Table 1. Major growth factors associated with wound repair

Growth factor	Produced in wound by	Responsive cells	Role in repair
TGF-β (1, 2, 3)	platelet neutrophil macrophage epithelial cell	macrophage fibroblast epithelial cell	Chemotatic to macrophage Inhibitor of proliferation Anti-inflammatory Regulator of differentiation Control matrix synthesis
PDGF (AA, AB, BB)	platelet neutrophil macrophage endothelial cell	neutrophil macrophage fibroblast endothelial cell	Chemotactic to neutrophil and macrophage Proliferation of fibroblast and endothelial cell
EGF	platelet macrophage epithelial cell	keratinocyte fibroblast	Re-epithelialization (keratinocyte migration)
TGF-α	platelet macrophage endothelial cell epithelial cell	keratinocyte fibroblast	Re-epithelialization (keratinocyte migration)
bFGF (FGF-2)	macrophage fibroblast endothelial cell	endothelial cell fibroblast smooth muscle cell	Angiogenesis Fibroblast proliferation
aFGF (FGF-1)	macrophage fibroblast endothelial cell	fibroblast endothelial cell epithelial cell	Proliferation and migration of fibroblast, endothelial cell and epithelial cell Angiogenesis
KGF (FGF-7)	macrophage fibroblast	epithelial cell keratinocyte	Proliferation of epithelial cell Differentiation of epithelial cell
IL-1	macrophage fibroblast keratinocyte	macrophage keratinocyte fibroblast endothelial cell	Inflammatory cell recruitment, matrix synthesis and remodeling
VEGF	platelet macrophage keratinocyte smooth muscle cell	endothelial cell	Angiogenesis (endothelial cell migration and proliferation)
IGF	platelet macrophage fibroblast	keratinocyte fibroblast	Proliferation of fibroblast and keratinocyte

From: Hart J, 1999

Table 2. Primer sets used for RT-PCR

mRNA template	Primer sequence (sense/anti-sense)	PCR product size (bp)
MMP-1	5′-ATTTCTCCGCTTTTCAACTT-3′	167
	5′-ATGCACAGCTTTCCTCCACT-3′	
MMP-9	5′-CATCTTCCAAGGCCAATCCTACTCC-3′	441
	5′-GATGCCATTCACGTCGTCCTTATGC-3′	
β-actin	5′-CGTCATACTCCTGCTTGCTGATCCACATCTGC-3′	870
	5′-ATCTGGCACCACACCTTCTACAATGAGCTGCG-3′	

From: S Holvoet et al., 2003

Table 3. Various growth factors in porcine placental extracts with different extraction solutions determined by ELISA

Placental extract (1 mg/ml)	bFGF (ng/ml)	EGF	PlGF
ddH_2O	24.35±1.98	10.39±1.56	2.53±0.47
0.1M NaCl	29.01±3.54	13.03±1.07	2.31±0.04
1M $MgCl_2$	33.52 ±2.64	23.23±2.53	3.40±0.34
2M Urea	86.46±2.36	50.36 ±1.65	8.96±0.24

Average ± SD.

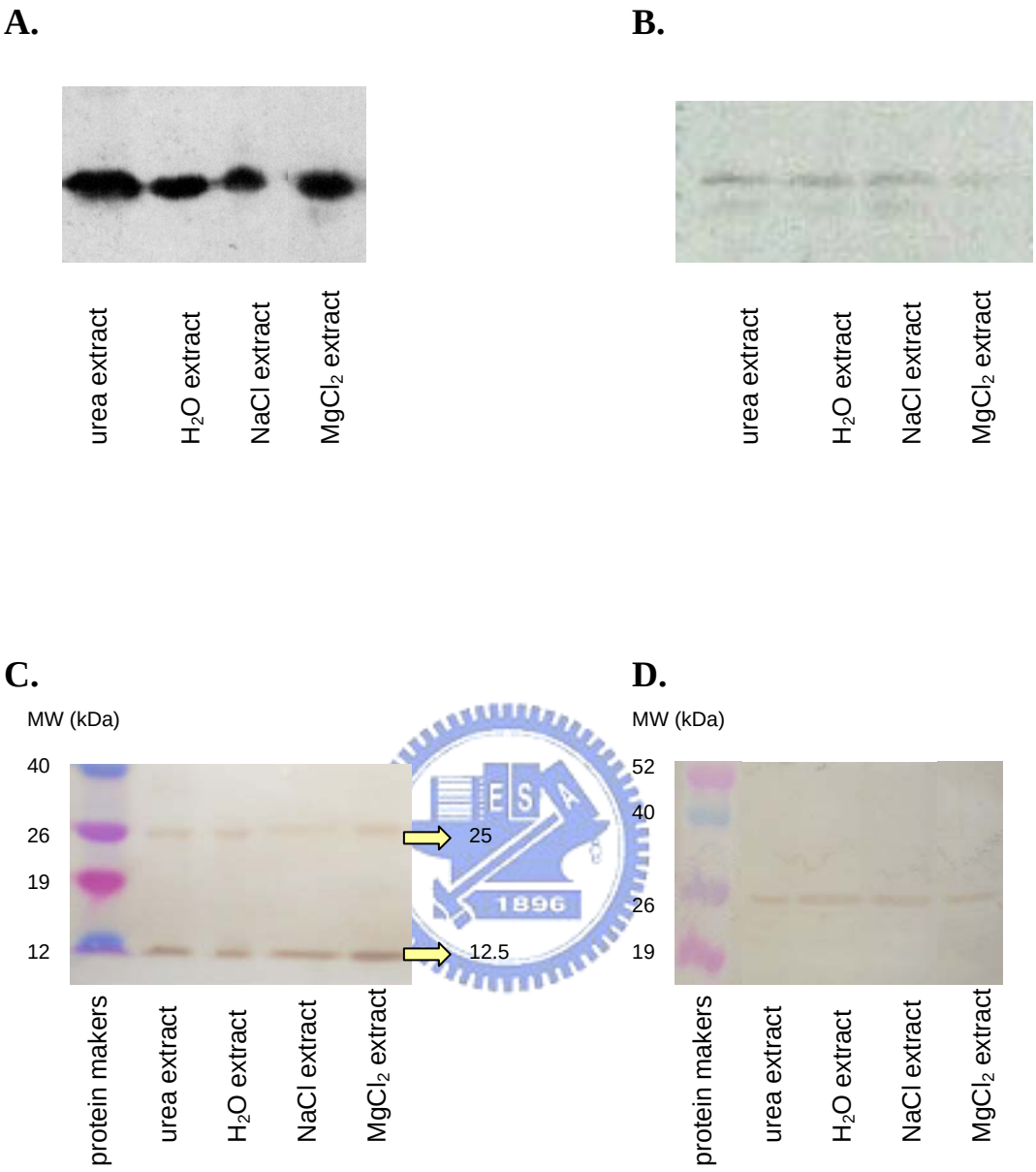
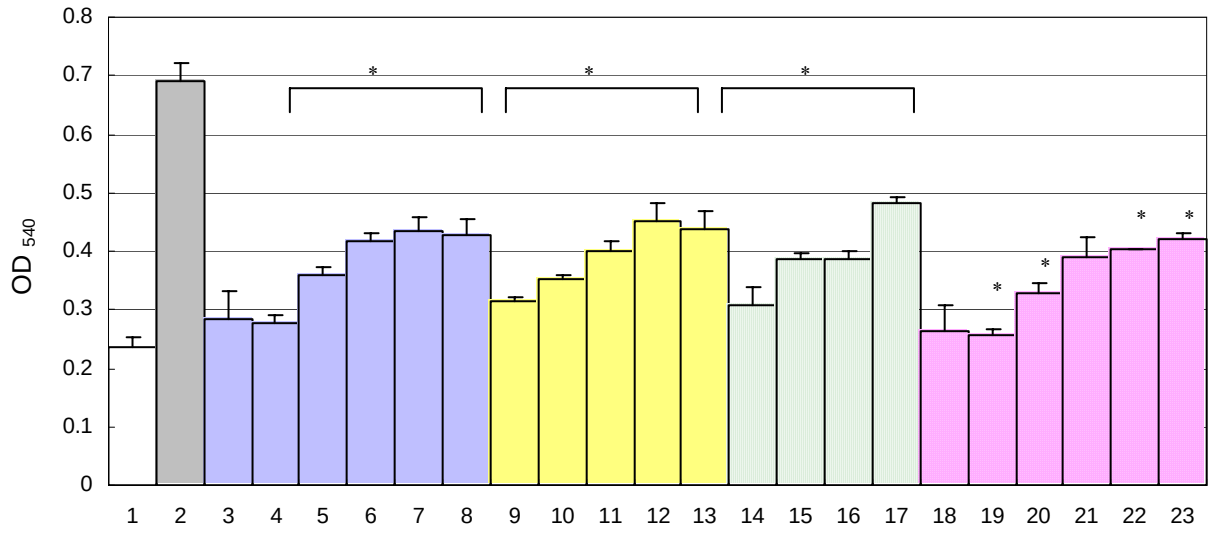
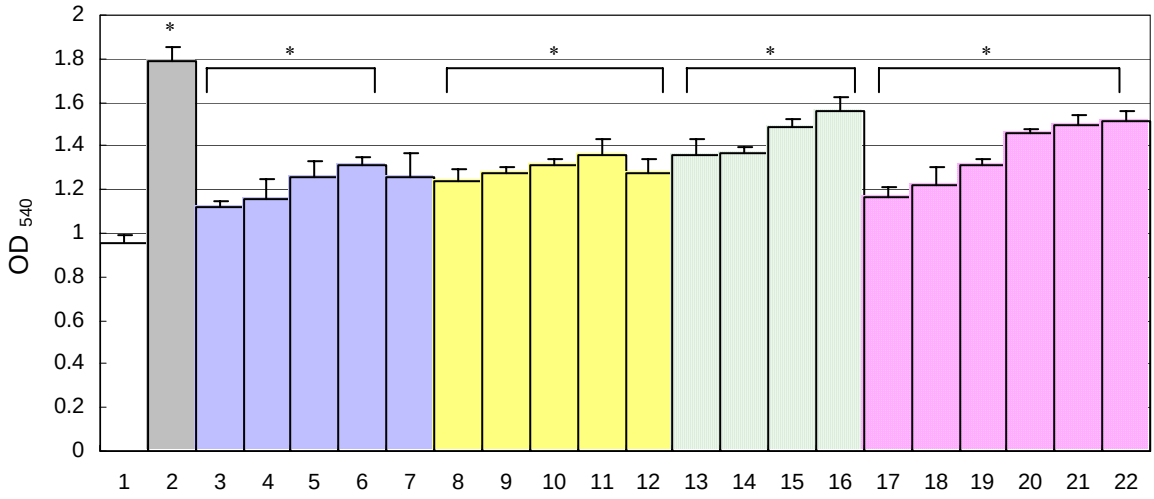


