INTRODUCTION

Over the last decade, liquid electron microscopy (liquid-EM) has grown from a curiosity to a fundamental tool in the repertoire of materials and life sciences research. Liquid samples are isolated from the column vacuum of the transmission electron microscope (TEM) using a variety of different strategies, the majority of which enclose the sample between two electron transparent membranes such as silicon nitride (SiN) or graphene. Thus, samples can move and react freely under observation, enabling real-time processes such as growth, degradation, and sample interactions to be observed at resolutions ranging from nanometers to a few angstroms. Although Liquid-EM has enabled new and exciting research, interpreting the results remains challenging, particularly due to the difficulty of de-coupling the effect of the electron beam itself from the native sample behaviour.

RADIOLYSIS AND LIQUID-EM

As the field of liquid-EM has grown, so has our understanding of the impact the electron beam. In all electron microscopy experiments the electrons interact with the sample. These interactions form the image or elemental signatures for chemical and elemental analysis, but also interact with the samples in detrimental ways such as causing displacement of atoms in the sample due to the high energy of the electrons. Liquids, normally absent in conventional electron microscopy, are particularly sensitive to the ionizing radiation of the electron beam. This radiation can lead to radiolysis, damage caused by bond breakage and decomposition of the molecules into radiolytic products. Subsequent chemical interactions between these radiolytic products and the sample and/or other components in the sample matrix can significantly influence the outcome and interpretation of experiments. [1-12]

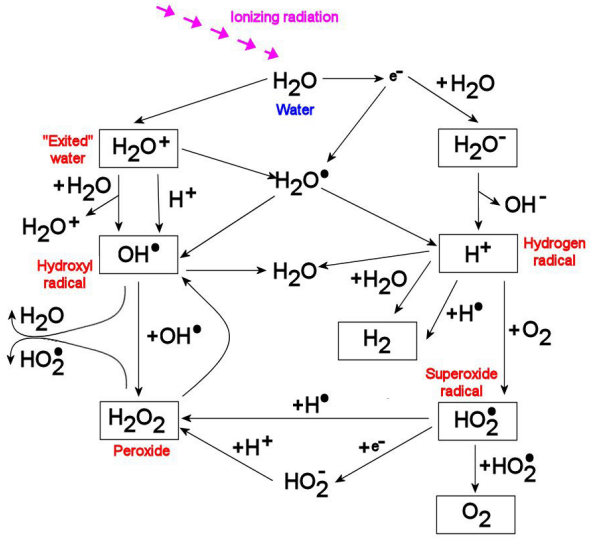

Figure 1: Radiolyric Species Produced by Ionization of Water.
Image from: https://www.mun.ca/biology/scarr/Radiolysis_of_Water.html

An example of the radiolytic cascade that can occur when a liquid is exposed to the ionizing radiation of the electron beam is shown in Figure 1. Here it is shown that even a relatively simple liquid, in this case water (H_2O), generates dozens of reactive radiolytic species that can interact with the sample and one another, introducing new chemical reaction pathways into the experiment. This can result in changes to the local pH, chemical and structure changes to the sample by reactive radical species, nucleation and growth of ions in solution, and many other unintentional processes. Thus, the electron beam should be regarded as a potentially active participant in any liquid-EM experiment [1-12].

ELECTRON DOSE CONSIDERATIONS AND LIQUID-EM

Since the electron beam itself causes radiolysis of the liquid or solvent used in a liquid-EM experiment, researchers have devoted considerable effort to understanding and quantifying how both the cumulative electron dose (total electrons delivered per given area) and the electron flux (electrons per area per unit time) influence the local

chemistry during an experiment. The cumulative electron dose, sometimes called the threshold dose, is generally limited by the degradation of the sample. For example, cryo-TEM experiments are generally not concerned with the electron flux but are constrained by the cumulative dose that the sample can be subjected to before it degrades and can no longer produce reliable structural information. Likewise, liquid-EM experiments must also consider the cumulative electron dose that a sample can withstand before it degrades to a point that useful information can no longer be acquired. However, the electron flux (dose rate) can have more significant implications on the immediate environment of a sample during a liquid-EM experiment due to the localized formation of reactive radiolytic species.

