The Radiobiological Effect of the TiO_2 – Cyclodextrin Suspension

Mihaela Corneanu
Genetics Engineering Dept.
Banat's University of Agricultural Sciences and Veterinary Medicine, 300645-Timişoara, Romania,
micorneanu@yahoo.com

Gabriel C. Corneanu, Aurel Ardelean
Life Sciences Dept.
"Vasile Goldis" Western University
310025-Arad, Romania
gabicorneanu@yahoo.com, aardelean@uvvg.ro

Carmen Lazău, Ioan Grozescu
I.N.C. –D.E.M.C. Timişoara
Timişoara, Romania
carmenlazau@yahoo.com
grozescu@icmct.uvt.ro

Nicoleta G. Hădărugă
Faculty of Food Processing Technology, Banat's University of Agricultural Sciences and Veterinary Medicine, 300645-Timişoara, Romania,
nico_hadaruga@yahoo.com

Daniel I. Hădărugă
Faculty of Industrial Chemistry and Environmental Engineering, "Politehnica" University of Timişoara,
300006-Timişoara, Romania,
daniel.hadaruga@chim.upt.ro

Lucian Barbu-Tudoran
Biology Dept.
Babeş-Bolyai University
Cluj-Napoca, România
lucianbarbu@yahoo.com

***Abstract* — The effect of a TiO_2 nanoparticle suspension (4 mg in 5 ml distilled water) doped or not with gold or iron, complexed or not with α- or β-cyclodextrin, on the mitotic division was studied. The treatment was performed in the M cellular mitotic cycle phase, in the meristematic tissue of Nigella damascena radicels, being analyzed the cytogenetic modifications. The cyclodextrin type and the ratio between cyclodextrin and titanium dioxide affect the percentage of normal cells and the chromosome aberration type. The TiO_2 complexed with α-cyclodextrin did not facilitate the reunion of the chromosome broken ends (absence of bridges in anaphase and in telophase). The titanium dioxide doped with iron, complexed with α- or β-cyclodextrin, also reduced the percentage of reunion of the broken ends of the chromosomes. These findings suggest the possibility of using doped or undoped TiO_2, complexed with α-cyclodextrin, in the anticarcinogen therapy.**

Keywords - TiO_2-Au, TiO_2-Fe, cyclodextrin, chromosome mutations.

I. INTRODUCTION

Although the bimetallic particles were obtained long ago, their characterization was effected at the end of the 20th century [1]. The conjugation of bimetallic nanoparticles of AuNP type with oligonucleotides facilitates their use in molecular biology experiments and nanobiotechnology [2]. Other types of AuNP biconjugate with peptides, lipids, enzymes, drugs or viruses, were also obtained with application in nanobiotechnology, nanomedicine and/or in gene therapy.

Cyclodextrins are cyclic oligosaccharides. Depending on the glucose moiety number, they are classified in: α-cyclodextrins (with 6 glucose units), β-cyclodextrins (with 7 glucose units), and other greather homologues. The complexes with pharmaceutical products are usually better accepted and tolerated by the organism; they protect the bioactive substances against degradation (oxygen, humidity) and reveal controlled release properties. They are non-toxic and stable at the human digestive enzyme action. They are used in the obtaining of some pharmaceutical products, food additives, in agriculture, a/o [3]. In recent papers, the effect of β-cyclodextrin conjugate with TiO_2 [4] or with different bioactive substances [5], [6] was analyzed, in different experimental conditions.

In the present study nanoparticles of TiO_2-Au and TiO_2-Fe, dispersed in α- or β-cyclodextrin, the ratio between TiO_2 and cyclodextrin being 1:1 or 1:2, were used. The experiments were performed at a radiobiological tester plant (*Nigella damascena* L.), the chromosome aberrations from the meristematic radicel tissue being used as radiobiological indices.

II. MATERIAL AND METHODS

A. Nanoparticle achievement

1) Achievement of undoped and doped TiO_2 nanocrystals.

