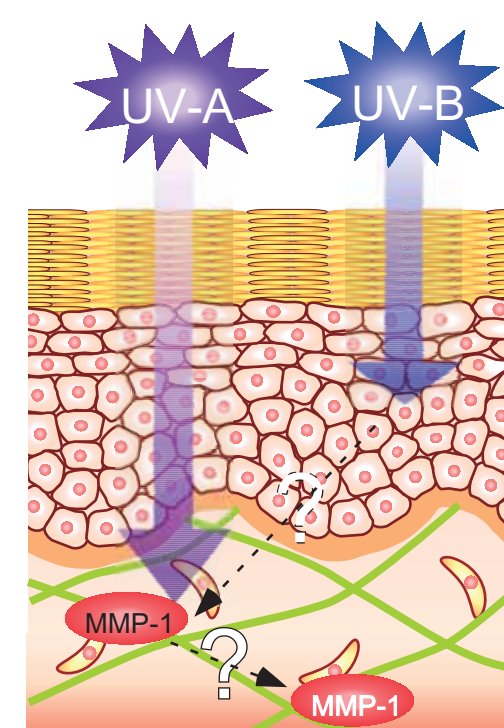


# Novel insights into dermal fibroblasts and extracellular matrix.

○Aska Sonoki, Megumi Konishi, Yuichiro Yoshitake  
(OPPEN COSMETICS CO., LTD.)

## Back ground

It is well known that ultraviolet (UV)-A irradiation causes dermal photo-aging and that MMPs are critical to the collagen degradation involved in that process. MMP-1 is one of the most important collagen-degrading enzymes produced by fibroblasts. However, it has been thought that MMPs cannot act on collagen without being activated by proteases formed mainly by keratinocytes because they are expressed as inactive precursor proteins. Previous studies have shown that MMP-1 is expressed in an inactivated form in monolayer culture, although its expression is induced in fibroblasts by UV-A. On the other hand, it has been reported that MMP-1 is activated in aged dermis in vivo. As a result, it has been thought that MMP-1 is activated by serine proteases produced by keratinocytes, for example plasmin and cathepsin G, and other MMPs in vivo. However, these mechanisms are not fully understood. More precisely understanding of mechanisms might contribute to exploring of new materials and improvement of our skins.



## Our suggestion

- In our skin, dermal fibroblasts may be capable of activating MMP-1 by themselves
- Embedded culture may demonstrate more properly and indicate newly features

Previous studies have shown that fibroblasts produce only pro-MMP-1 after UV-A irradiation in monolayer culture. However, fibroblasts in skin in situ are surrounded by extracellular matrix (ECM). It has also been suggested that there are some differences in fibroblast behavior in monolayer culture and when embedded in ECM. Therefore, it is conceivable that ECM-embedded fibroblasts might reflect what is actually going on in the skin regarding MMP-1 production. Actually, 3-D collagen lattice embedding fibroblasts is used as a dermis model. Many previous studies showed the lattice size decreased by cell activation factors and we could evaluate the activation capacity of them from lattice contraction. In this study, we embedded fibroblasts in a collagen lattice after UV-A irradiation and investigated the condition of the collagen lattice and MMP-1 production. The results suggest that MMP-1 can be activated in collagen-embedded fibroblasts and that the collagen lattice is diminished similar to photo-aged skin.

