Histological and analytics studies on the formation and composition of the incremental lines of rat dentin by melatonin medication

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Purpose, Material and Methods

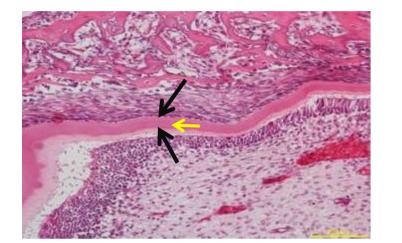
The purpose of the present study is to examine the relationship between the formation and composition of f incremental lines in tooth dentin and the role of melatonin by histological and analytics studies.

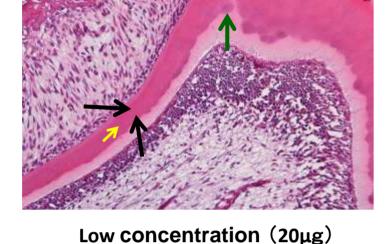
In this experiment, 5, 6, and 7 day old SD rats were used. These rats were divided into four groups: 1) a control group (0.5% alcohol content drinking water); 2) a low concentration group (0.5% alcohol +20µg/ml melatonin content drinking water); 3) a moderate concentration group (0.5% alcohol + 100µg /ml melatonin content drinking water); 4) a high concentration group (0.5% alcohol + 1000µg /ml melatonin content drinking water). The animal protocol was approved by the Animal Care and Use Committee of Meikai University. The specimens were observed and analyzed using a light microscopy, a scanning electron microscopy(SEM), a SEM-EDS analysis, a laser Raman microprobe spectrometry, and an electron-probe microanalyzer (EPMA). Staining: HE staining, azan staining and alkaline phosphatase (ALP) staining.

Results and Discussion

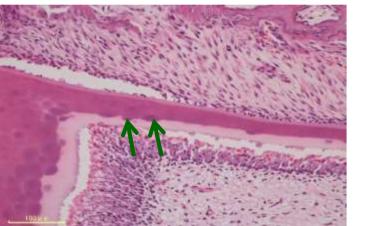
- In the control group, two dark-staining incremental lines of hematoxylin and one lightstaining layer were observed in incisor dentin.
- In the low melatonin concentration group, the light-staining layer was thin. In the moderate and high melatonin concentration groups, this layer disappeared. The number and size of calcospherites in predentin increased in proportion to the concentration of melatonin administered.
- By the result of HE staining and SEM observation, it was considered that the calcification of dentin was promoted by melatonin.

5 days old, daytime, decalcification specimens, HE staining, incisor





Control

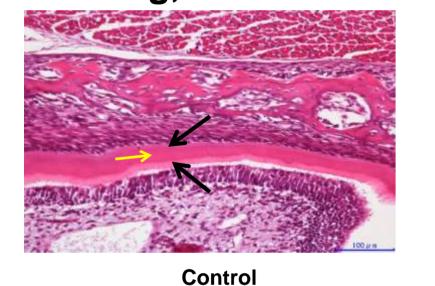


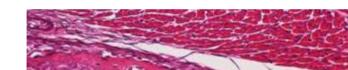
Moderate concentration (100µg

High concentration (1000µg

Black arrows: dark-staining incremental lines, yellow arrows: light staining layer, green arrows: calcospherites.

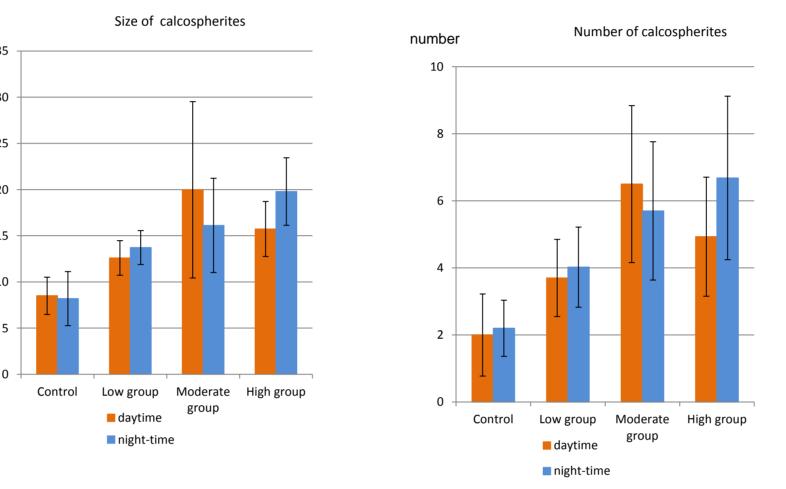
6 days old, night-time, decalcification specimens, HE staining, incisor





