**RNA Sequencing of Rat MSCs**

**Aim:** Compare the RNA expression profile among surface, chemical, and biological mediated MSC differentiation of rat MSCs.

**1. Validation of Rat MSCs**

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| --- | --- | --- | --- | --- | --- | --- |
| **Group** | **Cell Type** | **Surface** | **Treatment** | **n** | **Cells** | **Media** |
| 1 | rMSCs | TCPS | MSCGM | 6 | 0.12M | 12mL |
| 2 | PT | MSCGM | 6 | 0.12M | 12mL |
| 3 | SLA | MSCGM | 6 | 0.12M | 12mL |
| 4 | mSLA | MSCGM | 6 | 0.12M | 12mL |

1. Cells will be plated at a density of 10,000 cells/cm2 (20,000 cells/well) in a 24-well plate.

2. Cells will be fed with MSCGM 24h after plating and every 48h after the initial media change.

3. After 7d, cells will be incubated with fresh MSCGM for 24h.

4. After 24hr, media will be collected and immunoassays performed for OCN, BMP2, and OPN.

5. Monolayers will be lysed in 0.05% Triton X-100.

6. DNA, protein, and alkaline phosphatase activity will be measured in the cell lysate.

7. If a surface effect is achieved, the following experiment will begin.

**2. MSC Culture for RNA Sequencing**

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| --- | --- | --- | --- | --- | --- | --- |
| **Group** | **Cell Type** | **Surface** | **Treatment** | **n** | **Cells** | **Media** |
| 1 | rMSCs | TCPS | MSCGM | 24 | 0.48M | 48mL |
| 2 | TCPS | OM | 24 | 0.48M | 48mL |
| 3 | TCPS | MSCGM + BMP2 | 24 | 0.48M | 48mL |
| 4 | PT | MSCGM | 24 | 0.48M | 48mL |
| 5 | mSLA | MSCGM | 24 | 0.48M | 48mL |
| 6 | Surface 9 (Titan) | MSCGM | 24 | 0.48M | 48mL |

1. Cells will be plated at a density of 10,000 cells/cm2 (20,000 cells/well) in a 24-well plate.

2. Cells will be fed with MSCGM 24h after plating and every 48h after the initial media change.

3. After 7d of cultures, cells will be incubated with fresh treatment media for 12h.

4. 8 wells of each group will be combined to make an n=3 for each group

5. RNA will be isolated using a TriZol extraction.

6. Samples will be sent to Genewiz for standard RNA sequencing.

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| **Pricing** | | | | | |
| **Item** | **Price / (units)** | **Units** | **Cost** | |
| Rat MSCs | $369/1M | 3.36M | $369.00 | |
| MSCGM | $219/500mL | 288mL | $126.14 | |
| Osteogenic Differentiation Medium | $199/201mL | 48mL | $47.52 | |
| 24 well plate | $1.75/plate | 7 | $12.25 | |
| OPN | $649/1440s | 51 | $22.99 | |
| BMP2 | $649/1440s | 51 | $22.99 | |
| OCN | $499/128s | 40 | $155.94 | |
| BSA | $5/96s | 51 | $2.66 | |
| DNA | $373/2000s | 51 | $9.51 | |
| Alkaline Phosphatase | $22/100s | 51 | $11.22 | |
| RNA Extraction | $0.90/sample | 18 | $16.20 | |
| RNA Sequencing |  | 18 | $5240.00 | |
| **Total Cost** | | | | $6036.42 | |