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Review

Advancement, applications, and future directions of 3D models in breast cancer research: a comprehensive review

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Abstract

3D models have popped up as indispensable tools for breast cancer study, they provide a closer semblance of the multiplex cellular and cancer tissue microenvironment as compared to ancient 2D cultures. Their utilization in BC research permits a better interpretation of hemostasis, cell-to-cell, and cell-to-extracellular matrix interactions, differentiation of cells, and tissue organization. 3D models qualify the exploration of numerous aspects regarding cancer progression, it also includes invasion of the tumor, cancer metastasis, and drug resistance, in a way that more precisely contemplates in vivo conditions. Hence, they provided a precise environment for research as compared to a complex in vivo host cell environment. This review highlights the importance of different 3D models in BC research, focusing on their capability to enumerate complex disease physio-pathological features. This review explains the variety of 3D models utilized in BC research, encompassing Multicellular Tumor Spheroids (MCTS), Three-Dimensional (3D) bioprinting, Organoid Models, Microfluidic technologies, Organ on chip models, 3D hydrogel models and in silico approaches for BC, challenges and future of 3D models.

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Introduction: Breast cancer belongs to a heterogeneous disorder, which has diverse entities with definite biological and clinical aspects [1]. It is a metastatic cancer that can be transferred to other organs like respiratory organs, bones, liver, and brain, which is mainly described for its incurability. Conformation of this disease at preliminary stages might be helpful and enhance the survival rate [2]. Breast cancer in women causes a significant health burden worldwide. It has become one of the most familiar sources of cancer in high and low-resource settings and is to blame for over a million out of ten million neoplasms recognized all over the world every year in both genders. In 2012, 1.7 million cases of BC were reported by GLOBOCAN and caused 522,000 deaths due to BC [3]. The process of cancer initiation and its continuation is still not clear. Cancer is a complex network of diseases which differ from person to person due to heterogeneity between the cells in the tumor. So, there is a need to develop clinical models to determine the biological process of cancer and drug efficacy in tumors. In-vivo techniques are used for pre-clinical studies of drug development based on in vivo cytotoxicity in 2D models, which fails to elaborate the tumor response against the drug in the body [4]. Three-dimensional (3D) models play a crucial role in basic and translational research, screening of drugs as well as cancer prediction [5]. Mostly, cancer research depends on experiments using 2D cultures of cells in vitro. Although, there are numerous limitations of two- dimensional cultures, for example hurdles in the interaction between the cellular and extracellular environment, morphological changes, division methods, and polarity. So, three-dimensional cultures are better to mimic the tumor environment in vivo [6]. Three-dimensional models are becoming more reliable because they provide a platform for analyzing cell-to-cell and cell-to-material interaction. 3D models play a remarkable role in mimicking TME for the initiation of treatment strategies and analysis of the mechanism of tumor formation, tumor growth, and metastasis. The results of 3D in-vitro models show good association with the in vivo studies as well as clinical results [4]. In this review, different three-dimensional (3D) models like Multicellular Tumor Spheroids (MCTS), Three-Dimensional (3D) bioprinting, Organoid Models, Microfluidic technologies, Organ chip models, 3D hydrogel models and in silico approaches explained for breast cancer. This review will also explain the challenges faced by these 3D models in BC research. The prospects of available 3D models in BC research in the future.

Multicellular Tumor Spheroids (MCTS) for BC: Monolayer cultures are extensively used in cancer research to examine cell growth regulation and death. However, compared with in vivo tumors, monolayer cultures are more prone to the cytotoxic effects of xenobiotics because they do not have the microenvironmental characteristics and cellular activity that occur in solids. Therefore, the 3D culture of multicellular spherical tumors (MCTS) has been introduced as a remarkable model to make more complete tumor assessments for treatment strategies [7]. MCTS has been used as a model for many experimental therapeutic studies, involving radiotherapy, chemotherapy, radioimmunotherapy, antibody and cell-based immunotherapy, hyperthermia, gene therapy, and photodynamic therapy. It has also been widely utilized in basic research proliferation regulations, vitality, energy and nutrient metabolism, intercellular interactivity, and extracellular matrix composition in the microenvironment [8]. Tumor spheroids with a defined number of cells are a good model for immunotherapy. Heterotypic spheroid has been applied to interrogate cell invasion of tumor and metastasis through examining the tumor cell and fibroblasts interaction, EC, and epithelial cells [9]. MCTS is the most typical organ-type cancer model. MCTS is manufactured by cells of tumor only or tumor cells combined with other types of scaffold or non-scaffold cells. MCTS is an efficient model because it mimics the peculiarity of tumor cells in vivo in terms of developmental kinetics, heterogeneity of cells, signal transduction path activity, and expression of genes. The MCTS model plays a significant role in culturing cancerous cells as well as in studying inflammatory breast cancer.

MCTS manufacturing techniques: Spheroids are developed through various scaffold and non-scaffold techniques. On-top and embedded matrix. Matrix encapsulation, bioreactors, and Micropatterned plates are scaffold-based techniques, and non-adherent surfaces, hanging drop and Magnetic levitation are free techniques that are utilized for the manufacturing of spheroids [10]. Each technique has some benefits as well as limitations [11].

In scaffold based MCT engineering, different matrix of extracellular biomaterials is used like hydrogel for the printing of cells which plays a vital role in the cell's growth, propagation, and its interaction in three dimensions (3D). Some hydrogels are designed to adopt a tissue engineering process. These include alginate, Gelatin, Gelatin Methacrylate, Collagen, Hyaluronic Acid, Collagen, Extra cell fiber, PEG [12]. Tissue engineering via scaffold has a vital end in the reconstruction of numerous tissues and organs and, in some instances, has been further facilitating clinical applications. Although, it shows promising results but still faces numerous challenges like biomaterial choice, rate of degradation, immune response, formation of fibrous tissue caused by degradation of scaffold, and mismatch of tissues are the most important obstacles that may affect its prime biological function. To overcome these issues many techniques have been established to manufacture scaffold-free tissues. Tissue engineering via scaffold-free methods contributes custom- shaped tissues with high throughput and trouble-free manner [13]. Tissue engineering through scaffold-free MCTs depends upon the use of prefabricated multicellular constituents as cell sheets as well as spheroids. It has a great competence to fuse into huge compatible structures which leads to the establishment of extracellular matrix and is considered a supreme accredit of without scaffold technique [14].

