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3D *in vitro* cancerous tumor models: Using 3D printers

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A B S T R A C T

Recently, magnetic Hyperthermia is one of the promising methods for cancer treatments. In this method by applying magnetic fields and generating heat, cancerous tissues are eliminated. The degree and pattern of generated heat in cancerous and adjacent non-cancerous tissues plays an important role on the outcome of the treatment. it is mainly affected by diffusion and distribution pattern of magnetic nanoparticles within the cancerous and non-cancerous tissues. Study the diffusion and distribution patterns of magnetic nanoparticle *in vivo* is difficult and costly in many cases and in some cases evaluating the amount of generated heat at cancer site is almost impossible. *In vitro* models for cancer tissues are alternatives for *in vivo* models. However, usual *in vitro* models could not resembling all the characteristics of a cancer tumor. In this hypothesis we propose that using 3D printers can provide a platform to fabricate a personalized *in vitro* cancer model which could simulate the most important features of the cancerous tissues (including shape and vascular network) and can be used to study magnetic hyperthermia in a simulated media of compatible to *in vivo* conditions.

Introduction and background

There are several methods for cancer treatment like radical mastectomy (surgery), chemotherapy, radiotherapy, or immunotherapy. In the last two decades minimally invasive alternative therapies have been developed, such as focused ultrasound high-intensity ablation, radiofrequency ablation and, hyperthermia. Hyperthermia means heating the body to increase its normal temperature. By increasing temperature, cell structure proteins denature which in turn leads to cell death. In this method, the main concern is local control of heat at the site of cancer tissue to avoid damaging healthy surrounding tissues. Magnetic hyperthermia is a solution to this problem [1–4].

Magnetic Hyperthermia is a promising method for controlling the heat at the site of a cancer tumor. In magnetic Hyperthermia, magnetic or in some cases gold nanoparticles are injected at the cancer site to diffuse and distribute in the tumor, and by applying an alternating magnetic field (AMF) heat would be generated [5–7].

The main issue in magnetic hyperthermia is the concentration and distribution pattern of nanoparticles at the tumor site which will affect the result of hyperthermia. To reduce the side effects and also the maximum efficiency, it is crucial to select the proper dose of nanoparticles to reach the optimum heat distribution profile. The magnetic nanoparticles properties and the properties of microenvironment which diffusion process occurs would change the concentration and distribution patterns of nanoparticles [8–11]. In order to study distribution profile of nanoparticles concentration and heat generation, a suitable model (microenvironment) with the most similar characteristic to the targeted cancerous tissue should be applied (See Scheme 1).

The most similar microenvironments to human cancer tissue are *in vivo* models that mostly are induced in animal bodies via injection of human cancerous cells. However, there are some drawbacks associated with these models such as high cost and time-consuming process, occupational hazards associated with the care and use of laboratory animals like injuries, allergies and bites and also working with animals needs a high level of hygienic maintenance. Besides, researchers should consider animal ethics in their studies and avoid using animals for clinical tests as much as possible. One of the main rights of animals is “need to avoid suffering”. In many experiments animals should be sacrificed in order to complete the study. this would infring their rights. In addition, following up the diffusion of nanoparticles and temperature tracking in *in vivo* models are not facile [9,11–13].

Due to aforementioned reasons, researchers have focused on *in vitro* cancer models to study hyperthermia and nanoparticles diffusion patterns especially in early stages of studies. The common *in vitro* microenvironments and models for hyperthermia studies include *in vitro* cell cultures, fluidic media, microfluidic devices as *in vitro* culture, dead tissue models and polymeric hydrogels. Every mentioned model gives researchers an opportunity to study the effect of some parameters on hyperthermia process, however, each suffers from some drawbacks and still a comprehensive model is needed [4,14–18].

Hypothesis

The biomimetic structures of a cancer model must mimic the

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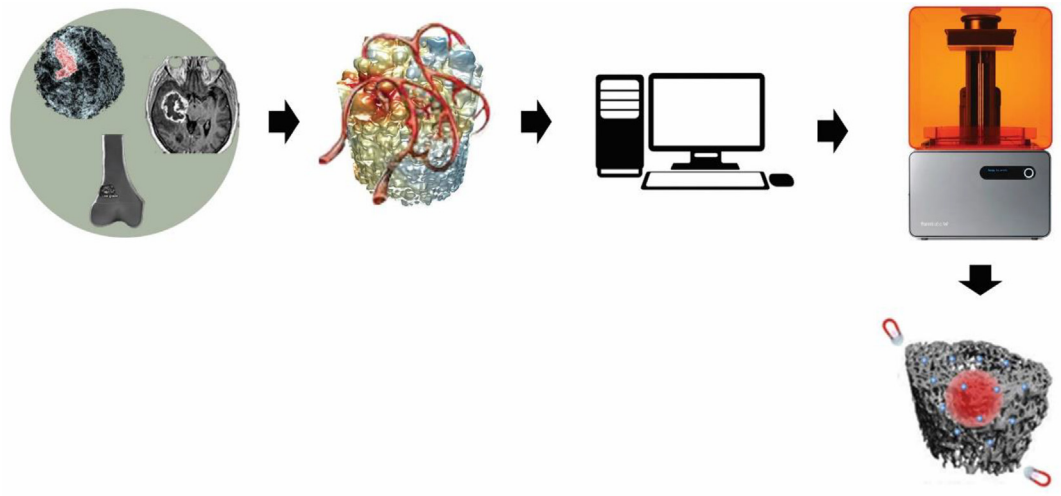

