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A review on three dimensional scaffolds for tumor engineering

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Abstract. Two-dimensional (2D) cell culture and in vivo cancer model systems have been used to understand cancer biology and develop drug delivery systems for cancer therapy. Although cell culture and in vivo model studies have provided critical contribution about disease mechanism, these models present important problems. 2D tissue culture models lack of three dimensional (3D) structure, while animal models are expensive, time consuming, and inadequate to reflect human tumor biology. Up to the present, scaffolds and 3D matrices have been used for many different clinical applications in regenerative medicine such as heart valves, corneal implants and artificial cartilage. While tissue engineering has focused on clinical applications in regenerative medicine, scaffolds can be used in in vitro tumor models to better understand tumor relapse and metastasis. Because 3D in vitro models can partially mimic the tumor microenvironment as follows. This review focuses on different scaffold production techniques and polymer types for tumor model applications in cancer tissue engineering and reports recent studies about in vitro 3D polymeric tumor models including breast, ewing sarcoma, pancreas, oral, prostate and brain cancers.

Keywords: polymeric scaffolds; 3D tumor models; cancer tissue engineering; *in vitro* cancer research

1. Introduction

Although a number of medications and new treatment methods have been developed for cancer treatment in recent years, the majority of current investigations are inadequate for completely treating cancer diseases (Hutchmer *et al*. 2010, Ricci *et al*. 2013). This situation can be explained with the fact that numerous points in especially cancer development have not been enlightened yet (Takei 2006, Da Rocha *et al*. 2014). Cancer cells have certain basic features such as autonomy, contact inhibition, apoptosis pressure, angiogenesis (Chwalek *et al*. 2014), immortality and metastasis (Hutchmer *et al*. 2010, Kim 2005). In studies that attempt to enlighten details in treatment and development process of this illness, employing 2D cell culture environments and experimental animal models is almost a must (Infanger *et al*. 2013). 2D systems (Fig. 1) have contributed to quite a few research studies so far with their features such as repeatability and easy accessibility (Xu *et al*. 2012); however completely imitating the real tissue is not possible due to lack of connective tissue in 2D systems (Chwalek *et al*. 2014, Song *et al*. 2014, Ferrarini *et al*. 2013).

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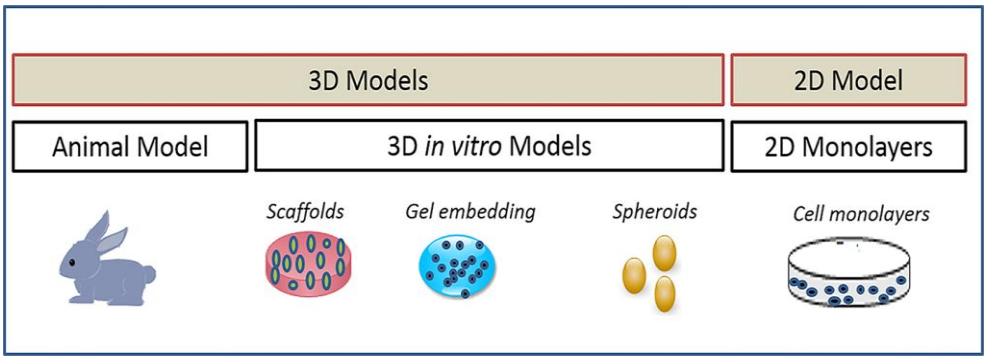

Fig. 1 Schematic illustration of different tumor models for cancer research

Recently in 3D animal models (Fig. 1), human tumor cells are injected into experimental animals and in this way, tumor development and metastasis processes are analyzed. As well as its being inconvenient, this method is also subject to ethical rules (Hutchmer *et al.* 2010, Chwalek *et al.* 2014). Moreover, although tumor formed in animal models have been thought to be promising, test results of the tumor models have not yielded adequate similarity to naturally formed tumor models (Nomikos *et al.* 2003, Burdett *et al.* 2010).

