Free Radical Biology and Medicine 107 (2017) 69–76

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review Article

Sensitive detection of DNA oxidation damage induced by nanomaterials☆

Andrew Collins[a,b,*], Naouale El Yamani[b,c], Maria Dusinska[c]

[a] University of Oslo, Department of Nutrition, Oslo, Norway
[b] NorGenotech AS, Skreia, Norway
[c] Norwegian Institute for Air Research, Department of Environmental Chemistry, Kjeller, Norway

ARTICLE INFO

Keywords:
Nanomaterials
Comet assay
DNA oxidation
Lesion-specific enzymes

ABSTRACT

From a toxicological point of view, nanomaterials are of interest; because – on account of their great surface area relative to mass - they tend to be more reactive than the bulk chemicals from which they are derived. They might in some cases have the potential to damage DNA directly, or could act via the induction of oxidative stress. The comet assay (single cell gel electrophoresis) is widely used to measure DNA strand breaks and also oxidised bases, by including in the procedure digestion with lesion-specific enzymes such as formamidopyrimidine DNA glycosylase (which converts oxidised purines to breaks) or endonuclease III (recognising oxidised pyrimidines). We summarise reports in which these enzymes have been used to study a variety of nanomaterials in diverse cell types. We also stress that it is important to carry out tests of cell viability alongside the genotoxicity assay, since cytotoxicity can lead to adventitious DNA damage. Different concentrations of nanomaterials should be investigated, concentrating on a non-cytotoxic range; and incubating for short and longer periods can give valuable information about the mode of damage induction. The use of lesion-specific enzymes can substantially enhance the sensitivity of the comet assay in detecting genotoxic effects.

1. Introduction

Hazard assessment of nanomaterials (NMs) presents particular challenges. Physicochemical properties of NMs differ significantly from those of corresponding bulk chemicals, mainly because of their high surface area relative to mass, which tends to increase reactivity. They are also better able to penetrate cellular and even, in certain cases, nuclear membranes (e.g. [1]), and so potentially have access to chromatin at all stages of the cell cycle. In addition to the possibility of direct, damaging interactions with DNA, NMs might cause oxidative damage – either as part of the cellular response to their presence, or as a secondary effect of inflammation induced by the particles.

The comet assay (single cell gel electrophoresis) [2] is a commonly used method for assessing DNA damage in many different cell types. Essentially, a single cell suspension is mixed with agarose and set on a glass slide or Gelbond film. Lysis in Triton X-100 removes membranes and soluble cell components, and high molarity NaCl strips histones from the DNA, leaving it as a series of supercoiled loops attached to a matrix – a structure known as a nucleoid. DNA breaks are detected by their ability to relax supercoiling, so that loops containing a single (or double) strand break (SB) are able to extend towards the anode during electrophoresis, forming a comet-like image as viewed by fluorescence microscopy. The % of DNA in the tail is proportional to the break frequency. Normally the mean or median % tail DNA from 50 or 100 comets is taken as the measure of DNA damage, and if required it can be converted to a break frequency using a calibration curve based on X- or γ-irradiation, which has a known breakage rate per Gray [3].

Other lesions than strand breaks can be detected by including incubation with a lesion-specific endonuclease in the assay, after lysis; net enzyme-sensitive sites are estimated by subtracting the % tail DNA after lysis alone (or with buffer incubation) from the % tail DNA after enzyme incubation. The first enzyme so used was EndoIII (endonuclease III, Nth) [4], which converts oxidised pyrimidines to breaks. Formamidopyrimidine DNA glycosylase (Fpg) recognises 8-Oxo-7,8-dihydroguanine (8-oxoG) and formamidopyrimidines (ring-opened purines) and is widely used to estimate DNA oxidation damage, for instance in white blood cells collected during human biomonitoring studies [5], as well as in genotoxicity testing. 8-Oxoguanine DNA glycosylase (OGG1), the mammalian analogue of Fpg, is occasionally used; it has a high specificity for oxidised purines, whereas Fpg also recognises some alkylated bases.

☆ This article is one of a series of papers on the subject of oxidative DNA damage & repair that have been published as a special issue of Free Radical Biology & Medicine to commemorate the Nobel Prize won by Prof. Tomas Lindahl. A detailed introduction and synopsis of all the articles in the special issue can be found in the following paper by Cadet & Davies [62].

* Correspondence to: University of Oslo, Department of Nutrition, PB 1046 Blindern, 0316 Oslo, Norway.
E-mail address: a.r.collins@medisin.uio.no (A. Collins).

http://dx.doi.org/10.1016/j.freeradbiomed.2017.02.001
Received 28 September 2016; Received in revised form 2 January 2017; Accepted 1 February 2017
Available online 02 February 2017

Table 1
Summary of published data (since 2011) on genotoxic effects of NPs in cultured cells, using lesion-specific enzymes to reveal oxidation damage to DNA. Cell lines were of human origin unless otherwise stated. Characterisation information was provided in all reports except those marked *.