Figure 1. **Various growth factors in placental extracts.** Porcine placental extracts were subjected to western blot, and blots were incubated with (A) anti-TGF-β1; (B) anti-bFGF antibodies. Human placental extracts were subjected to western blot, and blots were incubated with (C) anti-TGF-β1; (D) anti-bFGF antibodies. Blots are representative of those obtained in three separate experiments, with similar results.



Lane 1: 0.5% CS (negative control)
Lane 2: 15% CS (positivecontrol)
Lane 3: 5 μg/ml H_2O extract
Lane 4: 10 μg/ml H_2O extract
Lane 5: 20 μg/ml H_2O extract
Lane 6: 50 μg/ml H_2O extract
Lane 7: 80 μg/ml H_2O extract
Lane 8: 100μg/ml H_2O extract
Lane 9: 5 μg/ml 1M $MgCl_2$ extract
Lane 10: 10 μg/ml 1M $MgCl_2$ extract
Lane 11: 20 μg/ml 1M $MgCl_2$ extract
Lane 12: 50 μg/ml 1M $MgCl_2$ extract
Lane 13: 80 μg/ml 1M $MgCl_2$ extract
Lane 14: 5 μg/ml 2M Urea extract
Lane 15: 10 μg/ml 2M Urea extract
Lane 16: 20 μg/ml 2M Urea extract
Lane 17: 50 μg/ml 2M Urea extract
Lane 18: 5 μg/ml H_2O extract (human placenta)
Lane 19: 10 μg/ml H_2O extract (human placenta)
Lane 20: 20 μg/ml H_2O extract (human placenta)
Lane 21: 50 μg/ml H_2O extract (human placenta)
Lane 22: 80 μg/ml H_2O extract (human placenta)
Lane 23: 100 μg/ml H_2O extract (human placenta)

Figure 2.A **Effects of various concentrations of placental extracts in different extraction solvents on NIH3T3 fibroblast proliferation.** Cells were incubated for 18 h in DMEM only in order to bring most of cells into G0 phase of cell cycle. Thereafter, medium was replaced with various doses of placental extracts in 0.5% CS and incubations were continued for 20 h. The percentages of proliferous cells were determined by MTT assay. Data are shown as means ± S.D. of triplicate determinations and representative of three experiments. *P<0.05



