

MERCERIZED DECELLULARIZED PARTICLES FOR SLOW RELEASE APPLICATIONS AND METHODS THEREOF

FIELD OF THE INVENTION

[1] This invention relates to mercerized decellularized formulations for slow, controlled, or sustained release applications. More specifically, this invention relates to mercerized decellularized particles and methods of providing slow, controlled, or sustained release of a compound of interest.

BACKGROUND OF THE INVENTION

[2] A controlled release of compounds is desired to elicit specific biological responses or to provide continued and time-resolved therapeutic treatments. It has widespread applications in bioengineering for drug and gene delivery. Apart from these, slow or controlled release of biomolecules are also desired in tissue engineering applications.

[3] Presently, microencapsulation, diffusion systems, dissolution systems, osmotic systems, ion-exchanges, gastric floating systems, bio-adhesive systems, and matrix systems are being used to manipulate release kinetics of biomolecules. These current strategies rely on several secondary components and external factors to achieve the desired functionality. Additionally, they pose several issues and are not optimum for slow release applications.

[4] Therefore, there is a need of a better solution to achieve the slow release functionality with a system that is reproducible and is capable of delivering biomolecules at the desired rate kinetics.

SUMMARY OF THE INVENTION

[5] The invention describes a method of providing a slow, controlled or sustained release of a compound of interest. The method mainly involves a step of incubating or mixing the compound of interest with mercerized decellularized cellulose particles for a predetermined incubation time. This step is followed by a step of adjusting or manipulating a physical or a chemical property of the mercerized decellularized cellulose particles, the compound of interest, or a mixture of both, to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.

[6] The invention also describes a method of providing a slow, controlled or sustained release of lidocaine, ibuprofen, RGD-motif, tryptophan, acetaminophen, diclofenac, gentamicin, hyaluronic acid, naproxen, albumin, lysozyme, somatostatin, and insulin. The method involves a step of incubating the target compound with

mercerized decellularized cellulose particles for a predetermined incubation time, where the predetermined incubation time may range from 1-96 hours. The method further involves a step of adjusting or manipulating a physical or a chemical property of the mercerized decellularized cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the target compound in the patient.

[7] The invention also describes cellulose particles for slow, controlled or sustained release of a pre-absorbed compound of interest. The cellulose particles may be mercerized and could be derived from a decellularized plant material known in the art such as apple, banana, mango or pear. A formulation that comprises or contains the cellulose particles recited above along with a physiologically acceptable component is also described.

BRIEF DESCRIPTION OF DRAWINGS

[8] Figure 1 shows a graph of lidocaine slow release from mercerized cellulose after a 24 h incubation with the lidocaine where the absorbance was measured at 290 nm.

[9] Figure 2 shows a graph of lidocaine slow release from mercerized cellulose particles for different pre-incubation times, where grey bar = 24 h, blue bar = 48 h, and pink bar = 72 h.

[10] Figure 3 shows a graph of lidocaine slow release from mercerized cellulose particles for different pre-incubation times where grey bar = 24 h, blue bar = 48 h, and pink bar = 72 h.

[11] Figure 4 shows a graph of lidocaine release from unmodified mercerized decellularized material and succinylated mercerized decellularized material at pH 6.8 and 7.2.

[12] Figure 5 shows a slow release of ibuprofen from 4.5% mercerized decellularized cellulose-based particles.

[13] Figure 6 shows a slow release of ibuprofen from 4.5% mercerized decellularized cellulose-based particles with the exponential fit beginning on day 5.

[14] Figure 7 shows a slow release of tryptophan from 4.5% mercerized decellularized cellulose-based particles.

[15] Figure 8 shows a slow release of tryptophan from 4.5% mercerized decellularized cellulose-based particles with the exponential fit beginning on day 2.

[16] Figure 9 shows slow release of RGD from 4.5% mercerized decellularized cellulose-based particles.

[17] Figure 10 shows standard curve for bovine serum albumin where the data points represent the mean of triplicate readings and standard error of the mean.

[18] Figure 11 shows standard curve for lysozyme where the data points represent the mean of triplicate readings and standard error of the mean.

[19] Figure 12 shows a standard curve for somatostatin-14 where the data points represent the mean of triplicate readings and standard error of the mean.

Figure 13 shows a standard curve for insulin where the data points represent the mean of triplicate readings and standard error of the mean.

[20] Figure 14 shows bovine serum albumin slow release from mercerized cellulose after a pre-incubation of at least 3 days for N = 3 samples at each timepoint.

[21] Figure 15 shows lysozyme slow release from mercerized cellulose after a pre-incubation of at least 3 days for N = 3 samples at each timepoint.

[22] Figure 16 shows somatostatin-14 slow release from mercerized cellulose after a pre-incubation of at least 3 days for N = 3 samples at each timepoint.

[23] Figure 17 shows insulin slow release from mercerized cellulose after a preincubation of at least 3 days for N = 3 samples at each timepoint.

[24] Figure 18 shows bovine serum albumin slow release modelled as an exponential decay function, represented by the exponential fit equation $y = 0.01058 + 0.34261e^{-t/1.89658}$ ($R^2 = 0.999$).

[25] Figure 19 shows lysozyme slow release modelled as a linear function, represented by the linear fit equation $y = -0.02241x + 0.16382$ ($R^2 = 0.919$).

[26] Figure 20 shows somatostatin-14 slow release modelled as an exponential decay function, represented by the exponential fit equation $y = -0.00243 + 0.03157e^{-t/3.0372}$ ($R^2 = 0.977$).

[27] Figure 21 shows insulin slow release modelled as a linear function, represented by the linear fit equation $y = -0.00191x + 0.02068$ ($R^2 = 0.975$).

- [28] Figure 22 shows validation of TRITC-HA fluorescence stability.
- [29] Figure 23 shows standard curve for TRITC-HA in 1X PBS at constant gain of 50, excitation of 552 nm and emission of 576 nm.
- [30] Figure 24 shows hyaluronic acid slow release from mercerized cellulose after an incubation of at least 3 days for N = 3 samples at each timepoint.
- [31] Figure 25 shows HA slow release modelled as an exponential decay function, represented by the exponential fit equation $y = -0.002 + 0.07281e^{-t/2.08322}$ ($R^2 = 0.999$). Error bars represent SEM.
- [32] Figure 26 shows standard curve for gentamicin at 400 nm in 1X PBS. The data points represent the mean of triplicate readings and SEM. The red line represents the linear fit, $y = 0.96107x + 0.06607$ ($R^2 = 0.997$).
- [33] Figure 27 shows gentamicin slow release from mercerized cellulose after a preincubation of at least 3 days for N = 3 samples at each timepoint.
- [34] Figure 28 shows gentamicin slow release modelled as a linear function, represented by the linear fit equation $y = -0.0033x + 0.02612$ ($R^2 = 0.970$).
- [35] Figure 29 shows a standard curve for acetaminophen at 300 nm.
- [36] Figure 30 shows a standard curve for diclofenac at 315 nm.
- [37] Figure 31 shows a standard curve for naproxen at 300 nm.
- [38] Figure 32 shows acetaminophen slow release from mercerized cellulose after a pre-incubation of at least 3 days for N = 3 samples at each timepoint.
- [39] Figure 33 shows diclofenac slow release from mercerized cellulose after a preincubation of at least 3 days for N = 3 samples at each timepoint.
- [40] Figure 34 shows naproxen slow release from mercerized cellulose after a preincubation of at least 3 days for N = 3 samples at each timepoint.

[41] Figure 35 shows acetaminophen slow release modelled as an exponential decay function, represented by the exponential fit equation $y = -0.000439725 + 0.2737e^{-t/2.25448}$ ($R^2 = 0.999$).

[42] Figure 36 shows diclofenac slow release modelled as an exponential decay function, represented by the exponential fit equation $y = -0.14094 + 0.39238e^{-t/5.62176}$ ($R^2 = 0.999$).

[43] Figure 37 shows naproxen slow release modelled as an exponential decay function, represented by the exponential fit equation $y = -0.03244 + 0.3893e^{-t/3.50212}$ ($R^2 = 0.999$).

[44] Figure 38 shows a standard curve for acetaminophen at 300 nm.

[45] Figure 39 shows standard curve for naproxen at 300 nm as the data points represent the mean of triplicate readings and standard error of the mean.

[46] Figure 40 shows acetaminophen slow release from mercerized cellulose after a pre-incubation of 3 days for $N = 3$ samples at each timepoint.

[47] Figure 41 acetaminophen slow release from succinylated cellulose after a pre-incubation of 3 days for $N = 3$ samples at each timepoint.

[48] Figure 42 shows naproxen slow release from mercerized cellulose after a pre-incubation of 3 days for $N = 3$ samples at each timepoint.

[49] Figure 43 shows naproxen slow release from succinylated cellulose after a pre-incubation of 3 days for $N = 3$ samples at each timepoint.

[50] Figure 44 shows acetaminophen slow release from mercerized cellulose modelled as a linear function, represented by the linear fit equation $y = -0.04054x + 0.24961$ ($R^2 = 0.97984$).

[51] Figure 45 shows acetaminophen slow release from succinylated material modelled as a linear function, represented by the linear fit equation $y = -0.04931x + 0.25868$ ($R^2 = 0.96473$).

[52] Figure 46 shows naproxen slow release from mercerized cellulose modelled as a linear function, represented by the linear fit equation $y = -0.04071x + 0.31019$ ($R^2 = 0.99880$).

[53] Figure 47 shows naproxen slow release from succinylated cellulose modelled as a linear function, represented by the linear fit equation $y = -0.04782x + 0.34118$ ($R^2 = 0.98768$).

DETAILED DESCRIPTION

[54] The following description is of preferred embodiments by way of example only and without limitation to the combination of features necessary for carrying the invention into effect.

[55] All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains. The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[56] Although various features of the present disclosure can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the present disclosure can be described herein in the context of separate embodiments for clarity, the present disclosure can also be implemented in a single embodiment.

[57] The following definitions supplement those in the art and are directed to the current application. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[58] **DEFINITIONS**

[59] In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[60] In this application, the use of "or" means "and/or" unless stated otherwise. The terms "and/or" and "any combination thereof and their grammatical equivalents as used herein, can be used interchangeably. These terms can convey that any and all combinations are specifically contemplated. The term "or" can be used conjunctively or disjunctively, unless the context specifically refers to a disjunctive use.

[61] Furthermore, use of the term "including" as well as other forms, such as "include", "includes," and "included," is not limiting.

[62] Reference in the specification to "some embodiments," "an embodiment," "one embodiment" "alternate embodiment", or "other embodiments" means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosures.

[63] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the present disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present disclosure.

[64] The term "about" in relation to a reference numerical value and its grammatical equivalents as used herein can include the numerical value itself and a range of values plus or minus 10% from that numerical value. The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. In another example, the amount "about 10" includes 10 and any amounts from 9 to 11.

[65] The term "slow release" or "sustained release" means slow release of a medication or chemical agent of interest over a period of time. The term "controlled release" means releasing a medication or chemical agent of interest over time in correlation with concentration. These relate to the tailorable delivery of compounds (e.g., drugs, proteins, nutrients, and other biologically active agents or biomolecules) at an effective level in response to time, concentration and stimuli.

[66] The term "mercerized" means a process in which natural cellulose fibers are swollen by immersion in concentrated aqueous NaOH solution and washed with water.

[67] The term "decellularized" means a process by which the extracellular matrix (ECM) of a tissue is isolated from its inhabiting cells, leaving an ECM scaffold of the original tissue, which can be used for various tissue engineering purposes.

[68] **EMBODIMENTS**

[69] In an embodiment of the invention a method of providing a slow, controlled or sustained release of a compound of interest is provided. A person skilled in the art would understand that the compound of interest may be selected from any known compounds, pharmaceutical substances, drugs or biomolecules that may have any therapeutic, diagnostic or medical benefit to a subject. It is understood that the subject could be a human or a non-human subject.