### UV-A-irradiated fibroblasts contracted lattice less !

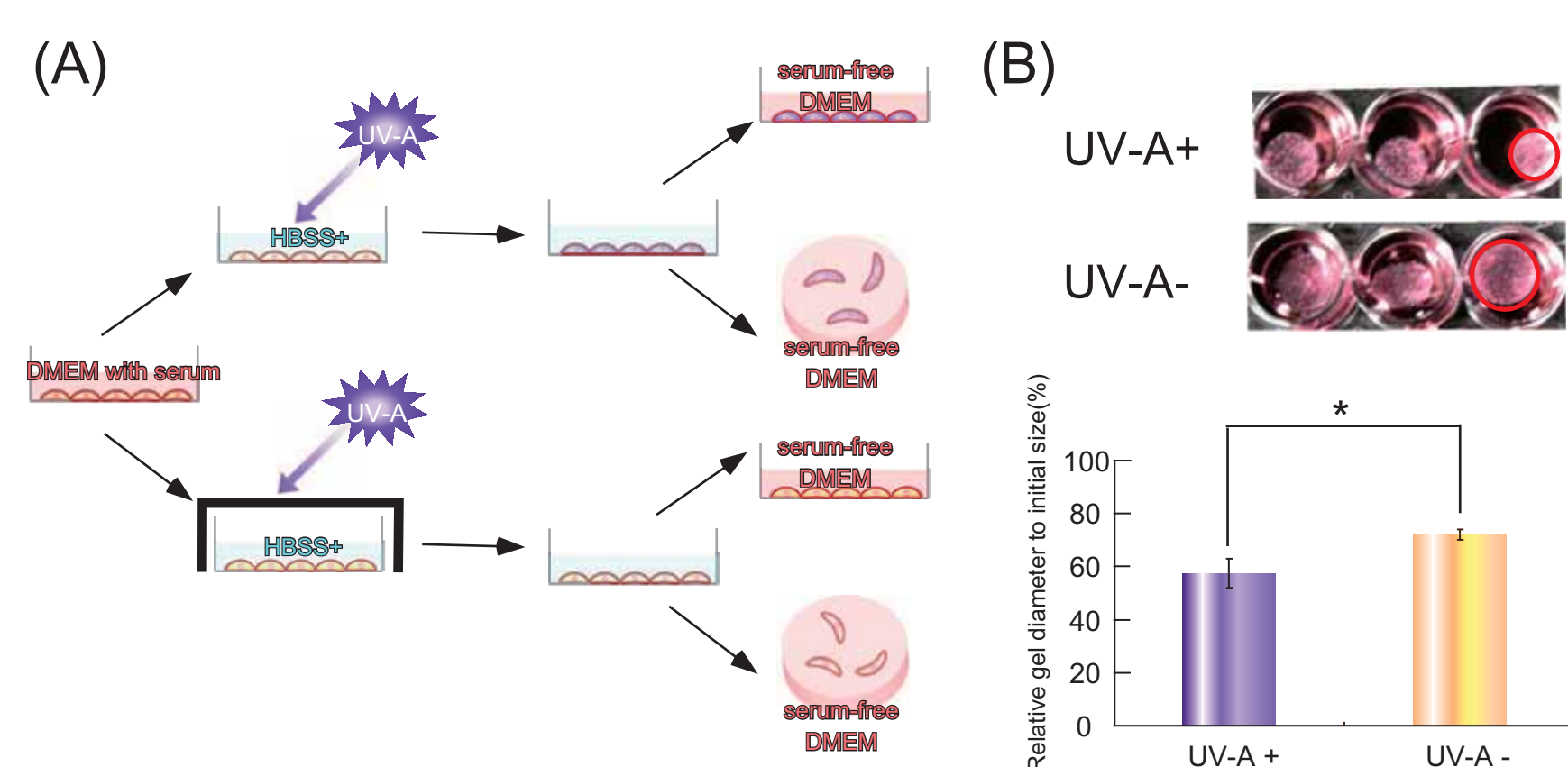


Fig. 1 Collagen lattice contraction assay of UV-A-irradiated fibroblasts  
(A) Collagen lattice construction method. Monolayer cultured fibroblasts were irradiated with UV-A in HBSS+ (Hanks balanced salt solution). After irradiation, cells were harvested by trypsin and embedded into collagen lattice or cultured on dishes.  
(B) Collagen lattice contraction assay. After detachment from dishes, lattice were incubated for 3 days. Lattices embedded with UV-A-irradiated (UV-A+) or non-irradiated (UV-A-) fibroblasts. Comparison of gel diameters; this graph indicates relative lattice size to initial size.\*p<0.05, n=3.

