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Preparation and Characterization of Hybrid Biomaterial based on PANI / $CaCO_3$ Nanoparticles for Photo Thermal Therapy of HeLa Cells Cancer.

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ABSTRACT

Conducting polyaniline (PANI) absorbs mild energy and transforms it into localized warmth to produce cell loss of life while encapsulation with inorganic materials. Amongst inorganic substances, $CaCO_3$ micro debris show case a high encapsulation efficiency and solubility in acidic media. Hybrid biomaterial turned into prepared based totally on $CaCO_3$-PANI-Cys nanoparticles (NP) for photo thermal therapy (PTT). The hybrid nanomaterial turned into synthesized through $CaCO_3$ and carboxymethyl cellulose in a chemical way. The characteristics have been examined using Ultraviolet spectrophotometer, FTIR and Scanning electron microscope. In-vitro anticancer activity of each compound in the direction of Hela cells strains was done using MTT assay in dark and irradiated conditions. Hybrid $CaCO_3$-PANI-Cys NP show low toxicity to most cancers cells in dark and increase with NIR laser. The consequences of this have a look at advice that hybrid $CaCO_3$-PANI-Cys NP with NIR laser can be used for huge medical packages and offer new drug recompense a chemotherapy drug.

Keywords: Hela Cancer; Photo thermal; Nanoparticles; PANI/$CaCO_3$

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INTRODUCTION

Cancer is the second leading cause of death that accounts for more than 25% of the deaths [1]. Currently, conventional cancer therapies including surgical excision, medical therapies such as chemotherapy and radiotherapy, and combination methods have their inherent drawbacks. Surgical excision usually fails to remove all cancerous cells resulting in serious morbidity [2]. In addition, surgery is limited to large numbers of tumors which are adjacent to critical tissue structures. Furthermore, the severe side effects of chemotherapy and radiotherapy make the patient lots of sufferings [3]. Because of the excessive specificity, low side outcomes, and exceptional efficacy, photo thermal therapy used mild power to burn for most cancers has been proposed as an appealing opportunity to conventional most cancers remedies [4]. Photo thermal interactions result from mild power conversion to warmth inside the tissue, probably supplying the sustained improved temperatures required for hyperthermia treatment options. Photo thermal harm is characterized by way of mitochondrial swelling, protein denaturation, and lack of birefringence, edema, whitening and tissue necrosis [5].

Tissue modifications are obvious inside min whilst temperatures attain 55–95°C. Conjugated polymers had been extensively explored by using many unique companies in biomedicine, displaying extremely good promise in packages which includes fluorescence imaging in addition to photodynamic remedy. Currently, they have exposed that many NIR-soaking up natural nanomaterial, consisting of some of conjugated polymers, with great picture balance, biodegradability or proper biocompatibility, and excessive photo thermal conversion performance, can function promising photo thermal marketers for most cancers remedy. Furthermore, the ones conjugated polymers may also function multifunctional drug providers for potential packages in mixture most cancers remedy [6, 7].

Polyaniline (PANI) a representatives from the family of conducting polymers. PANI is distinguished by easy synthesis and high environmental stability. Polyaniline consists of monomer units built from reduced (*y*) benzoid and oxidized (1-*y*) quinoid blocks as shown in Figure (1) [8].

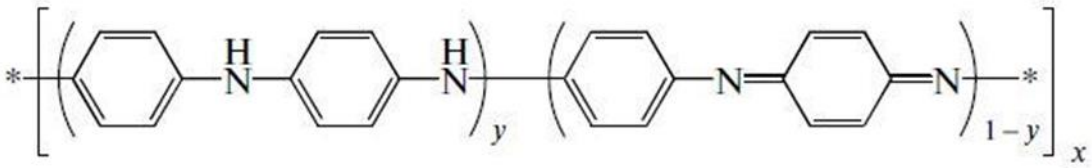

Fig 1: The structure of the polyaniline chain [8].

The polymer spine shape is conjugated (alternating unpaired and double bonds), is one of the maximum studied conjugated accomplishing polymers because of its exciting residences including environmental stability, appropriate electric conductivity, low price, and smooth chemical and electrochemical synthesis [9].

There are many works dealing with the biocompatibility of PANI; however, there is little information about the modified PANI to improve biocompatibility. For this reason, we present here an easy method that involves the modification of the polymer using cysteine amino acid. Calcium carbonate ($CaCO_3$) has been extensively used inside the improvement of hybrid natural-inorganic substances. Commonly because of the residences $CaCO_3$ of compound: biocompatibility, massive precise vicinity, hierarchical shape, and mesoporosity [10]. This inorganic fabric can adsorb or encapsulate compounds and supply them in regions within acidic pH, that's located in stable new plastic tissues [11]. Bioinorganic substances own key residences for drug transport, including a massive surface area and nano porous structure, which allow loading ofiCaCO3microparticlesiwith numerous pills. This precise assets of sure nanomaterial has been exploited to kill most cancers cells [12]. The hybrid substances used to synthesize superior micro debris should comprise severe traits, together with a specific length and uniform form; suitable solubility in an aqueous dispersion or solution, an excitation wavelength out to be among 650 nm and 950 nm to keep away from adverse the encompassing tissues, excessive photo balance, and non-cytotoxic outcomes [13].

