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(54) **INJECTABLE SHEAR-THINNING HYDROGEL CONTAINING POLYPEPTIDE THERAPEUTIC AGENT FOR ENHANCED TUMOR THERAPY**

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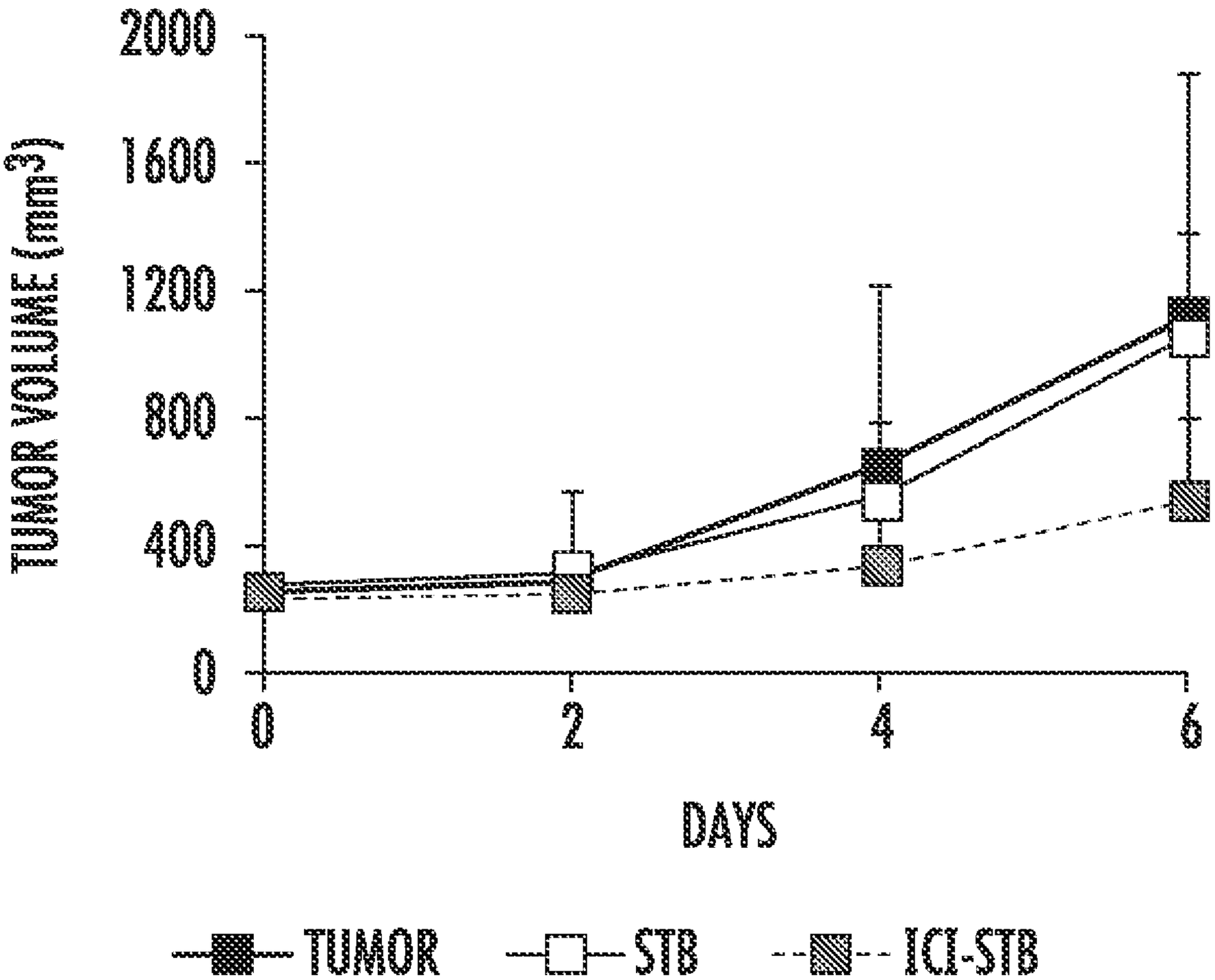
(57) **ABSTRACT**

We have developed novel shear-thinning biomaterials using silica nanoparticles, gelatin-based polymers and polypeptides such as anti-PD-1 antibodies. Shear-thinning biomaterial technology offers enables polymers and drugs loaded inside such polymers to be easily delivered directly through catheters into target area for use, for example, in cancer therapy and immunotherapy. When a force above a certain threshold is applied to inject such materials, they "thin" and behaves as a semi-solid, allowing the material to readily flow through a catheter. When the force is removed, the material instantly becomes a soft solid with significant cohesive properties that prevent it from dislodging or breaking up.



