

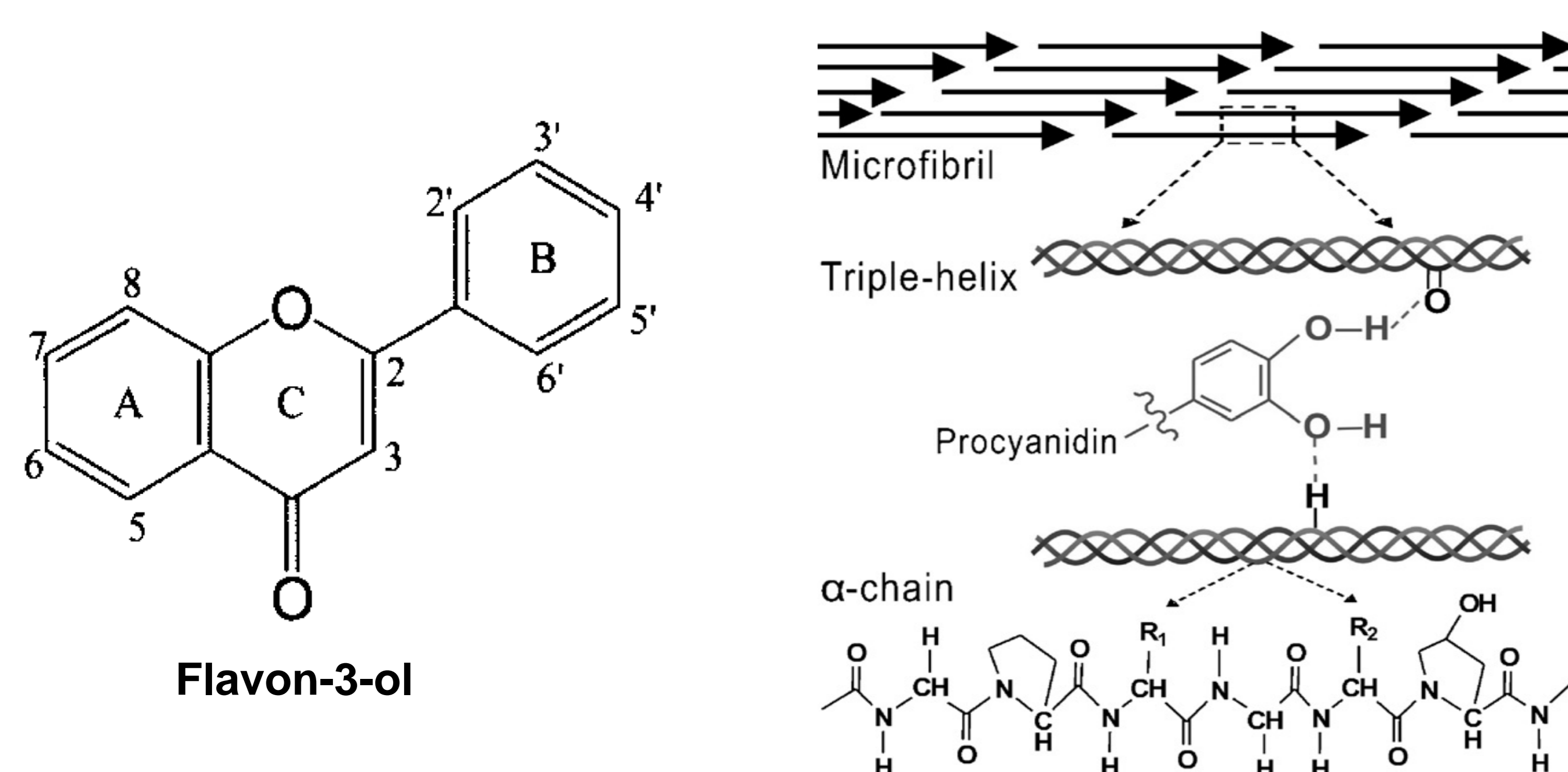
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## Background

The oxidative stimulus induced by Triethylene-glycol-dimethacrylate (TEGDMA) due to the activation of signaling pathways by ROS can induce cellular and molecular responses in odontoblast cells. These responses could be involved both in *de novo* synthesis and in the subsequent secretion and activation of pulp MMPs, which could be released into the dentinal fluid and contribute to the degradation of the resin–dentin interface.

