



(51) International Patent Classification:

A61K 9/16 (2006.01) *B82Y 40/00* (2011.01)
B05D 5/06 (2006.01)

(21) International Application Number:

PCT/US2015/034007

(22) International Filing Date:

3 June 2015 (03.06.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/007,125 3 June 2014 (03.06.2014) US

(71) Applicant: UNIVERSITY OF SOUTH FLORIDA
[US/US]; 3802 Spectrum Blvd., Suite 100, Tampa, FL
33612 (US).(72) Inventor: PYAYT, Anna; 4202 E. Fowler Ave ENB118,
Tampa, FL 33647 (US).(74) Agents: RISLEY, David, R. et al.; Thomas / Horstemeyer,
LLP, 400 Interstate North Parkway, Suite 1500, Atlanta,
GA 30339 (US).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,

DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,
TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, KM, ML, MR, NE, SN, TD, TG).

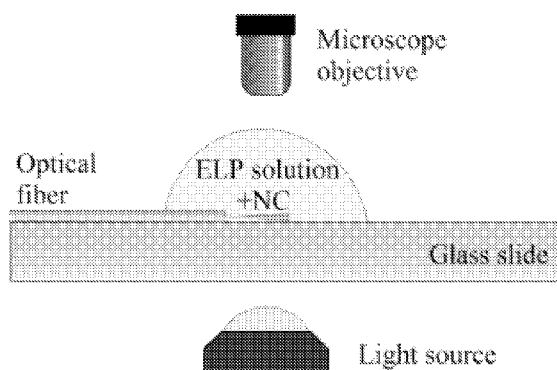
Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))

(54) Title: THERMOSENSITIVE LIQUIDS FOR VISUALIZING HEAT GENERATION ON A MICRO-SCALE

**FIG. 1**

(57) Abstract: In one embodiment, a thermosensitive liquid for visualizing heat generation includes elastin-like polypeptides suspended in an aqueous liquid, wherein the elastin-like polypeptides undergo a phase transition at a lower critical solution temperature in which they become insoluble and turn from translucent to opaque, thereby visually indicating that a temperature within the thermosensitive liquid has reached the lower critical solution temperature.

THERMOSENSITIVE LIQUIDS FOR VISUALIZING HEAT GENERATION ON A MICRO-SCALE

5 Cross-Reference to Related Application

This application claims priority to co-pending U.S. Provisional Application serial number 62/007,125, filed June 3, 2014, which is hereby incorporated by reference herein in its entirety.

10 **Background**

Photothermal cancer therapy has received increased attention as a potential alternative therapeutic approach to surgery, chemotherapy, and radiotherapy. It utilizes photothermal agents as transducers that transform energy harvested from light to heat. To date, various types of plasmonic nanoparticles have been synthesized as effective photothermal agents with tight control on their size and shape, tunable optical opportunities, and biocompatibility.

In photothermal therapy, cell death is initiated by either an elevated temperature or the associated shock wave via apoptosis, necrosis, or direct ablation. For an effective cancer treatment, the temperature in the tumor must be raised

above 42°C. However, temperatures above 45°C are not desirable because of the potential side effects (burns, blisters, discomfort, and pain) as well as the damage to surrounding healthy tissue. Therefore, a deep understanding of the parameters that control temperature, such as the concentration of the photothermal agent and the
5 light intensity influence, is necessary.

Controlling such parameters requires visualization of the heat generation on a micro-scale. Although thermographic cameras are great instruments for temperature visualization on a large scale, their resolution and compatibility with microscopy are limited since they operate at wavelengths as long as 14 μm . This makes it difficult to
10 resolve heating profiles generated by plasmonic particles with microscale spatial resolution. It can therefore be appreciated that it would be desirable to have a means for visualizing heat generation on a micro-scale.

Brief Description of the Drawings

15 The present disclosure may be better understood with reference to the following figures. Matching reference numerals designate corresponding parts throughout the figures, which are not necessarily drawn to scale.

Fig. 1 is a schematic diagram of a first experimental setup used to test an elastin-like polypeptide (ELP) solution.

20 Figs. 2A and 2B are micrographs showing heating profiles for experiments conducted using the setup of Fig. 1.

Figs. 3A-3O are micrographs sequentially illustrating a change of a heating profile in response to increasing laser intensity.

Fig. 4 is schematic diagram of a second experimental setup used to test an
25 ELP solution.

Figs. 5A-5C are micrographs showing the heating profiles for experiments conducted using the setup of Fig. 4.

Fig. 6 is schematic diagram of a third experimental setup used to test an ELP solution.

5 Figs. 7A-7C are micrographs showing the heating profiles for experiments conducted using the setup of Fig. 6.

Fig. 8 is a schematic diagram of a fourth experimental setup used to test an ELP solution.

10 Figs. 9A-9C are scanning electron microscope (SEM) images showing three different concentrations of gold nanocages used in experiments conducted using the setup of Fig. 8.

Figs. 10A-10C are micrographs showing the heating profiles for the experiments conducted using the setup of Fig. 8.

15 Detailed Description

As described above, it would be desirable to have a means for visualizing heat generation on a micro-scale. Disclosed herein are thermosensitive liquids that can be used for this purpose. In some embodiments, the liquids comprise a solution of elastin-like polypeptides (ELPs). As described below, the translucence of such solutions changes in response to localized photothermal heat generation by plasmonic nanoparticles. The heating profiles visualized using such solutions provide important insight into the heating dynamics of such particles, which is essential for optimization of parameters for photothermal cancer therapy. While the solutions can be used to monitor the heat generated by plasmonic nanoparticles, it is noted that

20 the solutions can more generally be used to monitor heat generated by any heating

25

element immersed in the solutions.

