Gears of the future

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CAPÍTULO 6

BIOMATERIALS FOR THE STUDY OF CANCER

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ABSTRACT: This chapter presents a review on the biomaterials currently used in the creation of three-dimensional culture media for the study of cancerous tumors. Three-dimensional tumor models are essential for *in vitro* research results to be faithful to *in vivo* reality, for the study and evolution of treatments and overall comprehension of cancer. Due to their high versatility, natural and synthetic polymers dominate this application. The main materials used, their applications, available commercial products, their advantages and disadvantages are presented. Some of the most cutting-edge research in this field and perspectives for the future of tumor models are discussed.

KEYWORDS: tridimensional tumor models, polymers, hydrogels, cell culture media.

1 I INTRODUCTION

Cancer is the second deadliest disease in the world. According to estimates by the World Health Organization (WHO) [1], cancer is the first or second leading cause of death before the age of 70 in 112 of 183 countries (Fig. 1). The same survey estimates 19 million new cases worldwide and 10 million deaths in 2020 [2], causing an economic impact of more than 1 trillion dollars every year [4]. The growing prominence of cancer as a leading cause of death reflects, in part, marked declines in cardiovascular diseases mortality rates in many countries [1,3].

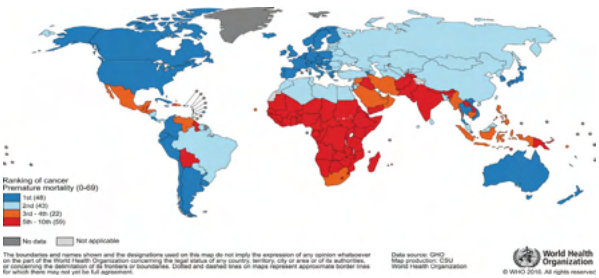

Figure 1 – Cancer's position in the mortality ranking of each country in 2019. Reproduced from [1].

Although the fatality rate has been falling by an average of 1.4% per year [2], this decline is very small compared to other diseases. This is explained by the little evolution of treatments over the years, despite the many researches and knowledge generated about cancer [4]. One

of the main reasons for this low evolution of treatments is the use of two-dimensional cell culture techniques to study tumors, which are three-dimensional tissues. This reduction in dimensional complexity ignores the effects of the microenvironment on tumor development, generating *in vitro* results that do not correspond to *in vivo* reality [5]. The recent development of biomaterials with controllable properties and new advanced manufacturing techniques allow the creation of three-dimensional cell culture media and the modeling of tumors more faithful to reality [4].

2 | TUMOR MODELS

Reliable tumor models are essential for performing detailed analyses, target identification, drug studies and personalized treatment planning [6]. Several types of models have already been developed, from creating cell lines *in vitro* to *in vivo* animal models. Cell culture models provide convenience and controllability, but often misrepresent the complex tumor microenvironment. Animal models, on the other hand, mimic better certain aspects of the microenvironment, but are inherently complex and the interpretation of their study results is not an easy task [6].

Currently, 3D models include spheroids, organoids, matrix scaffolds, tumor-on-chips and printed tissue constructions. This generation of models attempts to address the limitations of current 2D models while providing mimicry of *in vivo* tumors. Spheroids and organoids are being adopted due to the simplicity and convenience of copying densely packed cells in the three-dimensional context [7]. Scaffolds (Fig. 2) allow the systematic study of microenvironments, including cell-cell and cell-matrix interactions via culture of various cell types [8,9]. Microfluidic models are able to control and impose various chemical and physical conditions on the tumor microenvironment [10].