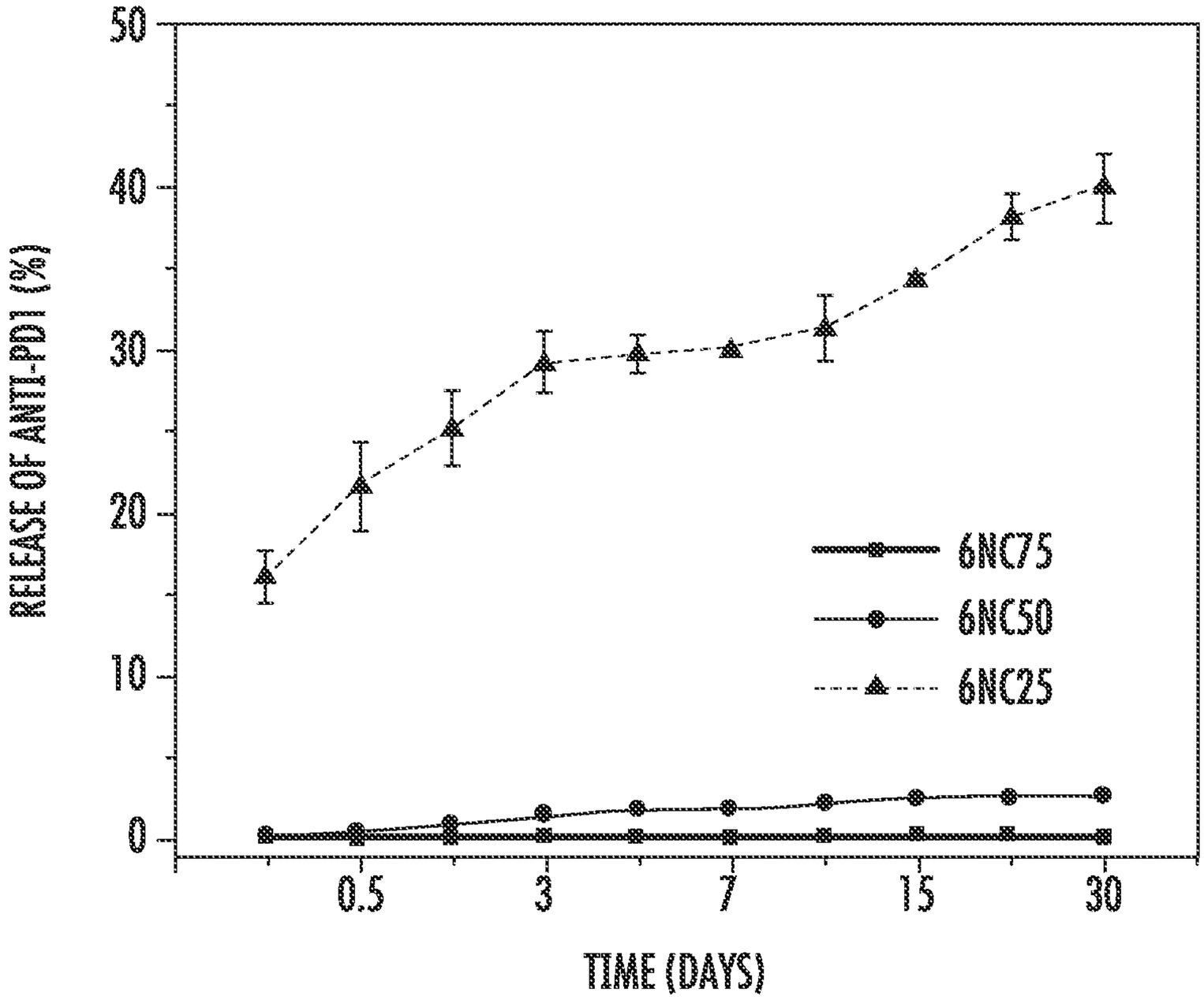

RELEASE OF ANTI-PD1 (%)
0
10
20
30
40
50
0.5
3
7
15
30
TIME (DAYS)
6NC75
6NC50
6NC25

FIG. 1A

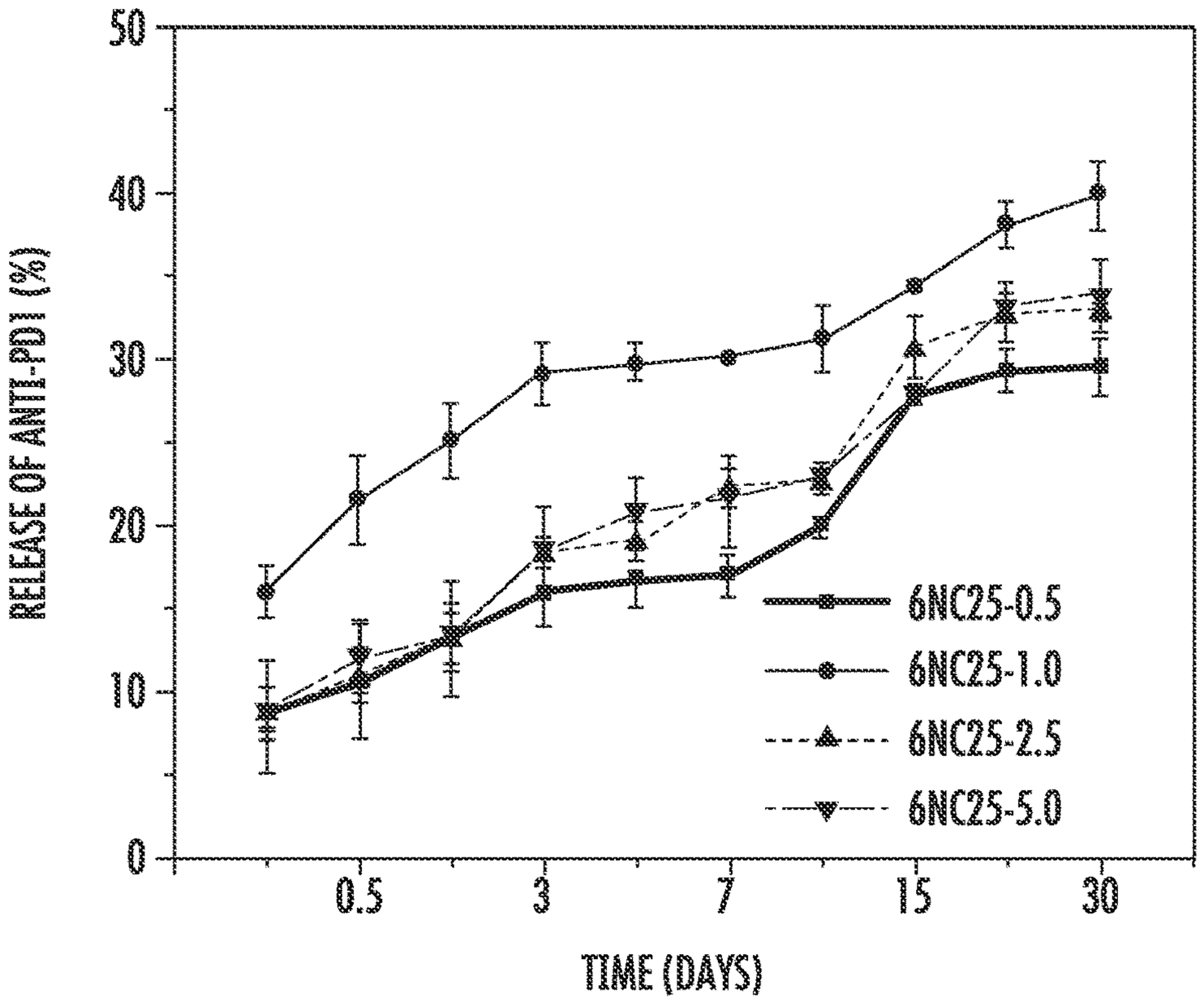

RELEASE OF ANTI-PD1 (%)
50
40
30
20
10
0
0.5
3
7
15
30
TIME (DAYS)
6NC25-0.5
6NC25-1.0
6NC25-2.5
6NC25-5.0

FIG. 1B

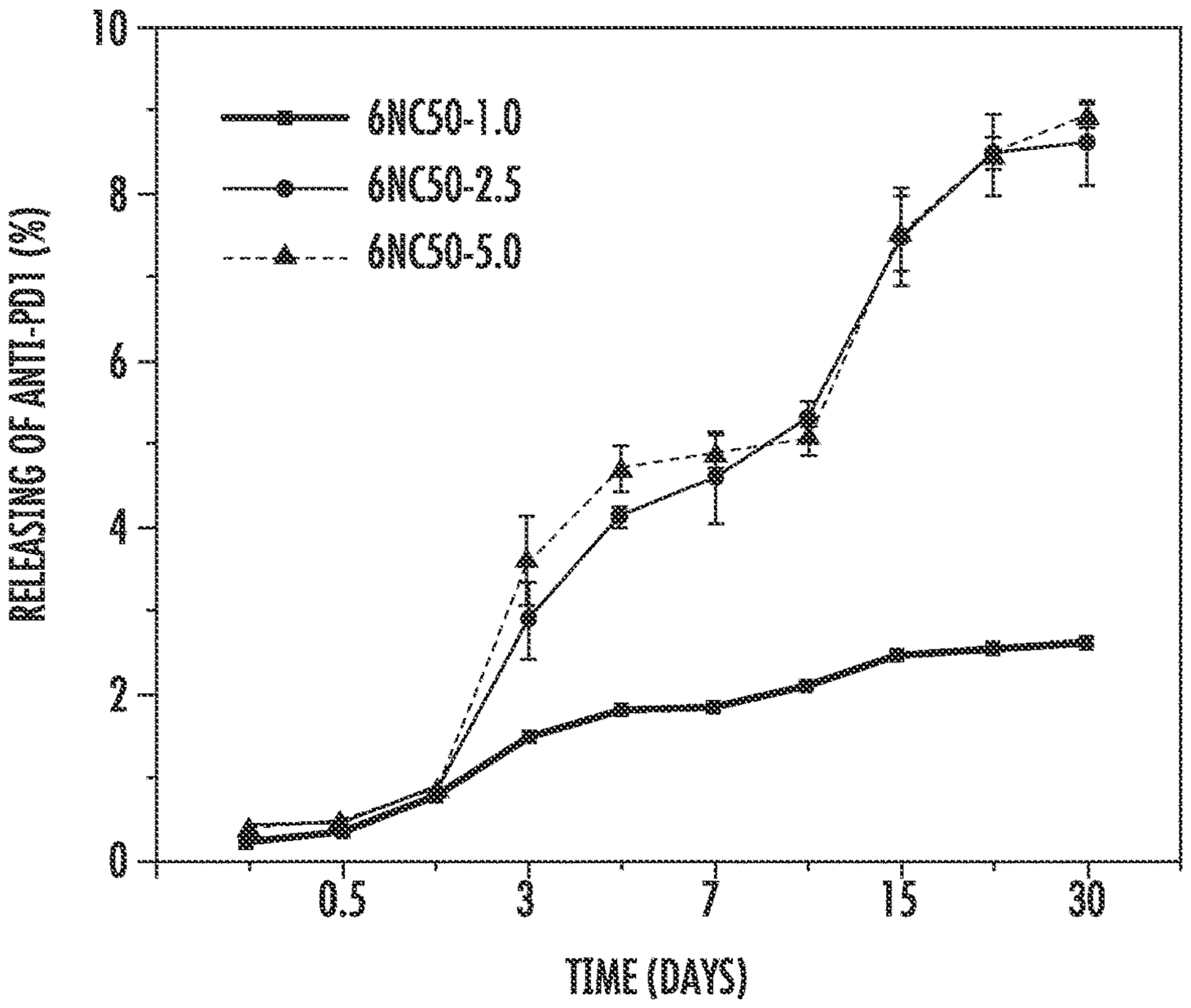

RELEASING OF ANTI-PD1 (%)
10
8
6
4
2
0
0.5
3
7
15
30
TIME (DAYS)
6NC50-1.0
6NC50-2.5
6NC50-5.0

FIG. 1C

