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Research paper

Cell growth underpinned by sepiolite

Javiera Cervini-Silva [a,b,c,*], María Teresa Ramírez-Apan [d], Stephan Kaufhold [e], Eduardo Palacios [f], Virginia Gomez-Vidales [g], Kristian Ufer [e], Paz del Angel [f], Ascención Montoya [f]

[a] Departamento de Procesos y Tecnología, Universidad Autónoma Metropolitana Unidad Cuajimalpa, Mexico
[b] Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA
[c] NASA Astrobiology Institute, USA
[d] Laboratorio de Pruebas Biológicas, Instituto de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, México City, Mexico
[e] BGR Bundesansaltfür Geowissenschaften und Rohstoffe, Stilleweg 2, D-30655 Hannover, Germany
[f] Direccion de Investigación y Posgrado, Instituto Mexicano del Petróleo, Mexico
[g] Laboratorio de Resonancia Paramagnética Electrónica, Instituto de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, México City, Mexico

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ABSTRACT

This paper reports the role of clay minerals, sepiolites, on the proliferation behaviour of human cancer cells. It reports as well on the proliferation of U251 (central nervous system, glioblastoma) and SKLU-1 (lung adenocarcinoma) cells by sepiolite bearing different extent of isomorphic substitution (*IS*), either because of the inclusion of Al^{3+}, $Fe^{3+,2+}$, or Ti^{4+} in Si structural sites (*IS* at the tetrahedral sheet, **T**) or that of Ni^{2+} in Mg structural sites (*IS* at the octahedral sheet, **O**). Studied sepiolites were originally from Ampandrandara, Madagascar; Cerro del Almodóvar, Spain; Deiva Forest, Italy; Eskidir, Turkey; Peguera, Falcondo Plant, Dominican Republic; Sepetciköyü, Turkey; Shimien, China; and Vallecas, Spain. Furthermore, obtained results for sepiolites were compared against those for clays (bentonites). Diffractograms showed characteristic patterns for sepiolite, with no evidence of significant accumulation of secondary phases. XRF data confirmed the incorporation of Al, Fe and, Ti; and Ni, consistent with *IS* at **T** and **O**. The effect of sepiolite on cellular proliferation was determined using the SRB protocol. All sepiolites induced inhibition or increment on the proliferation response of U251 or SKLU cells, depending on the sepiolite; however no correlation between proliferation against composition or microporosity properties became evident. Most notably, sepiolite from Sepetciköyü, Turkey, owning the highest microporosity (evidenced by surface area σ_s) of the sepiolite series, 343 $m^2\,g^{-1}$, exerted the highest proliferation response for U251 and SKLU-1 cells, namely, 100% inhibition and 22.8 $\pm$ 12.1% increase, respectively. Sepiolites from Ampandrandara, Sepetciköyü, and Deiva Forest, owing very low contents of Al ($Al_2O_3 \leq 0.2\%$) and variable σ_s yielded the highest inhibition in U251 cells proliferation, best accounted for by growth was limited by specific-adsorption mechanisms in which structural changes associated to Al-for-Si *IS* at **T** favoured the adsorption of metabolic growth components [epidermal growth factor receptor (EGFR)], thereby inhibiting the development of primary glioblastomas. On the other hand, increments (%) in SKLU-1 cells proliferation did not correlate with microporosity (measured σ_s values), yet two data clusters were identified, higher and lower data values, i.e., 22.8 ≤ % increment ≤ 39% ($\sigma_s = 83$, 220, or 343 $m^2\,g^{-1}$) and 6.9 ≤ % increment ≤ 14.2% ($96 \leq \sigma_s \leq 266$). The second group was composed by Ampandrandara, Sepetciköyü, and Deiva Forest, generating surface sites that catalyze the overexpression of activin A. So, the growth behaviour for both U251 and SKLU-1 cells was affected by Al at **T** *via* Al-for-Si *IS* if proceeded to a small degree. In all, however, the overall chemical composition lacked to serve as predictor for growth. Structural considerations supported the idea that controlled cell growth by sepiolite was not limited by the retention of small solutes at inner surfaces. Finally, whether variations in microporosity exerted changes in the cell proliferation behaviour was strongly dependent if the phyllosilicate was a clay mineral (sepiolite) or a clay (bentonite).

