



(51) International Patent Classification:

A61K 9/70 (2006.01) *A61K 38/16* (2006.01)
A61K 47/42 (2006.01) *A61L 15/32* (2006.01)

(21) International Application Number:

PCT/IL2012/050079

(22) International Filing Date:

8 March 2012 (08.03.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/450,694 9 March 2011 (09.03.2011) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

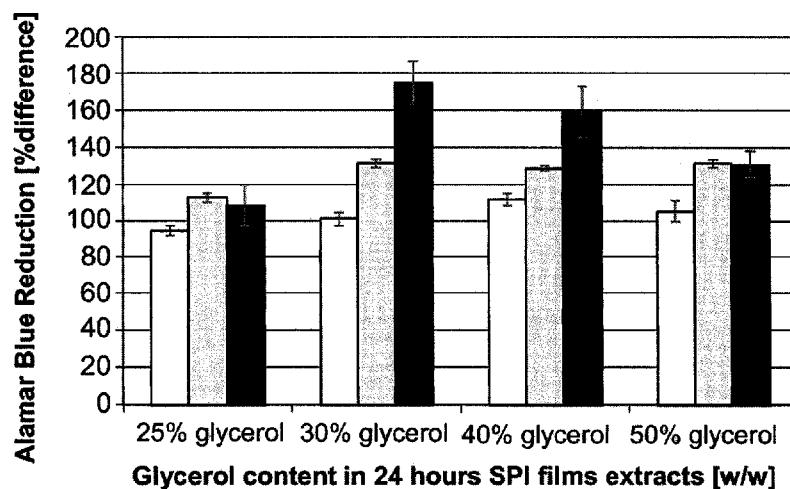
Published:

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

— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: SOY PROTEIN-BASED STRUCTURES AND USES THEREOF

FIG. 20A



(57) Abstract: Solution cast, crosslinked soy protein structures, characterized by mechanical and physical properties that are suitable for biomedical applications, and by a controllable release profile of bioactive agents therefrom are disclosed. Methods utilizing these structures and processes of preparing same are also disclosed.

SOY PROTEIN-BASED STRUCTURES AND USES THEREOF

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to material science and, more particularly, but not exclusively, to soy protein-based structures and uses thereof as drug-eluting systems.

Soybean is one of the most important and widely consumed legume crops in the world. It is composed of approximately 38 % proteins, 30 % carbohydrates, 18 % oil and 14 % minerals, other materials and water. Soybeans are widely consumed in the food industry in the form of inexpensive high-protein soy meals, soy oil, soy milk and tofu. Soybean protein has been explored mainly in the polymer, food and agriculture fields. The use of soybean protein as a food source is still increasing due to its functional and nutritional value, availability and low price. The carbohydrate and oil components of the soybean can be removed for obtaining soy protein isolate (SPI, at least 90 % protein).

Soy protein can be used for various applications. In the materials industry, soy protein was studied as an adhesive and as a "green" plastic [Kumar *et al.*, *Industrial Crops & Products*, 2002, 16(3), 155-172]. The soy protein matrix possesses the following characteristics: Low price, which makes it economically competitive; Non-animal origin, thus carrying no risk of transmissible diseases which poses a danger in human- and animal-derived products; Good water resistance and relatively long storage time and stability; and Degradation into natural components. These characteristics make soy protein favorable over the various types of biodegradable polymers and natural proteins used for biomedical applications.

Soybean-based products have also been reported as promoting tissue regeneration, such as new bone growth. Furthermore, these materials integrate into the blood clot and stimulate collagen deposition and therefore have a high potential for wound healing applications. They stimulate cells to produce new tissue, with no need for expensive growth factors [Santin M. and Ambrosio L., *Expert Review of Medical Devices*, 2008, 5(3), 349-358].

Protein films and adhesives are usually processed in water or extruded under low-moisture conditions. Several methods have been used to prepare films from soy

protein, including solvent casting [Kunte *et al.*, *Cereal Chemistry*, 1997, 74(2), 115-118], extrusion [Vaz, C.M., *et al.*, *J. Applied Polymer Science*, 2005, 97(2), 604-610], spinning in coagulating buffer [Rampon *et al.*, *J. Food Science*, 1999, 64(2), 313-316] and thermal compaction [Ogale *et al.*, *J. Food Science*, 2000, 65(4), 672-679].

5 Solvent casting of polymers is performed by a three step process: preparation of aqueous protein solutions, casting and drying by solvent evaporation. The structure and properties of the formed film can be modified by changing various parameters during preparation, such as the pH and temperature of the solution, amount of protein, drying conditions (temperature, humidity and duration) and heat treatment after film formation.

10 The film's properties can also be modified by additives such as plasticizers and crosslinking agents. During the solvent evaporation step, crosslinking occurs through intermolecular covalent disulfide (S-S) bonds and through secondary, hydrophobic and hydrogen interactions [Okamoto S., *Cereal Foods World*, 1978, 23(5), 257-262]. The resulting soy protein films have moderate mechanical properties compared to

15 commonly used films based on synthetic polymers, similarly to other protein films [Krochta J.M. and De Mulder-Johnston C., *Food technology*, 1997, 51(2), 61-74].

Degradation and weight loss profiles of the soy-protein films in aqueous medium were studied [Rhim *et al.*, *J. Agricultural and Food Chemistry*, 2000, 48(10), 4937-494; Vaz, C.M., *et al.*, *Polymer Degradation and Stability*, 2003, 81(1), 65-74;

20 Silva *et al.*, *J. Materials Science: Materials in Medicine*, 2005, 16(6), 575-579] and it was found that swelling occurs after the initial stage (several hours) of small chains and plasticizer leeching, and a constant slow degradation phase continues for approximately one month. This is followed by a third stage of erosion and massive weight loss.

Enzymatic degradation of soy protein films was also studied. Degradation of

25 crosslinked soy protein melt-processed films in collagenase buffer was found to be slightly higher than in a regular aqueous solution [Vaz, C.M., *et al.*, *Polymer Degradation and Stability*, 2003, 81(1), 65-74], but pepsin and pancreatin were found to be very effective in degrading soy protein films. Various modes of crosslinking were found to be effective in reducing the degradation rate [Chen *et al.*, *Biomaterials*, 2008,

30 29(27), 3750-3756].

