Chemosphere 149 (2016) 57–61

Contents lists available at ScienceDirect

Chemosphere

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

Role of bentonite clays on cell growth

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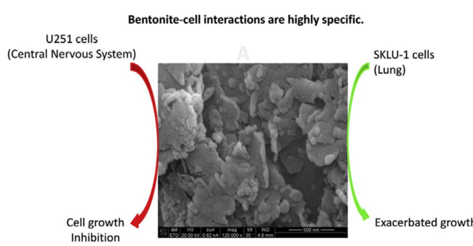
HIGHLIGHTS

- Interactions between bentonite and cell surfaces were highly specific.
- Bentonites inhibit the development of high-grade gliomas.
- Bentonites promote the proliferation of SKLU-1 cells.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 June 2015
Received in revised form
7 October 2015
Accepted 18 January 2016
Available online 3 February 2016

Handling Editor: Frederic Leusch

Keywords:
Cell proliferation response
Swelling

ABSTRACT

Bentonites, naturally occurring clays, are produced industrially because of their adsorbent capacity but little is known about their effects on human health. This manuscript reports on the effect of bentonites on cell growth behaviour. Bentonites collected from India (Bent-India), Hungary (Bent-Hungary), Argentina (Bent-Argentina), and Indonesia (Bent-Indonesia) were studied. All four bentonites were screened in-vitro against two human cancer cell lines [U251 (central nervous system, glioblastoma) and SKLU-1 (lung adenocarcinoma)] supplied by the National Cancer Institute (USA). Bentonites induced growth inhibition in the presence of U251 cells, and growth increment in the presence of SKLU-1 cells, showing that interactions between bentonite and cell surfaces were highly specific. The proliferation response for U251 cells was explained because clay surfaces controlled the levels of metabolic growth components, thereby inhibiting the development of high-grade gliomas, particularly primary glioblastomas. On the other hand, the proliferation response for SKLU-1 was explained by an exacerbated growth favoured by swelling, and concomitant accumulation of solutes, and their hydration and transformation *via* clay-surface mediated reactions.

1. Introduction

Bentonites are naturally occurring clays ubiquitously found on Earth. Bentonites are produced industrially because of their high adsorbent capacity stemming from a high exchange capacity and swelling capacity, as well as a low hydraulic conductivity (Allo and

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http://dx.doi.org/10.1016/j.chemosphere.2016.01.077
0045-6535/

Murray, 2004; Hanuláková et al., 2013; Sidhoum et al., 2013). Little is known, however, on the effects of bentonites on cell growth. Cell responses to bentonite surfaces have been recently reviewed (Nones et al., 2015). Bentonite-based formulations have been tested for drug delivery (Salcedo et al., 2012; Nones et al., 2015); treatment of human osteosarcoma, proliferation, migration, expansion, adhesion, penetration, spreading, and differentiation (Haroun et al., 2009); and wound healing (Sandri et al., 2014; Nones et al., 2015). Besides, expandable clays induced the in-vitro production of collagen fibres, serving as basis to postulate that these clays trigger a protective mechanism based on the absorption of allergen and an improved skin barrier function (Emami-Razavi et al., 2006; Nones et al., 2015). This manuscript reports on the effect of bentonites on cell growth behaviour. Four bentonites sharing various amounts of structural Fe, ranging from 1.1 to 15.7% weight, were studied herein (Table 1).

2. Materials and methods

2.1. Samples

Bentonite samples studied herein, namely, India (Bent-India), Hungary (Bent-Hungary), Argentina (Bent-Argentina), and Indonesia (Bent-Indonesia) were kindly provided by bentonite-producing companies thus exact localities cannot be mentioned (Kaufhold et al., 2008). Bentonite samples were first referred as B11, B14, B22, and B28, respectively (Kaufhold and Dohrmann, 2008; Kaufhold et al., 2008; Dohrmann and Kaufhold, 2009; Kaufhold et al., 2010a, b; Kaufhold et al., 2012; Kaufhold and Dohrmann, 2013; Kaufhold et al., 2013). All samples were used as received. Clays subject to study tested negative for bacterial growth.

2.1.1. Specific surface area (S_{BET})

S_{BET} determinations were conducted by N_2 adsorption using a 5 point BET method, placing 30 mg of the sample inside a Micrometrics Gemini III 2375 surface area analyser. S_{BET} values for Bent-India, Bent-Hungary, Bent-Argentina, and Bent-Indonesia were 66, 130, 31, and 72 $m^2\ g^{-1}$, respectively.