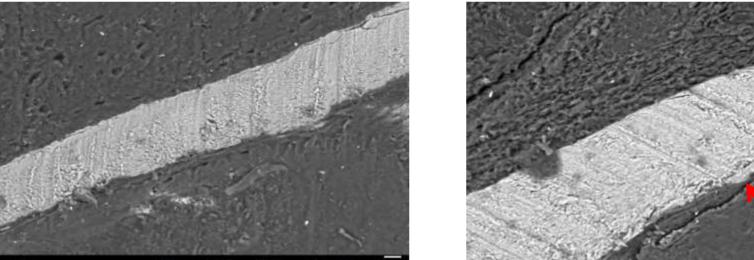
- In the moderate concentration group, ALP activity was high. It was suggested that the formation of the dentin was promoted by melatonin.
- In the moderate concentration group, a strong expression of ALP activity was observed.
- In EPMA analysis, Ca and P content were greater in the melatonin treated group.
- By laser Raman microprobe spectrometry, the full widths of half maximum intensity of the peak of PO_4^{3-} were narrower in the melatonin treated groups.
- It is considered that melatonin participates in the formation of incremental lines and the calcification mechanism of dentin

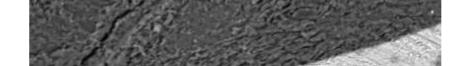
Number and size of calcosperites



Calcospherites in predentin were measured on specimens of the HE staining.