1. Introduction

Clays and clay minerals have been recognized for exerting positive effects on human health and for having great potential for the implementation in medical applications. For instance, bentonites are industrial clays that can be used in a wide variety of medical applications, such

* Corresponding author at: Departamento de Procesos y Tecnología, Universidad Autónoma Metropolitana, Av. Vasco de Quiroga 4871, Col. Santa Fe Cuajimalpa, CDMX C.P. 05348, Mexico.
E-mail address: jcervini@correo.cua.uam.mx (J. Cervini-Silva).

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as drug delivery (Salcedo et al., 2012; Nones et al., 2015); treatment of osteosarcoma, proliferation, migration, expansion, adhesion, penetration, spreading, and differentiation (Haroun et al., 2004); wound healing (Sandri et al., 2014; Nones et al., 2015); also bentonites induce the *in-vitro* production of collagen fibres, triggering a protective mechanism based on the adsorption of allergen and an improved skin-barrier function (Emami-Razavi et al., 2006; Nones et al., 2015). A related study reported on the *in vitro* screening of four bentonites (collected from Argentina, Hungary, India, and Indonesia) against two human-cancer cell lines [U251 (central nervous system, glioblastoma) and SKLU-1 (lung adenocarcinoma)] supplied by the National Cancer Institute (USA), showing in the former and latter case growth inhibition and increment, respectively. Cell proliferation was explained by highly specific interactions between bentonite and cell surfaces (Cervini-Silva et al., 2016a). First, bentonite surfaces served as binding site for biomolecules active in the proliferation of gliomas, particularly primary glioblastomas (Wells, 1999; Mendelsohn and Baselga, 2000). Second, the structural features proper of bentonites, namely swelling and expansion of the interlayer spacing, and concomitant accumulation of solutes, and their hydration and transformation, contributed to exacerbate the growth of SKLU-1 cells by favouring the overexpression of activin A (Matzuk et al., 1992). The current study reports on the effect of clay minerals on cell growth, and compared the obtained results to those reported in a related paper showing the role of bentonite clays on cell proliferation (Cervini-Silva et al., 2016a).

Clays and clay minerals are different. First, the term "clay" refers to materials with ≤2 μm in equivalent spherical diameter (e.s.d.; Moore and Reynolds, 1997; Bergaya and Lagaly, 2006), whereas according to the Joint Nomenclature Committee (JNC), clay minerals refer to phyllosilicates making up the fine-grained fraction of rocks, sediments, and soils (Bergaya and Lagaly, 2006). Second, both phyllosilicates can accumulate solutes, and facilitate their hydration and transformation. In particular, clays and clay minerals studied herein (bentonites and sepiolites, respectively) present key differences between their surface physicochemical properties. Most notably, bentonites are comprised by smectites, which are swelling (expandable) clays. Stemming from such swelling behaviour in bentonites is the presence of surface-reactive sites not found in clay minerals. That is to say, bentonites and sepiolites will show different surface-mediated reaction mechanisms. In addition, bentonites and sepiolites present different stacking characteristics, which are limiting for transport processes and mineral stability. Upon weathering the structure of sepiolite is disrupted due to dislocations of the inverted sites in the tetrahedral sheet (Table 1). Finally, bentonites and sepiolites studied herein own a significant difference between the total number of reaction sites (Table 2), with the latter phyllosilicate group owning a significantly larger number of adsorption sites, most of which are located at internal surfaces (e.g., for sepiolite from Vallecas, specific surface area: 322; external surface area 148; microporous area: 174 $m^2\ g^{-1}$; García-Romero and Suárez, 2010).