In the following disclosure, various specific embodiments are described. It is to be understood that those embodiments are example implementations of the disclosed inventions and that alternative embodiments are possible. All such
5 embodiments are intended to fall within the scope of this disclosure.

ELPs are derived from natural elastin and are composed of repeated blocks of penta-peptide, with Val-Pro-Gly-X-Gly being its prominent amino acid repeat, where “Val” is valine (2-amino-3-methylbutanoic acid), “Pro” is proline (pyrrolidine-2-carboxylic acid), “Gly” is glycine (aminoacetic acid), and “X” is a guest residue that
10 can be any amino acid except proline. ELPs undergo a phase transition at their lower critical solution temperature (LCST). Specifically, ELPs remain soluble in an aqueous solution when the temperature is below the LCST, but self-assemble and become insoluble when the temperature reaches or exceeds the LCST. When the ELPs become insoluble, they turn from translucent to opaque, thereby visually indicating a
15 temperature that meets or exceeds the LCST.

ELPs can be used to create thermosensitive solutions that can, in turn, be used to visualize localized heat generation by elements immersed in the solutions, such as plasmonic nanoparticles (e.g., gold nanocages). Such solutions can comprise a mixture of one or more ELPs and a phosphate buffered saline (PBS)
20 solution. In addition to ELPs, other thermosensitive materials can be used to form thermosensitive solutions. For example, thermochromic pigments can be used that change color with changes in temperature.

As noted above, ELP solutions can be used to observe localized heating on the micro-scale. Several experiments were conducted to demonstrate how changes
25 in the spatial distribution of gold nanocages change heating profiles for the same

level of light intensity. First, gold nanocages were evenly dispersed in a PBS ELP solution and exposed to 30 mW of laser radiation using the experimental set up shown in Fig. 1. As illustrated in this figure, a droplet of ELP solution containing gold nanocages (NC) was deposited on a glass slide positioned below a microscope objective and above a light source. The solution comprised a 5 μ l of a nanocage stock suspension (1.8 nM) mixed with a 50 μ l ELP solution. An optical fiber was used to deliver near infrared (NIR) laser light to the nanocages within the solution.

Fig. 2A is a micrograph showing the solution between activations of the laser and Fig. 2B is a micrograph showing the solution after the laser had been turned on for 10 seconds. As is evident from Fig. 2B, heating primarily occurred in and around the beam cone emerging from the optical fiber. Ten seconds after the laser was turned on, the ELPs became insoluble and opaque to visible light. The large dark spot corresponds to the volume where the ELP molecules had undergone this phase transition. When the laser was turned off, however, the ELPs underwent a phase transition in the opposite direction and became soluble and transparent (Fig. 2A). This process took one to two seconds while the heat dissipated to the environment.

The changes in transmission of the thermosensitive liquid can be extracted from the images. For example, the histogram tool from a photo editing program such as PhotoshopTM can be used to create a calibration curve for the quantitative measurement of the local temperature. Since the maximum opacity in the experiment of Fig. 2 was reached at 39°C, further increases in temperature cannot be determined using this method. However, the experiments were performed at 23°C and the increase in temperature could be tracked up to as high as 15 degrees, which, in vivo, would translate to an increase from 37°C to as high as 52°C.

Fig. 3 includes multiple sequential micrographs that illustrate changes of the

heating profile with the gradual increase of the laser power. The power of the laser was gradually changed from approximately 1 mW in Fig. 3A to approximately 29 mW in Fig. 3O with a step interval of 2 mW. At low intensities, the affected area was small and mostly traced the shape of the laser beam, as shown in Figs. 3A-3C. In these figures, the temperature increased by just 2 to 3 degrees. The laser beam was absorbed within several hundreds of microns from the fiber because of the very strong absorptive properties of the gold nanocages. As the laser power was increased, however, the heating increased and the visual difference between the locally heated solution and the surrounding solution likewise increased. As a result, the shape of the affected area became more oval (see Figs. 3D-3O).

The heating profile reached a steady state, with the energy delivered from the fiber and the heat dissipation being balanced with each other, when the laser power was kept constant. This occurred within one to two seconds for lower powers and within ten seconds for higher powers. Even though the diameter of the laser beam was the same for all the powers used in the experiments, the size of the heated area was dependent on the power level. This means that the higher the laser power is used for photothermal cancer therapy, the higher the local temperature increase and also the larger the area affected by the treatment.

An alternative design of the nano-heating experiment is shown in Fig. 4. Again, a droplet of ELP solution was deposited on a glass slide positioned below a microscope objective and above a light source. In this case, however, the gold nanocages were attached to the tip of the optical fiber that was used to deliver NIR laser light instead of being mixed in the solution. The surface density of the gold nanocages on the fiber tip was approximately 225 nanocages/ μm^2 . Adhesion of the gold nanocages to the tip of optical fiber was quite strong, since they could not be

noticeably washed away even after multiple flushing of the optical fiber.