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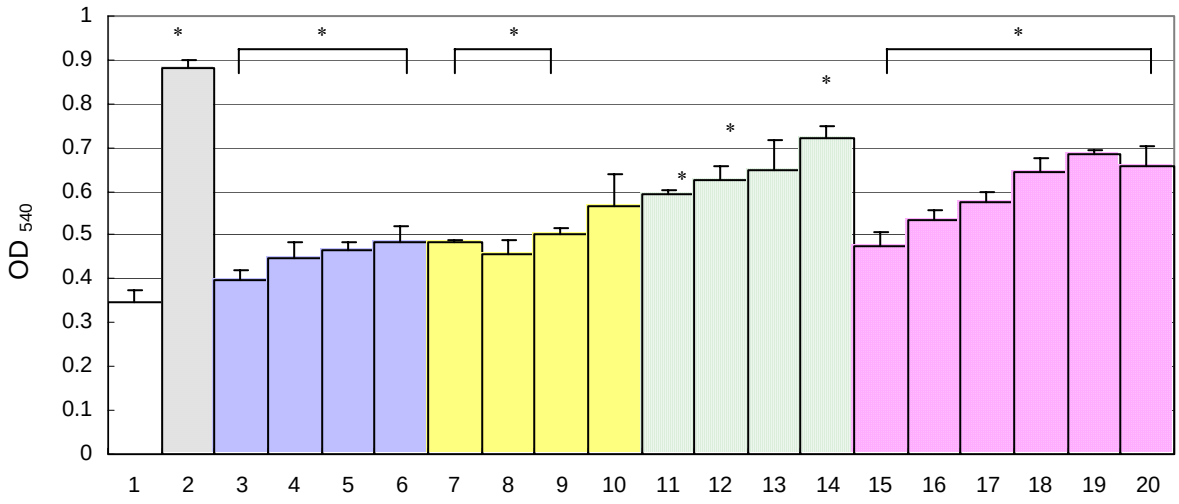
Lane 6: 50 μg/ml H_2O extract
Lane 7: 80 μg/ml H_2O extract
Lane 8: 5 μg/ml 1M $MgCl_2$ extract
Lane 9: 10 μg/ml 1M $MgCl_2$ extract
Lane 10: 20 μg/ml 1M $MgCl_2$ extract
Lane 11: 50 μg/ml 1M $MgCl_2$ extract
Lane 12: 80 μg/ml 1M $MgCl_2$ extract
Lane 13: 5 μg/ml 2M Urea extract
Lane 14: 10 μg/ml 2M Urea extract
Lane 15: 20 μg/ml 2M Urea extract
Lane 16: 50 μg/ml 2M Urea extract
Lane 17: 5 μg/ml H_2O extract (human placenta)
Lane 18: 10 μg/ml H_2O extract (human placenta)
Lane 19: 20 μg/ml H_2O extract (human placenta)
Lane 20: 50 μg/ml H_2O extract (human placenta)
Lane 21: 80 μg/ml H_2O extract (human placenta)
Lane 22: 100 μg/ml H_2O extract (human placenta)

Figure 2.B **Effects of various concentrations of placental extracts in different extraction solvents on HaCaT keratinocyte proliferation.** Cells were incubated for 18 h in DMEM only in order to bring most of cells into G0 phase of cell cycle. Thereafter, medium was replaced with various doses of placental extracts in 0.5% FCS and incubations were continued for 24 h. The percentages of proliferous cells were determined by MTT assay. Data are shown as means ± S.D. of triplicate determinations and representative of three experiments. *P<0.05



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Lane 8: 10 μg/ml 1M $MgCl_2$ extract
Lane 9: 20 μg/ml 1M $MgCl_2$ extract
Lane 10: 50 μg/ml 1M $MgCl_2$ extract
Lane 11: 5 μg/ml 2M Urea extract
Lane 12: 10 μg/ml 2M Urea extract
Lane 13: 20 μg/ml 2M Urea extract
Lane 14: 50 μg/ml 2M Urea extract
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Lane 17: 20 μg/ml H_2O extract (human placenta)
Lane 18: 50 μg/ml H_2O extract (human placenta)
Lane 19: 80 μg/ml H_2O extract (human placenta)
Lane 20: 100 μg/ml H_2O extract (human placenta)

Figure 2.C **Effects of various concentrations of placental extracts in different extraction solvents on CPAE cell proliferation.** Cells were incubated for 18 h in DMEM only in order to bring most of cells into G0 phase of cell cycle. Thereafter, medium was replaced with various doses of placental extracts in 0.5% FCS and incubations were continued for 24 h. The percentages of proliferous cells were determined by MTT assay. Data are shown as means ± S.D. of triplicate determinations and representative of three experiments. *P<0.05

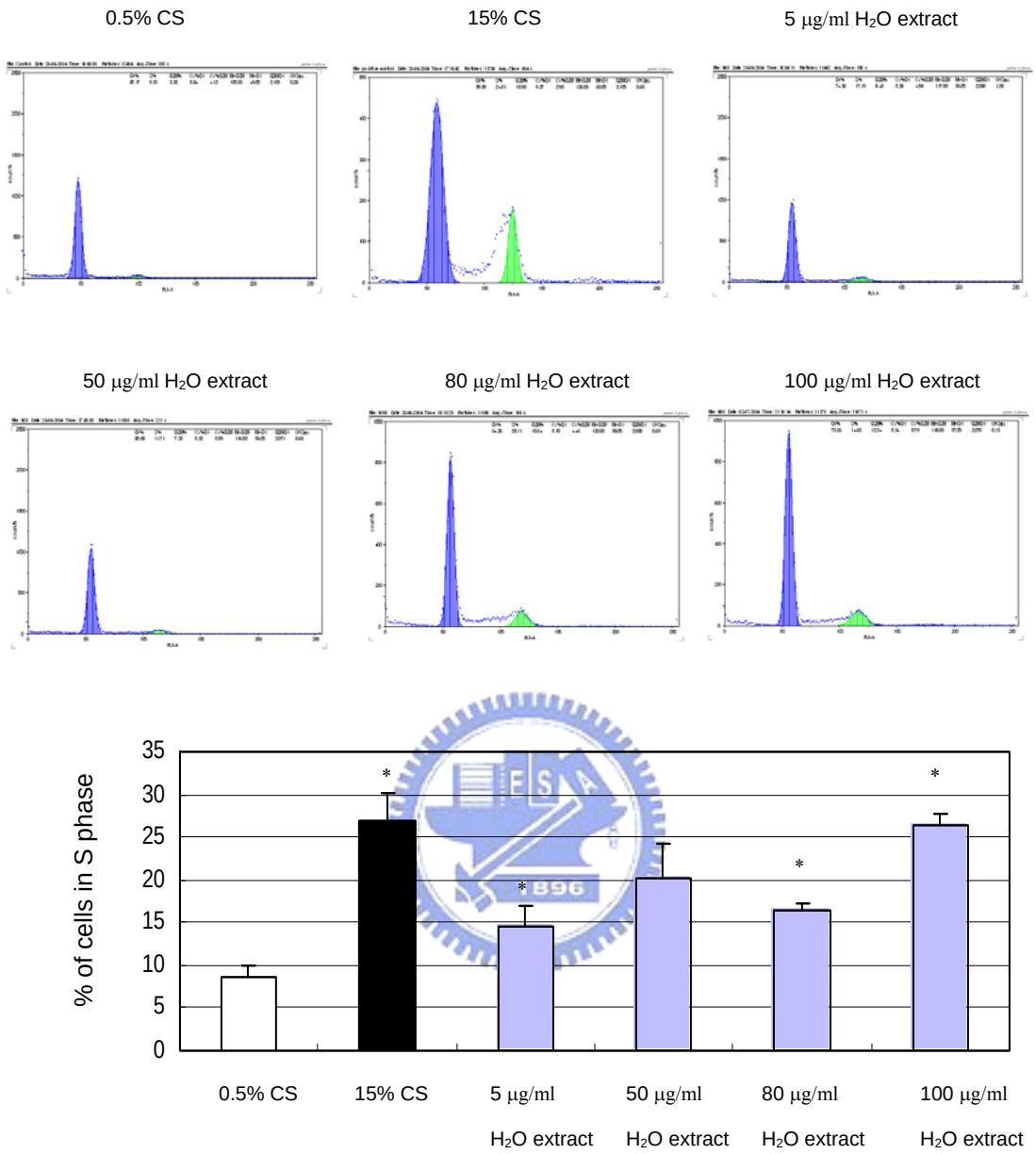


Figure 3. **Flow cytometric analysis of the effect of different concentrations porcine placental extracts on cell cycle of fibroblasts.** After incubation with 50 μg/ml different porcine placental extracts for 20 h, cells were harvested and stained with propidium iodide and analyzed for S phase of cell cycle. Data represent as means of duplicates ± S.D. *P<0.05