### Lattice contraction(miniaturization) depended on collagen degradation

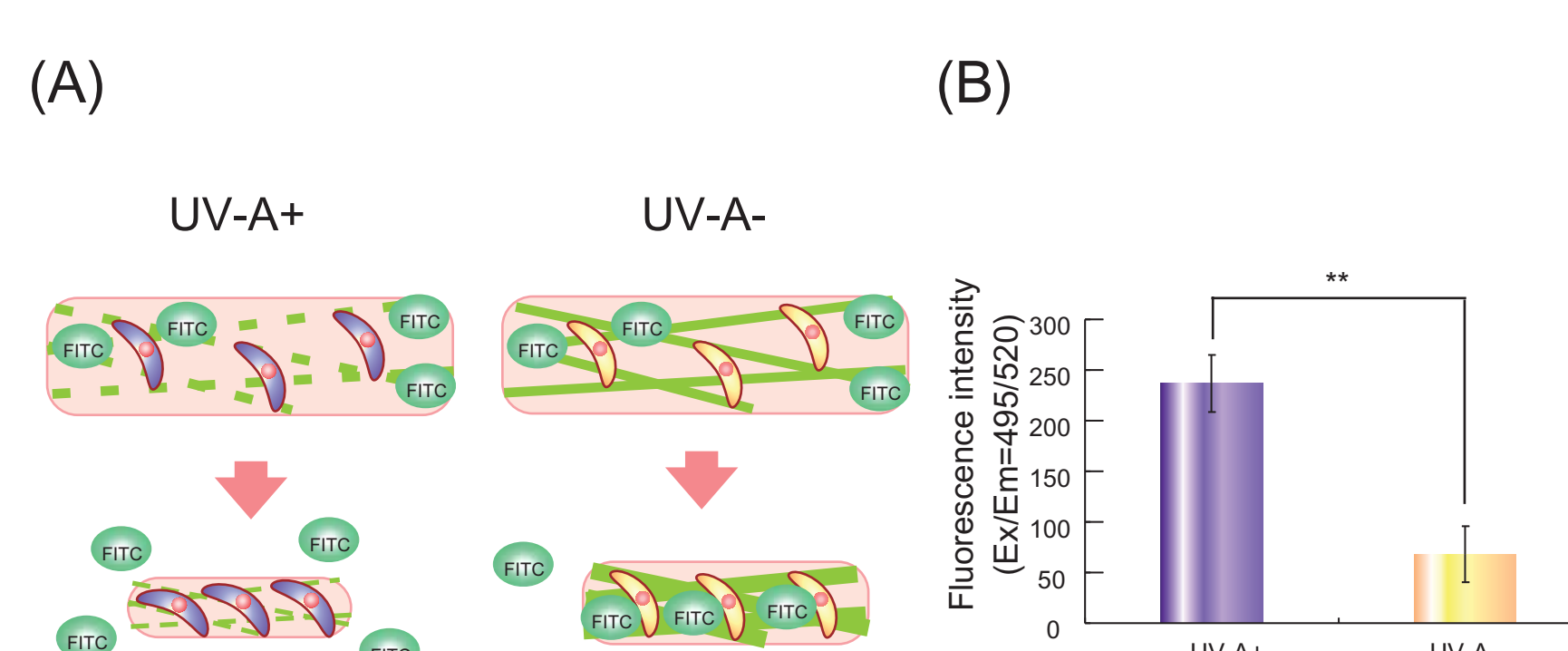


Fig. 2 The analysis of collagen degradation using FITC-conjugated collagen  
(A) FITC-conjugated collagen lattice shows the amount of degraded collagen by detection of FITC fluorescent in conditioned medium.  
(B) Larger amount of FITC was released to the medium of lattice embedding UV-A-irradiated cells. \*\*p<0.01, n=3.

### MMP-1 contributed to collagen degradation in lattice embedding UV-A-irradiated fibroblasts

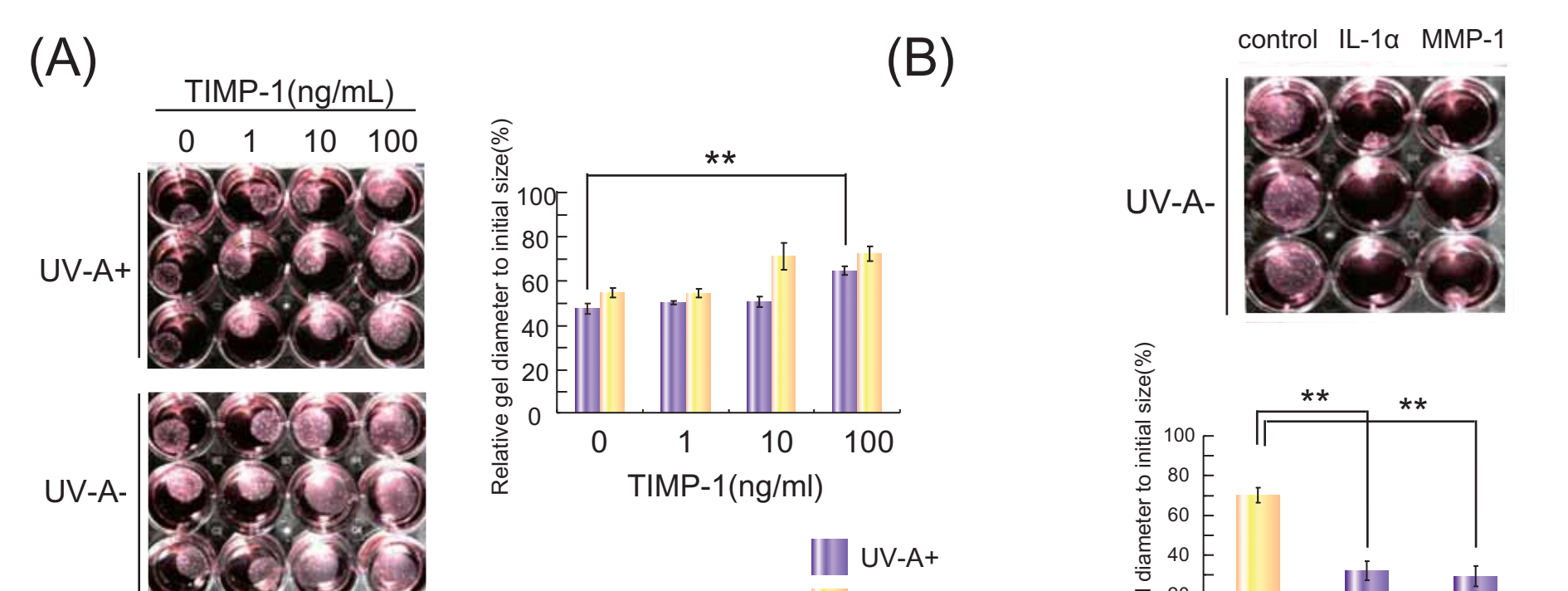


Fig. 3 Inhibition and promotion of collagen lattice contraction by TIMP-1 and MMP-1 activators  
(A) Collagen lattice contraction assay. TIMP-1 inhibited the lattice contraction(miniaturization) according to the concentration.  
(B) Collagen lattice contraction assay. IL-1 $\alpha$ , MMP-1 inducer, and MMP-1 recombinant protein promoted lattice miniaturization. \*\*p<0.01, \*p<0.05, n=3.