Scaffold-Based MCTS developing techniques

On-top and embedded matrix techniques: Cells are seeded either on the matrix surface or with a liquid matrix so that the cells are ingrained in the matrix after gelation. In these two methods, firstly wells of the tissue culture plate are coated with a matrix layer, like Matrigel, methyl cellulose, etc. The cooled Matrigel was applied to the pre-chilled well surface and gelled at 37˚C. On the other hand, use a hot micropipette tip to dispense 1% hot solution of agarose into the well, which thickens at room temperature. While in the case of the on-top matrix technique, cells are seeded into the wells to form a single-cell suspension. Then shake gently during incubation at 37˚C [15,16]. In the matrix-embedded process, cells suspended in a chilled liquid matrix are distributed into pre-

coated matrix wells and incubated at 37°C. During this time, the cells are incubated in the matrix after gelation [11].

Matrix Encapsulated tumor spheroids: The encapsulation of matrix is successfully done by microfluidic devices and hydrogels. In the case of hydrogel microfiber development based on the laminar flow-based different phase coordinated flow system. In ancient times hydrogel-based microfibers developed via spinning methods. The module of the hollow and solid fiber can be controlled through different flattest [17]. Although different designs of microfluidic devices with the same basic principle can be used, cell suspension droplets are encapsulated in a hydrogel shell that forms microcapsules. The middle capillary contains calcium free solution while the outer capillary tube contains solution of hydrogel [18]. When the suspended cells are mixed with the hydrogel drops in the calcium bath, it forms cell microcapsules through gelation. The tumor cells in the microcapsules aggregate to form a matrix-encapsulated sphere. Solution without any calcium behaves like a barrier to prevent the diffusion of calcium within the cell. On the hydrogel contained in the outermost tube, if it gels prematurely, it may flood the equipment. Prefabricated hydrogels reduce the risk of destruction of microfluidic devices because they do not need calcium for gelation [19]. For encapsulation, peptide solution is thoroughly blended with cellular suspension which is followed by gelation at physical temperature. One of the major advantages of this method is the uniform fabrication and yield of homogenous spheroids [20].

Bioreactors for Tissue Engineering: Bioreactors are usually used to produce tumor spheroids at large scale. In this approach, cells are grown in multicellular spherical bulk form. Two types of bioreactors are used for tissue engineering, spinner and rotating flasks [21]. The spinner flask is used to grow cells in a liquid medium as a suspension culture. Essentially, these are stirred bioreactors in which a paddle wheel mixture keeps the cells in suspension. The stirrer ensures the equal distribution of oxygen as well as nutrients. It is believed that the fluid movement facilitates the mass transportation of nutrients and unwanted materials from the spheroids. Also observed that the cells remain non-contacted with the substrate. Spinning flask culture technique is extensively used for several multicellular tumor spheroids cultures. The Stationary scaffolds are placed in a suspended flask, and the cells move on the surface of the scaffold via mobile liquid. Shear forces develop due to continuous movement of stirrer which may have adverse effect on the physiology of cells. Other methods have been used, such as roller tubes and rotary vibrators, with different levels of success. In the gyratory rotation technique, the cell suspension is placed in an Erlenmeyer flask containing a particular medium amount. Then the flask is rotated in the rotating incubator till spheroids of the desired size are obtained. Although the static medium of the liquid overlay culture can be used to study a single sphere, the rotating flask can grow more spheres in a dynamic suspension. Preferably, a 96-well plate is used to monitor the growth and co-cultivation of individual spheres [22]. There are many advantages of using a spinner flask its use is very simple, it gives massive production, and culture for a long time, culture conditions can be controlled dynamically, and different cell lines can be cultured at the same time. But this technique has some special equipment and faces problems due to shear forces [9].

Micro- patterning plates for tumor spheroids Micropattern plates are used to produce a huge number of spheroids with uniform dimensions which makes them supreme for high-level screening. In this protocol, in the first step the glass plate is coated with 3-trimethoxysilyl polymethacrylate (TMS-PA), and then coated with a uniform hydrogel layer, like polyethylene glycol dimethyl methacrylate Acrylate (PEGDMA). Polydimethylsiloxane (PDMS) is printed with microparticles and crossed with PEGDMA light to form a microwell. Pre-coating with TMS-PA assures covalently attachment between the hydrogel microwell and the glass plate [23]. In the direct photographic structure, ultraviolet radiation (360-480nm, 800 mW for 50s) will generate reactive species of oxygen, which leads to the separation of the protein-repellent part of the molecule grafted on the substrate, and the ECM protein binds to the substrate more firmly [24]. The benefit of direct light patterning is that it does not need etching, such as micro-contact printing. The micropatterned hydrogel can be used as an efficient and multifunctional biomaterial platform, which is very suitable for many biomedical applications, like muscle scaffolds and bio electrode material [25].

Scaffold-free MCTS developing techniques

Ultra- Low attachment microplates: 96- Well ultra-low fixed U-bottom microplates are used to develop and analyze the three-dimensional tumor spheroids. These plates are processed with a neutrally charged hydrophilic coating covalently bonded to the surface of the polystyrene pores, which facilitates the growth of rigid spheroids, dense aggregates, or loose aggregates in different tumor cell lines of humans. According to the total number of cells separated from the single-cell suspension, transfer 1000 cells/ml to the ultra-low fixation plate/bottle. Incubate the cells with 5% CO2 at 37°C. culture. The tumor spheres formed in the 96-well U-shaped bottom ULA plate have a morphology similarity to that of agar growth spheroids and immunohistochemical staining, which can be used to analyze tumor cell migration and invasion capabilities [26].

Hanging Drop method: Hanging drop technique is utilized for the cultivation of MCTs in a reproducible way. This method can be applied to many cell lines, avoiding possible material artifacts or interference from uncontrolled mechanical forces, and open new possibilities for co-cultivating MCTS from mixed cell populations. Polystyrene is used to build array plates and manufacture by injection molding. To control the pitfall of the conventional hanging drop process in the handling of liquid and inversion of the substrate, each cell culture site has an opening (1.6mm diameter) that can pass through the substrate from the bottom platform (3mm diameter and 0.5mm high). The cell culture sites are organized in 384- well plate [27]. The cells in the suspension aggregate spontaneously to form spheres under the action of gravity. The petri dish has phosphate-buffered saline (PBS) to prevent the droplets from drying out. Currently array plates are replaced with petri dishes [28]. Definite-sized Spheroids can be cultivated through this technique. It is one of the most time-consuming ways to grow MCTS. Another disadvantage of this method is that it affects cell viability, which is due to the high osmotic pressure caused by the evaporation of the medium from the droplets. To avoid rapid evaporation of the medium, a relatively large number of droplets (e.g., 15-30 µl) should be sprayed. However, this

limits the number of ink droplets. A sphere can be made that can be obtained in a specific area [29].

