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BC nanofibres: *In vitro* study of genotoxicity and cell proliferation

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ABSTRACT

Nanomaterials have unusual properties not found in the bulk materials, which can be exploited in numerous applications such as biosensing, electronics, scaffolds for tissue engineering, diagnostics and drug delivery. However, research in the past few years has turned up a range of potential health hazards, which has given birth to the new discipline of nanotoxicology. Bacterial cellulose (BC) is a promising material for biomedical applications, namely due its biocompatibility. Although BC has been shown not to be cytotoxic or genotoxic, the properties of isolated BC nanofibres (NFs) on cells and tissues has never been analysed. Considering the toxicity associated to other fibre-shaped nanoparticles, it seems crucial to evaluate the toxicity associated to the BC-NFs.

In this work, nanofibres were produced from bacterial cellulose by a combination of acid and ultrasonic treatment. The genotoxicity of nanofibres from bacterial cellulose was analysed *in vitro*, using techniques previously demonstrated to detect the genotoxicity of fibrous nanoparticles. The results from single cell gel electrophoresis (also known as comet assay) and the *Salmonella* reversion assays showed that NFs are not genotoxicity under the conditions tested. A proliferation assay using fibroblasts and CHO cells reveals a slight reduction in the proliferation rate, although no modification in the cell morphology is observed.

1. Introduction

The development of artificial materials with biomimetic behaviour is essential for tissue engineering purposes. Scaffolds based on nanofibres (NFs) mimic the natural extracellular matrix and its nanoscale fibrous structure. Several approaches have been described in order to achieve materials based on nanofibres from synthetic or natural polymers (Ma et al., 2005; Ashammakhi et al., 2007).

Bacterial cellulose (BC), secreted by *Gluconacetobacter xylinus*, has been presented as a biocompatible scaffold for the engineering of cartilage and blood vessels, wound dressing, guided tissue regeneration, among other applications (Astley et al., 2003; Entcheva et al., 2004; Svensson et al., 2005; Tabuchi and Baba, 2005; Czaja et al., 2007; Teeri et al., 2007; Andrade et al., 2008; Backdahl et al., 2008; Maneerung et al., 2008; Rambo et al., 2008). BC has unique characteristics including high purity, high crystallinity and remarkable mechanical properties, due to the uniform ultrafine-fibre network structure, the high planar orientation of the ribbon-like fibres when compressed into sheets, the good chemical stability, and the high water holding capacity (Svensson et al., 2005). Several materials based on bacterial cellulose, recognized as non-genotoxic and non-cytotoxic, have been commercialized (Schmitt et al., 1991; Jonas and Farah, 1998).

Since nanomaterials have unusual properties, not found in the bulk material, such as high surface reactivity and ability to cross-cell membranes, concerns about their safety and toxicology emerged. The impact of nanostructural features in the interaction of a material with cells and tissues is dependent on the size, chemical composition, surface structure, solubility, shape, and on the supramolecular structural organization (Barnes et al., 2008). A major concern with fibres is their carcinogenic potential. There is sufficient evidence that all forms of asbestos (generic term for a group of six naturally occurring fibrous silicate minerals) are carcinogenic and co-carcinogen to man (Speit, 2002; Dopp et al., 2005). Moreover, recent studies described the toxicity of materials associated to size or shape; namely, the toxicity of carbon nanotubes (Donaldson et al., 2006; Poland et al., 2008) and the size-dependence toxicity of gold or ferric oxide nanoparticles was reported (Pan et al., 2007; Backdahl et al., 2008; Wang et al., 2009).

The toxicity associated with inhaled fibres such as asbestos has been described. Inhaled fibres may be toxic, particularly when they are "long, thin and durable" (Donaldson et al., 2006). Asbestos

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fibres are dangerous because the fibres split lengthwise, producing thin fibres that can enter the lungs, being "moderately durable" once there (Speit, 2002). Although cellulose fibres, from wood pulp and textile fabric, are used without "significant concern", cellulose fibres share similar features with asbestos, including the needle-like shape and biopersistence. Moreover, the inflammatory responses of respirable cellulose fibres (wood pulp) using animal models were already reported (Cullen et al., 2000). In light of these results, it seems crucial to evaluate the toxicity of the BC nanofibres. It must be remarked that, although BC cannot be enzymatically degraded in the human body, the inflammatory processes may actually degrade cellulose to some extent. Given the current focus of BC as a promising biomaterial with a variety of applications, it is relevant to evaluate not only the toxicity of BC membranes or scaffolds, but also of its degradation products, including BC nanofibres.