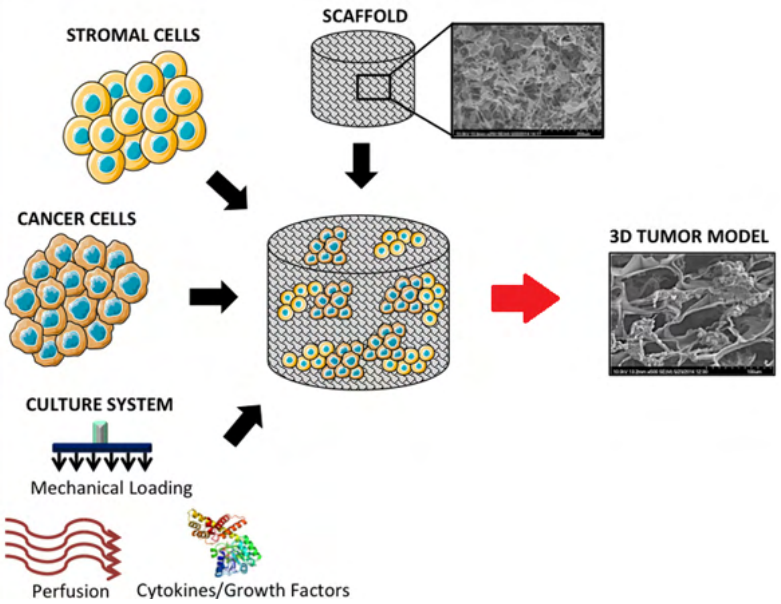

Figure 2 – Creation of a 3D tumor model using a scaffold. Adapted from [9].

3 | BIOMATERIALS CURRENTLY USED

Materials used as culture medium should mimic the tumor-specific extracellular matrix and composition, as well as aspects of cancer-related changes in tissue mechanics, structure, and interstitial pressure [8].

Although ideal for replicating the cell-matrix interactions of the native tumor microenvironment, scaffolds of natural materials suffer from batch-to-batch variability and can only be manufactured within limited ranges of mechanical stiffness, degradation rate, porosity, and number of sites for cell adhesion. [9].

To overcome the limitations of natural materials, research has been done on developing synthetic materials with a superior capacity for structural complexity and adjustable physical properties over a wider range. They provide the possibility to project the extracellular matrix characteristics specifically for the type of cell or tumor, allowing the study of microenvironmental signals in a highly controlled manner. However, the factors that still limit its use are the manufacturing processes that can be cytotoxic and the limited bioactivity of most synthetic materials [9].

Among the materials already applied, there is a predominance of natural and synthetic polymers. Table 1 presents some of the most used, their origin and available commercial products:

Naturals			
Materials	**Origin**	**Comercial products**	**Ref.**
Hyaluronic acid	Polysaccharide composed of repeating units of d-glucuronic acid and n-acetyl-d-glucosamine.	-	[12,13]
Agarose	Polysaccharide composed of d-galactose and 3,6-anhydro-alpha-l-galactopyranose isolated from red algae.	-	[14–17]
Alginate	Block copolymer derived from brown algae composed of guluronate and manuronate.	AlgiMatrix™, BioVision®	[18–20]
Type I collagen	Isolated from bovine or porcine skin and tendons.	Chondrex®	[21–23]
Basement membrane matrix	Mixture of basement membrane proteins isolated from Engel-breth-Holm-Swarm mouse sarcoma [EHS).	Matrigel®, MaxGel™	[24–26]
Cell-derived arrays	Matrix deposited by cells seeded in vitro.	-	[27,28]
Decellularized Tissues	Cells removed from an entire tissue or organ, keeping the matrix structure and composition intact.	-	[29–31]
Synthetics			
Material	**Origem**	**Produtos comerciais**	**Ref.**
Poly[acrylamide) [PA)	Bis-acrylamide cross-linked acrylamide monomers.	Acrigel®	[32,33]
Poly[lactic-co-glycolic acid) (PLGA)	Linear copolymer of lactic acid (LA) and glycolic acid (GA).	-	[8,34,35]
Poly(lactic acid) (PLA)	Polymer made up of lactic acid molecules.	-	[8,36]
Poly(glycolic acid) (PGA)	Highly crystalline, straight-chain glycolic acid polymer.	-	[8,36]
Poly(ethylene glycol) (PEG)	Ethylene glycol repeating units	-	[37]

Table 1 - Biomaterials for three-dimensional culture medium

3.1 Hyaluronic acid (HA)

A natural polysaccharide consisting of d-glucuronic acid and n-acetyl-d-glucosamine units, forming a linear copolymer [37]. As the main component of the extracellular matrix, HA is expressed in several organs, mainly in the skin [38]. The CD44 differentiation cluster protein is considered to be the main HA receptor, and the CD44-HA interaction is activated in many signaling pathways and involved in many biological processes, such as inflammation, wound healing, morphogenesis and cancer development [39,40]. As a 3D culture medium, it requires chemical modification for cell attachment via integrins and its mechanical adjustability is limited if not modified [18–20].