Undoped and doped titanium dioxide nanocrystals were synthesized by the sol-gel route, using the precursors: titanium isopropoxide, isopropyl alcohol, distilled water, nitric acid, gold (III) chloride trihydrate and ferrous nitrate. 5 ml of titanium isopropoxide (in drops) were mixed with 30

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ml of isopropyl alcohol, under continuous stirring. After a few minutes of stirring, distilled water was added, continuously controlling the solution pH with nitric acid in order to avoid the precipitation. In the case of iron-doped and gold-doped TiO_2 ions, after the adjustment of the pH (2.5 for Au, 5.0 for Fe), the previously prepared solutions, namely iron nitrate and gold (III) chloride trihydrate were added under continuous stirring. In both cases, the gel was dried and washed in order to remove the secondary reaction products. The calcination was achieved in the oven, at a temperature of 250ºC for undoped TiO_2 and 500ºC for Au or Fe doped TiO_2. The achieved nanocrystals based on titanium dioxide were used for complexation with cyclodextrins.

2) Achievement of complexes based on TiO_2 with α-cyclodextrin or β-cyclodextrin.

α- and β- cyclodextrins used for the co-crystalization with the previously attained titanium dioxide were of analytical purity (Fluka Chemie AG). In order to perform the complexation/co-crystalization, different solvents were used as suspension medium. In 1 ml distilled water, 0.054 g of α-(α-CD) or 0.067 g of β-cyclodextrin (β-CD) was dissolved, at a temperature of 50ºC. 0.075 g of undoped and doped TiO_2 was added to the solution, under continuous and vigorous stirring for 30 minutes at 50ºC. For the completion of the complexes crystallization, the solution was cooled and kept for 12 hours at a temperature of 5ºC, followed by filtration and drying of the achieved nanocrystals. The obtained materials were characterized by X-ray diffraction (XRD), scanning/transmission electron microscopy (SEM/TEM) and energy dispersive X-ray analysis (EDX).

Biological experiment

The experiment was performed *in vivo*, on the radiogenetic tester species *Nigella damascena* (Fam. *Ranunculaceae*). This species, used for a long time, is for its features: small somatic chromosome number different morphologically (2n=12), synchronized cell mitotic cycle, centromeres situated near the nuclear envelope, and absence of the bioactive substances in seeds [7], [8], [9]. The seeds with radicels of about 10 mm length were treated in the mitotic phase of the cellular cycle (for 2 hours), with a suspension of TiO_2-Au or TiO_2-Fe, complexed or not with α-cyclodextrin, or with β-cyclodextrin (4 mg TiO_2-Me in 5 ml distilled water). After treatment, the radicels were harvested for the cytogenetic investigations.

B. *Cytogenetic investigations*

The chromosome aberrations were analyzed in the radicular meristematic tissue, on squash preparation type, stain with a Carr solution. The normal and aberrant phases of the mitotic cycle were analyzed, as well as the metabolic and structural modifications of the chromosomes and of the mitotic spindle. Although in the literature there are numerous papers about the environmental factors' effect at the chromosome level, regarding the nanoparticles' effect at the chromosome level the information is poor. The investigation performed at the Chinese hamster ovary, with TiO_2 particles, in the presence or absence of the UV light, evidenced that these not enhance the chromosomal aberrations' frequency [10]. Similar results were obtained by other authors, in the genotoxicity tests performed in Chinese hamster ovary, as well as in experiments on aquatic organisms (*Daphnia magna*, *Onchorhynchus mykiss* and green algae *Pseudokirchneriella subcapitata*), acutely exposed to the ultrafine TiO_2 particle-types [11], or in experiments in fish (*Nothobranchius rachovi*), at which the mitotic index was analyzed in different organs and variants [12], [13].

III. RESULTS AND DISCUSSIONS

A. *Characterization of the nanoparticles*

The XRD patterns (Fig. 1) analyses present the crystallization as anatase form of the undoped/Au or Fe doped TiO_2, even if the calcination temperatures for the TiO_2 doping surpass the value of 250ºC. The presence of the dopant in the crystalline network of the titanium dioxide prevents the transition of phases from anatase to rutile. From the diffraction spectra it is noticed that the dopants did not present separate peaks, which means that they are distributed uniformly in the crystalline network. From the surface morphology (SEM) it can be observed that the TiO_2-Au and TiO_2-Fe nanospheres' dimensions range between 10 and 20 nm (Fig. 2, a and b). EDX microprobe provided a semiquantitative elemental analysis of the surface indicating the Ti, O, Au and Fe presence (Fig. 2, c and d).

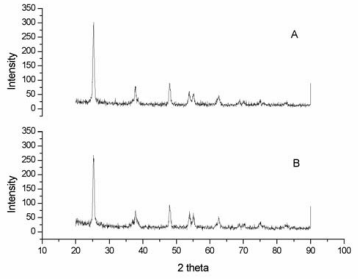

Figure 1. X-ray pattern of TiO_2 doped: (A) – with Au ions; (B) – with Fe ions.

