Chapter 6

CONCLUSIONS AND FUTURE PERSPECTIVES
CONCLUSIONS AND FUTURE PERSPECTIVES

The central goal of this doctoral dissertation was to study the feasibility of liver tissue engineering using a novel vascularized acellular liver derived bioscaffold. The assessment of alternative hepatic cell sources was also sought by specifically investigating the use of hFL progenitor cells and hAFS cells in liver cell therapies and liver tissue engineering.

The major implication of this work is the simple method of generating a biodegradable, biocompatible, vascularized scaffold with the equal amount of complexity as that seen in nature and with broad potential for liver and other organs bioengineering. This method may also reach beyond liver organ engineering as the perfusion decellularization process has been applied successfully to other organs such as heart, lung, kidney, and vascularized segments of small intestine with success.

Efficient re-cellularization of the liver bioscaffold with primary hepatocytes was also pursued. Generation of liver tissue using an optimized perfusion cell seeding process and bioreactor pre-conditioning yielded a considerable tissue mass expressing hepatic associated functions with 3D tissue formation in vitro. This seeding method provides the opportunity to adequately bioengineer liver and other organs, using the same type of bioscaffolds. The ability of these whole liver matrices to be densely re-cellularized by perfusion seeding may yield new insights into the mechanisms of tissue morphogenesis and regeneration, and ultimately lead to the development of solid organs for transplantation.

The use of liver derived matrix for hFL progenitor cell seeding opens new possibilities in the generation of hepatocytes in vitro for drug discovery, cell therapies and liver organ engineering. Our results indicate that hFL engraft, expand and are able to differentiate under certain conditions in the liver bioscaffold.
Finally, the multipotency and described characteristics and *in vivo* engraftment potential demonstrated by hAFS cells makes them of capital importance in human disease. The ability to differentiate these cells in hepatic progenitor cells converts them also in a potentially vital asset for liver cellular therapies and liver tissue engineering.

Our future perspectives with these organ bioscaffolds are vast. They provide a new tool to study cells seeded in an authentic 3D environment that mimics the native organ matrices allowing also for the constant perfusion of oxygen and nutrients, something elusive until now. Further investigation of long-term cultures and cell differentiated function maintenance in these bioscaffolds is also being pursued. Moreover, the use of bioengineered livers allows the testing of new drugs for liver metabolization and cytotoxicity.

We also expect that in the near future, both hFL cells and amniotic fluid stem cells will become a relevant, vital tool in stem cell research, tissue engineering, and regenerative medicine. Thus, the combination of the liver bioscaffold technology with liver stem/progenitor cells will potentially enable the generation of 3D liver tissue with a patent vascular network. These bioengineered organs will be capable of being directly anastomosed and transplanted, presenting new therapeutical strategies for patients with end-stage liver disease.