Using 3D culture models instead of 2D, significantly influences protein expression (Da Rocha *et al.* 2014, Chwalek *et al.* 2014) cell proliferation (Kim 2005), differentiation (Infanger *et al.* 2013), and metabolism (Xu *et al.* 2012, Song *et al.* 2014). Although animal studies are limited in their ability to resolve independent aspects of the human tissue microenvironment that contribute to disease progression and metastasis, they inherently still provide tissue content. However, animal models which include transplantation of human cancer cells into mice introduce species-dependent differences in cell communication (Ferrarini *et al.* 2013) and they are also confined to immunocompromised animals, necessarily eliminating the role of immune response and it is decisive in cancer progression (Nomikos *et al.* 2003, Benien and Swami 2014).

The creation of multiple 3D matrices (Fig. 1) and scaffolds which would be seeded with various types of cells in the laboratory can be used as *in vitro* tumor model (Horch *et al.* 2013). As a by-product, these new technologies also turned out to be attractive for other areas of research. Instead of rebuilding organs, the primary aim is to analyze the mechanisms of angiogenesis (Loessener *et al.* 2014), tumorigenesis, tumor spread, and suggest potential ways of fighting cancer cell growth with anti-cancer drugs (Sharma *et al.* 2014).

Due to disadvantages of 2D and 3D systems that have been used in the cancer research field so far, a new biomedical field has been developed in order to create ex vivo tumor models. In these studies, tissue engineering scaffolds have been made of polymers with various methods and activities of tumor cells added onto these scaffolds have been analyzed. The purpose of this article is to present different polymer types and various scaffold production techniques currently applied in different tumor models.

1.1 2D systems

Currently, for a number of illnesses, experimental models are developed to understand the mechanisms that have a major role in the advent of the illness in question (DelNero *et al.* 2013,

Gill and West 2014, Wu and Swartz 2014). With its repeatability, cost-efficiency and easy-access, 2D models are quite popular contributing majorly to many studies; however, the biggest disadvantage is that they lack of extracellular matrix (ECM) (Da Rocha *et al.* 2014, Kim 2005). This disadvantage makes it difficult for 2D models to create successful cancer tissue models. To illustrate it, breast cancer cases can be considered. 80% of breast volume in this cancer type consists of ECM (Drife and Angeli 1986). Therefore, it is impossible to understand the interaction mechanisms of cells with neighboring ones and cell migration in tumor metastasis (Bissell and Radisky 2001, Weaver *et al.* 1997, Talukdar *et al.* 2011).

1.2 3D systems

In order to investigate cancer formation and develop treatments to withstand this mechanism, the models to be developed are required to be as close to the original tissue as possible. Tumor formation is a process that is controlled by micro environmental conditions (Weaver *et al.* 1997, Fischbach *et al.* 2007). Due to disadvantages of 2D systems and animal models' ethical questions, to comprehend tumor formation and metastasis mechanisms, in vitro 3D systems, which are physiological, biological and pathological systems that include tissue and cells, are being developed (Alemany and Semino 2014, Xu *et al*. 2014).

Tissue engineering strategies provide a potent tool box for cancer research and they can overcome limitations of 2D systems (Benien and Swami 2014, Horch *et al.* 2013). There is still more to do to re-create the molecular architecture of the human cancer cell niche one to one and the dynamic mechanisms of the signaling milieu between ECM components and cancer cells. However, imitating these complex physiological phenomena under reproducible conditions allows a more reliable preclinical evaluation of anti-cancer drug candidates (Xu *et al.* 2012, Nomikos *et al.* 2003).

Models that can be considered to be 3D are multi-cell globules (spheroids), gel-embedded systems and tissue engineering scaffolds (Alemany and Semino 2014). When produced in form of three dimensional spheroids, a number of tumor cell strains have been observed to transform into antineoplastic agents and have higher resistance to radiation (Frankel *et al.* 1997). In the studies, it has been observed that spheroids between 20 and 1,000 μm release extracellular matrix and resist chemotropic medications more than 2D systems (Petersen *et al.* 1992). On the other hand, in gel-embedding systems, cells are cultured in hydrogel that imitates extracellular matrix. However, because the hydrogel pores are too small, cell immigrations and cell-to-cell interactions are limited. As a conclusion to this situation, life expectancy of cells is short (Sutherland *et al.* 1986, Fong *et al.* 2014).

