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Original article

Cytotoxic effect of ferrimagnetic glass-ceramic nanocomposites on bone osteosarcoma cells

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ABSTRACT

This work pointed out the anti-cancer effect of ferrimagnetic glass ceramic nanocomposites (CaO-ZnO-Fe_2O_3-SiO_2), which contain high amount of magnetite (~60%), crystallite size <100 nm, and different nucleating agents on bone cancer Saos-2 cells. The cell viability was inhibited by FH and FW to <50% and <25%, respectively, with/without magnetism, and both also reduced mitochondrial transmembrane potential (ΔYm), with/without magnetism (no influence of magnetism). Histone deacetylase (HDAC) activity was inhibited by FH, FW, and FHPNT, with/without magnetism. FHP3/magnetism resulted in HDAC inhibition.

In absence of magnetism, FH and FW increased both necrotic and apoptotic cell death, while FW/magnetism induced late apoptosis. DNA fragmentation was increased by FH- and FW-treatment, with/without magnetism. At the same time, FW and FH/magnetism can efficiently induce the intrinsic apoptotic pathway in Saos-2 cells, whereas FW with/without magnetism and FH/magnetism enhanced cytochrome-C release. Similarly, caspase-7 activity was elevated by FH and FW, with/without magnetism. However, the presence of P_2O_5 in the composition of the nanocomposites inhibited their apoptotic properties and diminished their anti-cancer activity.

1. Introduction

Hyperthermia is a promising therapeutic modality that resulted in cancer cells death by increasing their temperature to a "high fever" [1]. In general, healthy tissues are less heated than the tumor tissues due to the poorly-developed blood vessels in the tumor mass that resulted in a low oxygen supply. The advantages of hyperthermia are mainly focused on the lack of side effects [2] and the safety for the nearby healthy tissues with normal and effective cooling by blood system [3]. Ferrimagnetic glass–ceramics (FGCs) are promising hyperthermic agents, particularly for the deeply seated tumors such as osteosarcoma. Implantation of a granular form of FGCs near to the tumors resulted in a strong bonded FGC-bone forming a biologically active and stable bone-like apatite complex around the tumors [4]. The exposure of this stable bound form to alternating magnetic field can cause efficient heating of tumor cells leading to death [4]. A variety of biomaterials that generate heat by magnetic hysteresis loss were previously studied [5–10], including FGCs [11–14].

Variable preparations of magnetite-glass ceramics were reported [2,15–17], where FGCs contained a maximum 45% wt of magnetite [17], which possessed a coercive force of 85 Oe and a saturated magnetization of 34 emu/g. Ebisawa et al. [2] prepared a FGC with a matrix of $CaO{\cdot}SiO_2$-based glass and wollastonite that contained 36% magnetite that showed ferrimagnetisms but no bioactivity [18]. It was concluded previously that heat generation is based mainly on the magnetic properties of the implant, the magnetic field parameters and the tissue characteristics [17]. Zinc ions play an essential role in the bone metabolism through stimulating bone formation and elevating bone calcium content, protein, and alkaline phosphatases activity [19]. The biomaterial, hardystonite ($Ca_2ZnSi_2O_7$), has a phase that possessed a better biocompatibility than hydroxyapatite, due to its better fracture,

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toughness and bending strength [19]. This work aimed to investigate the therapeutic effect of some selected FGCs compositions based on crystallization of wollastonite or hardystonite with high quantity (~60%) of magnetite on cancer cells. These FGCs nanocomposites were previously prepared and studied by our group with several modifications [20–22], and in the present study we investigated FGCs in vitro effect on bone cancer Saos-2 cells.

2. Material and methods

2.1. Preparation of the FGCs nanocomposites

The glass was designed to crystallize ~60% Magnetite: 40% wollastonite or hardystonite and to study the effect of adding Na_2O, P_2O_5, and TiO_2, separately, or in a mixture of them on the crystallization sequence and the magnetic properties [20–22]. Mixtures of the following constituents were prepared from Ca_2CO_3, SiO_2, Fe_2O_3, ZnO and B_2O_3 (as H_3BO_3). TiO_2, Na_2O (as Na_2CO_3) and P_2O_5 (as $NH_4H_2PO_4$) were added above 100%. The chemical compositions were shown in Table 1. To obtain a glass–ceramic, a melting step was necessary to get the magnetite nucleation in a liquid-derived amorphous phase.