3.2 Agarose

Polysaccharide isolated from red algae, composed of d-galactose and 3,6-anhydro-alpha-1-galactopyranose. Due to its structure similarity with the extracellular matrix, this natural polymer supports cell adhesion with chemical modifications, while providing adequate permeation of oxygen and nutrients for cell growth [41]. Cell adhesion on agarose substrates can be improved by modifying the surface stiffness, using the fabrication of nano/microstructures and by blending with other polymers. It can be tuned to emulate brain and corneal tissues [42,43], where mechanobiology plays an important role in adjusting the behavior of cell signaling. It has a high water absorption capacity, which is useful for cell growth, differentiation and proliferation. The thermal gelling properties of its hydrogels allow its properties to be customized when blending with synthetic biomaterials, such as to form interpenetrating networks and copolymers [41].

3.3 Alginate

As one of the naturally occurring anionic polysaccharides, alginate gels have been used to encapsulate a wide variety of cells. They are generated after gelation with some divalent cations with controllable stiffness and flexibility [45]. The addition of calcium or other divalent cations is not harmful to cells at low concentrations, which avoids the toxicity of some catalysts or solvents during gelation [46]. Furthermore, alginate scaffolds are structurally similar to the extracellular matrix, with controllable diameters, porosity, and stiffness [47]. They can be processed under mild conditions and the culture system is easy to reproduce, convenient to handle and suitable for large-scale cultivation [48].

3.4 Type I Collagen

The presence of collagen throughout the connective tissue makes it one of the most studied biomolecules in the extracellular matrix. This species of fibrous protein is the main component of skin and bone and represents around 25% of the total dry weight of mammals [49]. The use of collagen-based biomaterials in the field of tumor engineering has grown intensely in recent decades. Several crosslinking methods were investigated and different combinations with other biopolymers were explored in order to improve tissue function. Collagen has a great advantage for being biodegradable, biocompatible, easily available and highly versatile [50].

3.5 Poly(lactic-co-glycolic acid) (PLGA)

Linear synthetic polymer composed of lactic acid and glycolic acid. PLGA scaffolds can be porous or fibrous and allow for precise control of biodegradability by adjusting the components ratio [8]. However, they have low mechanical properties adjustability and their biodegradation decreases the pH around cultured cells [8,34].

3.6 Poly(ethylene glycol) (PEG)

Non-toxic, flexible, water-soluble polymer synthesized by ring-opening polymerization of ethylene oxide (C_2H_4O) to produce a wide distribution of molecular weights. PEG-based hydrogels are inert in terms of triggering cell signaling pathways, but can be equipped with biochemicals such as proteolytic degradation sites and biological functionalities in a controlled manner. The rigidity of such hydrogels can be precisely adjusted independently of their proteolytic sensitivity and cell-ligand density [36].

3.7 Basement membrane matrix

Extracellular matrix secreted by the Engelbreth–Holm–Swarm mouse sarcoma cell line. It contains components of the human cell matrix, such as collagens, laminin, fibronectin, tenascin, elastin and a series of proteoglycans and glycosaminoglycans, similar to the basement membrane [52]. It allows the reproduction of cooperative interactions between epithelia and mesenchyme that occur during the development and culture of organotypic skin cells. This material creates a three-dimensional environment that enhances cell proliferation, promoting the growth and migration of many types of cells, including human embryonic stem cells, neural stem cells, neurons, glia, astrocytes, fibroblasts, hepatocytes, and keratinocytes [53].

3.8 Cell-derived matrices

Matrices deposited by cells seeded *in vitro*, such as fibroblasts [54] and stem cells derived from human adipose tissue [55]. They provide a convenient and highly physiological assay system for measuring and correlating cell morphology, proliferation, apoptosis tendency, and drug response in high-throughput formats [54]. Preserve the original composition and microstructure of the original cell type [11].