Indeed, although *in vivo* studies demonstrate the BC biocompatibility (Helenius et al., 2006), and lack of mutagenicity (Schmitt et al., 1991), no reports are available on the BC nanofibres toxicity. Although BC is not expected to be degraded *in vivo*, safety concerns makes this study mandatory. It is well accepted that *in vitro* studies using cell systems are valuable tools to clarify the cellular mechanisms involved in genototoxic effects, including DNA damage (Speit, 2002; Dusinská et al., 2004). Therefore, the aim of this study is to evaluate the genotoxicity of cellulose nanofibres at cellular level using the single cell gel electrophoresis and the *Salmonella* reversion assays. The cell proliferation in the presence of nanofibres was also evaluated. These tests are useful as a screening tool for setting priorities because they are an inexpensive and a quick way to help single out substances that should be targeted for further testing. Furthermore, these assays were already used to demonstrate the genotoxic effect of asbestos fibres in mammalian cells *in vitro* (Speit, 2002; Dusinská et al., 2004).

2. Materials and methods

2.1. Bacterial strain, cells and culture medium

The cellulose was produced by *G. xylinus* (ATCC 53582), purchased from the American Type Culture Collection, grown statically in Hestrin and Schramm (1954) medium, pH 5 at 30 °C, 5 days.

In the *Salmonella* reversion assay, four strains of *Salmonella tryphimurium* (Dr. B.N. Ames, Biochemistry Department, University of California, Berkeley, USA) were utilized, namely, TA97a [*his* D6610, *rfa*, Δ *uvr*^{B}, *bio*$^-$, pKM101 (Ap^R)], TA98 [*his* D3052, *rfa*, Δ *uvr*^{B}, *bio*$^-$, pKM101 (Ap^R)], TA100 [*his* G46, *rfa*, Δ *uvr*^{B}, *bio*$^-$, pKM101 (Ap^R)], and TA102 [*his* D428, *rfa*, pKM101 (Ap^R), pQA1 (Tt^R)] (Levin et al., 1982; Maron and Ames, 1983).

The proliferation assays were performed using mouse embryo fibroblasts 3T3 (ATCC CCL-164), grown in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% newborn calf serum (Invitrogen), and Chinese Hamster Ovary (CHO), grown in DMEM media supplemented with 10% fetal bovine serum (Invitrogen), both culture medium were supplemented with penicillin/streptomycin (1 μg/ml) (Sigma–Aldrich, St. Louis, USA) and the incubation was at 37 °C, in a fully modified air containing 5% CO_2. The same conditions were used to grow CHO cells in Comet assay. The cell viability was assessed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay, obtained from Invitrogen.

2.2. Production of BC nanofibres

The production of bacterial cellulose was performed by growing *G. xylinus* in Hestrin–Schramm medium, pH 5. After inoculation, the culture (100 ml) was incubated, first with agitation during 8 h, and then statically at 30 °C, for 5–7 days. BC pellicles were purified in a 4% NaOH solution at 70 °C, for 90 min. BC was then neutralised by thoroughly washing with water. Finally, BC pellicles were lyophilised prior to use.

The nanofibres production, by acidic and/or ultrasonic treatment, was based on previous works (Roman and Winter, 2004; Zhao et al., 2007). The acid hydrolysis was performed as follows: 20 mg of dry BC was sliced in small pieces and 2 ml of H_2SO_4 (50%, v/v) was added. The mixture was kept at 40 °C, for 2 h with vigorous stirring. To stop the hydrolysis, 10 ml of cold water was added and the cellulose was recovered by filtration, using a membrane with a 0.45 μm pore size. Then, the cellulose was washed out with 20 ml of water and the recovered pellet was resuspended in 10 ml of water. This suspension was treated by sonication at 40 W (Branson Ultrasonic Disruptor, Sonifier II/W450) for 10 min (samples were maintained on ice during sonication). Then, the NFs suspension was centrifuged (1 h, 15,000 rpm), and the pellet resuspended in water and sonicated again, in the same condition, for another 10 min. The yield of the process was evaluated by quantifying the total sugar in the samples, using the phenol-sulphuric method (Dubois et al., 1956).

2.3. TEM analysis

The NFs obtained were stained with uranyl acetate and analysed by transmission electronic microscopy (TEM, Zeiss 902A Orius SC 1000; 50 kV).

