Mechanical Stretching Inhibits Monocytes/Macrophage Proliferation and Activation on Collagen-Based Tissue Engineering Substrates

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Introduction
The interactions between tissues, cells and biomaterials has been largely investigated to provide suitable tissue engineering constructs. However, the impact of the host response towards tissue engineering constructs remains a neglected area of research. In particular, insufficient information is available clarifying the interactions between inflammatory cells and substrates undergoing mechanical stimuli (1). The present work has evaluated the effect of stretching on the proliferation and activation of monocytes/macrophages when adhering on collagen, one of the most commonly utilised substrates for cardiovascular tissue engineering applications.

Materials and Methods
Silicone elastomer membranes were activated in a plasma barrel etcher (Polaron PT7150) in an oxygen plasma and coated with a 0.02% v/v collagen type I in PBS solution. The samples were washed three times, dried at room temperature and UV sterilised. The coating stability was assessed before and after stretching at 1 Hz for 5 days by a stretching apparatus in Dulbecco-Modified Eagles’ Medium (DMEM) at 37°C by Picrosirius Red staining and scanning electron microscopy (SEM). Mononuclear cells were freshly isolated from peripheral human blood from 6 healthy donors. The collagen coated silicone elastomer membranes were preconditioned with fresh human plasma and seeded with 2x10^6 cells/membrane in 5 ml of DMEM. Cells were allowed to adhere for 1 h, the surfaces were then washed to remove non-adherent cells and 10% foetal bovine serum-enriched DMEM added. Experiments were performed at 1 and 5 days under either 16 % strain, 1 Hz frequency or static conditions at 37°C, 5% CO₂, 95% air. Immunostaining with CD68 and MAC387 antibodies was performed and counted by epi-fluorescence microscopy (x40 magnification). Six different fields/sample were counted and data were expressed as number of cell/field ± standard deviation (SD).

Results
Positive immunostaining for CD68 and MAC387 was observed under both static and stretching conditions. Macrophages underwent proliferation on collagen in static conditions. The number of cells observed after 1 day incubation under stretching was not significantly different from that found after 1 h incubation in static conditions (Table 1).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Time (h)</th>
<th>Number of Cells/Field ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static</td>
<td>1</td>
<td>12±6</td>
</tr>
<tr>
<td>Static</td>
<td>24</td>
<td>227±66</td>
</tr>
<tr>
<td>16 % Strain, 1 Hz</td>
<td>24</td>
<td>34±20</td>
</tr>
</tbody>
</table>

Table 1. Macrophage proliferation on collagen substrates under static (1 and 24h) and stretching conditions (24 h)

Discussion and Conclusions
Immunostaining positively identified the cells as activated macrophages. The similar cell levels observed after 1 h incubation and 1 day after stretching clearly showed that the mechanical stimuli inhibited cell proliferation and activation rather than affecting their adhesion properties. The release of cells from the substrate was also ruled out by the stability of the coating over 5 days of stretching. The data of this work seems to suggest that stretching conditions may inhibit the inflammatory potential towards scaffolds for vascular tissue engineering.

References

Disclosures
The authors have no conflict of interest related to this work.