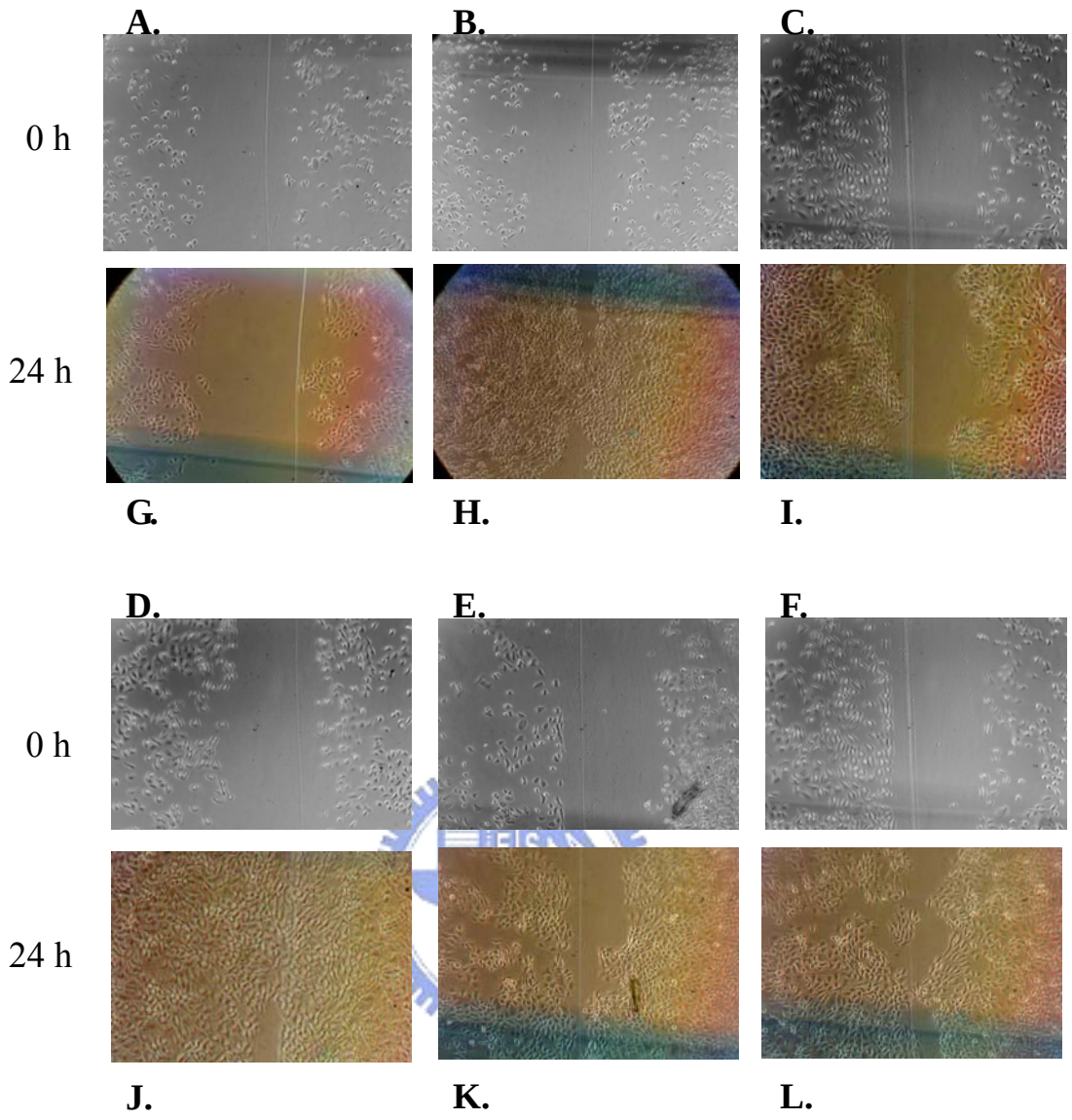


***Figure 4.A* Effects of porcine placental extracts on wound healing of HaCaT cells.** In this assay, cell migration from the edges of "scrape-wounded" monolayers to cover the denuded surface. Cell monolayers were scraped with a P-200 pipette tip and treated with placental extracts. Wounded cultures were allowed to re-epithelialize for 24 h in the presence of placental extracts. Wells were photographed at 0 h (A-F) and 24 h (G-L) adjacent to a reference line drawn on the bottom of the plate. (A, G) control; (B, H) 10% FCS; (C, I) 50 μg/ml H_2O extract; (D, J) 50 μg/ml NaCl extract; (E, K) 50 μg/ml $MgCl_2$ extract; (F, L) 50 μg/ml urea extract (original magnification ×100).