2.4. Evaluation of cellulose nanofibres mutagenicity by Salmonella reversion assay

Four *S. tryphimurium* strains were used to study the potential mutagenicity effect of the cellulosic NFs. The procedure was to some extent modified from the original description by Kado et al. (1986). This assay was performed in miscrosuspension with or without S9 mixture (Moltox™, North Carolina, USA), using 0.1, 0.5 or 1.0 mg/ml of NFs suspension. The negative control (NC) was distilled water, and the positive controls (PC) employed were: 0.1 μg/plate 4NQO (4-nitroquinoline 1-oxide) for the TA97a and TA98 strains; 5.0 μg/plate sodium azide for the TA100 strain; and 0.5 μg/plate mytomicyn C for the TA102 strain. Briefly, 105 μl of a mixture containing the NFs suspension and cell suspension (10^9 cells/ml) were incubated at 37 °C for 90 min. Then, 2.5 ml of molten Top agar (0.6% bacto-agar and 0.5% NaCl) was added, before plating in a Petri dish containing minimal agar (1.5% agar, Vogel-Bonner E medium). The His$^+$ revertant colonies were counted after 72 h of incubation at 37 °C. All experiments were repeated at least three times with three replicas. The mutagenicity of cellulose NFs was evaluated according to the following parameters: the maximum number of revertants in the presence of the NFs should be 2-fold or more relative to the negative control; a dose-dependent increase in the number of revertants should be observed (Mortelmans and Zeiger, 2000).

2.5. Proliferation assays

The proliferation assays were performed *in vitro* as follows: 1 ml of the CHO or mouse embryo fibroblast 3T3 cell suspension (10^4 cells/ml) was seeded in a 24-well polystyrene plate (TPP, Switzerland). The cells were allowed to adhere for 4 h. Before the addition of cellulose NFs, the medium with non-adherent cells was removed and the NFs containing medium (to a final concentration of 1, 0.5 or 0.1 mg/ml) was added. A control without NFs was carried out. The cellular growth at 0, 24, 48 and 72 h of incubation was evaluated by MTT assay, a colorimetric test that gives a measure of the mitochondrial activity. The effect of NFs on the cell morphology was evaluated by microscopic observation using a Nikon Eclipse TE300 Inverted Microscope.

2.6. Evaluation of cellulose nanofibres genotoxicity by single cell gel assay (comet assay)

The DNA integrity was evaluated by alkaline single cell gel assay (also kwon as comet assay) using CHO cells grown in the presence of different NFs concentration.

In this assay, 2 ml of CHO cell suspension (10^5 cells/ml) were seeded on a 6-well polystyrene plate (TPP, Switzerland). After 16 h, the medium was refreshed with medium containing the NFs (0.1, 0.5 or 1 mg/ml). Cells were incubated with NFs suspension during 48 h. Hydrogen peroxide (100 mM) and water were used as positive and negative controls, respectively. The alkaline comet assay was performed as described by Singh et al. (1988). Briefly, cells were trypsinized from 6-well polystyrene plate, and resuspended in 50 μl of medium. The cell viability was determined in a Neubauer counting chamber using the trypan blue exclusion test. A volume of 10 μl of the cellular suspension were embedded in 0.5% low-melting-point agarose and plated on agarose-coated microscope slide. Then, the slides with cells were treated with lysis solution (2.5 M NaHO, 0.1 M EDTA, 0.010 M Tris, 1% Triton X-100, 10% DMSO, adjusted to pH 10) for 12 h at 4 °C, rinsed with distilled water, and placed in the electrophoresis buffer (0.3 M NaOH, pH 13 and 0.001 M EDTA), for 20 min to allow DNA unwinding. Following electrophoresis (30 min, at 25 V and 300 mA), the slides were neutralised with 0.4 M Tris buffer (pH 7.5) and stained with ethidium bromide (20 mg/ml). The slides were analysed through fluorescence microscopy (Nikon Eclipse TE300 microscope equipped with a Nikon E600 camera, 0.488 μm/pixel). At least 300 cells per condition tested were analysed.

The DNA damages were evaluated by image analysis using the "Comet Assay IV version 4.2" image analysis system. Data collected from each cell included tail length (TL), tail migration (TMi), percent tail DNA (TI), and tail moment (TM), which correspond the product of the comet length and the amount of DNA in the tail (Olive and Durand, 1992).

2.7. Statistic analysis

The one-way analysis of variance (ANOVA) was applied to statistics evaluation of the comet scores and to the proliferation assays results. The post-test Tukey–Kramer Multiple Comparisons test was used to compare the scores of the samples and positive control, the analysis were performed using GraphPad Prisma v 3.05.