This study aimed to generate biocompatible, hierarchical hybrid nanomaterial, composed of PANI modified with l-cysteine and CaCO3, that is capable of incorporate the lively photo thermal nanomaterial and then investigated its activity on cancer cell lines.

EXPERIMENTAL SECTION

Materials

Aniline (C6H5.NH2) purity 98% from (HOPKIN&WILLIAMS) was distilled, Ammonium persulphate $(NH4)_2S_2O_8$ purity98% from (HIMEDIA-India) was used without further purification, L-Cysteine hydrochloride monohydrate ($C_3H_7NO_2S.HCL.H_2O$) purity98% from (HIMEDIA-India), carboxymethyl cellulose, Calcium chloride dehydrate ($CaCl_2$) (from BDH- England) was used without any purification , Sodium carbonate monohydrate (Na_2CO_3), NH4OH, and Dimethyl sulfoxide (DMSO) $(CH_3)_2SO$ purity 99.6% (BDH-England). All aqueous solutions were prepared using distill water.

Synthesis of PANI/$CaCO_3$ Hybrids Materials

Synthesis of PANI Nanoparticles

The synthesis of PANI nanoparticle done based on the Neira-Carrillo, etal[14] with some modification. To prepare the PANI nanoparticles, in the beginning, aniline is distilled. after which 7immol of aniline had been combined with 20imL of distilled water and saved underneath a magnetic bar stirrer for 30 min. Then, the combination turned into cooled to zero ◦C. An aqueous solution of ammonium peroxy disulfate 7mmol turned into dissolved in 80imL of freshly distilled water and brought to the aniline solution. The reaction combination stirred for 1 h to finish the polymerization. the consequent PANI was accumulated on a filter out paper 0.2 µm and washed with distilled water, and dried underneath a vacuum for 1 day. The PANI rinsed withi0.1 M NH_4OH solution after which accumulated and dried as defined within the preceding sentence to achieve emeraldine base, the deprotonated shape of PANI.

Synthesis of PANI-Cys

The polymer functionalized by way of nucleophile addition. The polymer was immersed right into a stirredi1 M aqueous solution of L-cysteine for twenty-four h at room temperature, after which filtered with 0.45µm filter out, washed with distill water then dried, and saved at room temperature. the solution A turned into synthesized primarily based ion $CaCO_3$ and carboxymethylcellulose CMC additives by way of blending 100mL of aqueous 0.025M $CaCl_2$ with 2 mL of CMC 5% W/V underneath a magnetic stirrer for 15imin. The solution B turned into organized by way of dissolving Na_2CO_3 in 100imL of aqueous approach to achieve ai0.025 M solution.

Synthesis of $CaCO_3$-PANI-Cys Hybrids Materials

PANI-Cys combined withi100 mL of solution B underneath magnetic stirrer till a homogenous suspension acquired. This suspension (solution B+PANI-Cys) turned into delivered to 100mL of solution A underneath ultra-sonication at 80 W for 10 min. $CaCO_3$-PANI-Cys substances had been filtered with 0.45iµm filter out and washed with distilled water.

Characterization

Fourieritransforminfra-red (FTIR) spectra were measured 8000 series (400 – 4000 cm^{-1}), in Department of Chemistry, College of Science, University of Baghdad. The FTIR was used to analyze the characterization of the PANI, $CaCO_3$ and $CaCO_3$-PANI-Cys in KBr pellets. UV-vis spectra were measured by using Shimadzu spectrophotometer UV 200-1100nm. UV-vis spectrometer was absorption spectra of the sample at wave length rang 200-1100 nm. From UV-Vis technique, we can estimate the different electronic transitions, which are associated to characteristic wavelengths (λ). The absorption was used to determine the optical energy gap and optical constant. Finally, SEM (GENEX, USA) analyses were conducted to determine the particle size and morphology.

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Cytotoxicity of Hybrid $CaCO_3$-PANI-Cys

To determine the cytotoxic effect, the MTT celli viability assay was conducted oni96-well plates[15]. Cell lines had been seeded at 1 × 104cells/well. After 24 hour a confluent monolayer achieved, cells have been treated with examined compound. Cellular viability was measured after 72 hrs. of remedy by way of casting off the medium, followed with addition 28 µL of 2 mg/mL solution of MTT and incubating the cells for 1.5 h at 37 °C. After putting off the MTT solution, the crystals ultimate inside the wells had been solubilized through the addition of one hundred thirty µL of DMSO accompanied via 37 °C incubation for 15 min with shaking. The absorbency determined on a micro plate reader at 492 nm (test wavelength); the assay accomplished in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:-

