Stem cell proliferation on 3D printed discs improved with low endotoxin and low molecular weight gelatin coating

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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 bridefield, 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.

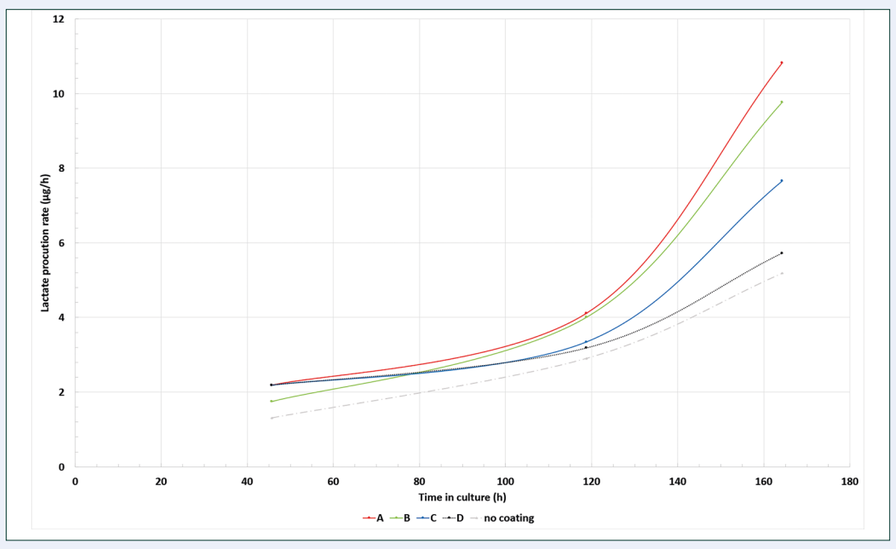
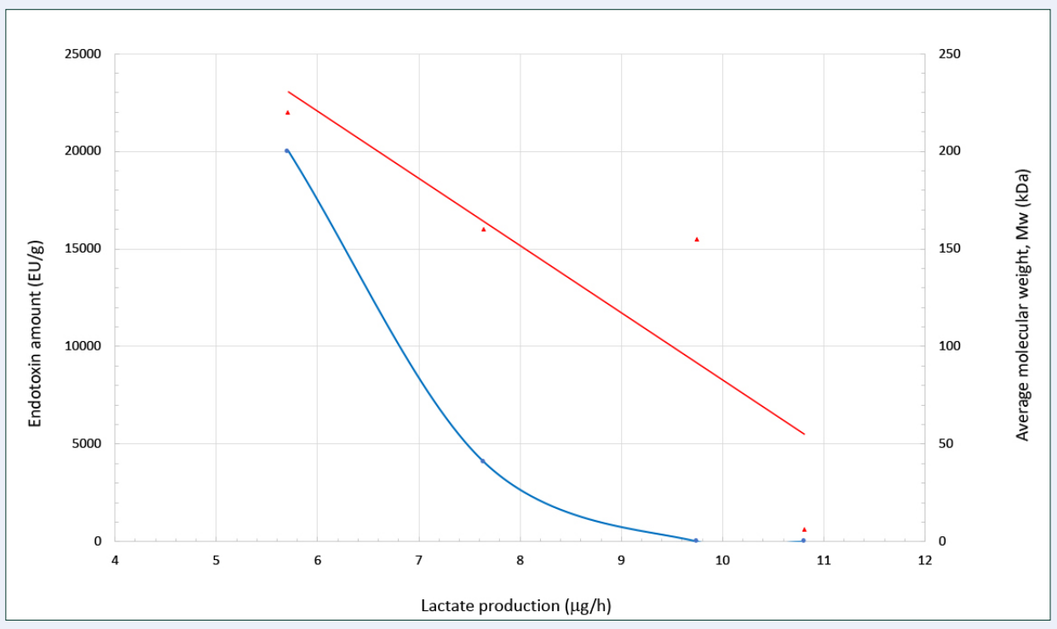


Table 1:
A. Rousselot X-Pure® 10HGP
Mw 6.5kDa. LPS <4EU/g
B: Rousselot X-Pure® 10P
Mw 155kDa. LPS <4EU/g
C: Sigma-Aldrich G1890
Mw 160kDa. LPS 4100EU/g
D: Fibronectin Sigma-Aldrich F4759
Mw 220kDa. LPS 20000EU/g