3.9 Decellularized Tissues

Decellularization is a technique in which an organ or tissue is chemically stripped of its cells, leaving behind an intricately structured extracellular matrix [56]. This type of scaffold retains tissue-specific matrix components and signaling molecules, in addition to allowing the spontaneous formation of three-dimensional colonies that histologically, molecularly and phenotypically resemble *in vivo* metastases [56]. Figure 3 illustrates the various existing decellularization processes:

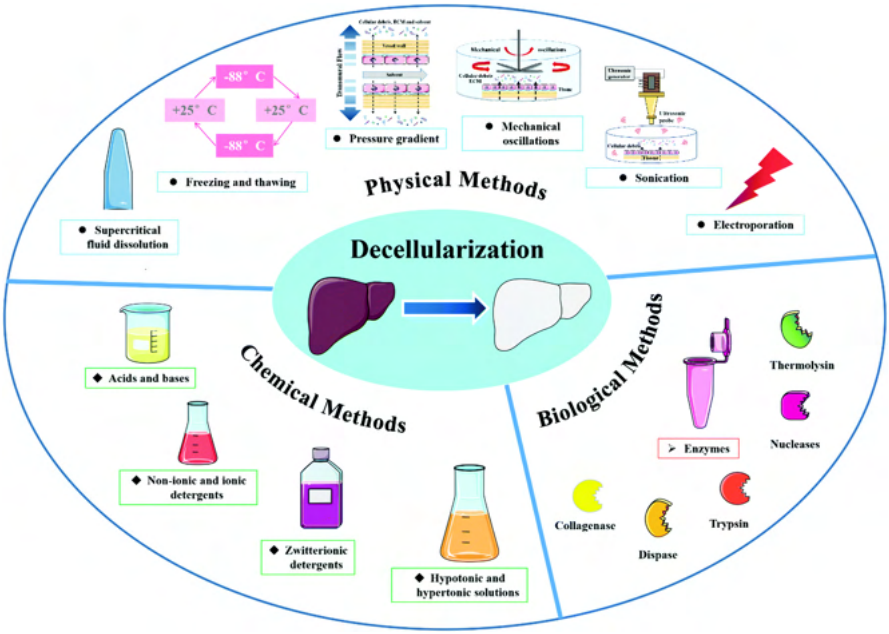

Figure 3 – Chemical, physical and biological methods of decellularization. Reproduced from [57].

4 I APPLICATIONS

4.1 Analysis of tissue dimensional effects

The exposure of cancer cells to 3D tissues significantly alters their signaling and gene expression due to a variety of mechanisms, including differential integrin engagement, cell-cell contact and tumor responses to the microenvironment. To directly identify the molecular mechanisms by which changes in cell-cell contact can influence cell behavior, it is possible to covalently couple adhesion molecules normally expressed in the cell membrane to hydrogels. Individual cells seeded in micropores (Fig. 4) interact with these proteins as if they were expressed by other cells. Such a system allows for the isolation and direct study of cell-cell contacts via the interaction of specific protein binding pairs [58].