SEM: backscattered electron images of ground sections



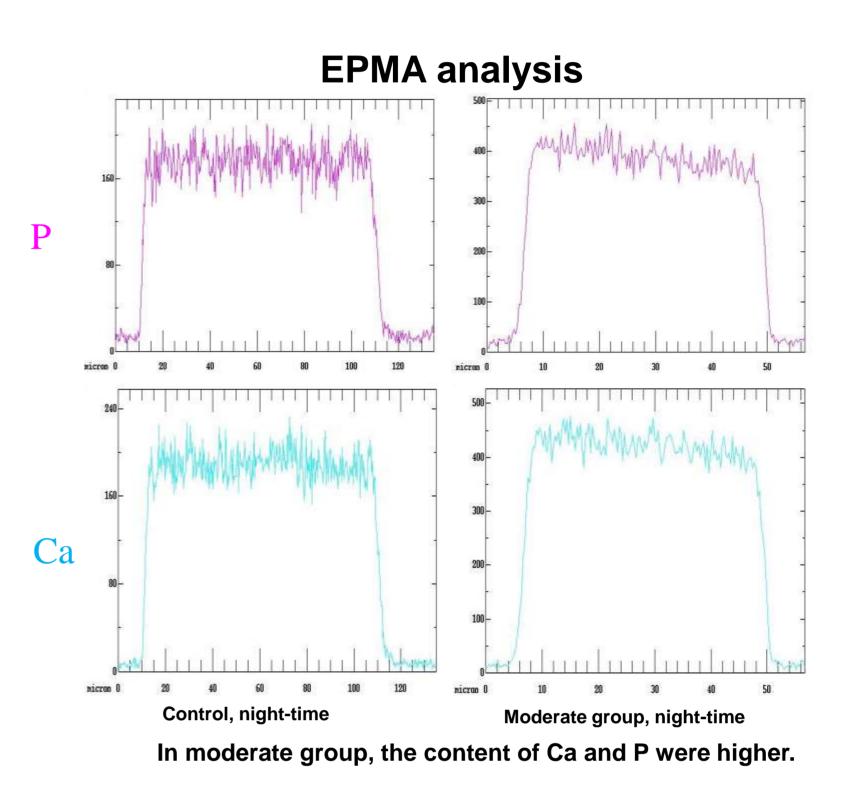


The number and size of

predentin increased . Red

arrow: calcospherites

calcospherites in

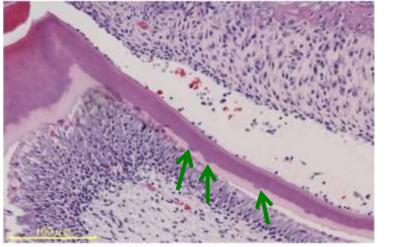


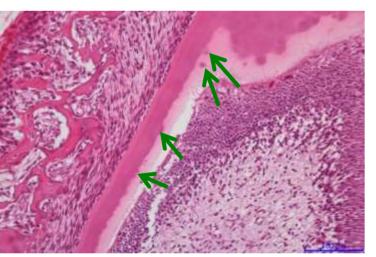
Results of laser Raman microprobe spectrometry

(Peak variation of the phosphate group PO_{4}^{3})



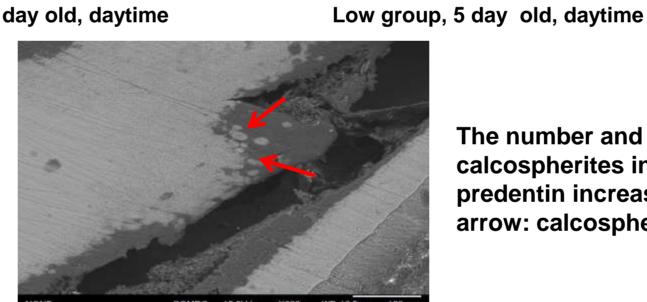
Low concentration (20µg)



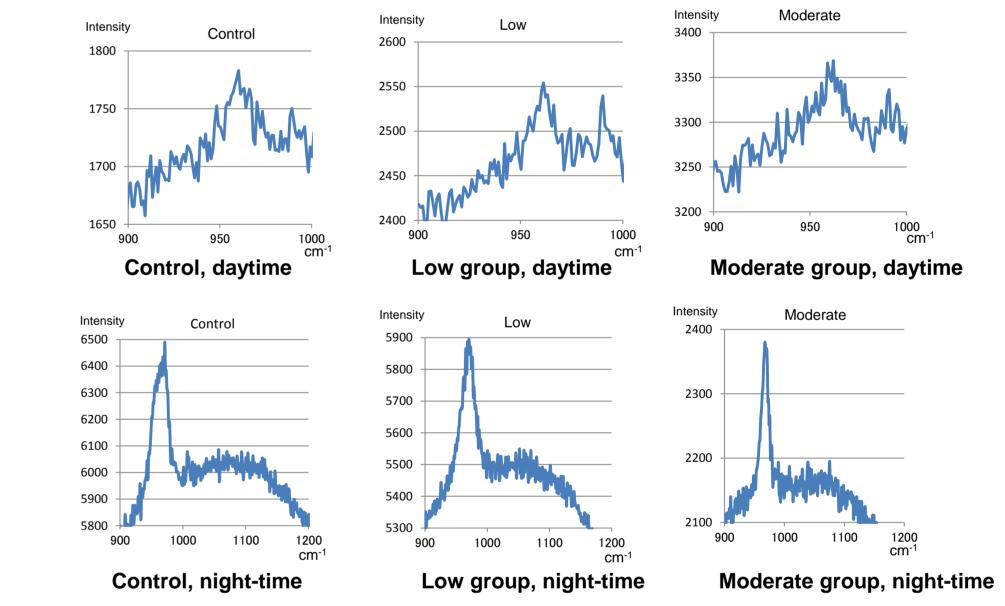


Moderate concentration (100µg) High concentration (1000µg) The number and size of calcospherites in predentin increased.

Control, 5 day old, daytime

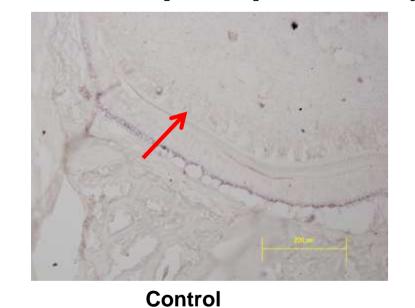


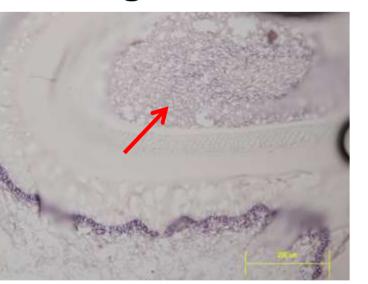
Moderate group, 5 day old, daytime



In the melatonin treated groups, the full widths of half maximum intensity were narrower.