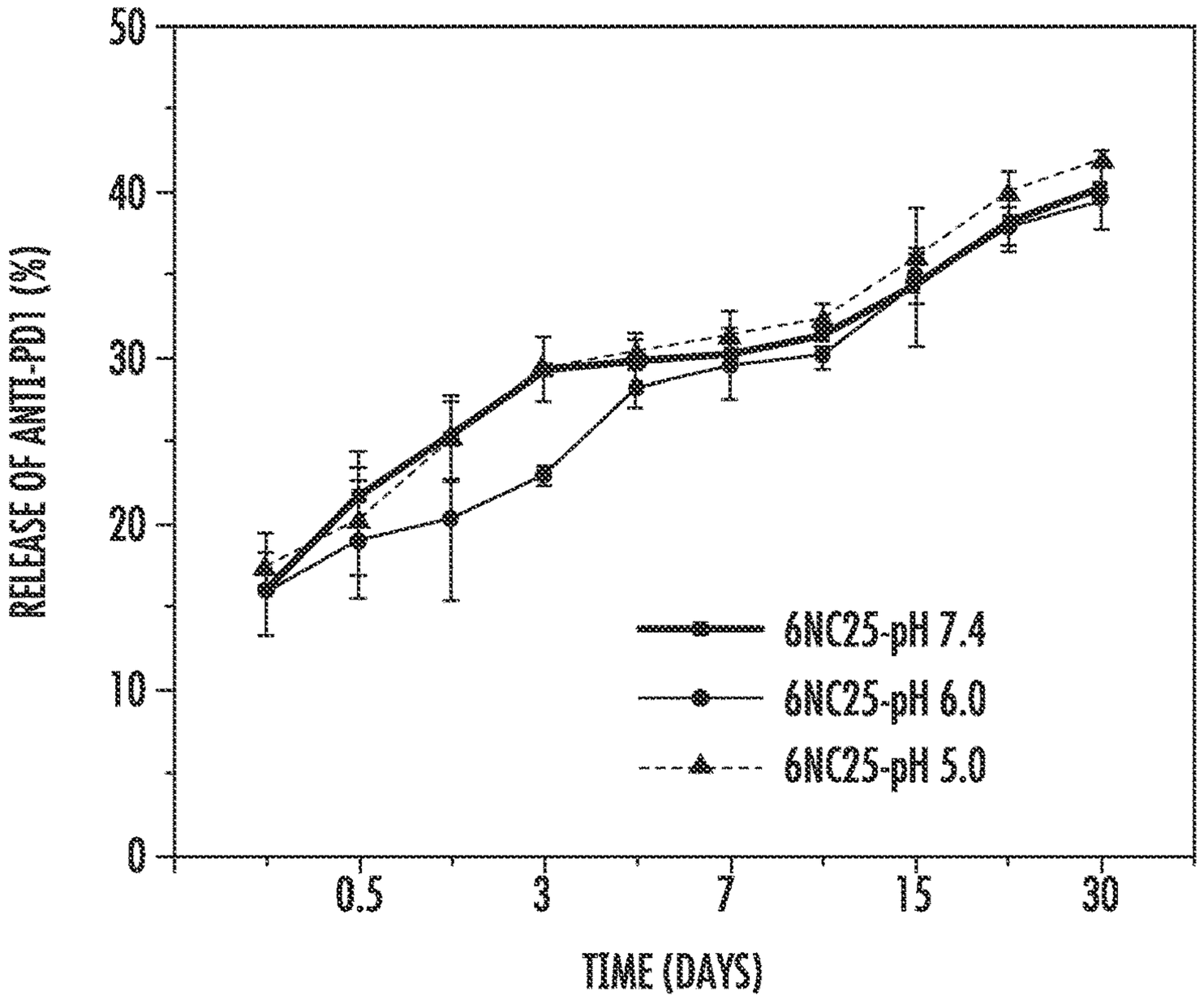

RELEASE OF ANTI-PD1 (%)
50
40
30
20
10
0
0.5
3
7
15
30
TIME (DAYS)
6NC25-pH 7.4
6NC25-pH 6.0
6NC25-pH 5.0

FIG. 1D

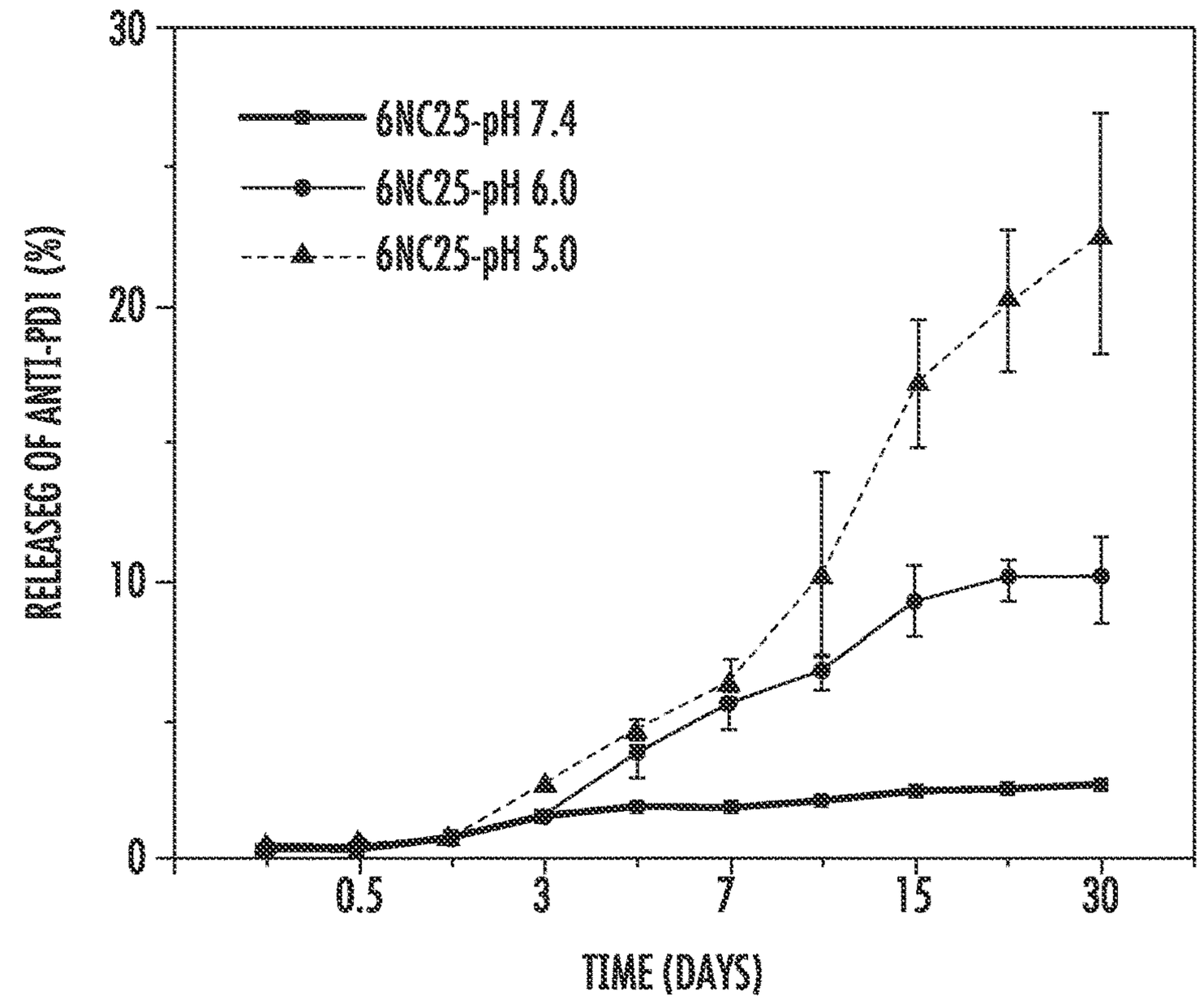

RELEASE OF ANTI-PD1 (%)
30
20
10
0
0.5
3
7
15
30
TIME (DAYS)
6NC25-pH 7.4
6NC25-pH 6.0
6NC25-pH 5.0

FIG. 1E

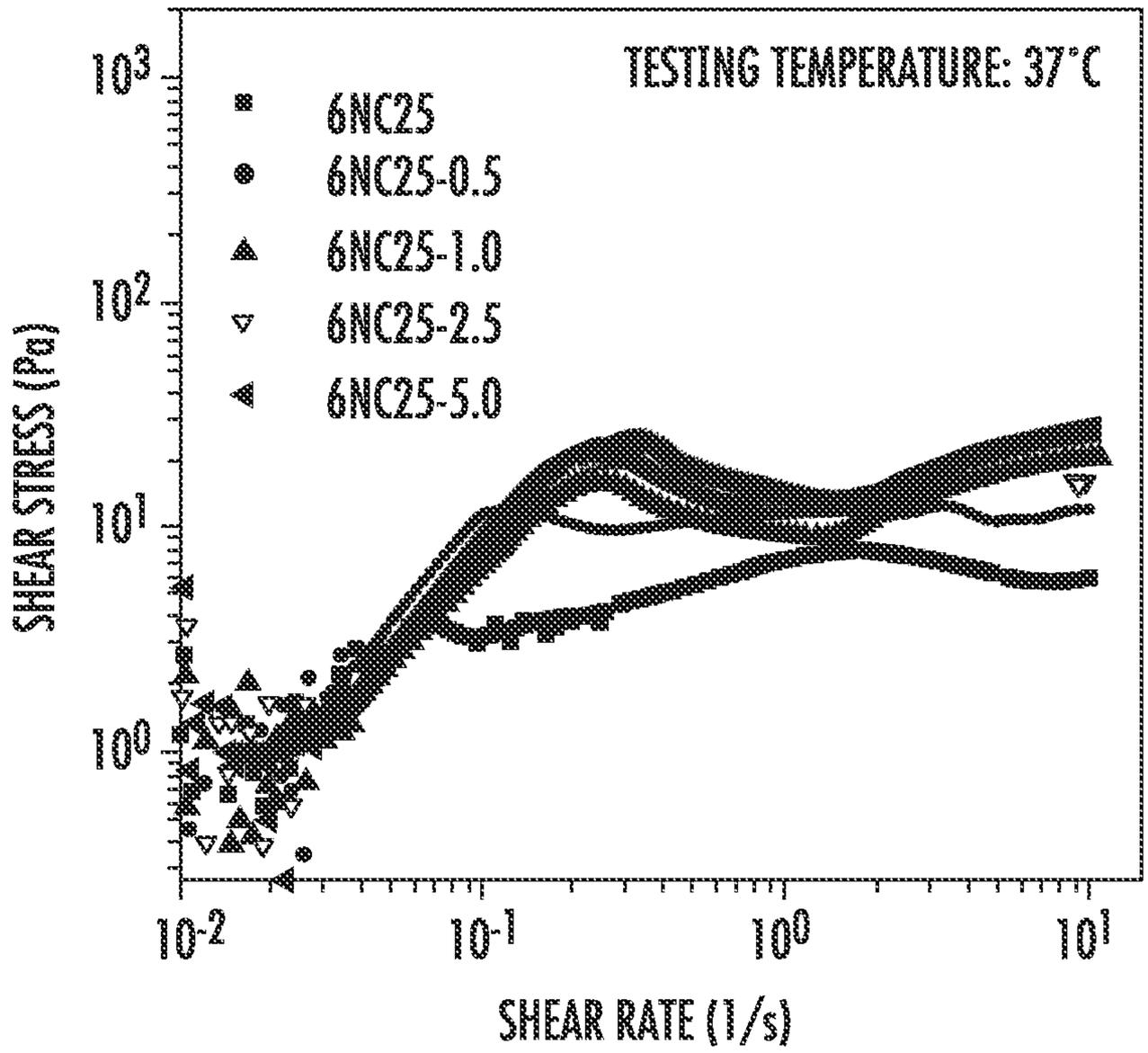

TESTING TEMPERATURE: 37°C
6NC25
6NC25-0.5
6NC25-1.0
6NC25-2.5
6NC25-5.0
SHEAR STRESS (Pa)
SHEAR RATE (1/s)