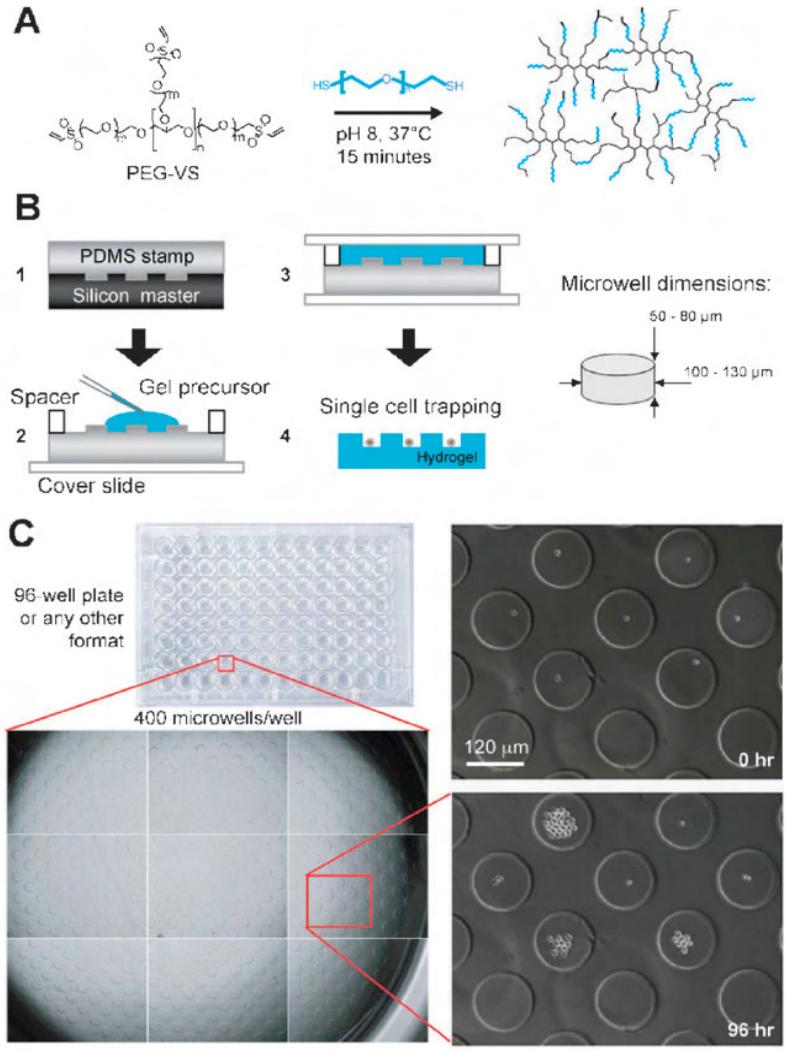

Figure 4 – Microporous plate for studies of disturbances in the hematopoietic stem cell microenvironment. a) Reaction of thioland vinylsulfone end groups on PEG precursors to form hydrogel matrices. b) plate manufacturing process. c) Matrix with 96 pores and tracking of cell behavior by time-lapse videomicroscopy. Reproduced from [59].

4.2 Study of cell-matrix interactions

Interactions with the extracellular matrix regulate gene signaling and expressions that underlie cellular processes during development, homeostasis, wound healing, and cancer invasion [60]. These interactions are particularly difficult to study due to their complex synergistic and antagonistic interactions *in vivo*. Experiments targeting integrins, a central family of cell surface receptors, have shown that integrin-matrix interactions are important regulators of cancer progression [61]. Thus, techniques that allow for specific

impartial interrogation of cell-matrix adhesion are needed to directly query the diversity of potential interactions [60].

To study the adhesion characteristics of the extracellular matrix of any cells of interest, Reticker-Flynn et al. [62] developed plates (Fig. 5) for deposition of array combinations by a DNA microarray identifier. Prior to deposition of molecules, slides are coated with a polyacrylamide hydrogel that can dry after immersion to remove any unpolymerized monomer. The dehydrated hydrogel works by binding molecules without requiring their chemical modification (Fig. 5a). To quantify cells attached to each dot, nuclei are stained according to standard fluorescence staining protocols and slides are imaged using an automated inverted epifluorescent microscope [61].

