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The Physical and Biological Properties of NanoTiO$_2$ Material

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ABSTRACT

The physical and biological properties of TiO$_2$ materials including NanoTiO2, micrometerTiO$_2$ and NanoTiO$_2$ tubes have been studied using scanning electron and infrared spectrometer, X-ray diffraction instrument as well as 3-(4,5- *dimethylthiazol* 2-*yl*)-2,5 *diphenyltetrazo-lium bromide* (*MTT*) *colorimetric method, respectively. These materials are prepared by chemical deposition and anode oxidation methods, respectively. The sizes of NanoTiO2 are* 80 *nm and* 1000 *nm, respectively, their infrared properties of absorption are different, the characteristic peaks of the former are* 1271, 1615, 2957 *and* 3422 *cm*$^{-1}$, *the latter are* 1645 *and* 2356 *cm*$^{-1}$. *The NanoTiO2 tubes can be formed by anode oxidation method, its diameters are between* 50 - 100 *nm, different NanoTiO$_2$ tubes contain different components of oxygen and titanium. In MTT experiment we discover the changes of properties of proliferation of the liver and chick embryo fibroblast cells under influences of NanoTiO$_2$ relative to those of the controlled groups, when small NanoTiO$_2$ suspension is added in these cultivated liquids of cell, but the influence of NanoTiO$_2$ on the proliferation of the person's liver cell is still very small, therefore, the toxicities of NanoTiO2 containing* 80 *nm and* 1000 *nm to these cells are still first score.*

Keywords: *NanoTiO$_2$, Micrometer TiO$_2$, NanTiO$_2$ Tube, Infrared and X-Diffraction Spectrum, MTT Method, Cell, Biological and Physical Property*

1. Introduction

At present, the Nanomaterials and Nanotechnology and Nanoinstrument have been greatly developed and applied extensively in the industry, agriculture, science and medicine as well as our living. A lot of Nanomaterials have been made in bulk in factory and were sold in markets. In such a case it is very necessary to know whether these Nanomaterials are safe, or speaking, have toxicity to the person's and animal's health [1-4]. This problem attracts scientist's and government' attentions in the world. Thus studying the safety or toxicity of the Nanomaterials has important significances in sciences and applications. In this paper we will study the physical and biological properties of NanoTiO$_2$.

TiO$_2$ is a kind of crystal, in which one titanium (Ti) atom combines with six oxygen (O) atoms, one oxygen atom links again three titanium atoms to form an octagon. Thus, TiO$_2$ belongs in the inclined crystalline system and is a semiconductor material. In this structure of crystal the width of forbidden band between the valence band and conductive band is between 3.0 eV - 3.2 eV. Therefore, TiO$_2$ can easily absorb the ultraviolet light with wavelength of 387 nm and414nm. Just so, single crystal TiO$_2$ can decompose water and other substances under action of the light. Hence TiO$_2$ is a good material of photocatalytic materials. Its photocatalytic theorem can be described as follows. Under action of incident light the electrons in the valence band transit into the conductive band (e^-), then the holes (h+) occur in valence band in TiO$_2$. This reaction is represented by $TiO_2 + h\nu \rightarrow TiO_2\left(e^-, h^+\right)$. These electrons and holes occurred are shifted on the surface of TiO$_2$ under action of an electric-field and can interact further with other materials through the following oxidation and reduction reactions:

$$h^+ + H_2 \rightarrow OH + H^+$$

$$h^+ + OH^- \rightarrow OH, \quad O_2 + e^- \rightarrow O_2^-$$

$$O_2 + H_2O \rightarrow OOOH + OH^-, \quad 2\cdot OOOH \rightarrow O_2 + H_2O_2,$$

$$OOOH + H_2O + e^- \rightarrow H_2O_2 + OH^-,$$

$$H_2O_2 + e^- \rightarrow OH + OH^-,.$$

In these processes a great number of free radicals of OH^- , O_2^- and OOOH are generated, which have strong ability of oxidation and can interact and react with plenty of materials, such as chloroform, PCBS, organic compounds, formaldehyde. Therefore, the NanoTiO_2 have extensively applications in medicine, agriculture and industry, containing chemical engineering, paint, paper, plastic, rubber, chemical fiber, electric-appliances, cosmetics and food packaging [4-7].