Inhibition rate = A-B/A

Where A and B are the optical density of control and the optical density of test,

Photo Thermal Therapy in Vitro

The laser specifications used in the experiment were determined at the laboratories of the laser Institute at the University of Baghdad. The presence of the laser was detected using a detector card and determining its power at a height of half a meter using photometer THOR LABS. As the previous assay, the cancer cells line was seeded on 96-well plates with density1 × 10^4 mL^{-1}. Then cells were incubated with CaCO3-PANI-Cys hybrid for 1 hrs. The treated cells with tested compound were irradiated using NIR wave length 805nm irradiation with a light fluency almost of 568mW/cm^2 for 10 and 30 min. furthermore, non-treated cancers cells were irradiated with the laser.

RESULTS AND DISCUSSION

Characterization

FTIR spectroscopy was used to determine the change in the chemical structure of pristine polymer after conjugated with CaCo3 and cysteine, The FTIR results of pristine polymer is shown in Figure(2).The PANI spectrum displayed all feature polyaniline absorption bands: 1557 cm^{-1}(assigned to –C=N- stretching vibration of quinonimine) and 1500 cm^{-1}(C=C stretching vibration of aromatic). additionally visible turned into the band at 1305 cm^{-1}corresponds to the stretching vibration of C-N, the band at 1150 cm^{-1}(aliphatic amines), which corresponded to the hoop stretching N-Q-N, wherein Q represents the quinoidiring. The extensive conduction band inside the variety of 1812 cm-1 to 2941 cm^{-1}changed into assigned to the digital transition within the unfastened providers of the polymer.

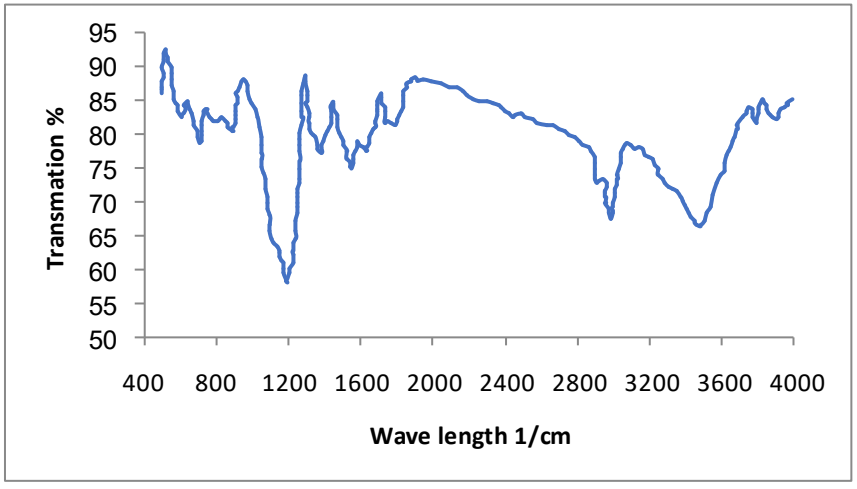

Fig 2: FTIR spectra of PANI

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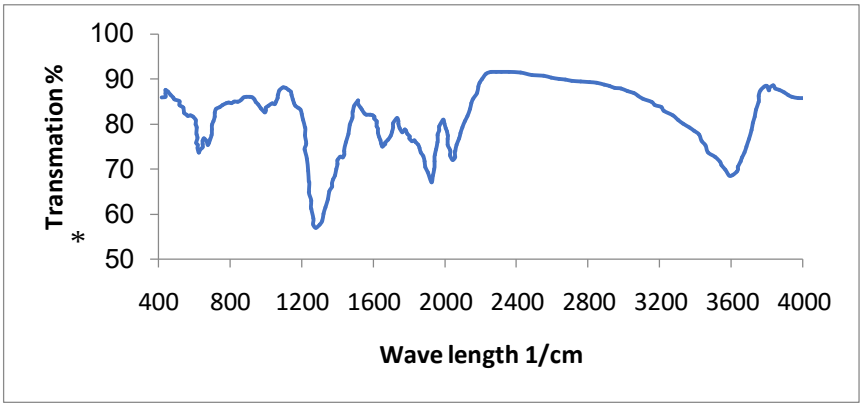

Fig 3: FTIR spectra of PANI-Cys

The FTIR spectrum of compound PANI-Cys confirmed extra absorption bands marked with an asterisk within the graph at ~645 cm^{-1}that might be assigned to the C-S linkage of the cysteine institution to the polymer As seen in Figure(3). Moreover, new bands at1665 $cm^{-1}$and 1314 cm^{-1}correspond to stretching of the C=O and C-O found in cysteine. The infrared spectrum found out that PANI-Cys exhibited new useful agencies in contrast with unmodified PANI, confirming powerful amendment of the polymer.