Magnetic Levitation Technique: This technique is used to manufacture the 3D cell culture. This technique uses superparamagnetic iron oxide nanoparticles as a modeling agent to guide cells to self-organize into spheres under the influence of magnetic force. Magnetic nanoparticle assemblage is used to encourage magnetic nanoparticle delivery; due to this property it is widely applicable to a variety of cells [30]. Trypsinize the cells are incubated along SPION overnight to ensure that they are taken up by the cells. Centrifuge the culture remove the supernatant and place the cell again in a fresh medium. Calculate the number of cells via a hemacytometer. A certain number of cells are seeded to grow the spheroids in the plate. During magnetic levitation place the lid insert, the lid of the well plate, and the magnetic drive. Place the cells at 37°C, 5% CO_2 and incubate for a few hours, and the sphere will begin to form. Depending on the size of the beads required, incubate the cells for 1 to 5 days. The cells labeled with SPION are pushed up and down by the force of a magnet. The aggregation of cells into spheres occurs within a few hours [31].

Three- Dimensional Bioprinting for BC: 3D bioprinting allows precise control of the matrix structure and achieves optimal performance consistent with improved cell performance [32]. 3D bioprinting provides a precise composition of the environment of the tumor with highly arranged spatial cell distribution and ECM other tumor which positively increases the self-assembling properties as well as functionality of tumor models. Specific biomaterials are used to build an extracellular matrix that supplies circumstances for the adhesion of cells, development, accretion of cells, relocation, and differentiation of cells [33].

3D Bio printing Approaches for BC: Inkjet printing, laser-assisted printing, and Extrusion- based printing are major technologies of Three- Dimensional Bioprinting. Inkjet bioprinting is also known as a drop-on-demand inkjet printer. It is a non-contact technique that applies droplet (picolitre) of biological material to a substrate to build two- dimensional and three-dimensional structures. Piezoelectric, thermal, and acoustic nozzles are different types of inkjet printers that are utilized for the production of droplets [34]. It has different ink chambers along different nozzles. The stator culture must be liquefied before printing so that the droplets can settle on the solid platform. During printing, thermal, acoustic, or piezoelectric actuators continuously pump a fixed volume of liquid onto the platform through the platform of a drop of ink. Before adding the next layer of droplets, the droplets must solidify into a predetermined geometric shape. The size of the droplet can be adjusted from 1 to 300 µl, and the deposition rate is 1 to 10,000 drops per second [35]. Laser-assisted printing system relay on laser-induced forward transfer (LIFT) and permits organic and inorganic printing with micron resolution. It consists of three sections, the first section consists of the pulsed laser source, the second section is composed of the target for printing biological material and the last section has a receptor substrate for capturing printed material [36]. It consists of two horizontal glass slides (co-planner). The upper donor slide is laminate with fine layer of absorbing layer, while the second layer consists of a thick layer of bio-ink for printing. This bio-ink is normally a sol (non-gel hydrogel precursor) with immersed cells [37]. Laser bioprinting (LAB) uses laser energy to volatilize the sacrificial layer and move the payload to the receptor substrate (nozzle-free bioprinting) [38]. Laser energy is intense on the donor glass slide which has laser-absorbing material that evaporates and deposits the biotin in the donor glass slide onto the target substrate. Its spatial resolution is usually high, and it has the ability to distribute some volume and precisely describe the geometry, but this is a slow process [39]. Extrusion-based 3D printing is a fast, paste-based prototyping method that permits the building of complex 3D structures. It enables effective and controllable printing of cell structures under physiological conditions. It allows the extrusion of solutions with higher viscosity, hydrogels, and suspension of colloidal [40]. It uses multiple nozzles to produce material under difficult conditions that may damage the cells. Two kinds of inks can be printed individually and then combined to create a core structure, where the core can be ceramic, such as alginate hydrogel [38]. In this technique, a small quantity of cell suspension is distributed on the plate form in a controllable way via nozzle by forces (shear), so that the encapsulated biological material is accurately deposited in a cylindrical shape with the desired irregular three-dimensional structure in the filaments. Cell death may occur due to shear forces. The large viscoelasticity of bioin decreases the holding as well as affects the viability of cell. However, there is a need to further improve the techniques for bioprinting of cells [39].

Method of Bioprinting: Two types of bio-fabrication methods are used in the bioprinting of cells. In two-step bio fabrication, scaffolds are three-dimensionally printed prior to cell seeding. Digital projection printing based on micromirrors has been used to create 3-D polyethylene glycol (PEG) scaffolds with wooden stake microstructures [41]. The elasticity of the scaffold can be changed by changing the concentration of PEG which affects the stiffness of isolated and examined cells. To analyze the cellular migration patterns of epithelial cells the breast cells are seeded on the scaffold. As compared to the 2D culture, 3D scaffold-based cellular culture explains the change in displacement, velocity, and path alignment which depends upon the different factors of scaffold like stiffness and existence of twist oncogene. This helps in further analysis of cancerous cells. In one- step bio fabrication cells are seeded on prefabricated scaffolds. Hence, it has some shortcomings in managing cell density, replicability, spatial control, and scalability. It provides a more operative way to fabricate three-dimensional tissue models with minimum input energy. This technique enhances cancerous co-culture model fabrication for the analysis of cell-to-cell interaction in a systematic way. By knowing the regulatory relationship between cancerous cells and their microenvironment highly productive and definitive drug screening can be done [42].

Organoid Model for Breast Cancer: Organoids are 3D structures and can be manufactured in embryonic and induced pluripotent stem cells and tumor cells, particularly in three-dimensional culture systems. Mature stem cells are fixed in the 3D matrix; it initiates the self-organization into epithelia of the related organ of origin [43]. These small three-dimensional tissue manufacture in the laboratory and are structurally and functionally like native organs. It has three features,

It has numerous types of mimic cells in vivo.
The cell organization is like the original tissues.
It works specifically for the native organ.

It acts as a bridge between traditional 2D in vitro and in vivo models and enhances clinical applications [44]. Organoids are different from other cultures because they can proliferate untransformed epithelial cells related to the manufacturing of many differentiated types of cells of the appropriate epithelium, however, they are also playing an important role in critical control of the same lineage cells for qualified studies of neoplastic cells. Finally, organoids have huge potential for medical implementations, treatment selection, and personalized diagnostics [45]. A study was conducted in which human breast organoids were engineered via CRISPR-Cas9 for breast tumor modeling. Organoids were developed from epithelial subsets of the breast which are categorized from ordinary reduction mammoplasties depending upon expression of CD49f and EpCAM. Then it is employed to mimic neoplasia [46].