3. Results and discussion

3.1. Production of BC nanofibres

G. xylinum synthesizes cellulose nanofibres with 40–50 nm width (the bacterial cellulose ribbons), which assemble in a static culture as a white gelatinous material (pellicle) on the surface of the culture liquid. The native cellulose consists of sets of parallel chains of β-1,4-D-glucopyranose units interlinked by intermolecular hydrogen bonds (Czaja et al., 2007). Several works describe the production of nanofibres from different cellulosic sources, using acid hydrolysis (Araki et al., 1999; Roman and Winter, 2004) or mechanic treatment (Zhao et al., 2007). These two approaches were used in order to extract NFs from BC. The acid hydrolysis was tested using a range of acid concentrations, temperatures and treatment time. Concentrations of H_2SO_4 superior to 50% resulted in extensive hydrolysis, yielding less than 20% of the material used (data not shown). The acid concentration is in fact the critical parameter in the acid hydrolysis approach. The use 50% H_2SO_4, for 2 h at 40 °C, yielded 50% of nanofibres. According to Zhao et al. (2007), sonication can also be successfully used to extract NFs from natural materials, including cellulose from wood, cotton, bamboo. This approach was also applied to BC. Using acid hydrolysis (50% H_2SO_4, 2 h, 40 °C) and sonication (20 min, 40 W), needle shaped cellulose NFs with 50–1500 nm length and 3–5 nm width, were obtained (Fig. 1).

3.2. Evaluation of cellulose nanofibres mutagenicity by Salmonella reversion assay

The purpose of the bacterial reverse mutation assay is to evaluate the mutagenicity of the cellulose NFs, by measuring its ability to induce reverse mutations at selected *loci*, in several bacterial strains. Having into account that mutations are essential for cancer formation, the reliable characterization of mutagenicity is mandatory, while characterizing the safety of a biomaterial. The Kado test (Kado et al., 1983) is a modification with improved sensitivity of the Ames test (Ames et al., 1972). This is a simple, quick and inexpensive mutagenicity test, required for safety testing of a variety of compounds, including drugs, medical devices, food additives, industrial chemicals and pesticides (McCann et al., 1975). Furthermore, the potential mutagenicity of BC and of some its derivates were already accessed using the Ames assay (Schmitt et al., 1991), therefore it was selected as a first approach in this work to investigate the possible mutagenicity of the cellulose NFs. The strains used were specially constructed to allow detection of mutagens acting via different mechanisms, namely frameshift mutations (TA97a and TA98 strains), base-pair substitution mutations (TA100 and TA102), detection of oxidative and alkylating mutagens and active forms of oxygen (TA102) (Hakura et al., 2005). Table 1 presents the results obtained with the different strains.

The reversion of the histidine phenotype in *Salmonella* strains is often adopted as a criteria for the classification of molecules as mutagenic. The results obtained in the presence of the cellulose NFs, without S9 mixture, correspond to the spontaneous reversion for each strain and are similar to those obtained to negative control (Table 1). In the presence of S9 mixture, an increase of revertant colonies per plate, for the TA98 and TA100 strains, is detected as compared with control; however, the increases was in each case <2-fold and does not appear to be dose-related. The results suggest that, under the conditions tested, the cellulose NFs does not present mutagenic behaviour, as described previously for BC and some fibrous BC-based materials (Schmitt et al., 1991).