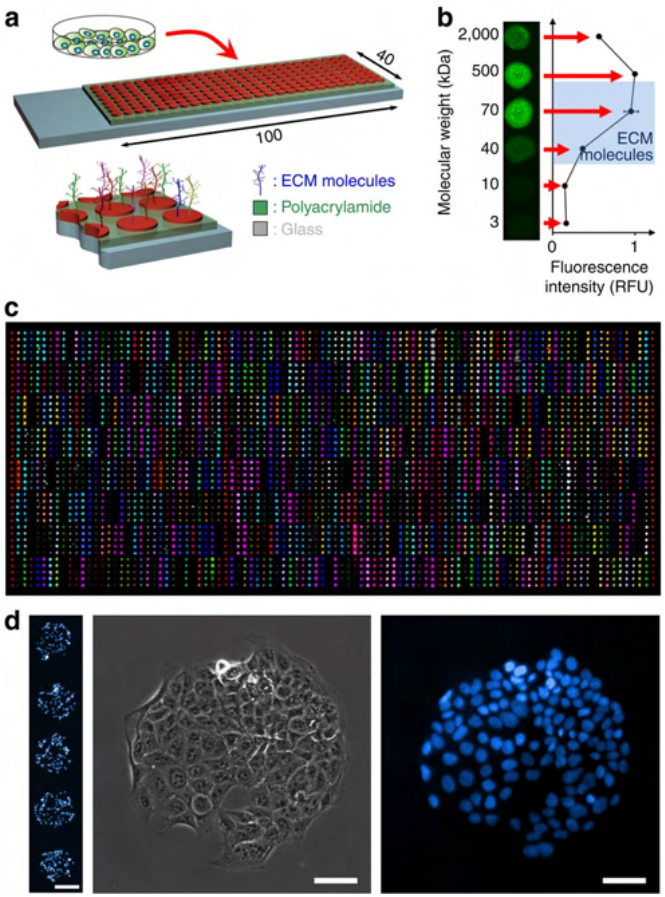

Figure 5 - (a) Extracellular matrix microarrays generated by locating nearly 800 unique combinations of molecules on polyacrylamide-coated glass sheets, followed by cell seeding. (b) Polyacrylamide works by trapping molecules of a wide range of molecular weights. (c) Presentation of all molecules by immunostaining (colored dots) or NHS-fluorescein markings (grayscale dots). [d) Representative images of cells adhered to the extracellular matrix. Reproduced from [62].

4.3 Tumor-stroma interaction modeling

The epithelial-mesenchymal transition is considered a crucial mechanism for the acquisition of malignant phenotypes by epithelial tumor cells. The reverse phenomenon, the mesenchymal-epithelial transition is also important as a process of reversion of cells to a differentiated state, when cancer cells colonize and grow at secondary sites. In addition to its role in cancer progression, the epithelial-mesenchymal transition can cause therapeutic failure, generating subpopulations of cells that show drug resistance or stem cell-like traits, i.e., tumor-initiating cells [63,64].

Cancer cell cultures in conjunction with cancer-associated fibroblast conditioned media isolated from tumor samples are commonly used models to study these transitions in vitro. Other models use cancer cells grown on extracellular matrix proteins or co-cultured with stromal cells directly. Data obtained using these *in vitro* models may explain the interaction between cancer cells and the extracellular matrix or between cancer cells and the stroma in inducing the transition [65,66].

Kim et al. (2015) [67] created a 3D co-culture model using colorectal tumor spheroids and colon fibroblasts cultured in collagen gel, representing the invasive margin of human tumors where the epithelial-mesenchymal transition actively occurs. In this model, cell-cell and cell-matrix interactions, proximity co-culture with fibroblasts and migration to the collagen matrix were implemented. It was found that normal fibroblasts are activated when co-cultured with 3D spheroids, but not with conventional two-dimensional culture. Co-culture allowed the visualization of an expression pattern of several genes related to epithelial-mesenchymal transition similar to that observed in tumors *in vivo* [67].

4.4 Microfluidic phenomena

During angiogenesis, blood vessel endothelial cells rapidly infiltrate avascular regions through vascular budding. Vascular endothelial cell growth factors can promote vessel dilation and angiogenic budding, but given the complex nature of vascular morphogenesis, additional signals are needed to determine, for example, which vessel segments sprout, which dilate and which remain quiescent. This process is critical to many normal and pathological processes, such as wound healing and tumor growth, but its initiation and control are still poorly understood [68].

Several approaches have been developed to mimic blood vessels by integrating microfluidic channels into polymeric materials, such as laser ablation [69], two-photon photolithography [70] and needle insertion [71]. Using microfluidic tissue, Song and Munn [68] found that blood flow shear stress attenuates endothelial cell germination in a nitric oxide-dependent manner and that interstitial flow, like that produced by extravasating plasma, directs endothelial morphogenesis. Furthermore, positive gradients of vascular endothelial cell growth factors initiate germination, but negative gradients inhibit it, promoting leaf-like migration instead, analogous to vessel dilation [68].

