STUDY OF MECHANICAL PROPERTIES AND POLYMORPHISM OF ROTAVIRUS VP6 NANOTUBES THROUGH ULTRASONICATION AND AFM

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Introduction.
Rotavirus viral protein VP6 has shown to self-assemble in tubular and spherical structures *in vivo* and *in vitro*[1,2]. Due to the great potential of VP6 nanotubes in nanotechnology[2], knowledge of their properties, as well as their manipulation possibilities are required. Here, a methodology of ultrasonication was developed as an alternative technique for both the characterization of its mechanical properties, such as limit length, and the production of novel VP6 structures.

Methods. VP6 nanotubes were produced and purified as described elsewhere[2]. Young Modulus was calculated using the Thin Shell Theory[3] through nanoindentations performed on immobilized tubes using atomic force microscope (AFM). For limit length studies, 10 µg of VP6 nanotubes were sonicated up to 180 s. Their final length was measured through transmission electron microscope images (TEM). For polymorphism assays, cycles of sonication with different amplitudes and pH were applied to 10 µg of nanotubes. Changes in morphology were monitored by TEM.

Results.
The produced nanotubes were scanned and indented using AFM (Fig. 1). They presented a Young Modulus of 0.4 GPa, which indicates they present lower elasticity in comparison with other proteic nanotubes.

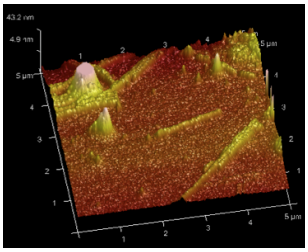

Fig. 1 Purified nanotubes observed using AFM.

On the other hand, ultrasonication produced a gradual decrease of VP6 nanotubes length through transversal cuts (Fig. 2). The minimum length in which VP6 remained as tube was 100-120 nm. Nevertheless, disassembled protein was also observed for 90 s of ultrasonication. In addition, nanotubes at pH 7 showed to be more resistant to ultrasonication than the ones at pH 5, in which low macrostructures were present post-sonication. Also, at pH 7, we were able to observe the formation of spiral structures at 1 and 2 % of sonication amplitude (A) (Fig. 2).

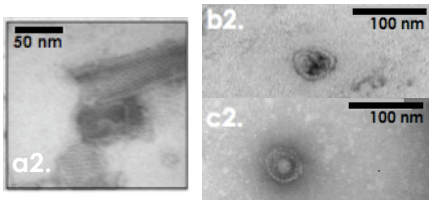

Fig. 2. TEM micrographs of purified nanotubes: *a2* after 90 s of sonication; *b2* after 9 s of sonication and 1% A at pH 7; *c2* after 9 s of sonication and 2% A at pH 7.

Conclusions.
The ultrasonication methodologies developed in the present work were useful to determine mechanical properties, such as limit length and to produce new structures of VP6 nanotubes. VP6 nanotubes were less elastic than other nanotubes. As a result, a limit length was not found but rather a minimum one before protein disassembly was observed. However, new un-described VP6 structures were found, although with a low prevalence.

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