Flavonoids are naturally occurring phenolic phytochemicals, which have been reported to possess several biological properties *in vitro*. The number and specific positions of hydroxyl groups and the nature of the substitutions determine whether flavonoids function as strong antioxidative or enzyme modulating agents.



Properties	Other Flavonoids	PDB-1 Experimental Flavonoid
Antioxidant	✓	✓
Dentin MMPs inhibitor	✓	✓
Collagen crosslinker	✓	✓
Dentin bond strength preservation	✓	✓
Low molecular weight	✗	✓
Ethanol-soluble	✓✗	✓
Non cytotoxic	✓✗	✓
Short application time	✗	✓

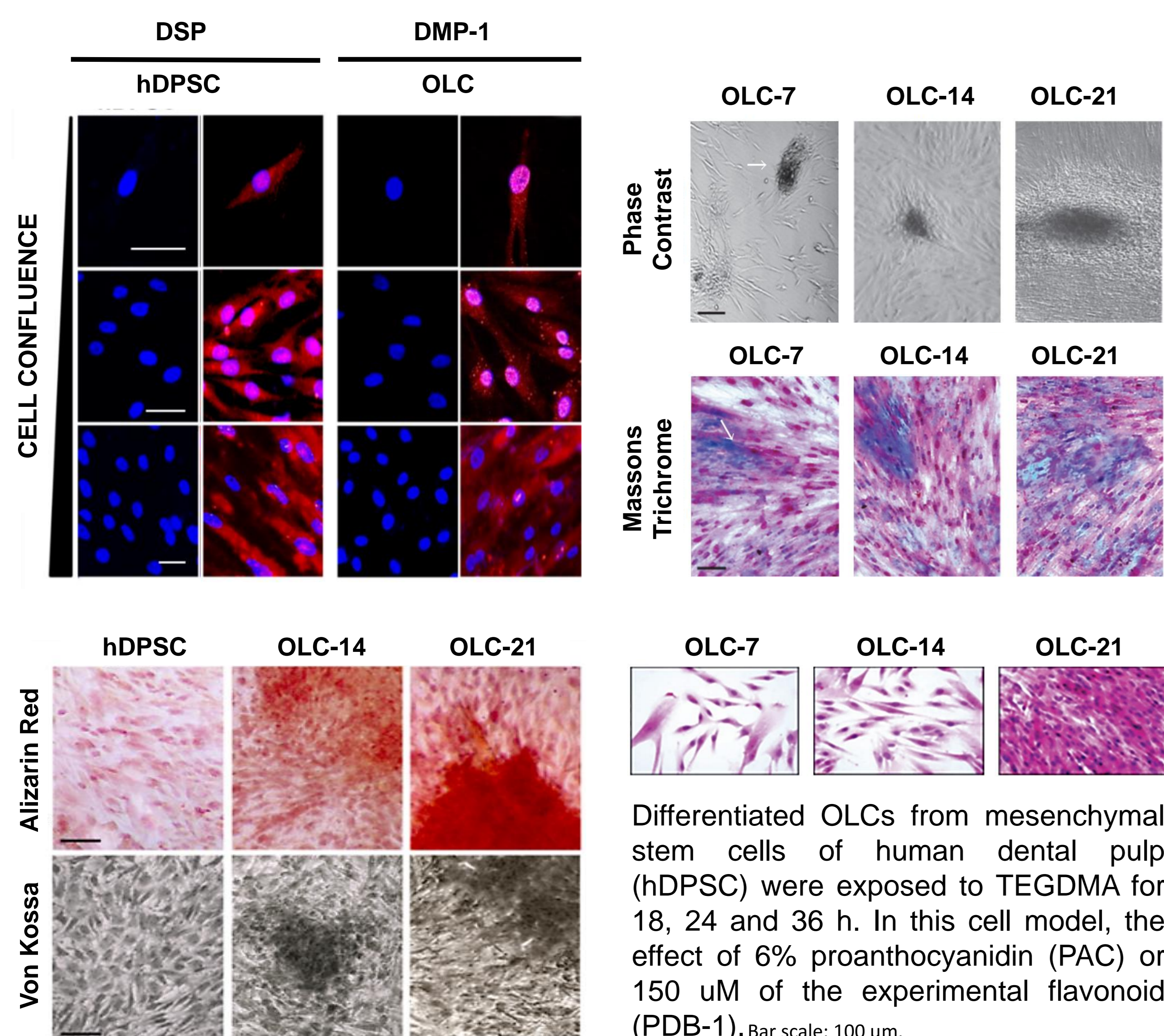
**Table 1. Flavonoids properties.** These antioxidants concerning the cell response, regulate the cellular redox homeostasis by controlling the intracellular levels of ROS, and as for the dentin, they act as MMPs inhibitors, collagen crosslinkers and bond strength preservers.