Fig. 5A shows the heating profile before the laser was turned on, Fig. 5B shows the heating profile after the laser (at a wavelength of 808 nm and a power of approximately 30 mW) had been turned on for 10 seconds, and Fig. 5C shows the heating profile after the laser had been turned off for 1 second. This temperature visualization method significantly changed the heating pattern. The heat generation occurred only on the surface of the core of the fiber (which was 8 μm in diameter), where the laser beam shined on the gold nanocages. The temperature distribution had a nearly spherical symmetry. This can be compared to the results shown in Fig. 3, in which the dark spot of insoluble ELPs is somewhat elongated due to the beam geometry. This demonstrates that, even in small numbers, the gold nanocages act as very efficient heaters since even a very thin layer of nanocages on an 8 μm core of the optical fiber can be used to heat a spherical volume that is approximately 500 μm in diameter by more than 15°C. At the same time, a high concentration of gold nanocages in a solution (or in tissue) results in very fast absorption of all the light in the first hundreds of microns. This means that the optimal concentration of the gold nanocages in a tumor may depend on the size of a tumor. The larger the size of a tumor, the deeper the light must propagate and the lower the nanocage concentration must be to insure uniform heating throughout the whole tumor. Therefore, there is delicate balance between providing enough nanocages in order to make the whole tumor sensitive to the laser radiation and keeping the concentration low enough so that the entire tumor will be somewhat transparent to light.

Fig. 5C shows the ELP solution one second after the irradiation had ceased.

Most of the ELP solution turned back to transparent state in that time frame.

However, in the area with the highest temperature (i.e., the optical fiber core directly heating the gold nanocages), some aggregated ELPs were still visible as a small dark bump on the optical fiber interface. Notably, an optical fiber having gold nanocages may be used as an ultra-small contact probe ("point heating source") with the ability of providing localized heating similar to much larger resistive heaters currently used for local tumor ablation.

The next experiment studied the plasmonic heating profile of an object located at distance from the laser source. This is important for photothermal cancer therapy, where gold nanocages are accumulated in a tumor and are exposed to external laser radiation. Most of the heating should occur near the gold nanocages since most of biological systems have low absorption at 808 nm. Fig. 6 is schematic diagram of a third experimental setup that demonstrated how remote heating occurs when the laser shines on an object coated with gold nanocages. In this setup, two optical fibers were immersed in ELP solution including a first optical fiber (left) that was coupled to the laser and a second optical fiber (right) positioned about 1 mm away whose sides were covered with gold nanocages. The surface density of the gold nanocages on the second optical fiber was approximately 225 nanocages/ μm^2 . Fig. 7A shows the heating profile before the laser was turned on, Fig. 7B shows the heating profile after 10 seconds of irradiation, and Fig. 7C shows the heating profile 1 second after the laser was turned off. From these figures, it can be appreciated that all of the heating occurred around the gold nanocages that covered the second optical fiber. Fig. 7C shows that the ELPs returned to their transparent state after the laser was turned off since the temperature around fiber decreased to room temperature. Based on this experiment, an important conclusion is that it is critical that the concentration of gold nanocages in a tumor should be significantly higher

than that in the surrounding healthy tissue. Otherwise, light shining from outside of the body on a deep tumor would heat all the healthy tissue on its path to the tumor.

A further experiment was conducted to characterize the heat generation and temperature increase with respect to the surface density of gold nanocages. The experimental setup that was used is illustrated in Fig. 8. In this setup, a glass slide was covered with three different concentrations of gold nanocages. Figs. 9A-9C are scanning electron microscope (SEM) images of the three different groups of nanocages with the first group (Fig. 9A) having a density of approximately 72 nanocages/ μm^2 , the second group (Fig. 9B) having a density of approximately 6 nanocages/ μm^2 , and the third group (Fig. 9C) having a density of approximately 0.2 nanocages/ μm^2 . The size of each individual nanocage was approximately 42 nm.

Figs. 10A-10C show the heating profiles for the three different gold nanocage concentrations after 10 seconds of irradiation. It can be appreciated from these figures that the heating profile was a function of the gold nanocage concentration.

For the maximum concentration of approximately 72 nanocages/ μm^2 , the light irradiation caused the ELPs to turn opaque and heat to spread across the entire substrate area observed under the microscope. The areas with higher temperatures were proportional to the concentrations of gold nanocages on the glass slide. For the lower concentrations of approximately 6 nanocages/ μm^2 and approximately 0.2 nanocages/ μm^2 , the heated volumes were much smaller.

Fig. 10 demonstrates that the heated volume is highly dependent upon the concentration of the gold nanocages, even when the diameter of the laser beam and the power are the same. Also, it is interesting to observe that even low surface coverage, such as just approximately 0.2 nanocages/ μm^2 , can still generate a local temperature increase of more than 10°C while being illuminated by relatively low

power (30 mW) laser radiation. This means that use of relatively low gold nanocages concentrations for the photothermal cancer therapy can result in great control over the heating region with a reasonable temperature increase in the affected area.

CLAIMS

Claimed are:

1. A thermosensitive liquid for visualizing heat generation comprising:
an aqueous liquid; and
elastin-like polypeptides suspended in the aqueous liquid, wherein the elastin-like polypeptides undergo a phase transition at a lower critical solution temperature in which they become insoluble and turn from translucent to opaque, thereby visually indicating that a temperature within the thermosensitive liquid has reached the lower critical solution temperature.
2. The thermosensitive liquid of claim 1, wherein the aqueous liquid is a phosphate buffered saline solution.
3. The thermosensitive liquid of claim 1, wherein the elastin-like polypeptides are composed of repeated blocks of penta-peptide, with Val-Pro-Gly-X-Gly being its prominent amino acid repeat, where "Val" is valine (2-amino-3-methylbutanoic acid), "Pro" is proline (pyrrolidine-2-carboxylic acid), "Gly" is glycine (aminoacetic acid), and "X" is a guest residue that can be any amino acid except proline.
4. A method for visualizing heat generation, the method comprising:
placing one or more heating elements within a thermosensitive liquid comprising elastin-like polypeptides suspended in an aqueous liquid, wherein the elastin-like polypeptides undergo a phase transition at a lower critical solution temperature in which they become insoluble and turn from translucent to opaque;

and

observing the phase transition of elastin-like polypeptides located near the heating elements as the heating elements raise the temperature of the elastin-like polypeptides above the lower critical solution temperature.