Scheme 1. the procedure of fabrication of cancer models using 3D printers: first the tumor structure is scanned, the following providing digital model and finally fabrication of the model using photocurable hydrogels.

chemical composition, morphological structure (shape and vascular network) and mechanical properties to native tumors. Furthermore, cancer tumors depending on cancer type and stage of disease possess different vascular network and mechanical properties thus the cancer models should be capable of tailoring such characteristics to be able to adapt to real cancerous tissues [14].

In recent years, polymeric hydrogels are at the center of attention in biological studies. The similarity of these structures to native tissues leads the researchers to use the hydrogels as *in vitro* models of human tissues. Polymeric hydrogels are one of those materials which have tunable mechanical properties by differing their composition and also, crosslinking density [23].

In order to mimic the vascular network of the cancerous tissues, porous hydrogels are applied. The interconnected pores in the hydrogel can simulate the movement of water through the hydrogel. There are myriad methods for fabrication of porous hydrogels however, most of these methods do not have the control over pores structure and morphology. In recent years additive manufacturing methods give us the ability to precise control over the geometry and physical structure of final product [19–21].

One of the other drawbacks of previously introduced hydrogel models based on synthetic polymers is the lack of chemical composition similarity with the native tumor tissue. In hyperthermia studies, especially in the diffusion process, the chemical composition of hydrogel affect the result of therapy since the interactions of MNPs with the microenvironment is influenced by the chemical composition of the media in which diffusion occurs [22].

Based on above-mentioned facts, we suggest a gelatin based hydrogel with biomimetic precise vascular-like network fabricated using additive manufacturing methods as an *in vitro* cancer model for studying hyperthermia process and nanoparticle distribution. This 3D model could mimic cancer tumor in three features: chemical structure, morphological structure (vascular network) and mechanical properties.

Evaluation of hypothesis

Cancer tissues have the same chemical structure as connective tissue, which its main component is collagen. Based on our study, Gelatin which is provided from collagen is the best material for simulating the chemical structure of native tissue. Gelatin is easy to process, has low cost and is simply available, but it has some properties like solubility in biological media and lack of ability of photocuring which is necessary for photocrosslinking of gelatin for fabrication of gelatin-based hydrogels [24]. A solution to this problem is the modification of gelatin by adding methacriloyl groups on the backbone of the protein. Gelatin methacriloyl which generally called Gelatin methacrylate (GelMA) is cross-linkable in the presence of a radical factor. The C═C bond in methacrylate groups breaks by light radiation and crosslinking would occur through this bonds [19,25–28]. Presence of a radical factor in reaction media is necessary for the crosslinking process because without these factors double bond breakage is impossible, therefore, photo-initiator selection as a radical factor would be a challenge. The photo-initiator must be soluble in aqueous media of gelatin methacrylate and should be reactive in the range of radiated light [29].

One of the other main parameters of cancer models is the microstructure and morphology of model. Morphology of cancer model would affect nanoparticle diffusion investigations. In cancerous tissues which are made from a colony of cells, there is a vast vascular network with fewer order forms than normal tissues to provide the nutrition to cancerous cells [30,31]. For presenting a model with the most similarity to the native tissue, simulating vascularization seems to be necessary. The hydrogel fabrication methods have a significant effect on the morphology and microstructure of the gels, so for mimicking the vascular networks of cancer tissues these techniques are reviewed to choose the best method [14,21,32,33].

3D fabrication techniques are classified into two categories: traditional and additive manufacturing methods.

Here we focused on three common traditional methods in fabrication of 3D hydrogel structures, these methods are solvent casting, freeze drying, and gas foaming. In solvent casting technology polymers are dissolving in their proper solvent then salt particles will be distributed in the solution. After that solvent evaporates and a composite of salt particles in the polymer will be made. Then the composites immersed in water and salt particles which uniformly distributed in the polymer matrix will be leached. The resulted porosity is due to salt particles but these porosities are not connected together so by using this method we are not able to simulate the vascular network of the tissue [21,34]. In the freeze-drying method the polymer solution is cooled down below its freezing temperature, this process separates the solvent and polymer and makes a two-phase system. Then via sublimation solvent evaporates, and this makes a highly porous polymeric structure with interconnected pores which are similar to the vascular network, but the structure of polymeric scaffold is not controllable in this method [21,35]. In the gas foaming method, the polymer matrix is mixed with gases such as carbon dioxide, nitrogen, or water at high pressures. Gas bubbles would be made in polymer and it causes a porous structure with pores sizes ranging from 100 to 500 μm and only 10–30% of pores are interconnected and we cannot trust this process for vascular

network simulation [21,36].