[70] The method involves a step of incubating the compound of interest with mercerized decellularized cellulose particles for a predetermined incubation time followed by a step of adjusting or manipulating a physical or a chemical property of the mercerized decellularized cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. For instance, adjusting or manipulating chemical properties like pKa value, pH value, etc. or adjusting or manipulating physical properties like crystal structure modification, adding a surface charge, succinylation etc. In some embodiments, the decellularized particles may be non-resorbable.

[71] In certain embodiments, the predetermined incubation time may range from 30 minutes to 100 hours. However, in some embodiments the incubation time may range from 1-96 hours. The incubation time allows the cellulose particles to interact with the compound of interest and promotes maximum absorption of the compound of interest.

[72] In certain embodiments, the method may additionally comprise a step of adjusting or manipulating a physical or a chemical property of the compound of interest to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. For instance, adjusting or manipulating size of the compound of interest.

[73] In certain embodiments, the method may additionally comprise a step of pretreating the cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest or to adjust the release kinetics or the release rate of the compound of interest. In certain embodiments, the method may additionally comprise a step of mixing, soaking or centrifuging the mercerized decellularized cellulose particles and the compound of interest to prepare a mixture prior to the step of incubating the compound of interest with mercerized decellularized cellulose particles. The mixing, soaking or centrifuging may also promote absorption of the compound of interest on to the cellulose particles. In certain embodiments, the mixing, soaking or centrifuging step occurs in the presence of a solvent, such as water, saline, or phosphate buffered saline (PBS).

[74] In certain embodiments, the method may additionally comprise adjusting or manipulating a physical or a chemical property of the mixture i.e. the mixture of mercerized decellularized cellulose particles and the compound of interest, to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. For instance, adjusting or manipulating pH of the mixture to trigger the release.

[75] In some embodiments, the method may comprise adjusting or manipulating the physical or the chemical property of the mercerized decellularized cellulose particles, the compound of interest and/or the mixture to control the release pattern or the release rate of the compound of interest. The method may also comprise adjusting or manipulating pKa of the cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. A person skilled in the art would understand that pKa is a number that describes the

acidity of a particular molecule. It may also be defined as the negative base -10 logarithm of the acid dissociation constant (K_a) of a solution.

[76] In some embodiments, the method may comprise adjusting or manipulating pH of the cellulose particles or a solution containing the cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. The method may additionally comprise a step of adjusting or manipulating the concentration of the cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.

[77] In some embodiments, the method may comprise a step of modifying the chemical structure of the cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. For instance, succinylating the surface of the cellulose particles. The method may also comprise a step of modifying the crystal structure of the cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.

[78] In some embodiments, the method may comprise modifying the chemical structure of the mercerized decellularized cellulose particles by succinylating the mercerized decellularized cellulose particles or by adding a charge on the mercerized decellularized cellulose particles. For instance, adding a positive or a negative charge on the surface of the cellulose particles. The method may additionally comprise a step of preparing a combination mixture of the modified mercerized decellularized cellulose particles and the unmodified mercerized decellularized cellulose particles followed by a step of adjusting or manipulating a physical or a chemical property of the combination mixture to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. For instance, preparing a mixture of modified and unmodified particles in a ratio of 1:1, 2:1, 3:1, 4:1 and so on. Alternatively, a mixture of the unmodified and the modified particles can be prepared in a ratio of 1:1, 2:1, 3:1 and so on. In some embodiments, the method may comprise preparing a combination mixture of the modified cellulose particles and the unmodified cellulose particles in a fixed ratio. In some embodiments, the combination mixture may have equal proportions of the modified and unmodified particles. In some embodiments, the proportions may comprise unequal proportions of the modified and unmodified particles.

[79] In certain embodiments, the method may comprise adjusting or manipulating the size of the compound of interest to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. For instance, the different concentrations of compound of interest may be used to trigger release of the compound of interest. Alternatively, the size of the compound may be adjusted to make the compound smaller to trigger release of the compound.

[80] In some embodiments, the method may comprise adjusting or manipulating hydrophilicity of the compound of interest to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. In certain embodiments, the method may comprise adjusting or manipulating the surface charge or adjusting or

manipulating the solubility of the compound of interest to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.

[81] In some embodiments, the method may comprise adjusting, altering or changing the predetermined incubation time to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. In certain embodiments, the method may comprise using varying incubation times to trigger the release of the compound of interest.

[82] In some embodiments, the method comprises adjusting or manipulating pH of the mixture of cellulose particles and the compound of interest to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. In some embodiments, the method comprises adjusting or manipulating pH of the combination mixture of modified and unmodified cellulose particles with or without the compound of interest to trigger release of the compound of interest.

[83] In some embodiments, the method further comprises measuring or quantifying the amount of absorption of the compound of interest in the mercerized decellularized cellulose particles by measuring or quantifying the fluorescence intensity or absorbance of the mixture. The fluorescence intensity or absorbance of the mixture may be quantified in the range of 200-600nm. In some embodiments, the method may comprise preparing a control saline solution and quantifying the amount of absorption of the control solution by measuring the fluorescence intensity or absorbance in the range of 200-600nm. This step is followed by a step of comparing the fluorescence intensity or absorbance of the mixture with the fluorescence intensity/absorbance of the control solution.

[84] In some embodiments, depending on the compound of interest and physical/chemical conditions, the method may trigger different release profiles. For instance, the compound of interest may show a steady state release profile, an exponential decay release profile or a combination thereof. In some embodiments, the method may comprise measuring / quantifying the amount of absorption of the compound of interest in the mercerized decellularized cellulose particles using a colorimetric detection assay or a quantification assay.

[85] In certain embodiments, the above-recited method and the various method steps may be employed for use in slow, controlled or sustained release of an anaesthetic, an antibiotic, an analgesic. The anaesthetic may be selected from any known anaesthetic in the art. In some embodiments the anaesthetic may be selected from one of lidocaine, procaine, chlorprocaine, prilocaine, tetracaine, bupivacaine, cinchocaine, ropivacaine, cocaine, benzocaine, cyclomethycaine, dimethocaine, piperocaine, proxycaine, artocaine, etidocaine, levobupivacaine, mepivacaine, trimecaine, saxitoxin, neosaxitoxin, tetrodotoxin, menthol, eugenol, and splianthol. The antibiotic may be selected from any known antibiotic in the art. In some embodiments the antibiotic may be gentamicin. The antibiotic may be for example gentamicin. The analgesic may be acetaminophen, diclofenac or naproxen.

[86] In some embodiments, the method may be employed to trigger slow, controlled or sustained release of an antibiotic such as gentamicin), or an analgesic, wherein the analgesic is selected from any known analgesic in the art. In some embodiment, the analgesic may be paracetamol, NSAIDs, COX-2 inhibitors, opioids, alcohol, cannabis, and adjuvants. In some embodiments, the method may be used for slow, controlled or sustained release of a biomolecule, wherein the biomolecule may be any biomolecule known in the art. The biomolecule may be selected from the group of protein, nucleic acid, vitamin or mineral, enzyme, glycoprotein, proteoglycan, lipid, sugar, carbohydrates, lignin, cellulose, hemicellulose, pectin, and hormones. The biomolecule may be a protein selected from albumin, lysozyme, somatostatin or insulin. The biomolecule in some cases may be hyaluronic acid.

[87] In some embodiments, the method may be used for slow, controlled or sustained release of a therapeutic substance, wherein the therapeutic substance for any therapeutic substance known in the art. In some embodiments, the therapeutic substance may be selected from the group of stem cells, growth factors, inhibitors, drugs, proteins, platelets, cofactors, and coenzymes. In some embodiments, the method may be employed for the slow, controlled or sustained release of an anti-inflammatory drug known in the art. In some embodiment, the anti-inflammatory drug may be NSAIDS or steroids.

[88] In some embodiments, the mercerized decellularized cellulose particles are derived, obtained or extracted from any plant tissue known in the art. In certain embodiments, the mercerized decellularized cellulose particles are derived, obtained or extracted from apple, banana, mango or pear.

[89] In some embodiments, a method of providing a slow, controlled or sustained release of lidocaine is provided. The method involves a step of incubating lidocaine with mercerized decellularized cellulose particles for a predetermined incubation time, where the predetermined incubation time may range from 1-96 hours. The method may further involve a step of adjusting or manipulating a physical or a chemical property of the mercerized decellularized cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of lidocaine in the patient. For instance, adjusting or manipulating pKa values, chemical structure alteration or modification, adding a positive or negative charge on the cellulose particles, specific concentration of cellulose particles i.e. 4.5% etc. The chemical or physical property adjustments noted hereinbefore may be employed to providing a slow, controlled or sustained release of lidocaine.

[90] In some alternate embodiments, a method of providing a slow, controlled or sustained release of ibuprofen is provided. The method involves a step of incubating ibuprofen with mercerized decellularized cellulose particles for a predetermined incubation time; where the predetermined incubation time may range from 1-96 hours. The method may further involve adjusting or manipulating a physical or a chemical property of the mercerized decellularized cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of ibuprofen in the patient. For instance, adjusting or manipulating pKa values, chemical structure alteration or modification, adding a positive or negative charge on the cellulose particles, specific concentration of cellulose

particles i.e. 4.5% etc. The chemical or physical property adjustments noted hereinbefore may be employed to providing a slow, controlled or sustained release of ibuprofen.

[91] In certain embodiments, cellulose particles for slow, controlled or sustained release of a pre-absorbed compound of interest are provided. The cellulose particles may be mercerized and derived from a decellularized plant material as the ones noted hereinbefore. In some embodiments, the cellulose particles may have a crystal structure. In some embodiments, the cellulose particles may be derived from any plant tissue known in the art. In some embodiments, the cellulose particles may be derived from any plant tissue of apple, banana, mango or pear.

[92] In some embodiments, a concentration of cellulose particles ranging from 1% to 7% by mass may be employed slow, controlled or sustained release of the pre-absorbed compound of interest. Any compound of interest noted hereinbefore maybe pre-absorbed on the cellulose particles. In some embodiments, a concentration of about 0.1% to about 20% of the pre-absorbed compound of interest may be pre-absorbed onto the cellulose particles.

[93] In certain embodiments, a change in pH of the cellulose particles or a solution containing the particles may trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. In some embodiments, a change in the pKa value of the particles triggers, alters, enables or affects the slow, controlled or sustained release of the compound of interest. In some a chemical modification of the particles triggers, alters, enables or affects the slow, controlled or sustained release of the compound of interest. In some embodiments, the cellulose particles may be succinylated to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest. The succinylated particles may show a greater absorbance of the compound of interest compared to the non-succinylated particles.

[94] In some embodiments, a modification to the crystal structure of the cellulose particles triggers, alters, enables or affects the slow, controlled or sustained release of the compound of interest. In certain embodiments, adding a charge on the particles triggers, alters, enables or affects the slow, controlled or sustained release of the compound of interest.

[95] In certain embodiments, the particles may be in the form of a dermal filler or could be employed to prepare a dermal filler. The dermal filler may be prepared with pre-absorbed compound of interest. In some embodiments, the cellulose particles may be used for management of pain, bone regeneration, soft tissue regeneration, regenerative medicine, angiogenesis, treatment of cancer, treatment of wounds, or nerve applications. In certain other embodiments, the cellulose particles may be used for production of growth factors, inhibitory factors, coagulation agents, or anticoagulation agents. In some embodiments, the cellulose particles may be used for slow release delivery of an anti-inflammatory drug, a therapeutic substance, an anaesthetic, an analgesic, or a biomolecule. A person skilled in the art would comprehend that any anti-inflammatory drug, any therapeutic substance, any anaesthetic, any analgesic, or any biomolecule known in the art can be employed in this invention.

[96] For instance, the anaesthetic may be selected from the group of lidocaine, procaine, chlorprocaine, prilocaine, tetracaine, bupivacaine, cinchocaine, ropivacaine, cocaine, benzocaine, cyclomethycaine, dimethocaine, piperocaine, proxycaine, articaine, etidocaine, levobupivacaine, mepivacaine, trimecaine, saxitoxin, neosaxitoxin, tetrodotoxin, menthol, eugenol, and splianthol. For instance, the analgesic may be selected from the group of paracetamol, NSAIDs, COX-2 inhibitors, opioids, alcohol, cannabis, and adjuvants. For instance, the biomolecule may be selected from the group of protein, nucleic acid, vitamin or mineral, enzyme, glycoprotein, proteoglycan, lipid, sugar, carbohydrates, lignin, cellulose, hemicellulose, pectin, and hormones. For instance, the therapeutic substance may be selected from the group of stem cells, growth factors, inhibitors, drugs, proteins, platelets, cofactors, and coenzymes. For instance, the anti-inflammatory drug may be selected from NSAID or steroid.

