CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction to Stem Cells 1
1.2 What are Stem Cells 2
1.3 Properties and Anatomic Location of Stem Cells 3
1.4 Maintenance of Stemness 4
1.5 Types of Stem Cells 10
  1.5.1. Embryonic Stem Cells 11
  1.5.2. Germinal Stem Cells 14
  1.5.3 Stem Cells from Umbilical Cord Blood 14
  1.5.4 Adult Stem Cells 15
    1.5.4.1 Mesenchymal Stem Cells 19
      1.5.4.1.1 Sources of Primary MSC 20
      1.5.4.1.2 Isolation of Primary MSC 20
      1.5.4.1.3 Surface Markers on MSC 22
      1.5.4.1.4 Basic Biology and Function of MSC 23
      1.5.4.1.5 Circulation and Niche of MSCs 27
      1.5.4.1.6 Differentiation 30
      1.5.4.1.7 Clinical Application of MSCs 31
    1.5.4.2 Limbal Stem Cells 36
      1.5.4.2.1 The Cornea 36
      1.5.4.2.2 Limbal Epithelial Stem Cells 36
      1.5.4.2.3 Evidence for the Location of LESC to the Limbus 38
      1.5.4.2.4 The LESC Niche 41
      1.5.4.2.5 Stromal Stem Cells 47
1.6. Scope and Aim of the Study 49
  1.6.1. Scope of the Study 49
  1.6.2 The Focus of the Thesis 50
CHAPTER 2

ISOLATION, CHARACTERIZATION AND DIFFERENTIATION POTENTIAL OF RAT BONE MARROW STROMAL CELLS

2.1. Introduction 51
2.2 Hypothesis 53
2.3. Aims 53
2.4. Material & Methods 53
  2.4.1. Preparation of Chemicals 53
  2.4.2. Sterility Check of Chemicals 53
  2.4.3. Source of Animals 54
  2.4.4. Isolation of Mononuclear Cells 54
  2.4.5. Cell Culture Conditions 55
  2.4.6 Colony Forming Assays 55
  2.4.7. Characterization of Mononuclear and Stromal cells 55
    2.4.7.1 Immunophenotyping 55
    2.4.7.2 Flow Cytometry 56
    2.4.7.3 Reverse-Transcription PCR 58
  2.4.8 Differentiation Potential 63
    2.4.8.1 Adipogenic Differentiation 63
    2.4.8.2 Osteogenic Differentiation 63
    2.4.8.3 Neural Differentiation 64
2.5. Results 64
  2.5.1 Isolation and Culturing of MSCs 64
  2.5.2 Characterization 66
    2.5.2.1 Flow Cytometry 66
    2.5.2.2 Immunocytochemistry 68
    2.5.2.3 Reverse-Transcription PCR 70
  2.5.3 Differentiation 71
    2.5.3.1 Adipogenic and Osteogenic Differentiation 71
    2.5.3.2 Neural Differentiation 72
2.6 Discussion 73
# CHAPTER 3

**ISOLATION, CHARACTERIZATION OF HUMAN BONE MARROW STROMAL CELLS AND THEIR DIFFERENTIATION POTENTIAL**

3.1. Introduction 79  
3.2. Hypothesis 80  
3.3. Aims 81  
3.4. Material and Methods 81  
   3.4.1. Preparation of Chemicals 81  
   3.4.2. Sterility check of Chemicals and Media 81  
   3.4.3. Source of Bone Marrow 81  
   3.4.4. Isolation of Bone Marrow Stromal Cells 82  
      3.4.4.1 Isolation of Bone Marrow Mononuclear Cells 82  
      3.4.4.2 Cell Counting 82  
   3.4.5. Cell Culture Conditions 83  
   3.4.6. Population Doublings 83  
   3.4.7 Colony-forming Assay 83  
   3.4.8. Differentiation 84  
      3.4.8.1 Adipocytic differentiation 84  
      3.4.8.2 Osteocytic differentiation 84  
      3.4.8.3 Neural differentiation 84  
   3.4.9. Characterization of Cells 85  
      3.4.9.1 Immunocytochemistry 85  
      3.4.9.2 Flow cytometry analysis 86  
      3.4.9.3 Microarray Experiment 88  
      3.4.9.4 RT-PCR 88  
3.5. Results 89  
   3.5.1. Isolation and Culturing of MSC-BM 89  
   3.5.2. Characterization of MSC’s 91  
      3.5.2.1 Flow Cytometry 91  
      3.5.2.2 Immunocytochemistry 94  
   3.5.3. Differentiation of MSC’s 95
3.5.3.1 Adipocytic and Osteogenic Differentiation 95
3.5.3.2 Neural differentiation 96
   3.5.3.2.1 RT-PCR 96
   3.5.3.2.2 Immunocytochemistry 97
   3.5.3.2.3 Microarray 97
      3.5.3.2.3.1 Quantity and Quality of RNA 97
      3.5.3.2.3.2 Quantity and Quality of cRNA 100
      3.5.3.2.3.3 Microarray analysis 101
3.6. Discussion 104