FIG. 2A

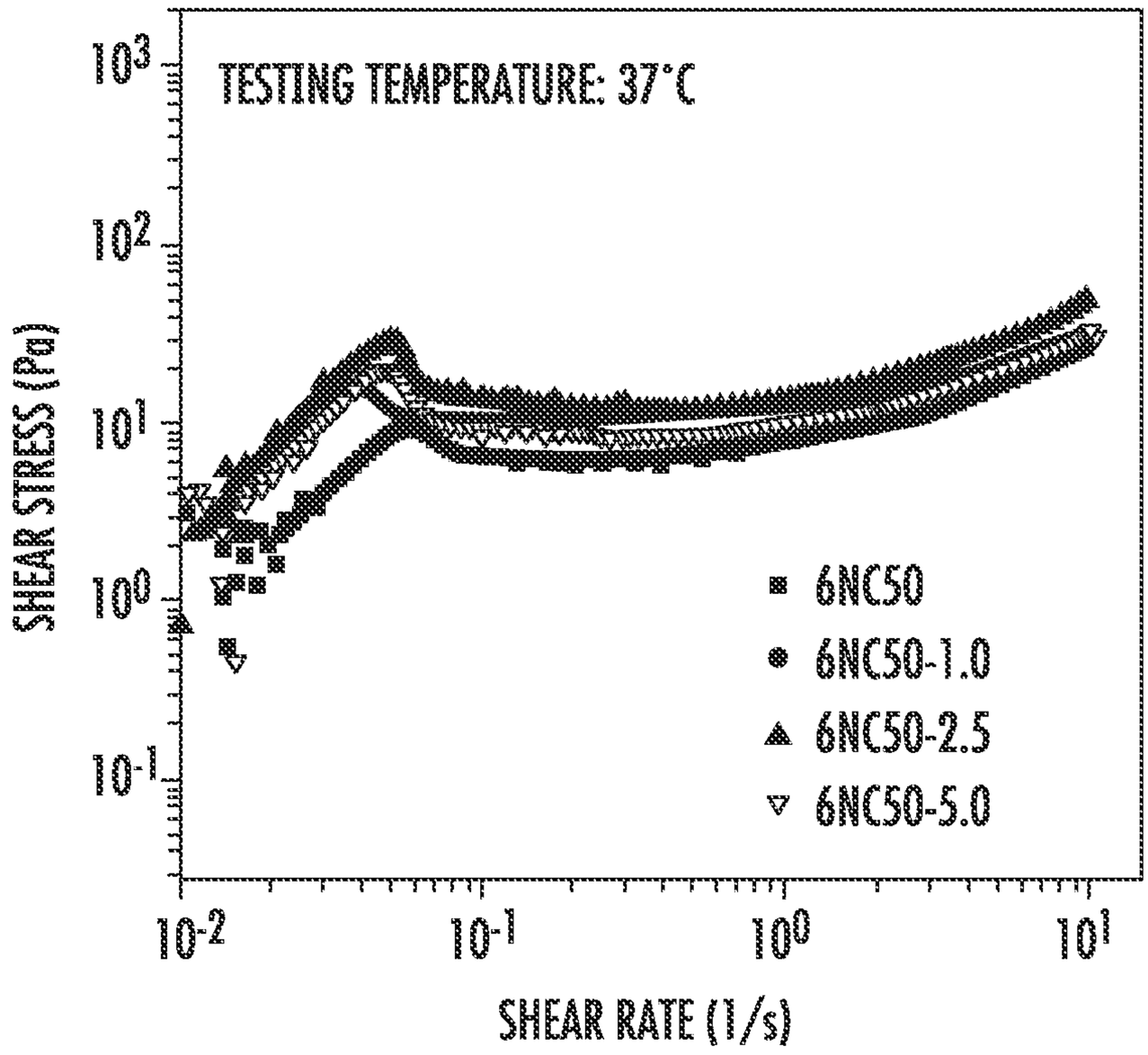

TESTING TEMPERATURE: 37°C
6NC50
6NC50-1.0
6NC50-2.5
6NC50-5.0
SHEAR STRESS (Pa)
SHEAR RATE (1/s)

FIG. 2B

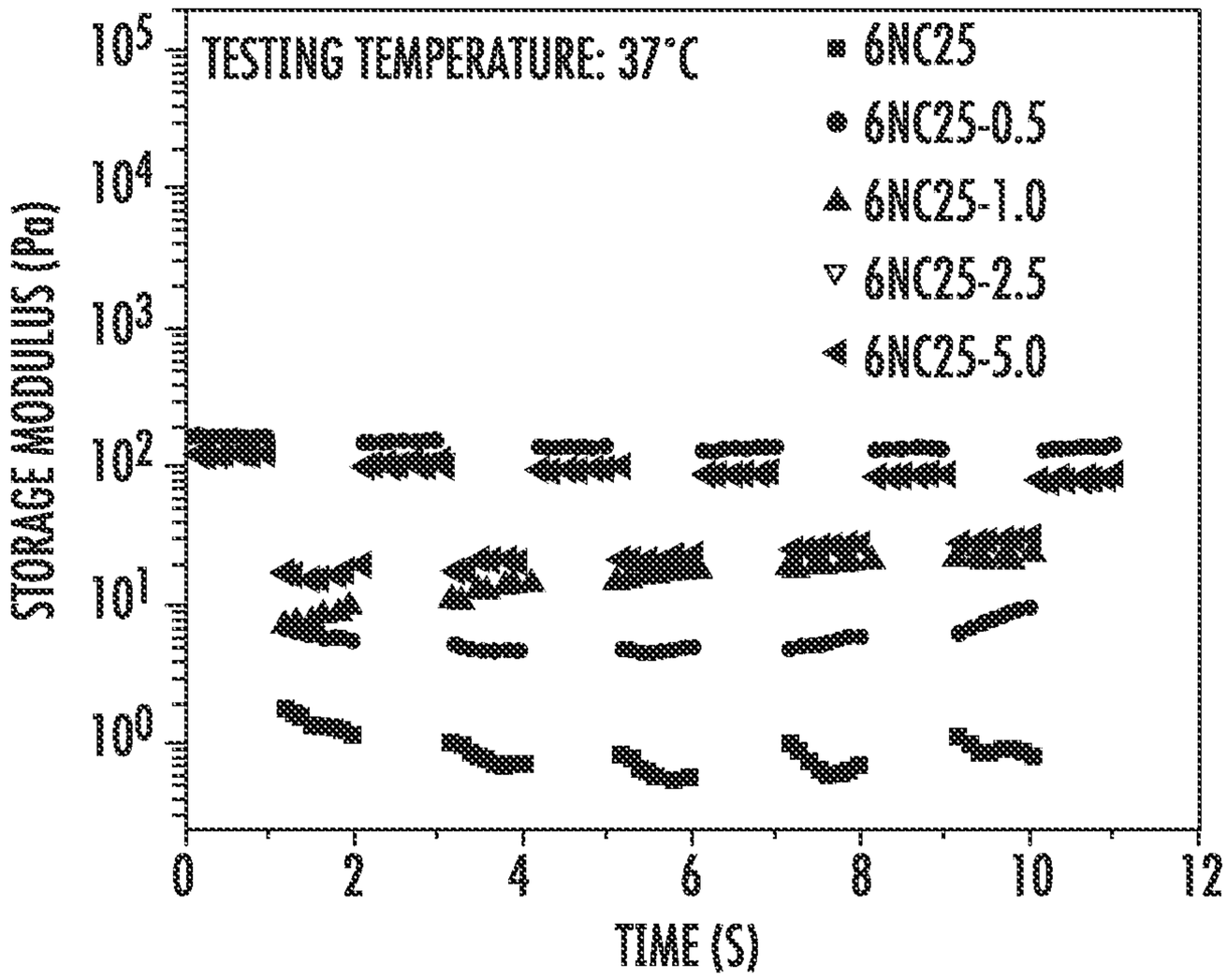

TESTING TEMPERATURE: 37°C
6NC25
6NC25-0.5
6NC25-1.0
6NC25-2.5
6NC25-5.0
STORAGE MODULUS (Pa)
10^5
10^4
10^3
10^2
10^1
10^0
0
2
4
6
8
10
12
TIME (S)

FIG. 2C

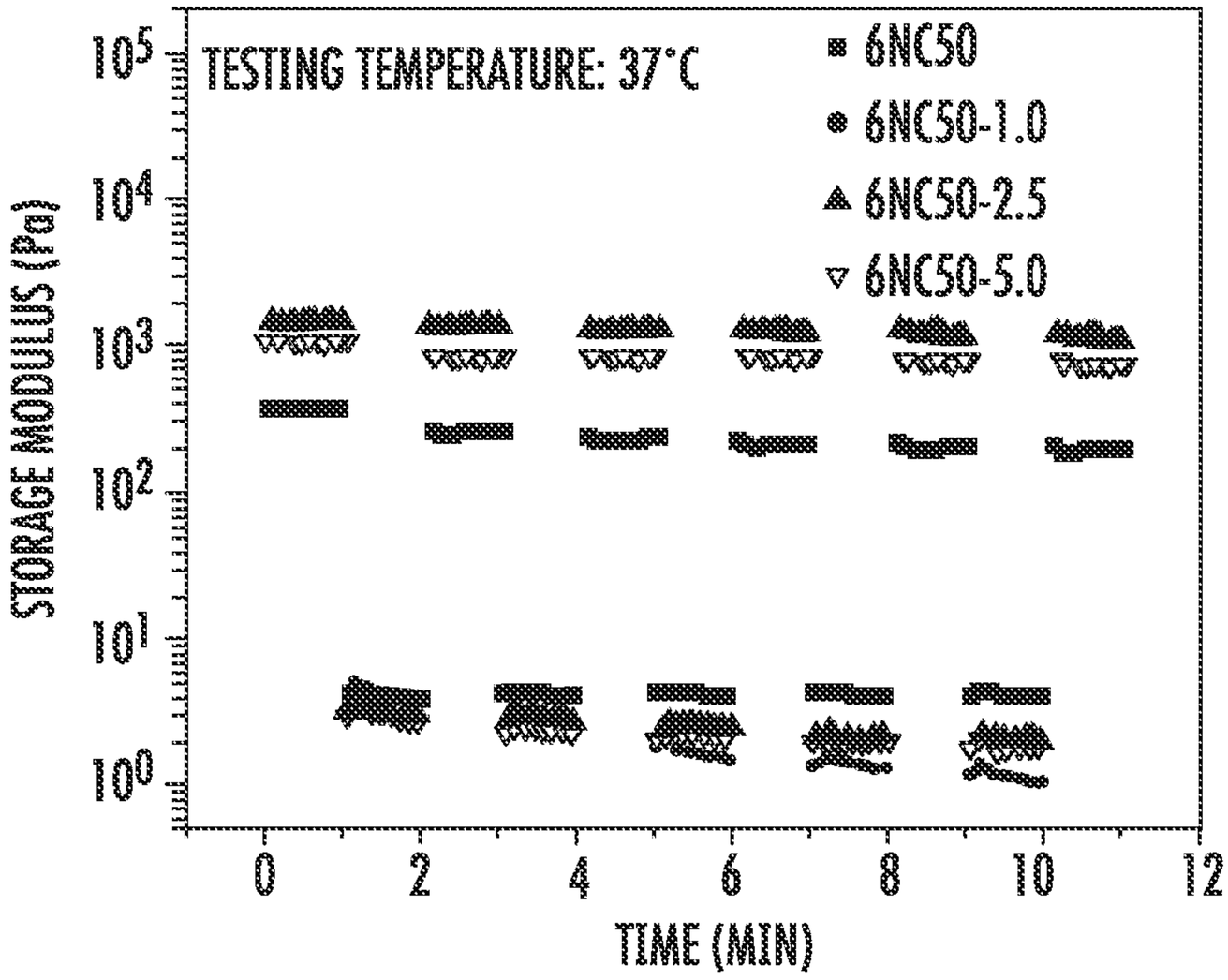

TESTING TEMPERATURE: 37°C
6NC50
6NC50-1.0
6NC50-2.5
6NC50-5.0
STORAGE MODULUS (Pa)
10^5
10^4
10^3
10^2
10^1
10^0
0
2
4
6
8
10
12
TIME (MIN)