In tissue engineering applications, polymeric scaffolds, which are produced with methods such as electrospinning, solvent casting-particle leaching, are now also used in *in vitro* tumor formation research. Polymeric scaffolds, as well as creating a suitable surface for cells to adhere, help forming cell-to-cell, cell-to-extracellular matrix interactions by their interconnected pores and thus, contributing to reaching desired tumor models (Fong *et al.* 2014). Moreover, another advantage of biomaterials produced with tissue engineering techniques is that, pore size, distribution and degradation time can be altered by using various techniques. By selecting the right scaffold production technique for the planned tumor model, natural or synthetic polymers can be used and targeted tumor architectural structure can be obtained (Petersen *et al.* 1992, Sutherland *et al.* 1986, Pampalini *et al.* 2007, Hutcmatcher *et al.* 2009, Loessener *et al.* 2010).

2. Scaffold production techniques used in tumor models

A number of physical features such as porosity, homogeneity, adequate mechanical strength that scaffolds need to possess pose differences depending on biomaterial production methods. In Table 1 polymers, production methods, cancer drugs and cell lines have been demonstrated.

Production methods and selected materials are significantly important steps because the preparation of the material that has similar features to targeted tissue depends on the preferred technological infrastructure of fabrication method and its success (Mota *et al.* 2015, Chen *et al.* 2012, Gualandi 2011, Fisher *et al.* 2006). In this section, in order to create various cancer models, scaffold production methods used in cancer tissue engineering applications are investigated.

Table 1 Different production method/polymer/cell types used to form *in vitro* tumor models

Scaffold type or production technique	Polymer	Tumor	Cell Line	Drug	Ref
Microsphere	Dextran, Sephadex G-50	Colon	HCT-116 and HepG2	5-Fluorouracil and 6-Bromoindirubin-3'-oxime	Skardal *et al.* (2015)
Microsphere	Polystyrene	Breast	MCF-7	-	Yang and Burg *et al.* (2015)
Microsphere	PLGA/PLA	Breast	MCF-7	-	Sahoo *et al.* (2005)
Electrospinning	(PCL)	Ewing sarcoma	TC-71	Doxorubicin	Fong *et al.* (2013)
Electrospinning	(PGA-TMC) /Gelatin	Pancreas	CD24+ CD44+	Oxaliplatin-gemcitabine	He *et al.* (2013)
Solvent-casting and particulate-leaching	PLGA	Oral	OSCC-3	LY294002	Fischbach *et al.* (2007)
Sphere-templated technique	Poly(2-hydroxyethyl methacrylate)	Prostate	LNCaP C4-2 and M12	-	Long *et al.* (2013)
Freeze drying	Chitosan/alginate	Brain	Human (U-87MG &U-118 MG)animal (C6)	-	Kievit *et al.* (2010)
Freeze drying	Chitosan	Breast	MCF-7	Tamoxifen	Dhiman *et al.* (2005)
Gas foaming particulate-leaching	(PLGA)	Breast	MDA-MB231	-	Pathi *et al.* (2011)
Solid Freeform Fabrication (SFF)	Polystyrene	Lymphoma	HBL2 Z138	-	Caicedo *et al.* (2011)

PLGA; poly(D,L-lactic-co-glycolide), PLA; poly(lactide), PCL; poly (ε-caprolactone), PGA-TMC; poly(glycolide-co-trimethylene carbonate).