Soy protein can be crosslinked by crosslinking and/or coupling agents, heat treatment, enzymatic treatment and irradiation. It has many reactive groups (e.g., -NH₂,

-OH and -SH) which enable crosslinking and coupling reactions. Low molecular aldehydes (e.g., formaldehyde, glutaraldehyde and glyoxal) react primarily with the free ϵ -amine groups of arginine, lysine and hydroxylysine residues of the protein, thereby forming intra- and inter-molecular crosslinks. Formaldehyde and glutaraldehyde are the most widely used agents, although concern for postimplantation cytotoxic effects following use of these two agents has been raised, due to monomer release from the crosslinked matrices [Huang-Lee *et al.*, *J. Biomedical Materials Research*, 1990, 24(9), 1185-1201; Van Luyn *et al.*, *J. Biomedical Materials Research*, 1992, 26(8), 1091-1110].

Crosslinking of soy protein can also be carried out by adding cysteine or Ca^{+2} ions.

Glyoxal is a potentially less toxic alternative for use in biomedical devices, as shown with collagen-based matrixes [Weadock *et al.*, *Artificial Cells, Blood Substitutes, and Biotechnology*, 1983, 11(4), 293 - 318].

Despite its functionality and good qualities, only a few groups have investigated the potential of soy, particularly soy protein, as a natural biomaterial for various applications. Soy protein-based thermoplastic reinforced with tricalcium phosphate was investigated for orthopedic biomedical applications [Vaz, C.M., *et al.*, *J. Macromolecular Science*, 2002, 41(1), 33-46]. These thermoplastics were found to be non-cytotoxic and even encouraged cell proliferation during *in vitro* tests [Silva *et al.*, *J. Materials Science: Materials in Medicine*, 2003, 14(12), 1055-1066]. Soybean-based materials were investigated for the purpose of bioactive bone fillers and wound dressing [Santin *et al.*, *Biomacromolecules*, 2007, 8(9), 2706-2711]. Two types of blends, poly(ethylene glycol)-soy protein hydrogel blends [Snyders *et al.*, *J. Biomedical Materials Research Part A*, 2007, 83A(1), 88-97] and chitosan-soy blends [Silva *et al.*, *J. Materials Science: Materials in Medicine*, 2005, 16(6), 575-579] were studied for wound dressing applications. Their investigation focused mainly on microstructural, physical and mechanical properties.

Additional Background art includes U.S. Patent Nos. 5,523,293, 5,543,164 and 6034198; U.S. Patent Application having Publication Nos. 2004/0081698, 2009/0010998, 2005/0196440 and 2010/0215774; WO 2009/005814; Lee *et al.*, *J. Food Science and Nutrition*, 2005, 10(1), 88-91; Vaz, C.M., *et al.*, *Materials Research*

Innovations, 2004, 8(3), 149-150; Reis *et al.*, *J. Materials Science: Materials in Medicine*, 2005, 16(6), 575-579; Santos *et al.*, *Tissue Engineering Part A*, , 2010 16(9), 2883-2890; Vaz, C.M., *et al.*, *Polymer*, 2003, 44(19), 5983-5992; Vaz, C.M., *et al.*, *Biomacromolecules*, 2003, 4, 1520-1529; Curt *et al.*, *Tissue Engineering: Part A*, 2008, 15(6), 1223-1232; Xu W, and Yang Y., *J. Materials Science: Materials in Medicine*, 2009, 20(12), 2477-2486; Zheng H., *J. Applied Polymer Science*, 2007,106(2); Rachelson and Zilberman, *Adv. Eng. Mater.*, 2009, 11, B122-B128; and Shifrovitch *et al.*, *J. Periodontol*, 2009, 330-337.

10 SUMMARY OF THE INVENTION

The present inventors have designed and successfully practiced solution cast, crosslinked soy protein structures and have surprisingly uncovered that these structures are characterized by mechanical and physical properties that are highly suitable for biomedical applications such as wound dressings, and are further characterized by a capacity to release bioactive agent therefrom in a controlled manner, rendering the soy protein structures highly suitable as local drug-delivery vehicles.

According to an aspect of some embodiments of the present invention, there is provided a composition-of-matter which includes crosslinked soy protein isolate and a plasticizer, wherein an amount of the plasticizer ranges from 25 to 100 weight percents of the weight of the soy protein isolate.

In some embodiments, the plasticizer is selected from the group consisting of glycerol, sorbitol, low molecular weight polyethylene glycol (PEG), polyvinyl alcohol (PVA) and any combination thereof.

In some embodiments, the plasticizer is glycerol.

In some embodiments, the amount of glycerol ranges from 25 to 80 weight percents of a weight of the soy protein isolate.

In some embodiments, the amount of glycerol ranges from 35 to 50 weight percents of a weight of the soy protein isolate.

In some embodiments, the amount of glycerol is 50 weight percents of a weight of the soy protein isolate.

In some embodiments, the amount of glycerol is 35 weight percents of a weight of the soy protein isolate.

In some embodiments, the soy protein isolate is chemically crosslinked.

In some embodiments, the chemically crosslinked soy protein isolate is obtained by contacting the soy protein isolate with a crosslinking agent.

5 In some embodiments, the crosslinking agent is selected from the group consisting of glyoxal, cysteine, formaldehyde, glutaraldehyde and polyglutaraldehyde.

In some embodiments, the crosslinking agent is glyoxal.

In some embodiments, the amount of glyoxal ranges from 0.1 to 2 weight percents of a weight of the soy protein isolate.

10 In some embodiments, the amount of glyoxal is 1 weight percent of a weight of the soy protein isolate.

In some embodiments, the composition-of-matter presented herein is thermally cured.

In some embodiments, thermal curing is effected by heat treatment of the crosslinked soy protein isolate.

15 In some embodiments, the soy protein isolate is crosslinked by thermal curing.

In some embodiments, thermal curing includes heat treatment of the soy protein isolate and the plasticizer.

In some embodiments, the thermal curing includes heat treatment which is effected at a temperature that ranges from 40 to 100 °C.

20 In some embodiments, the heat treatment is effected at a temperature that ranges from 60 to 80 °C.

In some embodiments, the thermal curing includes heat treatment which is effected for a time period that ranges from 12 to 48 hours.

25 In some embodiments, the composition-of-matter presented herein is characterized by at least one of:

a tensile strength that ranges from 5 to 30 MPa;

a Young Modulus that ranges from 50 to 600 MPa (e.g., 100 to 300 MPa);

a maximal strain that ranges from 50 to 300 percents; and

30 a water vapor transmission rate (WVTR) that ranges from 1000 to 4000 grams/m²/day.

In some embodiments, the water vapor transmission rate (WVTR) ranges from 2000 to 3000 grams/m²/day.

In some embodiments, the water vapor transmission rate (WVTR) ranges from 2000 to 2500 grams/m²/day.