FTIR of the hybrid compound turned into additionally accomplished. , the FTIR spectrum of the hybrid nanomaterial exhibited robust absorption bands at 1447 $cm^{-1}$and 719 cm^{-1}, resulting from the presence of CaCO3and the excessive amount of carbonate within the CaCO3-PANI-Cys that keep away from the visualization of PANI-Cys absorption bands that corresponds to the CaCO3 As seen in Figure(4).

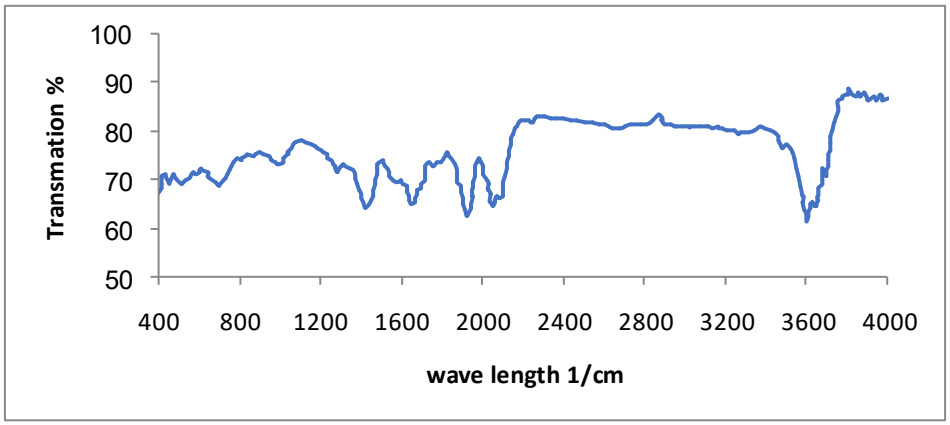

Fig 4: FTIR spectra of $CaCO_3$-PANI-Cys

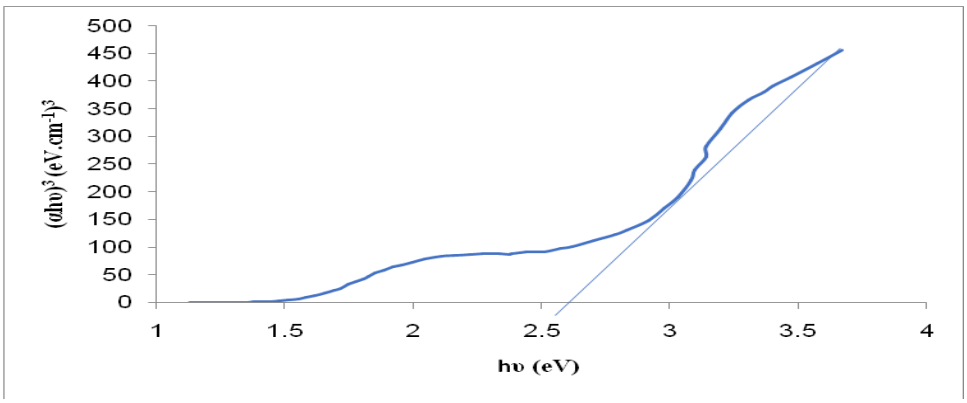

Fig 5: Determination the optical energy gap for $CaCO_3$-PANI-Cys

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The optical properties for $CaCO_3$-PANI-Cys have been investigated by using UV-visible absorbance and transmittance spectra, in the region of 200-1100nm. The optical energy gap has been evaluated as seen in Figure(5).

Figure(5). shows the plot of $(\alpha h\upsilon)^3$ versus hυ of $CaCO_3$-PANI-Cys.Tauc [16] put the empirical equation between the optical energy gap and energy of incident photon which is

$$\alpha h\upsilon = A(h\upsilon - E_g)^r$$

where A is a constant, hυ is the energy of incident photon, and r is the order of optical transition depending on the nature of electronic transition.

The plot is linear indicating the in direct band gap nature of the hybrid composites. Extrapolation of the line to the hυ axis gives the band gap its equal to 2.6 eV.

The morphology of the CaCO3 was also analyzed via SEM at 7 Ph. The CaCO3 had been stable, agglomerated spheres, the shape of agglomerated it's like cauliflowerwithdiameter∼30 nm Figure(6).