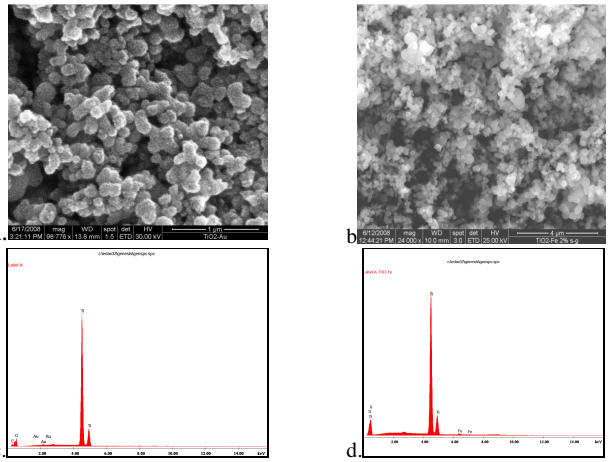

Figure 2. SEM micrographs of : a. TiO_2-Au, b.TiO_2-Fe, EDX spectra of: c. TiO_2-Au, d. TiO_2-Fe

From the SEM analyses of TiO_2 complexes with α- or β-cyclodextrin (Figs. 3-5), the needle-like, parallelepiped shape of the crystals (or co-crystals) for the undoped TiO_2/α-CD and β-CD complexes can be seen. The crystals have much smaller dimensions compared to those of the raw materials (pure α- or β-CD), the length being in the range of 10-100 nanometers, and the width of only a few tens of nanometers. In the case of complexes TiO_2-Au/α-CD, the crystals present similar morphologies and dimensions to the undoped TiO_2, but these are more agglomerated. In the case of TiO_2-Au/β-CD complexes, the crystal morphology is different; the crystals are more uniform, of smaller dimensions, agglomerated, of rhomboidal (generally polyhedral) form. The approximate dimensions of the crystals are between tens and hundreds of nanometers.

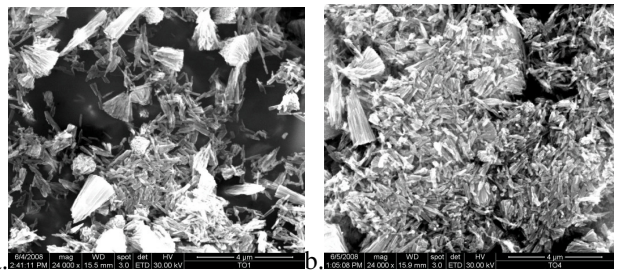

Figure 3. SEM image for undoped TiO_2-CD:a. TiO_2/α-CD b. TiO_2-β-CD

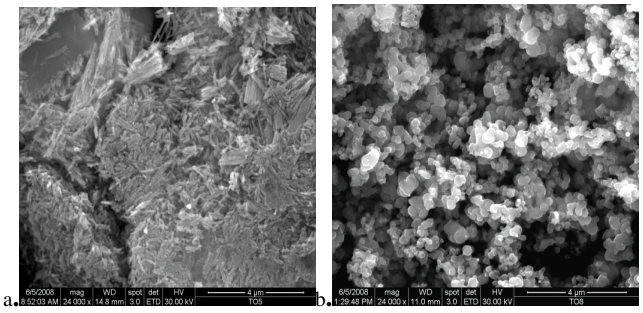

Figure 4. SEM image for TiO_2-Au-CD:a. TiO_2-Au/α-CD b. TiO_2-Au/β-CD

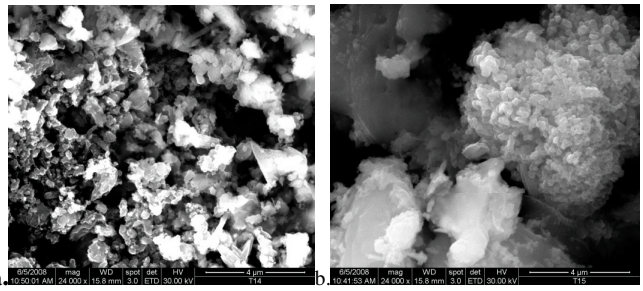

Figure 5. SEM image for TiO_2-Fe-CD: a. TiO_2-Fe/α-CD b. TiO_2-Fe/β-CD

B. Percentage of the normal and aberrant mitotic phases

The percentage of the normal cells, in all experimental variants, recorded inferior values in comparison with untreated Control. In the experimental variants, the percent