According to another aspect of some embodiments of the present invention, there is provided a composition-of-matter which includes a crosslinked soy protein isolate, whereas the composition-of-matter is characterized by at least one of:

a tensile strength that ranges from 5 to 30 MPa;

a Young Modulus that ranges from 50 to 600 MPa (e.g., 100 to 300 MPa);

a maximal strain that ranges from 50 to 300 percents; and

a water vapor transmission rate (WVTR) that ranges from 1000 to 4000 grams/m²/day, or from 2000 to 3000 grams/m²/day, or from 2000 to 2500 grams/m²/day.

In some embodiments, the composition-of-matter further includes a plasticizer.

In some embodiments, the amount of the plasticizer ranges from 25 to 100 weight percents of a weight of the soy protein isolate.

In some embodiments, the plasticizer is selected from the group consisting of glycerol, sorbitol, low molecular weight polyethylene glycol (PEG), polyvinyl alcohol (PVA) and any combination thereof.

In some embodiments, the plasticizer is glycerol.

In some embodiments, the amount of glycerol ranges from 25 to 80 weight percents of a weight of the soy protein isolate.

In some embodiments, the amount of glycerol ranges from 35 to 50 weight percents of a weight of the soy protein isolate.

In some embodiments, the amount of glycerol is 50 weight percents of a weight of the soy protein isolate.

In some embodiments, the amount of glycerol is 35 weight percents of a weight of the soy protein isolate.

In some embodiments, the soy protein isolate is chemically crosslinked.

In some embodiments, the chemically crosslinked soy protein isolate is obtained by contacting the soy protein isolate with a crosslinking agent.

In some embodiments, the crosslinking agent is selected from the group consisting of glyoxal, cysteine, formaldehyde, glutaraldehyde and polyglutaraldehyde.

In some embodiments, the crosslinking agent is glyoxal.

In some embodiments, the amount of glyoxal ranges from 0.1 to 2 weight percents of a weight of the soy protein isolate.

In some embodiments, the amount of glyoxal is 1 weight percent of a weight of the soy protein isolate.

5 In some embodiments, the composition-of-matter is thermally cured.

In some embodiments, the thermally cured isolate is obtained by heat treatment at a temperature that ranges from 40 to 100 °C.

In some embodiments, the thermally cured isolate is obtained by heat treatment at a temperature that ranges from 60 to 80 °C.

10 In some embodiments, the heat treatment is effected for a time period that ranges from 12 to 48 hours.

According to another aspect of some embodiments of the present invention, there is provided a composition-of-matter which includes crosslinked soy protein isolate and a plasticizer, wherein the amount and type of the crosslinking and the plasticizer are selected such that the composition is characterized by at least one of:

a tensile strength that ranges from 5 to 30 MPa;

a Young Modulus that ranges from 50 to 600 MPa (e.g., 100 to 300 MPa);

a maximal strain that ranges from 50 to 300 percents; and

20 a water vapor transmission rate (WVTR) that ranges from 1000 to 4000 grams/m²/day, or 2000 to 3000 grams/m²/day.

In some embodiments, the crosslinking includes chemical crosslinking by a crosslinking agent.

In some embodiments, the crosslinking includes thermal curing.

25 In some embodiments, the composition-of-matter further includes a bioactive agent incorporated therein.

In some embodiments, the content of the bioactive agent ranges from 0.1 to 10 weight percents of a weight of the soy protein isolate.

30 In some embodiments, the bioactive agent is selected from the group consisting of an antimicrobial agent, an analgesic agent, an anesthetic agent, an anti-inflammatory agent, a cell proliferation agent, an immunosuppressive agent, a clotting factor, an osseointegration agent, a growth factor, a genetic agent, a hormone, a vitamin, a mineral, an antibody and a antiproliferative agent.

In some embodiments, the composition-of-matter presented herein is capable of releasing the bioactive agent upon contacting the composition with a physiological medium over a time period of at least 2 hours.

In some embodiments, 30 to 70 percents of the bioactive active agent are released during 6 hours of the contacting and the remaining of the bioactive agent is released over at least 30 days of the contacting.

In some embodiments, the bioactive agent is an antimicrobial agent.

In some embodiments, the antimicrobial agent is selected from the group consisting of ampicillin, ampicillin-sulbactam, augmentin, cefazolin, cefotaxime, cefotetan, cefoxitin, ceftriaxone, cephalixin, ciprofloxacin, clavulanic acid, dicloxacillin, gentamicin, imipenem, metronidazole, piperacillin, tazobactam, ticarcillin and combinations thereof.

In some embodiments, the composition-of-matter presented herein is identified for use in treating an infection associated with a pathogenic microorganism.

In some embodiments, at least 90 percents of the bioactive agent are released during two days from the contacting.

In some embodiments, the bioactive agent is an analgesic agent.

In some embodiments, the analgesic agent is selected from the group consisting of a non-steroidal anti-inflammatory drug (NTHE), a COX-2 inhibitor, an opiate, a morphinomimetic and combinations thereof.

In some embodiments, the analgesic agent is selected from the group consisting of aspirin, ibuprofen, bupivacaine, naproxen, celecoxib, rofecoxib, morphine, codeine, oxycodone, hydrocodone, dihydromorphine, pethidine, buprenorphine, tramadol, venlafaxine, paracetamol, flupirtine, derivatives and combinations thereof.

In some embodiments, the composition-of-matter presented herein is identified for use in the treatment of local pain.

In some embodiments, the composition-of-matter presented herein is in a form selected from the group consisting of a film, a strip, a wound dressing, a bandage, a poultice, a compress, a fascia, a pack, a plaster, a pledget, a cataplasm and a patch.

According to another aspect of some embodiments of the present invention, there is provided a composition-of-matter which includes crosslinked soy protein isolate and a plasticizer, and further includes a bioactive agent incorporated therein, wherein

the amount and type of the crosslinking and the plasticizer are selected such that the composition is characterized as capable of releasing the bioactive agent upon contacting the composition-of-matter with a physiological medium over a time period of at least 2 hours.

5 In some embodiments, the crosslinking includes chemical crosslinking by a crosslinking agent.

 In some embodiments, the crosslinking includes thermal curing.

 In some embodiments, the thermal curing is effected at a temperature that ranges from 40 to 100 °C.

10 In some embodiments, the thermal curing is effected at a temperature that ranges from 60 to 80 °C.

 In some embodiments, the thermal curing is effected for a time period that ranges from 12 to 48 hours.

 In some embodiments, 30 to 70 percents of the bioactive active agent are released during 6 hours from the contacting and the remaining of the bioactive agent is released over at least 30 days of the contacting.