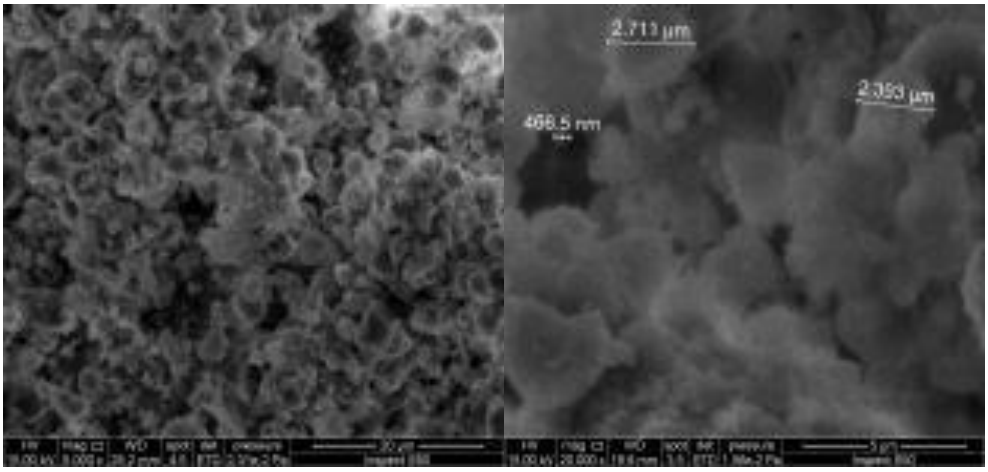

Fig 6: SEM images of $CaCO_3$

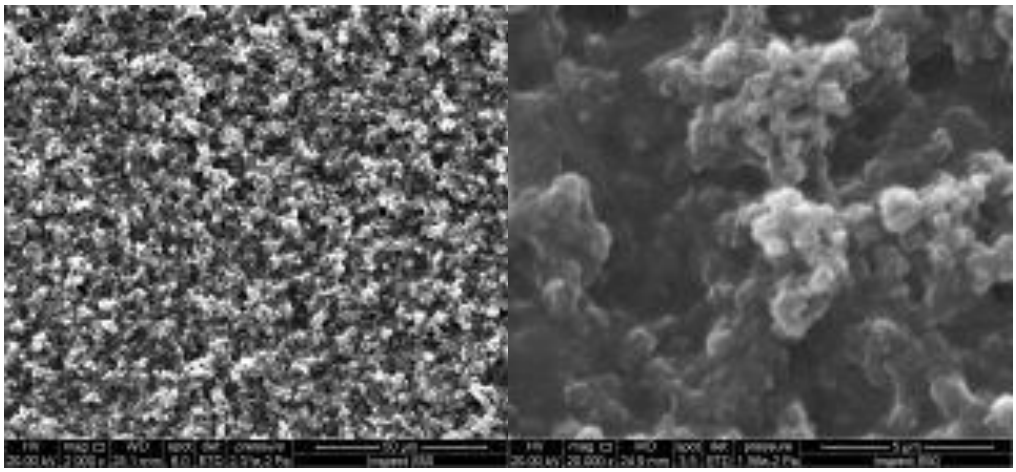

Fig 7: SEM images of $CaCO_3$-PANI-Cys biomaterial.

The agglomeration of $CaCO_3$ ought to arise because of the direct electrostatic interplay among the carboxylate of the carboxymethylcellulose(CMC) with the $CaCO_3$, wherein the debris have a tendency to agglomerate strongly. This technique is ruled by way of the surface unfastened power at some point of the

aqueous interfacial technique [60]. Those agglomerates exhibited an elongated morphology, starting from 1 µm to 500 nm in duration and ~100 nm extensive. The nanoparticles displayed a smooth surface with described and a round to ovular form.

The morphology of the brand new hybrid biomaterial composed of CMP-PANI-Cys turned into analyzed via SEM Figure(7). The hybrid biomaterial became ovular form with an abnormal surface because of the presence of the polymer and a particle size among 0.5 µm to 2 µm. evaluation of the SEM pictures permit to the proposition that CaCO3 microspheres with an average diameter of 5 µm acted as a scaffold, offering several micro- or Nano pores for the insertion of polymer nanoparticles.

The hybrid CaCO3-PANI-Cys changed into smaller than the CaCO3. The reduced CaCO3-PANI-Cys size might be attributed to the electrostatic interplay among the amino corporations (-NH2) of PANI-Cys and the carboxylate institution from CaCO3.

Cytotoxicity of Hybrid $CaCO_3$-PANI-Cys Material

The effect of $CaCO_3$-PANI-Cys on HeLa cells viability was investigated using the MTT test. The MTT analysis results for HeLa cells is displayed in Figure(8).