Formation of Breast Cancer Organoids: A three-dimensional culture system for the formation of organoids has Matrigel or basement membrane extract as a substitute for ECM and a special medium for culture. Dulbecco's improved Eagle's medium (ADMEM)/F12, penicillin or streptomycin, primocin, GlutaMAX, HEPES, B27, N2, EGF, FGF10, FGF7, hepatocyte growth factor (HGF), Wnt3A, Noggin, R-spondin-1, gastrin, prostaglandin E2, nicotinamide, neuregulin 1, N-acetylcysteine, Y27632 (a Rho kinase inhibitor), A-83- 01 (a transforming growth factor-beta inhibitor), and SB202190 (a p38 inhibitor) are the major components of culture medium of organoids. Mediums vary in components depending upon the type of organoids [47, 48]. A study was conducted in which Hans Clevers, *et al*. accentuated that Neuregulin 1 was important for better creation as well as long-lasting expansion for tumor organoids. Wnt3A was not necessary for culture. EGF is a two-fold sword: small amounts prevent proliferation while large amounts result in the damaging of organoids and a moderate loss of three-dimensional organization. A Higher concentration of SB202190 was effective for breast tumor organoids. The organoid breast cancer cell lines are compatible with the parent tumor in shape, histopathology, receptor status of the hormone, the status of human epidermal growth factor 2, mutational panorama, and CAN DNA [47]. The carcinoma organoid technique is also employed to inspect the complex interactivity between genetic changes and specific factors in the process of carcinogenesis. In short, cancer organoids having contrasted carcinogenic alterations represent specific dependence on niche elements. This contributes an efficient platform to analyze the relationship between genetic changes and cancerous microenvironment through carcinogenesis [49].

Organoid biobank for Breast cancer: The biobank of cancerous organoids is a depository of patient-derived tumor xenograft (PDTX) from various types and subtypes of cancer. In biobanks, organoids can be transferred and cryopreserved such as immortal cell lines [49]. Norman Sachs, *et al*. explain the biobank of breast cancer. Breast tissues are isolated either mechanically or enzymatically. Then these cells are planted on breast cancer organoid culture and the medium allow the efficient production of organoid along long-lasting expansion greater than 20 passages. Add ROCK (Rho-linked coiled-coil has protein kinase) to permit the proliferation of tumor epithelial cells for a longer time period [43].

Microfluidic technology for BC: Microfluidic models work in an efficient way to determine the highly complex phenomena occurring in the microenvironment under several manageable biochemical and biophysical factors. The formation of these features is technologically impossible with traditionally employed assay [50]. Microfluidic devices use channels in three-dimensional structures to enhance the area available to be laminated with antibodies. Cancer cells (captured from blood) utilized nickel-based micro-pillars immobilized functional super-magnetic beads to build a capture area inside microfluidic devices [51]. Henriette L. Lanz, *et al*. conducted a study in which they employed triple-negative cell lines (MDA-MB-453, MDA-MB-231 and HCC1937) of breast cancer as a model. The cell lines depend upon the BRCA1 and P53 genes which are the primary sources of breast cancer, the cell lines seeded in the microfluidic organo-plate. Microfluidic plate form permits culturing of 96 infused micro tissues by utilization of a limited amount of substrate. At last, they estimate the densities of the seed, the composition of the Extracellular matrix as well and their bio-mechanical situation [52]. A breast cancer model was developed in 2018 that imitates DCIS structure and allows a culture of multiple types of cells at a time. Cells of DCIS use metabolic pathways, and DCIS highly depend upon glycolysis to initiate their growth. TFG-β has an important role in breast cancer, it suppresses the tumor in the early stages while it enhances metastasis the in the later stage of breast cancer. Hence, the DCIS model used to find the probability of target cancerous cells depends upon the microenvironment [53]. However, microfluidic models provide a great plate form for breast cancer analysis in an efficient way.

Organ on a Chip Model for BC: Organ on a chip model is employed to analyze the circumstances in metastasis to get valuable facts about the determinants of the ailments [54]. Analysis of a full organ cannot be modeled yet on a chip, therefore, organ on a chip model is used to mimic the most essential functions of tissues or organ parts to meet the needs of specific applications and achieve hierarchical cells through careful design, structure, cell population and its dynamic microenvironment. To date, various tissues, as well as organs, have been conveniently customized to replicate the relative working subunits including the brain, heart, lung, intestine, liver, kidney, blood vessels, and musculoskeletal system [55].

Breast cells on chip platform: Breast cells are obtained from the epithelium lining duct with the width of 700 to 30 µm. Grafton *et al*. manufactured a breast cell on a chip platform in order to stimulate breast cancerous cells via lithography technique. They employed a monolayer of non-tumor breast epithelial cells to simulate duct channels. The outer surface of PDMS hemi-channels is laminated with laminin and membrane held near to the channel. They guided submicron superparamagnetic particles that have previously been shown to reach tumor cells in the breast. This research provides a platform for future breast cancer models [56].

Disease Modeling via Organ on a Chip Device: The physiological activities of the human body are supervised by the organ system to maintain homeostasis. In order to examine these systems under physiologically relevant conditions, the concept of the organ-on-chip platform was developed about two decades ago and has since been revised and updated [57]. The advent of this technology allows us to better understand the unregulated cellular response during the progress of diseases that alter homeostasis. However, many animal models are applied to deal with these shortcomings,

imitation of human feedback and interpretation of results show malfunction in various pathologies like a murine model. They are unable to recapitulate pathological and physiological responses in the human body. So, there is a need for more sophisticated models to imitate the pathology of organs and tissues in humans in a better way [58]. Tumor models on a chip can be used for preclinical drug screening because they are designed for high throughput testing of cancer drugs and other biological factors. Microfluidic MOC (multi-organ on chip) has multiplex tissues and organ models interconnected to the physiological sequence. It has multiple benefits to investigate the drug's pharmaceutical and pharmacodynamics along with metabolism and toxicity [59].

Three- Dimensional Hydrogel models for BC: Hydrogel is a three-dimensional polymeric matrix it gets expands with increased water content, which is related to the original Extra Cellular Matrix. Hydrogel is obtained from either natural source for example collagen, fibrin and HA (Hyaluronic acid) or from artificial manner for example Polyethylene glycol and PAAm. Both types of hydrogels are utilized for three-dimensional cellular mechanical microenvironment which directly affects cellular behavior like binding with other cells, cleavage region, progression, differentiation of cells as well as their migration [60]. Three dimensional models usually used collagen, Matrigel, and alginate for their construction. A mixture of more than one gel is prepared for gel composite to change the concentration, structure, and mechanical characteristics of gel, it has further effects on the adhesion of tumor cells and their emigration abilities [61]. Many hydrogels have been used to manufacture the three-dimensional cell culture. For culture development, hydrogels have mechanical energy to enhance cellular immobilization, tissue attachment as well as formation. Hydrogels must have large porous for transportation of oxygen and nutrients [62].