2.1 Microsphere production method

Microspheres are micro carriers which are between 1-1000 μm micrometers. Microspheres or microparticles have a wide variety of applications that range from medical field to biochemical sciences (Kataria *et al.* 2011). Microspheres were used as cell carrying scaffolds for three dimensional growth of cancer cells (Sahoo *et al*. 2005). In terms of biomedical applications, many types of drugs are included in microspheres for instance small molecules, proteins, and nucleic acids and are applied easily using a syringe needle. Their features such as biocompatibility, bioavailability and sustained release are some of their advantages. Microspheres can be porous or non-porous, also be biodegradable in body or not (Kim *et al.* 2006). Microsphere production technique should be selected considering parameters such as polymer type, medication (protein, peptide, etc.) treatment time and usage purpose (Saralidze *et al.* 2010). The selected method must not influence stability and biological activity of the drug negatively or change it during processing time. Also, the microspheres must be of desired size and medication loading ratio must be high. While microspheres exhibit free flowing, aggregation or sticking must not be allowed and final product must not be allowed to possess toxic matter or remains of it (Saralidze *et al.* 2010, Tiwari and Verma 2011, Padmanabhan and Kyriakides 2015). Several techniques of producing microspheres have been reported in published literature, such as solvent diffusion, spray drying, spray congealing, coacervation phase separation, polymerization and emulsion solvent evaporation (Tiwari and Verma 2011).

2.2 Electrospinning

Various polymers such as natural, synthetic, and hybrid substances are used to obtain ultrafine fibers. Cell attachment, drug loading, and mass transfer properties can be improved by the high

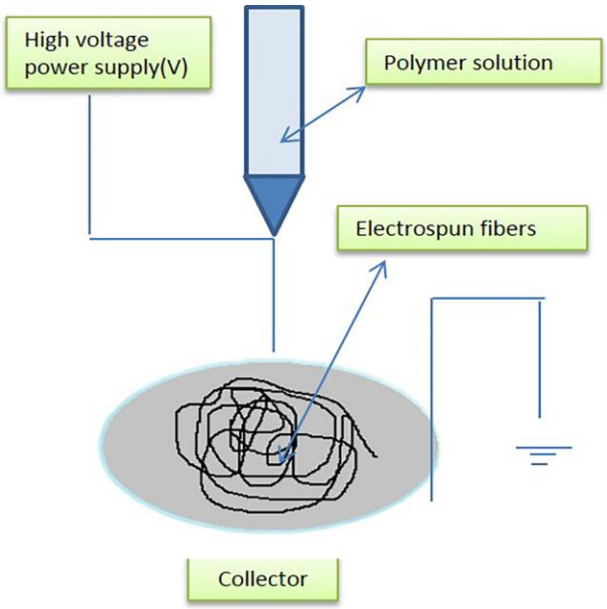

Fig. 2 Schematic illustration of electrospinning

surface to volume ratio of electrospun fibers. A wide variety of drugs for example, from proteins to antibiotics and anticancer agents, DNA, RNA, living cells, and various growth factors have been integrated into electrospun fibers (Hu *et al.* 2014). A distinguishing characteristic of cancer tissue engineering concerns the use of 3D scaffolds as they support adhesion dependent cells and allow cells to grow and differentiate in 3D structure, representing more *in vivo*-like conditions (Skardal *et al.* 2010).

In electrospinning method, polymer solution is kept in a syringe. When high voltage is released on polymeric solution, the droplet is expected to form cone and later spinning procedure is expected to take place (Hasan *et al.* 2014). On Fig. 2 electrospinning mechanism is exhibited. In this system, solution drop is influenced by two different force groups: electrical forces that move the drop and spin, and surface tension force that stops the drop from falling. When electrical forces deal with surface tension forces, polymeric solution is spun and the product is gathered in the form of nanofibers in accumulation panel (Fong *et al.* 2013, He *et al.* 2013).

2.3 Solvent-casting and particulate-leaching

With solvent-casting and particulate-leaching methods, it is possible to produce scaffolds with controlled by pore size. This method enables production without particular equipment; however, it has the disadvantage of not being able to select the size of particles required to gather materials with high porosity while sustaining sufficient mechanical properties and also producing a thick material as leaching out the particles from a large volume is demanding (Janik and Marzec 2015, Ma 2004).

