## Contents

Abstract I  
Resumen IV  
Resum VII  
Abbreviations X  

### 1. Introduction  
1.1 Cell-protein-material interaction 2  
1.2. Role of the culture dimensionality 6  
1.3. Sandwich-like culture 10  

### 2. Objectives  
21  

### 3. Materials and Methods  
3.1 Materials 24  
3.2 Materials fabrication 25  
3.3 Materials characterisation 28  
3.4 Cell culture 30  
3.5 Biological characterisation 32  
3.6 Statistical Analysis 38  
3.7 References 38  

### 4. Cell morphology and adhesion  
4.1 Introduction 40  
4.2 Materials and methods 41  
  4.2.1 Materials 41  
  4.2.2 Cell culture 42  
  4.2.3 Biological characterisation 42  
4.3 Results and discussion 43  
  4.3.1. Role of the ventral substrate in cell morphology 43  
  4.3.2 Effect of non-adhesive dorsal contact on cell morphology 48  
  4.3.3 Cell fate within sandwich culture with different dorsal stimulation 51  
4.4 Conclusions 63
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 References</td>
<td>64</td>
</tr>
<tr>
<td><strong>5. Cell migration</strong></td>
<td>69</td>
</tr>
<tr>
<td>5.1 Introduction</td>
<td>70</td>
</tr>
<tr>
<td>5.2 Materials and methods</td>
<td>71</td>
</tr>
<tr>
<td>5.2.1 Material</td>
<td>71</td>
</tr>
<tr>
<td>5.2.2 Cell culture</td>
<td>72</td>
</tr>
<tr>
<td>5.2.3 Biological characterisation</td>
<td>73</td>
</tr>
<tr>
<td>5.3 Results and discussion</td>
<td>73</td>
</tr>
<tr>
<td>5.3.1 Phenomenology of cell migration within sandwich environments</td>
<td>73</td>
</tr>
<tr>
<td>5.3.2 Characterisation of the migrating cells</td>
<td>76</td>
</tr>
<tr>
<td>5.3.3 Ventral ECM remodelling during cell migration</td>
<td>81</td>
</tr>
<tr>
<td>5.3.4 Role of the ventral substrate in cell migration</td>
<td>83</td>
</tr>
<tr>
<td>5.3.5 Role of dorsal stimuli in cell migration</td>
<td>87</td>
</tr>
<tr>
<td>5.4 Conclusions</td>
<td>88</td>
</tr>
<tr>
<td>5.5 References</td>
<td>89</td>
</tr>
<tr>
<td><strong>6. Induced C2C12 differentiation</strong></td>
<td>95</td>
</tr>
<tr>
<td>6.1 Introduction</td>
<td>96</td>
</tr>
<tr>
<td>6.2 Materials and methods</td>
<td>97</td>
</tr>
<tr>
<td>6.2.1 Material</td>
<td>97</td>
</tr>
<tr>
<td>6.2.2 Cell culture</td>
<td>97</td>
</tr>
<tr>
<td>6.3 Results and discussion</td>
<td>98</td>
</tr>
<tr>
<td>6.3.1 Material characterisation</td>
<td>98</td>
</tr>
<tr>
<td>6.3.2 Initial spreading and morphology; dorsal and ventral cues</td>
<td>99</td>
</tr>
<tr>
<td>6.3.3 Dorsal stimuli enhance myogenic differentiation</td>
<td>102</td>
</tr>
<tr>
<td>6.3.4 Integrin expression and cell signalling</td>
<td>106</td>
</tr>
<tr>
<td>6.3.5 Dorsal integrins and cell differentiation</td>
<td>110</td>
</tr>
<tr>
<td>6.3.6 Release of paracrine factors</td>
<td>114</td>
</tr>
<tr>
<td>6.4 Conclusions</td>
<td>115</td>
</tr>
<tr>
<td>6.5 References</td>
<td>117</td>
</tr>
</tbody>
</table>
7. Spontaneous cell differentiation  
  7.1 Introduction  
  7.2 Materials and methods  
    7.2.1 Material  
    7.2.2 Cell culture  
  7.3 Results and discussion  
    7.3.1 C2C12 differentiation  
    7.3.2 hMSCs differentiation  
  7.4 Conclusions  
  7.5 References

8. Conclusions