Cross-linking and printing ability of Hydrogel material for 3D printing: For three-dimensional printing, the physiochemical property of hydrogel is very crucial. The printability of hydrogel-based material depends upon the rheological characteristics and physiochemical cross-linking properties. The cross-linking mechanism can be done before or after printing in the physical method [63]. The viscosity of the polymer solution depends upon the hydrogel concentration. In order to increase the printing ability alginate solution, usually uses 3% alginate and 9% methylcellulose to build ink material. This material is valid for the three-dimensional printing and bioprinting process. The methylcellulose enhanced the viscosity of the solution. Methylcellulose excretes out of the scaffold and produces a structure with larger elasticity, porosity, and stability [64].

In- Silico Approaches for breast cancer: In silico approaches are widely used in breast cancer identification, characterization and mutated gene expression. Different bioinformatic platforms like CGAP (Cancer Genome Anatomy Project) and GEO (Gene Expression Omnibus) are used for functional analysis of the genome. Shen et al. used different bioinformatics tools to identify the cancerous gene of the breast. vNorthern (virtual Northern) SAGE digital gene expression displayers are utilized to check the expression of genes in benign and malignant tissues of breast [65]. The Cancer metastatic in silico model represents functionally similar mathematical models that include many features about progress of the disease. Computer models are much more versatile like cancerous cells. It includes statistical models, multi-scale models, continuous models, and discrete and agent-dependent models. These models elaborate on the different aspects of cancerous cells. Sometimes, a combination of in silico and in vitro models is utilized to predict feedback of different drugs 54. Different In silico models for cancer are shown in Figure 1. Continuous & agent-based models for interaction between microenvironment of tumor and tissues. Mathematical cancer models like molecular characteristics of abnormal genes and molecular signaling pathways and gene expression. Network models depict biochemical, metabolic & signal response networks which are essential for tumorigenesis. It includes the dynamic and constrained depend on methods to build such network.

In silico Tools for BC System Biology: Online databases allow the smooth retravel of high throughput information, which proved an efficient way to create different cancer models. This enables the researchers to investigate the diseases globally which was impossible in the past table no.1 [66].

Challenges & future perspective: Three-dimensional platforms provide simple, real and controlled conditions for the integration of cells and other factors like extracellular environment, development, and biochemical signals to stimulate the natural environment in a preferable manner which is highly supportive to growth as well as progression of tumor. They are also crucial in determining biological mechanisms or processes which result in abnormalities observed in cancerous cells. The formation of 3D engineered models involved the utilization of biomaterials like hydrogel and various types of cells as a co-culture, this allows the cells to adopt their natural form and obtain cell-to-cell as well as cell-to-ECM interactions. Bioprinting and microfluidics are reliable methods for manufacturing of three-dimensional in vitro models because they are capable of controlling biochemical components, cells & flow. Despite significant and regular success in developing three-dimensional models, some main problems and limitations are still related to existing models. Various models that used long-lasting 2D cultures of adapted cancerous cell lines, they do not show the accurate pathology of underlying disease. Various bioengineered models showed only morphological similarities with their natural environment. It is essential to restore the phenotypic resemblance and heterogeneity. When artificial matrixes are used, there is a lack of comprehensive studies of signal transmission in the microenvironment. In the future, there is a need to refine the physiological difficulties through innovative chemical materials and techniques, including the types of cells, matrix constitution and secular and spatial characterization of soluble factors. Eventually, as tumor models became more complex, computational models, biological techniques, real-time imaging, recognition and examining technologies are urgently needed [67]. Like, hydrogel precursor cross-linking in the presence of uniformly spread of cancerous cells which causes cells entrapment in the porous channels [68]. In order to develop a huge tumor- like assemblage, single state cells that are trapped at initial stage, must multiply inside the matrix and move through channel by amoeba or mesenchymal motion [69]. A quantitative understanding of combined motion of cancerous cells in 3D environment is needed. In vitro models lack the suitable media for all kinds of cells during co-culturing of cells [70]. This problem can be solved by spatially integrating essential

growth factors, cytokines and matrix metallopeptidases into tumor section that enhance the cells growth in each section. These constituents can be inactivated in hydrogels or enclosed in transporters to excrete them in a sustainable and controlled manner. The problem of 3D bioprinting in clinical applications like reproducibility and regularity of bioprinting structures and the use of biocompatible substrates to preserve complete molecular elements and cells, have not yet been resolved. Today, one of the main limitations of bioprinting is to make bioprinting more and more adaptable in terms of its ability to bio print various bio ink. The second main obstruction is that to get integrated vascular structures, that is crucial factor for the cells to proliferate and metastasis in cancerous cells. Another challenge is to develop appropriate technology to monitor the cell function in the structure in real time 3-D bioprinting creates opportunities for complex and heterogeneous tumor models, leading to the discovery of the potential for drugs and cancer therapies in clinical applications. With the passage of times, a quick improvement in three-dimensional models will allow further in-depth analysis of breast cancer as well as techniques to develop effective drugs against breast cancer.

Conclusion: This review has described different three-dimensional in-vitro and in silico models which have revolutionized breast cancer investigation. It offers a more illustrative platform for tumor behavior analysis, drug response, and disease proliferation. Each 3D model has pros and cons, and their applications, challenges, and future directions. 3D models have a special aptitude to mimic complex tumor microenvironments as compared to traditional 2D cultures. 3D models are able to recreate cell heterogeneity and organization allowing the in vivo representation. It also provides a piece of comprehensive knowledge about metastatic behavior, drug screening, and development. Besides these, 3D models are facing some challenges regarding protocols standardization and reproducibility, and multiplex experimental hurdles. 3D models have come up as strong tools in the research of bc, also remit 2D culture limitations.

