FORM 2

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(See section 10 and rule 13)

Provisional Specification

Title: A nano-hybrid conjugate system of a theranostic agent

Name of the Applicant:

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The following specification describes the invention.

Technical field

The present disclosure relates to theranostics. More particularly, the present disclosure provides a multifunctional nano-hybrid cancer theranostic agent with integrated functions/ modalities of targeted imaging and localized cancer treatment.

Background

'Theranostics' [**thera**(peutics) + (diag)**nostics]** is a concept that allows combined diagnosis and treatment of a disease. In short, it's a portmanteau of diagnostics and therapeutics. This concept was initially proposed by Mr. John Funkhouser of Cardiovascular Diagnostics, USA, in August 1998. This concept evolved over a period of time and its highest level of technical advancement is seen in the domain of cancer nanomedicine, popularly called 'Cancer Nanotheranostics'. A nanotheranostic agent *per se* has triple features namely a nano-sized particle, a diagnostic agent and a therapeutic agent which are expected to exert multiple functions.

The two phototherapeutic approaches are photothermal therapy (PTT) and photodynamic therapy (PDT). The combination of PDT and PTT approach helps in enhancing the anti-cancer efficiency. They usually employ various photosensitizers (PSs) depending on its ability to convert light and produce localized hyperpyrexia or reaction oxygen species (singlet oxygen) to kill the cancer cells. PSs are used both as imaging agent and light-activated therapeutic agent. Some popular PSs include Photofrin, Indocyanine Green (ICG), 5-Aminolevulinic acid (5-ALA) and others. Fundamentally, the molecules electronic state and its transition between them is the key for PTT/ PDT. In any case, photosensitizers employed must be safe and non-toxic or minimally toxic in absence of light source, as its accumulation in non-specific/ non-irradiated areas of the body causes little or no systemic toxicity.

Developing a targeted and multifunctional cancer nano-hybrid theranostic agent offers various advantages related to diagnosis and treatment of cancer. Several nano-hybrids were explored in the past, but they all are associated with various challenges/ limitations including but not limiting to poor dispersion of the

nanocomposite, slow degradation of the nanocomposite, non-specific targeting, lack of desired stability, poor imaging with low resolution and lack of ability to contrast in NIR imaging. Therefore, designing and fabricating a targeted and multifunctional nano-hybrid cancer theranostic agent with integrated functions/ modalities of targeted imaging and localized cancer treatments are the need of the hour. Additionally, the information disclosed in this background section is only for enhancement of understanding of the general backdrop of the disclosure and should not be taken as an acknowledgement or any form of suggestion that this information forms the prior art already known to a person skilled in the art.

Objectives

First and foremost objective of the present disclosure is to provide a synergistic multifunctional cancer nanotheranostic agent.

Second objective of the present disclosure is to provide a process for preparing the synergistic multifunctional cancer nanotheranostic agent.

Summary

One or more shortcomings of conventional system for treatment and/or management of orofacial cancer are overcome and additional advantages are provided through the synergistic multifunctional cancer nanotheranostic agent as claimed in the present disclosure. Additional features and advantages are realized through the techniques of the present disclosure. Other embodiments and aspects of the disclosure are described in detail herein and are considered a part of the claimed disclosure.

Accordingly, the present disclosure provides a nano-hybrid conjugate system of a theranostic agent; and is also disclosed is a process for preparing a nano-hybrid conjugate system, comprising steps of preparing gold nanoparticles and titanium dioxide nanoparticles followed by functionalization of respective nanoparticles; and conjugating the functionalized gold nanoparticles with functionalized titanium dioxide nanoparticles by coupling reaction to obtain a conjugated and synergistic cancer theranostic agent; and is also disclosed is a kit comprising of nano-hybrid conjugate system of functionalized and conjugated gold and titanium dioxide nanoparticles; infrared light source for irradiation; and an instruction manual

having instructions to use the kit; and is also disclosed is a nano-hybrid conjugate system for imaging and treatment of orofacial cancer.

The foregoing summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative aspects, embodiments, and features described above, further aspects, embodiments, and features will become apparent by reference to the drawings and the following detailed description.

Brief description of the accompanying drawings

The features of the present disclosure will become more fully apparent from the following description and appended claims, taken in conjunction with the accompanying drawings. Understanding that these drawings depict only several embodiments in accordance with the disclosure and are therefore not to be considered limiting of its scope the disclosure will be described with additional specificity and detail through use of the accompanying drawings.