[97] In some embodiments, a formulation that comprises or contains the cellulose particles recited above along with a physiologically acceptable component. A person skilled in the art would understand that any physiologically acceptable component known in the art could be employed to prepare the formulation.

[98] **EXPERIMENTAL DATA**

[99] Without wishing to be bound by theory or experimental results, the following paragraphs describe the nature of the invention by way of examples only. The experiments, or specific examples of materials, ingredients, and formulations described should not be construed as limiting the scope of the invention. A person skilled in the art would readily understand and appreciate that other materials not specifically described also form part of the invention.

[100] During the experimental design phase, there were many details that needed to be accounted for in order to capture the slow release pattern properly and to avoid having skewed results. Initially, diffusion based experiments were conducted. The diffusion across semi-permeable dialysis membranes was used to investigate the slow release properties. This method was highly dependent on evaporation effects, surface area contact and solvent to solute ratios. Although at face-value, the diffusion method appeared to replicate in vivo situations, the fact was that it was a highly synthetic system. It included several other complications such as affinity for adsorption of the compound of interest onto the dialysis membrane which itself led to inaccurate readings.

[101] During the material processing, the material became increasingly dilute and occupied a larger volume as more washing was conducted. Upon incubation with a salt solution such as phosphate buffered saline (PBS), the material reverted to a denser format. It was further observed that salt release and water absorption were slowly changing with each subsequent washing and centrifugation round. Similarly, during material processing, after the material is neutralized, it was found that the pH would then drift away from the neutral state as more alkali solvent was released from the mercerization process. As a result, multiple rounds of neutralization were needed to ensure

a pH plateau was achieved. In addition, the decellularized scaffolds themselves exhibited slow release kinetics. For instance, decellularized regenerated cellulose that was immersed in dimethylacetamide and ethanol displayed slow release properties as well. Interestingly, when pieces of this material were cut to include an “asymmetric protrusion” that resembled a gear, the material began to spin/rotate without external forces as the slow release of the absorbed chemicals were being released into a surrounding bath. Upon summing up these observations, it was found that the slow release of a given compound of interest was occurring and could be exploited in several different ways. It was hypothesized that the compounds of interest slowly diffuse from the cellulose and/or can be released with pH changes, ion gradients, and solution polarity

[102] It was observed that switching to centrifugation based assays eliminated these issues and allowed for direct measurement via absorbance spectroscopy of the amount of lidocaine that was released from the material mixture. However, the most significant challenge to obtaining accurate results was realized when the potential of pH as a control release “switch” was investigated. It was found that lidocaine absorbance was highly dependent on the pH. As such, if the pH drifted over the course of an experiment, false readings for how much lidocaine was released would occur. After accounting for all these effects, the inventors were able to obtain reproducible results, and this allowed for conclusions to be drawn. It is pertinent to note that the decellularized material provides the necessary structural support and the slow release are both features of the same base material.

[103] **SLOW RELEASE TESTING**

[104] The base materials used in this investigation were mercerized, decellularized cellulose materials as described in related international publication PCT/CA2021050783. U.S. Patent 7,649,089 B2 describes biodegradable oxidized cellulose esters and their uses as microspheres, however, the cellulose esters described therein are biodegradable, synthetic, and involve a different cellulose chemistry.

[105] In the present testing, specifically, 4.5% cellulose-based material by mass was diluted in phosphate buffered saline (PBS). The test samples contained 0.3% lidocaine HCl whereas the controls were complimented by an equal amount of PBS.

[106] As an initial step, the pH was adjusted to 7.2, and quantification was accomplished by measuring the absorbance of the extracts at 290 nm. The extracts were collected after centrifugation of 0.4 mL samples in microcentrifuge tubes at 21,100 x g for 15 min in order to assess the slow release properties of the drugs of interest, namely lidocaine, after certain incubation times (24 h, 48 h, and 72 h). Following the analysis, an equal volume of PBS (150 µL) was added to replenish the volume removed for the absorbance reading.

[107] Figure 1 shows a graph of lidocaine slow release from mercerized cellulose after a 24 h incubation with the lidocaine where the absorbance was measured at 290 nm. N = 3 samples per condition. It is pertinent to note that the bars represent the mean and the error bars represent the standard error of the mean.

[108] It was observed that the amount of lidocaine released from the particles followed an exponential decay. In order to assess whether different pre-incubation times affected the release of the lidocaine, incubation times of 48 h and 72 h were also investigated. A Two-Way ANOVA revealed that there was no significant difference between the pre-incubation times; moreover, there were no significant interaction effects. However, it was observed that there were significant differences with the measurement times at a significance level of 0.05. It is to be noted that $N = 3$ for each sample set and measurement.

[109] Figure 2 shows a graph of lidocaine slow release from mercerized cellulose particles for different pre-incubation times, where grey bar = 24 h, blue bar = 48 h, and pink bar = 72 h. The absorbance was measured at 290 nm and $N = 3$ samples per condition. The bars represent the mean and the error bars represent the standard error of the mean.

[110] In order to fit the slow release data the exponential decay function $y = y_0 + A_1 e^{-t/\tau}$ was used. Each pre-incubation time had an R^2 value of 0.99 for the exponential fit. The fitting enabled the determination of a time constant (τ) for the release where the mean time constant and standard error of the mean was 3.20 ± 0.54 days, and the resultant half-life was 2.22 ± 0.37 days. This release timescale is highly relevant for dermal filler pain relief and related applications.

[111] Figure 3 shows a graph of lidocaine slow release from mercerized cellulose particles for different pre-incubation times where grey bar = 24 h, blue bar = 48 h, and pink bar = 72 h. The absorbance was measured at 290 nm where $N = 3$ samples per condition. Exponential fits for the respective sample set were overlaid as dashed lines. It is to be noted that data points represent the mean and standard error of the mean.

[112] At the conclusion of the experiment, the total amount of lidocaine was added and compared to the initial amount of lidocaine in the samples (1.2 mg in the 0.4 mL samples). It was found that in each case all the lidocaine was removed. The three pre-incubation times yielded a mean total lidocaine mass that was not significantly different from the 1.2 mg theoretical value with a one sample T-test at the significance level of 0.05 ($P = 0.2162$).

[113] CONTROLLING SLOW RELEASE

[114] In addition to adjusting or manipulating the concentration of the cellulose and pH modifications, chemical modifications can also be used to alter release properties of compounds of interest. For instance, a succinylated mercerized decellularized sample was tested and compared to the control sample. The succinylation functionalized the decellularized particles with a carboxylate group. The introduction of the charge on the surface can be mediated by altering the pH of the solution, and therefore, a control pH can conceivably be used as a "switch" for modulating release properties of a compound of interest. Accordingly, a plant-based biomaterials can

be used to be able to slowly deliver therapeutics in addition to providing structural/architectural support to the compound of interest.

[115] In order to directly compare the release of lidocaine from the mercerized formulation and the succinylated formulation, both the pH and the mass density of the formulations were held constant, which made it possible to compare the release of lidocaine from the unmodified and the succinylated materials. The intermediate concentrations were set to 5.1% with a pH of 7.3. The intermediate 5.1% stocks were combined with stock lidocaine (xylocaine) to give final concentrations of 3.6% cellulose-based material and 0.3% lidocaine. In the controls, the lidocaine volume (0.45 mL for a 1.5 mL mixture) was replaced with dH₂O where N = 3 samples (0.4 mL each) were centrifuged at 14800 rpm for 5 minutes. The absorbance of 150 μ L of the supernatant was recorded at 290 nm for each sample. These absorbance readings of the supernatant after centrifugation (A_{290 nm}) are provided in table below.

Table I

	Control	Lidocaine	Corrected
Unmodified	0.957 $\pm$ 0.007	1.787 $\pm$ 0.027	0.828 $\pm$ 0.028
Succinylated	0.820 $\pm$ 0.020	1.924 $\pm$ 0.060	1.104 $\pm$ 0.020

[116] A two sample t-test revealed that the unmodified and the succinylated formulations were significantly different (P = 0.0014, N = 3). It was found that the succinylated sample had an absorbance that was 1.33 ± 0.05 x greater than the unmodified material. Therefore, it appears that inclusion of a charge on the cellulose, as mediated by the succinylation at physiological pH, increases hydrophilicity and can therefore increase the solubility of the lidocaine in the aqueous phase.

[117] It is well-known that modulating the diffusion of drugs across biomembranes is intricately linked to pH and pK_a values. The pH of the solutions determine the mass fraction of non-ionized forms of drugs. In this instance, lidocaine has a pK_a of 7.8 where the protonated form of lidocaine is more hydrophilic. The neutral form of lidocaine is substantially more hydrophobic than the charged counterpart. Therefore, unique tuning properties around the transition point could be a distinct advantage for drug delivery systems. The ability of the drug to pass across hydrophobic membranes in the body is related to the pK_a of the drug, charge state of the drug as well as the pH of the surrounding environment. In addition to the release of drugs, the mercerized decellularized cellulose also enables slow modulation of pH.

[118] Accordingly, various possible combinations of unmodified and modified cellulose materials can be used in different ratios to elicit different desired effects. Therefore, the present inventors investigated the ratios of 1:1, 2:1, and 1:2 of the unmodified and succinylated formulations at a mass density of 3.4% for each cellulose-based

component respectively. The test samples had a lidocaine concentration of 0.3% and two different pH values were investigated i.e. 6.8 and 7.2.

[119] Figure 4 shows a graph of lidocaine release from unmodified mercerized decellularized material and succinylated mercerized decellularized material at pH 6.8 and 7.2. The absorbance was measured at 290 nm and N = 3 samples per condition. The bars represent the mean and the error bars represent the standard error of the mean.

[120] A two-way ANOVA revealed that the two pH values were not significantly different, nor were the mixture ratios at that particular concentration. The rationale behind choosing and testing this range of pH values was that this pH range is highly relevant for dermal filler procedures and injections. Interestingly, basic pH changes solubility of the lidocaine as hydrophilicity is related to the protonation and charge of the lidocaine. Since, another layer of complexity is added when the drug has to pass through hydrophobic membranes in the body; thus, tunable properties around the pKa are very important considerations for drug delivery and slow release applications

[121] **ADDITIONAL TESTING**

[122] The present inventors further investigated the slow release of lidocaine from mercerized material at pH 3, 5, 7, and 9. Additionally, the slow release pattern of lidocaine from different concentrations of mercerized materials, in particular 3.5% and 4.5% was investigated. To ascertain the impact of chemical modifications on the slow release properties of mercerized particles, slow release of lidocaine from succinylated mercerized cellulose was repeatedly tested with increased N values,.

[123] Additionally, the slow release pattern of ibuprofen, tryptophan, and small peptides from mercerized material was investigated to ascertain whether different molecules followed a pattern similar to lidocaine release. Several slow release pre-loading investigations were also conducted by the inventors. For instance, the length of time the samples need to be incubated to achieve an initial release plateau which was ascertained by studying the 24h, 48h, 72h data and by doing a short time series study every hour i.e. 1h, 2h, 3h, 4h, etc.

[124] Additionally, the molecule size dependence of the slow release pattern was evaluated. The cellulose type and concentration were kept fixed and the release molecule or the compound of interest size was varied by using different sterics and different molecular weights.

[125] As shown above, lidocaine slow release can be achieved using the decellularized mercerized cellulose particles. Likewise, other drugs such as the non-steroidal anti-inflammatory drug ibuprofen can be delivered using the cellulose-based particles. Furthermore, it was contemplated by the inventors that small molecules such as short peptides or amino acids could be potential candidates for controlled delivery.