5 | RESEARCH OPPORTUNITIES

The number of publications about tumor models is increasing rapidly (Fig. 7). However, numerous challenges must be met to open up opportunities for the next generation of models. Although many reconstitute the main components of tumor tissues, they are still significantly limited in mimicking the full spectrum of microenvironmental pathophysiologies. Next-generation tumor models should provide recapitulation of molecular signaling pathways in tumorigenesis and drug resistance, which is essential for identifying molecular targets and testing innovative compounds capable of effectively inhibiting these targets [6].

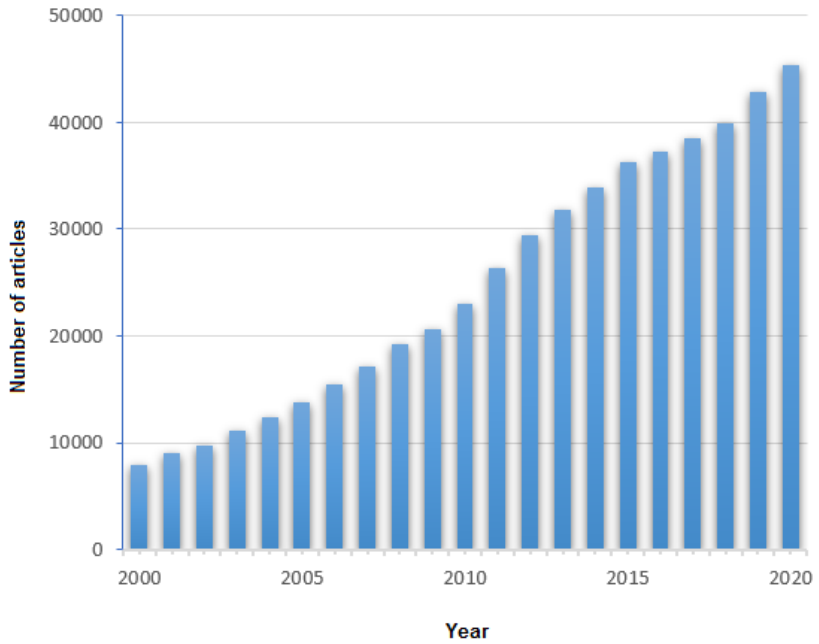

Figure 7 – Scientific publications on tumor models from the year 2000 to 2020. Source: Search in the Pubmed database for publications with the keywords “tumor model”.

5.1 Immune Cells and Specific Subpopulation Models

A major challenge is associated with the recreation of immunosuppressive and inflammatory aspects of the microenvironment. With the advent of immunotherapies, reliable tests to postulate and test new hypotheses to improve anti-tumor immunity in the microenvironment are highly demanded. However, recapitulating the immunosuppression of the microenvironment is extremely difficult. Several attempts to incorporate immune cells have been reported, but it is still in an early stage for wider use. However, given the clear contribution of the immune system to tumor progression and the emphasis on the development of therapies aimed at the immune system, incorporating immunological

components into 3D tumor models is a research area with great potential for development [10,72,73].

5.2 Pancreatic tumors: mimicry of pathophysiology

Pancreatic cancer has the highest mortality rate of all major cancers. For all stages combined, the relative 5-year survival rate is only 10% [74]. The complexity of recapitulating the entire tumor spectrum in a 3D model system is further complicated by the number of biological systems that are activated in the pancreatic tumor microenvironment. For example, recent studies have shown a reciprocal relationship in which the local action of the coagulation and fibrinolytic systems is a significant feature of the pathophysiology of this type of cancer, which has consequences on the disease progression and severity [75]. Systematic studies in different cell lines or patients with distinct oncogenic alternations are necessary to understand the relations between mutations and gene expressions and their contributions to disease prognosis [6].

6 I CONCLUSION

Three-dimensional cell culture media allow the creation of tumor models that are much more reliable, controllable and faithful to reality. This fidelity is extremely important for the results of *in vitro* research to bring innovations to i*n vivo* treatments. Many biomaterials are already being used for this purpose, but few commercial products are available on the market. Although polymers dominate this type of application, the great advances in the development of ceramic scaffolds for bone tissue engineering indicate that this class of materials also has great potential for application in tumor engineering. There is a consensus that microfluidic models will be the most researched model in the coming years, given their wide range of applications and versatility.

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