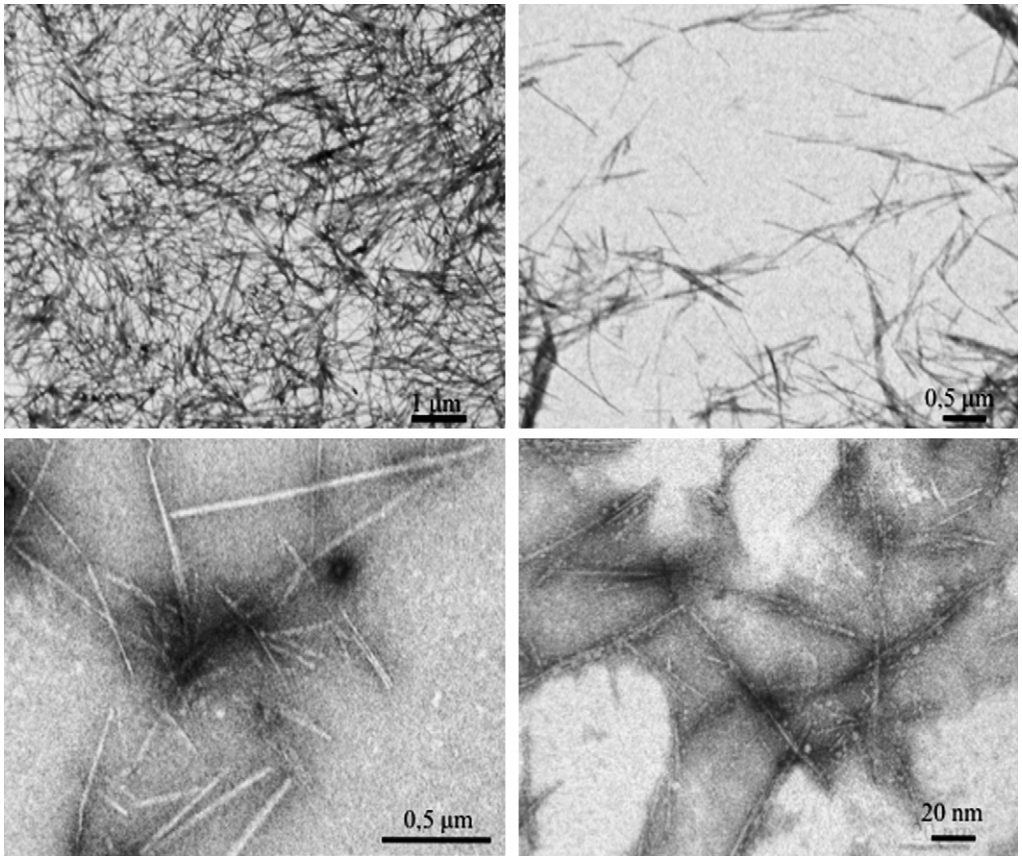

Fig. 1. TEM image of cellulose nanofibres (50 kV; Zeiss 902A Orius SC 1000).

Table 1
Results obtained in *Salmonella* reversion assay.

	Revertant colonies/plate ± SD (without S9)				Revertant colonies/plate ± SD (with S9)			
	TA97a[a]	TA98[a]	TA100[a]	TA102[a]	TA97a[a]	TA98[a]	TA100[a]	TA102[a]
PC	540 ± 54	389 ± 17	1531 ± 183	1026 ± 36	191 ± 21	195 ± 76	485 ± 14	2356 ± 196
NC	143 ± 17	36 ± 6	228 ± 18	350 ± 27	93 ± 8	16 ± 4	82 ± 44	958 ± 20
0.1	124 ± 6	31 ± 6	235 ± 9	327 ± 12	93 ± 4	20 ± 1	133 ± 7	691 ± 61
0.5	132 ± 14	43 ± 2	220 ± 2	327 ± 13	91 ± 10	20 ± 1	112 ± 14	656 ± 35
1.0	147 ± 12	42 ± 4	225 ± 7	333 ± 18	108 ± 7	26 ± 4	112 ± 33	859 ± 109

PC: positive control: 0.1 μg/plate of 4NQO to TA97a and TA98, 5.0 μg/plate sodium azide to TA100 and 0.5 μg/plate mytomicyn C to TA102; NC: negative control: H_2O; SD: standard deviation.
[a] Strain.

3.3. *In vitro proliferation assay*

The cellular morphology and proliferation may be affected by the presence of nanostructural patterns. Several studies analysed the proliferation of different cell lines on BC membranes, confirming its non-toxicity and applicability as scaffold for cell proliferation. However, depending on the cells used, the effect of the biomaterial on the proliferation rate and the cell morphology may be quite different (Sanchavanakit et al., 2006). Several studies showed that the cytotoxicity of a nanomaterial is many times cell-specific (Cullen et al., 2002). Recently, De Nicola et al. (2007) reported that, although carbon nanotubes do not present cytotoxic effect on human leukemic U937 cells, the proliferation rate is deeply altered. Moreover, Bottini et al. (2006) showed that the same nanotubes refereed above induce apoptosis in T lymphocytic cells, suggesting that cytotoxicity may be cell-specific. In addition, it has been reported that asbestos fibres inhibits the growth of CHO cells (Speit, 2002), and yet the same fibres stimulate the proliferation of different kinds of cells, *in vitro*, including fibroblasts (Bernstein et al., 2005). Taking in consideration the evidence of contradictory, cell-specific effects arising from the interaction cell-biomaterial, the evaluation of the NFs effect on proliferative rate was performed both with CHO cells and fibroblasts. In both cases, the proliferation was about 15–20% lower in the presence of NFs, after 72 h of cell culture, irrespective of the concentration used (Fig. 2). The lower proliferation rate may stem from the insolubility of NFs and their slow deposition on the polystyrene plate. It is known that cell proliferation is dependent on characteristics of material surface, such as it roughness. In addition, it was also described that cell proliferation on BC membrane is slower than on the cell culture plate

