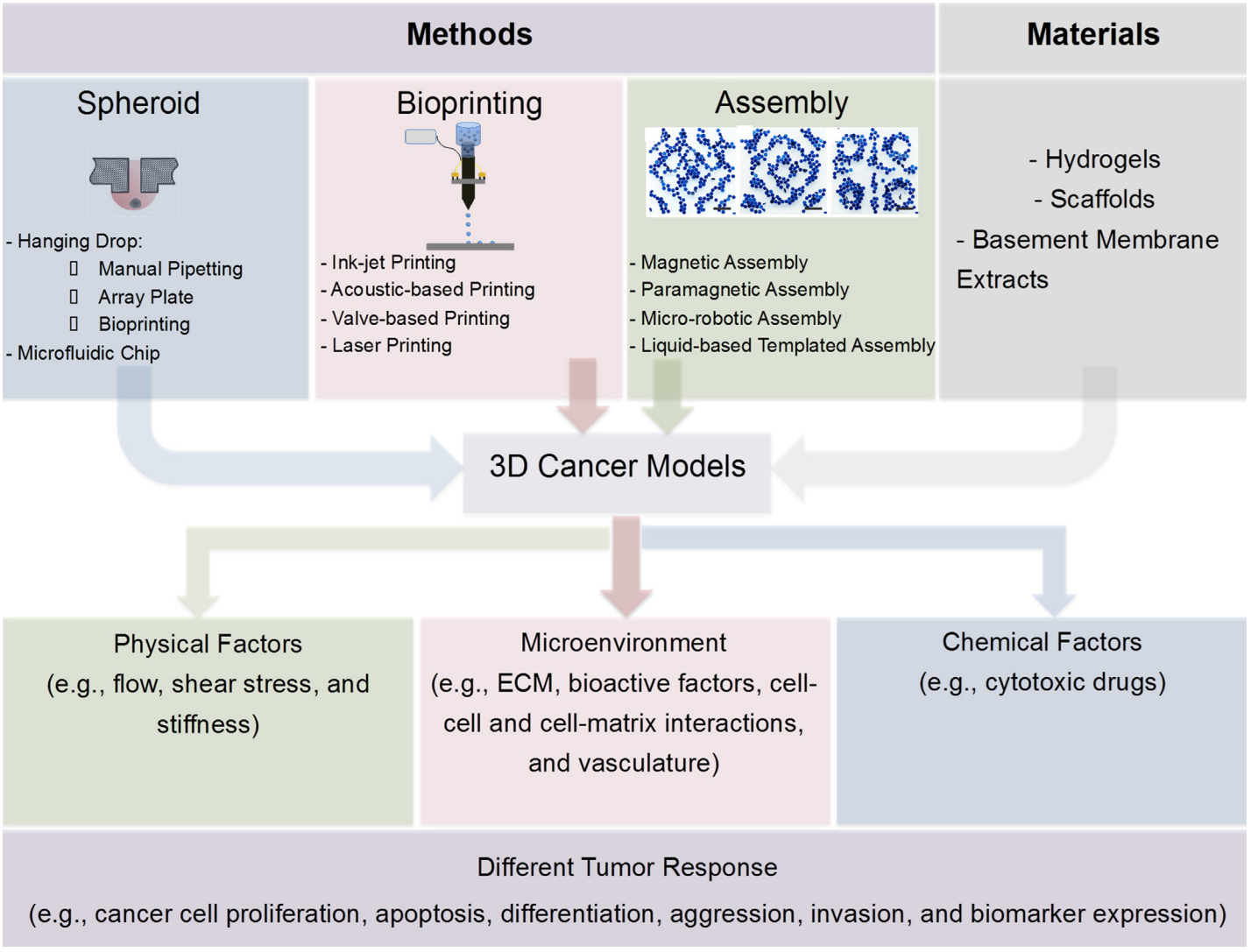

FIGURE 1

Methods and materials used to engineer 3-D cancer models. To generate 3-D cancer models various technologies are used including spheroids, bio-printing, and assembly. These technologies are implemented using numerous kinds of materials such as hydrogels, scaffolds, and basement membrane extracts. Controlling physical and chemical factors and mimicking the native microenvironment results in tumor responses such as cancer cell proliferation, aggression, and invasion.

months as compared to standard drugs and placebo controls [18]. Furthermore, in many clinical trials, drug testing is also hampered by the limited enrollment of cancer patients [19]. Such drawbacks limit the development of new drugs for cancer patients.

Two-dimensional (2-D) *in vitro* cancer models and small *in vivo* animal models are used conventionally for drug testing and screening [20,21]. However, because of the difficulty in recapitulating the natural tumor microenvironment in 2-D culture as well as the cost and issues associated with animal models, both approaches have become less attractive for routine drug testing. New three-dimensional (3-D) *in vitro* cancer models have emerged as an alternative approach to conventional methods and have shown the potential to recapitulate the natural microenvironment of tumors in a relatively simple and inexpensive way when compared to conventional methods [22–29]. In this article, we review the significance and limitations of different tumor models used in the literature for drug testing. We also discuss various approaches that are currently available for generating 3-D tumor models such as spheroids, hanging drop, bio-printing, and magnetic levitation. In addition, we evaluate the effect of materials (e.g., basement membrane matrix, hydrogels, and scaffolds) and physical parameters (e.g., stiffness, morphology, flow, and shear stress) on the growth, invasiveness, differentiation, and regulation of biomarker expression of cancer cells. Finally, we highlight future directions for 3-D cancer models toward applications in anti-cancer drug development.

Strengths and limitations of 2-D *vs.* 3-D tumor models

Cancer cells are routinely cultured on 2-D plastic substrata in the pharmaceutical industry [30]. In 2-D tumor models, cancer cells are grown as a monolayer and do not mimic the native tumor environment [21,31]. The cells sit on a flat 2-D surface with almost half of the cell's surface directly bound to plastic substrata. Cancer cells grown on a 2-D surface lose certain signaling pathways that are important in defining cell's natural response in terms of growth, metabolism, and differentiation [31–34]. In one study, human breast tumor cell line (T4-2) derived from phenotypically