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Atomic force microscopy of collagen structure in bone and dentine revealed by osteoclastic resorption

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Abstract

Mineralised tissues such as bone consist of two material phases: collagen protein fibrils, secreted by osteoblasts, form model structures for subsequent deposition of mineral, calcium hydroxyapatite. Collagen and mineral are removed in a three-dimensional manner by osteoclasts during bone turnover in skeletal growth or repair. Bone active drugs have recently been developed for skeletal diseases, and there is revived interest in changes in the structure of mineralised tissues seen in disease and upon treatment. The resolution of atomic force microscopy and use of unmodified samples has enabled us to image bone and dentine collagen exposed by the natural process of cellular dissolution of mineralised matrix. The morphology of bone and dentine has been analysed when fully mineralised and after osteoclast-mediated bone resorption, and compared with results from other microscopy techniques. Banded type I collagen, with 66.5 ± 1.4 nm axial D-periodicity and 62.2 ± 7.0 nm diameter, has been identified within resorption lacunae in bone and 69.4 ± 4.3 nm axial D-periodicity and 140.6 ± 12.4 nm diameter in dentine substrates formed by human and rabbit osteoclasts, respectively. This observation suggests a route by which the material and morphological properties of bone collagen can be analysed in situ, compared with collagen from non-skeletal sites, and contrasted in diseases of medical importance, such as osteoporosis, where skeletal tissue is mechanically weakened.

Keywords: Atomic force microscopy (AFM); Collagen; Resorption lacunae; Osteoclast; Dentine; Bone

1. Introduction

The structure of mineralised tissues, such as bone, is continually modified by cellular processes in response to changes in the mechanical stress to

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which the skeleton is exposed during everyday life. Bone can be considered as a nanostructured composite material comprising two compartments—the protein component, type I collagen, is produced by osteoblastic cells, thus forming a model for the subsequent deposition of calcium phosphate mineral, hydroxyapatite. Mineralised bone is removed by osteoclasts during bone dissolution in skeletal growth and repair after, for example, bone fracture. With the advent of effective therapies for common skeletal diseases such as osteoporosis, and the need for disease-modifying drugs for osteoarthritis, there has been renewed interest in understanding the structure–function relationship of nature's 'smart' materials in both health and disease and identifying any changes that might occur as a consequence of treatment.

A key initiator of the skeletal response to tissue damage (fracture), alterations in the amount of exercise (strain) or systemic changes (hormonal imbalance) is the osteoclast. These cells are relatives of tissue macrophages, the professional phagocytes that fight infection, and function solely to dissolve (resorb) bone and other mineralised tissues. They do this by an extra-cellular process, secreting acid to dissolve the mineral phase and cathepsin and metalloproteinase enzymes to degrade type I and other minor collagens and non-collagenous proteins. During this process, they form three-dimensional excavations in the bone surface—in vivo these are called Howship's lacunae and when studied in vitro, resorption lacunae or 'pits'. This natural process has the side benefit of providing a glimpse into the internal structure of skeletal tissue, without having to resort to technical procedures (such as tissue fixation, embedding, coating, staining or sectioning) and this feature can be utilised to examine the way collagen is laid down during osteoblastic synthesis in health and disease or following drug treatment. Traditionally, analysis of these processes has been performed after histochemical or immunological staining and followed by reflection or confocal optical or scanning electron microscope (SEM) imaging (as in Fig. 1). In this paper, we have utilised atomic force microscopy (AFM) to obtain quantitative high-resolution images of bone collagen in situ within resorption lacunae without the need for prior preparation.

A number of reports have appeared in the literature concerning AFM imaging of collagen in bone and dentine following artificial removal of calcium using mild acidification or chelation with EDTA [1–3]. These studies generally have been descriptive, aimed at relating collagen structure seen by AFM with that observed by transmission electron microscopy after fixation and staining, or to the process of in vitro collagen fibrillogenesis [4–6]. There have been some reports on the material properties of mineralised tissues at the bulk scale using AFM 'nanoindentation' of bone and tooth samples retrieved from patients and relating these to normal and diseased tissue structure (for example, Ref. [7]). Attempts at 'single molecule' mechanics have been made with bone collagen (such as reported by the Hansma group, [8,9]), but none have analysed these parameters in the face of natural bone degradation as seen after osteoclastic resorption. We have only found one report [10] where the consequences of osteoclastic resorption upon a mineralised substrate in vitro have been examined—however, the image resolution was poor in this publication and the authors did not comment upon collagen structure; indeed they used AFM as a metrology tool to measure the depth of resorption lacunae. In contrast, there is a substantial body of literature examining the detailed structure of the collagenous extra-cellular matrices in non-skeletal tissues, such as in the sclera and cornea [11–13].

Data is presented herein demonstrating the improved resolution of AFM imaging for the detailed structural analysis of bone and dentine collagen that is exposed by the natural, cellular dissolution of bone matrix. In summary, the morphology of native, fully mineralised, bone and dentine substrates have been characterised before and after osteoclast-mediated bone resorption by local probe techniques and compared with the gold standard analytical techniques used by bone cell biologists [14]. Using AFM, banded type I collagen approx. 66.5 ± 1.4 nm axial D-period spacing and 62.2 ± 7.0 nm fibril diameter has been identified in resorption lacunae formed in bone and 69.4 ± 4.3 nm axial D-periodicity and