In situ second-harmonic-generation imaging of collagen fibers produced by standing-cultured osteoblasts

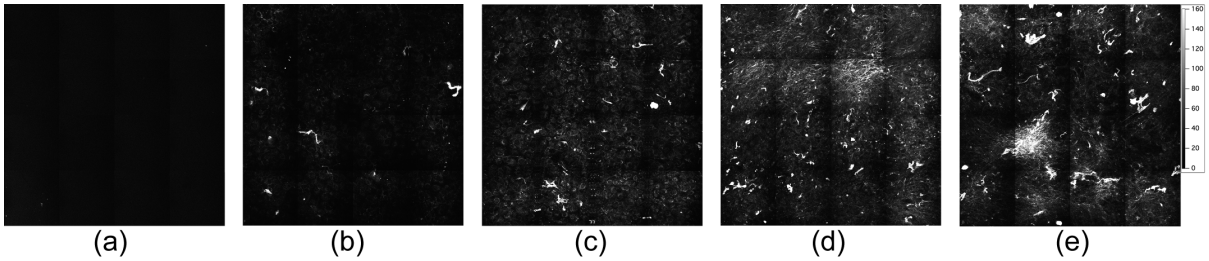
Takeshi Yasui, Eiji Hase, Takeo Minamikawa, and Katsuya Sato
Tokushima University
2-1, Minami-Josanjima, Tokushima, Tokushima 770-8506, Japan
E-mail: yasui.takeshi@tokushima-u.ac.jp http://femto.me.tokushima-u.ac.jp/eng/

In bone formation and regeneration in tissue engineering, it is important to monitor the density and structure of collagen fibers in bone because collagen fibers play an important role in constructing bone tissue as an extracellular matrix and the structural maturity of them, namely collagen maturity, is related with bone mineralization. In this article, we visualized the temporal evolution of collagen fibers produced by standing-cultured osteoblasts during a culturing period of 4 weeks *in situ* by using SHG microscope equipped with a 19 fs near-infrared pulsed light to enhance the SHG image contrast [1].

We used the mouse osteoblast cell line MC3T3-E1, which was provided by the RIKEN-BRC through the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science and Technology, Japan. The cells were cultured with α-MEM medium to which was added 10% FBS until the cultured cell concentration reached 80% confluent. We used a chamber made of silicone rubber for cell culturing. After the cells were seeded onto the chamber, they were cultured in the medium with an osteoblast-inducing reagent. This medium was α-MEM medium to which was added 1% ascorbic acid, 0.2% hydrocortisone, and 2% β-glycerophosphate. For sterilization, we also added 1% penicillin and streptomycin solutions. A single layer of osteoblasts adhered onto the bottom of the chamber produced a thin layer of collagen fibers (thickness < 10 μm) for the culturing period.

Figure 1 shows time-series SHG images of the collagen fiber distribution in one and the same sample at (a) 0 day, (b) 1 week, (c) 2 weeks, (d) 3 weeks, and (e) 4 weeks after starting the standing culture. The sample at 0 day, which is before producing the collagen fibers, indicated no SHG signals. In Fig. 1(b), the distribution of the collagen fibers appeared as circular shapes, implying that the collagen fibers were produced and stored in the osteoblasts. In Fig. 1(c), a similar distribution was confirmed; however, SHG light was more intense than that in Fig. 1(b), implying that the produced collagen fibers accumulated in the osteoblasts and their density increased. In Fig. 1(d), thin collagen fibers appeared as a network structure outside the osteoblasts in addition to the circle-like collagen distribution inside them. In Fig. 1(e), the collagen fibers became thicker, and the density of collagen fibers outside the osteoblasts increased locally. Such behavior of the collagen fibers during the standing culture was reasonably in agreement with previous researches based on the stained images.

[1] E. Hase *et al.*, "*In situ* time-series monitoring of collagen fibers produced by standing-cultured osteoblasts using a second-harmonic-generation microscope," *Appl. Opt.* **55**, 3261-3267 (2016).



(a) (b) (c) (d) (e)

Fig. 1. *In situ* time-series SHG images of the collagen fiber. (a) 0 day, (b) 1 week, (c) 2 weeks, (d) 3 weeks, and (e) 4 weeks after starting the standing culture.