## Objective

To evaluate the effect of two flavonoids (proanthocyanidins and PDB-1) on the MMPs expression by exposure to TEGDMA in human odontoblast-like cells (OLCs).

## Methods and results

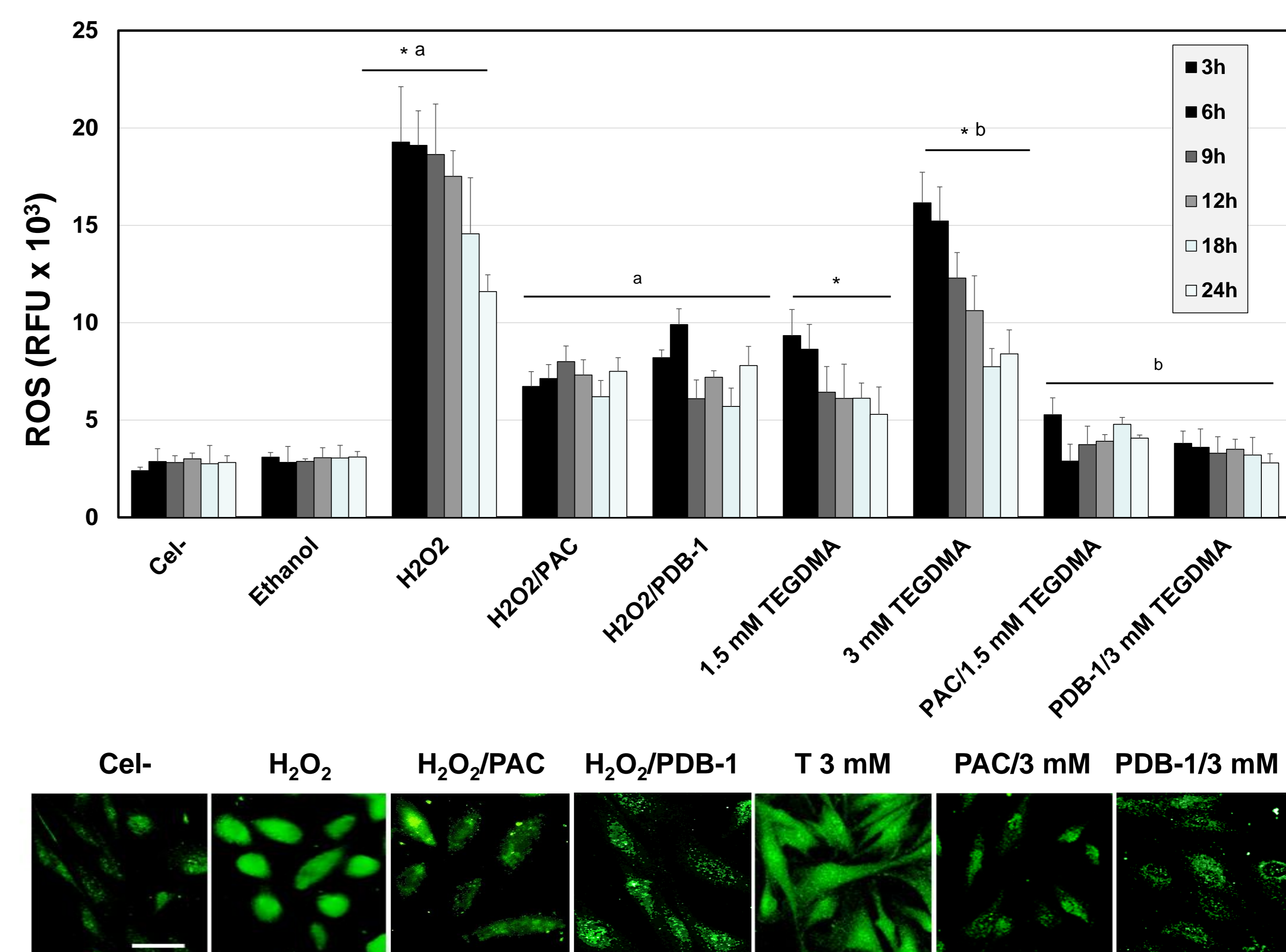
Based on a model previously published by our research group, Pulp mesenchymal stem cells were treated with odontogenic induction medium. The differentiated OLCs highly expressed odontogenic markers dentin sialoprotein (DSP) and dentin matrix protein-1 (DMP-1), and produce mineralized extracellular matrix.