For the NanoTiO_2, which is different from that of bulk TiO_2due to its scale to become very small, such as, it has larger rate of surface area versus volume about 70 m^2/g, its surface energy raises, the ratio of number of atom between the surface and interior increases, the number of the coordination of atoms in it is lowered, when compared with that in bulk [3-4]. Thus the physical and chemical activities as well as unstablities of NanoTiO_2 are increased correspondingly. Meanwhile, it is also quite difficultly resolved in water, but when a lot of NanoTiO_2 are collected together, they can combines and concretes each other to form gluey state in water. These gluey states of the NanoTiO_2 are easily spitted, if some electrolytes are added into this solution, or the PH value of solution is changed. Meanwhile, it can also form some new bases or chemical compound, such as, TiOH, K_2O-6 TiO_2 or $K_2Ti_6O_{13}$, etc., with water molecules or ions, involving OH, NH_2, COOH, C = O, through attraction interaction between the particles with charges on the surface. Therefore, it is very necessary to investigate in-depth the physical and biological properties of NanoTiO_2.

2. Experimental Method

We prepare three kinds of TiO_2 materials containing the NanoscaleTiO_2, microscale TiO_2 and NanoTiO_2 tubes by using chemical deposition and anode oxidation methods, respectively. Their features of structures are measured using Scanning Electron Spectrometer (SEM), respectively, its infrared properties of absorption and X-diffraction spectra are measured using a Nicolet Nexus 670-FT-IR spectrometer with resolution of 4 cm^{-1} and X-ray diffraction spectrometer, respectively. The proliferation and toxicity of NanoTiO_2 to the person's liver and chick embryo fibroblast (CEF) cells are determined by MTT colorimetric method [8].

So-called MTT colorimetric method [8] is just a very effective way checking the states of activity and proliferation of the cells. In this way the coloration substance used is MTT. The MTT is an abbreviation of 3-(4, 5-dimethylthiazol 2-yl)-2, 5 diphenyltetrazolium bromide, which is a sort of dye accepting hydrogen atom. In the mitochondrion of cell, the externally applied yellow tetrazolium salt 3-(4, 5-dimethylthiazol 2-yl)-2, 5 diphenyl-tetrazolium bromide (MTT, Amersco) will be reduced and become further, under the action of dehydrogenase of amber acid, as a blue insoluble formazan form, which is eventually deposited in the cell after this reaction. But the dead cell has not this function and effect. The dimethylsulfoxide (DMSO) added can resolve the blue insoluble formazan. The quantity of the blue insoluble formazan produced after the resolution is proportional with the number of cell participated in this process. Thus we can determine indirectly the number of the cells through measuring the strength of absorption of the light with determinate wavelengths in this case. The strength of light can be measured and collected by enzymic immunoassay instrument and spectrophotometer. Then we can determine the number of proliferation and activity of cells or of biological factors in the cells, thus we can assess the safety or toxicity of NanoTiO_2 to the cell, etc., according to the toxicology. The advantages of the method measuring this security or toxicity is fast and accurate, and have higher sensitivity and very good repeatability. Therefore, we here utilize this method to assess the influences of NanoTiO_2 on the proliferation states of person's liver cell (L-20) after it interacts with the NanoTiO_2 by traditional biological technique. The experimental process in this method is as follows.

2.1. Cell Growth

The person's liver and primary chick embryo fibroblast (CEF) cells are prepared, their secondary cultures are grown in the 5% CO_2 enriched incubator with temperature of 37˚C. The person's liver and CEF cells were grown in RPMI1 640(Hyclone,American) supplemented with 5% fetal calf serum (FCS, Biological Industries, BaiAn, China). Microscopic inspection to them verifies that the cells are not contaminated from third passage, and so forth.