Figure 1: shows FTIR spectrum of titanium dioxide nanoparticles.

Figure 2: shows DLS report of carboxylate functionalized gold nanoparticles.

Figure 3: shows DLS report of gold-titanium dioxide nanoparticle conjugate.

Figure 4: shows UV-Vis spectra of (a) titanium dioxide, (b) titanium dioxide nanoparticles with lambda max at 400 nm.

Figure 5: shows UV-Vis spectra of citrate capped gold nanoparticles non functionalized.

Figure 6: shows UV-Vis spectra of (a) carboxylate functionalized gold nanoparticles (b) Absorption spectra of gold-titanium dioxide nanoparticle conjugate.

Figure 7: shows schematic representation of mechanism of action for theranostic functionalized gold-titania nanoparticles.

The figures depict embodiments of the disclosure for purposes of illustration only. One skilled in the art will readily recognize from the following description that alternative embodiments of the formulation of the present disclosure may be employed without departing from the principles of the disclosure described herein.

Detailed description

Before explaining any one embodiment of the present disclosure by way of drawings, experimentation, results, and pertinent procedures, it is to be understood that the disclosure is not limited in its application to the details as explained in below embodiments set forth in the following description or illustrated in the drawings, experimentation and/or results. The disclosure is further capable of other embodiments which can be practiced or carried out in various ways. As such, the language used herein is intended to be given the broadest possible scope and meaning; and the embodiments are meant to be exemplary and not exhaustive. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Definitions:

The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

The present disclosure is in relation to a composition comprising a nano-hybrid conjugate system of a theranostic agent.

In another embodiment of the present disclosure, the nano-hybrid conjugate is synergistic and multifunctional cancer theranostic composition.

In yet another embodiment of the present disclosure, the composition comprises of functionalized gold nanoparticles conjugated with functionalized titanium dioxide nanoparticles.

The present disclosure is in relation to a process for preparing a nano-hybrid conjugate system, comprising steps of preparing gold nanoparticles and titanium dioxide nanoparticles followed by functionalization of respective nanoparticles; and conjugating the functionalized gold nanoparticles with functionalized titanium dioxide nanoparticles by coupling reaction to obtain a conjugated and synergistic cancer theranostic agent.

In another embodiment of the present disclosure, the gold nanoparticles are functionalized using pamoic acid. Similarly, the titanium dioxide nanoparticles are functionalized using (3-aminopropyl) triethyoxysilance.

In yet another embodiment of the present disclosure, the coupling reaction is carried out using (1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide) and (N-hydroxysuccinimide).

The present disclosure provides a kit comprising of nano-hybrid conjugate system of functionalized and conjugated gold and titanium dioxide nanoparticles; infrared light source for irradiation; and an instruction manual having instructions to use the kit.

The present disclosure provides a nano-hybrid conjugate system for imaging and treatment of orofacial cancer.

In still another embodiment of the present disclosure, the conjugated system is irradiated with near infrared light to exert cytotoxic activity.

Additionally, the disclosure is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope of the present invention. On the contrary, it is to be clearly understood that various other embodiments, modifications, and equivalents thereof, after reading the description herein in conjunction with the drawings and appended claims, may suggest themselves to those skilled in the art without departing from the spirit and scope of the presently disclosed and claimed invention.

Example 1: Method of preparation:

Gold nanoparticles were prepared and functionalized using sodium borohydride (NABH) method. Briefly, the gold chloride was reacted with sodium borohydride. The gold nanoparticles spontaneously form upon addition of 0.1M sodium hydroxide solution. Titania dioxide nanoparticles (appro. 97 nm) were procured from SRL chemicals. Titania dioxide nanoparticles were functionalized with ATPES method. Briefly, the nanoparticles are reacted with 2mM ATPES solution overnight, centrifuged. The pellet is then dried and stored for cross linking. Both the functionalized gold and titania oxide nanoparticles were cross linked with PSSA-co-MA. The extent of cross linking was verified using optical methods, isothermal calorimetry and mass spectroscopy.