 In some embodiments, the bioactive agent is an antimicrobial agent.

 In some embodiments, at least 90 percents of the bioactive agent are released during two days from the contacting.

20 In some embodiments, the bioactive agent is an analgesic agent.

 In some embodiments, the composition-of-matter is characterized by at least one of:

 a tensile strength that ranges from 5 to 30 MPa;

 a Young Modulus that ranges from 50 to 600 MPa (e.g., 100 to 300 MPa);

25 a maximal strain that ranges from 50 to 300 percents; and

 water vapor transmission rate (WVTR) that ranges from 1000 to 4000 grams/m²/day, or from 2000 to 3000 grams/m²/day, or from 2000 to 2500 grams/m²/day.

 In some embodiments, the composition-of-matter presented herein is a solution cast composition.

30

In some embodiments, the composition-of-matter is obtained by casting an aqueous solution includes the soy protein isolate, the plasticizer and a crosslinking agent, if present.

5 In some embodiments, the concentration of the soy protein isolate in the aqueous solution ranges from 3 to 7 percents weight per volume.

In some embodiments, the solution has a pH that ranges from 6 to 10.

In some embodiments, the pH is 7.2.

In some embodiments, the solution cast composition is further heated to a temperature that ranges from 25 °C to 70 °C.

10 In some embodiments, the temperature is 55 °C.

In some embodiments, the composition-of-matter is substantially devoid of non-SPI polymers.

In some embodiments, the composition-of-matter is substantially devoid of formaldehyde.

15 In some embodiments, the composition-of-matter is substantially devoid of a filler.

In some embodiments, the composition-of-matter is for use as wound dressing.

In some embodiments, the composition-of-matter is use in the treatment of a wound.

20 According to another aspect of some embodiments of the resent invention, there is provided a method of treating a wound, which is effected by contacting the wound with the composition-of-matter presented herein.

According to another aspect of some embodiments of the resent invention, there is provided a use of the composition-of-matter presented herein in the manufacture of a product for treating a wound.

25 According to another aspect of some embodiments of the resent invention, there is provided a process of preparing the composition-of-matter presented herein which is effected by:

providing an aqueous solution containing soy protein isolate at a concentration that ranges from 3 to 7 percents weight per volume;

30 a plasticizer in an amount that ranges from 25 to 100 weight percents of a weight of the soy protein isolate;

an crosslinking agent, if present, in an amount that ranges from 0 to 2 weight percents of a weight of the soy protein isolate ; and

a bioactive agent, if present, in an amount that ranges from 0.1 to 10 weight percents of a weight of the soy protein isolate,

5 heating the solution to a temperature that ranges from 25 °C to 70 °C;

casting the solution onto a mold or a surface; and

drying the solution at room temperature and ambient humidity for a time period that ranges from to thereby obtain the composition-of-matter.

In some embodiments, the process further includes, following the drying,
10 heating the composition-of-matter to a temperature that ranges from 40 °C to 100 °C.

In some embodiments, the temperature ranges from 60 to 80 °C.

In some embodiments, the heating is effected for a time period that ranges.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which
15 the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

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BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and
25 for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs .1A-C present bar graphs showing tensile strength tests (Figure 1A),
30 maximal strain (FIG. 1B) and Young's modulus tests (FIG. 1C), measured for exemplary SPI film samples prepared with, 5 % w/v SPI content, 1 % glyoxal crosslinking agent, cast from high temperature solutions (55 °C, 24 hours) and thermally

cured at 80 °C for 24 hours, showing the effect of various glycerol and bioactive agent contents on mechanical parameters;

FIGs. 2A-C present bar graphs showing the effect of duration of heat treatment ("thermal" crosslinking or thermal curing) for 1, 2, 6 and 24 hours on SPI films, according to some embodiments of the present invention, prepared at 70 °C from 5 % w/v SPI solutions and further containing 1 % glyoxal as a chemical crosslinking agent and 50 % glycerol as a plasticizer, wherein FIG. 2A shows the effect on tensile strength, maximal strain in FIG. 2B and Young's modulus in FIG. 2C;

FIGs. 3A-C present bar graphs showing tensile strength tests (FIG. 3A), maximal strain (FIG. 3B) and Young's modulus tests (FIG. 3C), measured for exemplary SPI film samples prepared with 5 % w/v SPI content, 50 % w/w glycerol plasticizer and 1 % w/w glyoxal crosslinking agent, and cast from high temperature solutions (70 °C, 24 hours), and showing the effect of heat treatment (thermal curing) at 60 °C, 80 °C and 100 °C;

FIGs. 4A-C present bar graphs showing tensile strength tests (FIG. 4A), maximal strain (FIG. 4B) and Young's modulus tests (FIG. 4C), measured for exemplary SPI film samples prepared with 4 %, 5 %, 7 % w/v SPI content, 50 % w/w glycerol plasticizer, and cast from high temperature solutions (70 °C and 55 °C, 24 hours), showing the effect of various SPI content and casting temperature on the tested parameters;

FIGs. 5A-C present bar graphs showing tensile strength tests (FIG. 5A), Young's modulus tests (FIG. 5B) and elongation at break tests (FIG. 5C), measured for two exemplary SPI film samples prepared with 5 % w/v SPI content and 50 % w/w glycerol plasticizer, wherein the films cast from high temperature and high pH solutions (70 °C, pH=10) are marked by solid black and films cast from low temperature and neutral pH solutions (55 °C, pH=7.2) are marked by checked bars, and the crosslinking method is indicated below the bars;

FIG. 6 presents comparative stress-strain curves of three exemplary 5 % w/v SPI film samples plasticized with 50 % w/w glycerol and cast as untreated films, crosslinked using 1 % glyoxal, and crosslinked with 1 % glyoxal and thermally treated at 80 °C for 24 hours as indicated therein;

FIGs. 7A-C present comparative bar-plots of tensile strength tests (FIG. 7A),

maximal strain (FIG. 7B) and Young's modulus tests (FIG. 7C), measured for exemplary SPI film samples prepared with 5 % w/v SPI content, 50 % w/w glycerol plasticizer, cast from high temperature solutions (55 °C, 24 hours) and thermal cured for 24 hours at 80 °C, showing the effect of various glyoxal amounts is 0 %, 0.5 %, 1 % and 2 % weight percents of the weight of the soy protein isolate (w/w relative to dry SPI weight).