[126] As a proof of concept, the slow release properties of tryptophan (Trp) and the RGD motif were investigated. As is well-known in the art, the RGD peptide also known as arginylglycylaspartic acid (Arg-Gly-Asp) is a common peptide that is responsible for cell adhesion to the extracellular matrix. Most importantly, cell adhesion proteins (integrins) recognize the amino acid sequence of RGD and bind to it. The RGD peptide is used for treatments ranging from cardiovascular diseases to cancer and has wide applications in diagnostics. It has widespread applications in bioengineering for drug and gene delivery and also in tissue engineering especially in bone, vascular, and ocular applications.

[127] **SLOW RELEASE PROPERTIES OF IBUPROFEN, TRYPTOPHAN AND RGD MOTIF**

[128] This experiment presents the slow release properties of ibuprofen from mercerized, decellularized, apple-derived, cellulose-based particles. The particles were used at the target concentration of 4.5%. The results shown below indicate that there was a prolonged initial steady state release followed by an exponential decay release profile.

[129] Moreover, the slow release properties of tryptophan from mercerized, decellularized, apple-derived, cellulose-based particles are described in detail below. The particles were used at the target concentration of 4.5%.

[130] Lastly, the slow release properties of the RGD motif from mercerized, decellularized, apple-derived, cellulose-based particles are presented below. The decellularized cellulose particles were used at the target concentration of 4.5%. It was observed that the RGD motif released rapidly and displayed a rapid release profile.

[131] The following materials were used to test the slow release properties:

- Mercerized decellularized cellulose-based plant-derived particles
- Ibuprofen: Supelco I1892 - 100G
- L- Tryptophan: Sigma - T8941-25G - 3 mg/mL stock in PBS
- RGD: BOC Sciences CAT No.: B2699-197-215
- PBS: Intermountain 1X - BSS-PBS-1X6

[132] Ibuprofen Set I: The pH of the mercerized intermediate particles was 7.3. The pH of the ibuprofen (Ibu) solution was initially 8.53 which was then corrected to 7.37. The bulk intermediate stock of the mercerized material was transferred to three 10 mL syringes each at a volume of 1.05 mL. An F/F luer lock connector was added to the syringe and a second syringe barrel was connected. The 0.45 mL of the Ibu stock solution was pipetted into the open barrel. The plunger was then placed in the barrel, the air was removed, and the solution was mixed 30X. Thereafter, 0.4 mL aliquots were transferred to three 2 mL microcentrifuge tubes and the material was incubated overnight in the fridge. The controls had an equal volume of PBS added in lieu of the Ibu.

[133] Ibuprofen Set 2: The mercerized stock was initially at a concentration of 6.62%. It was then diluted to an intermediate concentration of 6.45% in PBS. The pH of the intermediate stock was 7.25 after adjustment with HCl. The pH of the Ibu solution was 7.02. The bulk intermediate stock was transferred to three 10 mL syringes, each at a volume of 1.05 mL. An F/F luer lock connector was added to the syringe and a second syringe barrel (5 mL) was connected. The Trp stock solution (0.45 mL) was pipetted into the open barrel. The plunger was then placed in the barrel, the air was removed, and the solution was mixed 30X. Thereafter, 0.4 mL aliquots were transferred to three 2 mL microcentrifuge tubes and the material was incubated overnight in the fridge. The controls had an equal volume of PBS added in lieu of the Ibu

[134] Tryptophan Set 1: The pH of the intermediate stock was 7.3. The pH of the Trp solution was 7.02. The bulk intermediate stock of the mercerized material was transferred to three 10 mL syringes, each at a volume of 1.05 mL. An F/F luer lock connector was added to the syringe and a second syringe barrel was connected. The 0.45 mL of the Trp stock solution was pipetted into the open barrel. The plunger was then placed in the barrel, the air was removed, and the solution was mixed 30X. Thereafter, 0.4 mL aliquots were transferred to three 2 mL microcentrifuge tubes and the material was incubated overnight in the fridge. The controls had an equal volume of PBS added in lieu of the Trp.

[135] Tryptophan Set 2: The mercerized stock was initially at a concentration of 6.62%. It was then diluted to an intermediate concentration of 6.45% in PBS. The pH of the intermediate stock was 7.25 after adjustment with HCl. The pH of the Trp solution was 7.02. The bulk intermediate stock was transferred to three 10 mL syringes, each at a volume of 1.05 mL. An F/F luer lock connector was added to the syringe and a second syringe barrel (5 mL) was connected. The 0.45 mL of the Trp stock solution was pipetted into the open barrel. The plunger was then placed in the barrel, the air was removed, and the solution was mixed 30X. 0.4 mL aliquots were transferred to three 2 mL microcentrifuge tubes, and the material incubated overnight in the fridge. The controls had an equal volume of PBS added in lieu of the Trp.

[136] RGD Set: The mercerized stock was initially at a concentration of 7.012%. It was then diluted to an intermediate concentration of 5.3% in PBS. The pH of the intermediate stock was 6.99. Upon dilution to 4.5% with PBS for the control, the pH was 7.04. The pH of the RGD solution was approximately 4-5, yet upon mixing with the mercerized material, the pH reached 6.94. As such, no pH adjustments were required. For the mixing, the bulk intermediate stock was transferred to a 10 mL syringe at a volume of 1.05 mL. An F/F luer lock connector was added to the syringe and a second syringe barrel was connected. The 0.45 mL of the RGD stock solution was pipetted into the open barrel. The plunger was then placed in the barrel, the air was removed, and the solution was mixed 30X. Thereafter, 0.4 mL aliquots were transferred to three 2 mL microcentrifuge tubes and the material was incubated overnight in the fridge. The controls had an equal volume of PBS added in lieu of the RGD.

[137] **DETAILS OF THE ASSAY**

[138] Ibuprofen Set: The material was centrifuged in the 2 mL microcentrifuge tubes for 15 min at max speed (21000 g). Thereafter, 150 μ L of the supernatant was collected and transferred to a 96 well plate. The fluorescence intensity was measured using an excitation of 260 nm and an emission of 288 nm. The fluorescence gain setting was set to autogain to avoid oversaturation. The controls were used to scale the readings and subtract off the background signal. After a 24 h pre-incubation, the readings were performed. After the readings, an equal volume: 150 μ L of PBS was added to the pellet and the tubes were vortexed to mix. The samples were returned to the fridge for subsequent readings the following day. This repeated until the signal was indistinguishable from the controls.

[139] Tryptophan set: The material was centrifuged in the 2 mL microcentrifuge tubes for 15 min at max speed (21000 g). Thereafter, 150 μ L of the supernatant was collected and transferred to a 96 well plate. The fluorescence intensity was measured using an excitation of 250 nm and an emission of 348 nm. The fluorescence gain setting was set to autogain to avoid oversaturation. The controls were used to scale the readings and subtract off the background signal. After a 24 h pre-incubation the readings were performed. After the readings, an equal volume: 150 μ L of PBS was added to the pellet and the tubes were vortexed to mix. The samples were returned to the fridge for subsequent readings the following day. This repeated until the signal was indistinguishable from the controls.

[140] RGD Set: In contrast to the previous slow release experiments where UV-Vis absorbance readings enabled detection of the analyte, RGD displayed no detectable difference in absorbance compared to the control PBS. Furthermore, a literature search was conducted to explore the possibility of performing fluorescence detection. It was noted that excitation and emission wavelengths were not found for RGD without fluorescent protein tags. Therefore, the BCA assay was used to detect the protein.

[141] Briefly, the BCA assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. This method combines the well-known reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using a unique reagent containing bicinchoninic acid. The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20–2000 $\mu\text{g/mL}$). It is pertinent to note that the BCA method is not a true end-point method; that is, the final colour continues to develop. However, following incubation, the rate of continued colour development is sufficiently slow to allow large numbers of samples to be assayed together.

[142] In addition, some limitations are present in the BCA assay that interact with the colour intensity of the reaction of the copper in the reagents. In the PIERCE BCA™ Protein Assay Kit–Reducing Agent Compatible protocol, it is mentioned that small concentrations of hydrogen peroxide could have a significant increase in the

colorimetric reaction of the BCA reagent and falsify the results. Also, the BCA assay has a maximum tolerance of glucose at a concentration of 10 mM. To avoid these issues, it was contemplated to prepare a control material without the RGD to serve as an appropriate blank subtraction.

[143] The material was centrifuged in the 2 mL microcentrifuge tubes for 15 min at max speed (21000 g). Thereafter, 150 μ L of the supernatant was collected and transferred to clean microcentrifuge tubes. Thereafter, 200 μ L of the BCA reagent was added to the wells of a 96 well plate. Following that, 20 μ L of the 150 μ L aliquot was added to the wells with the BCA reagent. Triplicate readings for each sample were performed i.e. N = 3 samples for the control and the test particles. The absorbance was then measured at 595 nm. After a 24 h pre-incubation the readings were performed, and subsequent daily readings were taken until the controls were no longer significantly different from the test samples. After the readings, an equal volume of 150 μ L of PBS was added to the pellet and the tubes were stirred with a pipette tip to mix. The samples were returned to the fridge for subsequent readings the following day.

[144] **SLOW RELEASE OF PROTEINS AND PEPTIDES**

[145] The inventors also tested the slow release of albumin, lysozyme, somatostatin, and insulin from mercerized decellularized apple-derived cellulose particles. It was found that the release profile of these proteins and peptides was dependent on the protein or peptide. Albumin and somatostatin exhibited an exponential decay release profile, while lysozyme and insulin had a release profile that was relatively more constant in comparison under refrigerated conditions.

[146] **Context**

[147] In many situations, a controlled release of compounds is desired to elicit certain biological responses or to provide continued and time-resolved therapeutic treatments. The mercerized material has displayed the ability to exhibit slow release properties, as evidenced by the requirement for multiple neutralization cycles. Thus, the slow release properties were investigated to gain insight into the release characteristics of albumin, lysozyme, somatostatin, and insulin from the cellulose-based material. Albumin and lysozyme can be broadly grouped into the larger class of proteins, while insulin and somatostatin are peptide growth factors. These represent an important class of compounds that may be commonly used for tissue engineering applications.

[148] **Methods**

[149] Standard curve preparation, detection, and quantification of proteins and peptides: Quantification of bovine serum albumin (BSA), lysozyme, somatostatin-14, and insulin was performed using the Pierce BCA Protein Assay

Kit as per the manufacturer's instructions, which recommends using a quadratic fit for protein quantification. Importantly, all standard curves were generated using the proteins and peptides themselves, rather than solely with BSA as is done typically. This ensures more accurate results, given that the BCA assay reacts with only four amino acids (cysteine, cystine, tryptophan, and tyrosine), which will greatly influence the signal depending on the protein or peptide used.

[150] A stock solution of bovine serum albumin (Sigma-Aldrich; Cat# A9418) in 1X PBS was prepared at 10 mg/mL at pH ~ 7, which was utilized to prepare the standard curve. Figure 10 shows standard curve for bovine serum albumin. The data points represent the mean of triplicate readings and standard error of the mean. The red line represents the quadratic fit, $y = -0.09451x^2 + 1.01972x - 0.00564$ ($R^2 = 0.999$).

[151] A stock solution of lysozyme (Thermo Scientific Chemicals; Cat# ICN10083110) in 1X PBS was prepared at 10 mg/mL at pH ~ 7, which was utilized to prepare the standard curve. Figure 11 shows standard curve for lysozyme. The data points represent the mean of triplicate readings and standard error of the mean. The red line represents the quadratic fit, $y = -0.19106x^2 + 1.51584x + 0.00529$ ($R^2 = 0.997$).

[152] A stock solution of somatostatin-14 (Thermo Scientific Chemicals; Cat# AAJ66168LB0) in 1X PBS was prepared at 1.0 mg/mL at pH ~ 7, which was utilized to prepare the standard curve. Figure 12 shows a standard curve for somatostatin-14 where the data points represent the mean of triplicate readings and standard error of the mean. The red line represents the quadratic fit, $y = -0.44933x^2 + 1.03616x + 0.00159$ ($R^2 = 0.994$).