5. The method of claim 4, wherein the aqueous liquid is a phosphate buffered saline solution.

6. The method of claim 4, wherein the elastin-like polypeptides are composed of repeated blocks of penta-peptide, with Val-Pro-Gly-X-Gly being its prominent amino acid repeat, where "Val" is valine (2-amino-3-methylbutanoic acid), "Pro" is proline (pyrrolidine-2-carboxylic acid), "Gly" is glycine (aminoacetic acid), and "X" is a guest residue that can be any amino acid except proline.

7. The method of claim 4, wherein placing one or more heating elements comprises placing one or more plasmonic nanoparticles within the thermosensitive liquid.

8. The method of claim 7, wherein the plasmonic nanoparticles are gold nanocages.

9. The method of claim 7, further comprising irradiating the plasmonic nanoparticles with near infrared light to cause them to increase in temperature.

10. The method of claim 9, wherein irradiating the plasmonic nanoparticles

comprises emitting the near infrared light from a tip of an optical fiber immersed in the thermosensitive liquid.

11. A method for visualizing heat generation, the method comprising:

placing one or more heating elements within a thermosensitive liquid comprising a thermochromic pigment, wherein the pigment undergoes a color change when heated to a particular temperature; and

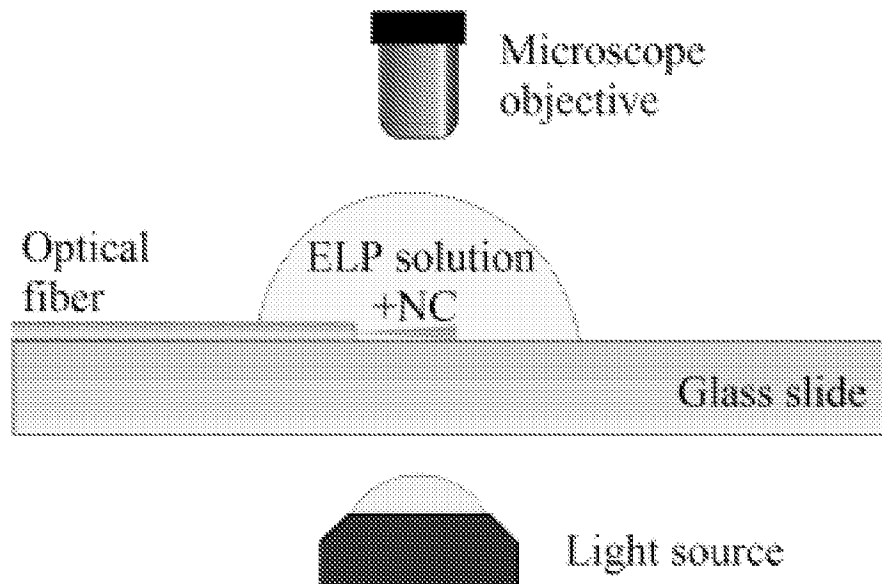
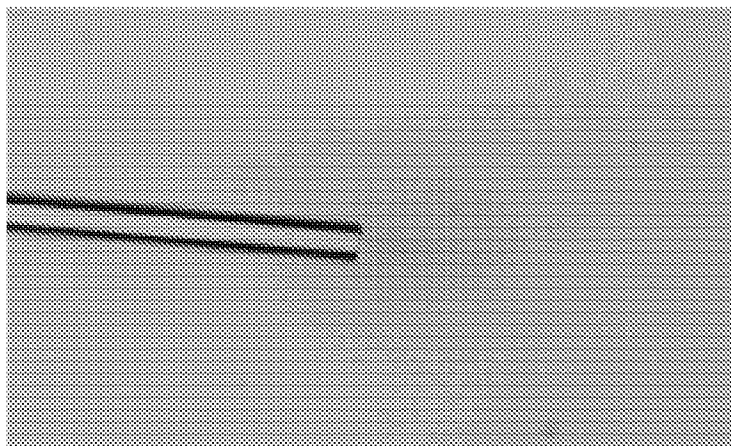
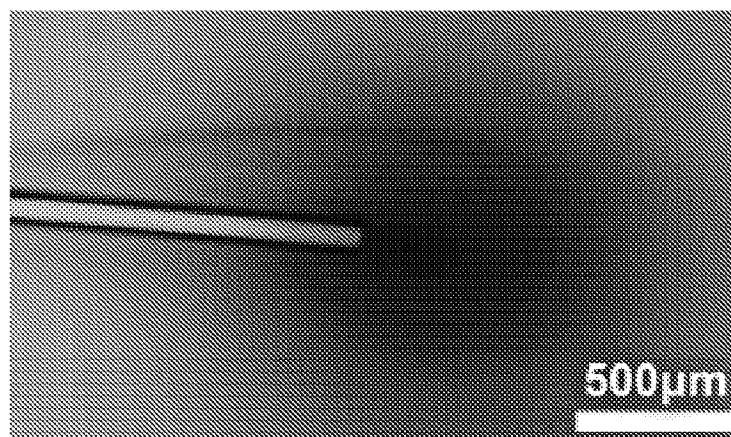
observing the color change at a location near the heating elements as the heating elements heat the pigment to the particular temperature.

