Introduction and purpose: The purity and composition of biomaterials used in cell culturing are important for efficient cell proliferation. Endotoxins are contaminants which can be present in biomaterials and can alter cell responses. We examined the proliferation efficiency on coatings of different biomaterials with different endotoxin levels and different molecular weight.

Methods: 3D printed and plasma activated acrylic discs were coated with type A gelatins (Rousselot® X-Pure® and Sigma G1890) with different endotoxin levels and average molecular weight as indicated in table 1. The gelatin coatings were compared to fibronectin (Sigma F4759) as the ‘standard’ coating material and to the uncoated (plasma activated) control. The discs were coated with 1 µg/ml of the indicated biomaterial in volume of 2 ml for 90 minutes at room temperature. After coating, HTERT AD MSCs were seeded in AdipoUp medium at a density of 9000 cells/disc in 75 µl. Culture and proliferation of cells were followed using lactate production of the cells. At day 7, cells are fully in the exponential phase and the differences among the different coatings are most pronounced. Lactate measurements in the culture media were performed at day 2, 5 and 7. The morphology of the cells was evaluated using a bridgefield, microscope at day 5. Endotoxin (LPS) levels were measured with the Hyglos EndoZyme assay and molecular weight distribution was analyzed using GPC.

Results:

Figure 1. Lactate production as measure for HTERT AD cell proliferation on 3D acrylic discs coated with different gelatins and fibronectin.

There is a clear influence of endotoxin and molecular weight on HTERT AD MSC proliferation. An inverse relation is observed between proliferation and both endotoxin levels and average molecular weight. This indicates that the use of endotoxin purified gelatins with a low average molecular weight results in an increased HTERT AD MSCs proliferation.

Figure 2. Influence of endotoxin levels and average molecular weight on lactate production.

Table 1:

<table>
<thead>
<tr>
<th></th>
<th>Rousselot X-Pure® 10HGP</th>
<th>Rousselot X-Pure® 10P</th>
<th>Sigma-Aldrich G1890</th>
<th>Sigma-Aldrich F4759</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mw (kDa)</td>
<td>6.5</td>
<td>155</td>
<td>160</td>
<td>220</td>
</tr>
<tr>
<td>LPS (EU/g)</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>4100</td>
<td>20000</td>
</tr>
</tbody>
</table>

Summary and conclusion

- Culture of HTERT AD MSCs on endotoxin purified gelatin coatings results in a 2 fold induced cell proliferation compared to fibronectin or non-coated conditions.
- Low endotoxin and low molecular weight (X-Pure® 10HGP) gelatin gave the best HTERT AD MSCs cell proliferation and is a very suitable gelatin for cell culturing.
- Based on cell density and morphology, coating with gelatin outperforms the other conditions and there is a preference for the hydrolyzed X-Pure® gelatin 10HGP for culture of HTERT AD MSCs.
- Coating with endotoxin contaminated gelatins results in a substantial leakage of the endotoxins during culture of the cells.
- The use of endotoxin purified gelatins should be standard practice in cell culture applications.