7 days old, night-time, decalcification frozen specimens, alkaline phosphatase (ALP) staining, molar

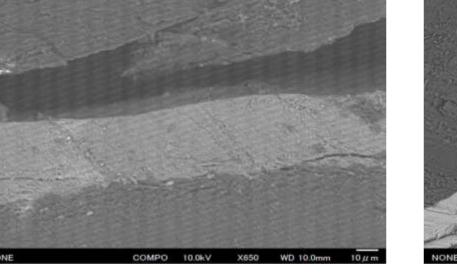




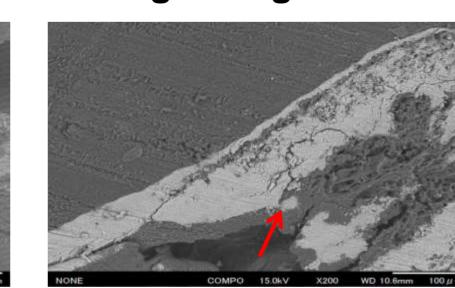
Low concentration (20µg)

In the moderate

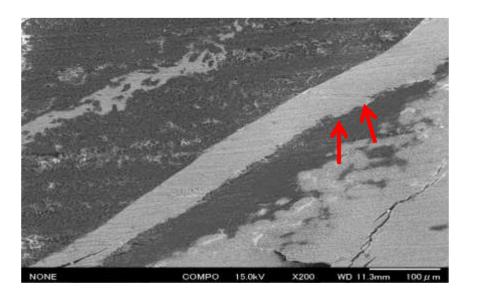
SEM: backscattered electron images of ground sections



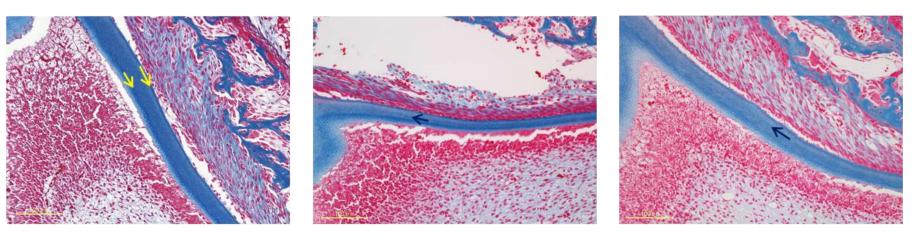
Control, 6 day old, night-time



Low group, 6 day old, night-time



5 day old, night-time, Azan staining, incisor



Low concentration

Control

Moderate concentration

Two dark-staining incremental lines were observed (arrow: yellow).

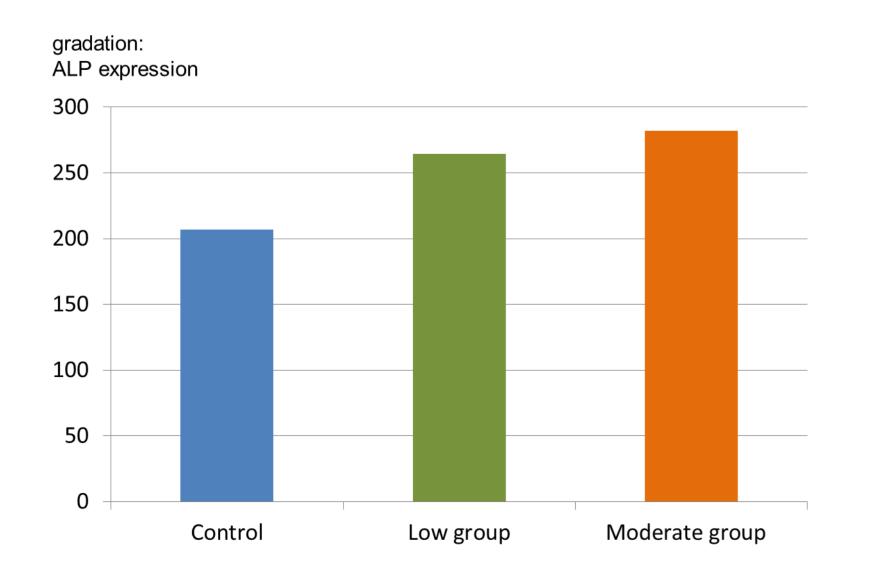
New incremental lines were observed (arrow: black).



concentration group, ALP activity was high. Red arrow: odontoblasts.

