6 Conclusion

It is clearly evident that ECM plays significant role in tumor cell behaviour. Specific ECM components append on cell surface receptors and stimulate peculiar response for cell polarity, growth, drug resistance or metastatic potential. In recent years, the tumor microenvironment has been recognized as a major contributor for tumor progression and become the focus of extensive research as a potential target for chemoprevention. It is now well recognized that to successfully study the pathobiology of human cancers, it is necessary to recreate appropriate tumor microenvironment; 3D tumor models perhaps could be applicable models to fulfil the need. Native ECM can be deconstructed and served as a clue for reconstructing artificial scaffolds for generation of 3D tumor models.

The present study was aimed predominantly to fabricate biocompatible 3D hydrogel scaffolds for lung tumor cells. The natural polymer chitosan was selected first as main component for it due to its biocompatibility, low toxicity, cell adhesiveness, ease of fabrication and low cost. Chitosan was widely accepted as a biomaterials in several tissue engineering application, moreover, it have a functional moieties in backbone which can be modified certainly. The scaffolds were fabricated as chitosan and chitosan-gelatine hydrogels. The parameters were optimized for the fabrication process and tumor cell culture. This initial studies confirms the biocompatibility of both the scaffolds and showed them as a tumor cell adhesive structure. These features made the underlying principle for modification of surface chemistry of scaffold that induce 3D microtumor formation.

The objective of the present study was to fabricate a cell-instructive 3D scaffold with emphasis on providing appropriate biochemical environment for lung tumor cells to form 3D tumor. Therefore, natural biopolymers dextran was selected along with chitosan and were modified for present work. The dextran was oxidized and chitosan was thiolated by grafting
Conclusion
cysteine. The Odex has aldehyde groups in its backbone, which reacted with the amine group of chitosan and formed polymer network structure. This self-gelling property made present model very easy to fabricate and eliminates the dependency of small chemical crosslinkers which otherwise should be removed after fabrication process. The hydrogel in this study can be produced in any shape and size without affecting the physiochemical properties which makes it versatile scaffolds for cell growth study to bioimaging. 3D micro porous structure could compartmentalize the cells and facilitate multiple tumor tissue formation.

The incorporation of biocompatible dextran demonstrated the reduction in hydrophobicity of scaffold which has improvised its mechanical property and significantly encouraged tumor tissue formation. The chemical structure of Odex have changed the surface chemistry of hydrogel that minimized the cellular adhesion, consequently allowing cells to adhere and colonize at specific places. Present study clearly demonstrates that not only 3D geometry could support the 3D tumor formation but, biochemical property is also very much required for the 3D micro tumor formation.

The growing tumor tissues were characterized by SEM and confocal microscopy for their shape, cytoskeleton organization, and ECM synthesis. The morphological change of cellular structure was clearly observed in the presence of Odex in hydrogel. The cellular shape and spreading parameters calculated by ImageJ have confirmed this morphological changes occurred at cellular level. Additionally, the internal cytoplasmic alignment was modified in accordance to the external signals and increased nuclear area in the present model suggest aggressive tumor phenotype. This observations specifies the mimicking features at individual external as well as internal cellular level in the microtumor. The change in cell morphology and random stress fibers further supported the contribution of scaffold in tumor formation. The transformation in cellular structure could alter the exposure of surface molecules involve in transforming external signals to the nucleus. Eventually, this leads to
greatly enhance or reduce the processes of proliferation, EMT, migration potential and response to the chemical molecules.

As the 3D tumors expand, there are nutrient and oxygen diffusion rate limitations occur, which can create hypoxic conditions. A similar observation was made in current study with elevated ROS level resembling to *in vivo* condition. The hypoxic condition can directly associated with expression of HIF-1α protein. The HIF-1α regulates process including EMT, invasion, migration, angiogenesis and drives the selection of metastatic phenotype. At the end, this study demonstrated the drug toxicity assay for microtumors and compared it with conventional 2D culture model. The drug toxicity assay clearly indicated that the tumor behavior was affected by geometry and biochemical microenvironment and presented an altered drug response compare to 2D culture.

Taken all together, this dissertation focuses on modification of dextran and chitosan polymer, fabrication of Oxidised dextran-thiolated chitosan based hydrogel, characterization of its physico-chemical properties and application as a scaffold for generating 3D tumor model. The present model could be more predictive model for initial drug toxicity studies and bridge the gap between existing 2D culture assays and in vivo models. Additionally, these hydrogels can be further studied for other cancer cells and stem cells plus can be developed as a tissue engineering construct.
7 Future perspectives

The current dissertation represented the MDC hydrogels and its essential biological characterization for inducing 3D microtumors. The future perspective of this study include evaluation of microtumors at molecular level. Further, the evaluation of microtumors for process of EMT, migratory and invasive potential of cells and stem cell population. All of these characteristics would be assessed and compared with in vivo tumors to validate the microtumor as an effective model to recapitulate native tumor microenvironment. In future, the scaffold can be potentially utilized for cancer disease modeling to understand fundamental questions regarding the effect of stiffness and cross-linking density on tumor phenotype and progression.

The present study demonstrated two-step method for developing 3D tumor including scaffold fabrication and cell seeding. However, it can be upgraded to one step by live cell encapsulation during fabrication and could also be explored for 3D printing of cells. We have utilized this scaffold for tumor cells, nevertheless, it could have applicability to use for other tissue engineering applications.

The present study showed a more predictive model compare to conventional 2D culture methods for initial drug screening assay. Nevertheless, several additional considerations are essential to expand the utility of such model. In particular, cancer is multi-scale process, involving a number of types of stromal cells and signalling pathways orchestrate at a time, reorienting such environment is essential for predictive drug screening. The biological advancement could include, 1) co-culturing of stromal cells with tumor cells to provide complex cell signalling environment, 2) Functionalization of devices with signalling molecules such as Growth factors or functional peptides could improve the bio-mimicking of tumor microenvironment. At the end, future motivation should be engineering models for
Future perspectives

High throughput screenings and easily accessible for clinicians and researchers of cancer biology field. Looking forward, engineering microenvironment based 3D cancer models could enhance both basic as well as translation cancer research and chemoprevention.