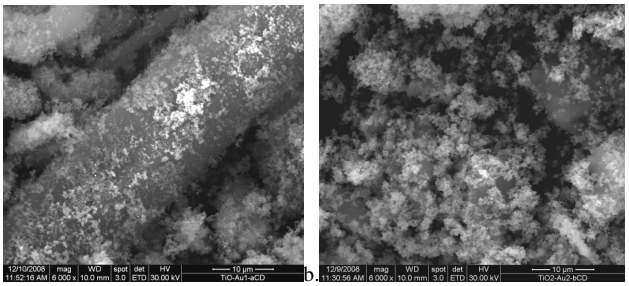

Figure 6. SEM image with TiO_2-Au nanoparticles on the: a. α-CD crystals surface b. β-CD crystals surface.

The EDX analyses of the obtained complexes with undoped/doped TiO_2 have indicated a higher concentration of Ti in the complexes, especially in the case of α-CD complexation, which can be explained by the fact that pure α-CD and its complexes are more soluble in the water system than β-CD or the corresponding complexes.

The TiO_2-Au or TiO_2–Fe nanoparticles are disposed on the surface of the α-CD or β-CD (Fig. 6). For the radicel treatment, the complex TiO_2-Me / cyclodextrin was suspended in distilled water, having as a result an aqueous solution of TiO_2-Au or TiO_2–Fe nanoparticles in α-CD or β-CD (Figs. 7, 8), without crystalline structure, easily absorbed by the cells.

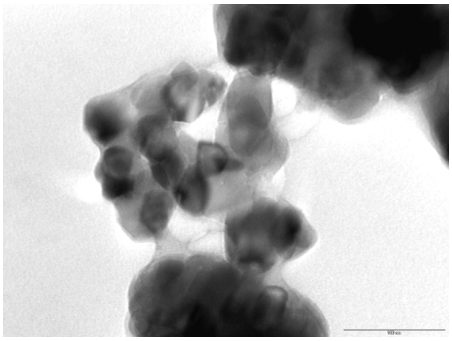

Figure 7. TEM image with a TiO_2-Au suspension in α-CD solution

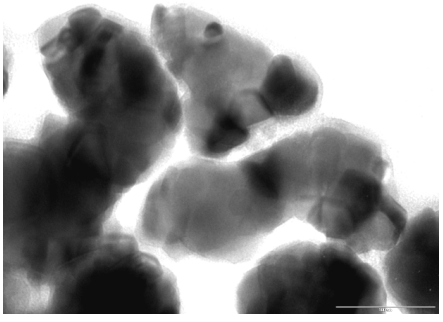

Figure 8. TEM image with a TiO_2-Au suspension in β-CD solution

of normal cells was dependent especially on the nanoparticle types (Table I). Thus, in the treatments with unconjugated TiO_2, the percentage of normal cell recorded values of about 96% (except for the variant with β-cyclodextrin in ratio 1:2),

92-94% in variants with Au-TiO_2 and around 95% in the variants with Fe-TiO_2.

The normal cell percentage in the presence of TiO_2 nanoparticles, without metals or cyclodextrin, recorded values similar to those recorded by the TiO_2 nanoparticles complexed with cyclodextrin α or β.

Because the cyclodextrin is a hydrosoluble substance and in an aqueous solution these crystals are dissolved, the shape of the cyclodextrin crystals does not influence their absorption in the plant meristem and implicitly the percentage of aberrant or normal cells in different mitotic phases, as well as the structural modifications of the chromosomes and of the mitotic spindle.