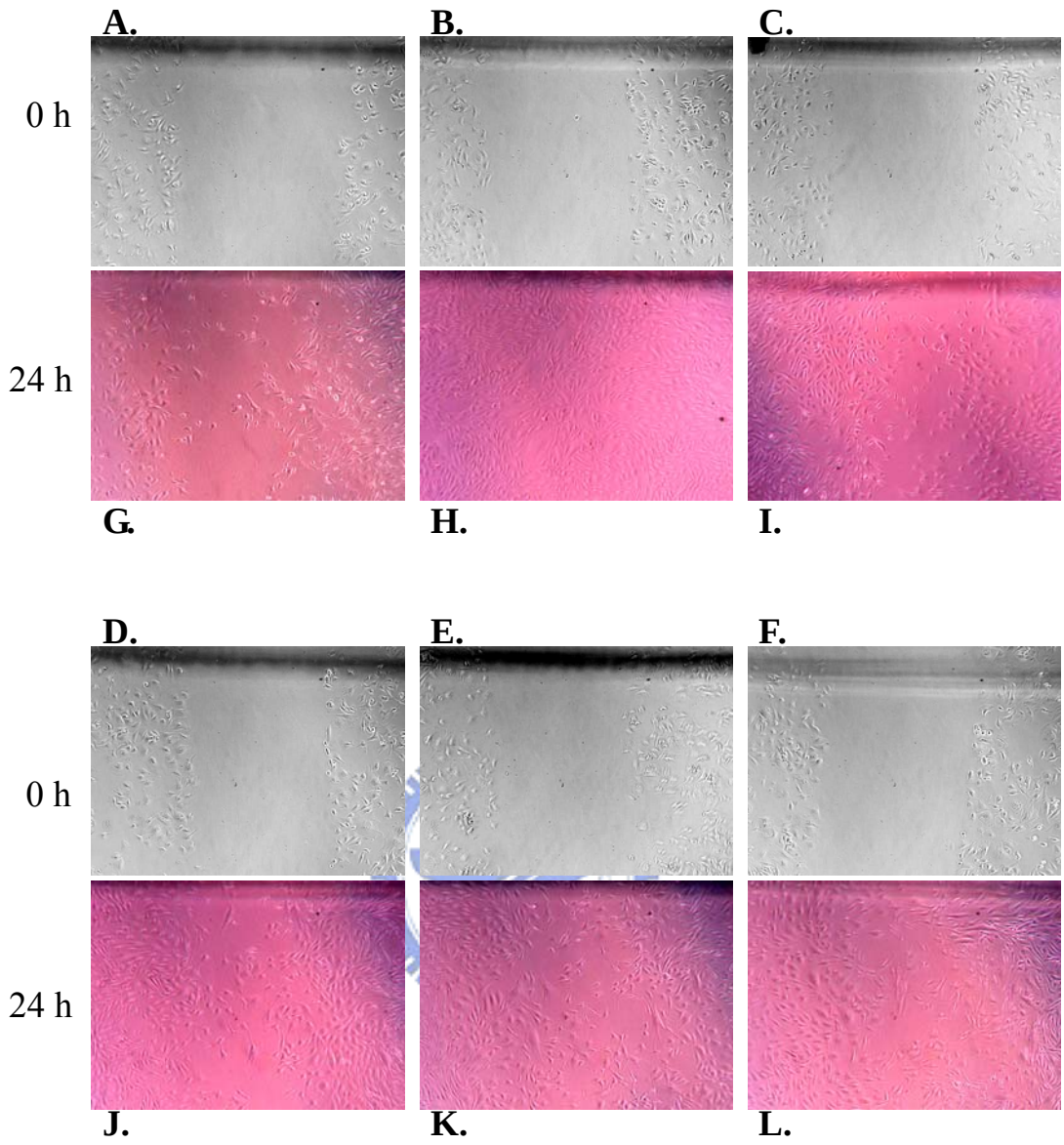
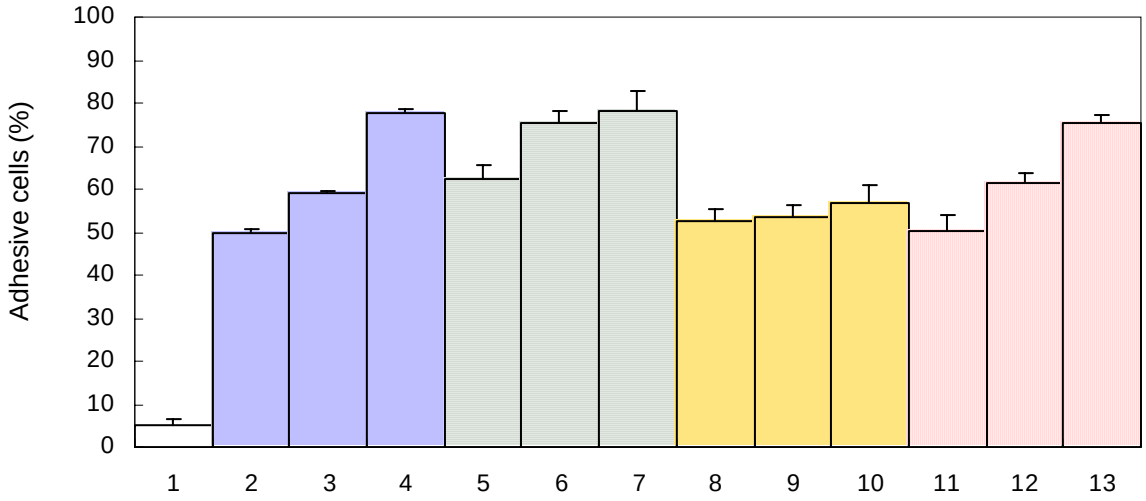
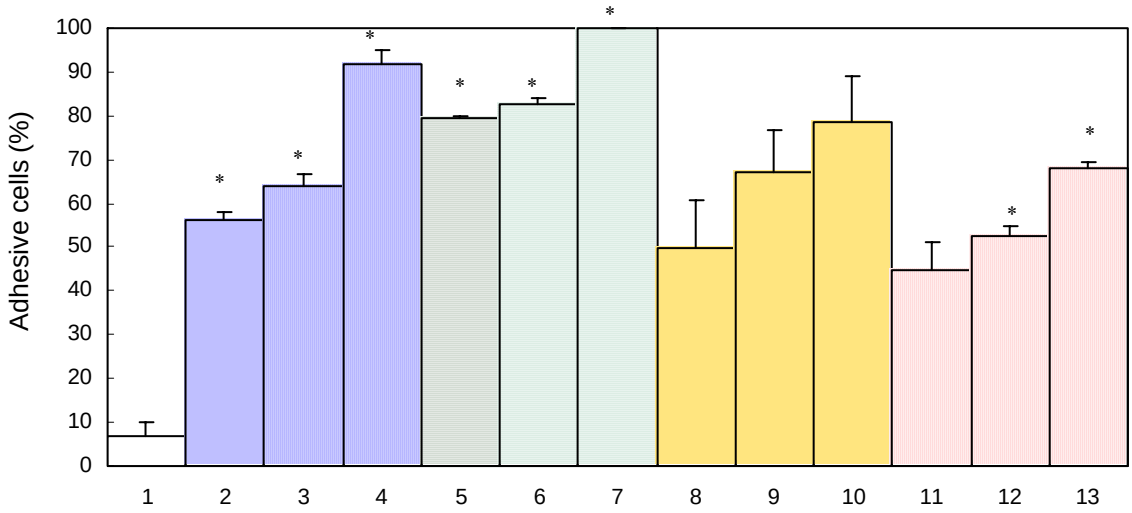


Figure 4.B **Effects of porcine placental extracts on wound healing of CPAE cells.** In this assay, cell migration from the edges of "scrape-wounded" monolayers to cover the denuded surface. Cell monolayers were scraped with a P-200 pipette tip and treated with placental extracts. Wounded cultures were allowed to re-epithelialize for 24 h in the presence of placental extracts. Wells were photographed at 0 h (A-F) and 24 h (G-L) adjacent to a reference line drawn on the bottom of the plate. (A, G) control; (B, H) 20% FCS; (C, I) 50 µg/ml H_2O extract; (D, J) 50 µg/ml NaCl extract; (E, K) 50 µg/ml $MgCl_2$ extract; (F, L) 50 µg/ml urea extract (original magnification ×100).