2.2. Crystallization and characterization of the FGCs nanocomposites

The prepared FGCs were submitted to differential thermal analysis (DTA) (SETRAM Instrumentation Reulation, Labsys ™ TG-DSC16), and powder X-ray diffraction (XRD)/Ni-filled Cu-Kα radiation by Pruker D8 [23]. The average XRD crystallite size for the intense peaks (220, 311, 400, 511 and 440) was calculated by Debye-Scherrer formula [D = kλ/B cos Θ]; D: particle size, λ Cu: 1.54 Å, k: constant, B: full half wide and 2 theta = 4° [23].

The prepared FGCs were observed under transmission electron microscope (TEM; Zeiss, Germany). In a maximum applied field of 20 kOe, their magnetic properties were evaluated by vibrating sample magnetometer (9600-1 LDJ, USA). The saturation magnetization (Ms), Remanent magnetization (Mr), and Coercivity (Hc) were evaluated from the resulted hysteresis loops [23].

2.3. Cell culture and treatments

Human osteosarcoma cell line (Saos-2; ATCC, Rockville, MD, USA) were cultured in ATCC-McCoy's 5a modified medium containing 10% fetal bovine serum (FBS), penicillin, and streptomycin. Cells were maintained in humidified air, 5% CO_2, and 37 °C. All experiments were repeated independently (n = 4). Cells were harvested after brief trypsinization. For experiments, actively growing Saos-2 cells were harvested, counted, cultured in 6-well plate (3×10^5 cells/well) and then incubated for 24 h. In each experiment, a fixed concentration of each nanocomposite (100 μg/ml) was used to treat Saos-2 cells and then the cells were exposed to a magnetic field for 30 min using a permanent magnet (0.5 T, 80 × 40 × 10 mm, nickel coated, Neotexx Company, Germany). After magnetic treatment, the cells were allowed to recover by 24 h incubation before being submitted to various evaluations. Wells of complete medium, nanocomposites, or magnetic treatment, with and without cells were compared with the treated cells.

2.4. Cytotoxicity

To evaluate the cytotoxic effect of the prepared FGCs on Saos-2 cells, in the presence and absence of magnetic field, 3,4,5 -dimethylthiazol-2,5-diphenyl tetrazolium bromide (MTT) technique was utilized [24]. The untreated cells were considered 100% viability.

2.5. Determination of mitochondrial transmembrane potential

The mitochondrial transmembrane potential ($\Delta\Psi_m$) was investigated by MitoTracker® Red CMX-Ros staining (Life Technologies, Carlsbad, USA). A total of 500 stained cells were visualized by Carl Zeiss automated fluorescence microscope with Zen 2011 software at 400× magnification.

2.6. Estimation of histone deacetylase activity

Histone deacetylase (HDAC) activity was evaluated in treated- and untreated-Saos-2 cells by a colorimetric kit (BioVision, USA) according to the manufacturer instructions.

2.7. Apoptosis and necrosis staining

To study the effect of different nanocomposites, with or without magnetic field, on the cell death mode apoptosis and necrosis, were analyzed by ethidium bromide/acridine orange (EB/AO) DNA staining [25,26].

2.8. DNA fragmentation

Assessing the DNA fragmentation allowed the estimation of the degraded DNA amount and it was assayed by previously reported

Table 1
Chemical composition of the prepared samples in wt%. In the lower part of the table, the magnetic properties obtained under a maximum magnetic field of 20 kOe.

Nanocomposites content		FW	FH	FHP	FHPNT	FHP3
Fe_2O_3		58.26	58.26	58.26	58.26	58.26
CaO		18.74	13.88	13.88	13.88	13.88
ZnO		–	10.07	10.07	10.07	10.07
SiO_2		20.09	14.88	14.88	14.88	14.88
B_2O_3		2.91	2.91	2.91	2.91	2.91
P_2O_5*		–	–	3	3	10
Na_2O*		–	–	–	3	–
TiO_2*		–	–	–	3	–
Magnetic properties of quenched samples	M_s(emu/g)	35.247	29.967	58.99	56.71	38.59
	M_r(emu/g)	1.36	0.074	4.634	4.129	3.32
	M_r/M_s	0.0386	0.0025	0.0785	0.0728	0.086
	Hc (Oe)	75.86		24.76	19.75	76.494
		7.21				

* P_2O_5 & Na_2O and TiO_2 were added above 100%.