TABLE I. THE NORMAL CELL PERCENTAGE IN DIFFERENT PHASES OF THE MITOTIC CYCLE

Experimental Variant	Normal cells in mitosis (%)	Normal cell percentage in mitosis phases				
		Prophase	*Prometaphase*	*Metaphase*	*Anaphase*	*Telophase*
Control	99.40	99.80	100.00	97.90	96.70	99.40
TiO_2	96.80	97.44	100.00	96.23	95.72	94.38
TiO_2-α 1:2	96.61	91.49	100.00	100.00	96.67	97.22
TiO_2-α 1:1	96.00	100.00	100.00	97.87	92.13	91.14
TiO_2-β 1:2	98.88	99.07	100.00	100.00	97.54	97.77
TiO_2-β 1:1	96.29	98.55	100.00	96.77	94.84	94.67
TiO_2-Au-α 1:2	94.28	98.66	100.00	94.34	90.22	95.36
TiO_2-Au-α 1:1	94.06	98.72	100.00	93.24	88.73	93.51
TiO_2-Au-β 1:2	94.28	93.88	100.00	91.43	84.95	97.20
TiO_2-Au-β 1:1	92.60	100.00	100.00	92.69	90.74	92.62
TiO_2-Fe-α 1:2	95.69	96.90	100.00	94.74	93.46	98.16
TiO_2-Fe-α 1:1	95.55	100.00	100.00	93.10	86.05	98.04
TiO_2-Fe-β 1:2	95.56	96.30	100.00	91.11	98.11	96.67
TiO_2-Fe-β 1:1	95.25	100.00	100.00	92.31	92.31	92.59

C. Structural modifications of the chromosomes

As a consequence of the free radicals action, the chromosomes are broken, which leads to structural chromosome aberrations. In different phases of the mitosis, or under influence of some substances, the broken ends of the chromosomes can be reunited or not. In the absence of the reunion processes, acentric fragments, minutes, a/o result (Figs. 9, 10, 12), and after the reunion of two brocken chromosomes, mainly bridges (Fig. 11), or arch result.

The BR index (anaphasic fragment frequency/anaphasic bridge frequency), points out the possibility of a substance or another factor to reunite of the broken ends of the chrfomosomes. A low BR index, suggests a substance with role in reunion of the broken ends of the chromosomes, having a radioprotective role [14]. A high BR index suggests that a substance not favor the reunion of the broken chromosome ends, the affected cells being eliminate from tissue (Table II).

The cyclodextrin type and ratio between TiO_2: cyclodextrin influenced the reunion or not of the broken ends of the chromosomes, and implicitly the aberration type (Table II). The absence of bridges in anaphase and telophase at variants with TiO_2-α-cyclodextrin, suggests that the α-cyclodextrin does not facilitate the chromosomal reunion processes of the chromosome broken ends. Thus, in variant TiO_2-α-cyclodextrin (ratio 1:2), 66.66% acentric fragments are present in anaphase and 11.11% in telophase, while the chromosomal bridges are absent. This feature can be used for the progressive elimination from organism of the cells with a high multiplication ratio (carcinogen cells). At a ratio of 1:1 between the two components, the percentage of acentric fragments is lower (15.73, and 8.86%), the reunion process of the broken ends being also absent.

TABLE II. THE STRUCTURAL AND METABOLIC MODIFICATIONS OF THE CHROMOSOMES

Experimental variant	Prophase (%)		Metaphase (%)			Anaphase			Telophase	
	PCC	*DCC*	*DCC*	*Spindle inactivation*	*Kinetochore Inactivation*	*Fragments/ 100 cells*	*Bridges/ 100 cells*	*BR index*	*Fragments/ 100 cells*	*Bridges/ 100 cells*
Control	0.09	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TiO_2	0.12	0.80	0.00	0.00	0.00	4.88	0.00	4.88	2.64	0.00
TiO_2-α 1:2	0.00	2.33	0.00	0.00	0.00	66.66	0.00	66.66	11.11	0.00
TiO_2-α 1:1	0.00	0.00	0.00	0.00	0.00	15.73	0.00	15.73	8.86	0.00
TiO_2-β 1:2	0.00	0.00	0.35	0.00	0.00	4.10	0.00	4.10	4.44	0.00
TiO_2-β 1:1	0.72	0.72	0.00	0.00	0.00	8.39	0.65	12.91	10.06	0.59
TiO_2-Au-α 1:2	0.00	0.00	1.79	0.00	0.00	4.28	0.35	12.23	1.34	2.46
TiO_2-Au-α 1:1	0.00	0.00	0.00	0.00	0.00	4.73	0.11	43.00	3.56	0.04
TiO_2-Au-β 1:2	4.08	0.00	0.00	0.57	0.57	5.38	1.08	4.98	2.80	2.80
TiO_2-Au-β 1:1	0.00	0.00	0.00	0.46	0.46	13.89	5.56	2.49	15.93	3.28
TiO_2-Fe-α 1:2	3.10	0.00	2.11	0.35	1.05	13.08	0.93	14.07	2.75	2.75
TiO_2-Fe-α 1:1	0.00	0.00	2.30	1.15	1.15	37.21	0.00	37.21	5.88	0.00
TiO_2-Fe-β 1:2	1.85	1.85	4.44	3.33	1.11	1.92	0.00	1.92	1.67	0.00
TiO_2-Fe-β 1:1	0.00	0.00	0.00	1.92	0.24	11.54	7.69	1.49	17.28	4.94