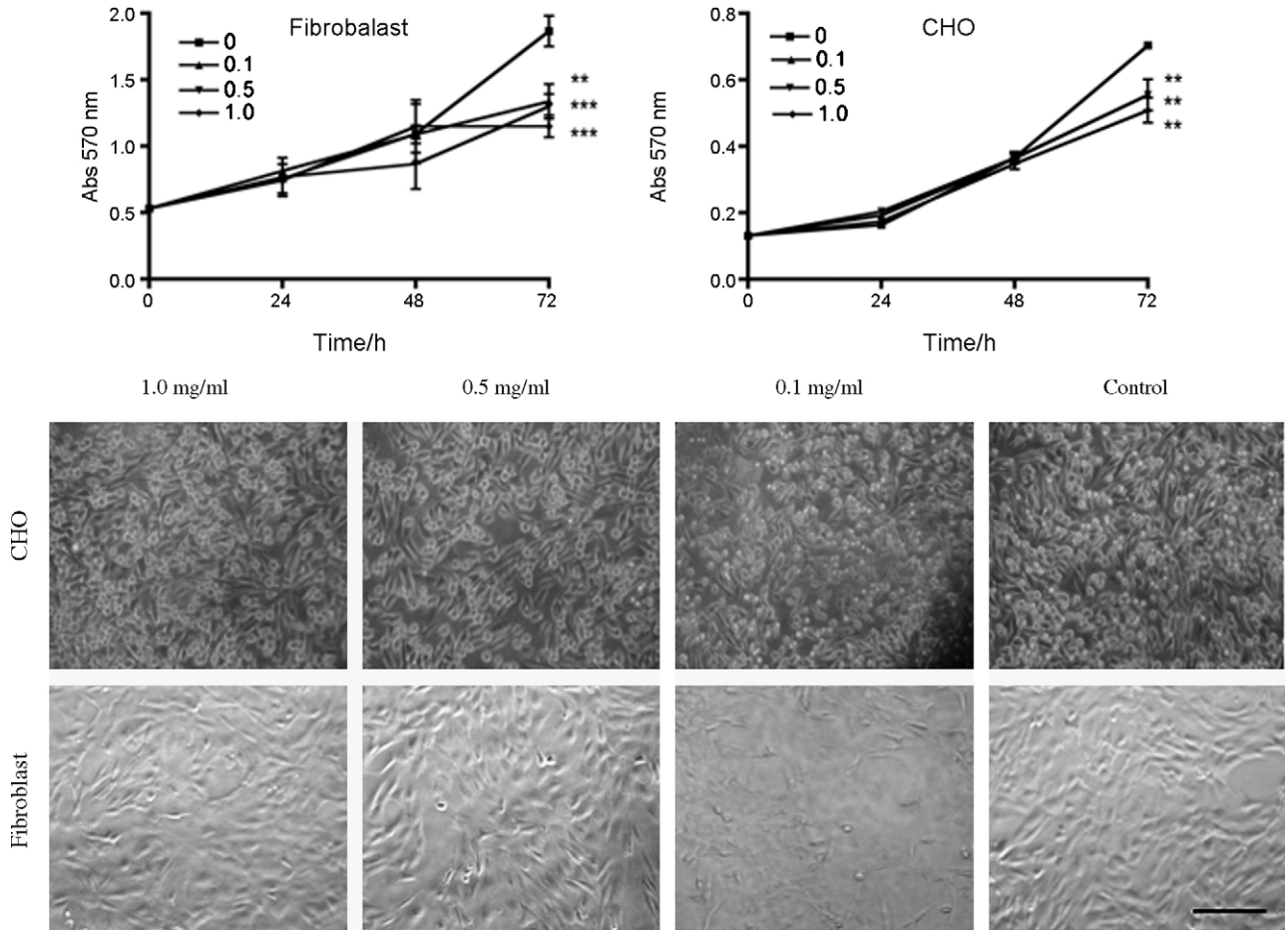

Fig. 2. MTT results from proliferation assays using mouse embryonic fibroblast 3T3 and CHO (mean ± SD; $^{**}P < 0.05$; $^{***}P < 0.005$). Image obtained by optical microscopy of fibroblasts grown in the presence of cellulose NFs during 72 h. Scale bar = 20 μm.

(Backdahl et al., 2006). However, the microscopic observations did not reveal differences in the cellular morphology.

