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# Fucan-coated silver nanoparticles synthesized by a green method induce human renal adenocarcinoma cell death

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ABSTRACT

Polysaccharides containing sulfated L-fucose are often called fucans. The seaweed *Spatoglossum schröederi* synthesizes three fucans, among which fucan A is the most abundant. This polymer is not cytotoxic against various normal cell lines and is non-toxic to rats when administered at high doses. In addition, it exhibits low toxicity against tumor cells. With the aim of increasing the toxicity of fucan A, silver nanoparticles containing this polysaccharide were synthesized using a green chemistry method. The mean size of these nanoparticles was 210 nm. They exhibited a spherical shape and negative surface charge and were stable for 14 months. When incubated with cells, these nanoparticles did not show any toxic effects against various normal cell lines; however, they decreased the viability of various tumor cells, especially renal adenocarcinoma cells 786-0. Flow cytometry analyses showed that the nanoparticles induced cell death responses of 786-0 cells through necrosis. Assays performed with several renal cell lines (HEK, VERO, MDCK) showed that these nanoparticles only induce death of 786-0 cells. The data obtained herein leads to the conclusion that fucan A nanoparticles are promising agents against renal adenocarcinoma.


## 1. Introduction

Brown algae produce a group of sulfated polysaccharides called fucans, which contain sulfated α-L-fucose as the main monosaccharide [1]. Due to the existence of homo- and heterofucans, IUPAC recommends that the term fucan be used to define a molecule that contains more than 90% of fucose, while polymers with less than 90% of fucose can be called fucoidans [2]. However, the nomenclature of fucans is not yet fully established. A classic example is fucoidan, a homofucan isolated from the seaweed *Fucus vesiculosus* whose name derives from the seaweed [3]. Homofucans are also synthesized by sea urchins and cucumbers [4].

Many studies have shown that algal fucans have antioxidant, antiproliferative, antitumoral, antiviral, antiangiogenic, anticoagulant, and antiadipogenic properties. A number of excellent reviews published in recent years provide a comprehensive description of the properties of fucans [1,5]. On the other hand, few studies have reported the synthesis of nanoparticles with this type of polysaccharide.

Our group has been studying the *Spatoglossum schröederi* brown seaweed and has shown that it synthesizes three different types of heterofucans, called fucan A [6], fucan B [7], and fucan C [8]. Fucan A corresponds to 80% of fucans synthesized by this seaweed [6]. It is a 21 kDa molecule composed of a core of β(1-3)-linked glucuronic acid units with branches at C-4 of α(1-3)-linked fucose trisaccharides. The majority of the fucose residues are usually substituted at C-4 with sulfate groups and some residues are substituted at C-2 with β(1-4)-linked xylose disaccharides, of which 50% are, in turn, sulfated. The proposed structure for fucan A is shown in Fig. 1. Fucan A was not found to be genotoxic, mutagenic, or cytotoxic against various normal cell lines [9]. In addition, it did not have a toxic effect on rats when applied at high doses, both in acute treatments

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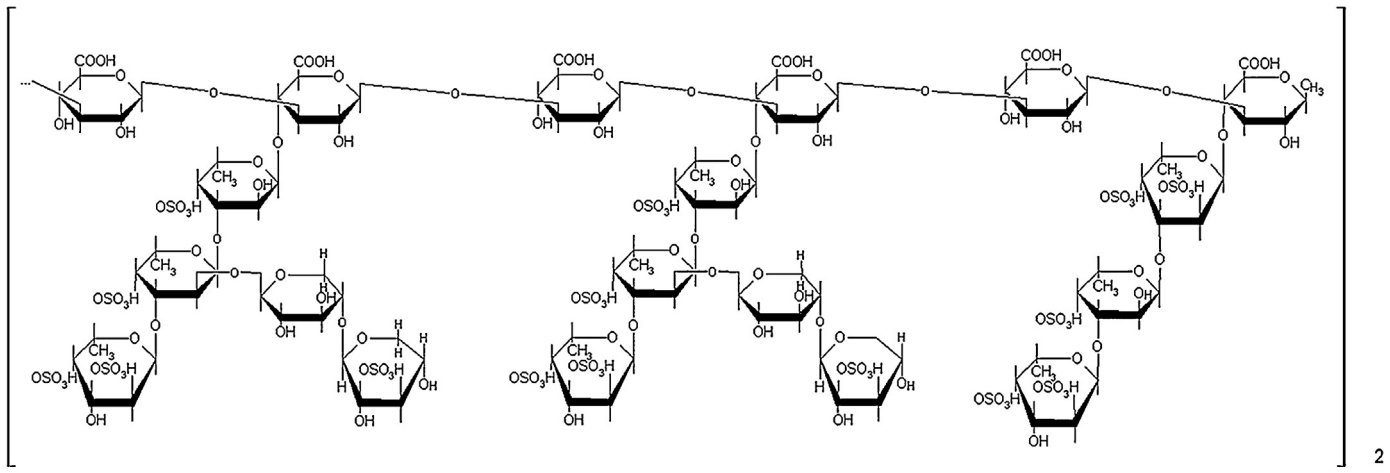

**Fig. 1.** Structure of fucan A of *S. schröederi* proposed by Leite et al. [6].

(seven days) and chronic treatments (90 days) [10]. On the other hand, fucan A was shown to have a toxic effect on various tumor cell lines, although moderate [9].