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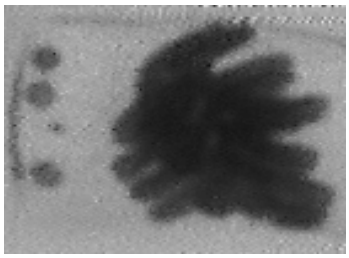


Figure 9. Acentric fragments in metaphase (TiO_2-Fe/α-CD, 1:1)

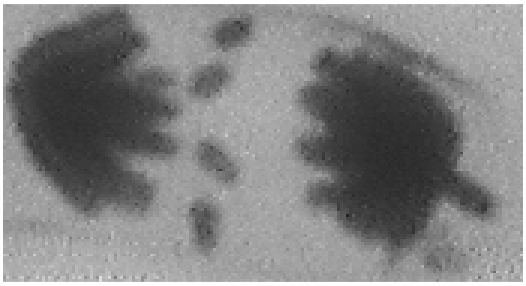


Figure 10. Acentric fragments in telophase (TiO_2-Fe/α-CD, 1:1)

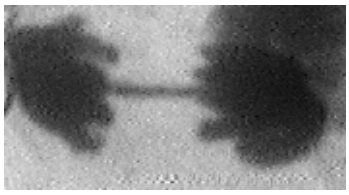


Figure 11. Bridge in anaphase (TiO_2-Fe/β-CD, 1:1)

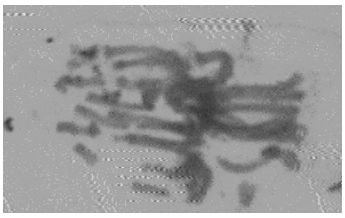


Figure 12. Kinetochore inactivation and chromosome break (TiO_2-Au/β-CD, 1:1)

The β-cyclodextrin presence induces a very low percent of reunion of the broken ends, only at a ratio of 1:1.

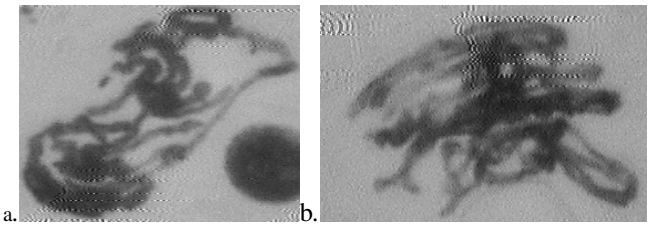
Complexed with cyclodextrin, the Fe-TiO_2 manifested the inhibition of the reunion process of the chromosomes' broken ends (the absence of the bridges or their presence in a lower percentage), the efficacy being dependent on the cyclodextrin type and the ratio TiO_2 : cyclodextrin.

The nanoparticles conjugated with gold or iron, independent of the cyclodextrin type presence and of the ratio TiO_2 : cyclodextrin, induced in a small percentage (0.57%-1.92%) the inactivation of the mitotic spindle, as well as the kinetochore inactivation (0.46-1.15%; Fig. 12).

The most stable phase from the cellular cycle was prometaphase, and the most sensitive one was anaphase, independent on the experimental variant (Table I). Although in the literature there are numerous papers about the environmental factors' effect at the chromosome level, regarding the nanoparticles' effect at the chromosome level the information is poor. The investigation performed at the Chinese hamster ovary, with TiO_2 particles, in the presence or absence of the UV light, evidenced that these not enhance the chromosomal aberrations' frequency [10]. Similar results were obtained by other authors, in the genotoxicity tests performed in Chinese hamster ovary, as well as in experiments on aquatic organisms (*Daphnia magna*, *Onchorhynchus mykiss* and green algae *Pseudokirchneriella subcapitata*), acutely exposed to the ultrafine TiO_2 particle-types [11], or in experiments performed in fish (*Nothobranchius rachovi*), at which the mitotic index (the metaphase number in 100 microscopic fields) was analyzed in different organs and experimental variants [12], [13]. The recorded values were dependent on the organ, being bigger in animals treated with TiO_2, in comparison with untreated Control. Other authors [15] analyzed the percentage of chromosomal aberrations induced by three commercial TiO_2 types in isolated human lymphocyte cultures. The percentage of chromosomal aberrations depended on the treatment type, the features of the used product, a/o. The anatase crystallization form induced a significant increase of the total chromosomal number, but without establishing a dose – response relation. The main types of recorded chromosomal aberrations were not presented.