[153] A stock solution of insulin (Sigma-Aldrich; Cat# I5500) in 1X PBS was prepared at 2.0 mg/mL at pH ~ 3, which was utilized to prepare the standard curve. Figure 13 shows a standard curve for insulin where the data points represent the mean of triplicate readings and standard error of the mean. The red line represents the quadratic fit, $y = -0.4264x^2 + 1.41153x + 0.00427$ ($R^2 = 0.998$).

[154] Mercerized material preparation and compound preparation

[155] Mercerized material was combined with the proteins or peptides at a final concentration of 4.5% mercerized material, all prepared using 1X PBS as the diluent, at a final pH of ~7 for BSA, lysozyme, and somatostatin-14, or at a final pH of ~3 for insulin. BSA was prepared in the mercerized material at a final concentration of 2 mg/mL, lysozyme at a final concentration of 2.5 mg/mL, somatostatin-14 at a final concentration of 0.33 mg/mL, and insulin at a final concentration of 0.61 mg/mL. Following syringe mixing, the mixture was incubated at 4°C for at least 3 days prior to the start of the experiment.

[156] Centrifugation method for release evaluation

[157] Centrifugation tests were used to quantify relative amounts of proteins/peptides released from the mercerized materials. For this assay, 0.5 mL of the sample was placed in a 2.0 mL microcentrifuge tube, and it was centrifuged for at least 15 minutes at max speed (21000 x g or 14800 rpm). 150 μ L of the supernatant was collected and analyzed. An internal control was required, as the mercerized material itself leached contaminants into the solution (not visible by eye) or acted as an enhancer. In other words, samples without the compound of interest, but the same amount and concentration of mercerized material were used as controls; simple water controls were insufficient. Note that BSA, lysozyme, and somatostatin-14 utilized mercerized material in 1X PBS at pH 7 as a control, while insulin utilized mercerized material in 1X PBS at pH 3 as a control. Once the assay was completed, samples were replenished with 150 μ L of 1X PBS, gently mixed with a pipette tip, and incubated at 4°C until the next sampling point.

[158] Figure 14 shows bovine serum albumin slow release from mercerized cellulose after a pre-incubation of at least 3 days for N = 3 samples at each timepoint. The bars represent the mean $\pm$ standard error of the mean. Figure 15 shows lysozyme slow release from mercerized cellulose after a pre-incubation of at least 3 days for N = 3 samples at each timepoint. The bars represent the mean $\pm$ standard error of the mean. Figure 16 shows somatostatin-14 slow release from mercerized cellulose after a pre-incubation of at least 3 days for N = 3 samples at each timepoint. The bars represent the mean $\pm$ standard error of the mean. Figure 17 shows insulin slow release from mercerized cellulose after a preincubation of at least 3 days for N = 3 samples at each timepoint. The bars represent the mean $\pm$ standard error of the mean. Figure 18 shows bovine serum albumin slow release modelled as an exponential decay function, represented by the exponential fit equation $y = 0.01058 + 0.34261e^{-t/1.89658}$ ($R^2 = 0.999$). Figure 19 shows lysozyme slow release modelled as a linear function, represented by the linear fit equation $y = -0.02241x + 0.16382$ ($R^2 = 0.919$). Figure 20 shows somatostatin-14 slow release modelled as an exponential decay function, represented by the exponential fit equation $y = -0.00243 + 0.03157e^{-t/3.0372}$ ($R^2 = 0.977$). Figure 21 shows insulin slow release modelled as a linear function, represented by the linear fit equation $y = -0.00191x + 0.02068$ ($R^2 = 0.975$).

[159] The amount of protein and peptide released appears to be highly dependent on the protein or peptide used. For BSA and somatostatin-14, the exponential decay function $y = y_0 + A_1e^{-t/\tau}$ was used to fit the slow release data to enable the determination of a time constant (τ) for the release. BSA had a mean time constant and standard error of the mean of 1.90 ± 0.13 days, and the resultant half-life was 1.31 ± 0.09 days. Somatostatin-14 had a mean time constant and standard error of the mean of 3.04 ± 2.07 days, and the resultant half-life was 2.11 ± 1.43 days.

[160] Meanwhile, the amount of lysozyme or insulin does not follow a typical exponential drug release trend or cannot be ascertained due to the limited number of data points. Instead, these were modelled as linear fits. From the data, it is apparent that both lysozyme and insulin do not exhibit burst release from the mercerized material,

unlike with BSA which is released at relatively high amounts in the first day. Meanwhile, despite somatostatin-14 also exhibiting an exponential decay for release as with BSA, it does not have a notable burst release as compared to BSA.

[161] At the conclusion of the experiment, the total amount of each compound was summed and compared to the initial amount in the samples. It was found that 84% of BSA, 50% of lysozyme, 46% of somatostatin-14, and 28% of insulin was released from the mercerized material.

[162] **SLOW RELEASE OF HYALURONIC ACID**

[163] The inventors then tested slow release of hyaluronic acid from mercerized decellularized apple-derived cellulose particles. It was found that the release profile of hyaluronic acid followed an exponential decay under ambient conditions.

[164] In many situations, a controlled release of compounds is desired to elicit certain biological responses or to provide continued and time-resolved therapeutic treatments. Our material has displayed the ability to exhibit slow release properties, as evidenced by the requirement for multiple neutralization cycles. Thus, the slow release properties were investigated to gain insight into the release characteristics of hyaluronic acid from the cellulose-based material.

[165] Hyaluronic acid can be broadly grouped into the larger class of glycosaminoglycans, which is an important class of compounds that makes up the extracellular matrix of many tissues. Hyaluronic acid is also prevalent as the base material for numerous dermal fillers, and is also utilized for other biomedical applications, such as viscosupplementation into articular joints for lubrication and pain relief in degenerative joint diseases such as osteoarthritis.

[166] **Methods**

[167] Standard curve preparation, detection, and quantification of hyaluronic acid

[168] Hyaluronic acid (HA) conjugated to a fluorophore, tetramethylrhodamine (TRITC), was utilized for the slow release experiments to facilitate ease of detection. TRITC-HA (TdB Labs) was dissolved in 1X PBS, and quantification was performed using an excitation of 552 nm and emission of 576 nm, with a constant gain of 50.

[169] The fluorescent signal of TRITC-HA was validated to be stable throughout the course of the experiment, as the standard curve following incubation of TRITC-HA for 2 days at 4°C and an additional 3 days at room temperature had negligible differences. Validation was also performed in parallel to the slow release experiments, where the fluorescent signal of a 0.45 mg/mL TRITC-HA control at day 4 closely matched that at day 0.

[170] Figure 22 shows validation of TRITC-HA stability. A standard curve for TRITC-HA in 1X PBS was generated at constant gain of 50, excitation of 552 nm and emission of 576 nm, following 2 days of storage at 4°C and an additional 3 days at room temperature (for a total of 5 days).

[171] Table II provides RFU of 0.45 mg/mL of TRITC-HA following incubation at room temperature.

Day	Normalized RFU to Day 0 Control (%)
0	100.00
1	100.14
4	98.80

[172] Figure 23 shows standard curve for TRITC-HA in 1X PBS at constant gain of 50, excitation of 552 nm and emission of 576 nm. The data points represent the mean of triplicate readings and SEM (not visible because error is small). The red line represents the exponential fit, $y = 43837.77454 - 43861.5561e^{-2.66443x}$ ($R^2 = 0.999$).

[173] Mercerized material preparation and compound preparation

[174] Mercerized material was combined with TRITC-HA and 1X PBS (negative control) at a final concentration of 4.5% mercerized material. A stock solution of 1 mg/mL TRITC-HA was prepared with 1X PBS and added to the mercerized material for a final concentration of 0.45 mg/mL TRITC-HA, and a final pH of ~7. Following syringe mixing, the mixture was protected from light and incubated at 4°C for at least 3 days prior to the start of the experiment.

[175] Centrifugation method for release evaluation

[176] Centrifugation tests were used to quantify relative amounts of hyaluronic acid released from the mercerized materials. For this assay, 0.5 mL of the sample was placed in a 2.0 mL microcentrifuge tube and protected from light. Samples were centrifuged for at least 15 minutes at max speed (21000 x g or 14800 rpm). 150 µL of the supernatant was collected and analyzed. Mercerized material prepared with PBS acted as an internal

control for this assay. Once the assay was completed, samples were replenished with 150 μ L of 1X PBS, gently mixed with a pipette tip, and incubated at room temperature, protected from light.

[177] Figure 24 shows hyaluronic acid slow release from mercerized cellulose after an incubation of at least 3 days for N = 3 samples at each timepoint. The bars represent the mean $\pm$ standard error of the mean.

[178] Figure 25 shows HA slow release modelled as an exponential decay function, represented by the exponential fit equation $y = -0.002 + 0.07281e^{-t/2.08322}$ ($R^2 = 0.999$). Error bars represent SEM.

[179] The amount of hyaluronic acid released followed an exponential decay. The exponential decay function $y = y_0 + A_1e^{-t/\tau}$ was used to fit the slow release data. Each pre-incubation time had an R^2 value of 0.999 for the exponential fit. The fitting enabled the determination of a time constant (τ) for the release. The mean time constant and standard error of the mean was 2.08 ± 0.07 days, and the resultant half-life was 1.44 ± 0.05 days. This release timescale is highly relevant for biomedical applications. At the conclusion of the experiment, the total amount of hyaluronic acid was summed and compared to the initial amount in the samples. It was found that after 4 days, 82% of hyaluronic acid was removed.

[180] SLOW RELEASE OF GENTAMICIN

[181] The inventors also tested the slow release of gentamicin from mercerized decellularized apple-derived cellulose particles. It was found that the release profile of gentamicin was relatively slow under ambient conditions.

[182] In many situations, a controlled release of compounds is desired to elicit certain biological responses or to provide continued and time-resolved therapeutic treatments. In the context of dermal fillers, the injection and the space filling action of the filler can lead to pain. Many commercial fillers include lidocaine (0.3%) as part of the formulation to help alleviate the pain. However, there is also potential to include antibiotics in dermal fillers to prevent bacterial infections, which may also lead to inflammation and other complications. The mercerized material has displayed the ability to exhibit slow release properties, as evidenced by the requirement for multiple neutralization cycles. Thus, the slow release properties were investigated to gain insight into the release characteristics of gentamicin from the cellulose-based material. Gentamicin can be broadly grouped into the larger class of antibiotics, which is an important class of compounds commonly used for the treatment of bacterial infections.

[183] Methods

[184] Standard curve preparation, detection, and quantification of gentamicin

[185] Gentamicin sulfate (TCI America; Cat# G03831G) was quantified using ninhydrin, which reacts with the primary and secondary amine groups present on gentamicin to produce a purple colour. Briefly, 1.25% ninhydrin stock solution was prepared in distilled water, and protected from light. 145 μ L of the analyte was combined with 242 μ L of 1X PBS, along with 116 μ L of 1.25% ninhydrin. The mixture was heated at 95°C for 15 minutes, and the absorbance was measured at 400 nm for each sample in technical triplicates.

[186] Figure 26 shows standard curve for gentamicin at 400 nm in 1X PBS. The data points represent the mean of triplicate readings and SEM. The red line represents the linear fit, $y = 0.96107x + 0.06607$ ($R^2 = 0.997$).

[187] Mercerized material preparation and compound preparation

[188] Mercerized material was combined with gentamicin and 1X PBS (negative control) at a final concentration of 4.5% mercerized material. A stock solution of 1 mg/mL gentamicin was prepared with 1X PBS and added to the mercerized material for a final concentration of 0.50 mg/mL gentamicin, and a final pH of 6.81. Following syringe mixing, the mixture was incubated at 4°C for at least 3 days prior to the start of the experiment.

[189] Centrifugation method for release evaluation

[190] Centrifugation tests were used to quantify relative amounts of gentamicin released from the mercerized material. For this assay, 0.5 mL of the sample was placed in a 2.0 mL microcentrifuge tube, and it was centrifuged for at least 15 minutes at max speed (21000 x g or 14800 rpm). 145 μ L of the supernatant was collected and analyzed as described above. Mercerized material prepared with PBS acted as an internal control for this assay. Once the assay was completed, samples were replenished with 150 μ L of 1X PBS, gently mixed with a pipette tip, and incubated at room temperature.