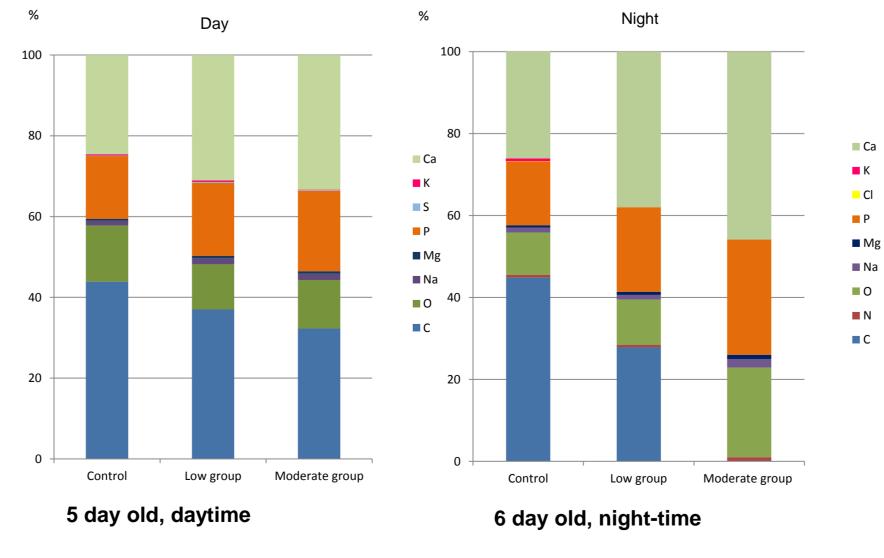
Moderate concentration (100µg)

Expression changes of ALP activity (average)



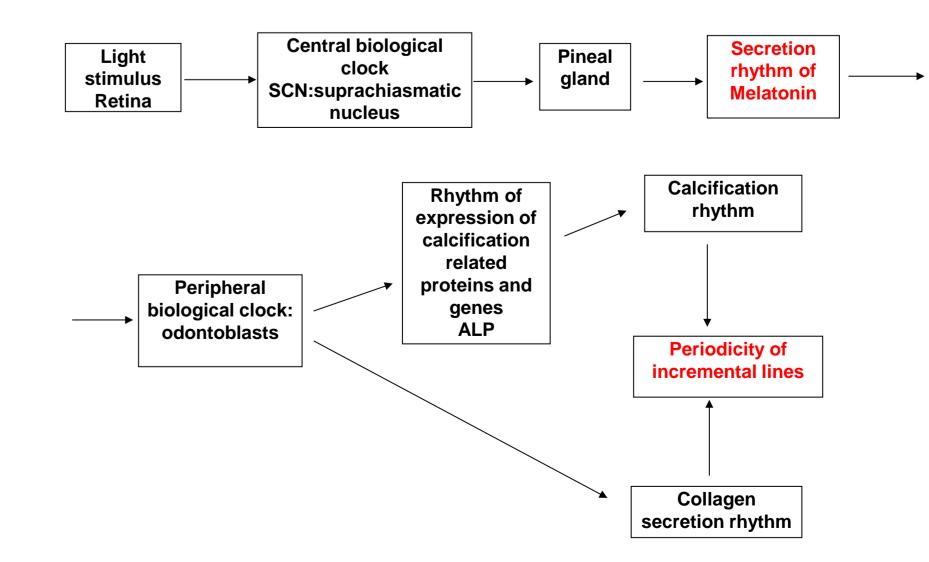
Moderate group, 6 day old, night-time

SEM-EDS analysis (Wt.%)



Ca and P content increased in the melatonin treated groups.

Periodicity of Incremental Lines in Dentin



It is suggested that melatonin may participate in the periodicity of the incremental lines of dentin.

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