Example 2: Characterization methods

Example 2a: FTIR analysis: The successful formation of nanoparticles was characterized by employing Fourier Transform Infrared (FTIR) analysis, using potassium bromide method. The resultant spectrum obtained is presented in **Figure 1**. The spectra showing results for non-functionalized titanium dioxide is significant for the successful synthesis of the particles. The slender smooth peak at 3581, 3010.98 are identified to indicate vibration corresponding to the presence of the O-H alcohol compound. The band at 2366.74 belongs to C-H stretching. The distinguished sharp peaks are observed at 1610.61 and 750 cm^{-1} these peaks appear as strong. Additionally, the band vibrations at 1269.20 cm^{-1} are representing C-O stretching for the compound class alkyl aryl ether. The sharp peak at 750 cm^{-1} represents the Ti-O band. The Ti-O-Ti band confirms the presence of titanium dioxide nanoparticles.

Example 2b: DLS analysis: As regards the validation of particle sizing of gold nanoparticles before and after conjugation with titanium dioxide nanoparticles was done by dynamic light scattering (DLS) experiment. The DLS tool is used to analyse and optimize the gold nanoparticles conjugation. The novel strategy of conjugating gold and titanium dioxide is characterized by DLS. The colloidal solution of gold nanoparticles comprised of surface-functionalized with pamoic acid produced the results as displayed in **Figure 2.**

According to the achieved results, the average particle size of the gold nanoparticles prior to conjugation is 20.75 nm. The value obtained for the Polydispersity index (PDI) being 0.6 insinuates a slight polydispersed nature of the colloidal solution. Considering the presence of two peaks in which the second peak indicates the presence of larger particles in the solution. This signifies the aggregation of the gold nanoparticles that might have occurred during the synthesis. However, peak 1 represents particles in the size range of 12.92 nm. It shows a higher light scattering intensity compared to larger particles. This is because particles have the ability to scatter light proportionate to their diameter to the sixth power. This strengthens the presence of a higher number of smaller colloidal gold nanoparticles being formed with a size average of 20.75 nm in the colloidal gold solution. The DLS report of functionalized gold nanoparticles after undergoing conjugation with titanium dioxide utilizing EDC and NHS functional cross linkers is given in the **Figure 3**. The report shows a PDI of 0.3 which is an optimum value representing the monodispersed nature of the colloidal solution. The size measured in the DLS technique is the hydrodynamic diameter which is not only related to the metallic core of the

nanoparticle but also the surface groups and the conjugated particles. The mentioned factors and the electric double layer thickness influence the size measured in DLS, making particle size bigger compared to macroscopic techniques. Therefore, as expected upon conjugation there is a considerable increase in particle size which is supporting evidence for successful conjugation of gold and titanium nanoparticles. The resultant size average of 1449 nm, the peak formed has a light scattering intensity of 100% further adding to the monodispersed nature of the solution. These results are supplementing as supportive evidence of the successful conjugation of gold and titanium dioxide nanoparticles. To further add that the size increase observed in the DLS report is due to the conjugation of particles. This experiment was followed by a UV-Vis absorption study.

Example 2c: UV-Vis Absorption Study: The given spectral data gives the absorption spectra of titanium dioxide **Figure 4**. In the study, these particles were synthesized by the sonochemical method to obtain a fine powder of milky white colour. This indicates that the titanium isopropoxide is reduced to form titanium dioxide nanoparticles following the texture and colour of the particles formed after functionalization. The properties were further studied using UV- Vis spectroscopy for non-functionalized sonochemical synthesized. As shown in **Figure 4(b)** with spectral range 300 nm to 800 nm establishes the formation of particles. Further, the UV-Vis absorption spectra of titanium dioxide are given in **Figure 4(a).** The peaks imply the immediate recombination amidst the valence band holes and the conduction band electrons.

Example 2d: Localized surface Plasmon resonance (LSPR): The gold nanoparticles are colloidal particles that have a characteristic optical property to exhibit localized surface plasmon resonance (LSPR) this is a resultant property of electron oscillation in the conduction band due to the incidence of light of a specific wavelength. The particles synthesized by citrate reduction synthesis exhibit a spectral peak as shown in **Figure 5 (a)**. According to the figure, the absorption maxima is at a wavelength of 534 nm which indicates the formation of gold nanoparticles. Moreover, the change in colour of the reagent from clear yellow to deep red is an indicator for the formation of gold nanoparticles.

