The effect of endothelial precursor cell-conditioned media on keratinocytes and 3D skin models

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Introduction

- Endothelial precursor cell-conditioned media (EPC-CM) derived from hESC has various secreted cytokines such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF).
- Recently, many studies have been conducted on the clinical efficacy of EPC-CM.
  - Improving the signs of skin aging such as pigmentation and wrinkles
  - Enhancement of normal wound healing
- The mechanism of action and effects of EPC-CM on cells and skin equivalents have been limited.
- In this study, the biological effects of the EPC-CM on keratinocytes and 3D human skin equivalents were evaluated.

Methods

- Primary human epidermal keratinocytes were isolated from the newborn and adult foreskin.
- The effect of EPC-CM on keratinocytes was examined using doubling time, colony forming efficiency.
- Macroscopic and histologic analyses were also performed to examine the effect of EPC-CM on 3D skin equivalents which were produced with fibroblasts and 2 types of keratinocytes, newborn and adult.
- Recovery effects of EPC-CM on ultraviolet B (UVB)-induced damage were evaluated on 3D skin equivalents.
  - When EPC-CM was treated after UVB irradiation, protein levels of procollagen I and matrix metalloproteinase (MMP-1) were measured using Western blot analysis.
  - Masson's trichrome staining was carried out to investigate the effect of EPC-CM on the collagen degradation by UVB in 3D skin equivalents.

Results

Doubling time and colony forming efficiency (CFE) of the newborn and adult keratinocytes from EPC-CM with DME HAM with 5% serum(DHc5%) versus DHc5%H only

Fold Change vs DHc5%H						
			Doubling time		CFE	
	Condition	Passage	Mean	SD	Mean	SD
	DHc5%H	P3	1	0	1	0
		P4	1	0	1	0
		P5	1	0	1	0
Newborn	DHc5%H: EPC-CM /DHc5%H	P3	1.02	0.03	0.97	0.11
		P4	1.05	0.03	1.16	0.05
		P5	0.98	0.00	1.10	0.10
Adult	DHc5%H: EPC-CM /DHc5%H	P3	1.08	0.05	1.01	0.13
		P4	1.05	0.01	1.35	0.10
		P5	1.31	0.07	1.01	0.05

Table 1. Comparisons of doubling time and CFE at each passage (P3 to P5) of the newborn and adult keratinocytes