2.2. The Nanotio$_2$ is Added into the Group Solutions

In our experiments, 4×10^5 cells per well are seeded in 60-well micro-culture plates and allowed to continually grow. The 60-well cells are added into the 100 μL/well foster liquids containing the fetal calf serum in which the 1 mL/250 mL insulin liquid is included. The NanoTiO_2s are added into these wells to study the influences of the NanoTiO_2 on the proliferation behavior of the person's liver and CEF cells. In this experiment these cells are separated as controlled and experimental groups, which are all 30 well. The NanoTiO_2s are assigned in the following rule. (a) the first or controlled group is 30 wells, which the 15 mL/ well foster liquid without the fetal calf serum is added into; (b) the second or experiment group 1 has 10 wells, in which the 5 μL NanoTiO_2 suspension

and 10 μL foster liquid without the fetal calf serum are added, the concentration of NanoTiO$_2$ achieves 43 μg/mL; (c) the third or experiment group 2 contains 10 wells, in which there is 10 μL NanoTiO$_2$ suspension and 5uL foster without fetal calf serum, the concentration of the NanoTiO$_2$ achieves 86 ug/mL; (d) the fourth or experiment group 3 contains 10 wells, in which 15 uL NanoTiO$_2$ suspension is added, the concentration of the NanoTiO$_2$ achieves 129 μg/mL. The above four groups are all placed into the CO_2 enriched incubator with 37˚C to develop about 24 hours. The cell proliferation was evaluated after 24 h.

2.3. Measurement of the Proliferation of the Person's Liver Cell

We observe and measure the proliferation of the person's liver cell (L-20) in above conditions by the MTT method [8-10] and calculate the proliferation rate of cell by using these experimental data.. In the calculation we should firstly measure the increased values of mitochondrial dehydrogenase activity as the number of cells increases. In such a case, 100 uL/ well MTT solution is prepared in PBS (5 mg/mL) and further diluted (10%v/v) in RPMI 1640. The cell growth medium is aspirated. In this case the 100 μL of MTT solution are added into each well in the above four groups. The cells are then further incubated for 4 h at 37˚C Excess of MTT solution is removed, after this the 100 μL/well DMSO is added into dissolve the blue crystals formed in the cells. The absorption strength of the DMSO solution and values of optical density (OD) of each well to the incident light with wavelength of 570 nm for the controlled and experimental groups are measured spectrophotometrically by DG3022 enzymicimmunoassay instrument, respectively. Finally we can find out the cell proliferation rate (CPR) for the person's liver cell by using the experimental data and the formula: CPR = $[(F_{exp} - F_{con})/F_{con}] \times 100\%$, here F_{exp} is the value of optical density of experimental group, F_{con} is the value of optical density of controlled group.

3. Experimental Results and Discussion

3.1. Properties of Microscale TiO$_2$ and NanoTiO$_2$ Tubes

The structures of two kind of TiO$_2$ using SEM are shown in **Figure 1(a)** and **(b)**, respectively. This figure shows that the NanoTiO$_2$ is 80nm, other is 1000nm. We collect their spectra of infrared absorption by 670 FT-IR instrument, which is shown in **Figure 2**. We see from this figure that their infrared absorptions are different not only the strengths and frequencies of peaks of absorption but also the amounts of peaks, the new peaks at 2422, 2956 and 1271 cm^{-1} occur in the NanoTiO$_2$, they are the characteristic peaks of NanoTiO$_2$. However, in micrometer TiO$_2$ there is only a small new peak at 2355 cm^{-1}. Only the peak at 1645 cm^{-1} is same for The NanoTiO$_2$ and

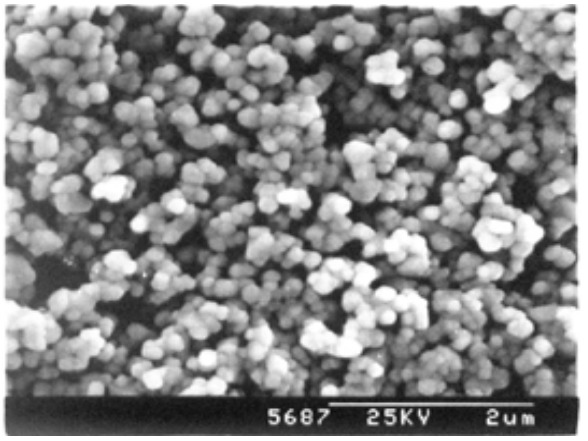

Figure 1. The images of SEM of TiO$_2$ powder. (a)The image of NanoTiO$_2$ powder; (b) The image of micrometerTiO$_2$ powder.