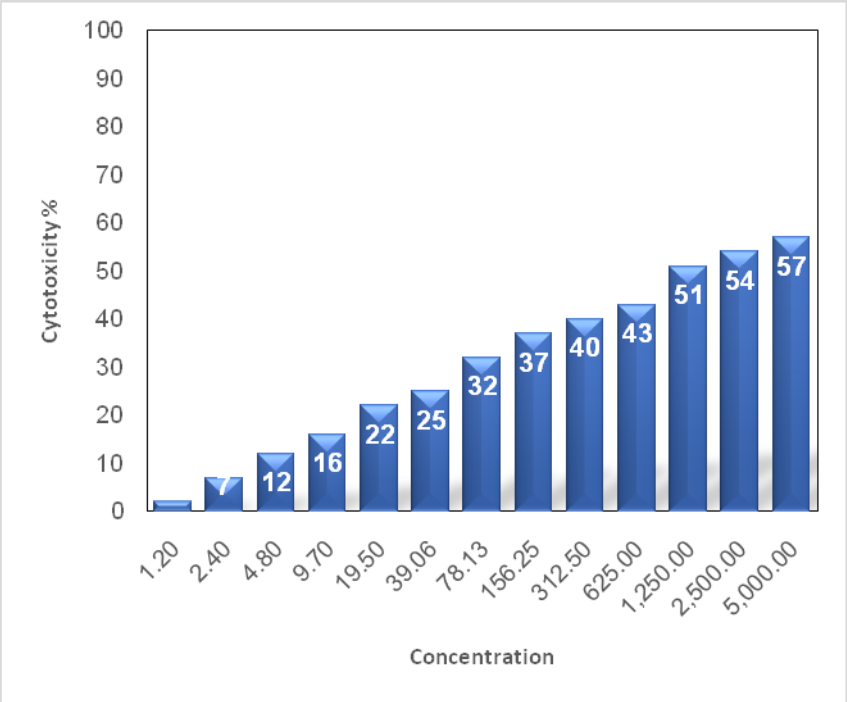

Fig 8: The cytotoxicity of HeLa cell with different concentrations of hybrid CaCO3-PANI-Cys biomaterial.

This assay showed that different concentrations of CMP-PANI-Cys were non toxic to cells treated for 24 hrs. , and then the toxicity begins to increase and reaches 57.13% at 5000µg/ml and 2.19% at 1.2µg/ml after 48h to 72h. Below these concentrations, the CMP-PANI-Cys does not negatively influence the cells viability.

Microscopic observations of HeLa cells Figure(9) support the previously discussed results demonstrating the changes in cell quantity and morphology induced by CMP-PANI-Cys dispersion. At the concentration of 1.2 µg/ml only rare and negligible cell damage can be observed. An increase of CMP-PANI-Cys concentration to 5000µg/ml undoubtedly influenced cell viability via decreasing cell numbers and simultaneous changes to their morphology.

The synthesis of inorganic substances with managed morphology and polymorphism has garnered attention and hobby of chemists because of the huge applicability of the substances [17]. One of the most

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plentiful bio minerals, CaCO3 has received prominence in lots of fields and was observed to be a perfect candidate for drug delivery as it has massive porosity and surface vicinity and may swiftly decompose below enormously slight situations. This biomaterial might be a capability service of numerous pills[18]. The consequences implied that this compound might be applied as quite drug however that the impact of excessive dosages have to be used whilst trying to maximize healing pastime by way of growing the concentrations.

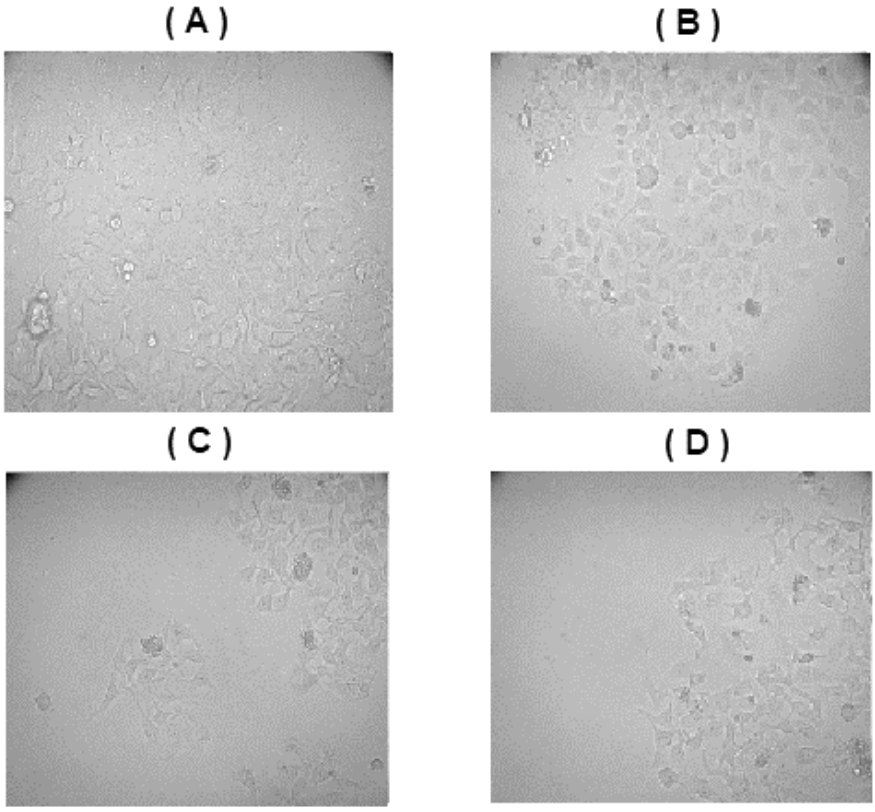

Fig 9: Microphotographs of HeLa cell line after the cytotoxicity test: (a) reference, (b) 9.7 µg/ml CaCO3-PANI-Cys, (c) 625 µg/ml CaCO3-PAIN-Cys, (d) 5000µg/ml CaCO3-PANI-Cys

