Applications of Synchrotron Infrared Microspectroscopy to the Study of Biological Cells and Tissues*

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Synchrotron infrared light is an ideal source for infrared microspectroscopy due to its high brightness and broadband nature. These characteristics permit the collection of high signal-to-noise spectra through small apertures (3-5 mm in the mid-infrared region) and optically dense samples (0.1 % transmission). Using the advantages of the synchrotron infrared source, we are able to *chemically* image biological samples that are too small and/or too thick to examine with a conventional globar source. At Beamline U4-IR at the National Synchrotron Light Source, we have imaged the protein (Amide I, 1650 cm^{-1}; Amide II, 1545 cm^{-1}) and lipid (2850 cm^{-1}) components of a living cell in the process of cell division. By imaging the lipid components, we observe a high concentration of lipids in the center of the dividing cell, in the region where the contractile ring responsible for the cleavage furrow is located. We have also chemically imaged dying cells and found significant broadening of the protein (Amide I and Amide II) bands and the formation of a sharp, carbonyl ester peak near 1740 cm^{-1}. This feature most likely signifies protein oxidation which occurs during necrosis. A second biological application of synchrotron infrared microspectroscopy is the study of hair and the effects of chemical treatment on the structure of hair. For the first time, the high spatial resolution achievable with the synchrotron infrared source allows us to study the three components of hair *individually*, i.e., the medulla, cortex, and cuticle. By imaging the lipid region, we find that the medulla contains a higher CH_2/CH_3 ratio than the cortex, suggesting a higher average lipid chain length in the medulla. In addition, we observed keratin oxidation in the cortex upon bleaching, represented by the appearance of an S=O feature at 1040 cm^{-1} in bleached hair. Bleaching also results in hydration of the cuticle, which we observe as an increase in the bound-water concentration at 3400 cm^{-1}. A third biological application that will be presented is the study of bone chemical composition and bone disease. In this case, the

synchrotron infrared source is necessary due to the highly absorbing mineral components in bone. In osteoarthritis, it has been demonstrated that the bone underlying the joint cartilage (subchondral bone) becomes thickened prior to cartilage breakdown. Thus, using synchrotron infrared microspectroscopy, we have examined the chemical composition of the subchondral bone in histologically normal and osteoarthritic monkeys. We find that the bone crystallinity, i.e., average crystal size, is similar in osteoarthritic and normal bone. However, the subchondral bone of osteoarthritic monkeys is significantly more mineralized than the normal bone, primarily due to an increase in carbonate concentration in the osteoarthritic bone.

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*The NSLS is supported by the Department of Energy under contract DE-AC02-98CH10886. The work on cells is supported by NATO Grant CRG 972179. The work on hair is done in collaboration with Yves-Rocher, Inc. The work on bone is supported by the NIH Grants 5T32-AG00194 (L.M.M.), RR01633 (M.R.C.) and RR08562 (C.S.S.).