FIGs. 8A-C present bar graphs showing tensile strength tests (FIG. 8A), Young's modulus tests (FIG. 8B) and elongation at break tests (FIG. 8C), measured for two exemplary SPI film samples prepared with 5 % w/v SPI content, 50 % w/w glycerol plasticizer and 1 % w/w glyoxal crosslinking agent, and cast from high temperature solutions (80 °C, 24 hours), wherein the results obtained for films cast from solutions at pH 6 are marked by solid white bars, films cast from solutions at pH 8.2 are marked by solid grey bars and films cast from solutions at pH 7.2 are marked by solid black bars, and the temperature of the cast solution is indicated below the bars;

FIG. 9 presents a scatter-plot of the water reuptake of SPI film samples as a function of time, wherein the films are prepared with 5 % w/v SPI content, plasticized using 50 % w/w glycerol and cast from solution at pH 7.2 and 55 °C, whereas samples of un-crosslinked films are marked by solid rectangles, thermally crosslinked (80 °C for 24 hours) films are marked with X, films crosslinked using 1 % w/w glyoxal are marked with solid rhombs and filmed crosslinked using both thermal treatment and glyoxal are marked by solid triangles;

FIG. 10 presents a bar graph showing the water vapor transmission rate (WVTR) measured for SPI film samples prepared with 5 % w/v SPI content, plasticized using 50 % w/w glycerol and cast from solution at pH 7.2 and 55 °C, wherein the crosslinking process is indicated from left bar to right bar as thermally and chemically crosslinked samples (1 % w/w glyoxal and thermal treatment at 80 °C for 24 hours), chemically crosslinked samples, thermally crosslinked samples, un-crosslinked samples and an aqueous solution designated as "open-cup";

FIG. 11 presents a scatter-plot of the weight loss profile of SPI film samples as a function of time, wherein the films are prepared with 5 % w/v SPI content, plasticized using 50 % w/w glycerol and cast from solution at pH 7.2 and 55 °C, whereas samples of un-crosslinked films are marked by solid rectangles, thermally crosslinked (80 °C for

24 hours) films are marked with X, films crosslinked using 1 % w/w glyoxal are marked with solid rhombs and filmed crosslinked using both thermal treatment and glyoxal are marked by solid triangles;

FIGs. 12A-B present scatter-plots of cumulative gentamicin release from SPI film samples as a function of time from SPI (5 % w/v SPI, pH 7.2, 55 °C, 50 % w/w glycerol and 1 % w/w glyoxal), wherein measurements obtained from films not treated with heat are marked by solid rectangles, and film samples thermally treated at 80 °C for 24 hours are marked by solid triangles, while FIG. 12A presents results obtained for films loaded with 1 % w/w gentamicin, and results obtained for films loaded with 3 % w/w gentamicin are presented in FIG. 12B;

FIGs. 13A-B present photographs of Petri dishes in which a culture of *Streptomyces albus*, an actinobacteria species of the genus *Streptomyces* has been grown and round samples of SIP films, according to embodiments of the present invention, have been placed upon to demonstrate a modified Kirby–Bauer disc diffusion test, showing a growth inhibition circles around SIP film samples prepared with 3 % gentamicin (FIG. 13A) and no growth inhibition around samples of SPI films prepared with no antibiotic agent as a control experiment (FIG. 13B);

FIGs. 14A-C present comparative histogram plots showing the effect of drug release on corrected zone of inhibition (CZOI) around the four types of exemplary gentamicin-eluting SPI films, as a function of pre-incubation time, as observed for three bacterial strains *S. albus* (FIG. 14A), *S. aureus* (FIG. 14B), and *P. aeruginosa* (FIG. 14C), wherein solid black bars represents results obtained with SPI films crosslinked with 1 % glyoxal and thermally treated at 80 °C for 24 hours containing 3 % gentamicin, diagonally striped bars represent results obtained with SPI films crosslinked using 1 % glyoxal containing 3 % gentamicin, solid grey bars represent results obtained with SPI films crosslinked with 1 % glyoxal and thermally treated at 80 °C for 24 hours containing 1 % gentamicin, and dotted bars results obtained with SPI films crosslinked using 1 % glyoxal containing 1 % gentamicin;

FIG. 15 presents a plot showing the cumulative amount of ibuprofen released during the first week from SPI films, prepared with various plasticizer contents and loaded with 3 % w/w ibuprofen, wherein rhombs represent films prepared with 25 % glycerol, rectangles represent 30 % glycerol, triangles represent 40 % and X represent

films prepared with 50 % glycerol, and the insert shows a magnification of the cumulative release profile in the first day;

FIGs. 16A-B presents bar graphs showing the percentage of counted cells compared to the number of seeded cells in the presence of the 24-hours (FIG. 16A) and the 24-to-48 hours (FIG. 16B) SPI film extractions tests at different cultivation times, wherein dotted bars represent results obtained with untreated SPI film samples, diagonally stripped bars represent results from films crosslinked with 1 % glyoxal and thermally treated at 80 °C for 24 hours, solid red bars represent results from films crosslinked using 1 % glyoxal containing 3 % gentamicin, and solid blue bars represent results obtained from control experiments;

FIGs. 17A-H present photographs of fibroblast cell cultures grown for 72 hours in the presence of 24 hours extracts of exemplary SPI film samples (FIGs. 17 A-D), and 24-to-48 hours extracts (FIGs. 17 E-H), wherein growth in the presence of extract of untreated SPI film samples are shown in FIGs. 17A and 17E, films crosslinked using 1 % glyoxal and containing 3 % gentamicin in FIGs. 17B and 17F, films crosslinked with 1 % glyoxal and thermally treated at 80 °C for 24 hours in FIGs. 17C and 17G, and controls in FIGs. 17D and 17H;

FIGs. 18A-B present the results obtained for the positive control experiment of fibroblast cultures growth for 72 hours, wherein FIG. 18A shows a photograph of the cell culture, and FIG. 18B is a bar-plot showing percentage of counted cells compared to the number of seeded cells in the presence of film components after 24 hours of cultivation, compared to the cytotoxic effects of glycerol and SPI on cell culture;

FIGs. 19A-C present photographs of fibroblast cell cultures grown for 5 days in the presence SPI film samples, wherein cells grown in direct contact with films crosslinked using 1 % glyoxal and containing 3 % gentamicin are shown in FIG. 19A, films crosslinked with 1 % glyoxal and thermally treated at 80 °C for 24 hours in FIG. 19B, and control experiment in FIG. 19C; and

FIGs. 20A-B present bar graphs showing the effect exhibited by the 24 hours (FIG. 20A) and the 24-to-48 hours (FIG. 20B) SPI films extractions, wherein the white bars represent Alamar-Blue reduction after 1 day cell cultivation, grey bars after 2 days and black bars represent readings after 3 days of cell cultivation.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to material science and, more particularly, but not exclusively, to soy protein-based structures and uses thereof as drug-eluting systems.