[191] Figure 27 shows gentamicin slow release from mercerized cellulose after a preincubation of at least 3 days for N = 3 samples at each timepoint. The bars represent the mean $\pm$ standard error of the mean.

[192] Figure 28 shows gentamicin slow release modelled as a linear function, represented by the linear fit equation $y = -0.0033x + 0.02612$ ($R^2 = 0.970$).

[193] The amount of gentamicin released does not follow a typical exponential drug release trend, or cannot be ascertained due to the limited number of data points. Instead, its release was modelled as a linear fit. From the data, it is apparent that gentamicin does not exhibit burst release from the mercerized material. Rather, after an initial plateau, a drop was observed. From a one-way ANOVA, there is a statistically significant decrease in the gentamicin released at days 3 - 4 compared to days 0 - 2. At the conclusion of the experiment, the total amount of gentamicin was summed and compared to the initial amount of gentamicin in the samples. It was found that after 4 days, 42% of gentamicin was removed from the mercerized material.

[194] **SLOW RELEASE OF ANALGESICS**

[195] The inventors then tested the slow release of acetaminophen, diclofenac and naproxen from mercerized decellularized apple-derived cellulose particles. It was found that the release profile of all analgesics was exponential decay under ambient conditions.

[196] In many situations, a controlled release of compounds is desired to elicit certain biological responses or to provide continued and time-resolved therapeutic treatments. In the context of dermal fillers, the injection and the space filling action of the filler can lead to pain. Many commercial fillers include lidocaine (0.3%) as part of the formulation to help alleviate the pain. Our material has displayed the ability to exhibit slow release properties, as evidenced by the requirement for multiple neutralization cycles. Thus, the slow release properties were investigated to gain insight into the release characteristics of acetaminophen, diclofenac and naproxen from cellulose-based material. Acetaminophen can be broadly grouped into the larger class of non-opioid analgesic and antipyretic agents, which are important classes of compounds commonly used for mild to moderate pain relief and temporary reduction of fever. Naproxen and diclofenac are non-steroidal anti-inflammatory drugs (NSAIDs), which on top of the same applications for acetaminophen, can also help relieve pain associated with inflammation. Acetaminophen and diclofenac can be administered in a wide range of ways, whereas naproxen is currently only available for oral administration.

[197] **Methods**

[198] Standard curve preparation, detection, and quantification of analgesics

[199] A stock solution of acetaminophen (Sigma; Cat# A5000) in 1X PBS was prepared at 2 mg/mL, with the pH verified at 7.02. UV-vis assays were performed using 1X PBS as a blank. The figure below shows the established standard absorbance curve for acetaminophen.

[200] A stock solution of diclofenac sodium salt (Thermo Scientific Chemicals; Cat# J6260906) in 1X PBS was prepared at 2.025 mg/mL at pH ~ 7. UV-vis assays were performed using 1X PBS as a blank. The figure below shows the established standard absorbance curve for diclofenac.

[201] A stock solution of naproxen sodium salt (Thermo Scientific Chemicals; Cat# J6310306) in 1X PBS was prepared at 5 mg/mL and the pH was verified at 6.96. UV-vis assays were performed using 1X PBS as a blank. The figure below shows the established standard absorbance curve for naproxen.

[202] Figure 29 shows a standard curve for acetaminophen at 300 nm. The data points represent the mean of triplicate readings and standard error of the mean (not visible because error is small). The red line represents the linear fit, $y = 0.78581x + 0.04918$ ($R^2 = 0.991$).

[203] Figure 30 shows a standard curve for diclofenac at 315 nm. The data points represent the mean of triplicate readings and standard error of the mean (not visible because error is small). The red line represents the linear fit, $y = 0.71688x + 0.0208$ ($R^2 = 0.999$).

[204] A stock solution of naproxen sodium salt (Thermo Scientific Chemicals; Cat# J6310306) in 1X PBS was prepared at 5 mg/mL and the pH was verified at 6.96. UV-vis assays were performed using 1X PBS as a blank. The figure below shows the established standard absorbance curve for naproxen. Figure 31 shows a standard curve for naproxen at 300 nm. The data points represent the mean of triplicate readings and standard error of the mean (not visible because error is small). The red line represents the linear fit, $y = 0.96107x + 0.06607$ ($R^2 = 0.997$).

[205] Mercerized material preparation and compound preparation

[206] Experiments were carried out using mercerized decellularized apple-derived cellulose particles. Mercerized material was diluted to 4.5% using 1X PBS and the stock solution of each compound (made in 1X PBS), such as that mercerized material was loaded at 2 mg/mL for acetaminophen, 1.68 mg/mL for diclofenac, and 2.5 mg/mL for naproxen, as well as a negative control using 1X PBS diluted to 4.5%. After the final mercerized material formulation was prepared with the compound of interest and diluted to 4.5%, the material was stored at 4°C for 3-4 days as a pre-incubation step prior to testing.

[207] Table III provides details about drug-loaded mercerized material used for assessing slow release properties.

Compound	Compound Concentration in MER	pH After Loading	Mercerized Concentration (%w/w)	Material
Acetaminophen	2.00 mg/mL	7.13	4.5%	
Diclofenac	1.68 mg/mL	7.20	4.5%	
Naproxen	2.50 mg/mL	6.93	4.5%	

[208] Centrifugation method for release evaluation

[209] Centrifugation tests were used to quantify relative amounts of the different compounds released from the mercerized materials. For the UV-vis assays, 0.5 mL of the mercerized material + compound samples was placed in a 2.0 mL microcentrifuge tube, which was centrifuged for 15 minutes at max speed (21000 x g or 14800 rpm). 150 µL of the supernatant was collected and analyzed. Once the assay was completed, samples were replenished with 150 µL of 1X PBS, gently mixed with a pipette tip, and incubated at room temperature.

[210] Figure 32 shows acetaminophen slow release from mercerized cellulose after a pre-incubation of at least 3 days for N = 3 samples at each timepoint. The bars represent the mean ± standard error of the mean.

[211] Figure 33 shows diclofenac slow release from mercerized cellulose after a preincubation of at least 3 days for N = 3 samples at each timepoint. The bars represent the mean ± standard error of the mean.

[212] Figure 34 shows naproxen slow release from mercerized cellulose after a preincubation of at least 3 days for N = 3 samples at each timepoint. The bars represent the mean ± standard error of the mean.

[213] Figure 35 shows acetaminophen slow release modelled as an exponential decay function, represented by the exponential fit equation $y = -0.000439725 + 0.2737e^{-t/2.25448}$ ($R^2 = 0.999$).

[214] Figure 36 shows diclofenac slow release modelled as an exponential decay function, represented by the exponential fit equation $y = -0.14094 + 0.39238e^{-t/5.62176}$ ($R^2 = 0.999$).

[215] Figure 37 shows naproxen slow release modelled as an exponential decay function, represented by the exponential fit equation $y = -0.03244 + 0.3893e^{-t/3.50212}$ ($R^2 = 0.999$).

[216] The amount of analgesic released followed an exponential decay for all compounds. The exponential decay function $y = y_0 + A_1e^{-t/\tau}$ was used to fit the slow release data to enable the determination of a time constant (τ) for the release. Acetaminophen had a mean time constant and standard error of the mean of 2.25 ± 0.28 days, and the resultant half-life was 1.56 ± 0.19 days. Diclofenac had a mean time constant and standard error of the mean of 5.62 ± 2.82 days, and the resultant half-life was 3.90 ± 1.96 days. Lastly, naproxen had a mean time constant and standard error of the mean of 3.50 ± 0.18 days, and the resultant half-life was 2.43 ± 0.12 days. This release timescale is highly relevant for dermal filler pain relief and related applications.

[217] At the conclusion of the experiment, the total amount of each compound was summed and compared to the initial amount in the samples. It was found that 69% of acetaminophen, 87% of diclofenac, and 83% of naproxen was removed from mercerized material.

[218] Table IV provides overview of slow release of analgesics from mercerized material.

Compound	Amount released (% of initial mass)	$\tau \pm \text{SEM}$ (days)	Half-life $\pm \text{SEM}$ (days)
Acetaminophen	69%	2.25 ± 0.28	1.56 ± 0.19
Diclofenac	87%	5.62 ± 2.82	3.90 ± 1.96
Naproxen	83%	3.50 ± 0.18	2.43 ± 0.12

[219] **SLOW RELEASE OF ANALGESICS FROM SUCCINYLATED CELLULOSE**

[220] The inventors then tested the slow release of acetaminophen and naproxen from mercerized decellularized apple-derived cellulose particles and the chemically modified counterpart consisting of succinylated mercerized decellularized apple-derived cellulose particles. It was found that the release profile of acetaminophen and naproxen was near linear under ambient conditions for both the mercerized and succinylated material.

[221] In many situations, a controlled release of compounds is desired to elicit certain biological responses or to provide continued and time-resolved therapeutic treatments. In the context of dermal fillers, the injection and the space filling action of the filler can lead to pain. Many commercial fillers include lidocaine (0.3%) as part of the formulation to help alleviate the pain. The mercerized material has displayed the ability to exhibit slow release properties, as evidenced by the requirement for multiple neutralization cycles. Thus, the slow release properties were investigated to gain insight into the release characteristics of acetaminophen and naproxen from cellulose-based material as well as the chemically modified counterpart consisting of a covalently linked succinic acid functional group.

[222] Acetaminophen can be broadly grouped into the larger class of non-opioid analgesic and antipyretic agents, which are important classes of compounds commonly used for mild to moderate pain relief and temporary reduction of fever. Naproxen is a non-steroidal anti-inflammatory drug (NSAID), which on top of the same

applications for acetaminophen, can also help relieve pain associated with inflammation. Acetaminophen can be administered in a wide range of ways, whereas naproxen is currently only available for oral administration.

[223] **Methods**

[224] Standard curve preparation, detection, and quantification of analgesics

[225] A stock solution of acetaminophen (Sigma; Cat# A5000) in 1X PBS was prepared at 6.5 mg/mL. UV-vis assays were performed using 1X PBS as a blank. Figure 38 below shows the established standard absorbance curve for acetaminophen. Figure 38 shows a standard curve for acetaminophen at 300 nm. The data points represent the mean of triplicate readings and standard error of the mean (not visible because error is small). The red line represents the linear fit, $y = 0.70681x + 0.01763$ ($R^2 = 0.996$). A stock solution of naproxen sodium salt (Thermo Scientific Chemicals; Cat# J6310306) in 1X PBS was prepared at 8 mg/mL. UV-vis assays were performed using 1X PBS as a blank. The figure below shows the established standard absorbance curve for naproxen.

[226] Figure 39 shows standard curve for naproxen at 300 nm as the data points represent the mean of triplicate readings and standard error of the mean (not visible because error is small). The red line represents the linear fit up to 2.5 mg/mL, $y = 1.03733x + 0.03394$ ($R^2 = 0.996$). The 3.0 mg/mL point was excluded due to poor linearity.

[227] Succinylated and mercerized material preparation and compound preparation

Experiments were carried out using succinylated and non-succinylated mercerized decellularized apple-derived cellulose particles.

[228] The cellulose materials were diluted to 3.5% using 1X PBS and the stock solution of each compound (made in 1X PBS), such as that the samples were loaded at 2 mg/mL for acetaminophen and 2.5 mg/mL for naproxen. For each set, a negative control was also prepared using 1X PBS to dilute the cellulose to 3.5% without the corresponding drug compound. After the final formulation was prepared with the compound of interest and diluted to 3.5%, the material was stored at 4°C for 3 days as a pre-incubation step prior to testing.

[229] **Table V.** Details about drug-loaded material used for assessing slow release properties.