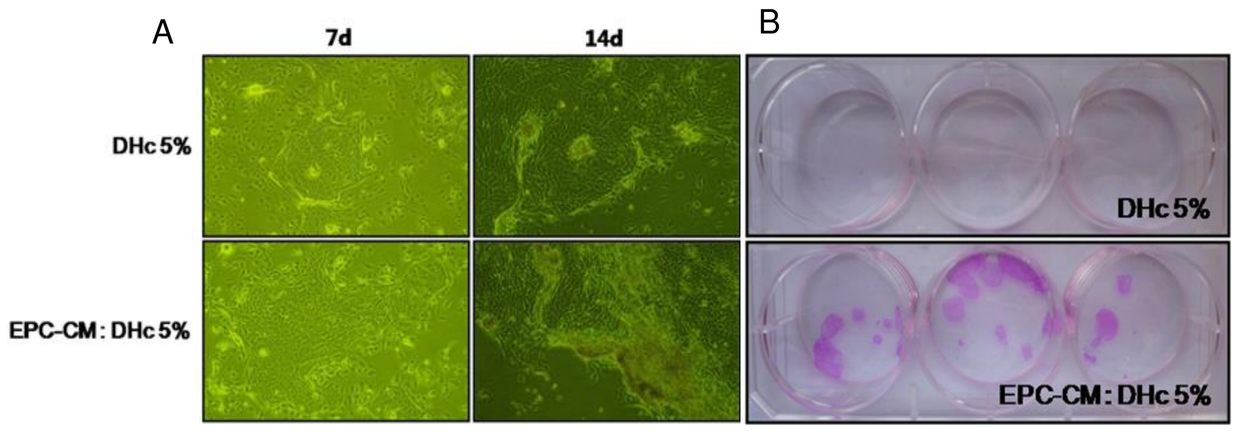

Fig.1. EPC-CM medium effect on keratinocytes. (A) Proliferation (B) CFE

Macroscopic and histological analyses of the 3D skin model

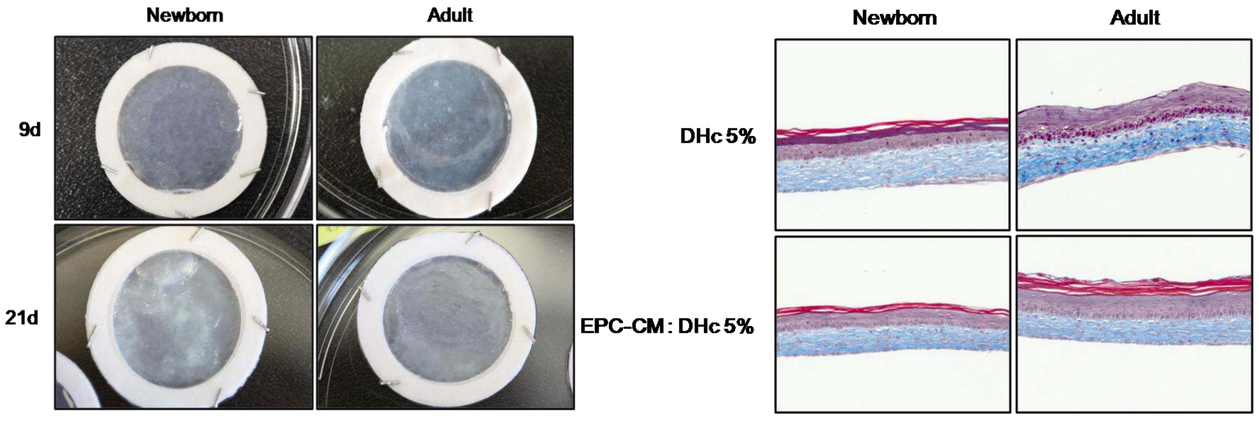

Fig.2. Tissue engineered skins produced with fibroblasts and 2 types of keratinocytes (newborn and adult). (A) Tissue engineered skin was visually observed after 9 and 21 days of culture at the air-liquid interface. (B) Pictures were taken after 21 days of culture at the air-liquid interface stained with Masson's trichrome staining.

The effect of EPC-CM on the protein levels of procollagen I and MMP-1 in 3D skin models

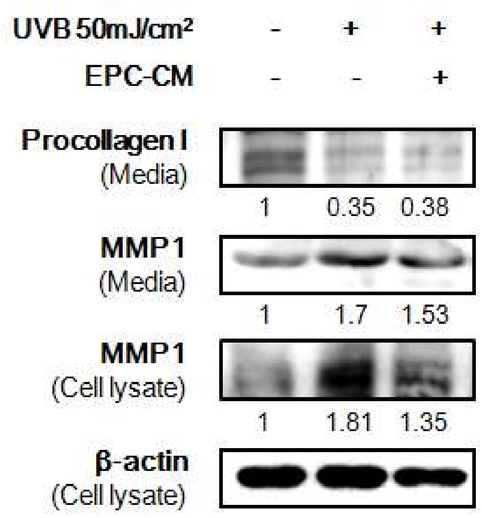

Fig.3. The effect of EPC-CM on the protein levels of procollagen I and MMP-1. The skin equivalents were irradiated with 50mJ/cm² of UVB and then treated with EPC-CM for 24 hrs. Procollagen I and MMP-1 expression was determined by Western blot analysis.

The effect of EPC-CM on the collagen degradation by UVB in 3D skin models

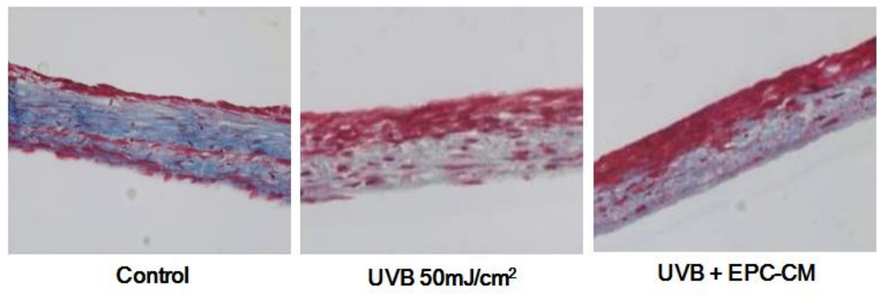

Fig.4. The effect of EPC-CM on the collagen degradation by UVB in 3D skin models. Collagen deposition was analyzed by Masson's trichrome staining and intensity of blue staining showed the amount of content of collagen. The images were taken at 400X magnification.

Conclusion

- When the keratinocytes of adult and newborn were used, it was confirmed that doubling time was maintained in each passage.
  - At the P4, EPC-CM showed higher colony forming abilities.
- When keratinocytes derived from adult skin were used, the horny layer was not observed when using the control medium, failing to form proper skin structures.
  - However, when EPC-CM was used, a proper structure including the horny layer was produced.
- In the study, anti-photoaging effect of EPC-CM was studied in 3D skin equivalents that were irradiated by UVB.
  - Protein level of MMP-1 increased by UVB irradiation was reduced by EPC-CM treatment.
  - As a result, EPC-CM effectively restored expression of MMP-1 that changed by UVB irradiation, but did not significantly affect procollagen I synthesis.
- When 3D skin models were post-treated with EPC-CM, the intensity of blue stain showing the expression of collagen was higher than that of UVB-treated group.
  - From these results, EPC-CM showed inhibitory effect of MMP-1 overexpression and collagen degradation in 3D skin models.

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