In the environment the replacement of elements into the structure of phyllosilicates (isomorphic substitution; *IS*) often takes place, because of their incorporation at either tetrahedral (Al^{3+}, $Fe^{2+,\ 3+}$, or Ti^{4+} substitute structural Si; **T**) or octahedral (Ni^{2+} substitute structural Mg; **O**) sheets, causing changes in the surface charge and, consequently, affecting biological processes *in lieu* of increasing repulsive forces between mineral and cell surfaces. This paper reports on cell growth by clay minerals as affected by *IS*, for which a series of eight sepiolites were screened against U251 (central nervous system, glioblastoma) and SKLU-1 (lung adenocarcinoma). Sepiolite [ideal formula $Mg_4Si_6O_{15}(OH)_2\cdot 6H_2O$], also known as *meerschaum* ("foam of the sea"), was selected as probe clay mineral because it owns a high specific surface area, microporous area and volume and external area, and large tunnels allowing for high diffusion rates (Sánchez del Río et al., 2006; Ovarlez et al., 2009; Dejoie et al., 2010; Giustetto and Wayhyudl, 2011). Studying the role of sepiolite on cell growth is significant because it is an industrial clay mineral, produced around the world at 1.2 m tons per year and used in a number of applications including the production of pet litter, animal feed stuff to roof parcels, construction and rheological additives, and other applications needing to replace long-fibre length asbestos, among others.

Table 1
Composition and specific surface area for the sepiolite series.

	CAL	VAL	VAL[a]	AMP	SHI	DFO	ESK	SEKO	FAL
σ_s ($m^2\ g^{-1}$)	163	266		184	96	83	157	343	220
Composition									
SiO_2	55.6	55.7	68.01 ± 2.2	52.1	61.6	52.6	49.7	55.2	50.19
TiO_2	0.1	0.05	0.31 ± 0.52	0	0.2	0	0	0	<0.001
Al_2O_3	2.7	1.07	2.01 ± 1.1	0.2	5.4	0.1	0.3	0.1	0.11
Fe_2O_3	0.6	0.2	0.64 ± 0.83	2.1	2.3	5.9	0.2	0	0.44
MnO	0.1	0.01	–	0.8	0.1	0	0	0	0.002
MgO	21.2	23.5	28.2 ± 1.1	22.2	12.8	20.7	24.1	24.9	15.2
CaO	0.7	0.05	0.29 ± 0.56	1.9	1.8	1	2.8	0.1	<0.005
Na_2O	0.2	0.06	0.34 ± 0.57	<0.01	0.1	<0.01	<0.01	<0.01	<0.01
K_2O	0.9	0.2	0.34 ± 0.57	0	0.4	0	0	0	0.014
P_2O_5	0	0.01	–	0.4	0.1	0	0	0	0.013
(Cl)		<0.002	–	–					0.065
(F)	<0.05	<0.05	–	–	0.1	0	0	0.1	0.09
As (ppm)	7	2	–	6	4	5	3	5	8
Ba	138	161	–	313	319	4	6	<4	5
Bi	<2	<5	–	<2	4	<2	<2	<2	18
Ce	<16	<50	–	1651	35	<16	<16	<21	<26
Co	11	<5	–	4	11	12	4	<3	11
Cr	<4	<10	–	10	105	16	81	10	131
Cs	3	<63	–	<3	<5	<3	<3	<3	<4
Cu	29	15	–	14	34	93	10	15	<11
Ga	4	<4	–	<2	8	<2	<2	<2	<3
Hf	<5	<14	–	<5	<5	<6	<5	<6	<10
La	<13	<38	–	1282	55	<13	<13	<17	<21
Mo	<2	<6	–	3	<2	<3	<2	<3	<4
Nb	6	<5	–	5	3	5	2	3	4
Nd	<12	<33	–	381	<50	<12	<12	<15	<18
Ni	7	7	–	21	115	760	1510	2497	126800
Pb	21	<6	–	20	17	6	6	6	16

[a] Analytical electron microscopy (mean values, wt%). $n = 17$. Data taken from Tables 4 and 5 in García-Romero and Suárez (2010).