Differentiated OLCs from mesenchymal stem cells of human dental pulp (hDPSC) were exposed to TEGDMA for 18, 24 and 36 h. In this cell model, the effect of 6% proanthocyanidin (PAC) or 150  $\mu$ M of the experimental flavonoid (PDB-1). Bar scale: 100  $\mu$ m.

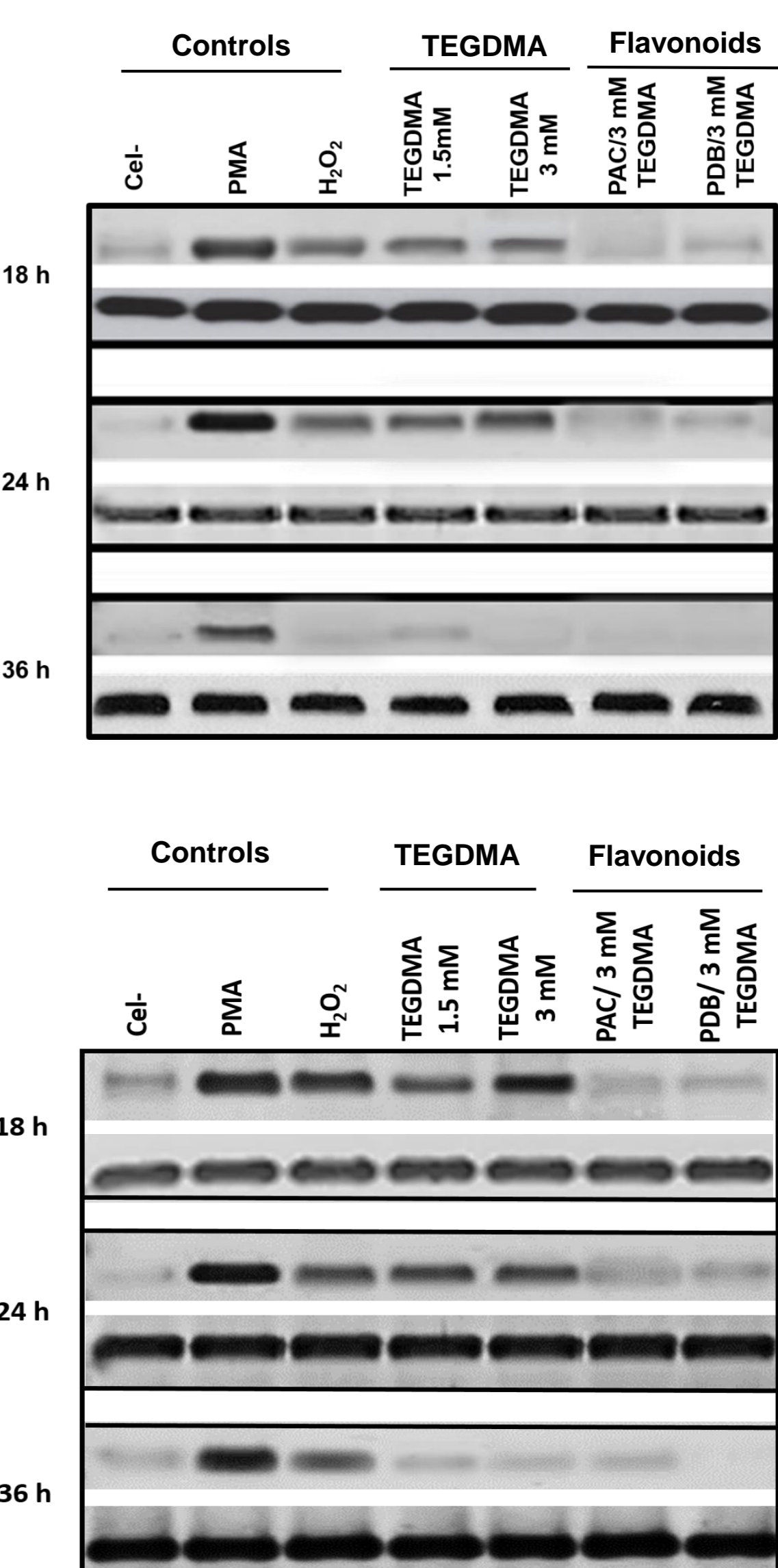
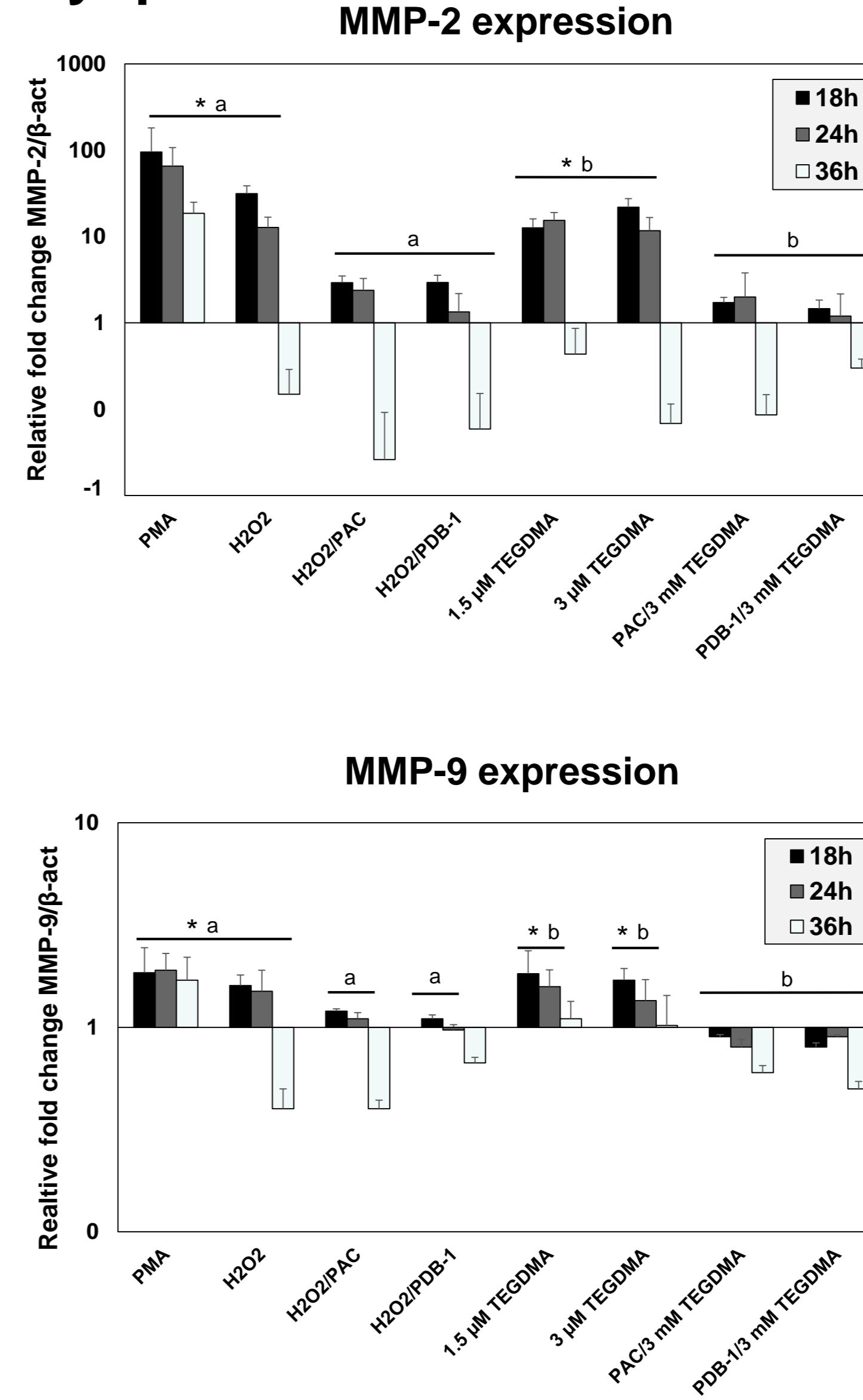
## Intracellular Reactive Oxygen Species (ROS)

ROS production induced by the exposure to TEGDMA of OLCs was evaluated using the 2,7-dichlorofluorescein probe. Increased levels of oxygen species in the cells oxidizes the reagent to a molecule with a higher fluorescence, which is quantified by spectrofluorometer and were observed by fluorescence microscopy.