Lane 1 : 1% BSA only (negative control)
Lane 2 : 10 μg/ml H_2O extract
Lane 3 : 50 μg/ml H_2O extract
Lane 4 : 100 μg/ml H_2O extract
Lane 5 : 10 μg/ml 0.1M NaCl extract
Lane 6 : 50 μg/ml 0.1M NaCl extract
Lane 7 : 100 μg/ml 0.1M NaCl extract
Lane 8 : 10 μg/ml 1M $MgCl_2$ extract
Lane 9 : 50 μg/ml 1M $MgCl_2$ extract
Lane 10: 100 μg/ml 1M $MgCl_2$ extract
Lane 11: 10 μg/ml 2M Urea extract
Lane 12: 50 μg/ml 2M Urea extract
Lane 13: 100 μg/ml 2M Urea extract

Figure 5.A **Porcine placental extracts mediated NIH3T3 fibroblasts adhesion.** After seeding and incubation, cells attached to the surfaces were quantified. Negative control was coated with 1%BSA. Data represent as means ± S.D. of triplicate determinations and representative of three experiments. All **P* value<0.05



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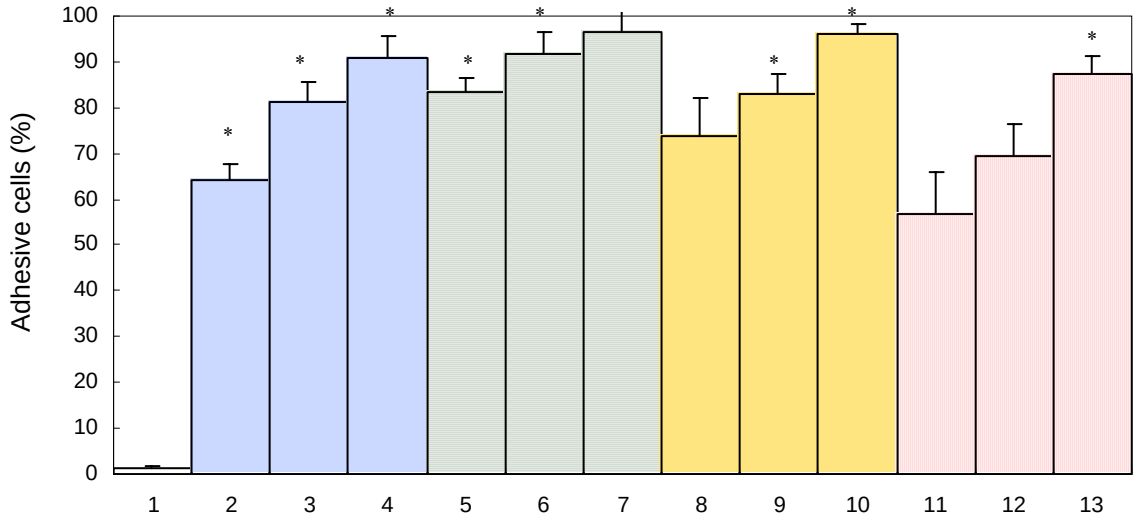
Lane 10: 100 μg/ml 1M $MgCl_2$ extract
Lane 11: 10 μg/ml 2M Urea extract
Lane 12: 50 μg/ml 2M Urea extract
Lane 13: 100 μg/ml 2M Urea extract

Figure 5.B **Porcine placental extracts mediated HaCaT keratinocytes adhesion.** After seeding and incubation, cells attached to the surfaces were quantified. Negative control was coated with 1%BSA. Data represent as means of duplicates ± S.D. **P*<0.05



Lane 1 : 1% BSA only (negative control)
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Lane 11: 10 μg/ml 2M Urea extract
Lane 12: 50 μg/ml 2M Urea extract
Lane 13: 100 μg/ml 2M Urea extract

Figure 5.C **Porcine placental extracts mediated CPAE epithelial cells adhesion.** After seeding and incubation, cells attached to the surfaces were quantified. Negative control was coated with 1%BSA. Data represent as means ± S.D. of triplicate determinations and representative of three experiments. **P*<0.05

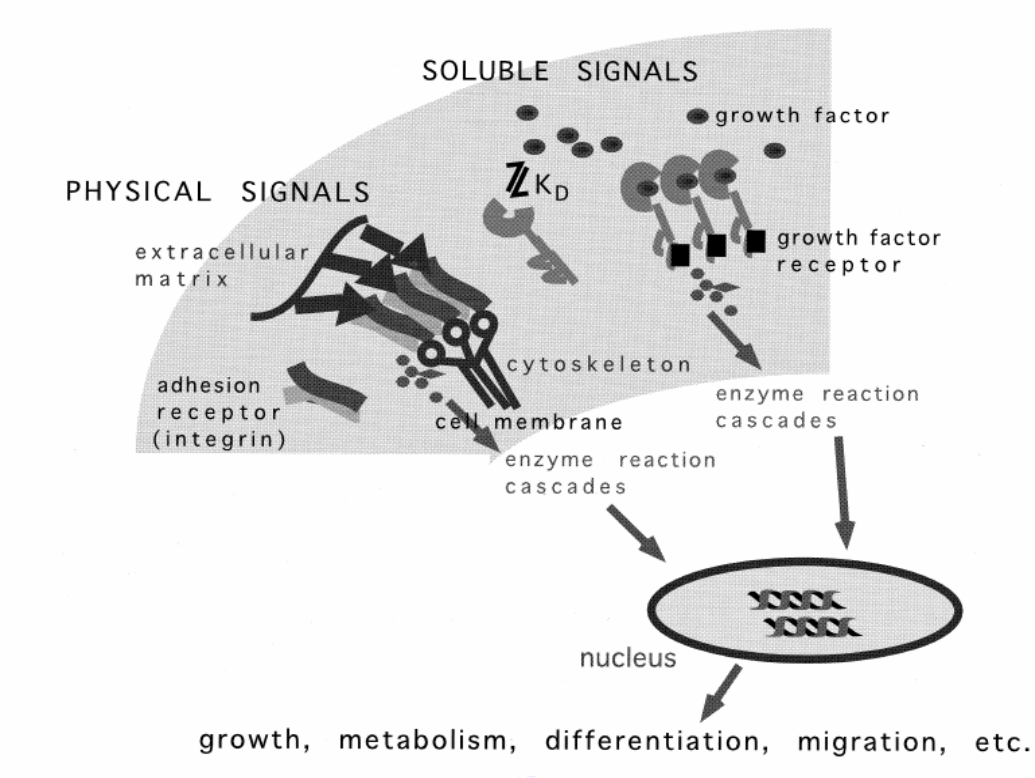

Figure 6. **Schematic of cell interaction with the external environment.** Transmembrane receptors bind ligands in the extracellular domain, and transmit signals to the interior of the cell. The receptor may be an enzyme itself which is activated on ligand binding, or it may increase its affinity for molecules which become active enzymes upon binding to the receptor. The boundaries between soluble ligands and physical ligands are not strict, as some growth factors are found primarily in matrix-bound form.