### MMP-1 was activated in the lattice without keratinocytes

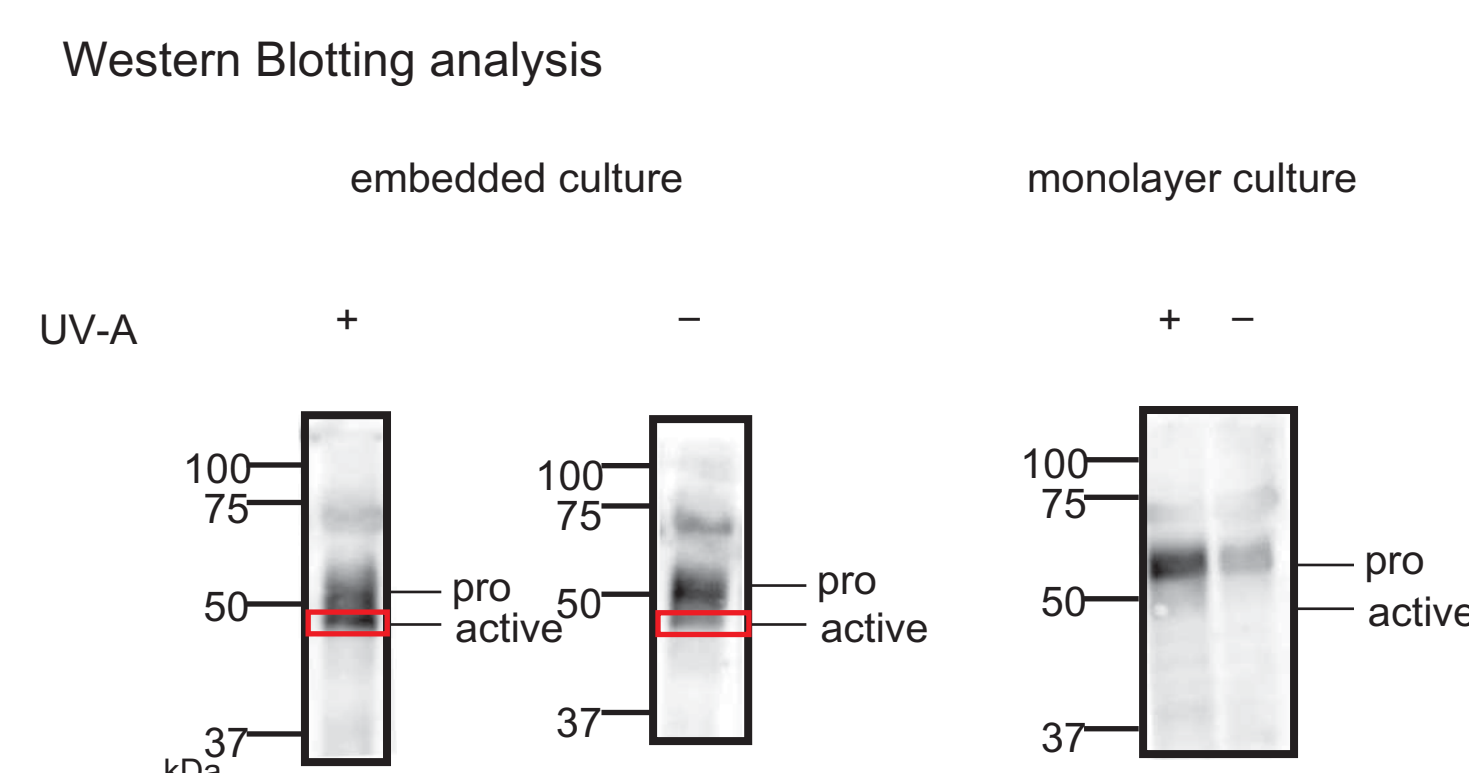


Fig. 4 Western Blotting analysis of MMP-1 state  
Western blotting analysis showed that activated MMP-1 existed in collagen embedded culture, although there is no active type MMP-1 in the medium from monolayer cultured fibroblasts. Especially, more MMP-1 was activated in lattice embedding UV-A irradiated cells comparing to the lattice with non-irradiated cells.

