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doi:10.1017/S1431927622006390

Microsc. Microanal. 28 (Suppl 1), 2022

Transmission Electron Microscopy Using Hitachi's HILEM IL1000™ Ionic Liquid as a Dispersion Agent for Fetuin-Mineral Complexes

Heather Rose Berensmann[1*], Jenna N. Toohey[2]

[1.] Hitachi High-Tech America Inc. Nanotechnology Systems Division, Clarksburg, MD USA
[2.] Department of Biochemistry, Westminster College, New Wilmington PA USA
[*] Contact Information: Heather.Berensmann@hitachi-hightech.com

Understanding the environment and organization of the bone matrix requires investigation into the growth of hydroxyapatite crystals within the collagen matrix. Non-collagenous proteins, such as fetuin, have shown to regulate the growth of this mineral within the highly crowed bone matrix. In order to further understand the structure and function of these mineral inhibitors, transmission electron microscopy (TEM) is utilized. However, observations of such calcium-phosphate bone minerals have shown to produce large conglomerates of clustered particles when drop-casted onto grids, making it challenging to image. To reduce conglomeration, Hitachi's HILEM IL1000™ Ionic Liquid was added to the sample aliquot prior to drop casting in order to act as a dispersion agent. This ionic liquid was developed for electron microscopy preparation, having properties such as high solubility, high ionic conductivity, nontoxic characteristics, and having a biological compatible pH of 7.4 [1]. The IL1000™ has also shown to preserve structural integrity while avoiding many of the difficulties encountered with traditional preparation techniques [1, 2].

Before the fetuin-mineral samples were imaged, they were first suspended in 40 μl of ethanol and sonicated for 20 minutes. The sample without ionic liquid was then drop casted as-is onto silicone monoxide grids. The other sample was then combined (1:1) with 20% (w/v) IL1000™ (diluted in ethanol) and drop casted onto lacey carbon grids. Since the grids were placed upon filter paper, the excess fluid was absorbed, leaving behind menisci containing the fetuin-mineral in the voids of the holey carbon. Both sets of grids were then imaged at either 100 kV or 120 kV in a Hitachi HT7800 TEM with a tungsten filament and an AMT NanoSprint15 direct mount camera.

The untreated sample, as shown in figure 1a, contained large chunks of the fetuin-mineral sample, roughly 2.5 μm across. When trying to image at higher magnifications (x200,000) as shown in figure 1b, the highly clustered assembly made it challenging to isolate individual needle-shaped hydroxyapatite particles. The large conglomerations also created difficulties locating a suitable area for selected area electron diffraction. Even when an area was discovered, as shown in figure 1c and 1d, the multilayer assortment produced various planes within the pattern. After suspending the sample with the ionic liquid, shown in figure 2a, the chunk size was reduced to less than 1 μm across and smaller particles were dispersed around it. Within the holy carbon voids, containing the ionic liquid meniscus, particles were further dispersed and better distributed, as seen in figure 2b. Making it easier than the untreated sample to observe individual hydroxyapatite particles at higher magnifications (x300,000) shown in figure 2c. This is turn made it less challenging to find suitable areas for diffraction without the interference of multiple planes, shown in figure 2d and 2e.

The results of this data demonstrate that Hitachi's HILEM IL1000™ Ionic Liquid is a suitable dispersion agent in drop casting techniques for fetuin-mineral complexes. By dispersing the particles in the ionic liquid, imaging and selected area electron diffraction capabilities with transmission electron microscopy were improved. With properties designed specifically for electron microscopy, the IL1000™ continues to improve various techniques for an abundance of applications [1, 2, 3].

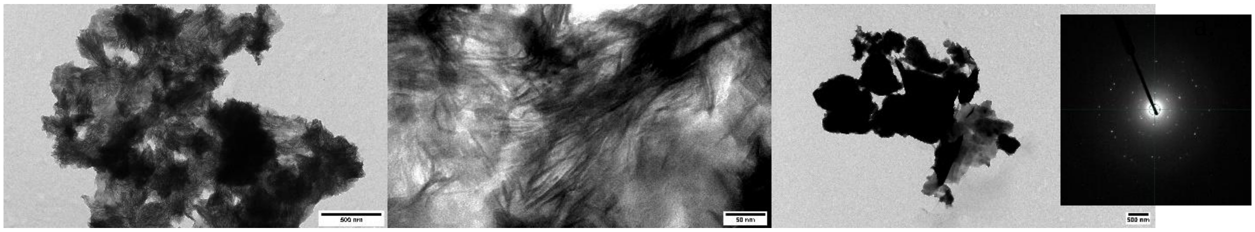

Figure 1. Untreated fetuin-mineral sample imaged at 100 kV. **(a.)** A large chunk of the fetuin-mineral sample, roughly 2.5 μm across, at x30,000 magnification. **(b.)** A cluster of needle-like hydroxyapatite particles at x200,000 magnification. **(c.)** The mineral sample where selected area electron diffraction was performed; the square box represents the general area where the pattern was collected. **(d.)** Diffraction pattern collected at a camera length of 0.8 m.