5 Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose
10 of description and should not be regarded as limiting.

The main goal in wound management is to achieve rapid healing with functional and esthetic results. An ideal wound dressing can restore the milieu required for the healing process, while simultaneously protecting the wound bed against bacteria and environmental threats. The dressing should also be easy to apply and remove. Most
15 modern dressings are designed to maintain a moist healing environment, and to accelerate healing by preventing cellular dehydration and promoting collagen synthesis and angiogenesis.

However, over-restriction of water evaporation from the wound should be avoided, since accumulation of fluid under the dressing may cause maceration and
20 facilitate infection. The water vapor transmission rate (WVTR) from the skin has been found to vary considerably depending on the wound type and healing stage, increasing from 204 g/m²/day for normal skin to 278 and as much as 5138 g/m²/day for first degree burns and granulating wounds, respectively. The physical and chemical properties of the dressing should therefore be adapted to the type of wound as well as to the degree of
25 wound exudation.

A range of dressing formats based on films, hydrophilic gels and foams are available or have been investigated. For example, thin semi-permeable polyurethane films coated with a layer of acrylic adhesive, such as Optsite[®] (Smith & Nephew) and Bioclusive[®] (J & J), are typically used for minor burns, post-operative wounds, and a
30 variety of minor injuries including abrasions and lacerations.

Bacterial contamination of a wound seriously threatens its healing. In burns, infection is the major complication after the initial period of shock, and it is estimated

that about 75 % of the mortalities following burn injuries are related to infections rather than to osmotic shock and hypovolemia. This has encouraged the development of improved wound dressings that provide an antimicrobial effect by eluting germicidal compounds such as iodine (Iodosorb[®], Smith & Nephew), chlorohexidime (Biopatch[®], J & J) or most frequently silver ions (e.g., Acticoat[®] by Smith & Nephew, Actisorb[®] by J & J and Aquacell[®] by ConvaTec). Such dressings are designed to provide controlled release of the active agent through a slow but sustained release mechanism which helps avoid toxicity yet ensures delivery of a therapeutic dose to the wound. Some concerns have been raised regarding safety issues related to the silver ions included in most products. Furthermore, such dressings still require frequent change, which may be painful to the patient and may damage the vulnerable underlying skin, thus increasing the risk of secondary contamination.

There is thus an increasing need to develop new biodegradable materials for use in wound healing applications.

As discussed hereinabove, soy protein, the major component of the soybean, is an advantageous natural biomaterial. However, very few research projects investigated this protein as a biomaterial.

The present inventors have envisioned that soy protein-based structures can be efficiently used as wound dressings and have designed such structures. The present inventors have studied the effect of various parameters on the mechanical and physical properties of such structures, and further prepared and studied drug-eluting soy protein-based structures and drug release profile thereof. The present inventors have surprisingly found that certain conditions and processing techniques can afford a composition-of-matter based on soy protein isolate that exhibits specific and highly desired properties which render the resulting structure highly useful in various medicinal applications, such as wound dressing.

As presented in the Examples section that follows, soy protein isolate (SPI, in which the carbohydrate and oil components of the soybean have been removed for obtaining soy protein (at least 90 %)), was investigated as a matrix for wound dressing applications. The plasticizer type and crosslinking method were found to affect the tensile properties of the SPI films; the degree of water uptake and the weight loss

profile. The water vapor transmission rate of the films was in the desired range for wound dressings (from 2000 to 2500 g/m²/day; e.g., about 2300 g/m²/day).

The antibiotic drug gentamicin was incorporated into the matrix for local controlled release and thus protection against bacterial infection. Homogenous yellowish films were cast from aqueous solutions. After crosslinking they combined high tensile strength and modulus with the desired ductility.

The gentamicin release profile exhibited a moderate burst effect followed by a decreasing release rate which was maintained for at least 4 weeks. Diffusion was the dominant release mechanism of gentamicin from crosslinked SPI films.

Thus, it was shown that an appropriate selection of the process parameters yielded SPI wound dressings with desired mechanical and physical properties and drug release behavior which protects against bacterial infection. These unique structures are thus potentially useful as burn and ulcer dressings, as well as in other drug-releasing applications.

It was further shown that an appropriate selection of the process parameters yielded SPI wound dressings with desired mechanical and physical properties and drug release profile which can be used as an effecting treatment of local pain, by releasing an analgesic agent such as bupivacaine or the NSAID ibuprofen over a period of a few days.

Hence, according to an aspect of some embodiments of the present invention there is provided a composition-of-matter which comprises a crosslinked soy protein isolate (SPI) and a plasticizer in an amount of 25-100 weight percents relative to the dry weight of the SPI. As discussed and presented hereinbelow, the present inventors have demonstrated that the high plasticizer content confers desirable mechanical properties and further confers desirable drug-release profile.

As used herein, the term "composition-of-matter" is used interchangeably with the term "soy protein structure", and encompasses any composition or structure that has a skeleton which comprises crosslinked soy protein isolate.

According to some embodiments of the present invention, the soy protein structure is biocompatible. As used herein, the term "biocompatible" and any adjective, conjugation and declination thereof, refers to a quality of a composition of not having

toxic or injurious effects on biological systems, also to the extent to which the composition does not elicits an immune or other response in a recipient subject.

According to some embodiments of the present invention, the soy protein structure presented herein is biodegradable. The term "biodegradable" and any
5 adjective, conjugation and declination thereof as used herein, refers to a characteristic of a material to undergo chemical and/or physical transformation from a detectable solid, semi-solid, gel, mucus or otherwise a localized form, to a delocalized and/or undetectable form such as any soluble, washable, volatile, absorbable and/or resorbable breakdown products or metabolites thereof. A biodegradable material undergoes such
10 transformation at physiological conditions due to the action of chemical, biological and/or physical factors, such as, for example, innate chemical bond lability, enzymatic breakdown processes, melting, dissolution and any combination thereof.

According to some embodiments of the present invention, the soy protein used to form the composition-of-matter presented herein, is a soy protein isolate (SPI). As
15 used herein, the term "soy protein isolate" refers to a highly refined or purified form of soy protein, which contains less than 10 % of non-protein mater on a dry basis. SPI is typically manufactured from defatted soy flour which has had most of the non-protein components, fats and carbohydrates removed. Soy protein isolate is generally accepted as safe and used in the food industry as a food additive or to increase protein content in
20 various foods products.