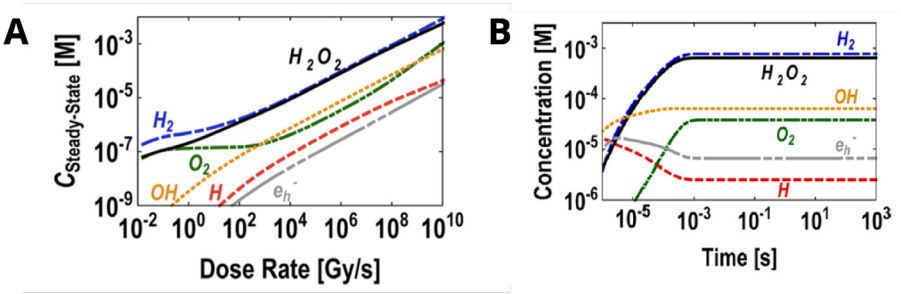

Figure 3: Electron Dose and Spatial Resolution for a Typical TEM Setup (1)

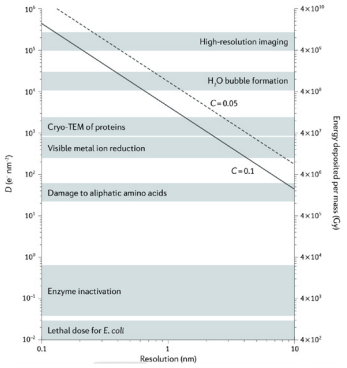

Figure 2: Concentration of Radiolytic Products in Water as a Function of Electron Dose (3)

Using a combination of computer modelling and data from radiation chemistry experiments, Schneider et al. calculated that as the electron flux increases, so does the concentration of radiolytic products (Figure 2A). However, for a given electron flux these products quickly reach a steady state (Figure 2B), enabling researchers to make assumptions about the identity and concentration of radiolytic products produced during the reaction and interpret the experimental results [3]. Because the electron flux influences the production rate and concentration of reactive radiolytic species generated by the beam, it can have immediate implications for liquid-EM experiments, even when imaging short durations. As an example, exposing oxides such as MgO and SiO2 to the same cumulative dose in water was investigated. This showed that MgO degraded within 10 minutes, while SiO2 stayed stable for 30 minutes or more [10].

The beam-generated species are different for each sample/solvent combination, which adds significant layers of complexity. For instance, performing a liquid-EM experiment with carbon nanotubes that uses water as the solvent, results in their rapid degradation due to the OH· radicals produced as the beam ionizes the surrounding water. However, exchanging water for ethanol, which does not produce OH· radicals, results in the carbon nanotubes remaining stable during the liquid-EM experiment [6].

The varying sensitivities samples have to these species becomes a limiting factor for achievable resolution. The chart in Figure 3 shows how typical levels of electron dose required for a given resolution leads to sample damage. The electron doses conventionally used for high-resolution imaging are significantly larger than the doses required to initiate nucleation of metal nanoparticles in solution, which is well beyond the critical dose of many liquid-EM

experiments. These high-resolution doses can also form hydrogen gas during liquid-EM experiments, which is undesirable because it leads to the formation of hydrogen bubbles [1].

MINIMIZING RADIOLYSIS EFFECTS IN LIQUID-EM

Because the critical electron dose, or flux, varies from experiment to experiment, researchers must test each condition individually. Although beam effects due to radiolysis cannot be eliminated, minimizing electron flux by using low electron dose conditions is one strategy to minimize radiolysis effects during liquid-EM experiments. As shown below in the equation for calculating dose rate when using scanning transmission electron microscopy (STEM), increasing the imaging area, achieved by decreasing magnification, is a simple method to reduce the dose rate during an in-situ experiment while keeping the beam current and other settings fixed.

Equation 1: $$Dose_{rate} = \frac{6.2415 \cdot 10^{18} (Beam\ Current)}{Area}$$

In experiments where reactive radiolytic species cannot be effectively managed using low dose methods radical scavenger additives and buffers have been successfully utilized to mitigate the detrimental interactions between radiolysis products and the sample [8,9].