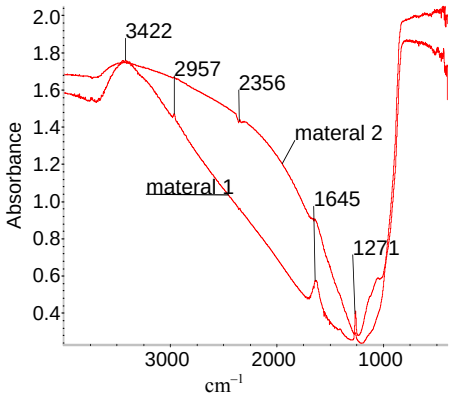

Figure 2. The spectra of infrared absorption for NanoTiO$_2$ (material1) and micro TiO$_2$ (material 2).

micrometerTiO$_2$. Obviously, this is due to the changes of structure and sizes of molecules of TiO$_2$, the new peaks in **Figure 2** indicate that there are many new radicals or base groups in NanoTiO$_2$. This is just the feature of Nanomaterials.

We also use SEM to measure the feature and size of NanoTiO$_2$ tubes formed by anode oxidation method, which are shown in **Figures 3-4**, which are born from the surface of titanium alloy in the solutions of hydrofluoric acid as well as phosphoric acid and hydrofluoric acid, respectively. From these figures we see clearly the occurrences of a great number of Nanotubes, which are distributed densely and nonuniformly on the surface, their sizes of diameter are different and between 50 nm and 100 nm.

In the meanwhile, we measure the X-diffraction spectrum of NanoTiO$_2$ tubes using X-ray diffraction instrument. These results for different components of oxygen and titanium are shown in **Figure 5**, where **Figure 5a** is the result obtained from solution of phosphoric acid and hydrofluoric acid in which the weight ratio of oxygen and titanium are 17.54% and 82.46%, respectively, their volume ratios are 38.91% and 61.09%, respectively, **Figure 5b** is the result obtained from solution of hydrofluoric acid in which the weight ratio of oxygen and titanium are 24.22% and 75.78%, respectively, their volume ratios are 48.90% and 51.10%, respectively. These results manifest that not only different NanoTiO$_2$ tubes can be obtained by different conditions in the anode oxidation but also the products of NanoTiO$_2$ tubes manufactured by this method are very pure and contain not impurities.

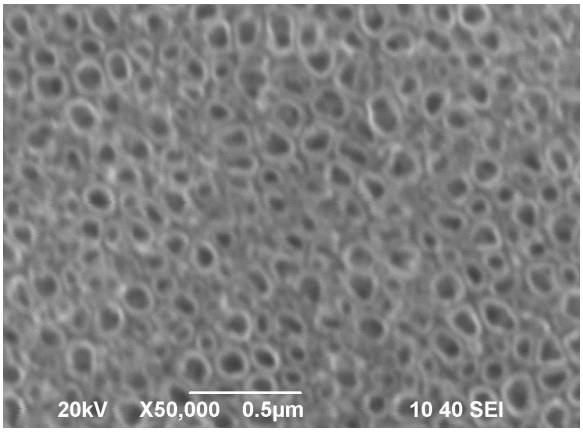

Figure 3. The images of SEM of NanoTiO$_2$ tube formed in the solution of hydrofluoric acid.

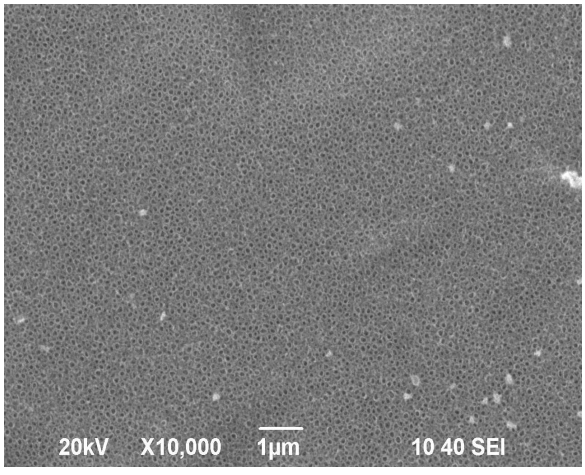

Figure 4. The images of SEM of NanoTiO$_2$ tube formed in the solution of phosphoric acid and hydrofluoric acid.