References

1. Weigelt B, Ghajar CM, Bissell MJ. The need for complex 3D culture models to unravel novel pathways and identify accurate biomarkers in breast cancer. *Adv Drug Deliv Rev*. 2014;69-70:42-51. doi:10.1016/j.addr.2014.01.001
2. Sun YS, Zhao Z, Yang ZN, et al. Risk Factors and Preventions of Breast Cancer. *Int J Biol Sci*. 2017;13(11):1387-1397. doi:10.7150/ijbs.21635
3. Lukong KE, Ogunbolude Y, Kamdem JP. Breast cancer in Africa: prevalence, treatment options, herbal medicines, and socioeconomic determinants. *Breast Cancer Res Treat*. 2017;166(2):351-365. doi:10.1007/s10549-017-4408-0
4. Bahcecioglu G, Basara G, Ellis BW, Ren X, Zorlutuna P. Breast cancer models: Engineering the tumor microenvironment. *Acta Biomater*. 2020;106:1-21. doi:10.1016/j.actbio.2020.02.006
5. Kang Y, Datta P, Shanmughapriya S, Ozbolat IT. 3D Bioprinting of Tumor Models for Cancer Research. *ACS Appl Bio Mater*. 2020;3(9):5552-5573. doi:10.1021/acsabm.0c00791
6. Kapałczyńska M, Kolenda T, Przybyła W, et al. 2D and 3D cell cultures – a comparison of different types of cancer cell cultures. *Archives of Medical Science*. Published online 2016. doi:10.5114/aoms.2016.63743
7. Ho WY, Yeap SK, Ho CL, Rahim RA, Alitheen NB. Development of Multicellular Tumor Spheroid (MCTS) Culture from Breast Cancer Cell and a High Throughput Screening Method Using the MTT Assay. *PLoS One*. 2012;7(9):e44640. doi:10.1371/journal.pone.0044640
8. Kunz-Schughart LA, Freyer JP, Hofstaedter F, Ebner R. The Use of 3-D Cultures for High-Throughput Screening: The Multicellular Spheroid Model. *SLAS Discovery*. 2004;9(4):273-285. doi:10.1177/1087057104265040
9. Lin RZ, Chang HY. Recent advances in three-dimensional multicellular spheroid culture for biomedical research. *Biotechnol J*. 2008;3(9-10):1172-1184. doi:10.1002/biot.200700228
10. Nath S, Devi GR. Three-dimensional culture systems in cancer research: Focus on tumor spheroid model. *Pharmacol Ther*. 2016;163:94-108. doi:10.1016/j.pharmthera.2016.03.013
11. Nath S, Devi GR. Three-dimensional culture systems in cancer research: Focus on tumor spheroid model. *Pharmacol Ther*. 2016;163:94-108. doi:10.1016/j.pharmthera.2016.03.013
12. Thakuri PS, Liu C, Luker GD, Tavana H. Biomaterials-Based Approaches to Tumor Spheroid and Organoid Modeling. *Adv Healthc Mater*. 2018;7(6):1700980. doi:10.1002/adhm.201700980
13. Norotte C, Marga FS, Niklason LE, Forgacs G. Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials*. 2009;30(30):5910-5917. doi:10.1016/j.biomaterials.2009.06.034
14. Ovsianikov A, Khademhosseini A, Mironov V. The Synergy of Scaffold-Based and Scaffold-Free Tissue Engineering Strategies. *Trends Biotechnol*. 2018;36(4):348-357. doi:10.1016/j.tibtech.2018.01.005
15. Allensworth JL, Evans MK, Bertucci F, et al. Disulfiram (DSF) acts as a copper ionophore to induce copper-dependent oxidative stress and mediate anti-tumor efficacy in inflammatory breast cancer. *Mol Oncol*. 2015;9(6):1155-1168. doi:10.1016/j.molonc.2015.02.007
16. Johansson E, Andersson L, Örnros J, et al. Revising the embryonic origin of thyroid C cells. *Development*. Published online January 1, 2015. doi:10.1242/dev.126581
17. Chung BG, Lee KH, Khademhosseini A, Lee SH. Microfluidic fabrication of microengineered hydrogels and their application in tissue engineering. *Lab Chip*. 2012;12(1):45-59. doi:10.1039/C1LC20859D
18. Alessandri K, Sarangi BR, Gurchenkov VV, et al. Cellular capsules as a tool for multicellular spheroid production and for investigating the mechanics of tumor progression in vitro. *Proceedings of the National Academy of Sciences*. 2013;110(37):14843-14848. doi:10.1073/pnas.1309482110
19. Mendes AC, Baran ET, Lisboa P, Reis RL, Azevedo HS. Microfluidic Fabrication of Self-Assembled Peptide-Polysaccharide Microcapsules as 3D Environments for Cell Culture. *Biomacromolecules*. 2012;13(12):4039-4048. doi:10.1021/bm301332z
20. KUNZ-SCHUGHART LA, KREUTZ M, KNUECHEL R. Multicellular spheroids: a three-dimensional *in vitro* culture system to study tumour biology. *Int J Exp Pathol*. 1998;79(1):1-23. doi:10.1046/j.1365-2613.1998.00051.x
21. Correction: A Versatile Bioreactor for Dynamic Suspension Cell Culture. Application to the Culture of Cancer Cell Spheroids. *PLoS One*. 2016;11(6):e0157289. doi:10.1371/journal.pone.0157289
22. Page H, Flood P, Reynaud EG. Three-dimensional tissue cultures: current trends and beyond. *Cell Tissue Res*. 2013;352(1):123-131. doi:10.1007/s00441-012-1441-5
23. Raof NA, Raja WK, Castracane J, Xie Y. Bioengineering embryonic stem cell microenvironments for exploring inhibitory effects on metastatic breast cancer cells. *Biomaterials*. 2011;32(17):4130-4139. doi:10.1016/j.biomaterials.2011.02.035
24. Peela N, Sam FS, Christenson W, et al. A three dimensional micropatterned tumor model for breast cancer cell migration studies. *Biomaterials*. 2016;81:72-83. doi:10.1016/j.biomaterials.2015.11.039
25. Park J, Choi JH, Kim S, Jang I, Jeong S, Lee JY. Micropatterned conductive hydrogels as multifunctional muscle-mimicking biomaterials: Graphene-incorporated hydrogels directly patterned with femtosecond laser ablation. *Acta Biomater*. 2019;97:141-153. doi:10.1016/j.actbio.2019.07.044
26. Sant S, Johnston PA. The production of 3D tumor spheroids for cancer drug discovery. *Drug Discov Today Technol*. 2017;23:27-36. doi:10.1016/j.ddtec.2017.03.002
27. Tung YC, Hsiao AY, Allen SG, Torisawa YS, Ho M, Takayama S. High-throughput 3D spheroid culture and drug testing using a 384 hanging drop array. *Analyst*. 2011;136(3):473-478. doi:10.1039/c0an00609b
28. Nagelkerke A, Bussink J, Sweep FCGJ, Span PN. Generation of multicellular tumor spheroids of breast cancer cells: How to go three-dimensional. *Anal Biochem*. 2013;437(1):17-19. doi:10.1016/j.ab.2013.02.004
29. Froehlich K, Haeger JD, Heger J, et al. Generation of Multicellular Breast Cancer Tumor Spheroids: Comparison of Different Protocols. *J Mammary Gland Biol Neoplasia*. 2016;21(3-4):89-98. doi:10.1007/s10911-016-9359-2
30. Haisler WL, Timm DM, Gage JA, Tseng H, Killian TC, Souza GR. Three-dimensional cell culturing by magnetic levitation. *Nat Protoc*. 2013;8(10):1940-1949. doi:10.1038/nprot.2013.125
31. Leonard F, Godin B. 3D in Vitro Model for Breast Cancer Research Using Magnetic Levitation and Bioprinting Method. *Methods in*