3.4. Evaluation of cellulose nanofibres genotoxicity by comet assay

The genotoxicity of a material may be measured by analysing the damages caused on DNA. The comet assay is based on the ability of negatively charged loops/fragments of DNA to be drawn through an agarose gel, in response to an electric field. The extent of DNA migration depends directly on the DNA damage present in the cells (Collins et al., 2008). The advantages of the comet assay, relative to other genotoxicity tests, include its high sensitivity for detecting low levels of both single and double stranded breaks in damaged DNA, the requirement for small numbers of cells per sample, flexibility, low cost, and ease of application (Collins et al., 1997, 2008). Moreover, the comet assay is arguably one of the most widely used tests for genotoxicity available, being already described as a reproducible assay to evaluate nanoparticles genotoxicity (Collins et al., 1997), and suggested as a diagnostic tool for clinical management of cancer (Collins et al., 2008). The nanomaterial's genotoxicity may result from a direct interaction with DNA, or from an indirect response caused by several factors, including surface stress through direct particle influences on DNA, the release of toxic ions from soluble nanoparticles, or generation of oxidative stress (Donaldson et al., 2006). It has been proposed that (oxidative) DNA damage plus structural and numerical chromosome aberrations are the most sensitive genetic endpoints for detection of asbestos-induced genotoxicity detectable by *in vitro* assay. The comet assay has indeed proven to be a sensitive test to detect genotoxic effect of asbestos fibres in mammalian cell *in vitro* (Speit, 2002; Dusinská et al., 2004). Therefore, cells grown in NFs-containing medium were analysed by the comet assay, in order to evaluate their genotoxicity. Cells grown on bacterial cellulose membrane were also tested as a control.

The DNA damages were evaluated by visual scoring and image analysis. Fig. 3 shows a representative image obtained for each NFs

