

## H36 The Estimation of Postmortem Submersion Interval (PMSI): Are Nails' Ultrastructure of Any Help?

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**Learning Overview:** The goal of this presentation is to provide new tools for the estimation of the PMSI in bodies recovered in freshwater.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing a scarcely investigated issue that can provide helpful information regarding the circumstances and time of the death.

Forensic pathologists face numerous challenges when dealing with corpses submerged for several days. In such cases, the pathologist encounters many difficulties in determining the time since death, as the submersion deeply alters the postmortem phenomena. Therefore, it is of great interest to be able to assess how long the corpse remained in water, regardless of the PMI. This data can be helpful to reconstruct the circumstances of the death. Several approaches have so far been proposed for the PMSI estimation. Methods involving the growth of biofilms, such as algal, bacterial, fungal, and protozoan species, have been reported in the literature with encouraging results. Another tested method used to estimate the PMSI involves measuring the degree of decomposition of the recovered human remains. In the present study, a novel approach was tested, consisting of examining the microscopic structure and the ultrastructure of the nail plate, experimentally submersed in freshwater. The structure of the nail unit has been well described in literature. The nail plate is the completely keratinized part of the nail unit and it is made up of three different layers that are well-bonded together. Nail matrix produces the nail plate that includes an average of 196 cell layers that are distributed among three tightly bound layers. Given its very low permeability to water, due essentially to the chemical composition of the nail containing residual lipids, the penetration of water into the nail plate is likely to be slow. These characteristics lead to the hypothesis that the changes in the nail structure due to submersion in water develop gradually, going through different stages.

To observe these modifications, the nail plate of the hallux was removed from five cadavers during forensic autopsies. Each nail was experimentally kept in freshwater at room temperature for defined time intervals: 3, 6, 9, 12, and 24 days. As a negative control, a dry hallux nail, removed as well from a cadaver, was used. As positive controls, the hallux nails of two cadavers pulled from the water, respectively, after 9 and 24 days, were examined. Each nail was formalin fixed for 24 hours, then dehydrated in a graded series of ethanols (60%, 80%, 95%, 100%). Then, after drying, each nail was longitudinally divided into two parts, to expose the internal structure of the nail, rather than the external surface. Each sample, previously carbon-coated using Cressington carbon coater 208c, was examined with a Scanning Electron Microscopy (SEM) Tescan® Mira3XMU, operated at 20kV.

The results showed that a relevant different pattern can be observed for each experimental submersion interval. High-power views indicate that the nails not submerged or submerged for the shortest interval (3 days) show a compact structure, with regularly aligned keratin fibers. After 6 days, some cracks are observed, and they appear considerably more extended after 9 days; after 12 days of submersion, the nail plate is characterized by the apposition of exogenous material, such as diatoms. After 24 days of submersion, the structure is totally altered, with extensive discontinuities and it is no longer possible to distinguish the different layers of keratin, which appear to be compacted and amorphous.

Such results suggest the usefulness of the ultrastructural observation of the nails using the SEM in the evaluation of the PMSI in bodies recovered in freshwater. Indeed, the observed changes seem to follow a gradual process. The characteristics pointed out in this presentation, even though they must be validated and confirmed by more research, appear promising and may be extremely useful in the forensic practice.

### Scanning Electron Microscopy, Nail, Postmortem Submersion Interval