FIG. 2D

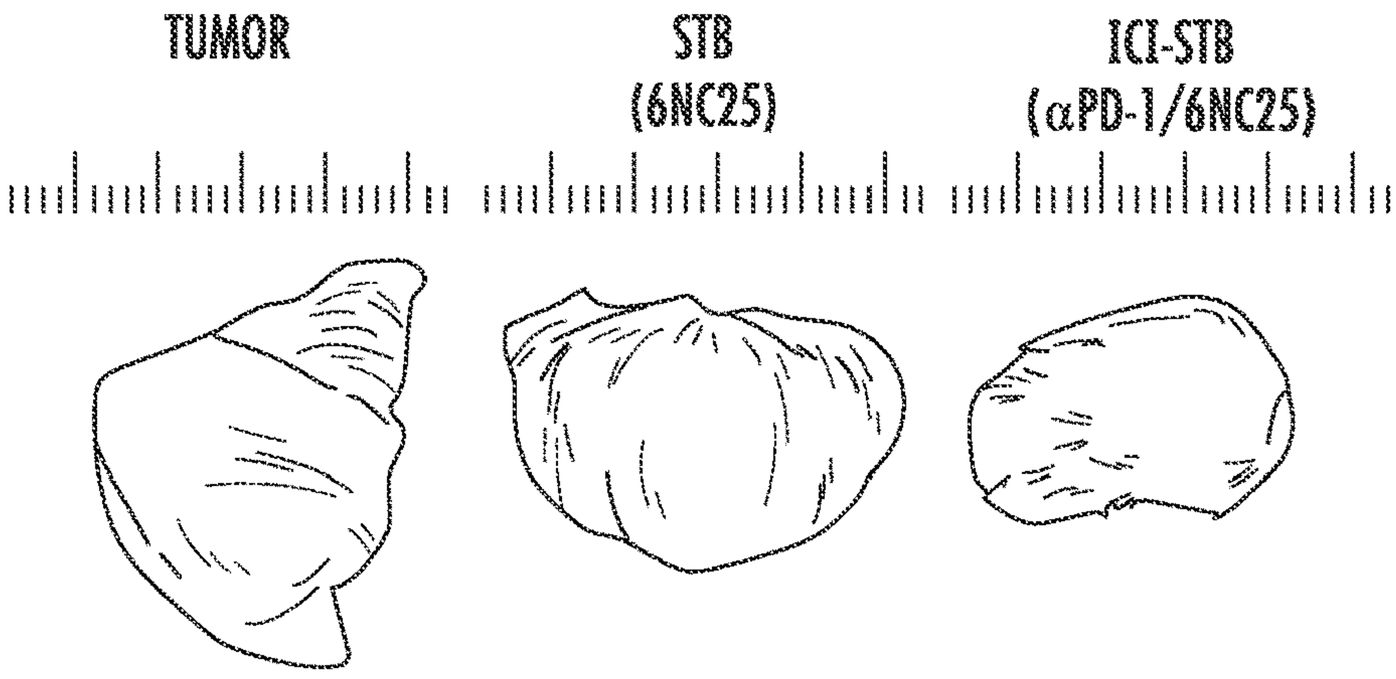


FIG. 3A

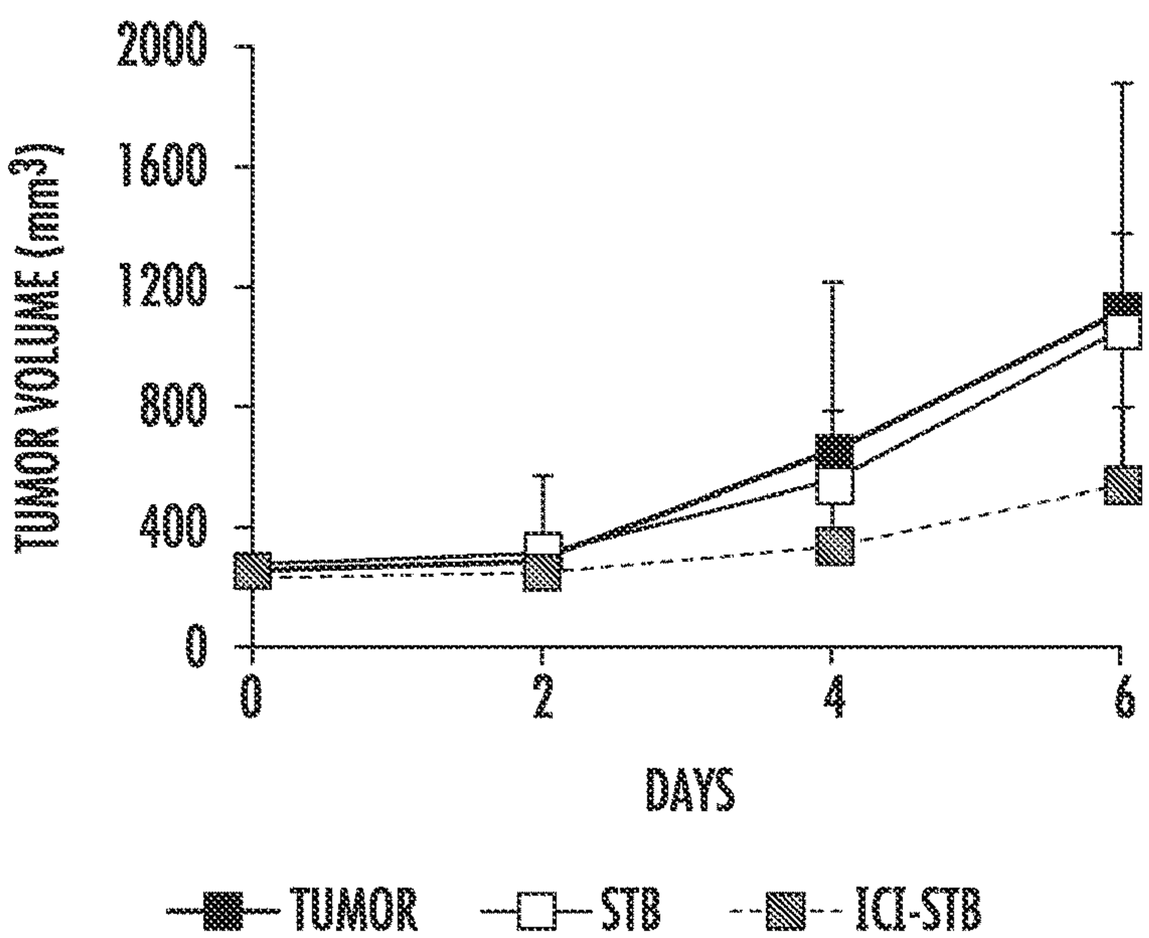


FIG. 3B

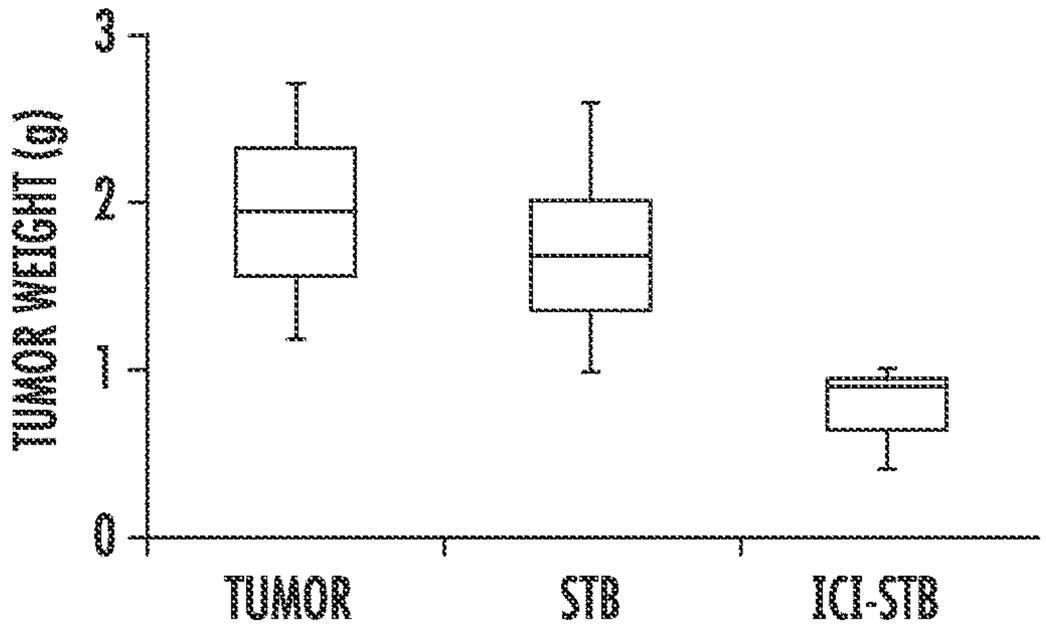


FIG. 3C

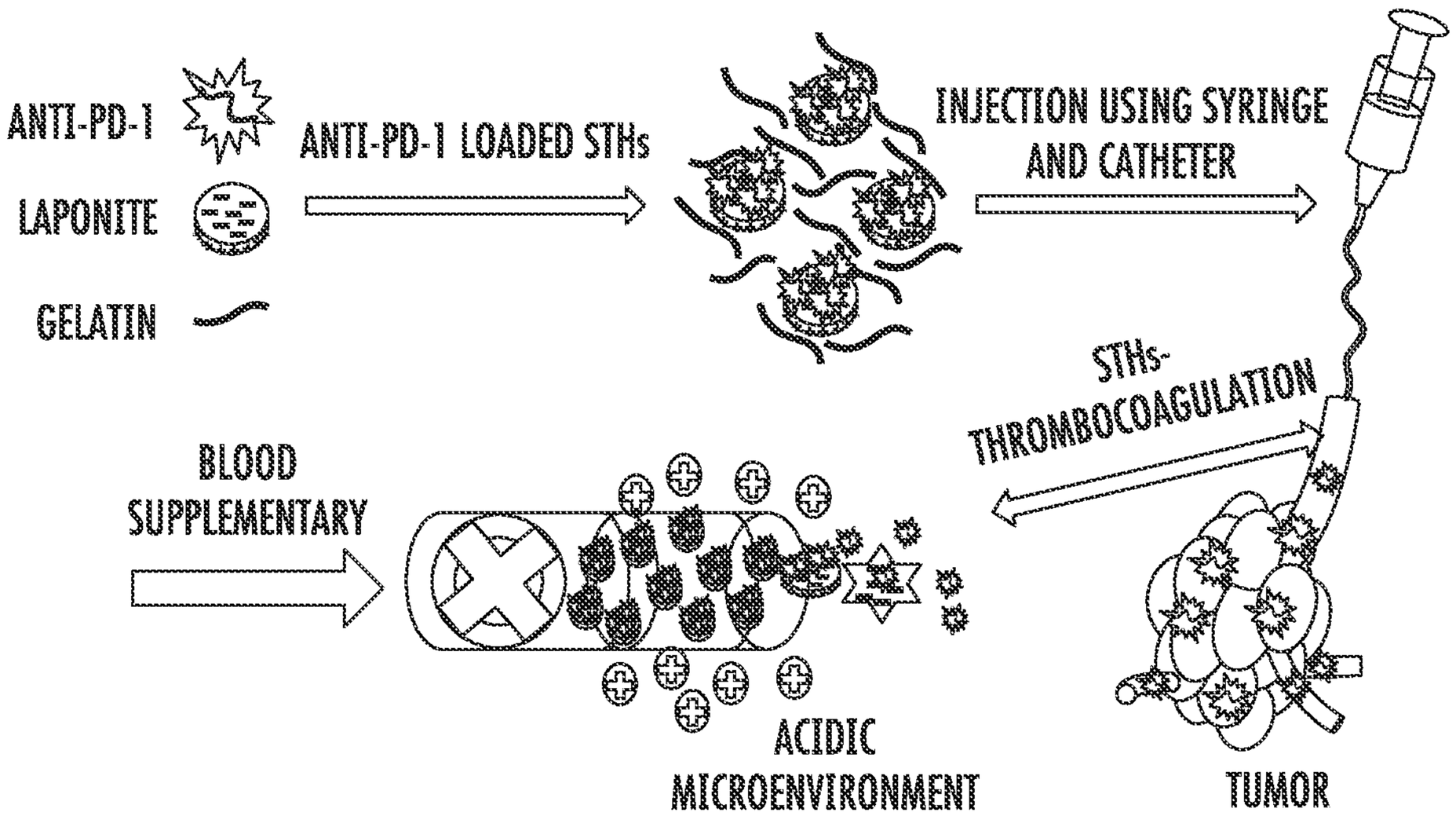

ANTI-PD-1
LAPONITE
GELATIN
ANTI-PD-1 LOADED STHs
INJECTION USING SYRINGE AND CATHETER
STHs-THROMBOCOAGULATION
BLOOD SUPPLEMENTARY
ACIDIC MICROENVIRONMENT
TUMOR

FIG. 4

INJECTABLE SHEAR-THINNING HYDROGEL CONTAINING POLYPEPTIDE THERAPEUTIC AGENT FOR ENHANCED TUMOR THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Ser. No. 63/250,441 filed on Sep. 30, 2021 and U.S. Provisional Patent Application Ser. No. 63/249,949 filed on Sep. 29, 2021. These provisional applications are incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with government support under Grant Numbers HL140951, HL137193, awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

[0003] The present invention relates to shear thinning biomaterials comprising bioactive agents and methods for making and using them.

BACKGROUND OF THE INVENTION

[0004] Shear-thinning hydrogels are non-Newtonian materials that behave as viscous fluids under shear stress and then recover solid-like properties upon elimination of the stress [1]. Due to these properties, injectable shear-thinning biomaterials (STB) are attracting attention as a group of self-healing materials that allow for fluent infusion and local equilibrium after approaching the final application site. In clinical applications, STBs can be delivered into the body using a needle or a general/microcatheters by manual pressure [2-4]. To optimize the clinical application, it is necessary to adjust the physical properties of STB according to the specific clinical situations. A number of physiological and clinical parameters affect the formulation design, including but not limited to (1) peri-tumoral pH (environmental pH of tumor) (2) Tumor cell density, (3) Extracellular matrix synthesis of Tumor, (4) Treatment duration, (5) Aggressiveness of tumor, (6) Tumor physiology including central necrotic lesion formation, (7) Accessibility of tumor (8), Drug solubility, and (9) Hydrophilicity of drugs/agents. By changing the physical properties, one can synthesize tailored hydrogels for specific clinical applications, such as embolizing a certain size of blood vessel, controlled drug release, and modulation of the stiffness of tissue engineering scaffolds.

[0005] The physical properties of conventional STBs can be modulated by a combination of several carbon-based, polymeric, and inorganic nanomaterials [5-8]. Several biomaterials, such as gelatin, hyaluronic acid, chitosan, collagen, and alginate have been previously used along with inorganic constituents to form STBs [14-17]. In particular, gelatin limits the adsorption of nonspecific proteins, enhanced hemolysis, and ultimately prolongs clotting time, demonstrating substantially improved hemocompatibility of STB in vitro [18]. Furthermore, the application of gelatin in tissue engineering and regenerative medicine has been approved by the Food and Drug Administration (FDA) [19-21]. Conventional STBs are prepared by mixing gelatin with synthetic clay nanoparticles, LAPONITE®, for hemostasis and endovascular embolization [18, 22]. These STBs exhibit strong shear-thinning behavior as well as biocompatible properties ranging from blood coagulation to minimized inflammatory response. Others have extended this work to implement STBs as embolic agents [23], functionalized scaffolds [24, 25], 3D-bioinks [26] and drug delivery systems [27]. Unfortunately, however, synthetic clay nanoparticles such as LAPONITE are crystallized nanoparticles and the size, surface chemistry are not easily tuned. In addition, the biocompatibility of LAPONITE in various in vivo uses needs to be further investigated, a fact which complicates its use in clinical applications.

[0006] For the reasons noted above, there is a need in the art for new shear-thinning materials and methods for making and using them.

SUMMARY OF THE INVENTION

