X-ray Microscopy and Biomedical Specimens

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Summary

Application of X-ray microscopy to biological specimens has been very limited to date, and is restricted to a few large synchrotron X-ray sources which need specimen exposure for a few seconds before an image is obtained. In diagnosis of infectious diseases, detection of microbial pathogens is performed using low resolution optical microscopy, Gram-stain, growth on selective media and polymerase chain reaction (PCR). These procedures require fixation - staining for optical microscopy, overnight incubation for growth of the pathogen on selective liquid or plated media, and complete cell lysis for PCR. We utilized a bench top source of single shot laser (nanosecond) plasma to generate X-rays in the "water window" similar to synchrotron facilities to image live hydrated cells (in 0.9% phosphate buffered saline). A 5µl aliquot suspension was placed on a small (5 mm^2) polymethylmethyacrylate (PMMA) coated photoresist and covered with a thin (100nm) SiN window. This sealed specimen was placed in vacuum close (2cm) to the laser-plasma point X-ray source. The X-ray emission spectrum was tuned for optical absorption by carbon-rich material to etch an image on the PMMA coated photoresist. The photoresist was then developed by immersion in solvent to remove the broken polymeric PMMA material and then scanned by an atomic force microscope to obtain a topographical image of differential X-ray absorption. We have applied high resolution X-ray microscopy to image various types of biomedical specimens to assess its usefulness in this area. The images that we obtained are similar to those observed by other direct microscopy techniques (1-4), such as optical, fluorescence or variations of electron microscopy. However, these images represent the status of the cell in its live hydrated and therefore natural dynamic state.

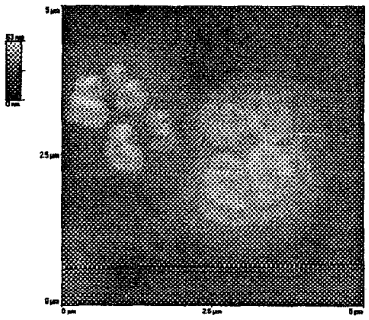

Fig.1. X-ray micrograph of live cells of *Neisseria gonorrhea* in 0.9% phosphate buffered saline.

The conventional concept of the infection process is for the microbe to colonize and establish small scale foci which enable it to hide from the host immune response. Based on optical microscopy, this model includes microbial cell division into two daughter cells, one division at a time. However, *in vivo*, such a model would be too slow to establish a high enough concentration to be pathogenic to the host tissue before the host immune response eradicates the causative pathogen. X-ray micrographs of *N.gonorrhea* (Fig.1) show round/oval shaped diplococci with distinct division septa as seen by standard optical microscopy. In addition, the X-ray images show an uniform layer of capsular material enclosing at least four distinct cellular units that appear to be products of recent simultaneous multi-planar cell divisions and some sub-cytoplasmic infra-structure within an approximately one

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cubic micron area using conventional microscopic methods, one cell of *N.gonorrhea* is believed to be 1 micron in diameter. This data suggests a faster multiplication rate for gonococci, and points to the process of cell division as a primary target in the early treatment of *N.gonorrhea* infections.

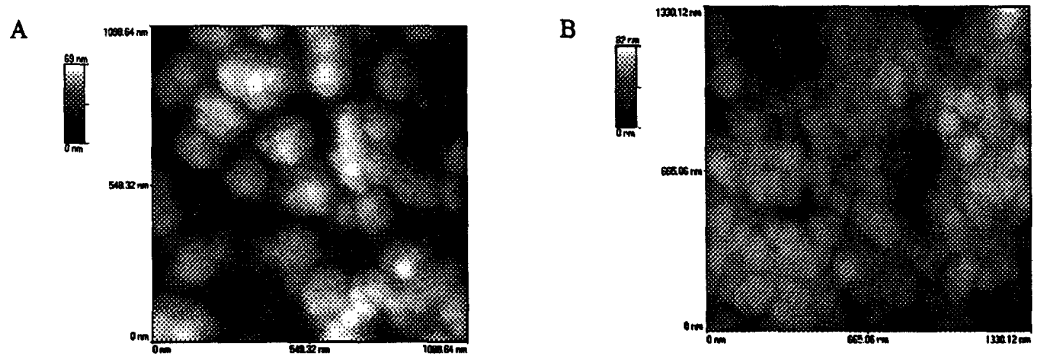

Fig. 2. X-ray micrographs of macromolecules. A. DNA. B. LPS.

Our x-ray images of the macromolecules DNA (Fig.2A) and LPS (Fig.2B) are the first such visual images seen. Their structures are usually determined using indirect chemically degradative techniques. The circular structures seen in the LPS image (Fig.2B) may represent the micelles formed by LPS when in solution. Such structures are similar to the electron micrographs of membrane vesicles (MVs) that are believed to be secreted by pathogenic bacteria such as *Pseudomonas aeruginosa* (Fig.3A) into the environment.

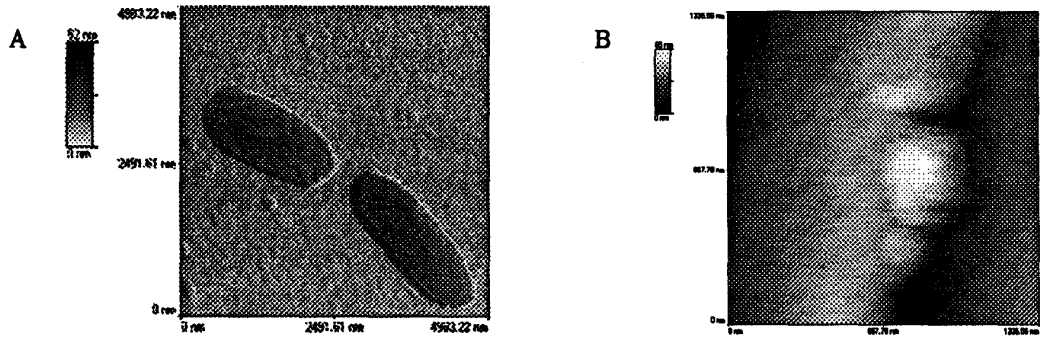

Fig.3. A. Control cell, no antibiotic treatment. B. in presence of gentamicin at 25μgml after 15 minutes.

These MVs are known to contain tissue degradative enzymes enveloped by membrane material with capability to fuse with epithelial cells. The degradative enzymes can thus severely damage the host defense structure and at the same time provide nutritional cell lysis products that the pathogen can use to proliferate. Fig.3B shows such membrane vesicle on the surface of cells damaged by exposure to an antibiotic. This antibiotic is known to accelerate the membrane vesicle formation and release as it damages the bacterial surface first. Chronic inflammation eg. in the lung, of Cystic Fibrosis patients due to colonization and infection by *Pseudomonas* leads to severe tissue damage followed by deteriorating lung function and death. Quick observation of structures like MVs in live bacteria can thus identify them as primary targets so that we can devise strategies to decrease their secretion and thereby minimize damage to lung tissue. As illustrated here, high resolution X-ray microscopy is very much suitable in the study of various types of fragile live biomedical specimens.

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