

**TITLE:** HEMOLYTIC AND CYTOTOXIC ACTIVITY OF THE BACTERIAL NANOCELLULOSE LOADED WITH PROTEASE

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**ABSTRACT:**

The bacterial nanocellulose (NCB) is a biopolymer excreted extracellularly by *Gluconacetobacter xylinus*. NCB's properties include non-toxicity, biocompatibility, low cost and easy production. One of the applications of bacterial cellulose is the use of the same in processes of tissue regeneration, associating biomolecules with antimicrobial activity, among them, bromelain. This protease can be extracted from the pineapple, on its stem, fruit and leaves. Bromelain is usually applied as an anti-inflammatory agent. The objective of the study was to associate the properties of these biomaterials, evaluating the hemolytic and cytotoxic action. Therefore, the *Gluconacetobacter xylinus* was grown in standard culture medium *Hestrin & Schramm* (HS). After 10 days of cultivation, NCB formation occurred. The biomaterial was removed and washed to remove the impurities. Thereafter, NaOH bleaching was carried out for 1.5 hours, washed to pH 7 and autoclaved. The bromelain concentration applied in the test was 30 mg / mL, in McIlvane buffer (pH 5). Loaded was performed by contacting the NCB in 1 mL of the bromelain solution. For hemolytic assays, the sample blood was washed three times in saline solution (0.9%) under centrifugation process at 2500 rpm for 10 minutes. After 3 times, 2% of blood was applied in bromelain solution and in the loaded system, which remained in contact for 30 minutes at 37 ° C, after the samples were collected and centrifuged at 2500 rpm for 10 minutes and the supernatant was measured in UV spectrophotometer (scanning process UV-VIS). For Cytotoxicity assays was applied L929 fibroblast. The cultures were maintained at 37°C in a CO<sub>2</sub> oven and the performed at 24, 48 and 72 hours. The readings were analyzed by MTS [3- (4,5-dimethylthiazol-2-yl) -5- (3-carboxymethoxyphenyl) -2- (4-sulfophenyl) -2H-tetrazolium] colorimetric assay. Both the NCB, the bromelain solution and the loaded sample showed hemolytic activity above the standard percentage (2%). For the cytotoxicity test, none of the samples showed activity. It is possible to conclude that although the materials tested have hemolytic activity, not a cytotoxic activity, but new tests will be necessary to validate the hemolytic activity methodology.

**Keywords:** bacterial nanocellulose, hemolytic activity, cytotoxicity, bromelain

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