CHAPTER 4
MESENCHYMAL CELLS FROM LIMBAL STROMA OF HUMAN EYE
4.1. Introduction 109
4.2. Hypothesis 110
4.3. Aims 110
4.4 Material and Methods 111
   4.4.1. Preparation of Chemicals 111
   4.4.2. Sterility Check of Chemicals and Media 111
   4.4.3 Source of Limbal Tissue 111
   4.4.4. Preparation of HAM 111
   4.4.5. Human Corneal Epithelial Medium 112
   4.4.6. Processing of HAM 113
   4.4.7. Explanting of Limbal Epithelial Tissue 113
   4.4.8. Culture of Limbal Epithelial Cells in HCE Medium 113
   4.4.9. Establishment of Stromal Cell Cultures 114
   4.4.10. Colony-forming Unit Assays 115
   4.4.11. Population Doublings 115
   4.4.12. Characterization 116
      4.4.12.1 Flow cytometry 116
      4.4.12.2. Immunocytochemistry 117
CHAPTER 5
GENE EXPRESSION PROFILE OF LIMBAL EXPLANT CULTURE DERIVED CELLS

5.1. Introduction

5.2. Hypothesis

5.3. Aim

5.4. Material and methods
   5.4.1. Preparation of Chemicals
   5.4.2. Sterility Check for Chemicals and Media
   5.4.3. Establishment of Cell Cultures
   5.4.4. Microarray
      5.4.4.1. Isolation of Total Cellular RNA
      5.4.4.2. Quality and Quantification of RNA
      5.4.4.3. Purification of RNA
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4.4.4. Quality and Quantity of RNA</td>
<td>146</td>
</tr>
<tr>
<td>5.4.4.5. cDNA and cRNA Preparation</td>
<td>148</td>
</tr>
<tr>
<td>5.4.4.6. Purification of the labeled RNA</td>
<td>150</td>
</tr>
<tr>
<td>5.4.4.7. Quality and Quantification of cRNA</td>
<td>151</td>
</tr>
<tr>
<td>5.4.4.8. Hybridization</td>
<td>151</td>
</tr>
<tr>
<td>5.4.4.9. Microarray Wash</td>
<td>153</td>
</tr>
<tr>
<td>5.4.4.10. Microarray Image and Data Analysis</td>
<td>153</td>
</tr>
<tr>
<td>5.4.5. Validation of Microarray</td>
<td>154</td>
</tr>
<tr>
<td>5.5. Results</td>
<td>156</td>
</tr>
<tr>
<td>5.5.1. Quantity and Quality of RNA</td>
<td>156</td>
</tr>
<tr>
<td>5.5.2. Quality and Quantity of cRNA</td>
<td>159</td>
</tr>
<tr>
<td>5.5.3. Microarray Data Analysis</td>
<td>160</td>
</tr>
<tr>
<td>5.5.4. Validation of Microarray</td>
<td>173</td>
</tr>
<tr>
<td>5.6. Discussion</td>
<td>175</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>181</td>
</tr>
<tr>
<td>LIMITATIONS</td>
<td>189</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>191</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>211</td>
</tr>
<tr>
<td>PUBLICATIONS &amp; PRESENTATIONS</td>
<td>217</td>
</tr>
</tbody>
</table>