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Comments on *Neutron diffraction studies of collagen in human cancellous bone* by Skakle & Aspden (2002)

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Comments are made on a paper by Skakle & Aspden [*J. Appl. Cryst.* (2002), **35**, 506–508] regarding the lateral spacing of collagen in human bone.

Skakle & Aspden (2002) report their determination of the lateral spacing of the collagen in human bone, both compact and cancellous. They question the accuracy of previous neutron diffraction studies of mineralized tissues. One objective of their study was to improve the determination of the diffraction line peak in the presence of considerable noise.

In prior studies, it was assumed that the signal cross section is a Gaussian embedded in noise. The two components are extracted as two functions using a least-squares program. The Gaussian peak is taken to be the peak of the diffraction line. Skakle & Aspden used a computer program that does not assume a shape for the line except that it be similar to a second-order polynomial. They found the lateral spacing of wet tissue human compact bone to be 1.230 nm, and 1.191 nm when dry. Lees *et al.* (1984) reported finding the lateral spacing for cow bone, of density 2.04 Mg m^{-3}, to be 1.24 nm wet and 1.16 nm dry. Skakle & Aspden did not cite the density of their material.

Lees (2003) showed that the lateral spacing d for compact bone collagen is strongly linear with the inverse wet density,

$$d = (0.75/\rho) + 0.871,\ R^2 = 0.98,$$

where ρ is the wet tissue density. There is a different line for the dry tissue spacing,

$$d = 1.467 - (0.639/\rho),\ R^2 = 0.95.$$

The slope of the wet tissue spacing is positive and that for the dry tissue is negative. The largest deviation for either line is less than 0.02 nm. Values for the lateral spacing are listed in Table 1 for both cow and human bone densities. The values of Skakle & Aspden are entered in the last column. The entries for bone of density 2.04 and 1.80 Mg m^{-3} are experimental; the others were obtained from the above equations.

Since the lateral spacing increases with decreasing wet tissue density, and 1.23 nm is a value smaller than that of the other wet tissues in Table 1, the tissue density should be greater than 2.04 Mg m^{-3}. The same argument is employed for dry tissues. When the lateral spacing *decreases* with decreasing density, and 1.191 nm is greater than all other dry tissue terms in Table 1, the wet density again should be greater than 2.04 Mg m^{-3}. Skakle & Aspden were unable to interpret their results because they did not account for density. The difficulty here is that human compact bone is less dense than cow bone. The data-extraction protocol must be consistent since it yields the strong linear dependence on the inverse wet density with an uncertainty less than 0.02 nm

Table 1
Comparison of the lateral spacing of collagen in bone of different densities.

Sample	2.04 Mg m^{-3}	2.01 Mg m^{-3}	1.95 Mg m^{-3}	1.80 Mg m^{-3}	Skakle & Aspden (2002)
Wet spacing (nm)	1.24	1.244	1.256	1.29	1.230
Dry spacing (nm)	1.16	1.149	1.14	1.11	1.191

Lees & Hukins (1992) demonstrated the successful use of X-ray diffraction to determine the lateral spacing of collagen in cow bone of wet density 2.01 Mg m^{-3}. Six adjacent samples of the same bone were obtained. The uncertainty between samples was $\pm$0.03 nm and the uncertainty within a single pattern was $\pm$ 0.01 nm. The lateral spacing (1.22 nm) compares with the calculated value of 1.244 nm, allowing for the uncertainty. No assumptions for the shape of the diffraction line were required. It would be valuable to compare the lateral spacing for the same single specimen by the various known methods in order to evaluate the contributions to the error by the source of neutrons and by the data-extraction process. If possible, the same data should be treated by several processes.

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