2.1.2. Cation exchange capacity (CEC)

CEC determinations were conducted using the Cu-Triethylenetetramine method (Meier and Kahr, 1999). CEC values for Bent-India, Bent-Hungary, Bent-Argentina, and Bent-Indonesia were 90, 82, 86, and 72.4 meq 100 g^{-1}, respectively.

2.1.3. Surface charge

Electrophoretic mobility determinations were conducted using a Zetasizer Nano ZS ZEN3600 (Malvern Instruments, UK). The suspensions were adjusted to 20% in weight and pH 7.4 using a phosphate buffer.

2.1.4. Layer charge density

Layer charge density values for selected bentonites were determined using the alkylammonium method (Lagaly, 1994) and reported elsewhere (Kaufhold et al., 2011). Experimental LCD values for Bent-India, Bent-Hungary, Bent-Argentina, and Bent-Indonesia corresponded to 0.32, 0.33, 0.35, and 0.32 eq FU^{-1}, respectively.

2.1.5. X-ray fluorescence (XRF)

X-ray fluorescence was performed using a PANalytical Axios and a PW2400 spectrometer. The samples were prepared by mixing them with a flux material and melting them into glass beads. To determine loss on ignition (LOI) 1000 mg of sample material was heated to 1030 °C for 10 min (Kaufhold et al., 2009).

2.1.6. X-ray diffraction (XRD)

XRD patterns were recorded using a PANalytical Pert PRO MPD diffractometer (Co-Kα ± radiation generated at 40 kV and 40 mA), equipped with a variable divergence slit (20 mm irradiated length),

Table 1
Chemical and mineralogical composition and surface and colloidal properties for bentonites.

Chemical composition

Bentonite	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	MnO	MgO	CaO	Na_2O	K_2O	P_2O_5	Sum
Bent-India	46.1	2.4	14.4	15.7	0.1	2.5	0.9	0.9	0.1	0.0	99.7
Bent-Hungary	45.8	2.1	16.8	9.9	0	2.2	1.6	1.6	0.7	0.4	99.6
Bent-Argentina	62.4	0.2	15.0	1.1	0	3.3	1.0	1.0	0.4	0.1	99.8
Bent-Indonesia	58.8	0.2	13.5	2.1	0	3.1	2.7	2.7	0.5	0.1	99.6

Mineralogical composition[a]

Bentonite	Smectite	Quartz	Kaolinite	Feldspar	Calcite	Cristobalite	Hematite	Ilmenite	Rutile/Anatase	Clinoptalite
Bent-India	89	1		0[b]	1		0	0	3	
Bent-Hungary	88	3	3	3	1				2	
Bent-Argentina	76	4		8	0	12				
Bent-Indonesia	69	2		4		8				17

Surface and colloidal properties

				Surface charge[c]			
Bentonite	σ_s ($m^2\ g^{-1}$)	CEC[a,d] (meq 100 g^{-1})	LCD[a,d] (eq FU^{-1})	[C] (ppm)	Ω (mS cm^{-1})	*Em* (μm cm $V^{-1}\ s^{-1}$)	Zeta potential (mV)
Bent-India	66	90	0.31	192	15.7 ± 0.5	−2.2 ± 0.2	−28.0 ± 3.1
Bent-Hungary	130	82	0.33	200	16.7 ± 0.1	−2.7 ± 0.04	−34.5 ± 0.5
Bent-Argentina	31	86	0.35	200	16.7 ± 0.9	−2.6 ± 0.05	−33.1 ± 0.6
Bent-Indonesia	72	72.4	0.31	200	17.2 ± 0.2	−2.2 ± 0.04	−28.6 ± 0.5

[a] Determined by Rietveld analysis. Data taken from Kaufhold and Dohrmann, 2008; Kaufhold et al., 2008; Dohrmann and Kaufhold, 2009; Kaufhold et al., 2010a, b; Kaufhold et al., 2012; Kaufhold and Dohrmann, 2013; Kaufhold et al., 2013.
[b] "0" refers to $0.1 \le x \le 0.49$.
[c] Data taken from Cervini-Silva et al., 2015b.
[d] CEC and LCD refer to cation exchange capacity and surface layer density, respectively. LCD refers to when swelling occurs when the bentonite expands beyond the original limit of 0.95 nm.