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PRODUCTION AND FUNTIONALIZATION OF P22 VIRUS-LIKE PARTICLES WITH CYTOCHROME P450 ACTIVITY

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Introduction. Since chemotherapy is one of the most used strategies for the treatment of cancer tumors, there is a great diversity of cytotoxic drugs (1). The majority of these compounds are administered as pro-drugs and therefore they need to be activated into the anticarcinogenic form, a task performed mainly by the cytochrome P450 (CYP) superfamily (2,3). The biomedical field is gaining recognition especially in the drug delivery systems based on nanoparticles (4). One of the strategies for increasing the *in situ* generation of the active form of the drug within a tumor is the use of virus-like particles (VLPs) which are excellent stable structures offering advantages as molecular cargo vehicles (5).
This study was focused to encapsulate *in vivo* CYP_{BM3} from *B. megaterium,* mutant "21B3" into bacteriophage P22 VLPs and reduce their immunogenicity by pegilation of the capsid surface.

Methods. For the *in vivo* encapsulation pBAD-CYP and pRSF-CP plasmids were expressed in *Escherichia coli* BL21 DE3 using L-arabinose and IPTG for the induction of CYP_{BM3} and the P22 capsid protein (CP), respectively. The purification was performed by a two-step method: after sonication, the cellular extract was ultracentrifuged at 215,000 *x g* using a sucrose cushion and the pellets were resuspended in PBS buffer. Then, a gel filtration chromatography was performed in a HiPrep 16/60 Sephacril S-500 HR column with an elution of 1 ml/min. The capsids containing CYP were chemically modified with poly(ethylene glycol) (PEG) and their stability and activity were characterized.

Results. The fractions corresponding to the correctly assembled VLPs were collected with a maximum peak at 62 min (Fig. 1A) and then concentrated by ultracentrifugation. The presence of both proteins (CYP=71.5 kDa, CP=46.6 kDa) was confirmed by a 12% SDS-PAGE gel (Fig. 1B). The concentrated samples were then analyzed by transmission electron microscopy (Fig. 2). The VLPs showed a diameter of 48 nm. Finally pegilation was carried out with PEG5000 by means of lysine residues of the capsid protein as a linker and the CYP P450 activity was confirmed using 2,6-DMP and H_2O_2 as substrates.

Conclusions. It is demonstrated that it is possible to produce VLPs with CYP activity *in vivo* and then modified by pegilation. Further analysis of the VLPs' immunogenicity will be performed.

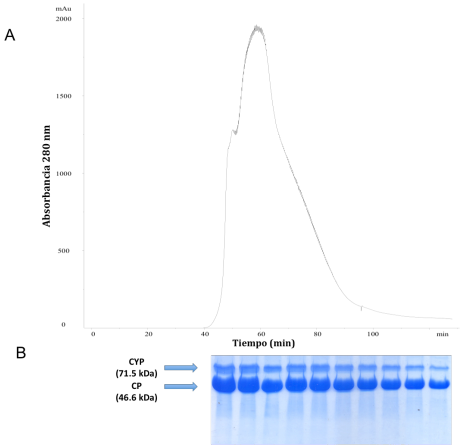

Fig. 1. A) Chromatogram of the VLPs obtained by ultracentrifugation with a maximum peak at 62 min. B) 12% SDS-PAGE of the fractions obtained by gel filtration chromatography, both proteins are shown.

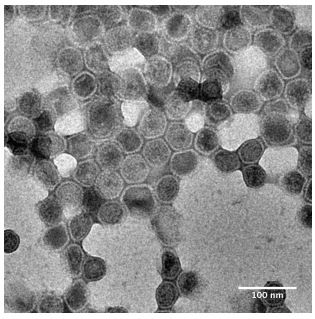

Fig. 2. Transmission electron microscopy of VLPs showing a very homogeneous population with a diameter of ≈48 nm. .

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References.

1. Al-Lazikani B,Banerji U,Workman P.(2012). *Nat Biotecnol.* 30:679-92.
2. Moen EL,Godley LA,Zhang W,Dolan ME.(2012). *Genome Med.* 4:90.
3. Sánchez-Sánchez L,Cadena-Nava RB,Palomares LA,Ruiz-Garcia J., Koay MST,Cornelissen JJMT,Vazquez-Duhalt R.(2014). *Enzyme Microb Tech.* 60:24-31.
4. Xu X,Ho W,Zhang X,Bertrand N,Farokhzad O.(2015). *Trends Mol Med.* 1-10.
5. Patterson DP,Schwarz B,El-Boubbou K,van der Oost J,Prevelige PE, Douglas T.(2012). *Soft Matter* 8:10158-10166