Cellulose	Compound	Compound Concentration	pH After Loading	Cellulose Concentration (%w/w)
Mercerized	Acetaminophen	2.00 mg/mL	7.09	3.5%

	Naproxen	2.50 mg/mL	7.14	3.5%
Succinylated	Acetaminophen	2.00 mg/mL	7.17	3.5%
	Naproxen	2.50 mg/mL	7.15	3.5%

[230] Centrifugation method for release evaluation

[231] Centrifugation tests were used to quantify relative amounts of the different compounds released from the mercerized and succinylated celluloses. For the UV-vis assays, 1 mL of the mixture was placed in a 2.0 mL microcentrifuge tube, which was centrifuged for 15 minutes at max speed (21000 x g or 14800 rpm). 150 μ L of the supernatant was collected and analyzed. Once the assay was completed, samples were replenished with 150 μ L of 1X PBS, gently mixed with a pipette tip, and incubated at room temperature. The same volume was used for the controls.

[232] **Results**

[233] Acetaminophen

[234] Mercerized cellulose: Figure 40 shows acetaminophen slow release from mercerized cellulose after a pre-incubation of 3 days for N = 3 samples at each timepoint. The bars represent the mean $\pm$ standard error of the mean.

[235] Succinylated cellulose: Figure 41 acetaminophen slow release from succinylated cellulose after a pre-incubation of 3 days for N = 3 samples at each timepoint. The bars represent the mean $\pm$ standard error of the mean.

[236] Naproxen

[237] Mercerized cellulose: Figure 42 shows naproxen slow release from mercerized cellulose after a pre-incubation of 3 days for N = 3 samples at each timepoint. The bars represent the mean $\pm$ standard error of the mean.

[238] Succinylated cellulose: Figure 43 shows naproxen slow release from succinylated cellulose after a pre-incubation of 3 days for N = 3 samples at each timepoint. The bars represent the mean $\pm$ standard error of the mean.

[239] Mercerized cellulose – Acetaminophen: Figure 44 Acetaminophen slow release from mercerized cellulose modelled as a linear function, represented by the linear fit equation $y = -0.04054x + 0.24961$ ($R^2 = 0.97984$).

[240] Succinylated cellulose – Acetaminophen: Figure 45 shows acetaminophen slow release from succinylated material modelled as a linear function, represented by the linear fit equation $y = -0.04931x + 0.25868$ ($R^2 = 0.96473$).

[241] Mercerized cellulose – Naproxen: Figure 46 shows naproxen slow release from mercerized cellulose modelled as a linear function, represented by the linear fit equation $y = -0.04071x + 0.31019$ ($R^2 = 0.99880$).

[242] Succinylated cellulose – Naproxen: Figure 47 shows naproxen slow release from succinylated cellulose modelled as a linear function, represented by the linear fit equation $y = -0.04782x + 0.34118$ ($R^2 = 0.98768$). The amount of acetaminophen and naproxen released from the mercerized material followed a linear function. While the succinylated material is also modelled as a linear function, there appears to be a plateau in release for both acetaminophen and naproxen at days 3 and 4. This release timescale is highly relevant for dermal filler pain relief and related applications. At the conclusion of the experiment, the total amount of each compound was summed and compared to the initial amount in the samples. It was found that 42% of acetaminophen and 46% of naproxen was removed from the mercerized material, while 43% of acetaminophen and 51% of naproxen was removed from the succinylated cellulose (SUC) material.

[243] **Table VI.** Overview of slow release of analgesics from mercerized and succinylated material.

Cellulose	Compound	Amount released (% of initial mass)
Mercerized	Acetaminophen	42%
	Naproxen	46%
Succinylated	Acetaminophen	43%
	Naproxen	51%

[244] **DISCUSSION ON RESULTS**

[245] Ibuprofen set: It was found that an extended initial plateau of release was observed. However, a significant decrease was observed by day 6 and an exponential decay profile was observed. The exponential decay function $y = y_0 + A_1 e^{-t/\tau}$ was used to fit the slow release data starting from the beginning of the decay on day 5. The exponential fit had an R^2 value of 0.98. The fitting enabled the determination of a time constant (τ) for the release. The time constant was 1.06 ± 0.285 days, and the resultant half-life was 0.74 ± 0.19 days. Including the initial plateau, the half life was ~ 5.5 days from the initial release measurement. A person skilled in the art would appreciate that this is a highly relevant timescale for delivering drugs of interest.

[246] The initial plateau differs from the previous lidocaine and tryptophan results. It was hypothesized that the non-polar character of the ibuprofen led to this moderately sustained initial plateau. Since, cellulose is made up of glucose, which contains polar hydroxyl moieties, and it extensively hydrogen bonds to itself intra- and inter-molecularly. As such there is often an outer hydration layer of water, yet the cellulose does not completely dissolve in water due to the strong hydrogen bonding within and between the cellulose chains, despite its high polarity. Since, the slow release study included the solvent PBS which is an aqueous solution, it was proposed that the non-polar Ibu may remain adsorbed to the mercerized material with a minimal enthalpic and entropic penalty when a sufficient amount of material is in the aqueous phase stemming from enthalpic and entropic contribution. Additional work is required to confirm this hypothesis. However, the different release profiles of Ibu compared to lidocaine are highlighted herein. Surprisingly, rather than a simple exponential decay function release profile, a reduction in the concentration is observed midway through the time-course study. Figure 5 shows a slow release of ibuprofen from 4.5% mercerized decellularized cellulose-based particles. Note: there was no statistically significant difference between day 0 and day 1 values. Each of the timepoints have $N = 3$ samples where (A) is Trial 1 and (B) is Trial 2. Figure 6 shows a slow release of ibuprofen from 4.5% mercerized decellularized cellulose-based particles with the exponential fit beginning on day 5.

[247] Tryptophan set: In the tryptophan set, it was found that an initial plateau of release was observed. After the plateau, a significant decrease occurred and an exponential decay profile was observed. The exponential decay function $y = y_0 + A_1 e^{-t/\tau}$ was used to fit the slow release data starting from the beginning of the decay on day 1. The exponential fit had an R^2 value of 0.93. The fitting enabled the determination of a time constant (τ) for the release. The time constant was 3.24 ± 0.44 days, and the resultant half-life was 2.24 ± 0.31 days and including the initial plateau the half-life was ~ 4 -5 days from the initial release measurement. This is a highly relevant timescale for delivering cues to host cells.

[248] It was observed that the initial plateau differs from the previous lidocaine results. It is hypothesized that the non-polar character of the tryptophan led to this briefly sustained initial plateau. Cellulose is made up of glucose, which contains polar hydroxyl moieties; nevertheless, it extensively hydrogen bonds to itself intra- and inter-molecularly. As such there is often an outer hydration layer of water, yet the cellulose does not completely dissolve in water due to the strong hydrogen bonding within and between the cellulose chains, despite its high polarity. Since, during the slow release study the solvent used was PBS which is an aqueous solution, it was

proposed that the non-polar Trp may remain adsorbed to the mercerized material with a minimal enthalpic and entropic penalty when a sufficient amount of material is in the aqueous phase stemming from enthalpic and entropic contributions. Figure 7 shows a slow release of tryptophan from 4.5% mercerized decellularized cellulose-based particles. Note: there was no statistically significant difference between the day 0 and day 1 values. Each of the timepoints have N = 3 samples wherein (A) is Trial 1 and (B) is Trial 2. Figure 8 shows a slow release of tryptophan from 4.5% mercerized decellularized cellulose-based particles with the exponential fit beginning on day 2.

[249] RGD set: In the RDG Set, it was found that fairly rapid release was observed. The exponential decay function $y = y_0 + A_1 e^{-t/\tau}$ was used to fit the slow release data. The exponential fit had an R^2 value of 0.95. The fitting enabled the determination of a time constant (τ) for the release. The time constant was 0.06 ± 0.01 days, and the resultant half-life was 0.04 ± 0.01 days. This is a highly relevant timescale for delivering temporary attachment cues to cells. It should be noted that the release measurement is a centrifugation “pull” and is a measure of the ease of release from the material; thus, the half-life in the body or an in vitro system is expected to be longer in the absence of centrifugation.

[250] The logarithm of the partition coefficient ($\log(P)$) is a measure of the hydrophilicity. Since, RGD has a $\log(P) = -3.016$, which is highly hydrophilic, and it is believed that the polar nature of the peptide is responsible for the high hydrophilicity. Cellulose is made up of glucose, which contains polar hydroxyl moieties; nevertheless, it extensively hydrogen bonds to itself intra- and inter-molecularly. As such there is often an outer hydration layer of water, yet the cellulose does not completely dissolve in water due to the strong hydrogen bonding within and between the cellulose chains, despite its high polarity. During the slow release study, the solvent was PBS, which is an aqueous solution and the RGD is highly soluble in the aqueous solvent. Therefore, it is hypothesized that this high degree of hydrophilicity is responsible for the rapid release of the peptide. It is believed that changing the hydrophobicity of the solvent or microenvironment could change the release kinetics. Figure 9 shows slow release of RGD from 4.5% mercerized decellularized cellulose-based particles. Note: Day 0 refers to 24 h after the sample was mixed. Each of the timepoints have N = 3 samples with N = 3 replicates for each. Values are mean $\pm$ standard error of the mean.

[251] Proteins and Peptides: Simple centrifugation extractions to measure the “pull” required to release a certain amount of protein/peptide and compare to different conditions or proteins/peptides is a valuable approach. Here, inventors were able to show that various proteins and peptides were slowly released from mercerized decellularized plant-derived cellulose.

[252] Hyaluronic Acid: Here, we were able to show that hyaluronic acid was slowly released from mercerized decellularized plant-derived cellulose. Note that as the hyaluronic acid used in this study was tagged with TRITC, release kinetics may be affected due to alterations in both the molecular weight and chemical properties, which will influence diffusion and interactions with the mercerized material.

[253] Gentamicin: Simple centrifugation extractions to measure the “pull” required to release a certain amount of drug and compare to different conditions or drugs is a valuable approach. Here, inventors were able to show that gentamicin was slowly released from mercerized decellularized plant-derived cellulose.

[254] Analgesics: Inventors were able to show that acetaminophen, diclofenac and naproxen were slowly released from mercerized decellularized plant-derived cellulose, with acetaminophen showing the shortest half-life (approximately a day and a half), and diclofenac the longest (almost 4 days).

[255] Analgesics with Succinylated Cellulose: Inventors were able to show that acetaminophen and naproxen were slowly released from mercerized decellularized plant-derived cellulose and succinylated mercerized decellularized plant-derived cellulose. Both the succinylated and mercerized cellulose had similar release profiles for acetaminophen and naproxen. Interestingly, there appears to be a plateau in release for the succinylated material at days 3 and 4, where drug release appears relatively constant. Therefore, it is possible that the succinylated material exhibits a fast release initially followed by slower release of these drugs, which is not observed for the mercerized material.

[256] **Conclusion:** Slow release profile of Lidocaine provided a great proof-of-concept study. It was also shown that the mercerized material was able to slowly release the NSAID ibuprofen. After an initial moderately sustained plateau release profile, a drop in the amount released was observed which then followed an exponential release. This proof of concept study provides preliminary data for the controlled slow release of drugs. This feature can be used to provide time-resolved delivery of select agents to local microenvironments within the body. In addition to the concentration of the cellulose-based particles and pH, the interactions with drugs of different polarity, and the polarity of the cellulose (native or modified) can be exploited to tune release properties.

[257] Likewise, it was shown that the mercerized material was able to slowly release the amino acid tryptophan. After an initial plateau release profile, an exponential release was observed. This proof of concept study provides preliminary data for the controlled slow release of amino acids and small peptides. This feature can be used to provide time-resolved cues to local microenvironments within the body.

[258] Lastly, it was shown that the mercerized material was able to release the RGD peptide. A rapid release was observed. This proof of concept study provides preliminary data for the controlled slow release of amino acids and small peptides. This feature can be used to provide time-resolved cues to local microenvironments within the body. This can be further exploited to study the release of the RGD motif and providing temporary but sufficiently long cues for cell attachment and growth.

[259] It was also found that the various proteins and peptides such as albumin, insulin, somatostatin and lysozyme were slowly released from the mercerized material over several days. This is highly relevant for biomedical applications.

[260] It was also found that hyaluronic acid was slowly released from the mercerized material over several days. This is highly relevant for biomedical applications.