Nanomaterials	Cytotoxicity/ viability test	Assays	Cells	Concentrations	Exposuretimes	Results	Ref.
TiO_2, < 25 nm anatase, < 100 nm rutile	MTT	Comet assay +Fpg	Hepatoma cells HepG2	1, 10, 100, 250 μg/ml	2, 4, 24 h	Anatase; SBs and Fpg-sites, all times, highest concentration. Rutile; not convincing.	[17]
TiO_2	MTT, NRU	Comet assay +Fpg	Epidermal cell line A431	0.008, 0.08, 0.8, 8, 80 μg/ml	6 h	SBs at 8 and 80 μg/ml; Fpg-sites at 0.8 μg/ml and above	[15]
TiO_2-Ag and ion exchange resin coated with Ag	MTT	Comet assay +Fpg	Mouse macrophage line J774A.1	0.25, 2.5, 5, 10 μg/ml	24 h	TiO_2-Ag: SBs at all doses, with Fpg-sites. Res-Ag: only SBs, from 2.5 μg/ml	[36]
MWCNT	MTT	Comet assay +Fpg	Alveolar epithelial cells A549	5, 10, 40, 100 μg/ml	2, 4, 24 h	Concentration dependent increase in SBs, all 3 times. No Fpg-sites.	[37]
TiO_2 Different samples: 3 anatase, 2 anatase/rutile	LDH, WST−1	Comet assay +Fpg	Colon cancer cells Caco−2	20 μg/ml	4 h	Mild DNA damage with anatase-rutile (1 sample)	[38]
Ag	TB, CFE, RGA	Comet assay +hOGG1	Embryonic kidney cells HEK 293	1, 25, 100 μg/ml	30 min	hOGG1 sites at all concentrations	[39]
Stainless steel, subway particles, MnO_2, Ag, CeO_2	No	Comet assay +Fpg	Alveolar epithelial cells A549	20, 40 μg/ml	4 h	Fpg interacts with NPs: → underestimation?	[28]
PLGA-PEO	RGA	Comet assay +Fpg	Lymphoblastoid cells TK6	0.12–75 μg/cm^2	2, 24 h	No Fpg-sites (or SBs)	[40]
TiO_2 (5) ZnO (2) MWCNT (2) Ag	method not specified	Comet assay +Fpg	Hepatoblastoma cells C3A	Various (< 1–20 μg/cm^2)	4 h	Fpg-sites with 3 TiO2 NMs Fpg-sites with 1 MWCNT NM	[27]
TiO_2	PI	Comet assay +Fpg	Lymphoblastoid cells TK6	0.12, 0.6, 3, 15, 75 μg/cm^2	2, 24 h	Fpg-sites at 75 μg/cm^2 (non-cytotoxic) (2 h, not 24 h)	[41]
			Monkey kidney fibroblasts Cos−1	0.12, 0.6, 3, 15, 75 μg/cm^2	2, 24 h	SBs at 75 μg/cm^2 (cytotoxic) at 2 h, increase at 24 h	
			Embryonic epithelial cells EUE	0.12, 0.6, 3, 15, 75 μg/cm^2	24 h	SBs at 75 μg/cm^2 depending on dispersion protocol	
TiO_2, Fe_3O_4, Fe_3O_4 oleic acid-coated, SiO_2, PLGA-PEO	PI, TB and CFE	Comet assay +Fpg	Lymphoblastoid cells TK6	0.12, 0.6, 3, 15, 75 μg/cm^2	2, 24 h	Fpg-sites at 75 μg/cm^2, 2 h (depends on dispersion protocol)	[42]
			Monkey kidney fibroblasts Cos−1	0.12, 0.6, 3, 15, 75 μg/cm^2	2, 24 h	SBs at 75 μg/cm^2, 2 h and 24 h	
			Embryonic epithelial cells EUE	0.12, 0.6, 3, 15, 75 μg/cm^2	24 h	SBs at 75 μg/cm^2, depending on dispersion protocol	
Polysaccharide cationic NP	TB	Comet assay +Fpg	Bronchial epithelial cells 16HBE14o-	156–5000 μg/ml	3 h	Fpg-sites at most doses but not SBs	[43]
MWCNTs +/- OH	MTT, LDH	Comet assay +Fpg	Alveolar epithelial cells A549	1, 5, 10, 20, 40 μg/ml	2, 4, 24 h	SBs at 40 μg/ml for MWCNTs, 5 μg/ml for MWCNT-OH. Increase with time. No Fpg-sensitive sites.	[44]
TiO_2, ZrO_2, Al_2O_3	Fluorescein/Et br	Comet assay +Fpg, EndoIII	Embryonic kidney cells HEK293 Peripheral blood lymphocytes	1, 10, 100 μg/ml	3 h	ZrO_2 and Al_2O_3; No DNA damage +/- Fpg. TiO_2: SBs at 100 μg/ml No DNA damage.	[12]
TiO_2 (rutile) ZnO ZnO coated Ag capped with Tween 20 MWCNT (2)	WST−1	Comet assay +Fpg	Kidney epithelial cells HK−2	Various (around LC20)	4 h	No Fpg-sites seen with any NMs	[45]
Ag 20 nm and 200 nm	CFE	Comet assay +Fpg	Hepatoma cells HepG2 Colon cancer cells HT29	10, 50, 100 μg/ml	2, 24 h	HepG2 especially sensitive; SBs and Fpg-sites at all doses, especially with 20 nm. Other cell types; also generally more damage with	[21]

(continued on next page)