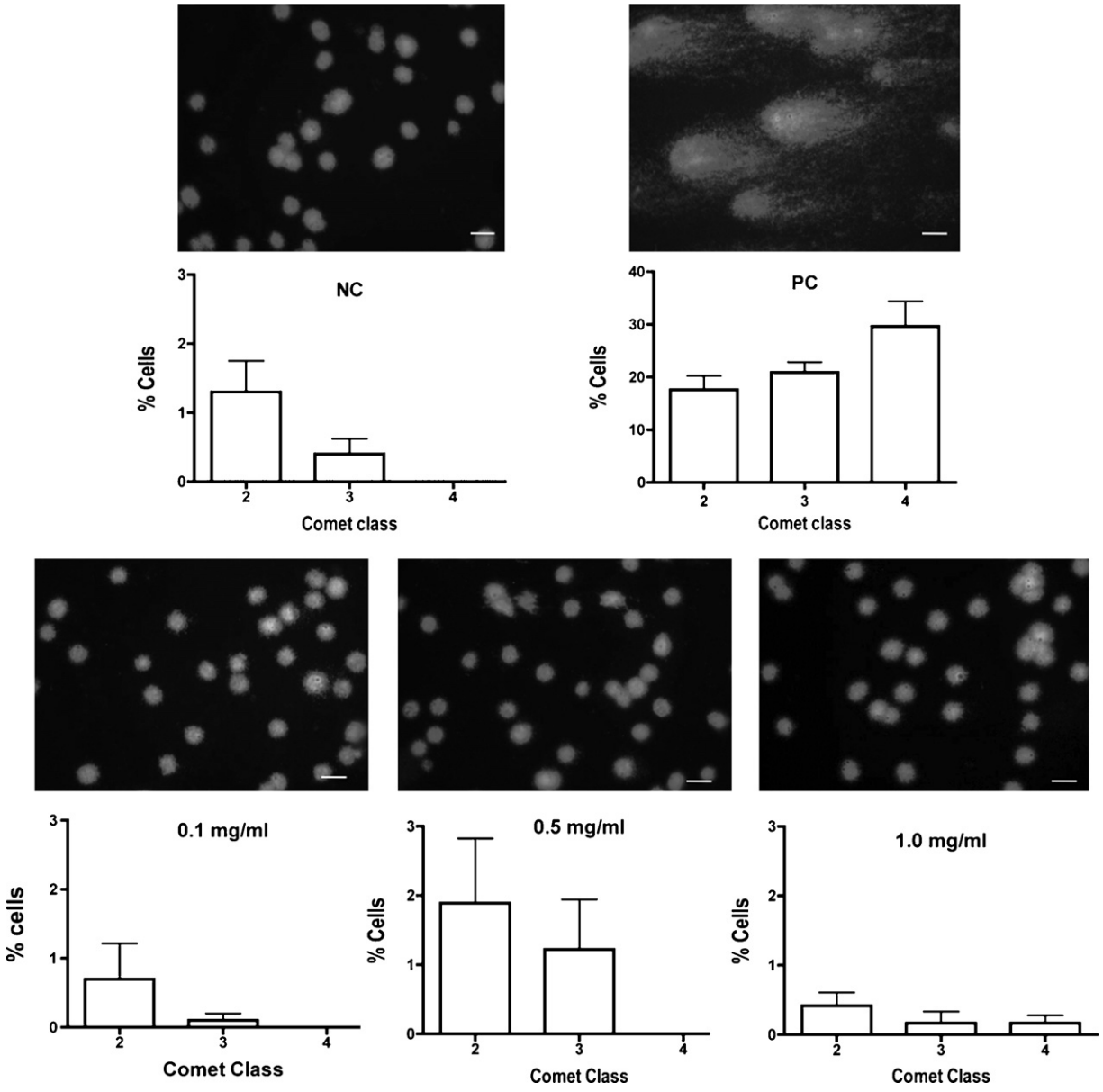

Fig. 3. Fluorescent microscopy images of ethidium bromide stained DNA and results from visual scoring in the comet assay. PC: positive control (H_2O_2); NC: negative control (H_2O); 0.1–1.0 NFs concentration in mg/ml. The images were scored and classified into five classes and given a value according to tail intensity, from 0 (no tail) to 4 (almost all DNA in the tail). Scale bar = 50 μm.

Table 2
Results from images analysis using the Comet Assay IV software (mean ± SD).

Sample	Tail length (μm)	Tail DNA (%)	Tail moment	Tail migration
0.1	17.78 ± 1.73	6.26 ± 1.20	0.66 ± 0.17	2.11 ± 0.82
0.5	21.25 ± 4.99	6.99 ± 3.48	1.03 ± 0.94	4.82 ± 4.25
1.0	15.88 ± 1.44	6.16 ± 1.78	0.71 ± 0.23	1.59 ± 0.55
NC	19.69 ± 3.31	6.88 ± 1.84	1.09 ± 0.67	3.94 ± 2.53
PC	101.36 ± 35.11***	49.06 ± 14.51***	25.42 ± 14.38***	83.11 ± 35.13***

PC: positive control (H_2O_2); NC: negative control (H_2O); 0.1–1.0 NFs concentration in mg/ml.
*** $P < 0.001$.

concentration tested (0.1, 0.5, and 1.0 mg/ml), negative and positive control and the results obtained from the visual score. The results show that the DNA damages in the presence of NFs are similar to the negative control for each NFs concentration used. Around 95% of cells present comet class 0 and 1, corresponding to no or insignificant DNA damage. The cell percentage showing comet class 2, 3 and 4 under different condition are in the graphics of Fig. 3, and represent around 5% of cell. Similar results were obtained with the cells grown on BC membranes (comet class 2: 4.7 ± 3.72; comet class 3: 1.3 ± 2.31, and comet class 4: 1.3 ± 1.53).

Regarding the comet parameters obtained from image analyses (Table 2), tail length (TL), tail % DNA (TI), tail moment (TM) and tail migration (TMi), the NFs did not induce DNA damages under the concentrations tested, since the negative control and samples with NFs present similar results, significantly (TL, TM, TI, TMi, $P < 0.001$) lower than the positive control. The same results were obtained for the cells grown on surface of BC membrane (data not shown); in fact, our results confirmed the previous reports describing the non-genotoxicity of BC (Schmitt et al., 1991).

Taking together the results from visual scoring and image analysis, it may be concluded that the cellulose NFs do not present genotoxicity, under the tested conditions. Since alkaline comet assay allows for detection of DNA strand breaks, cross-links and alkali-labil sites induced by a series of physical and chemical agents it may be concluded that NFs do not induce those damages in DNA.

4. Conclusion

This work presents the first evaluation of the potential genotoxicity of nanofibres extracted from bacterial cellulose. Regarding the results of *Salmonella* reversion and comet assays, cellulose NFs did not present genotoxicity under the conditions tested, as already described for bacterial cellulose membrane. The cell culture systems have been shown to be valuable tools in fibre genotoxicity testing. Unlike *in vivo* studies, secondary inflammatory effects do not affect *in vitro* findings. Induction of DNA damages has been demonstrated for various types of asbestos fibres in several cell systems including CHO cell lines, which was not observed for BC nanofibres. Nevertheless, further studies must be performed in order to comprehensively characterize the toxicology of cellulose-based materials, since small modification in the material could result in drastic changes in cell–material interactions. Work in progress includes the interaction of BC nanofibres with macrophages and *in vivo* assays.

Conflict of interest statement

Nothing to declare.

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