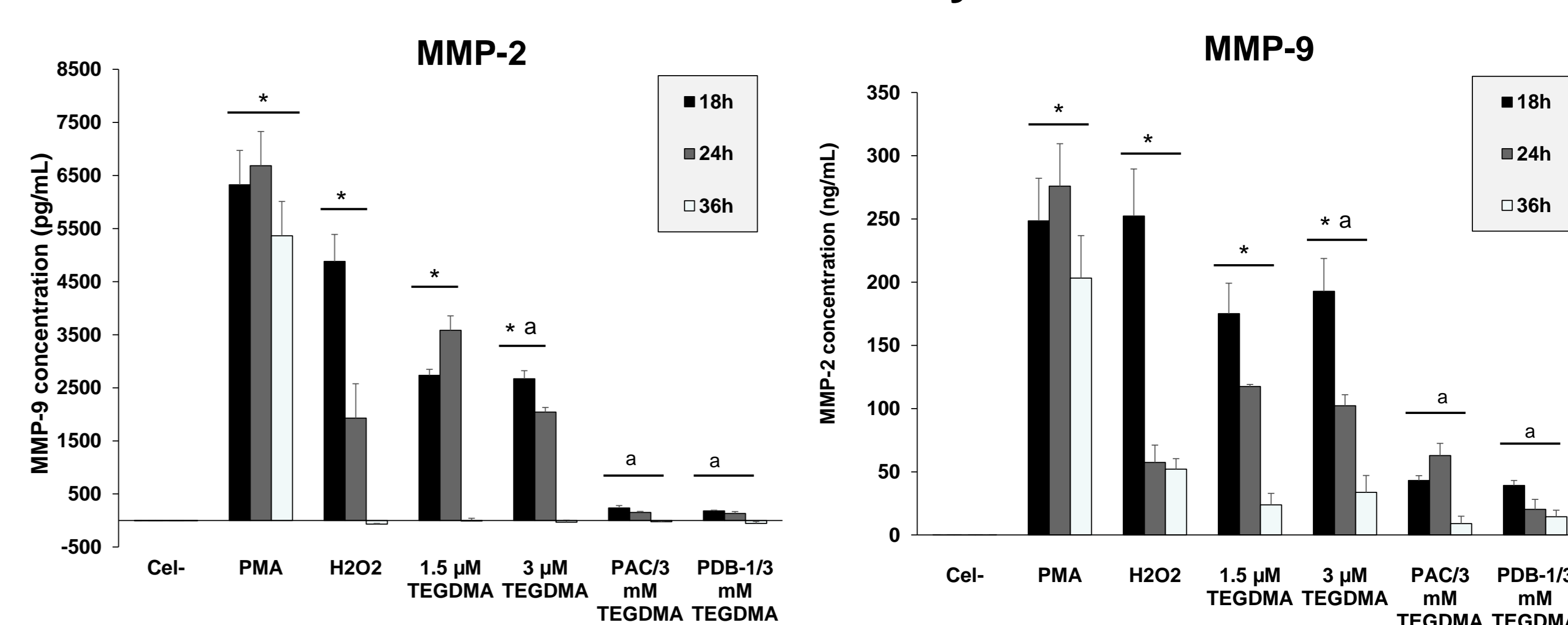


**Detection of ROS by fluorescence microscopy.** The presence of oxidizing species (green) is confirmed by exposure to TEGDMA for 6 h, similar to H<sub>2</sub>O<sub>2</sub>. Positive effect of flavonoids as an antioxidants. Bar scale: 100  $\mu$ m.

