ABSTRACT

The first step of understanding the efficacy of any new drug designed are *in-vitro* models. They are also elementary in comprehending or deciphering any new pathway or their components. The most habitually explored methods to understand these processes are flat-surface techniques also known as two-dimensional culture techniques (2D techniques). However, recent reports have suggested more than half of the drugs fail clinical trials, making drug discovery a costly process with low success rates. Reasons for failure being the cells in the monolayer culture are not able to mimic *in-vivo* cellular organization. They are very uniformly spread unlike the *in-vivo* conditions. The receptors on the cell surfaces are exposed only on one side of the cells. These reasons lead to high attrition rates in drug discovery, thus, there is an urgent need to develop new and precise techniques to improve the success rates in drug testing. One of the ways to achieve that is designing of better pre-clinical models, that can better recapitulate the *in-vivo* biology and microenvironmental factors. Animal cell culture techniques have been around since the 20th century. To overcome these limitations of 2D cell-culture techniques, there was the introduction of three-dimensional (3D) culture platforms in the early 21st century. The spheroid like structure mimics the heterogenous and the hypoxic environment of *in-vivo* culture better as compare to monolayer culture. They give us the flexibility to understand cell-cell and cell-matrix interactions better. They also allow compartmentalization of different cells, that permit spatially separated cells in close proximity. Hence, these platforms can be better prescribed as high throughput drug screening platforms as compared to 2D platforms. Conventional bulk hydrogels were observed not to replicate the property of the natural tissue, moreover, single cell-cell interaction was difficult to study in macroscale platform. As an alternative to bulk coculture methods, miniaturization techniques were used to used generate microscale hydrogels having their own microenvironment. Various techniques have been exploited for generation of cell-laden microgels including droplet microfluidics, bioprinting, micromoulding, stop-flow lithography to name a few.

Cancer, still being one of the diseases with high mortality rates have been extensively studied using 3D platforms in the hope of having better pre-clinical models. The key factors for 3D model to be suitable for studying tumor properties are (i) formation of nutrient, oxygen and metabolic waste diffusive gradients (ii) heterogenous layer of cells (iii) control over the hydrogel degradability. The existing platforms lack (i) reproducibility, as most of the microenvironments are cell or tissue derived, (ii) easy read-out and (iii) assay validation, due

to lack of relevant data to be compared with. The goal of this thesis work was to be able to develop a platform that could address some of the limitations present in the field.

Chapter 1, introduces the 3D cell culture, it gives a comparison between 2D and 3D platforms, summarizing the significance of 3D platform. It reviews the existing 3D models in brief and the different materials and techniques used to generate the platform.

Chapter 2 focuses on designing the platform, which can be used as 3D *in-vitro* cell culture. 3D platforms established till date lack control over precise spatial distribution of cells in the 3D scaffold. Alginate-based simple micron sized platform using droplet microfluidics technique that closely mimics the tumor architecture is developed. The real time monitoring strategy using pH-sensitive carbon dot nano-sensors removes the need of additional end-point assays for monitoring cellular growth. This platform gives the flexibility to modulate its properties according to the requirement of the model to be designed. It provides spatial control, generates hypoxia in a short span as well as better the cellular characteristics to yield reliable results in drug screening.

To establish the 3D model developed in Chapter 2 for studying cancer, cells with modulated oncogene expression was studied. One of the major causes for cancer is mutation in oncogenes and tumor-suppressor genes. In **chapter 3**, genetic modification and its effects in the encapsulated cells were studied using the platform developed. The target chosen to demonstrate the application of the developed platform is a deubiquitinating enzyme, Ubiquitin Specific Peptidase 37 (USP37). This is found to be elevated in different cancers and has been reported to play a role in regulation of various processes like cell cycle regulation, oncogenesis and metastasis. Transiently modified cell lines are difficult to study in the existing 3D platforms as they generally take 2-3 weeks to prepare. The platform was utilized to study the difference is the transiently altered cells with growth curve as readout. The difference could be observed by culturing the cells in the 3D platform and simply reading the fluorescence of the carbon nanodot sensors at various time points.

The platform developed in Chapter 2 was further modified to understand another basic cellular process, migration. Migration of cells has a role to play in various conditions, be it an immune response, or response to a stimulus for progression of a disease. **Chapter 4,** explores migration of cancer cells using a collagen-alginate model. Collagen was introduced in the microbead composition to make the system more relatable to the extracellular matrix of cells. To validate that the migration of cells was being observed upregulated MMP9 cells were

encapsulated in the microscaffolds. Increase in the number of ‘cluster of cells’ observed after 48 hours inside the microscaffold as compared to the control cells was the readout indicating the invasiveness of the encapsulated cells.

Developing a platform that can preserve the functional behavior of the cells is of high clinical relevance in the field of tissue engineering. Historically, hepatocytes have been one of the primary cells whose functionality is very difficult to maintain in 2D over longer period of time. Culturing them in 2D is cumbersome as they eventually de-differentiate and lose their morphology and function. An attempt was made to preserve the functionality of primary rat hepatocytes in **Chapter 5.** Using the same droplet microfluidic technique for the generation of microscaffolds but introducing decellularized matrices alongwith collagen and alginate to see which composition supports the survival of hepatocytes. Further, to check if this model can be used for toxicity assays, the microscaffolds were administered with drugs which are known as potent inducers of drug induced liver cytotoxicity. These studies conclude that this model can be a probable liver model for drug toxicity studies. **Chapter 6**, discusses the conclusion and future outlook of the thesis.