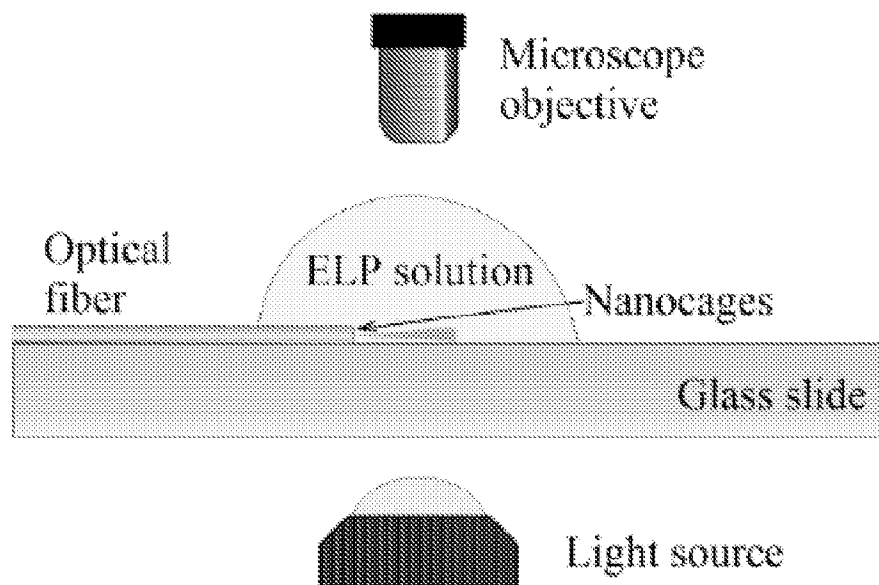
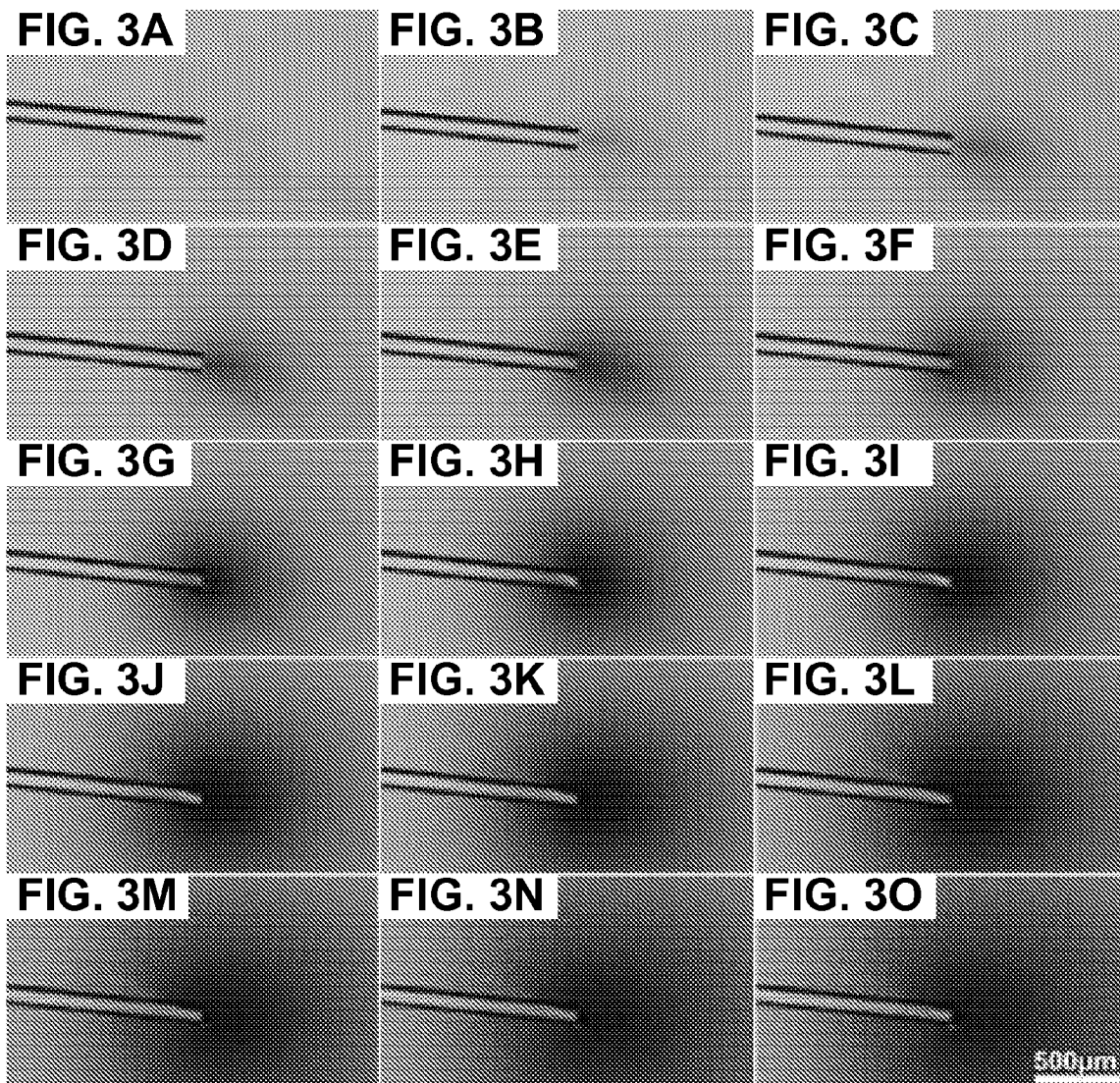
12. The method of claim 11, wherein placing one or more heating elements comprises placing one or more plasmonic nanoparticles within the thermosensitive liquid.

13. The method of claim 12, wherein the plasmonic nanoparticles are gold nanocages.

14. The method of claim 12, further comprising irradiating the plasmonic nanoparticles with near infrared light to cause them to increase in temperature.