This technique involves producing a polymer in solution and adding porogen particles of a specific diameter to produce a uniform suspension. Porogens are not soluble in polymer solution (Sachlos and Czernuszka 2003, Loh and Choong 2013). Later this solution is poured on to a non-sticking surface and the solvent is extracted thus a hard polymeric substance in membrane form is obtained. When this structure interacts with any solvent in which porogen can be solved, porogen leaves the structure (Fig. 3). The composite can be immersed in water to dissolve salt and sugar. For paraffin, hexane is preferred (Chern *et al.* 2013). However, there are difficulties in the removal of the porogens because of inadequate interconnectivity. Olah *et al*. prepared porous PCL scaffolds by solvent casting and particulate leaching technique with NaCl as the porogen. If the porogen content was 33%, the interconnectivity was not assured and there was residual salt in the scaffold. If the salt content was higher than 33%, the interconnectivity of the pores were efficient, which provided the fully removal of the porogen from the final product (Olah *et al.* 2006).

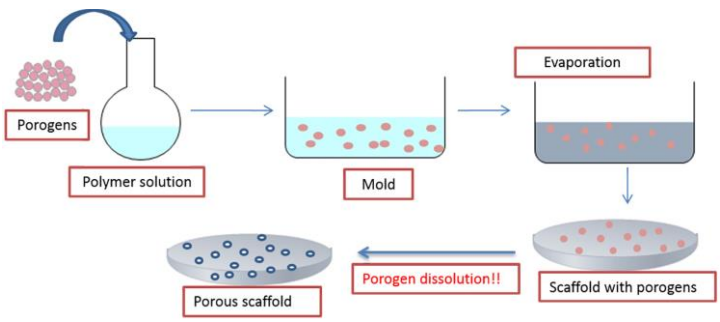

Fig. 3 Schematic illustration of solvent-casting and particulate-leaching

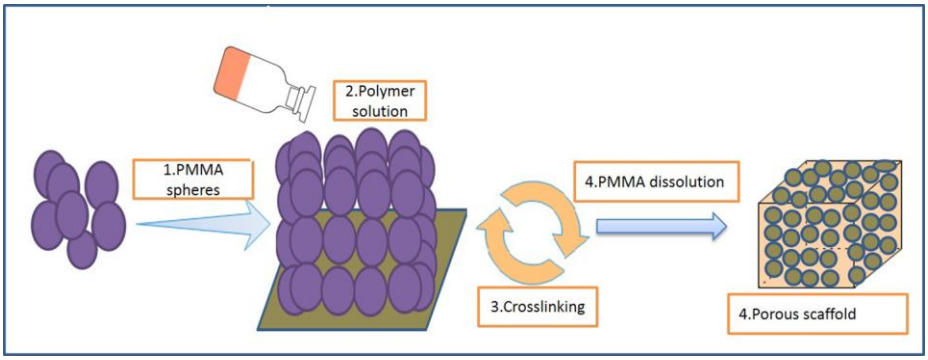

Fig. 4 Schematic illustration of sphere templated production

2.4 Sphere-templated production

In this method, various spheral structures are lined up and a three dimensional cavernous structure is obtained. Following heat treatment, spheres are bound to each other on connection points. Then, polymer solution is poured to sphere-templated three dimensional material. After polymers are cross-linked with different methods, this composite structure is cleared off micro-spheres with the help of a solvent that spheres can dissolve but the polymer structure cannot. Fig. 4 exhibits sphere-templated production set up. In this way, porous structure is obtained. Furthermore, in this situation, pore diameters can be adjusted using spheres. Poly (methyl methacrylate) (PMMA) spheres is commonly used to obtain 3D structure in literature (Long *et al.* 2013).