Studies have shown that the synthesis of silver nanoparticles with different molecules potentiates the activity of these molecules that are applied in areas such as cosmetics [11], medicine [12], and materials engineering [13]. Therefore, one way of increasing the antiproliferative activity of fucan A is the synthesis of silver nanoparticles with fucan A. In the present study, silver nanoparticles with fucan A from the *S. schröederi* (C. Agardh) Kützing brown alga were synthesized using a green method, i.e., a method not harmful to the environment. These nanoparticles were subsequently characterized and tested as antiproliferative agents in various tumor and normal cells.

## 2. Materials and methods

### 2.1. Materials

6-diamidino-2-phenylindol (DAPI), MTT (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide), standard sugars were purchased from Sigma Chemical Company, St. Louis, MO, USA. Cell culture medium components (Dulbecco's Modified Eagle Medium-DMEM), trypsin and newborn calf serum (FCS) were obtained from Cultilab (Campinas, Brazil). L-glutamine, sodium bicarbonate, sodium pyruvate and phosphate buffered saline (PBS) were purchased from Invitrogen Corporation (Burlington, ON, USA). All other solvents and chemicals were of analytical grade.

### 2.2. Chemical analysis and monosaccharide composition of fucan A

Total sugars, sulfate, protein, phenolic compounds and glucuronic acid were determined as previously described [6–9].

The samples were hydrolyzed with 2 M HCl for 2 at 100 °C and sugar composition was determined using a LichroCART® 250-4 column (250 mm × 40 mm) packed with Lichrospher® 100 NH2 (5 μm) coupled to a LaChrom Elite® HPLC system from VWR-Hitachi equipped with a refractive index detector (RI detector model L-2490). The sample mass used was 0.2 mg and analysis time was 25 min. The chromatogram was compared to those of sugar references: arabinose, fructose, fucose, galactose, glucose, glucosamine, mannose, and xylose.

### 2.3. Agarose gel electrophoresis

Agarose gel electrophoresis of the acidic polysaccharides was performed in 0.6% agarose gel (7.5 cm × 10 cm × 0.2 cm thick) prepared in 0.05 M 1.3-diaminopropane acetate buffer pH 9.0, as previously described [8]. Aliquots of the polysaccharides (about 50 μg) were applied to the gel and subjected to electrophoresis. The gel was fixed with 0.1% cetyltrimethylammonium bromide solution for 2 h, dried, and stained for 15 min with 0.1% toluidine blue in 1% acetic acid in 50% ethanol. The gel was, then, destained with the same solution without the dye. Representative image of three independent experiments is shown.

### 2.4. Green synthesis of silver nanoparticles with fucan A (NFucA)

The process of green synthesis of NFucA involves reducing agents that convert silver ions into nanoparticles. Initially, the synthesis of NFucA was performed using a 0.001 M solution of silver nitrate combined with a 10 mg/mL solution of fucan A (in a 1:9 ratio). The solutions were mixed in a 250 mL Erlenmeyer flask previously protected from light with aluminum paper. The resulting solution was homogenized by gentle shaking for one hour. The AgNPs were centrifuged at 10,000 × *g* for 15 min at 25 °C and the precipitates were collected, freeze-dried, weighed, and stored in conical tubes protected from light.

### 2.5. Characterization of the nanoparticles

#### 2.5.1. Sample digestion

Analytical scales were used to weigh 100 mg of sample in Teflon cups (total 200 mg of sample; 100 mg of each cup), to which 7 mL of 65% nitric acid purified by sub-boiling distillation (Berghof, Eningen, Germany) and 1 mL of 30% hydrogen peroxide (Merck, Darmstadt, Germany) were added. The digestion cups were subsequently closed and digestion was performed in a microwave digester (Start D, Milestone, Italy) using 6 stages and a power of 1100 W: (1) 5 min at 70 °C; (2) 2 min at 70 °C; (3) 3 min at 120 °C; (4) 2 min at 120 °C; (5) 10 min at 170 °C; (6) 15 min at 170 °C and lastly 30 min of ventilation before the removal of the rotor from the microwave. The digested content was quantitatively made up to 15 mL using deionized water and filtered through a 0.45 μm membrane. The analyses were conducted in duplicate and analytical blanks were performed by conducting the procedure in the absence of sample.