15. The method of claim 14, wherein irradiating the plasmonic nanoparticles comprises emitting the near infrared light from a tip of an optical fiber immersed in the thermosensitive liquid.

**FIG. 1****FIG. 2A****FIG. 2B**

**FIG. 4**

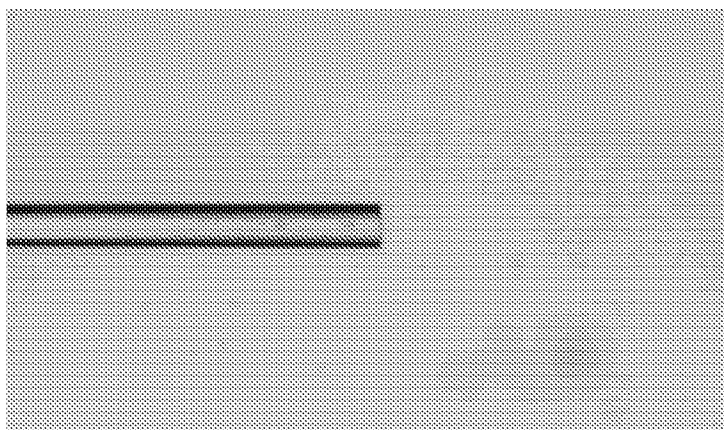


FIG. 5A

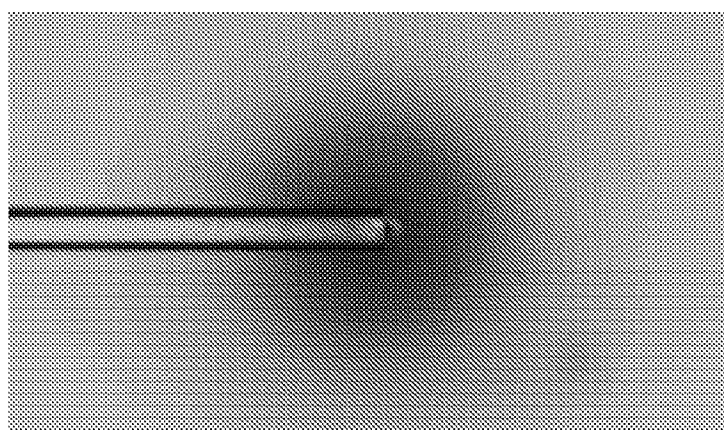


FIG. 5B

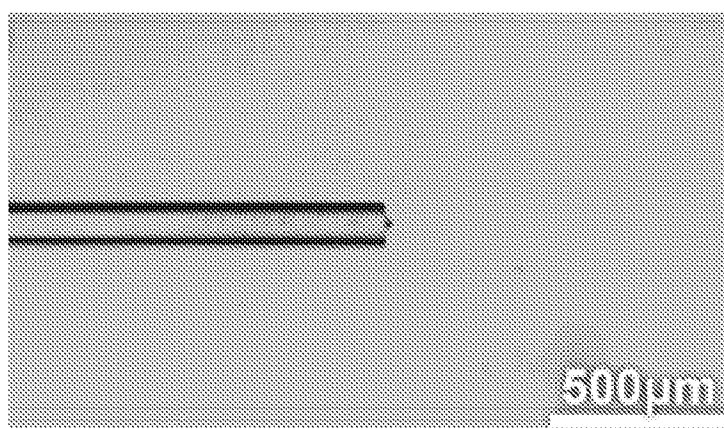
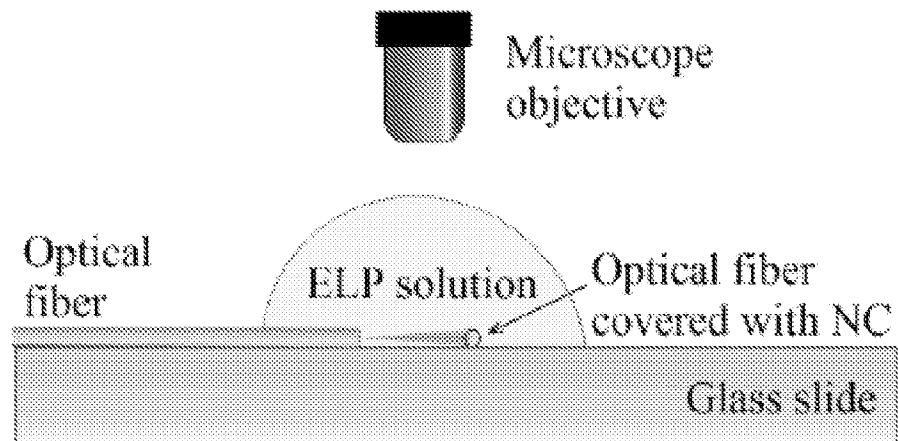
