Chapter 7

Conclusions and future scope of work

Loss of skeletal mass occurs due to various conditions such as high-energy accidents, trauma, congenital defects, and myopathies (Kwee and Mooney 2017). Some of these myopathies prove to be fatal as there are no current treatments available for such diseases for example in case of muscular dystrophy patient's average survival age ranges from 25-26 (Passamano et al. 2012). This survival age decreases by ten years for patients in India (Duraiswamy 2009). The number of cases concerning age-related skeletal muscle loss is bound to increase as World Health Organisation estimates doubling of population in the age group of greater than 60 by 2050 ("Ageing and Health" 2018). Eighty percent of the cancer cases are associated with skeletal muscle loss (Dewys et al. 1980). Skeletal muscle loss amounting to greater than 20% of the mass impedes skeletal muscle regenerative capabilities. Current treatment options for skeletal muscle loss are limited to muscle flap transfer, which is associated with lack of donors, donor site morbidity and inefficient functional recovery of the muscle (Ma et al. 2011). Tissue engineering has emerged as a possible solution by providing ways for fabrication of skeletal muscle substitutes. Conventional tissue engineering approaches face various challenges: choice of a biomaterial which supports proliferation, differentiation, and fusion of myoblasts to form myotubes, mimicking structural organisation of skeletal muscle tissue and recapitulation of innervation and vascularisation of skeletal muscle tissues (Bian and Bursac 2008). Various enabling technologies like 3D bioprinting, microfabrication-based techniques and optogenetics are necessary to adjuvant the conventional tissue engineering strategy. The present thesis offers three strategies in order to help fabrication of in vitro skeletal muscle tissue including bioprinting, optogenetics and micropatterning. Bioprinting and micropatterning based strategies have great potential to mimic the structural organisation of skeletal muscle. Optogenetics based technology enables dynamic stimulation and control over the artificial skeletal muscle tissue. Biomaterials like sodium alginate, gelatin

and hydrolysed collagen were suitably processed with C2C12 cells to prepare a bioink, which was used to print skeletal muscle like models. Due to the favourable properties exhibited by bioink components, the printed 3D constructs showed excellent cell viability of greater than 90% after 14 days of in vitro culture. The cells formed physical networks for communication. In total, a cost-effective and straightforward strategy with potential for skeletal muscle development was successfully created by combining inexpensive bioprinting system and novel bioink composition.

The thesis delivers optically sensitive C2C12, HepG2, and NIH-3T3 cells by incorporating optogenetic tool channelrhodopsin using lipofectamine based transfection method. The transfected cells were found to be expressing green fluorescence protein confirming the transfection of the cells. The thesis demonstrates that the microchannel flowed plasma process of micropatterning could be used as a strategy to mimic the organized alignment and cellular organisation of the skeletal muscle tissue. The micropatterned environment was able to pattern and align the actin cytoskeleton of the C2C12 cells. The alignment and elongation of myoblasts due to micropatterns provides the prerequisites for mimicking the skeletal muscle organization. Thus, the application of the simple micropatterning technique was useful to regulate the orientation and behaviour of C2C12 cells. The thesis applies novel methods to study the influence of micropatterning on differentiation and alignment of primary myoblasts derived from satellite cells; the micropatterned environment influenced the transfer of alignment information between the primary myoblasts leading to alignment and regulation of myotubes size. These observations are necessary for revealing key information regarding the formation of skeletal muscle tissue.

The future prospective of the current work may be as described in the following text section. The bioink used in the current study could be used to print multiple cell types,

including vascular endothelial cells and neurons along with myoblasts to obtain a vascularised or innervated skeletal muscle tissue. The bioink used in this study includes sodium alginate, gelatin and hydrolysed collagen type I, although alginate provides printability of bioink it has been associated with lack of in vivo stability due to ion exchange with body fluids. This problem can be overcome by using various chemical based crosslinking strategies targeting hydroxyl and carboxyl groups with processes like acetylation, phosphorylation and sulfation. UV based crosslinking can also be applied to stabilize the in vivo degradation of alginate. Such crosslinked alginate can be combined to optimize a bioink with better in vivo stability, this will also be reflected in the corresponding bioprinted constructs. The application of hydrolysed collagen type-I as a source of cell adhesive bioink component can be done in other bioprinting applications. Bioprinting of skeletal muscle constructs can be combined with mechanically conditioning by providing different regimes of mechanical stimulus to train the artificial skeletal muscle tissue to better acclimatize in harsh wound environment. Such an in vitro model can also be used to study the exercising of skeletal muscle tissue and the resultant adaptive responses due to the exercise.

The optically sensitive HepG2, NIH3T3, and C2C12 cells can be utilized to create in vitro models with cationic imbalances, which may replicate various diseased states in hepatic and skeletal muscle tissues. Co-culture models involving the optically sensitive cells can be used to fabricate light controlled physiological and pathological models for developing therapeutic strategies to enhance skeletal muscle regeneration. The optically sensitive skeletal muscle cells could be 3D bioprinted in gelatin alginate and hydrolysed collagen based bioink to fabricate 3D printed optically sensitive in vitro skeletal muscle model. This optically sensitive skeletal model could be combined with PDMS micropillar in order to obtain force based functional output on optical stimulation. Such in vitro

models have great potential for applications in bioactuation, non-invasive drug delivery, multifunctional implants and high throughput drug screening.

The Microchannel flowed plasma process of micropatterning could be used as platform to incorporate skeletal specific ECM materials like Laminin in the APTES region in order to better mimic the in vivo environment. Such micropatterned environments could be used to study the influence of micropatterning on sarcomere patterning, calcium dynamics and contractility of primary myotubes, under the influence of electrical stimulus and optical stimulus. The micropatterning procedure also provides an ideal environment with physiologically relevant micropattern widths for coculture of optically sensitive neurons and skeletal muscle myotubes in order to develop neuromuscular junction models. The microchannel flowed plasma process of micropatterning could be applied to create micropatterned platforms for facilitating experiments on cell-cell interaction, cellular migration, drug screening, in vitro disease models and toxicity assays for a wide variety of cells.