Differentiation of human MSCs into hepatic and cholangiocyctic cells by various concentration of collagen type I

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Human Adipose Tissue-Derived Mesenchymal Stem Cells 배양시 효율적인 Extracellular Matrix의 중용

The Extracellular Matrix Affected Proliferation and Cell Adhesion of Human Adipose Tissue Derived Mesenchymal Stem Cells in vitro

**Conclusion:**
- The cell morphology can be changed faster in the FN coated culture plates than that in the non coated culture plates.
- Because Proliferation and adhesion with FN can enhance the expansion, the culture within a FN coated plate is needed to encourage hAD-MSCs to proliferate *in vitro*. 
Liver composed of various extracellular matrix (ECM), and Collagen type I (Col I) is ECM which supports most hepatocytes.

Also, in hepatic differentiation study of mesenchymal stem cells, Col I has been used to improve efficient hepatic induction.

However, the standard concentration of Col I has not yet been established.
To examine effective concentration of Col I for hepatic or cholangiocytic induction of mesenchymal stem cells.
Isolation of hADSCs

Chopping

Tx with collagenase for 30min

Isolation of fat supernatant

Precipitated MSC

Cell purification

Cultivation
Hepatic & Cholangiocytic Differentiation


Step 1 Conditioning Stage
- Activin A
- bFGF
- EGF
- nicotinamide

Step 2
- bFGF
- HGF
- EGF

Step 3
- OSM or BMP
- Dex or DMSO

Undifferentiated hADSC
- 3 days
- 7 days
- 10 days

- Two different Concentrations of Collagen I were coated on culture plate as two groups
  - group I: 5μg/cm² Vs. group II: 500μg/cm²
Different Conc. of HGF & EGF

Control of Hepatic Differentiation by Activin/TGFβ Signaling

Differentiated cells were analyzed by RT-PCR analysis with endodermal and hepatic progenitor markers (Foxa2, HNF4a, & CK19) and stained by immunocytochemistry assay with C-Met, CK19 & CK7 antibodies.

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Primary human adipose tissue derived stem cells

on day 1 after plating

on day 2 after plating

Scale bar = 200\(\mu\text{m}\)
Rapid endodermal induction by Activin A

SMAD4/p-SMAD2/3

Scale bar = 100μm
Hepatic and Cholangiocyctic induction by high concentration of Col I with HGF, bFGF and EGF on 10 days (cell density=1X10^4 cells/cm^2)

Hepatic induction

HGF(50ng/ml), EGF(20ng/ml)

Cholangiocyctic induction

HGF(20ng/ml), EGF(50ng/ml)

Scale bar = 200μm, 100μm, respectively.
RT-PCR analysis

**Step 1**
Conditioning Stage

- Activin A
- bFGF
- EGF

**Step 2**

- bFGF
- HGF
- EGF
- nicotinamide

**Step 3**

- OSM or BMP
- Dex or DMSO

**Timeframes**

- 3 days
- 7 days
- 10 days
### Immunocytochemistry assay

<table>
<thead>
<tr>
<th></th>
<th>c-Met</th>
<th>CK19</th>
<th>CK7</th>
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<tbody>
<tr>
<td>Hepatoblasts</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cholangiocytes</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</table>
Hepatic and Cholangiocyctic induction on collagen coated plate without FBS

Hepatic induction

Col I 5μg/cm²

Col I 500μg/cm²

Cholangiocyctic induction

Col I 5μg/cm²

Col I 500μg/cm²

Scale bar = 200μm
Differentiated cells on plates with high concentrations of Col I (500μg/cm²) were more expressed hepatic progenitor gene compared to low concentration of Col I (5μg/cm²).

Cell morphology via light microscope was more exchanged as a result of hepatic progenitor cells close the polygon (the polygonal shape) on high concentration of Col I.

Also, in cholagiocytic induction group, cell migration was appeared as a result of circle formation.

In hepatic and cholangiocyctic induction, CK19 and CK7 as bipotency hepatic progenitor markers were more expressed on groups using high concentration of Col I.
In hepatic and cholangiocyctic induction using high concentration of Col I, cells were effectively differentiated into hepatoblasts.

These cells could be expected as good cell therapy sources for liver disease patients and bile duct disease.