[0007] As discussed below, we have developed an immune checkpoint inhibitor (ICI) loaded injectable shear thinning biomaterial (STB) using silicate nanoplatelet (LAPONITE XLG) and gelatin-based polymers. Injectable STB is one class of hydrogels which can be injected by application of shear stress during injection and rapidly self-healing after removal of stress. STBs have attracted considerable attention in biomedical fields, especially for delivery of drugs and cells and for endovascular embolization in a minimally invasive manner due to its ability to be easily applied through a syringe and undergo a fast sol-gel transition at the target sites. LAPONITE (LAPONITE XLG) is a synthetic disk-like silicate nanoparticles with a diameter of 25 nm and a thickness of about 1 nm. The surfaces of LAPONITE are negatively charged while their slices are positively charged. These unique characteristics not only allow this clay to form a hydrogel with shear-thinning property but also endow them promising potential to deliver various drugs by adsorbing them through the electrostatic interaction. Herein, we described a strategy to utilize the injectable STB containing LAPONITE and gelatin to enhance tumor therapy by combining checkpoint blockade and endovascular embolization. As shown in Scheme 1 (FIG. **4**), PD-1-blocking antibody (anti-PD-1) molecules are firstly adsorbed on the surfaces of LAPONITE through electrostatic interaction, which subsequently delivered to vasculature near tumor through syringe in the form of STBs. Anti-PD-1 are released from STBs and then enter the interior of tumor.

[0008] The invention disclosed herein has a number of embodiments. Embodiments of the invention include, for example, a composition of matter comprising a gelatin, silicate nanoparticles and a polypeptide, which is typically a therapeutic agent such as an immune checkpoint inhibitor antibody (e.g. Anti-PD-1). In such compositions, amounts of the gelatin, the silicate nanoparticles and the polypeptide are selected to form a shear thinning hydrogel. In certain embodiments of the invention, the composition comprises from about 1% (w/w) to about 5% (w/w) gelatin (e.g., ranging anywhere from about 1% (w/w) to about 2% (w/w) to about 3% (w/w) to about 4% (w/w) to about 5% (w/w) gelatin) (in other words, ranging between any two of the preceding numerical values), and from about 1% (w/w) to about 5% (w/w) silicate nanoparticles (e.g., ranging anywhere from about 1% (w/w) to about 2% (w/w) to about 3%

(w/w) to about 4% (w/w) to about 5% (w/w) silicate nanoparticles). In certain embodiments of the invention, the composition comprises from about 0.5% (w/w) to about 85% (w/w) gelatin and silicate nanoparticles (e.g., ranging anywhere from about 0.5% (w/w) to about 1% (w/w) to about 2% (w/w) to about 5% (w/w) to about 10% (w/w) to about 25% (w/w) to about 50% (w/w) to about 75% (w/w) to about 85% (w/w) gelatin and silicate nanoparticles). In certain embodiments of the invention, the ratio of the silicate nanoparticles to the gelatin is from about 1.0 to about 0.1 (e.g., ranging anywhere from about 1.0 to about 0.9 to about 0.8 to about 0.7 to about 0.6 to about 0.5 to about 0.4 to about 0.3 to about 0.2 to about 0.1). In certain embodiments, the gelatin is methacrylated gelatin (GelMA), acrylated gelatin, or thiolated gelatin. In certain embodiments of the invention, the composition comprises about 0.5% (w/w) to about 99% (w/w) water (e.g., ranging anywhere from about 0.5% (w/w) to about 1% (w/w) to about 2% (w/w) to about 5% (w/w) to about 10% (w/w) to about 25% (w/w) to about 50% (w/w) to about 75% (w/w) to about 90% (w/w) to about 95% (w/w) to about 97.5% (w/w) to about 99% (w/w) water). In certain embodiments, the composition comprises from about 0.01% (w/w) to about 20% (w/w) of the polypeptide therapeutic agent (e.g., ranging anywhere from about 0.01% (w/w) to about 0.02% (w/w) to about 0.5% (w/w) to about 1% (w/w) to about 2% (w/w) to about 5% (w/w) to about 10% (w/w) to about 15% (w/w) to about 20% (w/w) of the polypeptide therapeutic agent). In certain embodiments, the polypeptide therapeutic agent is selected from the group consisting of cytokines, antibodies, anticancer enzymes, tumor antigens, and pro-apoptotic proteins or peptides. In certain embodiments, the polypeptide therapeutic agent is selected from the group consisting of IL-2, IL-12, IFN-γ, TNF-α, Trastuzumab, Pertuzumab, Bevacizumab, Rituximab, Atezolizumab, Durvalumab, Nivolumab, caspase-3, recombinase (Cre), L-asparaginase, RNase A, DNase I, cGAMP synthase, granzyme B, catalase, OVA, TRP2, Hpg10025-33, p-AH1-A5, MAGE-A3, p53, cytochrome c, KLAKLAKKLAKLAKGG, PTEN, and Saporin. Typically, these compositions further comprise a pharmaceutical excipient selected from the group consisting of a preservative, a tonicity adjusting agent, a detergent, a viscosity adjusting agent, a sugar and a pH adjusting agent. In some embodiments of the invention, the composition further comprises a human cancer cell. The compositions of the invention include one or more polypeptide therapeutic or other agents. In illustrative embodiments of the invention, the therapeutic agent is anti-PD-1 antibody.