D. Metabolic modifications of the chromosomes

The metabolic modifications of the chromosomes affected especially the condensation degree of the chromatin fibre and the synchronisation of this process, and others (Table II). Their presence reveals the action of a stress or an experimental factor which makes the interphase cells enter into mitosis abruptly.



a. b.

Figure 13. a. PCC in prophase (TiO_2-Fe/β-CD, 1:2); b. DCC in metaphase (TiO_2-β-CD, 1:1)

The main metabolic modifications are premature chromosome condensation (PCC; Fig. 13a.), or delay in chromosome condensation (DCC; Figs. 13b, 14), as well as gaps (the uncoloured regions on the chromosome length), differences in condensation degree of the chromatin fibers in eu- and heterochromatin regions of the chromosomes, resulting a banding aspect of the chromosomes (met especially in metaphase), the parallel disposition of the chromatin fibers in prophase, a/o. PCC and DCC were met in a low percentage (0.72-4.08%), especially in variants treated with β-cyclodextrin, independent of the presence or absence of the chelated metal (gold or iron).

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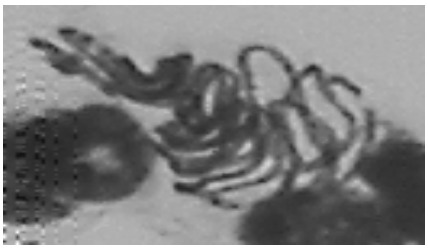

Figure 14. DCC in metaphase (TiO_2-β-CD, 1:1)

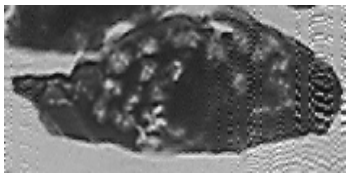

Figure 15. Lyses areas in interphase nucleus (TiO_2-Au/β-CD, 1:1)

The presence of some lyses areas in nucleus in different percentage in almost all treated variants (Fig. 15) underline the presence of an exobiotic factor in the cell.

IV. CONCLUSIONS

The undoped and doped TiO_2 nanocrystals used in this experiment presented the *anatase* form crystallization, the TiO_2-Au and TiO_2-Fe nanospheres presented dimensions ranging between 10-20 nm.

The TiO_2-Me nanoparticles, complexed or not with α- or β-cyclodextrin, presented a different morphology, dependent on the doped metal and cyclodextrin type. The TiO_2-Me nanocrystals are present at the surface of cyclodextrin crystals.

In an aqueous solution, cyclodextrin crystals are dissolved and TiO_2-Me are suspended in a cyclodextrin solution, being thus absorbed in the meristem cells.

Doped or undoped TiO_2 nanocrystals, complexed with α- or β-cyclodextrin, induced both metabolic and structural modifications at the chromosome and mitotic spindle level, depending on the chelated metal, the cyclodextrin type and the ratio TiO_2: cyclodextrin (1:2 or 1:1).

As the cyclodextrin is a hydrosoluble substance, the different form of crystals of the complexed TiO_2-Me-cyclodextrin, did not influence the percentage of aberrant or normal cells in different mitotic phases, as well as the structural or metabolic modifications of the chromosomes and of the nuclear spindle.

The cyclodextrin type and ratio between TiO_2: cyclodextrin influenced both the reunion process of the chromosome broken ends, and the chromosome aberration type.

The α-cyclodextrin induced a high acentric fragment percentage in anaphase and telophase, and did not facilitate the reunion processes of the chromosome broken ends (the absence of bridges from cells). For this reason the variant TiO_2~α-cyclodextrin (ratio 1:2), can be used for the progressive elimination, from organism, of the cells with a high mitotic multiplication rate (carcinogen cells).

The TiO_2 conjugated or not with a metal (gold or iron), complexed or not with a cyclodextrin, induced, in a low percentage, the metabolic modification of the chromosomes, their compaction degree in prophase and metaphase being affected (**PCC** and **DCC**), as well as the inactivation of the kinetochore or mitotic spindle.

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