Molecular Biology. 2016;1406:239-251. doi:10.1007/978-1-4939-3444-7_21
32. Zhou X, Zhu W, Nowicki M, et al. 3D Bioprinting a Cell-Laden Bone Matrix for Breast Cancer Metastasis Study. *ACS Appl Mater Interfaces*. 2016;8(44):30017-30026. doi:10.1021/acsami.6b10673
33. Zhang B, Luo Y, Ma L, et al. 3D bioprinting : an emerging technology full of opportunities and challenges. *Biodes Manuf*. 2018;1(1):2-13. doi:10.1007/s42242-018-0004-3
34. Zhang X, Zhang Y. Tissue Engineering Applications of Three-Dimensional Bioprinting. Published online 2015. doi:10.1007/s12013-015-0531-x
35. Wang X, Ao Q, Tian X, et al. 3D Bioprinting Technologies for Hard Tissue and Organ Engineering. *Materials*. 2016;9(10):802. doi:10.3390/ma9100802
36. Guillotin B, Souquet A, Catros S, et al. Biomaterials Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. *Biomaterials*. 2010;31(28):7250-7256. doi:10.1016/j.biomaterials.2010.05.055
37. Sorkio A, Koch L, Koivusalo L, Deiwick A, Miettinen S. Human stem cell based corneal tissue mimicking structures using laser-assisted 3D bioprinting and functional bioinks. *Biomaterials*. Published online 2018. doi:10.1016/j.biomaterials.2018.04.034
38. Ashammakhi N, Hasan A, Kaarela O, et al. Advancing Frontiers in Bone Bioprinting. 2019;1801048:1-24. doi:10.1002/adhm.201801048
39. Kang Y, Datta P, Shanmughapriya S, Ozbolat IT. 3D Bioprinting of Tumor Models for Cancer Research. *ACS Appl Bio Mater*. 2020;3(9):5552-5573. doi:10.1021/acsabm.0c00791
40. Ji S, Guvendiren M. Recent Advances in Bioink Design for 3D Bioprinting of Tissues and Organs. 2017;5(April):1-8. doi:10.3389/fbioe.2017.00023
41. Soman P, Kelber JA, Woo J, et al. Biomaterials Cancer cell migration within 3D layer-by-layer microfabricated photocrosslinked PEG scaffolds with tunable stiffness. *Biomaterials*. 2012;33(29):7064-7070. doi:10.1016/j.biomaterials.2012.06.012
42. Knowlton S, Onal S, Yu CH, Zhao JJ, Tasoglu S. Bioprinting for cancer research. *Trends Biotechnol*. 2015;33(9):504-513. doi:10.1016/j.tibtech.2015.06.007
43. Sachs N, Clevers H. ScienceDirect Organoid cultures for the analysis of cancer phenotypes. *Curr Opin Genet Dev*. 2014;24:68-73. doi:10.1016/j.gde.2013.11.012
44. Xu H, Lyu X, Yi M, Zhao W, Song Y, Wu K. Organoid technology and applications in cancer research. Published online 2018:1-15.
45. Clevers H, Tuveson DA. Organoid Models for Cancer. :223-236.
46. Dekkers JF, Whittle JR, Vaillant F, et al. 1,2,4 *,. Published online 2019.
47. Kopper O, Gerhardus R, Vries J, Cuppen E, Clevers H. Resource A Living Biobank of Breast Cancer Organoids Resource A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity. *Cell*. 2018;172(1-2):373-382.e10. doi:10.1016/j.cell.2017.11.010
48. Puca L, Bareja R, Prandi D, et al. cancer phenotypes. *Nat Commun*. Published online 2018:1-10. doi:10.1038/s41467-018-04495-z
49. Fan H, Demirci U, Chen P. Emerging organoid models: Leaping forward in cancer research. *J Hematol Oncol*. 2019;12(1):1-10. doi:10.1186/s13045-019-0832-4
50. Bersini S, Jeon JS, Dubini G, et al. A microfluidic 3D invitro model for specificity of breast cancer metastasis to bone. *Biomaterials*. 2014;35(8):2454-2461. doi:10.1016/j.biomaterials.2013.11.050
51. Pratt ED, Huang C, Hawkins BG, Gleghorn JP, Kirby BJ. Rare cell capture in microfluidic devices. *Chem Eng Sci*. 2011;66(7):1508-1522. doi:10.1016/j.ces.2010.09.012
52. Lanz HL, Saleh A, Kramer B, et al. Therapy response testing of breast cancer in a 3D high-throughput perfused microfluidic platform. *BMC Cancer*. 2017;17(1):1-11. doi:10.1186/s12885-017-3709-3
53. Ayuso JM, Gillette A, Lugo-Cintrón K, et al. Organotypic microfluidic breast cancer model reveals starvation-induced spatial-temporal metabolic adaptations. *EBioMedicine*. 2018;37:144-157. doi:10.1016/j.ebiom.2018.10.046
54. Caballero D, Kaushik S, Correlo VM, Oliveira JM, Reis RL, Kundu SC. Organ-on-chip models of cancer metastasis for future personalized medicine: From chip to the patient. *Biomaterials*. 2017;149:98-115. doi:10.1016/j.biomaterials.2017.10.005
55. Zhang YS, Zhang YN, Zhang W. Cancer-on-a-chip systems at the frontier of nanomedicine. *Drug Discov Today*. 2017;22(9):1392-1399. doi:10.1016/j.drudis.2017.03.011
56. Grafton MMG, Wang L, Vidi PA, Leary J, Lelièvre SA. Breast on-a-chip: Mimicry of the channeling system of the breast for development of theranostics. *Integrative Biology*. 2011;3(4):451-459. doi:10.1039/c0ib00132e
57. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. *Nat Biotechnol*. 2014;32(8):760-772. doi:10.1038/nbt.2989
58. Maschmeyer I, Lorenz AK, Schimek K, et al. A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. *Lab Chip*. 2015;15(12):2688-2699. doi:10.1039/c5lc00392j
59. Subia B, Dahiya UR, Mishra S, et al. Breast tumor-on-chip models: From disease modeling to personalized drug screening. *Journal of Controlled Release*. 2021;331(September 2020):103-120. doi:10.1016/j.jconrel.2020.12.057
60. Huang G, Wang L, Wang S, et al. Engineering three-dimensional cell mechanical microenvironment with hydrogels. *Biofabrication*. 2012;4(4). doi:10.1088/1758-5082/4/4/042001
61. Wang C, Tang Z, Zhao Y, Yao R, Li L, Sun W. Three-dimensional in vitro cancer models: A short review. *Biofabrication*. 2014;6(2). doi:10.1088/1758-5082/6/2/022001
62. Hwan S, Young K, Kim B, Hwan J. Hydrogel-Based Three-Dimensional Cell Culture for Organ-on-a-Chip Applications. Published online 2017. doi:10.1002/btpr.
63. Khalil S, Nam J, Sun W. Multi-nozzle deposition for construction of 3D biopolymer tissue scaffolds. *Rapid Prototyp J*. 2005;11(1):9-17. doi:10.1108/13552540510573347
64. Luo Y, Lode A, Gelinsky M. Direct plotting of three-dimensional hollow fiber scaffolds based on concentrated alginate pastes for tissue engineering. *Adv Healthc Mater*. 2013;2(6):777-783. doi:10.1002/adhm.201200303
65. Shen D, He J, Chang HR. In silico identification of breast cancer genes by combined multiple high throughput analyses. *Int J Mol Med*. 2005;15(2):205-212. doi:10.3892/ijmm.15.2.205
66. Edelman LB, Eddy JA, Price ND. In silico models of cancer. *Wiley Interdiscip Rev Syst Biol Med*. 2010;2(4):438-459. doi:10.1002/wsbm.75
67. Xu X, Farach-Carson MC, Jia X. Three-dimensional in vitro tumor models for cancer research and drug evaluation. *Biotechnol Adv*. 2014;32(7):1256-1268. doi:10.1016/j.biotechadv.2014.07.009
68. Nicodemus GD, Bryant SJ. Cell encapsulation in biodegradable hydrogels for tissue engineering applications. *Tissue Eng Part B Rev*. 2008;14(2):149-165. doi:10.1089/ten.teb.2007.0332
69. Wolf K, Friedl P. Molecular mechanisms of cancer cell invasion and plasticity. *Br J Dermatol*. 2006;154 Suppl:11-15. doi:10.1111/j.1365-2133.2006.07231.x
70. Holliday DL, Speirs V. Choosing correct breast cancer cell line for breast cancer research. *Breast cancer research*. 2011;13(4):1-7.