Based on above-mentioned facts, traditional methods are not able to control the matrix structure and they are not reliable for mimicking vascular network of a native tissue. So we have focused on recent advances in 3D fabrication which could help us in constructing more complex models of cancer tissue.

Additive manufacturing methods

Additive manufacturing is a group of methods which constructing 3D models based on the three- dimensional computer-aided design data. One of the most common techniques of rapid prototyping is additive layer manufacturing or 3D printing. 3D printing gives us the ability to precise control over the model, by finely tuning and defining structure geometries and mechanical properties. So by these capabilities, we can fabricate complex structures like native tissues [21]. 3D printing methods are also categorized in groups, based on the process of manufacturing the 3D platforms. In this paper, we focused on the 3D printing methods which most adapted to our work and the material which we have chosen.

Inkjet and extrusion 3D printing

These printing techniques are based on common 2D printing methods and in both of them there is a cartridge that is connected to the nozzle of the printer and the ink of printing or in the case of our study the prepolymer solution is saved in it. The nozzle is controlled electronically and the pattern of every layer which formerly had been designed in the computer is creating with moving the nozzle. In inkjet printing after transferring the prepolymer to the head of the printer by a pump, by piezoelectric or thermal actuation the ink comes out of a nozzle to lie on a substrate as droplets. Extrusion printing is the modified form of inkjet printing. In this method, a prepolymer with high viscosity come out of the nozzle with the pressure of a piston or screw or even by pneumatic pressure. The output ink of extrusion printer is like a continuous stream. The prepolymer solution in this printers could be cured by both heat and light and a wide range of material uses in this printers. In these techniques controlling over the structure is very difficult and it seems the printing of very complex tissues is not possible and just a simple model of tissues is able to be printed [37].

Laser-assisted printing

Laser-assisted printing originated from laser direct-write printing method. The system is composed of three layers at the top of the substrate. The first layer against the laser beam is called the donor layer which response to laser stimulation. The second layer is the energy absorbing layer which is at the top of the ink solution (third layer). After applying a laser pulse a small area of the donor layer vaporizes and creating a high-pressure bubble at the interface of the ink layer and makes a jet of ink which falls into the collector slide. Ink at the collector slide crosslinked and the whole structure will print layer by layer upon a pattern to form a 3D structure. This technology has high cost and could support solutions with high viscosity [38].

Photolithography

Photolithography techniques use light or photons to transfer the geometric shapes of a mask to a light-sensitive surface it means the technique uses light for photopolymerization. The advantages of these techniques are temporal control of reaction kinetics, low heat production and in case of using bio-ink the cell's distribution in the polymer matrix is good. The main photolithography methods are mask-based and stereolithography [21,33,38,39].

Based on the ability of photocuring of gelatin-methacrylate it seems the best technique for the fabrication of 3D structures by this material is photolithography technique. For modeling cancer tissue and all its complexity by mask-based technique, we have to use a large number of masks to construct the structure layer by layer and base on the pattern of every layer a different mask is needed these matters make this technique time consuming and expensive [19].

One of those printers with the ability to construct complex structures with a layer by layer photo-curing method is Stereolithography. For fabrication of a structure based on stereolithography technology following steps should be taken: At the first step, the pattern of printing must be designed in a CAD format. Then the 3D designed pattern must be loaded to the software of printer on the computer. The software slices the 3D structure design into 2D layers and the thickness of each layer could be altered. Then by data transport to the SLA apparatus, the pattern of each layer will form on the resin which is in the resin tank by a UV laser by curing the polymer. The laser is controlled by a step motor that moves in three directions. The first layer sticks on the platform of apparatus. When curing of resin complete the platform goes up and the second layer will cure base on the pattern of the structure by a UV laser. This process repeats until the 3D structure completed [33].

In this hypothesis we suggest the use of stereo-lithography technology for replicating the vascular network of cancerous tissues due to its high speed, low cost and high accuracy which is mainly depend on the resolution of the printer's laser. For making the most similar model to the real tumor we are going to use μCT and MRI images of tumors. To make this images usable in 3D printer, they should be converted to CAD files [23].

The parameters of the printer that affect the properties of final structure are the time of curing of each layer, laser resolution, number of layers and the thickness of each layer, post cure conditions and wavelength of UV laser [19,40].

Consequence of hypothesis

After making a 3D structure of cancer tumor *in vitro* researchers could do experiments on the ways of cancer treatment with fewer cost and time and the use of animal models would be limited and animal ethics would no longer violated. Also, magnetic hyperthermia treatment could be done with fewer casualties and it could be more effective and more accurate. Due to use of hydrogels with different degree of crosslinking, we could have tailoring structure with different mechanical properties then different stage of cancers could be simulated with controlling material, printer and condition of process. Finally, the most important subject is personalized medicine which by using this technique, clinicians could have a unique model of every patient tumor with their unique individual features and the treatment process could be more effective.

Conflict of interest

None.

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