### Serine proteases might activate MMP-1

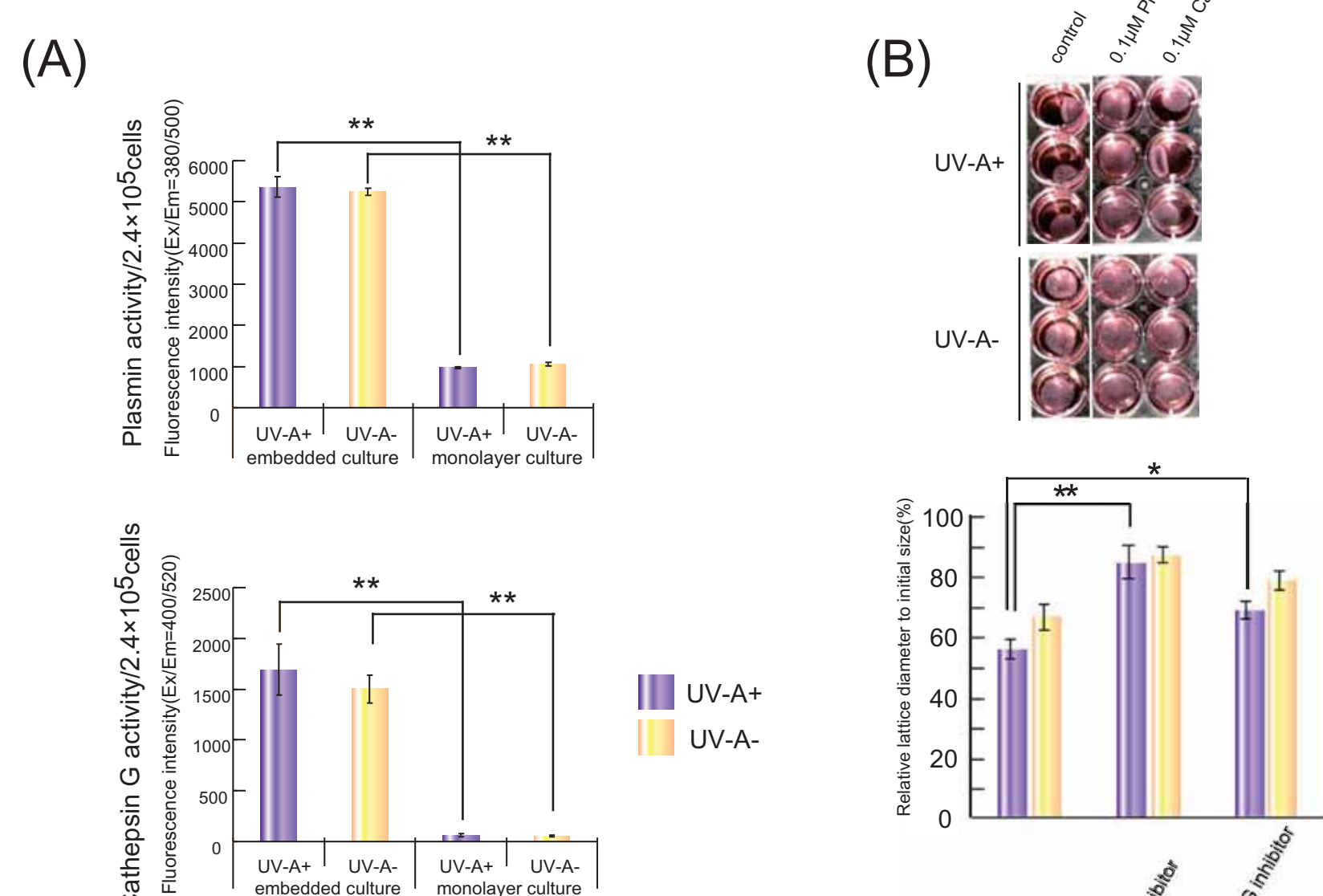


Fig. 5 Involvement of serine protease activity to MMP-1 activation  
(A) Serine protease activity in the conditioned medium of embedded cultured cells and monolayer cultured cells. Both proteases activities were significantly increased in collagen embedded culture compared with monolayer culture. However there was no meaningful difference between irradiated and non-irradiated cells.  
(B) Collagen lattice contraction assay. Each Plasmin inhibitor and cathepsin G inhibitor inhibited lattice miniaturization.