[0009] In some embodiments of the invention, amounts of the components are such that when disposed in an environment having a pH of 7.4, less than 10% or less than 5% of agent is released from the thinning hydrogel over a period of 15 days; and when disposed in in an environment having a pH of 5.0, more than 5% or more than 10% is released from shear thinning hydrogel over a period of 15 days. In certain embodiments of the invention, the shear thinning hydrogel requires an injection force of at least 5 newtons but less than 30 newtons to extrude the shear thinning hydrogel from a 2.4Fr Catheter/1 cc syringe.

[0010] Another embodiment of the invention is a method of making a shear-thinning biocompatible composition disclosed herein comprising combining together spherical silica nanoparticles, gelatin and a small therapeutic molecule such as anti-PD-1 antibody, and optionally a pharmaceutical excipient so as to form a shear-thinning biocompatible composition. In certain embodiments of these methods, a surface property of the spherical silica nanoparticles, a median diameter of the spherical silica nanoparticles, a relative amount of spherical silica nanoparticles; and/or a relative amount of gelatin or the like is selected to tune or modulate one or more rheological properties or polypeptide release profile of the shear-thinning biocompatible composition. By modulating the mechanical properties of the compositions of the invention in this manner, embodiments of the invention can be tailored for use in a variety of different clinical applications.

[0011] Another embodiment of the invention is a method of delivering a shear-thinning biocompatible composition disclosed herein to a preselected site (e.g. an in vivo location comprising cancer cells). Typically, such methods comprise disposing the composition in a vessel having a first end comprising an opening and a second end (e.g. a catheter); applying a force to the second end of the vessel, wherein the force is sufficient to liquify the composition; and then delivering the composition out of the vessel through the opening and to the preselected site. In some embodiments, the vessel comprises a syringe loaded with the composition, the syringe configured for fluid communication with a needle and/or a catheter tube. Embodiments of such methods include treatment regimens that use a shear-thinning biocompatible composition disclosed herein to deliver therapeutic polypeptides such as antibodies. In certain embodiments, the present disclosure is directed to method of treating a solid tumor in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a shear-thinning biocompatible composition disclose herein. In certain embodiments, the solid tumor is selected from a blood vessel tumor, brain tumor (e.g., meningioma or glioblastoma), spinal tumor, carotid body tumor, liver cancer, lung cancer, neuroendocrine tumor, renal tumor, pancreatic tumor or prostatic tumor. In certain embodiments, the solid tumor is selected from skin cancer (e.g., melanoma, mast cell tumor, squamous cell carcinoma, or basal cell tumor), breast cancer, sarcomatous tumor (e.g., fibrosarcoma, leiomyosarcoma, rhabomyosarcoma, osteosarcoma, or chondrosarcoma), and lymphomatous tumor. In certain embodiments, the composition is administered to a localized area in need of treatment. In certain embodiments, the composition provides sustained release of a therapeutically effective amount of the polypeptide therapeutic agent to the localized area. In certain embodiments, the localized area comprises a solid tumor. In certain embodiments, the composition provides tumor embolization and sustained release of a therapeutically effective amount of the polypeptide therapeutic agent to the tumor.

[0012] Other objects, features and advantages of the present invention will become apparent to those skilled in the art from the following detailed description. It is to be understood, however, that the detailed description and specific examples, while indicating some embodiments of the present invention, are given by way of illustration and not limitation. Many changes and modifications within the scope of the present invention may be made without departing from the spirit thereof, and the invention includes all such modifications.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. **1**A shows anti-PD-1 released from STHs with different Laponite/gelatin ratios in PBS (pH=7.4). FIG. **1**B and FIG. **1**C show anti-PD-1 released from various compositions loaded with different antibody concentrations in PBS (pH=7.4). FIG. **1**D and FIG. **1**E show release profile data from various compositions comprising anti-PD-1 antibody loaded STBs under different pH conditions.

[0014] FIG. **2**A and FIG. **2**B shows shear stress data from various compositions of STBs with different antibody concentrations. FIG. **2**C and FIG. **2**D show the mechanical properties of various compositions of STBs with different antibody concentrations.

[0015] FIG. **3**A shows photographs of tumors treated with embodiments of the invention. FIG. **3**B provides data from an in vivo study assessing direct-contact anti-cancer effect of anti-PD-1 antibody loaded STBs. FIG. **3**C shows data from a tumor weight study on the day of sacrifice.

[0016] FIG. **4** shows Scheme 1, an illustration of dual functions of injectable STHs to enhance tumor therapy by combining the anti-PD-1 delivery and endovascular embolization.

DETAILED DESCRIPTION OF THE INVENTION

[0017] In the description of embodiments, reference may be made to the accompanying figures which form a part hereof, and in which is shown by way of illustration a specific embodiment in which the invention may be practiced. It is to be understood that other embodiments may be utilized, and structural changes may be made without departing from the scope of the present invention. Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. Many of the aspects of the techniques and procedures described or referenced herein are well understood and commonly employed by those skilled in the art. The following text discusses various embodiments of the invention.

[0018] Shear-thinning biomaterial (STB) technology offers unique properties enabling a solid polymer and drugs loaded inside to be easily delivered directly through means such as catheters into a target area. In view of this, we have developed a class of novel shear-thinning biomaterials using silica nanoparticles and gelatin-based polymers. Embodiments of the invention include, for example, a shear-thinning biocompatible composition of matter comprising silica nanoparticles, gelatin and a polypeptide such as an antibody. Among inorganic composite compositions, silica is classified as "Generally Recognized As Safe (GRAS)" FDA, and is considered one of the most biocompatible materials. In view of this, silicate nanoparticles have been employed in pharmaceutical, cosmetic and food industries as active ingredients or rheological modifiers due to their uniform particle size with electrical surface charges and bioactive properties [9, 10]. In addition, the size, structure, surface properties of silica nanoparticles can be easily tuned, properties which allow silica nanoparticles to provide a class of materials that are useful in a wide variety of biomedical applications.

[0019] The compositions of the invention can include further constituents such as additional polymers, excipients, therapeutic agents and the like. For example, compositions of the invention can include one or more Food and Drug Administration (FDA) approved or cytocompatible polymers. Such polymers include alginate, chitosan, collagen, hyaluronic acid (HA), chondroitin sulfate (ChS), dextrin, gelatin, fibrin, peptide, and silk. Synthetic polymers such as poly(ethylene glycol) (PEG), poly(ethylene oxide) (PEO), poloxamer (Pluronic®) (PEO-PPO-PEO), polyoxamine (Tetronic®) (PEO-PPO), poly(vinyl alcohol) (PVA), poly (lactic-co-glycolic acid) (PLGA), poly(glycolic acid) (PGA), poly(lactic acid) (PLA), polycaprolactone (PCL), poly(L-glutamic acid) (PLga), polyanhydrides, poly(N-isopropylacrylamide) (PNIPAAm), polyaniline and the like can also be included in compositions of the invention. As is known in the art, preparations of hydrogels can be made to include either chemically or physically crosslinked materials.

[0020] Certain embodiments of the compositions of the invention include, for example a pharmaceutical excipient such as one selected from the group consisting of a preservative, a tonicity adjusting agent, a detergent, a viscosity adjusting agent, a sugar and a pH adjusting agent. For compositions suitable for administration to humans, the term "excipient" is meant to include, but is not limited to, those ingredients described in Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins, 21st ed. (2006) the contents of which are incorporated by reference herein.

[0021] Optionally, the compositions of the invention include one or more polypeptide therapeutic agents such as an immune checkpoint inhibitor or the like. In a working embodiment of the invention disclosed herein, the polypeptide therapeutic agent is anti-PD-1 antibody. Compositions of the invention can be formulated for use as carriers or scaffolds of therapeutic agents such as drugs, cells, proteins, and bioactive molecules (e.g., enzyme). As carriers, such compositions can incorporate the agents and deliver them to a desired site in the body for the treatments of a variety of pathological conditions. These include, for example, infectious and inflammatory diseases (e.g. Parkinson's disease, bacterial and antimicrobial infection, diabetes and the like) as well as cancers (e.g. colon, lung, breast, ovarian, lymphoma cancers and the like). In addition, as scaffolds, compositions of the invention can provide a flexible dwelling space for cells and other agents for use in tissue repair and the regeneration of desired tissues (e.g. for cartilage, bone, retina, brain, and, neural tissue repair, vascular regeneration, wound healing and the like). Moreover, embodiments of the invention can include immunomodulatory agents useful for immunotherapy in order to, for example, modulate components of the immune system. Certain illustrative materials and methods that can be adapted for use in such embodiments of the invention are found, for example in Hydrogels: Design, Synthesis and Application in Drug Delivery and Regenerative Medicine 1st Edition, Singh, Laverty and Donnelly Eds; and Hydrogels in Biology and Medicine (Polymer Science and Technology) UK ed. Edition by J. Michalek et al.

[0022] Embodiments of the invention include, for example, a composition of matter comprising a gelatin, silicate nanoparticles and a polypeptide. In such compositions, amounts of the gelatin, the silicate nanoparticles and the polypeptide are selected to form a shear thinning hydrogel. In certain embodiments of the invention, the composition comprises from about 1% to about 5% gelatin, and from about 1% to about 5% silicate nanoparticles. Typically, these compositions further comprise a pharmaceutical excipient selected from the group consisting of a preservative, a tonicity adjusting agent, a detergent, a viscosity adjusting agent, a sugar and a pH adjusting agent. In some embodiments of the invention, the composition further comprises a human cancer cell. In illustrative embodiments of the invention, the polypeptide is an anti-PD-1 antibody.

[0023] In some embodiments of the invention, the composition is disposed within a vessel (e.g. a catheter) selected for its ability to facilitate a user modulating one or more rheological properties of the composition. Certain illustrative materials and methods that can be adapted for use in embodiments of the invention are found, for example in Biomedical Hydrogels: Biochemistry, Manufacture and Medical Applications (Woodhead Publishing Series in Biomaterials) 1st Edition; Steve Rimmer (Editor). As shown in FIGS. **2**A-**2**D, illustrative embodiments of the invention, the components of the composition are selected to provide desirable rheological properties.