2.5 Freeze drying

Freeze drying is another technique to fabricate porous scaffolds. This technique uses the principle of sublimation as a basis (Alizadeh *et al.* 2013). As a first step, polymer is dissolved in a solvent. Later, the solution is frozen and solvent is discharged by lyophilization under the high vacuum that fabricates the scaffold with high porosity and interconnectivity. The freezing rate controls pore size; smaller pores can be produced by faster freezing rates (Offeddu *et al.* 2015). To create a homogenous 3D pore structure, controlled solidification in a single direction has been used. Alizadeh *et al*. dealt with obtaining homogeneous pore structure. Gelatin/chitosan scaffolds

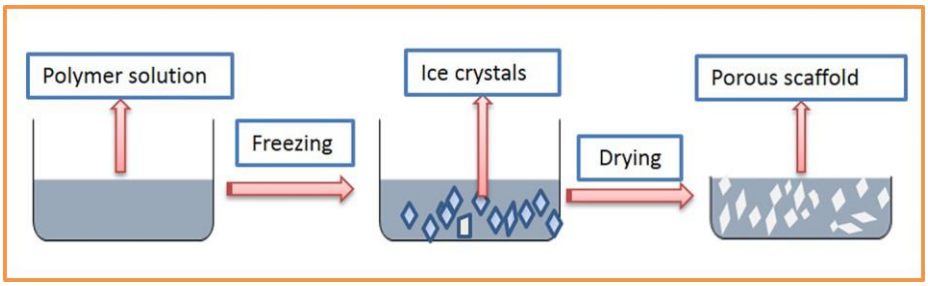

Fig. 5 Schematic illustration of freeze drying technique

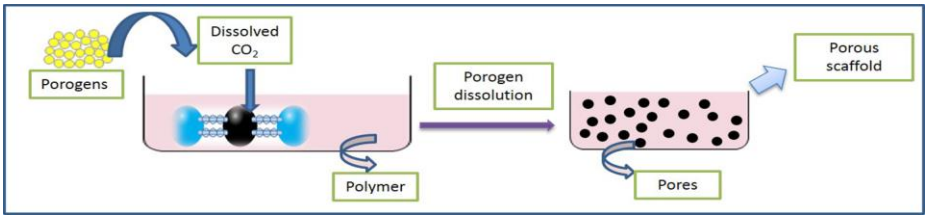

Fig. 6 Schematic illustration of gas foaming- particulate leaching

were prepared by freeze-drying in combination with particulate leaching. The incorporation of the particulate leaching method enhanced pore orientation and homogeneity in pore size (Alizadeh *et al.* 2013). One of the biggest advantage of freeze drying technique is that, it does not require high temperature or separate leaching step. Nevertheless, the disadvantage of this technique is long processing time and smaller pore size. A schematic diagram for scaffold fabrication by freeze drying technique is demonstrated in Fig. 5.

2.6 Gas foaming - particulate-leaching

In gas foaming-particulate leaching method, high pressure carbon dioxide gas is used for the fabrication of highly porous scaffolds (Fig. 6). The amount of gas dissolved in the polymer affects the porosity and porous structure of these scaffolds. The polymer is saturated with carbon dioxide at high pressure. As a result, dissolved carbon dioxide becomes unstable. Pore nucleation is created. The significant expansion of polymeric volume and decrease in polymeric density is caused by these pores. After completion of foaming procedure, a three dimensional porous structure is formed.

Sugar, salt and wax can be used to control the porosity of the scaffolds. In foaming process, the polymer fuses around the porogen to create a continuous polymeric matrix, and also entraps any other molecule which is present in the mixture. Polymer and porogen mixture are exposed to high pressure until they are saturated with carbon dioxide. Followed by foaming process, porogen is removed and a highly interconnected pore structure is obtained (Kundu and Kundu 2013).

2.7 Solid freeform fabrication (SFF)

Scaffolds have been produced by using different conventional methods for tissue engineering applications such as, solvent casting, particulate leaching, gas foaming, fiber meshes/fiber bonding, phase separation, melt molding, emulsion freeze drying, solution casting, and freeze drying. Although conventional scaffold production techniques are relatively simple, these methods do not enable to control the size, shape, distribution, or interconnectivity of pores. Furthermore, to dissolve the synthetic polymers, organic solvents have been used such as chloroform or methylene chloride for the conventional techniques. The disadvantage of these organic solvents for the conventional production techniques is toxicity.

