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LS-4-P-3332 Exploring the ultrastructure of bacterial inclusion bodies (IB) with therapeutical interest

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IBs are highly pure, mechanically stable and biocompatible protein deposits commonly observed during recombinant protein production processes in microbial hosts [1, 2]. Since these sub-micron particles retain biological activity, several potential applications in biomedicine for bacterial IBs have recently emerged [3]. In this regard, it has been demonstrated that these protein deposits, when formed by proteins with therapeutic interest can actually rescue challenged cells in culture [4]. The present study explores the electron microscopy methods to obtain information of fine architecture (both size and shape, and surface morphology) of bacterial Inclusion Bodies (IBs). In addition, IB biological activity is studied through immunolocalization techniques of the particle forming protein (GFP in our IB model).

The analyses have been performed using scanning electron microscopy (ultrastructure, freeze-fracture and immunolocalization in entire IBs) and transmission electron microscopy (ultrastructure of entire IBs with negative staining and ultrathin sections of IBs in Epon resin, immunolocalization of GFP in entire IBs and in ultrathin sections of Lowicryl HM20 resin).

The fine architecture and the high content of protein of IBs were evaluated by SEM and TEM using ultrastructural morphology and immunolocalization (Figure 1). The ultrastructure also revealed differences in porosity between IBs, showing from high compact structures to highly porous IBs. In summary, this study reports several EM methods to obtain ultrastructural data of IBs characterized with conventional and non-conventional microscopy techniques and help to understand the mechanism of IB-mediated protein drug delivery for therapeutic use.

References

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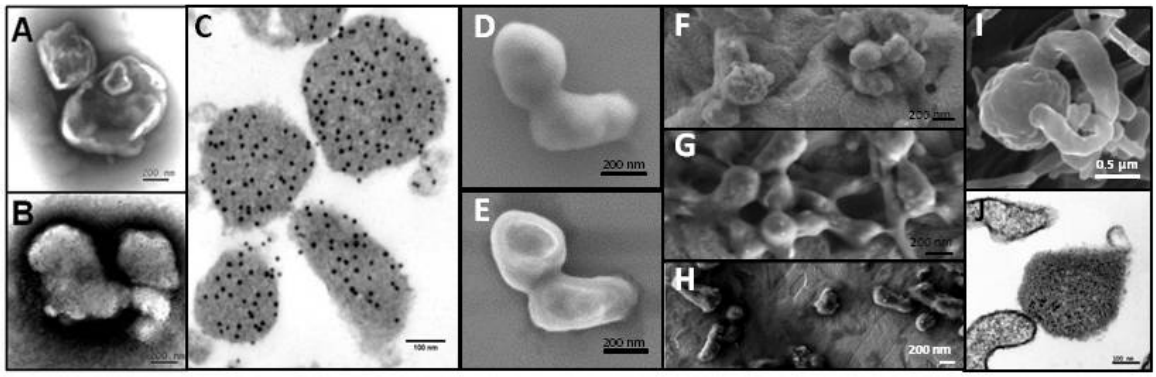

Fig. 1: Representative micrographs of IBs: A) TEM negative staining; B) TEM immunolabelling of GFP in entire IB; C) TEM immunolabelling of GFP in Lowicryl section; D-E) SEM morphology using 2 different secondary detectors; F-H) SEM freeze-fracture using different substrates (Cu, Si, Au); and IBs-HeLa cell surface contacts observed with SEM (I) and TEM (J).