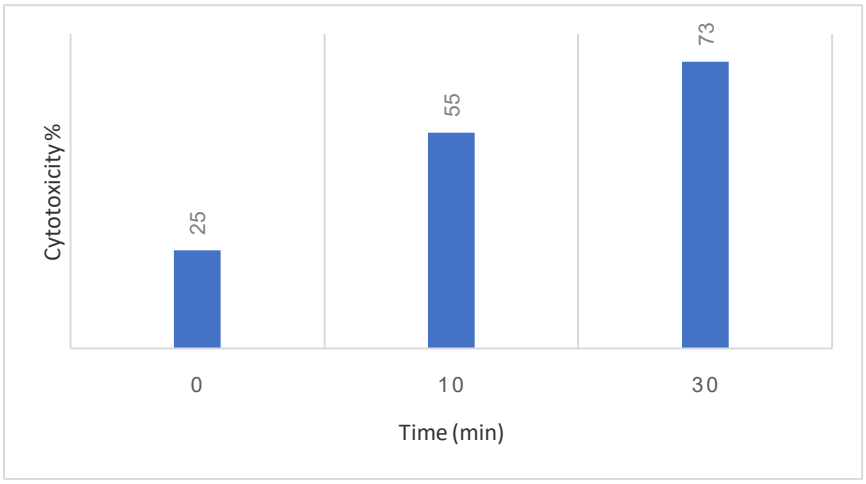

Fig 10: Effect of laser with concentration 10 µg/ml of CaCO3-PANI-Cys on HeLa cells. Control without laser then 10 min and 30 min irradiated.

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Photo Thermal Therapy in Vitro

Cells irradiated only with the NIR laser for 10min without nanoparticles then 10min, 30 min and control cells with concentration 10 µg/ml of CMP-PANI-Cys hybrid nanoparticles. This result demonstrated that the NIR laser alone did not induce cell death at the assayed doses Figure(10).

Alternatively, cells incubated with CaCO3-PANI-Cys and irradiated with NIR laser for 10 min showed cytotoxicity 55% and for 30 min showed cytotoxicity 73%. These results indicated that the CaCO3-PANI-Cys with high photo thermal conversion efficiency and photo stability could be used as a photo thermal agent as seen in Figure(11).

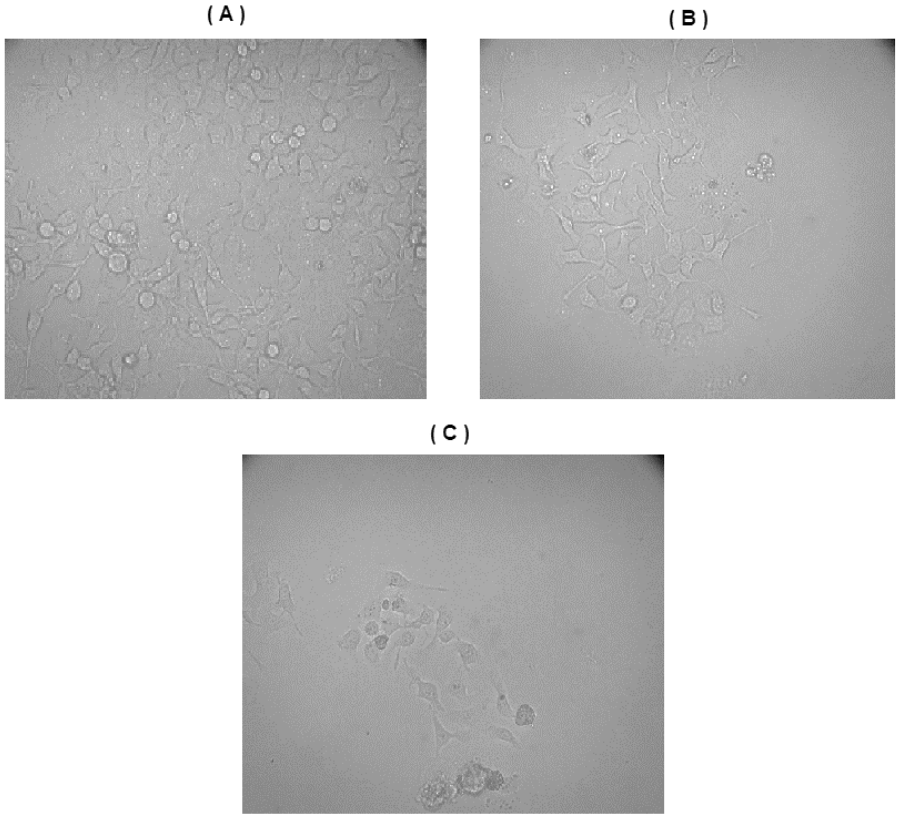

Fig 11: The cell image of laser with concentration 10 µg/ml of CaCO3-PANI-Cys on HeLa cells. Control without laser then 10 min and 30 min irradiated