### Dermal fibroblasts are capable of activating MMP-1 by themselves

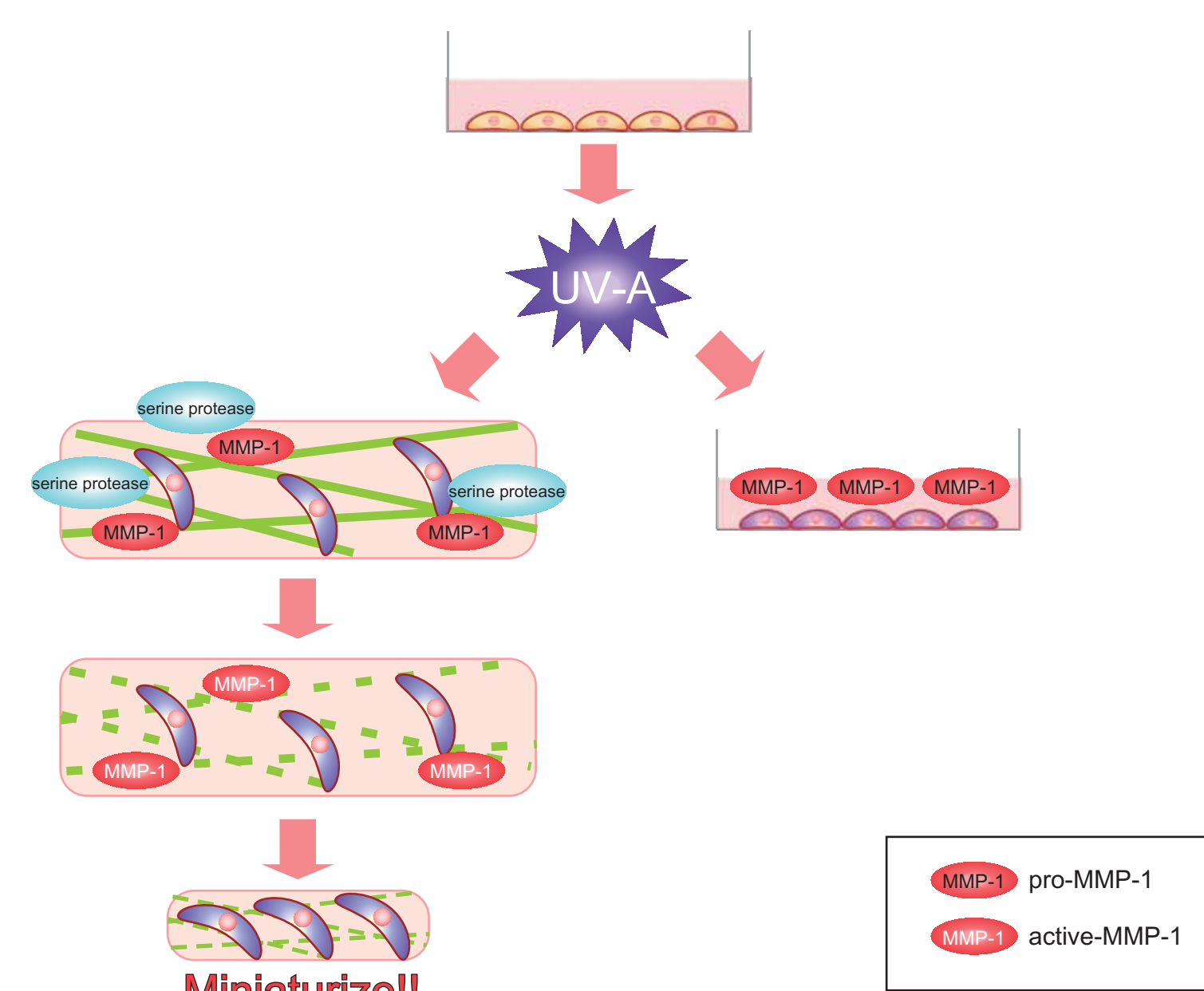


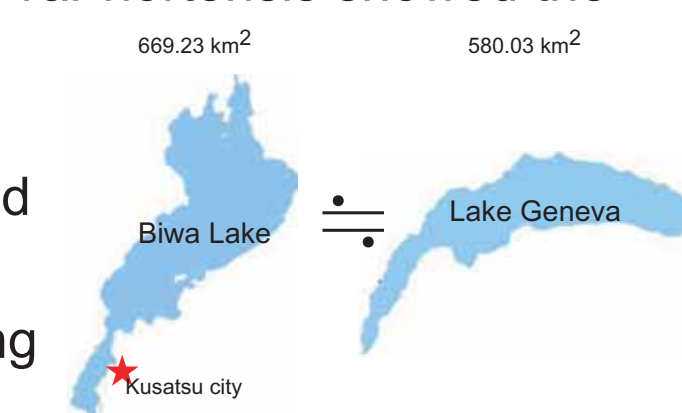
Fig. 6 Mechanisms of collagen lattice miniaturization and MMP-1 activation  
Fibroblasts express serine protease, such as plasmin or cathepsin G, in collagen embedded lattice. When UV-A irradiation induces MMP-1 expression, MMP-1 might be activated by serine proteinase. Activated MMP-1 degrades collagen fibers in the lattice. As a result, collagen lattice was miniaturized.

## Results of material screening

*Commulina communis* var *hortensis*, which is a variant from "Asiatic dayflower" (*Commulina communis*), a special product in Kusatsu city. These flowers are larger than "Asiatic flower" and the dye made from these flowers colors blue. This dye has been used for drawing a rough sketch of "Yuzen", and it has supported Japanese kimono culture.



On the other hand, *Commulina communis* var *hortensis* have been used as a natural medicine. It has been known that the extract of aerial parts of *Commulina communis* var *hortensis* showed the properties of antioxidants, radical scavengers and inhibitors of  $\alpha$ -glucosidase. We have also reported that the flower extract of *Commulina communis* var *hortensis* has whitening effect.



