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# Temporal observation of osteoblastic mineralization by Raman imaging

Aya Hashimoto[1], Liang-da Chiu[1], Tomohiko Ikeuchi[1], Katsumasa Fujita[1], Masahide Takedachi[2], Yoshinori Yamaguchi[1, 3], Satoshi Kawata[1, 4], Shinya Murakami[2], Eiichi Tamiya[1, 4]

[1] Department of applied physics, Graduate School of Engineering, Osaka University,
[2] Department of Periodontology, Graduate School of Dentistry, Osaka University,
[3] Department of Physics, Faculty of Science, East China University of Science and Technology,
[4] Photonics Advanced Research Center, Osaka University
E-mail: hashimoto@ap.eng.osaka-u.ac.jp

## 1. Introduction

Mineralization is main function of osteoblasts. In the mineralization process, osteoblasts initiate production of hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$), which is major component of bone. Then, through several biological processes they calcify surrounding extracellular matrix and finally bone tissue is formed. Previous researches have shown that various biomolecules are involved in the process of osteoblastic mineralization. Although traditional bio logical assays greatly contribute to understanding bone formation, they are unable to follow up the osteoblastic mineralization process since they are destructive techniques. Therefore a nondestructive and quantitative measurement technique is required to further increase our understanding of the osteoblastic mineralization process. Raman imaging is a non-destructive and label-free technique that provides contrast images based on vibrational frequencies that are derived to target molecules. Thus, Raman imaging enables time-lapse observation of the dynamics of the biomolecules in a living specimen without any preliminary preparations. In this study, we observed the osteoblastic mineralization process in the culture of mouse osteoblasts by consecutive Raman imaging

## 2. General Instructions

We induced differentiation of KUSA-A1, a mouse mesenchymal stem cell line, into osteoblasts and monitored the differentiation and mineralization process by Raman imaging. The Raman measurements were taken every 4 hours. HA Raman band was used as a marker for the osteoblastic mineralization since HA Raman signal was gradually strong by progression of the processes [1]. As the result, we revealed that HA was produced in where β-carotene was localized in osteoblastic mineralization process. In the Raman images, first, Raman signal of β-carotene was detected from minute spots in the tissue and then, HA Raman signal appeared around the minute spots after disappearance of the β-carotene signal. Previous researches have reported that β-carotene has a direct stimulatory effect on the differentiation of osteoblasts. From our result and the previous study, it is suggested that β-carotene is an important biomarker of initial site of the mineralization. Furthermore, decrease of Raman signals which are assigned to cytochrome c and protein was observed. This indicates that cytochrome c was released from the mitochondrial intermembrane space into the cytoplasmic matrix and the intracellular protein was degraded by apoptosis. This results corresponded to previous report that most of the osteoblasts dies by apoptosis to regulate physiologic bone remodeling process after the mineralization process.

## 3. Conclusions

The monitoring of mineralization-related molecules in the same osteogenic lineage cells was successfully accomplished. It was revealed that HA was produced in where β-carotene was localized. In addition, the decrease of cytochrome c and protein Raman signals, which is associated with apoptosis, was observed after the mineralization process. Raman imaging serves as a powerful tool to reveal the mechanism of osteoblastic mineralization in an in-vitro culture of osteogenic lineage cells.

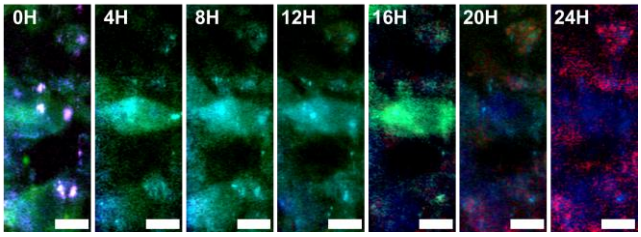


**Figure. Raman images of mouse osteoblasts.** The Raman images were reconstructed from the distribution of the Raman signal at 750, 956, 1526 and 2940 $cm^{-1}$ assigned to cytochrome c, HA, β-carotene and protein, respectively. In the image, cytochrome c, HA, β-carotene and protein are shown in green, red, magenta and blue, respectively. Scale bar: 10 μm.

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## References

[1] A. Hashimoto, L. D. Chiu, K. Sawada, T. Ikeuchi, K. Fujita, M. Takedachi, Y. Yamaguchi, S. Kawata, S. Murakami, E. Tamiya, J. Raman Spectrosc. **45** (2014) 157.