Thus, many researchers agree that conventional scaffold production techniques have limitations and there is a need for advanced scaffold fabrication methods to overcome the limitations of conventional methods. Solid freeform fabrication (SFF) is an alternative technique to produce scaffolds with very fine structures and complex geometries using computer-aided design (CAD) data acquired from medical images of patients (Seol *et al.* 2012, Leong *et al.* 2003, Liu *et al.* 2007,

Hutchmer *et al.* 2004). This method controls the scaffold design parameters, such as the size, shape, distribution, and interconnectivity of pores (Leong *et al.* 2003).

In solid free fabrications, scaffolds are produced using data transferred from the computer such as magnetic resonance imaging (MRI), computer-aided design (CAD) and computed tomography (CT) (Chern *et al.* 2013). Scaffold formatting is made considering section geometry. SFF can be categorized into stereolithography (SL), fused deposition modeling (FDM), and selective laser sintering (SLS) (Seol *et al.* 2012, Hollister 2005, Morissette and Lewis 2000).

3. Different tumor models with polymeric scaffolds

Today, a number of 3D experimental models have been developed for the purpose of investigating the responsible mechanisms in formation of many cancer types. In this section bone, breast, oral, prostate and brain tumor models produced by using different scaffolds is demonstrated.

3.1 Breast tumor model

Yang *et al*. have observed tumor formation by creating various systems for breast cancer applications. In this study, they established heterocellular tumour spheroids (HTSs) an in vitro tissue engineered model: i.e., a tissue test system that combined heterocellular tumour spheroids, polymeric microcarriers and adipocytes, an abundant stromal cell type in breast tissue, to investigate the behaviour of breast cancer cells in response to different environmental stimuli in a more relevant 3D microenvironment. Microscope screenings, Western blot and Gelatin Zymography analysis results suggest that adipocyte existence influences the tendencies of cancer cells (Yang and Burg *et al.* 2015). In these systems, effects of adipocytes and polymeric microcarriers on the differentiation of breast tumor cells (MCF-7) have been investigated. The results of their study revealed that the engineered microenvironment could affect breast cancer cell proliferation, differentiation and migration. Multi-cellular interactions and changes in micro-environmental stiffness are factors that lead to this situation (Yang and Burg *et al.* 2015).

In their study, Sahoo *et al*. suggested a method of fabrication of large porous micro-particles using PLGA/PLA polymers which can be used as a scaffold for cell growth. Modifying the solvent evaporation method, porous PLGA/PLA microparticles were formed. Microparticles which include hydrophilic polymers were also integrated in their matrix structure. PLA microparticles with poly (vinyl alcohol) (PVA) in the matrix structure (PLA-PVA) treated with serum before cell seeding exhibited more successful cell adhesion and growth than other formulations of microparticles. Their results showed that PVA incorporated in the internal matrix structure of micro-particles plays an important role for cell adhesion and growth. MCF-7 cells were shown to grow into a tissue-like structure on micro-particles in about 5 days post-seeding (Sahoo *et al.* 2005).

3.2 Ewing sarcoma tumor model

In their study, Fong *et al*. have created porous scaffolds using electrospinning method and poly (Ɛ-caprolactone) (PCL) polymer. Scaffold was incubated with cancer cells (TC-71) and created 3D cancer model was compared to 2D model. The effect of produced micro environment on cancer

cells was investigated. In terms of morphological and biochemical features, experiment results of 3D in vitro models were closer to animal models experiment results compared to traditional 2D models (Fong *et al.* 2013).