Table 1. Different in silico databases and software for BC system biology

Resources	Data bases	Functions
Ensemble UCSC Genome browser EVEP	Data of genome sequence, Annotation of the genome Analysis, classification of coding & non-coding sections	Detailed information about genome Investigation and classification of variants in coding and noncoding region
Genomic annotation data Element of gene	Entrez Gene database GOA UniProt Genomic review	specific sequenced genome information comprehensive data about protein function and annotation
Biochemical pathway & functional relationship	Genes & Genome Kyoto Encyclopedia (KEGG) GO SEED Meta Cyc Bio Cyc Transport DB	investigate the organism functions at the cell level, molecular level, and at gene level create networks at the genomic scale contains different metabolic pathways and enzymes of multiple organisms information about the transporter present on the cytoplasm membrane
Regulatory sequence	EPD (Eukaryotic Promoter Database) TRED (Transcriptional Regulatory Element Database)	Regulatory sequences are available
Protein interactivity networks	Interacting protein database Molecular INTeraction database Mammalian protein-to-protein interactivity database	Provide information about protein interactivity
Genomic scale data Transcriptomics	GEO (Gene Expression Omnibus) SMD (Stanford Microarray Database)	used for Genomic scale data and Transcriptomics
Proteomics	Proteomics identifications database	
Software for visualization and management of data	Cystoscape Gaggle	used for visualization and management of data
Bibliography database	PubMed	contains all published data about medical sciences
Structure and function-based prediction of protein	I-Tasser	Provide structure-based function annotation of secondary protein
Drug designing	Molecular Docking	Interaction between protein and ligand
Three-dimensional molecular visualization	PyMol	Helps in the visualization of molecular docking

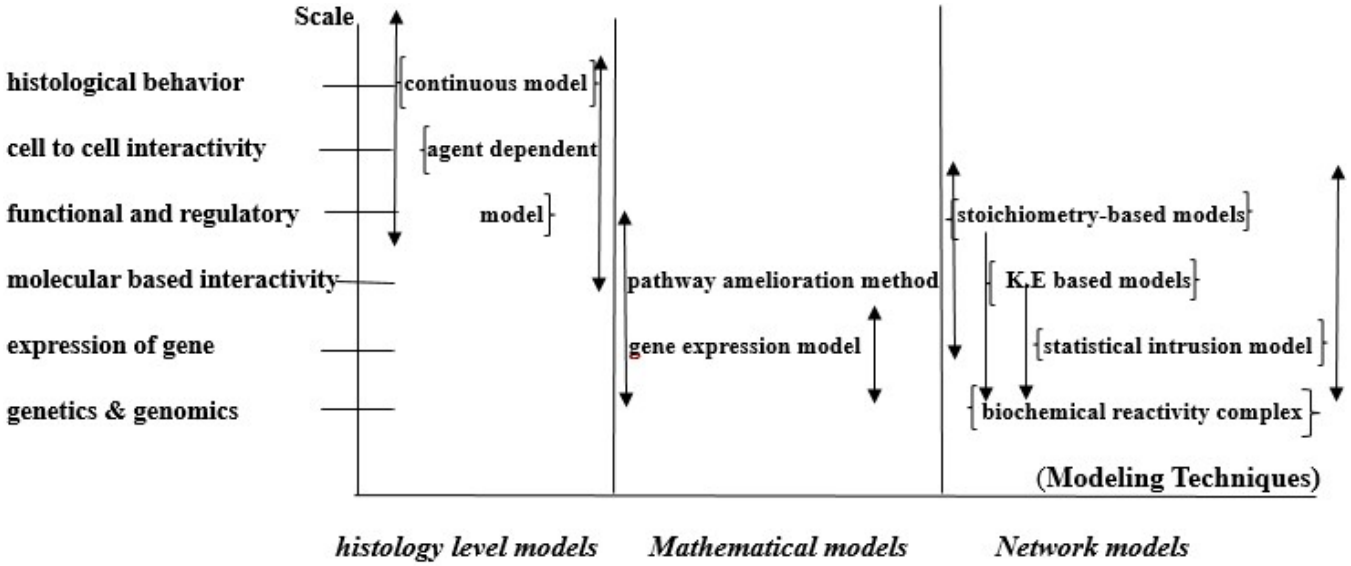

Fig 1. Biologically developed scale and modeling techniques.