### C. communis var hortensis inhibited collagen degradation

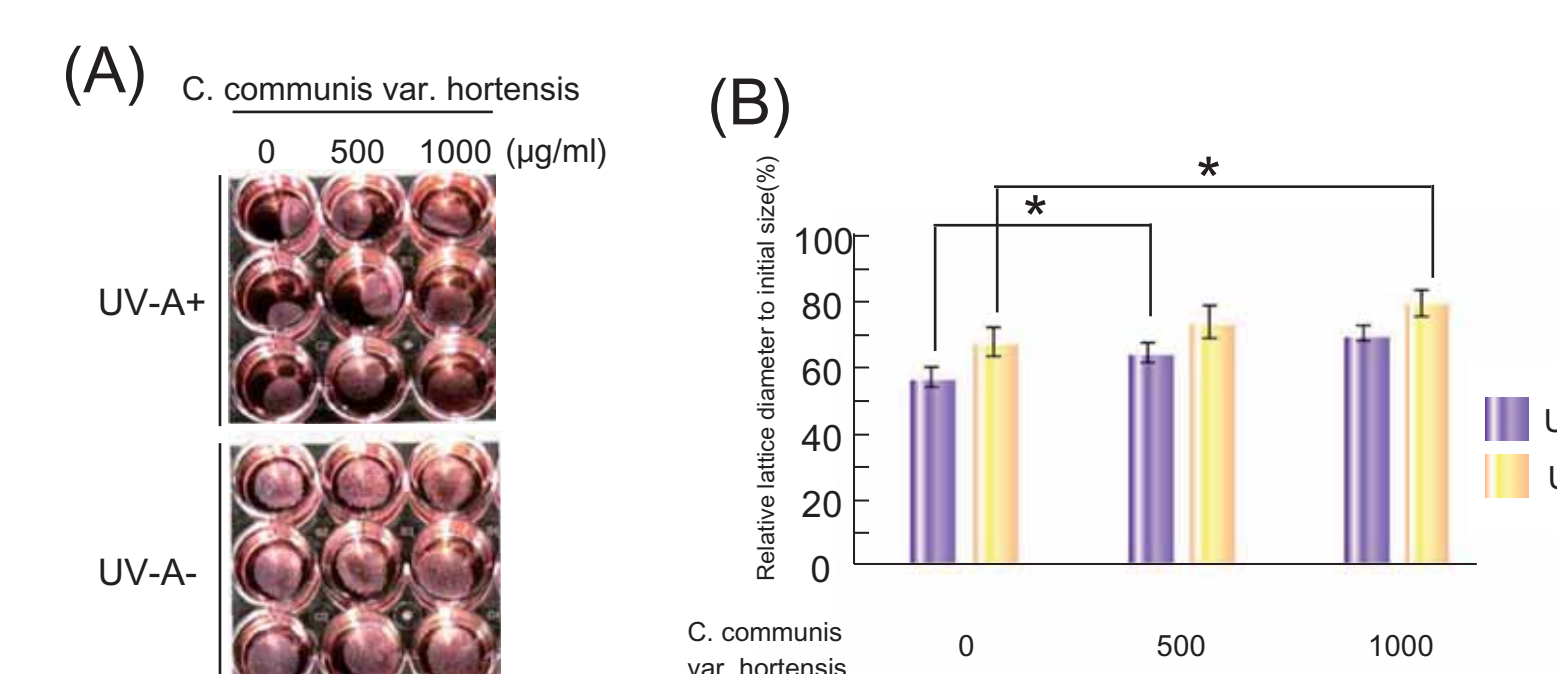


Fig. 7 The effect of *C. communis* var. *hortensis* extracts to collagen lattices  
(A) Collagen lattice contraction assay. *C. communis* var *hortensis* extracts were added into the lattices. (B) Comparison of gel diameters; this graph indicates relative lattice size to initial size.\*p<0.05, n=3

### C. communis var hortensis inhibited MMP-1 and MMP-3

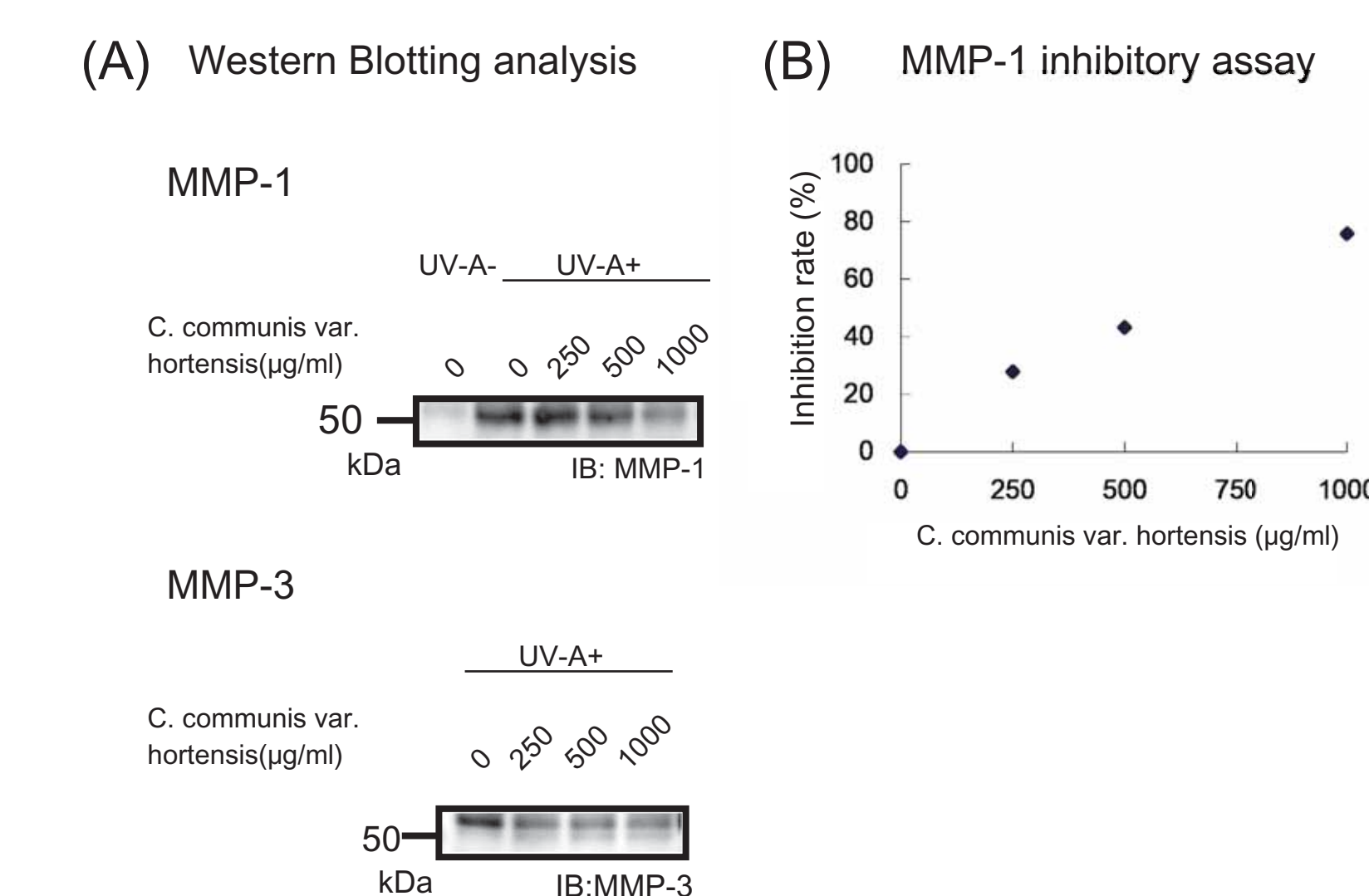


Fig. 8 Effects of *C. communis* var. *hortensis* extracts to MMPs  
(A) Western Blotting analysis showed *C. communis* var *hortensis* inhibited the expression of MMP-1 and MMP-3.  
(B) In vitro MMP-1 inhibitory assay showed *C. communis* var *hortensis* inhibited the activity of MMP-1