From: Griffith LG, 2000

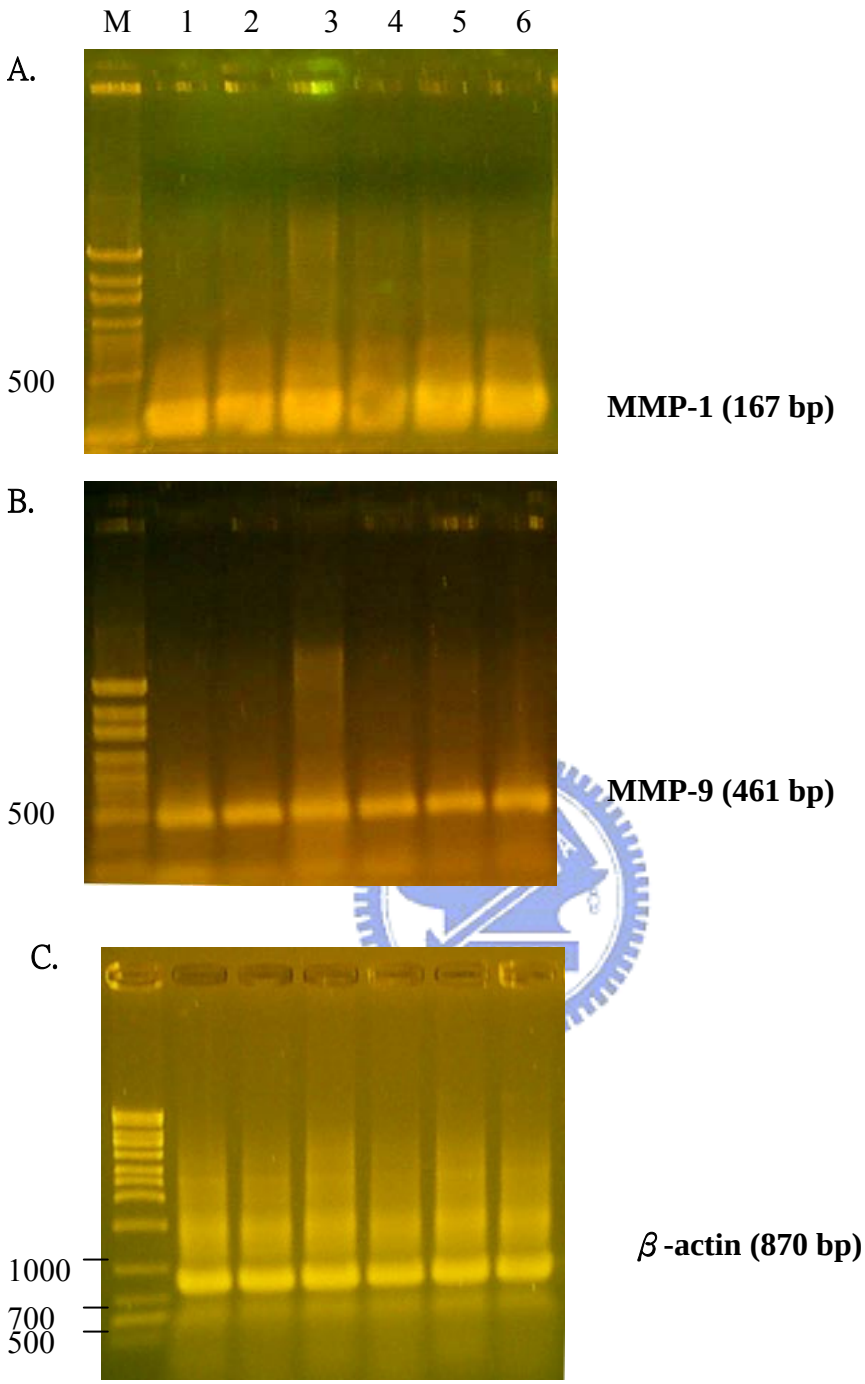

Figure 7. MMP-1 and MMP-9 expression wasn't regulated at its mRNA level. HaCaT cells were treated with in serum-free medium for various durations. Total RNA was isolated from cell layer at 0, 12, 24, 36, or 48 h (M: DNA ladder; Lane 1: control; Lane 2-6: 0, 12, 24, 36, 48 h) and analyzed by RT-PCR. (C) β-actin was monitored as a loading control.

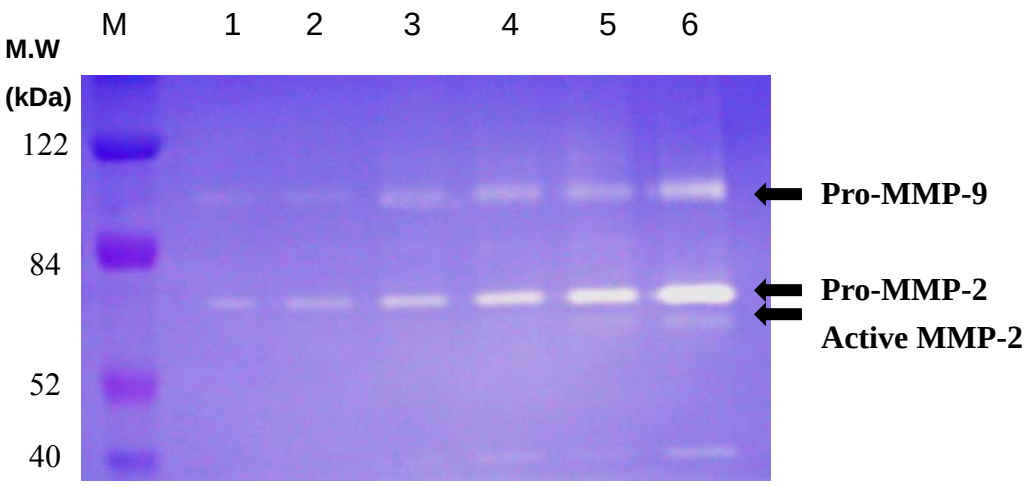

Figure 8. **Expression time course of gelatinase activities of porcine placental extracts using gelatin zymography on HaCaT keratinocytes.** The gelatinase activities were visualized on a non-reducing 10% SDS-PAGE containing 0.1% gelatin. After electrophoresis, gels were incubated in 2.5% Triton X 100 to move SDS, then in reaction buffer at 37°C overnight. Staining was performed with Coomassie Blue R 250. Lane 1: control; Lane 2-6: 0, 12, 24, 36, or 48 h