According to some embodiments of the present invention, the soy protein structures presented herein are substantially devoid of polymers which are not soy protein. For example, the composition-of-matter presented herein is devoid of polysaccharides such as chitosan, cellulose and the likes. By "substantially devoid of
25 polymers which are not soy protein" it is meant less than 1 %, less than 0.5 %, less than 0.1 %, less than 0.01 %, including absolute 0, are present in the composition-of-matter or in the soy protein isolate used for its preparation.

Plasticizer:

The present inventors have shown that the use of the soy protein structures
30 presented herein as, e.g., a primary wound dressing platform, requires the addition of a non-volatile plasticizer to guarantee the mechanical durability, i.e. the elasticity and strength of the structure, during and after the casting process, and while in use. The

present inventors have also shown that a plasticizer has an additional effect on the release profile of agents or solutes incorporated in the soy protein structure. Typically a plasticizer counterbalances the stresses that are induced in the casting process, such as shrinking, as the plasticizing effect of water in the films decreases upon drying, heating and curing.

In some embodiments of the present invention, the plasticizer belongs to those plasticizers which are approved by many official pharmacopoeias and local regulatory authorities (e.g., polyalcohols).

Exemplary plasticizers which can be used in soy protein structures according to some embodiments of the present invention, include polyols, glycerol, sorbitol, maltitol, xylitol, mannitol, erythritol, polyvinyl alcohol (PVA), polyglycerol, glycerol trioleate, tributyl citrate, acetyl tri-ethyl citrate, glyceryl triacetate, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, polyethylene oxide, ethylene glycol, diethylene glycol, polyethylene glycol (PEG), low molecular weight PEG such as PEG 200 and PEG 400, and combinations thereof.

In some embodiments, suitable plasticizers include, but are not limited to, glycerol, special grades of non-crystallizing aqueous sorbitol and sorbitol/sorbitan solutions, and combinations thereof. In addition, propylene glycol and low molecular weight polyethylene glycol (PEG 200, PEG 400 etc.) are contemplated.

The type and concentration of plasticizer(s) in the film is related to the desired mechanical performance and other aspects such as drug release profile, i.e. the possible interactions with the bioactive agent, the film's size, thickness and shape, the end use of the product and the anticipated storage conditions and the product's biocompatibility.

The choice of the proper formulation of a soy protein structure with respect to the protein/plasticizer combination, relates to the physical stability of the structure during manufacture, storage and ultimately during use. Therefore, a rational design of a soy protein structure requires analytical tools that allow the performance-related test parameters to be assessed. Differential scanning calorimetry (DSC) and dynamic mechanical thermal analysis (DMTA) can be used to monitor phase transitions and elastic moduli indicating molecular soy protein/plasticizer interactions and their effect on structure elasticity, i.e. to evaluate plasticizer effectiveness and compatibility.

A suitable plasticizer should interact with the soy protein molecules in such a way as to reduce effectively the glass transition temperature (T_g) of the soy protein structure without inhibiting the formation of crystallites that stabilize the three dimensional structure thereof. Glycerol combines some advantages of high plasticizing effect, a sufficient biocompatibility and a low volatility with the ability to interact specifically with the soy protein, thus allowing for the formation of a stable thermoreversible structure. Without being bound by any particular theory, it is assumed that the glycerol's plasticizing capability results from direct interactions with the soy protein and from its hygroscopic nature, which allows for an additional indirect moisturizing effect.

Sorbitol is an indirect plasticizer, mainly acting as a moisturizing agent with water being the effective plasticizer. Gradual differences of various grades of non-crystallizing sorbitol solutions in their plasticizing capability and their compatibility with protein are the result of differences in the amount of byproducts, namely hydrogenated oligosaccharides and sorbitol anhydrides, i.e. sorbitans. Sorbitol grades with a high amount of sorbitans, such as Anidrisorb, may be used effectively as plasticizers, according to some embodiments of the invention, owing to a certain direct plasticizing effect.

According to some embodiments of the present invention, hydrogenated oligosaccharides such as maltitol in combination with glycerol are also contemplated as effective plasticizers in the soy protein structures used as edible structures, since they augment the taste and chewability and assist in the rapid dissolution of the soy protein based structure upon chewing, thus improving the mouthfeel.

Considering plasticizing capability, propylene glycol (PEG) is also a suitable plasticizer. However, owing to its high solvent power for proteins, it has a slightly opposite effect on the formation of the structure that has to be compensated for by adjusting the processing parameters. Liquid polyethylene glycols can be used in combination with glycerol or propylene glycol.

In terms of content, the ratio by weight of dry plasticizer to dry soy protein determines some of the mechanical properties of the film and usually varies from 0.25:1 to 1.0:1 plasticizer:soy protein (w:w) (from 25 % w/w to 100 % w/w).

As demonstrated in the Examples section that follows below, glycerol and/or sorbitol have been shown to act as effective plasticizers at a content of 30, 50 and 80 weight percents relative to the weight of the dry soy protein isolate.

According to some embodiments of the present invention, glycerol is in an amount of 25 to 80 percents by weight relative to a weight of the SPI.

As demonstrated in the Examples section that follows below, glycerol has been shown to act as effective plasticizers at a content of 25, 30, 35 and 50 weight percents relative to the weight of the dry soy protein isolate. An improved performance was observed when a content of glycerol of 35 and higher weight percents relative to the weight of the dry soy protein isolate was used.

According to some embodiments of the present invention, glycerol is in an amount of 25 to 50 weight percents relative to a weight of the SPI.

According to some embodiments of the present invention, glycerol is in an amount of 35 to 50 weight percents relative to a weight of the SPI.

In some embodiments, the amount of glycerol is 50 % by weight relative to a weight of the SPI.

In some embodiments, the amount of glycerol is 35 % by weight relative to a weight of the SPI.

It is to be noted that in cases where biocompatibility of the soy protein structure is desired, high glycerol content is less preferred. The showing that a desired mechanical performance of the soy protein structure is achieved at glycerol concentrations of 50 weight percents relative to a weight of the SPI, while not adversely affecting the biocompatibility, represents an advantageous feature of the structures described herein. Further, the showing that a desired mechanical performance of the soy protein structure is achieved at glycerol concentrations lower than 50 weight percents (e.g., 35 weight percents) relative to a weight of the SPI, at which concentrations the biocompatibility is even enhanced (compared to the 50 % glycerol content), represents another advantageous feature of the structures described herein.

According to some embodiments of the present invention, the soy protein structures are substantially devoid of a filler, or alternatively, substantially devoid of particulate solid matter dispersed therein. By "substantially devoid of a filler" it is

meant less than 1 %, less than 0.5 %, less than 0.1 %, less than 0.01 %, including absolute 0.