On the other hand, we determine the solubility of the NanoTiO$_2$ in some liquids . Experiments show that the NanoTiO$_2$ is insoluble in the unorganic (as D-Han'k liquid) and organic (as DMSO) solutions and alcohols (95%) and t1 640 foster liquid without fetal calf serum, etc.

3.2. The Biological Properties of the NanoTiO$_2$

We measured the values of optical density (OD) in MTT experiment of liver cell and changes of proliferation of CEF by MTT method [8].

a) The OD values for the controlled group (30 wells) obtained are as follows,

0.37, 0.31, 0.41, 0.46, 0.54, 0.45, 0.44, 0.45, 0.42, 0.43, 0.50, 0.43, 0.51, 0.54, 0.41, 0.39, 0.52, 0.47, 0.46, o.31, 0.54, 0.38, 0.42, 0.47, 0.34, 0.41, 0.42, 0.44, 0.46.

Therefore, the average value of the OD of the controlled group is 0.43 + 0.05

b) The OD values for the experimental groups are as follows. The OD values of the experimental group 1 (10 wells) are 0.41, 0.44, 0.41, 0.62, 0.35, 0.42, 0.77, 0.49, 0.54, 0.45. Thus, its average value of OD is 0.49 + 0.09, its PRC value is +13.95%.

The OD values of the experimental group 2 (10 wells) are 0.44, 0.59, 0.40, 0.56, 0.40, 0.46, 0.32, 0.20, 0.43, 0.35. Then its average value of OD is 0.42 + 0.08, its PRC value is –2.32%.

The OD values of the experimental group 3 (10 wells) are 0.45, 0.44, 0.42, 0.46, 0.46, 0.40, 0.45, 0.58, 0.35, 0.32.Then its average value of OD is 0.43 + 0.05, its PRC value is 0.0%.

3.3. Discussion of the Results

From above results we can find out the average value of OD for the experimental group which is 0.45 + 0.07, its CPR value is +0.02%. This shows that the NanoTiO$_2$ in fluences not basically the proliferation of the person's liver cell (L-20).