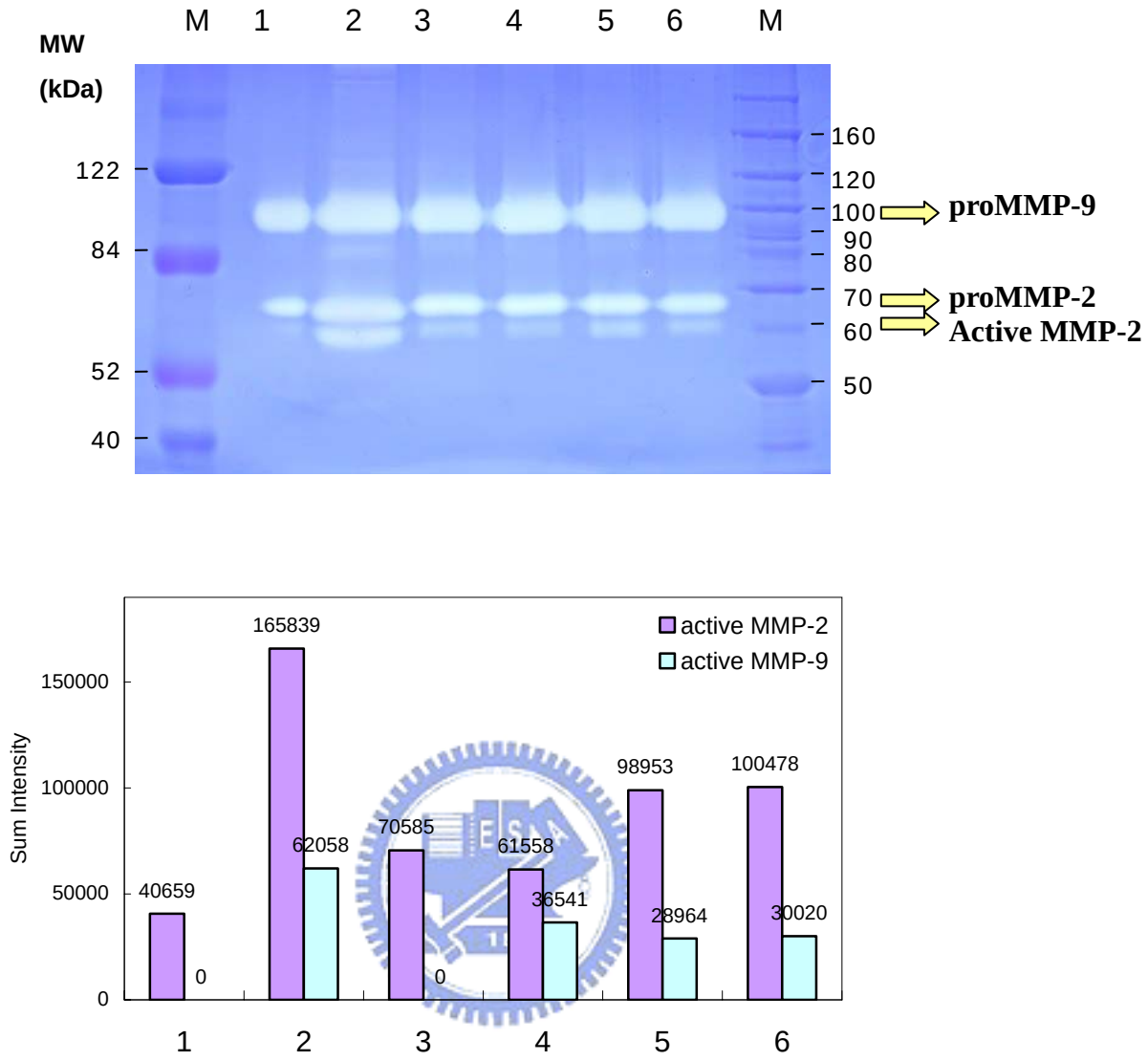

***Figure 9.A* Expression of gelatinase activities of different porcine placental extracts using gelatin zymography.** HaCaT cells treated with 50 μg/ml different porcine placental extracts stimulated MMP-2 activation after 48 h. M: protein standard maker, Lane1-6:control (DMEM only), 10% FCS, H_2O extract, 0.1M NaCl, 1M $MgCl_2$, 2M urea. Data are representative of those obtained in two separate experiments, with similar results.

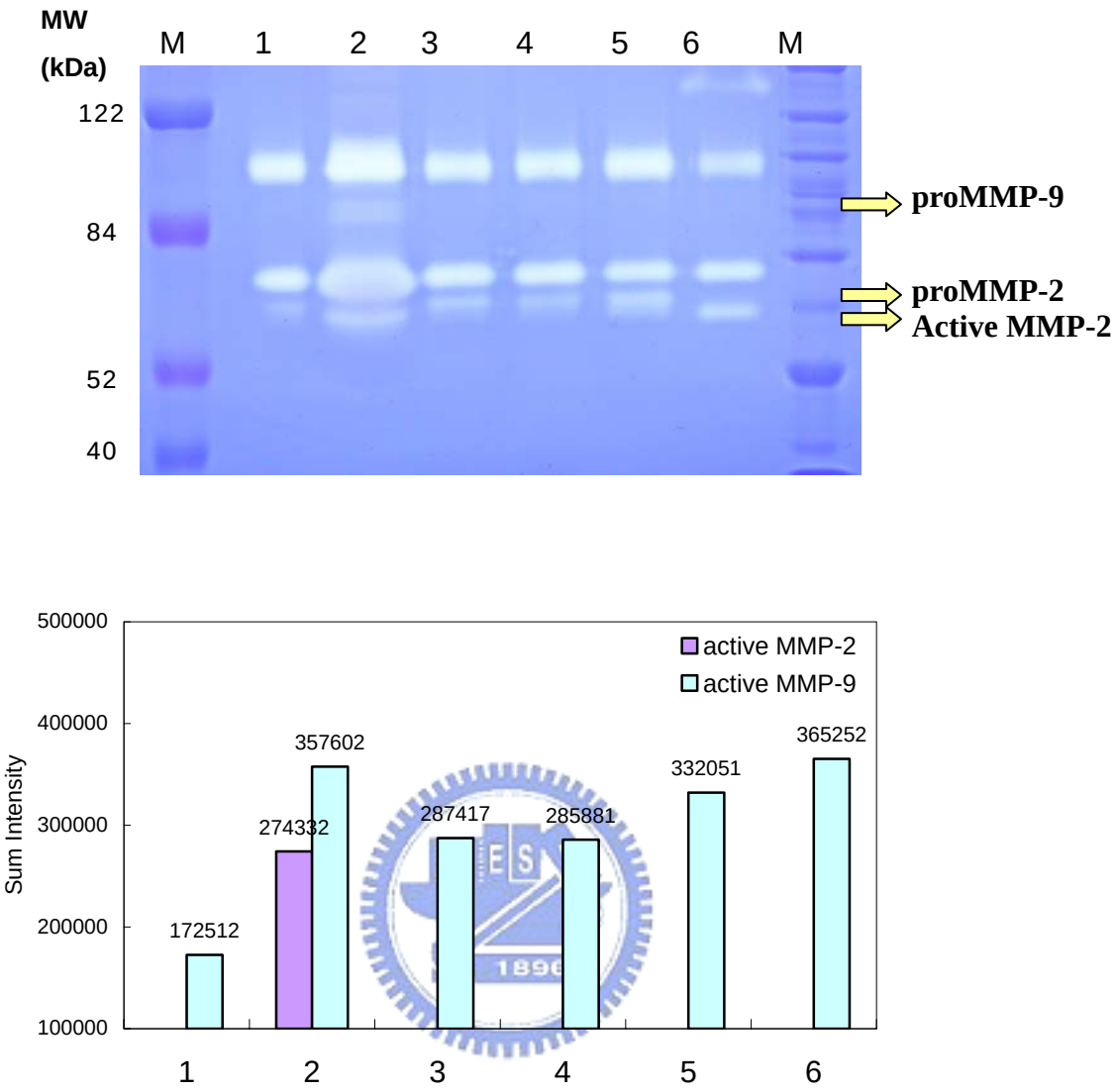

***Figure 9.B* Expression of gelatinase activities of different human placental extracts using gelatin zymography.** HaCaT cells treated with 50 μg/ml different human placental extracts stimulated MMP-2 activation after 48 h. M: protein standard maker, Lane1-6:control (DMEM only), 10% FCS, H_2O extract, 0.1M NaCl, 1M $MgCl_2$, 2M urea. Data are representative of those obtained in two separate experiments, with similar results.