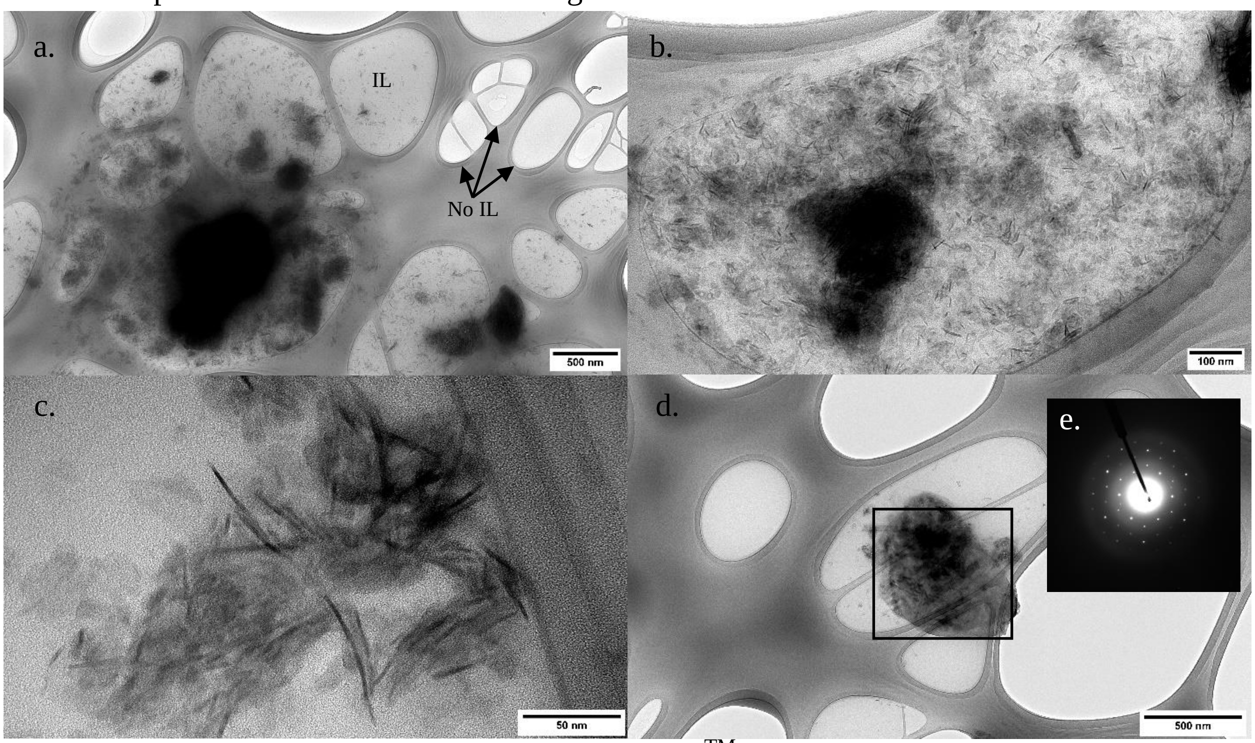

Figure 2. Fetuin-mineral treated with HILEM IL1000™ Ionic Liquid, imaged at 120 kV. **(a.)** An overview at x20,000 magnification, of the lacey carbon grid with the ionic liquid menisci being the darker voids and the lighters voids being where the ionic liquid menisci have broken. The chunk size being less than 1 μm across and smaller particles dispersed around it. **(b.)** Fetuin-mineral within the ionic liquid menisci at x80,000 magnification **(c.)** Individual needle-like hydroxyapatite particles at x300,000 magnification. **(d.)** The mineral sample where selected area electron diffraction was performed; the square box represents the general area where the pattern was collected. **(e.)** Diffraction pattern collected at a camera length of 0.8 m.

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https://doi.org/10.1017/S1431927622006390 Published online by Cambridge University Press