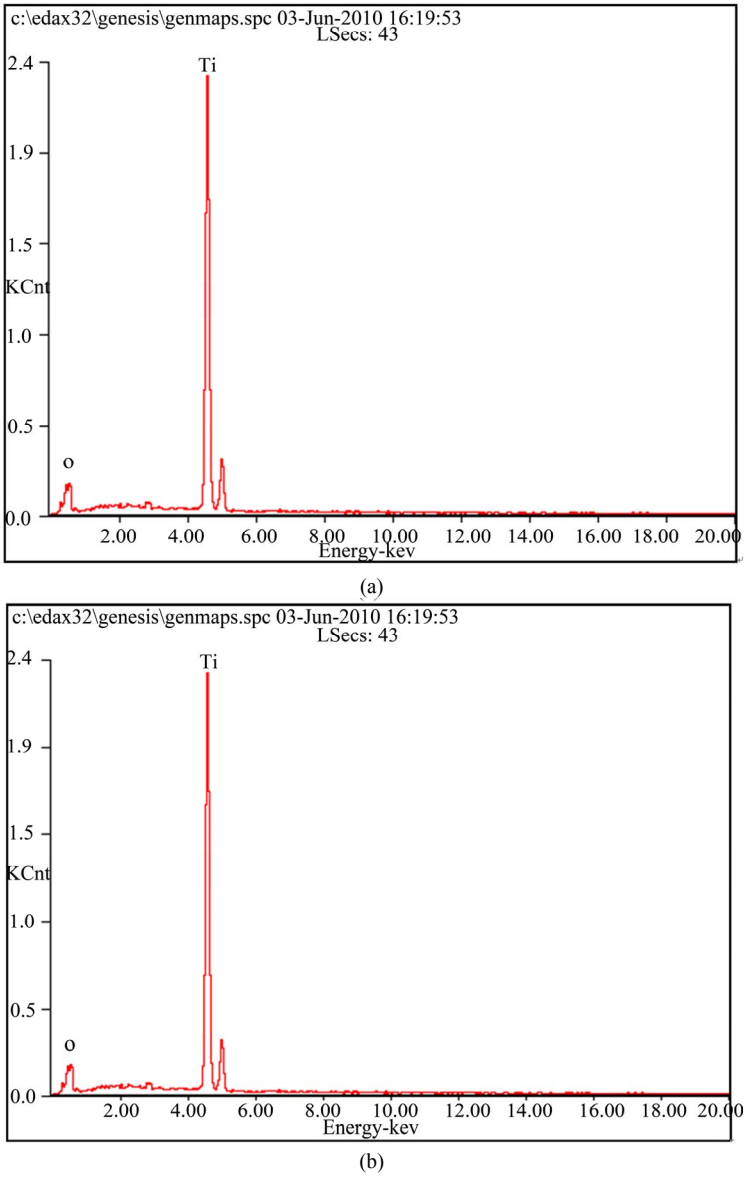

Figure 5. X-diffraction spectra of NanoTiO_2 tubes with different components of oxygen and titanium in different conditions.

In order to verify above result we further study and measure the changes of the proliferation behavior of the chick embryo fibroblast (CEF) cell by above MTT method [8-10] , when the NanoTiO_2 of 80 nm and the TiO_2 of 1000 nm are added into these cells, respectively. We obtain their OD values and find correspondingly out the relative cell proliferation rate (RCPR) of chick embryo fibroblast by the formula, $(F_{exp}/F_{con}) \times 100\%$. The OD values and corresponding RCPR values and evaluation of their toxicity scores in the cases of interaction times of 24 hours and 48 hours are shown in **table 1** and **2** for the six samples in the two groups, respectively, where 100% group denotes this case in which the NanoTiO_2 samples are not added into the water, the 50% group denotes that the 50% water is added into the sample, 25% group denotes that 75% water is added into the sample. From the table 1 and 2 we see that the degrees of toxicity for the chick embryo fibroblast cell are all first score. This shows that the toxicities of both the NanoTiO_2 and TiO_2 of 1000 nm to the cells are all lower. Therefore, the NanoTiO_2 is safe in the living systems.

4. Conclusions

In this paper we investigated the physical and biological properties of TiO_2 materials including NanoTiO_2, micrometerTiO_2 and NanoTiO_2 tubes using scanning electron and infrared spectrometer and X-ray diffraction instrument as well as MTT colorimetric method, respectively.

Table 1. The OD values, Relative cell proliferation Rate (RCPR) and toxicity Score of CEF cell after 24 hours.

Groups	24hours		
groups	OD value(X+S)	RCPR (%)	Score
100% T1	0.298±0.006,	98.3	1
50% T1	0.298±0.006,	98.8	1
25% T1	0.2995±0.007	99	1
100% T2	0.276±0.007	91.4	1
50% T2	0.286±0.003	94.7	1
25% T2	0.294±0.009	97.4	1
Control group	0.302±0.009	100	0

Table 2. The OD values, Relative cell proliferation Rate (RCPR) and toxicity Score of CEF cell after 48 hours.

Groups	48hours		
groups	OD value(X+S)	RCPR (%)	Score
100% T1	0.309±0.01	90.6	1
50% T1	0.320±0.003	93.8	1
25% T1	0.327±0.008	95.9	1
100% T2	0.307±0.007	90.0	1
50% T2	0.319±0.003	93.5	1
25% T2	0.325±0.008	995.3	1
Control gronp	0.341±0.009	100	0

These materials used are prepared by chemical deposition and anode oxidation methods, respectively. The sizes of NanoTiO_2 are checked using scanning electron spectrometer and 80nm and 1000 nm, respectively, diameters of the NanoTiO_2 tubes are between 50 - 100 nm. The infrared properties of absorption for the NanoTiO_2 and micrometerTiO_2 are different, the characteristic peaks of the former are 1271 cm^{-1}, 1615 cm^{-1}, 2957 cm^{-1} and 3422 cm^{-1}, the latter are 1645 cm^{-1} and 2356 cm^{-1}. Meanwhile, we can obtain different components of oxygen and titanium in the NanoTiO_2 tubes in different conditions by the anode oxidation method. In MTT experiment we discover the changes of properties of proliferation of the liver and chick embryo fibroblast cells under influences of NanoTiO_2 relative to those of the controlled groups, when small NanoTiO_2 suspension is added in these cultivated liquids of cell, but the influence of NanoTiO_2 on the proliferation of the person's liver cell is still very small, therefore, the toxicities of NanoTiO_2 containing 80 nm and 1000 nm to these cells are still first score.

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