### C. communis var hortensis inhibits collagen degradation multilaterally

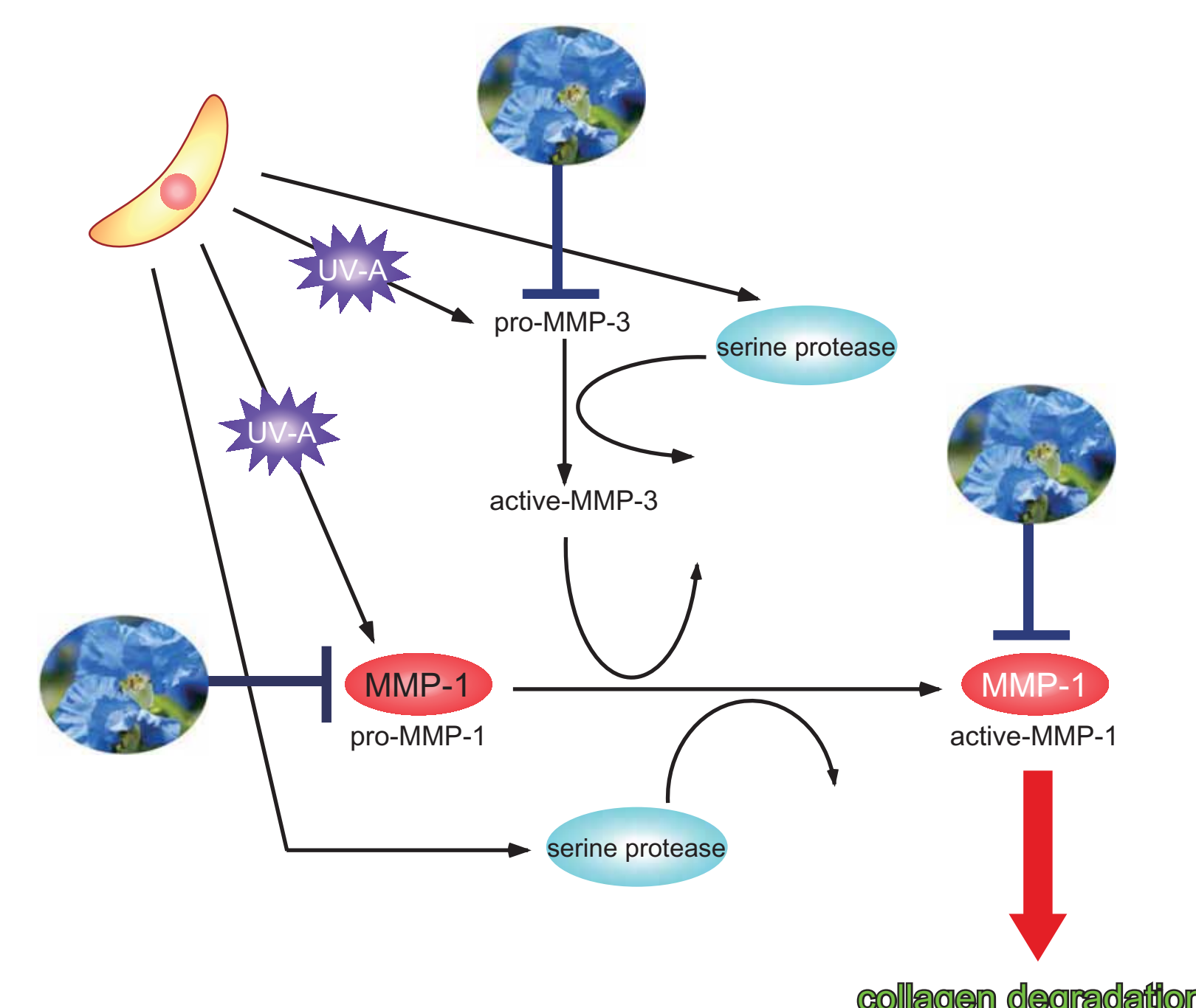


Fig. 9 Inhibitory mechanisms of collagen lattice miniaturization by *C. communis* var *hortensis* extracts  
Collagen degradation pathway and inhibitory mechanisms of *C. communis* var *hortensis*. It is thought that *Commulina communis* var *hortensis* inhibits collagen degradation in three terms at least, which MMP-1 expression, MMP-1 activity, and MMP-3 expression. In this method, we might be able to evaluate whole effect and magnify the capacity of some materials.

## Conclusion

### Known

- Collagen lattice contraction was promoted by cell activity.
- Active-MMP-1 is not produced by fibroblasts only.



### New

- Collagen degradation causes the miniaturization of lattice when fibroblasts are irradiated with UV-A and this phenomenon depends on MMP-1 activity.
- Pro-MMP-1 is activated in collagen lattice without keratinocytes.
- Plasmin and Cathepsin G are thought to be two of MMP-1 activators.
- *C. communis* var. *hortensis* inhibited collagen degradation.