The previous study of photothermic therapy found that cancer cells required a lower laser radiation power to achieve the temperature rise for cell destruction. The results proved that even the lower doses of CaCO3-PANI-Cys can still enhance killing effect using radiation However, The results suggested that large cluster of CaCO3-PANI-Cys lead to rise of temperature, then to vaporize adjacent water molecules which creating tiny bubbles that quickly expand and burst, ripping apart of cancer cells but not the normal cells[19]. Therefore, the present study suggest that using of NIR with CaCO3-PANI-Cys has potential future in medical application, and strong candidate for cancer treatment

CONCLUSIONS

Polymers have gained a splendid region inside the biomedical area as substances for the fabrication of numerous gadgets and for tissue engineering programs. Hybrid CaCO3-PANI-Cys substances composed of PANI nanoparticles functionalized with L-cysteine have been synthesized and successfully integrated into CaCO3. The CMC became used to maintain the form, composition, and long time balance of hybrid substances and to supply the burden close to the most cancers cellular, which turned into launched within the low pH regions that the cells set off. FTIR showed that PANI-Cys integrated into the Nano pores of CaCO3. SEM commentary

of CaCO3 confirmed described edges, a easy surface, and a spherical to ovular form starting from of 500nm to 5µm in size. In vitro research of the hybrid CaCO3-PANI-Cys biomaterials did result in modifications in cellular viability on the studied concentrations without laser, however the mixture of a laser within the NIR area and CaCO3-PANI-Cys markedly growing cytotoxicity. In precis, we concluded that the consequent hybrid biomaterial can be used a PTT agent for most cancers ablation.

Declaration of interest

The authors declare that there are no conflicts of interest. The authors alone are responsible for the content and writing of the paper

REFERENCES

[1] Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RG, Barzi A, Jemal A. a cancer journal for clinicians 2017, 67(3):177-193.

[2] Lane RJ, Khin NY, Pavlakis N, Hugh TJ, Clarke SJ, Magnussen J, Rogan C, Flekser RL. Future Oncology 2018, 14 (7):647-663.

[3] Yin S-Y, Wei W-C, Jian F-Y, Yang N-S. Evidence-Based Complementary and Alternative Medicine 2013, 2013, Review Article .

[4] Xu L, Cheng L, Wang C, Peng R, Liu Z. Polymer Chemistry 2014, 5(5):1573-1580.

[5] Rossmann C, Haemmerich D. Critical Review in Biomedical Engineering 2014, 42(6).

[6] Qian C-g, Chen Y-l, Feng P-j, Xiao X-z, Dong M, Yu J-c, Hu Q-y, Shen Q-d, Gu Z. Acta Pharmacologica Sinica 2017, 38(6):764.

[7] Qiu H, Tan M, Ohulchanskyy TY, Lovell JF, Chen G. Nanomaterials 2018, 8(5):344.

[8] May S. Ibraheem, Salma M. Hassan. International Journal of Basic and Applied Science 2015, 4(2): 28-39

[9] Ohlan A, Singh K, Chandra A, Dhawan SK. ACS applied materials & interfaces 2010, 2(3):927-933.

[10] Feoktistova N, Rose J, Prokopović VZ, Vikulina AS, Skirtach A, Volodkin D. Langmuir 2016, 32(17):4229-4238.

[11] Kumari A, Singla R, Guliani A, Yadav SK. EXCLI journal 2014, 13:265.

[12] ud Din F, Aman W, Ullah I, Qureshi OS, Mustapha O, Shafique S, Zeb A. International journal of nanomedicine 2017, 12:7291.

[13] Ott A: Disstrenation in tital Synthesis and application of hybrid materials based on plasmonic nanoparticles. Humboldt-Universität zu Berlin: 2016.

[14] Neira-Carrillo A, Yslas E, Marini YA, Vásquez-Quitral P, Sánchez M, Riveros A, Yáñez D, Cavallo P, Kogan MJ, Acevedo D. Colloids and Surfaces B: Biointerfaces 2016, 145:634-642.

[15] Sulaiman GM, Jabir MS, Hameed AH. Artificial cells, nanomedicine, and biotechnology 2018:1-13.

[16] Salma M. Hassan, A.F. Sultan, Falah A-H. Mutiak. Iraqi Journal of Physics 2016, 14(31): 161-168.

[17] Bhattacharya P, Du D, Lin Y. Journal of The Royal Society Interface 2014, 11(95):20131067.

[18] Bande F, Arshad SS, Bejo MH, Kamba SA, Omar AR. Journal of Nanomaterials 2015, 16(1):207.

[19] Lu Y, Peterson JR, Luais E, Gooding JJ, Lee NA. Journal of Nanomaterials 2015, 16(1):389.