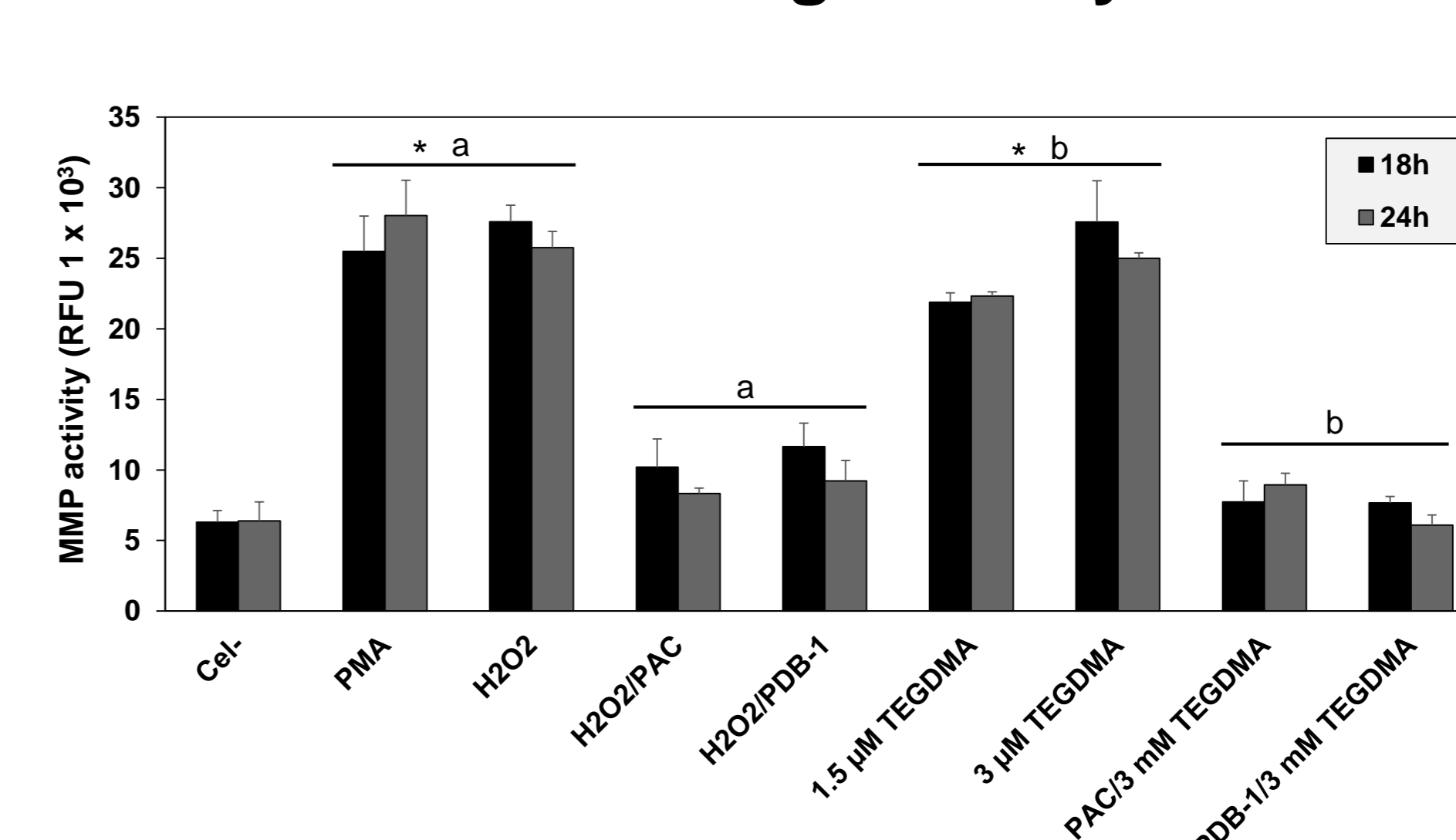
## Evaluation of MMPs expression by qPCR



## Detection of MMP-2 and -9 secreted by ELISA



## Quantification of the gelatinolytic activity of secreted MMPs



The MMPs enzymes present in the supernatants of the cells exposed at H<sub>2</sub>O<sub>2</sub> and TEGDMA showed a high activation capacity, which was controlled by flavonoids.

## Conclusion

The results demonstrate a relationship between the expression, secretion and activation of MMP-2 and MMP-9 and ROS increase induced by exposure to TEGDMA. PDB-1 had an inhibitory effect similar to that demonstrated by the PAC at the transcriptional and protein levels. Both flavonoids could be a therapeutic strategy to control factors that induce the MMPs activation and could improve the adhesive interface stability.