The UV Vis absorbance analysis was conducted for gold nanoparticles with carboxylate functionalization using pamoic acid. Then the functionalized gold nanoparticles were put through the conjugation reaction with titanium dioxide. The resultant spectral peaks are distinguished for the covalent interaction of gold and titanium dioxide nanoparticles, as

in **Figure 6.** It can be observed that there is an increase in absorption maxima value of wavelength from 349 nm to 546 nm before and after conjugation respectively. Investigations show that there is an increase in peak absorbance wavelength along with particle size for uneven particles such as gold associated with titanium dioxide nanostructure. Upon conjugation, with titanium dioxide particles there is a notable red shift in the peaks formed. This suggests an increase in particle size. Further, the absorption peak provides optical density (OD) values of 2.6 and 0.5 before and after conjugation respectively, showing linear association to the concentration of particles in the solution. The reduction in OD value is characteristic of aggregation of gold nanoparticles due to coupling reaction, also supporting the increase in particle size. Therefore concluding the successful conjugation of gold titanium nanoparticle complex.

Accordingly, the present disclosure provides synthesized and functionalized gold and titanium nanoparticles to fit the necessities. The particles were put through conjugation reaction to form the conjugated theranostic system having large potential in theranostic applications. The size-regulated synthesis of gold nanoparticles was achieved by regulating the pH. The titania nanoparticle synthesized by sonochemical method and the presence was confirmed by FTIR analysis. The resultant functionalization of gold and titania was characterized by UV-Vis spectroscopy. The novel conjugation method was brought out using functional cross-linkers. The findings from the DLS report imply favourable results with an increase in particle size. In summary, shows schematic representation of mechanism of action for theranostic functionalized gold-titania nanoparticles, see **Figure 7**.

Insofar as the description above and the accompanying drawing disclose any additional subject matter that is not within the scope of the single claim below, the inventions are not dedicated to the public and the right to file one or more applications to claim such additional invention is reserved.

While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

We claim:

1) A nano-hybrid conjugate system of a theranostic agent.
2) The system as claimed in claim 1, wherein said nano-hybrid conjugate is synergistic and multifunctional cancer theranostic composition.
3) The system as claimed in claim 2, wherein said composition comprises of functionalized gold nanoparticles conjugated with functionalized titanium dioxide nanoparticles.
4) A process for preparing a nano-hybrid conjugate system, comprising steps of:
    a) preparing gold nanoparticles and titanium dioxide nanoparticles followed by functionalization of respective nanoparticles; and
    b) conjugating the functionalized gold nanoparticles with functionalized titanium dioxide nanoparticles by coupling reaction to obtain a conjugated and synergistic cancer theranostic agent.
5) The process as claimed in claim 4, wherein said gold nanoparticles are functionalized using pamoic acid.
6) The process as claimed in claim 4, wherein said titanium dioxide nanoparticles are functionalized using (3-aminopropyl) triethyoxysilance.
7) The process as claimed in claim 4, wherein said coupling reaction is carried out using (1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide) and (N-hydroxysuccinimide).
8) A kit comprising of:
    a) nano-hybrid conjugate system of functionalized and conjugated gold and titanium dioxide nanoparticles;
    b) infrared light source for irradiation; and
    c) an instruction manual having instructions to use the kit.
9) A nano-hybrid conjugate system for imaging and treatment of orofacial cancer.

10) The nano-hybrid conjugate system as claimed in claim 9, wherein said conjugated system is irradiated with near infrared light to exert cytotoxic activity.

Dated this 18th day of February 2022

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ABSTRACT

Title: A nano-hybrid conjugate system of a theranostic agent and a method thereof.

Cancer that develops in any part of the mouth or oral cavity is said to be oral cancer. While there are many modalities to treat oral cancer, but, they all suffer with various problems/ limitations. Accordingly, the present disclosure provides a nano-hybrid conjugate system of a theranostic agent for treatment of oral cancer. Particularly, the nano-hybrid conjugate system of the present disclosure comprises of functionalized gold and titanium dioxide nanoparticles that are conjugated by coupling reaction using (1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide) and (N-hydroxysuccinimide). The synergistic nature of the combination of functionalized gold and titanium dioxide nanoparticles is vital to show the intended therapeutic activity.

Accompanying figure is Figure No. 7