PEG-based Tumor Millibeads for Three-dimensional Cancer Cell Culture

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Abstract:

Models that mimic the pathophysiology of human cancers are critical to advancing cancer research and drug discovery. In this research, we have developed a novel cancer model (called, 'tumor millibead model') through the use of poly(ethylene glycol)(PEG)-based hydrogel biomaterial for three-dimensional (3D) cancer cell culture, which can be potentially used in the drug discovery and development process. Whereas most current cancer research relies on two-dimensional (2D) cultured cells or animal models, the use of biomaterials to create tissue-engineered cancer models provides advantages in replicating the tumor microenvironment. Animal and 2D cell culture models have some inherent limitations in their ability to mimic the native human cancer pathophysiology. 2D models lack the complexity of the 3D extracellular microenvironment, an important player in the tumorigenic progression. Animal models are physiologically different from the microenvironmental conditions of the human body and, hence, frequently fail to provide accurate information about the future clinical efficacy of the tested drugs. Therefore, there is a clear need for the development of cancer models which can provide physiologically and clinically relevant information about drug action and efficacy. In order to address this problem, the emerging field of cancer tissue engineering is focused on incorporating the principles of cancer biology in tissue engineering to create 3D *in vitro* engineered cancer tissue through the use of hydrogel biomaterials, facilitating the investigation of tumorigenic phenomena and testing efficacy of new anti-cancer drugs.

In order to create the tumor millibead model reported in the study, we have developed a novel method for the fabrication of millimeter-scale hydrogel spheres (called 'hydrogel millibeads') using a surface-tension based, liquid-liquid dual phase system. This technique allows the creation of highly uniform, PEG-based hydrogel spheroids, which are significantly larger in size compared to PEG-based hydrogel microspheres obtained by previous fabrication methods. In addition, the ability to culture cancer cells within the hydrogel millibeads provides a unique cancer model which can mimic the millimeter-sized tumors in the human body.

Briefly, poly(ethylene glycol diacrylate) (PEGDA) precursor solution of a fixed volume (2, 5 or 10 µl) was pipetted on the surface of an oil phase solution in a Petri dish, where the precursor droplet floated at the air-oil interface due to surface tension differences between air, oil and aqueous phase. The droplets were crosslinked in presence of light of wavelength 365-700 nm for 10 seconds to form hydrogel millibeads. They were subsequently harvested and washed with DMEM to remove all oil. The hydrogel millibeads obtained were larger in size, more uniform in shape with better ease of size control and consistency between fabrication batches. The swelling behavior and diffusion characteristics of hydrogel millibeads were correlated to their initial fabrication size. Human breast cancer MCF7 cells were encapsulated within PEGDA millibeads to generate 'tumor millibeads', which were maintained in long-term 3D culture. Fluorescence imaging and SEM imaging revealed the spherical morphology and uniform

distribution of cells in tumor millibeads. Live/Dead staining revealed the presence of a necrotic core of cells surrounded by healthy viable cell layers at the periphery of the millibeads, which is a prominent feature observed in many native and *in vivo* tumors.

Overall, in this study, we have demonstrated a novel technique for the fabrication of hydrogel millibeads which can support long-term 3D cancer cell culture. Important characteristics of the native tumor microenvironment, including tumor heterogeneity, necrotic core formation and 3D cancer cell morphology, are closely simulated within the tumor millibeads. In addition, use of the PEG-based hydrogel system allows for the biochemical and mechanical modifications of the model engineered tissue to match the properties of native cancer tissue in the human body. Biochemical modifications include the use of PEG-peptide or PEG-protein conjugates that allow cellular adhesion, degradation and remodeling of the engineered tissue. Mechanical modifications include tuning of the matrix stiffness to match the native stiffness of cancer tissue by controlling relative PEG concentration, crosslinking density and chain length. This engineered tumor model could be potentially used to obtain physiologically and clinically relevant information from future drug studies. The millibead fabrication technique can be scaled up to create engineered 3D cancer tissue models for high-throughput drug-testing applications. Hydrogel millibeads can also be potentially used to encapsulate suitable biomolecules or therapeutics for controlled delivery at the site of interest. Hence, PEG-based hydrogel biomaterials can significantly contribute to the creation of useful cancer models, and thereby advance cancer research, and drug discovery and development.