[0024] Another embodiment of the invention is a method of delivering a shear-thinning biocompatible composition disclosed herein to a preselected site (e.g. an in vivo location where an individual has experienced trauma or injury or exhibits a pathology such as a cancer). Typically, such methods comprise disposing the composition in a vessel having a first end comprising an opening and a second end (e.g. a catheter); applying a force to the second end of the vessel, wherein the force is sufficient to liquify the composition; and then delivering the composition out of the vessel through the opening and to the preselected site. Embodiments of such methods include treatment regimens that use a shear-thinning biocompatible composition disclosed herein to deliver a therapeutic agent as shown, for example, in FIGS. **3**A-**3**C.

[0025] Yet another embodiment of the invention is a method of making a shear-thinning biocompatible composition disclosed herein comprising combining together silica nanoparticles and gelatin, a polypeptide (e.g. anti-PD-1 antibody) and optionally a pharmaceutical excipient so as to form a shear-thinning biocompatible composition. In certain embodiments of these methods, a surface property of the silica nanoparticles, a median diameter of the silica nanoparticles, a relative amount of silica nanoparticles; and/or a relative amount of gelatin or the like is selected to tune or modulate one or more rheological properties or polypeptide release profile properties of the shear-thinning biocompatible composition. By modulating the mechanical properties of the compositions of the invention in this manner, embodiments of the invention can be tailored for use in a variety of different clinical applications. In this context, a wide variety of art accepted materials and methods can be adapted for use in embodiments of the invention, for example those disclosed in U.S. Patent Publication Nos.: 20050227910, 20100120149, 20120315265, 20140302051 and 20190290804; and Lee, Biomaterials Research volume 22, Article number: 27 (2018); Thambi et al., J Control Release. 2017 Dec. 10; 267:57-66. doi: 10.1016/j.jconrel.2017.08.006. Epub 2017 Aug. 4; and Gianonni et al., Biomater. Sci., 2016, the contents of which are incorporated by reference.

Illustrative Materials and Methods of the Invention

[0026] As an illustrative working embodiment of the invention, we developed an anti-PD-1 antibody loaded injectable shear thinning biomaterials (STB) using silicate nanoplatelet (LAPONITE XLG) and gelatin-based polymers. Shear-thinning biomaterial technology offers unique properties enabling drugs loaded inside to be easily delivered directly through needles or catheters into target area. We focused on the fact that shear thinning biomaterial is 1) injectable and easy to apply, and 2) exhibits high localization due to the high mechanical stability after injection. Therefore, we loaded anti-cancer drug, anti-PD-1 antibody into the shear thinning material to treat solid tumor.

[0027] We tested three different STB compositions (Gelatin 4.5%/LAPONITE 1.5% [6NC25]; Gelatin 3.0%/LAPONITE 3.0% [6NC50]; Gelatin 1.5%/LAPONITE 4.5% [6NC75]) for the ICI-loading biomaterials and anti-PD-1 antibody release profile was analyzed for 30 days (FIG. **1**A). The release ratio of anti-PD-1 from STBs was sharply decreased with the increase of LAPONITE content in the STBs. As shown in FIGS. **1**B and **1**C, when the antibody concentration was adjusted ranging from 0.5 μg/mg STB to 5.0 μg/mg STB, the release rate of anti-PD-1 was remarkably increased from 1 ug/mg STB (2.58±0.05%) to 5.0 ug/mg STB (8.92±0.14%) in the case of 6NC50 (FIG. **1**C). While the highest release of anti-PD-1 was observed at the antibody concentration of 1 ug/mg STB in the case of 6NC25 (FIG. **1**B). PBS with different pH values (7.4, 6.0, and 5.0) was used to evaluate its influence on the release of anti-PD-1(FIGS. **1**D and **1**E). The results show that the release of anti-PD-1 was greatly improved in the acidic conditions.

[0028] As shown in FIGS. **2**A and **2**B, the similar shear stress-shear rate curves were observed in 6NC25 loading anti-PD-1 with different concentrations, indicating no significant influence on shear-thinning behavior of 6NC25 and 6NC50 after loading of antibody at 37° C. Storage modulus reflects the recovery from liquid-like behavior to solid-like behavior after application of high strain (100%) and low strain (1%). FIGS. **2**C and **2**D showed that storage moduli of STBs loading anti-PD-1 with different concentrations after five cycles of high (100%) to low (1%) oscillatory strain amplitudes. In the case of 6NC25 and 6NC50, the storage modulus during 100% strain oscillations was slightly lower than the initial values during high strain after five cycles, indicating rapid recovery of the organic-inorganic hybrid network consisting of LAPONITE and gelatin. These results show that the both shear-thinning performance and storage moduli of STBs remain stable under physiological temperature regardless of loading anti-PD-1 with different concentrations.

[0029] Finally, in vivo anti-cancer efficacy of ICI-STB was analyzed in a melanoma tumor model made by subcutaneous injection of B16F10 melanoma tumor cells into C57Bl/6J mice (FIG. **3**A). In gross, STB (6NC25) itself did not show anti-cancer efficacy, while tumor size decreased in anti-PD1 loaded 6NC25. On the tumor growth curve measured by caliper every two days, anti-PD1 loaded STB group was found to inhibit melanoma growth (FIG. **3**B). Interest-

ingly, median tumor weights of ICI-STB (anti-PD1 loaded 6NC25) was less than others in tumor weight (FIG. **3**C).

[0030] In summary, we developed a novel shear-thinning biomaterial with the composite of anti-PD-1 antibody, gelatin and biocompatible silica nanoparticles with selected desirable properties. By tuning the properties of silica nanoparticles such as particles size and composition of gelation/silica nanoparticles, the mechanical and rheological properties can be carefully adjusted to meet different requirements. All the compositions used in these experiments are injectable through different sized catheters. The rheological tests showed rapid recovery and mechanical stability with shear-thinning characteristics. Gelatin-Silica nanoparticles-based STBs display superior biological stability, body temperature extrudability, shear-thinning behavior, and rapid network recoverability. These attractive physicochemical properties are favorable for easy administration in vivo, and a gelatin-Silica nanoparticle-based STB may hold great potential in drug delivery, endovascular embolization, tissue regeneration, bioprinting and for other biomedical applications.

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[0084] All publications mentioned herein (e.g. the references numerically listed above, and U.S. Patent Publication 20180104059) are incorporated herein by reference to disclose and describe aspects, methods and/or materials in connection with the cited publications.

[0085] The Appendix that is included with this provisional application provides invention disclosure that is formatted for publication in a journal. This disclosure shows various aspects and embodiments of the invention. This Appendix is incorporated by reference herein.

1. A shear thinning hydrogel composition comprising:

a gelatin;

silicate nanoparticles; and

a polypeptide therapeutic agent; wherein:

the gelatin, the silicate nanoparticles and the therapeutic agent form a shear thinning hydrogel.

2. The composition of claim **1**, wherein the composition comprises:

from about 1% (w/w) to about 5% (w/w) gelatin; and

from about 1% (w/w) to about 5% (w/w) silicate nanoparticles.

3. The composition of claim **1**, wherein

when disposed in an environment having a pH of 7.4, less than 10% or 5% of agent is released from the shear thinning composition over a period of 15 days; and

when disposed in in an environment having a pH of 5.0, more than 5% or 10% is released from shear thinning hydrogel over a period of 15 days.

4. The composition of claim **1**, wherein the composition comprises from about 0.5% (w/w) to about 85% (w/w) gelatin and silicate nanoparticles.

5. The composition of claim **1**, wherein the ratio of the silicate nanoparticles to the gelatin is from about 1.0 to about 0.1.

6. The composition of claim **1**, wherein the gelatin is methacrylated gelatin (GelMA), acrylated gelatin, or thiolated gelatin.

7. The composition of claim **1**, further comprising about 0.5% (w/w) to about 99% (w/w) water.

8. The composition of claim **1**, wherein the composition comprises from about 0.01% (w/w) to about 20% (w/w) of the polypeptide therapeutic agent.

9. The composition of claim **1**, wherein the polypeptide therapeutic agent is selected from the group consisting of cytokines, antibodies, anticancer enzymes, tumor antigens, and pro-apoptotic proteins or peptides.

10. The composition of claim **9**, wherein the polypeptide therapeutic agent is selected from the group consisting of IL-2, IL-12, IFN-γ, TNF-α, Trastuzumab, Pertuzumab, Bevacizumab, Rituximab, Atezolizumab, Durvalumab, Nivolumab, caspase-3, recombinase (Cre), L-asparaginase, RNase A, DNase I, cGAMP synthase, granzyme B, catalase, OVA, TRP2, Hpg10025-33, p-AH1-A5, MAGE-A3, p53, cytochrome c, KLAKLAKKLAKLAKGG, PTEN, and Saporin.

11. The composition of claim **1**, wherein the polypeptide therapeutic agent comprises an anti-PD-1 antibody.

12. The composition of claim **1**, wherein the shear thinning hydrogel requires an injection force of at least 5 newtons but less than 30 newtons to extrude the shear thinning hydrogel from a 2.4Fr Catheter/1 cc syringe.

13. The composition of claim **1**, further comprising a human cancer cell.

14. The composition of claim **1**, further comprising a pharmaceutical excipient selected from a preservative, a tonicity adjusting agent, a detergent, a viscosity adjusting agent, a sugar and a pH adjusting agent.

15. The composition of claim **1**, wherein the composition is disposed within a catheter.

16. A method of delivering a composition to a preselected site comprising:

disposing the composition in a vessel having a first end comprising an opening and a second end, the composition comprising a gelatin, silicate nanoparticles, and a polypeptide therapeutic agent, wherein the gelatin, the silicate nanoparticles and the therapeutic agent form a shear thinning hydrogel;

applying a force to the second end of the vessel, wherein the force is sufficient to liquify the composition;

delivering the composition out of the vessel through the opening and to the preselected site.

17. The method of claim **16**, wherein the site is an in vivo site.

18. The method of claim **16**, wherein the site is at an in vivo location comprising cancerous cells.

19. The method of claim **16**, wherein the vessel is a catheter.

20. A method of treating a solid tumor in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a composition comprising a gelatin, silicate nanoparticles, and a polypeptide therapeutic agent, wherein the gelatin, the silicate nanoparticles and the therapeutic agent form a shear thinning hydrogel.

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