3.3 Pancreas tumor model

In their study, the purpose of He *et al.* was to tissue-engineer a pancreatic cancer model that could readily cultivate a pancreatic tumor by using electrospun scaffold of poly (glycolide-co-trimethylene carbonate) and gelatin. The scaffold supported *in vitro* tumorigenesis of cancer stem cells for up to 7 days without inducing apoptosis. Moreover, the scaffold turned into a native-like mature pancreatic tumor in 8 weeks *in vivo* and it exhibited accelerated tumorigenesis as well as a higher incidence of tumor formation than the traditional model. Use of cancer stem cells together with a well-defined scaffold greatly reduces the variability associated with the traditional model, which uses a heterogeneous tumor cell population and poorly defined Matrigel. The scaffold model was a platform for investigating the antitumorigenesis mechanism of novel chemotherapeutic drugs with a special focus on cancer stem cells (He *et al.* 2013).

3.4 Oral tumor model

In oral cancer research, 3D artificial tumor model has been created in *in vitro* environment by using scaffold produced by solvent-casting and particulate leaching method. In this study, poly (lactide-co-glycolide) (PLGA) polymer was used and cancer cells were used to develop artificial model. Fischbach *et al.* have evaluated tumor models' angiogenic capacity created by measuring Vascular endothelial growth factor, interleukin-8 and basic fibroblast growth factors (VEGF, IL-8 and bFGF). In this study, the growth factors of 2D, 3D artificial or *in vitro* model and *in vivo* model were investigated to compare the models. The measured rates of growth factors have been observed to be close to 3D artificial model and *in vivo* model. Also, tumor models developed in 2D and 3D systems were later implanted into *in vivo* environment and at the end of determined procedures, tumor volumes and weights were measured. The experiment results indicate that in animal group 3D tumor model was implanted, tumor growth (volume and growth) was observed to be higher (Fischbach *et al.* 2007).

3.5 Prostate tumor model

As well as its being one of the most common cancer types among men, prostate cancer is also the second most fatal cancer type. Among men, one in every six have been encountered to have prostate cancer case. Long *et al.* have created an in vitro 3D tumor model using scaffold of poly 2-hydroxyethyl methacrylate (pHEMA) polymer produced by sphere template technique. Prostate cancer cell lines were added on to scaffold. Proliferation of prostate cancer cells within the 3D scaffold was demostrated quantitatively by a PicoGreen DNA assay. The sphere-templated polymeric scaffold system and the techniques developed in these studies were proposed to be applied in the emerging field of tissue-engineered biomaterial based cancer models (Long *et al.* 2013).

3.6 Brain tumor model

Despite numerous researches in cancer treatment and developed treatments, only 17 to 43 % of the patients with brain tumor (glioma) have been observed not to live more than 2 years after diagnosis (Mirimanoff *et al.* 2006). In order to create this fatal tumor in *in vitro* environment, Kievit *et al*. have produced 3D tissue scaffolds and developed an artificial model using chitosan and alginate because traditionally used 2D models are insufficient in imitating the tumor structure (Kievit *et al.* 2010). In this study, chitosan/alginate scaffolds were created by freeze-drying technique and later crosslinked with $CaCl_2$ solution (Li *et al.* 2005). Three-dimensional (3D) culture systems are projected to bridge the gap between *in vitro* and *in vivo* cancer models (Leong *et al.* 2003). Multi-cell globules (spheroids), gel-embedded systems and tissue engineering scaffolds mimick the structure of the tumor microenvironment (Kievit *et al.* 2010, Faute *et al.* 2002, Kenny *et al.* 2007).

4. Conclusions

The use of polymeric scaffolds for to develop *in vitro* tumor models is another method to understand cancer mechanism. Although currently there is only small number of polymers and scaffold production methods used to obtain 3D tumor models, different models that are ranging from ewing sarcoma to brain cancer models have been utilized. Basically, 2D both monolayer culture models have limitations to investigate the mechanism of cancer biology. In contrast to these models, the conditions of 3D tumor models can be modified in many ways. Although, there are few studies about *in vitro* tumor models created with scaffolds, the results of the experiments are promising.

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