EXPLOITING RADIOLYSIS TO DRIVE CHEMICAL REACTIONS

An alternative approach to minimizing the electron flux to suppress the formation of radiolytic species is to actively exploit the electron beam-induced radiolysis to control and drive chemical reactions, using it as a tool to study mechanistic pathways. A common application studied using this method is the nucleation and growth of metallic nanomaterials. Ionizing radiation from the electron beam decomposes water molecules in the solvent, resulting in a cascade of solvated electrons and reactive oxygen species that drive reduction of metal ions in solution [2,4-5,7,8]. To identify the radiolytic products produced during a given experiment, researchers typically utilize data obtained from radiation chemistry experiments (such as pulse radiolysis studies) and correlate these with their measured or estimated electron flux to extrapolate the reactive species generated by a specific liquid or sample [3,8]. Careful tuning of the electron flux enables researchers to control the reactive environment and observe nucleation and growth process and study the resulting nanoparticle particle morphology. An example of this type of study (Figure 4) shows the nucleation and growth of a gold nanoparticle at low doses (growth limited, Figure 4A-E) and high dose regimes (diffusion limited, Figure 4F-J), respectively. The dependence on the electron flux in the growth rate and structural morphology is clearly observed by the significant inflection in the graph shown in Figure 4K when the dose rate is increased from 0.74 electrons/Å^{2}s to 2.8 electrons/Å^{2}s [7].

Thus, exploiting the electron beam to change the growth conditions by altering the local concentration of reactive products to drive nucleation and growth by reducing metal ions in solution enables different mechanistic pathways to be observed directly.

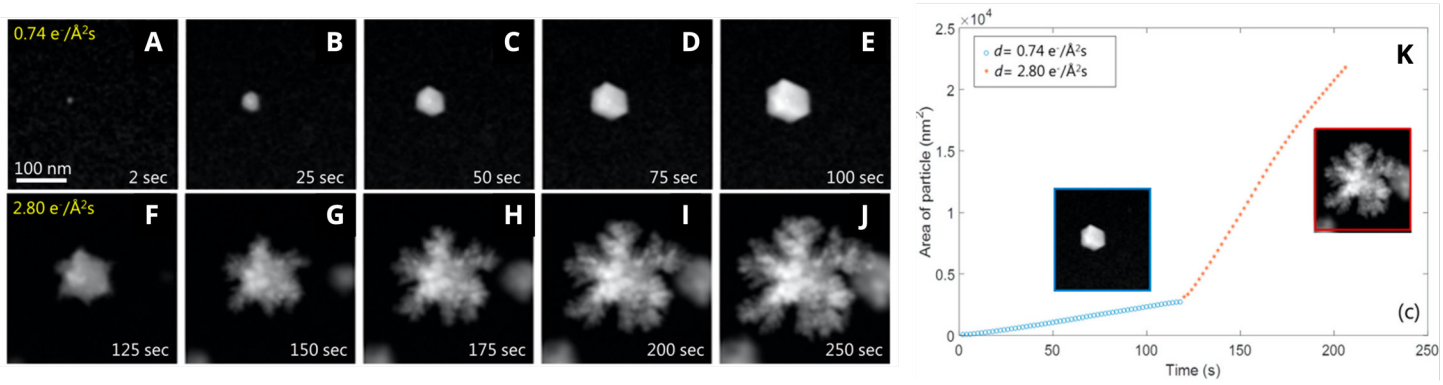

Figure 4: Exploring Growth Mechanisms by Controlling Electron Dose Rate (Flux). The growth mechanism changes from growth limited to diffusion limited as the flux is increased [7].

CONCLUSIONS

The electron beam can significantly impact the interpretation of results obtained during liquid-EM experiments and therefore must be carefully considered when designing in situ liquid-EM experiments. Accurate, reproduceable dose measurements are a key requirement for successful liquid-EM experiments and robust data reporting. Protochips' AXON Dose module enables accurate, quantifiable tracking and recording of the cumulative electron dose across the entirety of a TEM session. Combined with AXON's powerful image stabilization, metadata embedment, and analysis tools, AXON Dose allows you to investigate and measure the true impact of the electron beam on your sample, in ways you never thought possible.

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