[261] It was found that gentamicin was slowly released from the mercerized material over several days. This is highly relevant for biomedical applications, as that is a typical timeframe for some antibiotic regimens.

[262] It was found that acetaminophen, diclofenac and naproxen were slowly released from the mercerized material over several days. This is highly relevant for biomedical applications, as this can be the desired timeframe for the action of analgesics and antipyretics.

[263] It was found that acetaminophen and naproxen were slowly released from the mercerized and succinylated material over several days. This is highly relevant for biomedical applications, as this can be the desired timeframe for the action of analgesics and antipyretics.

[264] These slow release properties of mercerized decellularized material described in the present application can be replicated using tablet and drug delivery as excipients. Furthermore, delivery of other drugs, growth factors, hormones, vitamins, minerals, chemicals, and nanoparticles could also be exploited. Additionally, by means of reverse application the absorption of particles for removal of unwanted agents in the body (micro absorbers) could also be studied.

[265] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS**WHAT IS CLAIMED IS:**

1. A method of providing a slow, controlled or sustained release of a compound of interest:
 - (a) incubating the compound of interest with mercerized decellularized cellulose particles for a predetermined incubation time; and
 - (b) adjusting a physical or a chemical property of the mercerized decellularized cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.
2. The method of claim 1, wherein the predetermined incubation time allows the cellulose particles to absorb the compound of interest and ranges from 1-96 hours.
3. The method of claim 1 or 2, wherein the method additionally comprises:
 - (c) adjusting a physical or a chemical property of the compound of interest to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.
4. The method of claim 1, wherein the method additionally comprises:
 - (d) pretreating the cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest or to adjust the release kinetics of the compound of interest.
5. The method of any one of claims 1-4, wherein the method comprises:
mixing, soaking or centrifuging the mercerized decellularized cellulose particles and the compound of interest to prepare a mixture prior to step (a).
6. The method of claim 5, wherein the mixing, soaking or centrifuging promotes absorption of the compound of interest on to the cellulose particles
7. The method of any one of claims 1-5, wherein the mixing, soaking or centrifuging step occurs in the presence of a solvent.
8. The method of claim 7, wherein the solvent is water, saline, or PBS.
9. The method of any one of claims 1-8, wherein the method additionally comprises:
 - (d) adjusting a physical or a chemical property of the mixture to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.

10. The method of claim 1 or 3, wherein the method comprises modulating the physical or the chemical property of the mercerized decellularized cellulose particles, the compound of interest and/or the mixture to control the release pattern of the compound of interest.

11. The method of claim 1, wherein step (b) comprises:
adjusting pKa of the cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.

12. The method of claim 1, wherein step (b) comprises:
adjusting pH of the cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.

13. The method of claim 1, wherein step (b) comprises:
adjusting concentration of the cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.

14. The method of claim 1, wherein step (b) comprises:
modifying chemical structure of the cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.

15. The method of claim 1, wherein step (b) comprises:
modifying crystal structure of the cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.

16. The method of claim 15, wherein modifying the chemical structure of the mercerized decellularized cellulose particles comprises: succinylating the mercerized decellularized cellulose particles.

17. The method of claim 15, wherein modifying the chemical structure of the mercerized decellularized cellulose particles comprises: adding a charge on the mercerized decellularized cellulose particles.

18. The method of claim 16 or 17, wherein step (b) additionally comprises:
preparing a combination mixture of the modified mercerized decellularized cellulose particles and the unmodified mercerized decellularized cellulose particles; and
adjusting a physical or a chemical property of the combination mixture to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.

19. The method of claim 18, wherein the combination mixture comprises the modified cellulose particles and the unmodified cellulose particles in a fixed ratio.

20. The method of claim 2, wherein step (c) comprises adjusting size of the compound of interest to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.
21. The method of claim 2, wherein step (c) comprises adjusting hydrophilicity of the compound of interest to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.
22. The method of claim 2, wherein step (c) comprises adjusting surface charge of the compound of interest to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.
23. The method of claim 2, wherein step (c) comprises adjusting solubility of the compound of interest to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.
24. The method of claim 1, wherein the method comprises adjusting / changing the predetermined incubation time to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.
25. The method of claim 4, wherein step (d) comprises:
adjusting pH of the mixture to trigger, alter, enable or affect the slow, controlled or sustained release of the compound of interest.
26. The method of claim 3, wherein the method further comprises:
measuring / quantifying the amount of absorption of the compound of interest in the mercerized decellularized cellulose particles by measuring/quantifying the fluorescence intensity or absorbance of the mixture in the range of 200-600nm.
27. The method of claim 26, wherein the method further comprises:
preparing a control saline solution and quantifying the amount of absorption of the control solution by measuring the fluorescence intensity or absorbance in the range of 200-600nm; and
comparing the fluorescence intensity or absorbance of the mixture with the fluorescence intensity/absorbance of the control solution.
28. The method of claim 9, wherein the compound of interest has a steady state release profile, an exponential decay release profile or a combination thereof.
29. The method of claim 3, wherein the method further comprises:
measuring / quantifying the amount of absorption of the compound of interest in the mercerized decellularized cellulose particles using a colorimetric detection assay or a quantification assay.

30. The method of claim 1, wherein the predetermined incubation time is in the range of 1-96 hours.

31. The method of any one of claims 1-30, for use in slow, controlled or sustained release of an anaesthetic, wherein the anaesthetic is selected from the group of lidocaine, procaine, chloroprocaine, prilocaine, tetracaine, bupivacaine, cinchocaine, ropivacaine, cocaine, benzocaine, cyclomethycaine, dimethocaine, piperocaine, proxycaine, articaine, etidocaine, levobupivacaine, mepivacaine, trimecaine, saxitoxin, neosaxitoxin, tetrodotoxin, menthol, eugenol, and splianthol.

32. The method of any one of claims 1-30, for use in slow, controlled or sustained release of an analgesic, wherein the analgesic is selected from the group of paracetamol, NSAIDs, COX-2 inhibitors, opioids, alcohol, cannabis, and adjuvants.

33. The method of claim 32, wherein the analgesic is acetaminophen, diclofenac or naproxen.

34. The method of any one of claims 1-30, for use in slow, controlled or sustained release of a biomolecule, wherein the biomolecule is selected from the group of protein, peptide, nucleic acid, vitamin or mineral, enzyme, glycoprotein, proteoglycan, lipid, sugar, carbohydrates, lignin, cellulose, hemicellulose, pectin, and hormones.

35. The method of claim 34, wherein the biomolecule is a protein selected from albumin, lysozyme, somatostatin, or insulin.

36. The method of claim 34, wherein the biomolecule is hyaluronic acid.

37. The method of any one of claims 1-30, for use in slow, controlled or sustained release of a therapeutic substance, wherein the therapeutic substance is selected from the group of stem cells, growth factors, inhibitors, drugs, proteins, peptides, platelets, cofactors, antibiotics, antipyretics and coenzymes.

38. The method of claim 37, wherein the antibiotic is gentamicin.

39. The method of any one of claims 1-30, for use in slow, controlled or sustained release of an anti-inflammatory drug, wherein the anti-inflammatory drug is NSAIDS or steroids.

40. The method of claim 1, wherein the mercerized decellularized cellulose particles are derived from a plant tissue.

41. The method of claim 1, wherein the mercerized decellularized cellulose particles are derived from apple, banana, mango or pear.

42. A method of providing a slow, controlled or sustained release of lidocaine comprising:
- (a) incubating lidocaine with a mercerized decellularized cellulose particles for a predetermined incubation time; and
 - (b) adjusting a physical or a chemical property of the mercerized decellularized cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of lidocaine in the patient.
43. A method of providing a slow, controlled or sustained release of ibuprofen comprising:
- (a) incubating ibuprofen with a mercerized decellularized cellulose particles for a predetermined incubation time; and
 - (b) adjusting a physical or a chemical property of the mercerized decellularized cellulose particles to trigger, alter, enable or affect the slow, controlled or sustained release of ibuprofen in the patient.
44. Cellulose particles for slow, controlled or sustained release of a pre-absorbed compound of interest, wherein the cellulose particles are mercerized and derived from a decellularized plant material.
45. The cellulose particles of claim 44, wherein the particles have a crystal structure.
46. The cellulose particles of claim 44, wherein the cellulose particles are derived from a plant tissue of apple, banana, mango or pear.
47. The cellulose particles of any one of claims 44-46, wherein the concentration of the cellulose particles ranges from 1% to 7% by mass.
48. The cellulose particles of any one of claims 44-47, wherein the concentration of the pre-absorbed compound of interest ranges from about 0.1% to about 20%.
49. The cellulose particles of any one of claims 44-48, wherein a change in pH of the particles triggers, alters, enables or affects the slow, controlled or sustained release of the compound of interest.
50. The cellulose particles of any one of claims 44-49, wherein a change in pKa value of the particles triggers, alters, enables or affects the slow, controlled or sustained release of the compound of interest
51. The cellulose particles of any one of claims 44-50, wherein a chemical modification of the particles triggers, alters, enables or affects the slow, controlled or sustained release of the compound of interest.
52. The cellulose particles of any one of claims 44-51, wherein succinylation of the particles triggers, alters, enables or affects the slow, controlled or sustained release of the compound of interest.

53. The cellulose particles of claim 52, wherein the succinylated particles show a greater absorbance of the compound of interest compared to the non-succinylated particles.

54. The cellulose particles of any one of claims 44-53, wherein a modification to the crystal structure of the particles triggers, alters, enables or affects the slow, controlled or sustained release of the compound of interest.

55. The cellulose particles of any one of claims 44-54, wherein adding a charge on the particles triggers, alters, enables or affects the slow, controlled or sustained release of the compound of interest.

56. The cellulose particles of any one of claims 44-55, wherein the particles are in the form of a dermal filler.

57. The cellulose particles of any one of claims 44-56, for use in the management of pain, bone regeneration, soft tissue regeneration, regenerative medicine, angiogenesis, treatment of cancer, treatment of wounds, or nerve applications.

58. The cellulose particles of any one of claims 44-57, for production of growth factors, inhibitory factors, coagulation agents, or anticoagulation agents

59. The cellulose particles of any one of claims 44-58, for use in the slow release delivery of an anti-inflammatory drug, a therapeutic substance, an antibiotic, an anaesthetic, an analgesic, an antipyretic or a biomolecule.

60. The cellulose particles of claim 59, wherein the anaesthetic is selected from the group of lidocaine, procaine, chlorprocaine, prilocaine, tetracaine, bupivacaine, cinchocaine, ropivacaine, cocaine, benzocaine, cyclomethycaine, dimethocaine, piperocaine, proxycaine, articaine, etidocaine, levobupivacaine, mepivacaine, trimecaine, saxitoxin, neosaxitoxin, tetrodotoxin, menthol, eugenol, and splianthol.

61. The cellulose particles of claim 59, wherein the analgesic is selected from the group of paracetamol, NSAIDs, COX-2 inhibitors, opioids, alcohol, cannabis, and adjuvants.

62. The cellulose particles of claim 59, wherein the analgesic is acetaminophen, diclofenac or naproxen.

63. The cellulose particles of claim 59, wherein the antibiotic is gentamicin.

64. The cellulose particle of claim 59, wherein the biomolecule is a protein selected from albumin, lysozyme, somatostatin, or insulin.

65. The cellulose particles of claim 59, wherein the biomolecule is hyaluronic acid.
66. The cellulose particles of claim 59, wherein the biomolecule is selected from the group of protein, peptide, nucleic acid, vitamin or mineral, enzyme, glycoprotein, proteoglycan, lipid, sugar, carbohydrates, lignin, cellulose, hemicellulose, pectin, and hormones.
67. The cellulose particles of claim 59, wherein the therapeutic substance is selected from the group of stem cells, growth factors, inhibitors, drugs, proteins, peptides, platelets, cofactors, and coenzymes.
68. The cellulose particles of claim 59, wherein the anti-inflammatory drug is NSAID or steroid.
69. A formulation comprising the cellulose particles of any one of claims 44-68 and a physiologically acceptable component.