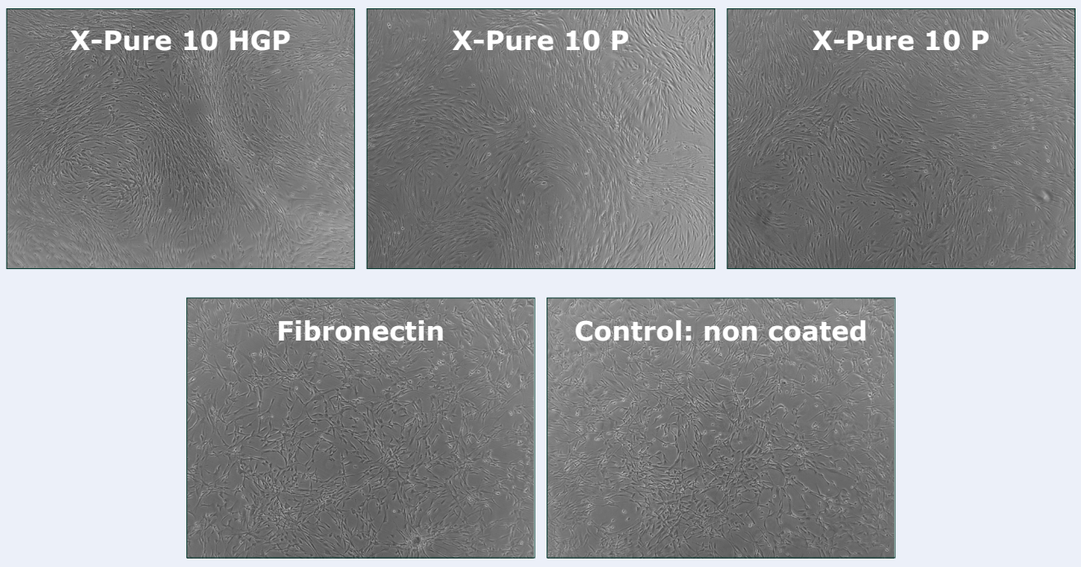
HTERT AD MSCs proliferation was visible on gelatin, fibronectin coated and uncoated 3D acrylic discs. When reaching the exponential phase (day 7), the cell proliferation on gelatin coated discs was much higher than on the fibronectin coated disc. The use of X-Pure® gelatin (A-B) resulted in a 2 fold enhancement of the proliferation at day 7 compared to fibronectin (D) coated or uncoated discs.

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



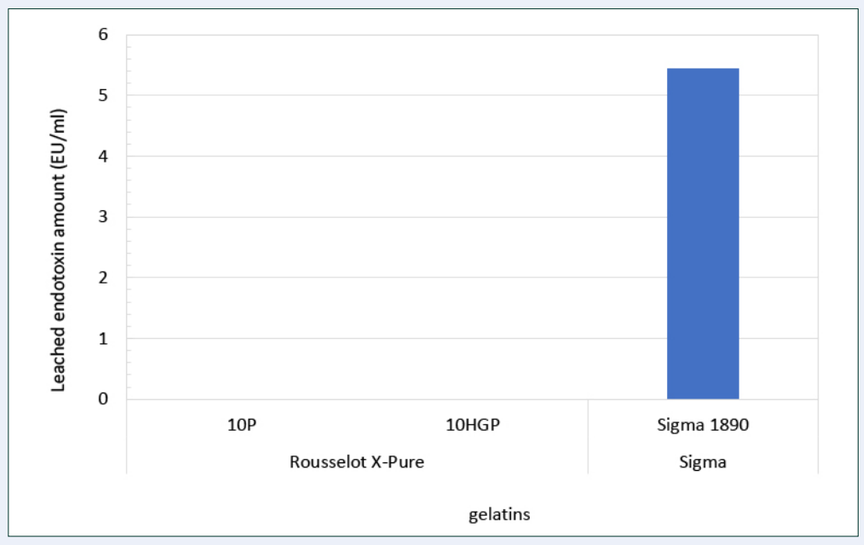
There is a clear influence of endotoxin and molecular weight on HTERT AD MSC proliferation. An inverse relation is observed between proliferation and both endotoxin levels and average molecular weight. This indicates that the use of endotoxin purified gelatins with a low average molecular weight results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

Figure 3. Cell morphology on 3D printed, coated acrylic surfaces



Evaluation of the cell morphology at day 5 of culture shows elongated and aligned cells with a spindle shaped like morphology on the discs coated with gelatin. In contrast, cells on the fibronectin or non-coated discs are visually less dense and show a more diverse and rather cuboidal or stellar shaped morphology.

Figure 4. LPS leaching in medium after coating



Coating with LPS contaminated gelatin Sigma 1890 leads to substantial endotoxin leaching into the culture medium. This implies that cells seeded on the coatings are exposed to endotoxin during culture. As a consequence, the endotoxin can influence proliferation and the phenotype of the HTERT AD MSCs cells, as extensively described in literature.

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

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