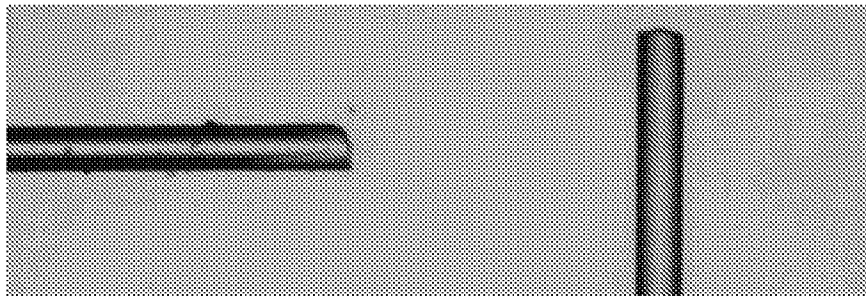
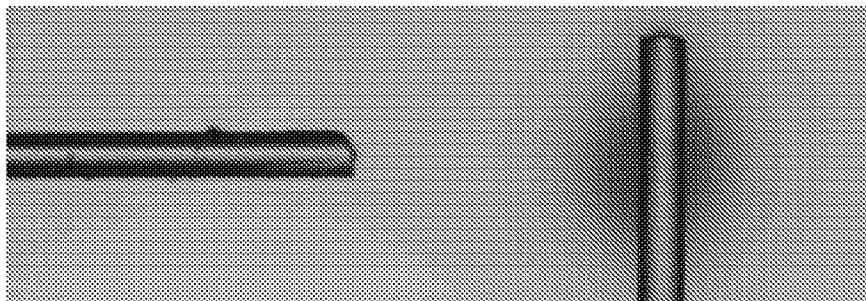
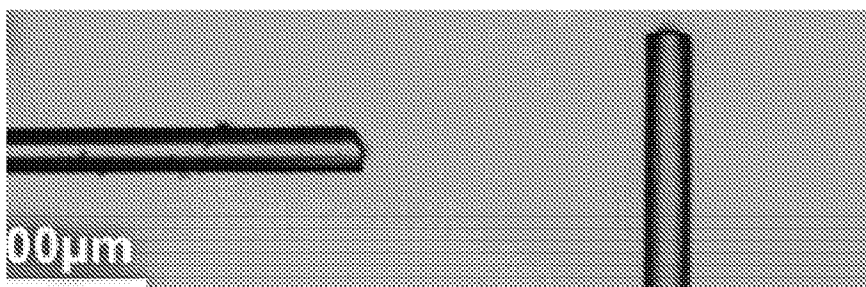
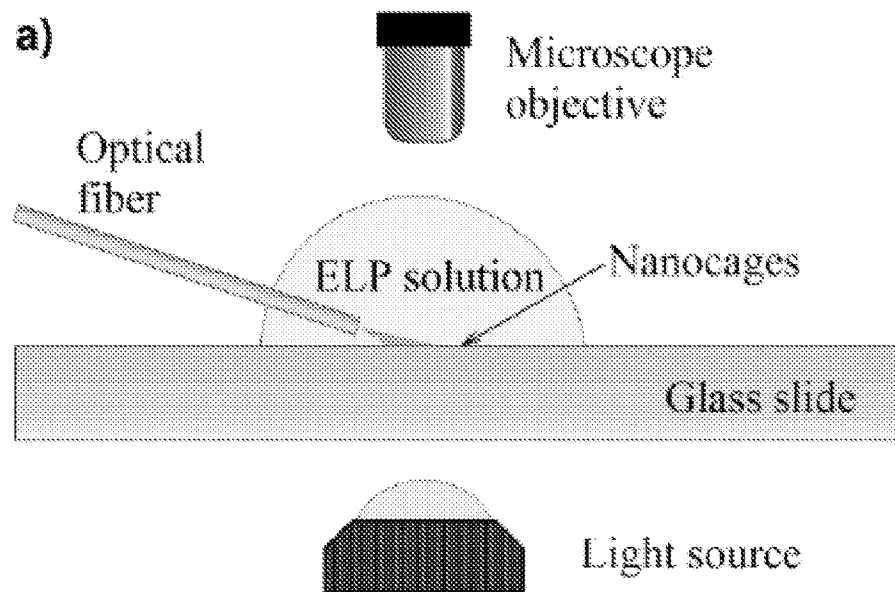
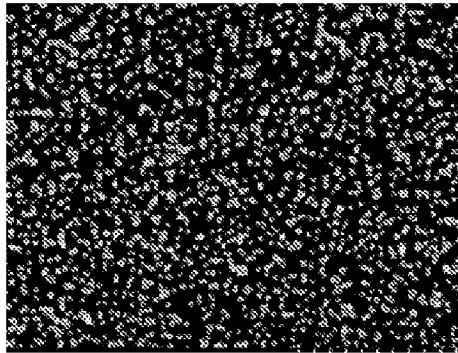
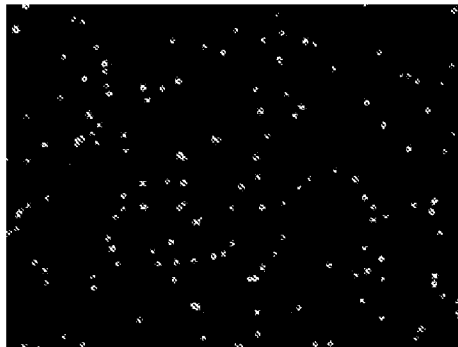
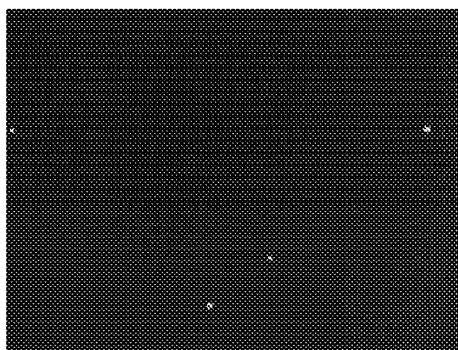
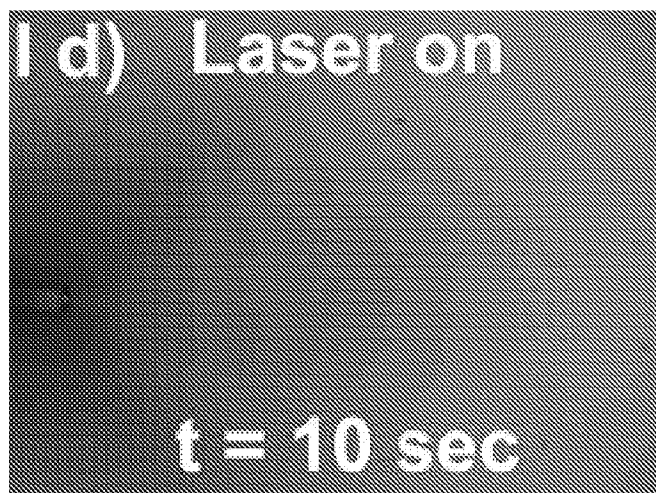
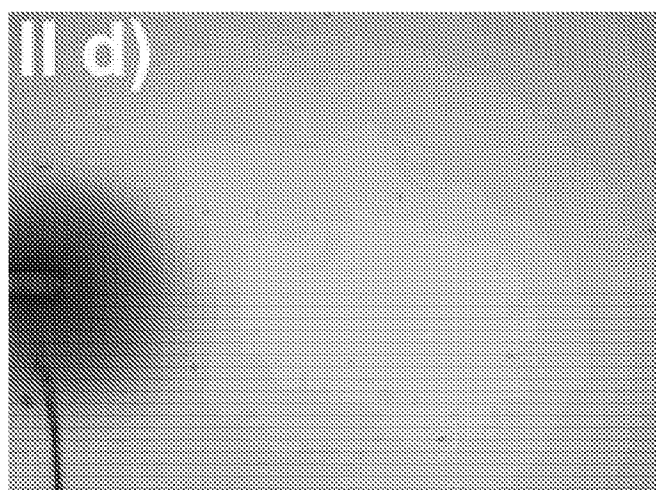
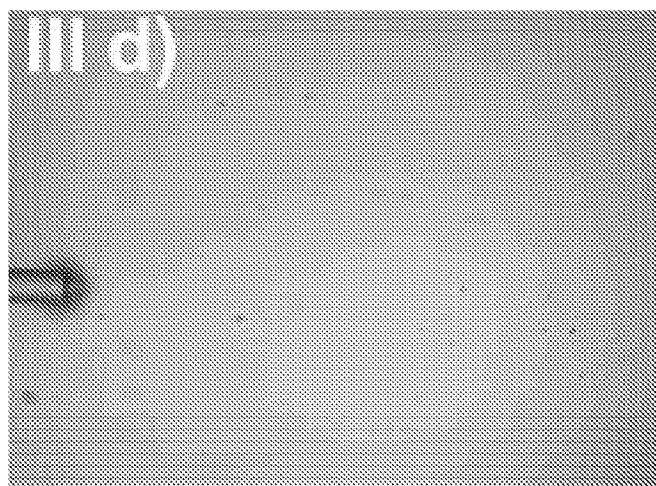


