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**3. Title:**

Nanotopography reveals metabolites that maintain the immunomodulatory phenotype of mesenchymal stromal cells during large-scale expansion.

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**Abstract**

*Mesenchymal stromal cells (MSCs) are multipotent progenitor cells that are immunomodulatory and thus of considerable therapeutic potential in transplant operations. However, MSCs rapidly differentiate once in culture, making their large-scale expansion for use in immunomodulatory therapies challenging. Although the differentiation mechanisms of MSCs have been extensively investigated using materials, little is known about how materials can influence paracrine activities of MSCs. Here, we show for the first time that nanotopography can control the immunomodulatory capacity of MSCs through decreased intracellular tension increasing oxidative glycolysis. We also use the nanotopography to identify bioactive metabolites that modulate intracellular tension, growth and immunomodulatory phenotype of MSCs in standard culture and during larger scale cell manufacture. Our findings show a novel route to support large-scale expansion of functional MSCs for therapeutic purposes.*

**5. Funder information:**

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**6. Date data set can be made public:** immediately.

**7. Restrictions:**  No restrictions.

**8. Ethical approval:** not required.

**9. preferred licence:** No preferred.

**Data folders**

**Figure 1**

**Figure 1.xlsx** contains works sheets for Figure 1c, d, e, f, h & i.

Figure 1c – flow cytometry data exported from FlowJo software to asses proliferation using CFSE. Histograms produced on FlowJo software from this data.

Figure 1d - flow cytometry data exported for FlowJo software to calculate proliferation index.

Figure 1e – cell counts and means calculated.

Figure 1f – Flow cytometry data exported from FlowJo software to assess MSC proliferation index.

Figure 1h - Flow cytometry data exported from FlowJo software to assess suppression of PBMC proliferation by MSCs cultured with Y27632. Proliferation index calculated.

Figure 1i – Fold changes calculated using flow cytometry data from Figure 1h.

**Figure 2**

**Figure 2.xlsx** contains works sheets for Figure 2c, d, f, g, h & i.

**Untargetted Metabolomics.xlsx** contains LC-MS data processed by Glasgow Polyomics facility.

Figure 2a – metabolites filtered from Untargeted Metabolomics.xlsx and used to generate heatmap using metabolanalyst.ca

Figure 2D – fold changes calculated from Untargeted Metabolomics.xlsx

Figure 2F – gating strategy for JC1 flow cytometry represented in SFig 3, using FlowJo software. Ratio MFIs and calculated fold changes in Figure 2.xlsx.

Figure 2g and h – MFIs calculated using flow cytometry and FlowJo software.

Figure 2i – flow cytometry data for JC1 staining from flowjo software.

**Figure 3**

**Figure 3.xlsx** contains works sheets for Figure 3b, c & d.

**13C Heavy Labelled Targetted Metabolomics.xlsx** contains LC-MS data processed by Glasgow Polyomics facility.

Figure 3b – generated using data from 13C Heavy Labelled Targetted Metabolomics.xlsx, Figure 3.xlsx contains calculations of fold changes.

Figure 3c – MFI values from flow cytometry, exported from FlowJo software. Background subtracted and means calculated in Figure 3.xlsx.

Figure 3d - Cell culture supernatants were collected, aliquoted and frozen at -80°C. Lactate levels in supernatants were quantified using the Lactate-Glo chemiluminescence assay (Promega), and luminescence measured using a Pherastar FS plate reader (BMG Labtech). Calculations in Figure 3.xlsx.

**Figure 4**

**Figure 4.xlsx** contains works sheets for Figure 4a, b & c.

Figure 4a – flow cytometry data exported from flowjo software. PBMC proliferation index calculated from CFSE stained cells MFI values.

Figure 4b – qRT-PCR data. Ct values and calculations for ∆∆CT calulations.

Figure 4c – MFI values generated from flow cytometry using flowjo software.

**Figure 5**

**Figure 5.xlsx** contains works sheets for Figure 5a, b, c & d.

Figure 5a – T cell proliferation index in MSCs cultured in metabolites.

Figure 5b – list of metabolites used to generate heatmap. Taken from Untargeted Metabolomics.xlsx.

Figure 5c – cell counts.

Figure 5d – Raw values generated from western blot.

**Figure 6**

**Figure 6.xlsx** contains works sheets for Figure 6c, d & e.

Figure 6c & d- flow cytometry data exported from flowjo software. PBMC proliferation index calculated from CFSE stained cells MFI values.

Figure 6e – MFI values from flow cytometry exported using flowjo software, isotype controls subtracted and fold change calculated.