FIG. 5C

**FIG. 6****FIG. 7A****FIG. 7B****FIG. 7C**

**FIG. 8****FIG. 9A****FIG. 9B****FIG. 9C**

**FIG. 10A****FIG. 10B****FIG. 10C**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US15/34007

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 9/16; B05D 5/06; B82Y 40/00 (2015.01)

CPC - A61K 47/48; B82Y 5/00; C07K 19/00, 14/78; G01N 21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K 9/16; C12N 5/04; B05D 5/06; C12N 1/14; B82Y 40/00 (2015.01)

CPC: A61K 8/64, 47/48; B82Y 5/00; C07K 19/00, 14/78, 2319/00, 14/78; G01N 21/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data), PatSeer, EBSCO, GoogleScholar, Google patents
thermosensitive liquid/fluid, Thermo responsive / thermo stimulus, Stimuli responsive polymer / SRP, Visualization of heat generation,
Elastin like polypeptides/ ELP, Phase transition, insoluble, Lower critical solution temperature/ LCST, translucent, opaque, PBS**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	HUANG, HC et al. Optically Responsive Gold Nanorod-Polypeptide Assemblies. Langmuir. 16 December 2008; Vol. 24, No. 24, p 14139-14144; abstract; page 2, section 1, paragraph 1; page 3, section 2, paragraph 1; figures 1b, 1c; 2c, 2d, 3	1-7, 9, 11-12, 14 ----- 8, 10, 13, 15
Y	CHEN, J et al., Facile synthesis of gold-silver nanocages with controllable pores on the surface. Journal of the American Chemical Society. 2006 Vol. 128, No. 46, pages 14776-14777;	8, 13
Y	CUBUKCU, E et al. Plasmonic Laser Antennas and Related Devices. Journal of Selected Topics in Quantum Electronics. IEEE. 24 December 2008, Vol.14, No.6, pages1448, 1461;	10, 15
P	KOGA, T et al. Fabrication of Thermo-Responsive Molecular Layers from Self-Assembling Elastin-Like Oligopeptides Containing Cell-Binding Domain for Tissue Engineering. Polymers 19 January 2015, Vol. 7, 134-146.	1-15
A	US 6,615,855 B2 (LOPEZ, G et al.) 9 September 2003; entire document	1-7
A	US 2012/0263793 A1 (VITALIANO, F) 18 October 2012; entire document	1-15
A	NATH N et al. Interfacial Phase Transition of an Environmentally Responsive Elastin Biopolymer Adsorbed on Functionalized Gold Nanoparticles Studied by Colloidal Surface Plasmon Resonance. Journal of the American Chemical Society. 2001 Vol. 123, No. 34, pages 8197-8202; entire document	1-15

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 July 2015 (28.07.2015)

Date of mailing of the international search report

25 AUG 2015

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774