A filler is often used to soften structures and bestow some mechanical properties thereto. A filler is typically a dispersion of particulate solid matter, such as a ceramic powder, a metal oxide powder and the likes, that is dispersed in the pre-crosslinked or pre-cured polymer solution or melt.

Crosslinked structure:

According to some embodiments of the present invention, the composition-of-matter presented herein is based on a crosslinked soy protein structure. The state of being crosslinked refers to the presence of intermolecular chemical interactions (e.g., covalent bonds, ionic bonds, hydrogen bonds, hydrophobic interactions, etc.) linking individual polymer chains; a state which confers some of the unique desired properties of the resulting structure, as presented hereinbelow.

The terms "crosslinking", "crosslinked" and other inflections thereof, are used herein to describe a soy protein in which links are formed between the polymeric chains of the protein synthetically, namely, in addition to any naturally occurring crosslinks in the protein (in accordance with the quaternary structure of the naturally occurring protein). Crosslinking of the protein can involve a curing process, such as thermal curing, and/or can be effected in the presence of certain reagents, referred to as "crosslinking agents", which promote chemical interactions between polymeric chains to effect "chemical crosslinking". Crosslinking affects physical and mechanical properties of a polymeric substance.

As used herein, the phrases "crosslinking agent" refers to a substance that promotes or regulates intermolecular interaction (e.g., covalent bonds, ionic bonds, hydrogen bonds, hydrophobic interactions or other form of interactions) between polymer chains, linking them together to create a network of chains which result in a more rigid structure. Crosslinking agents typically feature more than one chemical functionality, for example, two or more double bonds (vinyls), two or more aldehyde groups (e.g., dialdehydes) or two or more amines, thereby allowing the crosslinking agent to form chemical bonds between two or more polymer molecules (chains).

According to some embodiments of the present invention, exemplary crosslinking agent include, without limitation, glyoxal, formaldehyde, glutaraldehyde,

polyglutaraldehyde, cysteine, dextran, citric acid derivatives, microbial transglutaminase and genipin.

While formaldehyde and glutaraldehyde are regarded as the most commonly used crosslinking agents for protein-based products, concerns related to the cytotoxic effects of these two agents have caused the present inventors to contemplate other, more biocompatible crosslinking agents.

As formaldehyde is regarded as an unsafe reagent which is typically ban from food and medical products which must come in contact with a living subject, according to some embodiments of the present invention, the composition-of-matter presented herein is substantially devoid of formaldehyde. Furthermore, the crosslinking agent is optionally a formaldehyde-free crosslinking agent. Thus, the phrase "formaldehyde-free crosslinking agent", as used herein, refers to a crosslinking agent that does not contain and/or generate formaldehyde during and/or after its use. By "substantially devoid of formaldehyde" it is meant less than 1 %, less than 0.5 %, less than 0.1 %, less than 0.01 %, including absolute 0 is present or is generated.

Hence, according to some embodiments of the present invention, the crosslinking agent is glyoxal. Alternatively, the crosslinking agent is cysteine. A mixture of glyoxal and cysteine is also contemplated.

Typically, the amount of a crosslinking agent used in the making of a soy protein structure, according to some embodiments of the present invention, ranges from 0.1 to 2 percents by weight relative to the weight of the dry SPI. In some embodiments, glyoxal is used at an amount of 1 weight percent relative to the weight of the SPI.

As used herein, the term "curing" includes an active procedure such as subjecting a composition to certain conditions, such as heating and/or irradiation. The phrase "thermal curing" refers to an active exposure of a composition at some point along the process of its manufacturing to heat which causes crosslinking to occur. Without being bound by any particular theory, it has been suggested that such exposure to heat promotes the formation of disulfide bonds between polymer chains via cysteine side-chains, to thereby effect crosslinking of soy protein molecules by heat treatment (thermal curing).

According to some embodiments of the present invention, the thermal curing process is carried out by exposing the soy protein isolate or the composition-of-matter

comprising same to heat treatment at elevated temperatures, typically at or below water boiling point (100 °C or less).

According to some embodiments of the present invention, thermal curing of the soy protein isolate or the composition-of-matter comprising same as presented herein, is performed by exposing it to a temperature in the range of from 40 °C to 100 °C, or to a temperature ranging from 60 °C to 80 °C, or to a temperature of 40 °C, 45 °C, 50 °C, 55 °C, 60 °C, 65 °C, 70 °C, 75 °C, 80 °C, 85 °C, 90 °C, 95 °C or 100 °C, including any intermediate value.

According to some embodiments of the present invention, the heat treatment or thermal curing, is effected for a time period that ranges from 12 hours to 2 days, including any intermediate value.

In some embodiments, thermal curing is effected at a temperature of 80 °C. In some embodiments, thermal curing is effected at a temperature of 80 °C for 24 hours.

In some embodiments, the crosslinked soy protein isolate is crosslinked by thermal curing, such that the thermally-cured SPI is mixed with the plasticizer to form the composition-of-matter.

In some embodiments, the composition-of-matter is subjected to thermal curing, such that, for example, an intermediate solution cast soy protein structure, afforded after the initial drying step at ambient (room) temperature, is subjected to elevated temperature as described herein.

According to some embodiments of the present invention, the composition-of-matter is crosslinked by one or both thermal curing and/or chemical crosslinking.

While reducing the present invention to practice, the inventors have found that optimal properties of the soy protein structure are obtained by combining two crosslinking approaches, thermal curing and chemical crosslinking.

Thus, in some embodiments, the crosslinked SPI in the composition-of-matter is chemically crosslinked as described herein, such that the composition-of-matter further comprises a crosslinking agent which provides the crosslinked SPI. In some of these embodiments, the composition-of-matter which comprises the chemically crosslinked SPI and the plasticizer is further crosslinked by means of thermal curing.

Other additives:

In addition to soy protein, plasticizer(s), the optional crosslinking agent(s), and optional bioactive agent(s) which are discussed hereinbelow, according to some embodiments of the present invention, the composition-of-matter as described herein can further comprises other optional components (additives). These include, for example, colorants and/or opacifiers, which may be used to confer a desired color and/or a desired finish, e.g., to allow a film made from soy protein, according to some embodiments of the present invention, to provide protection from light and/or to mask the unpleasant look of a wound.

Solution cast:

According to some embodiments of the present invention, the composition-of-matter is formed by a solution cast processing technique using an aqueous solution of the soy protein isolate and the other ingredients. Such processing technique confers some of the unique desired properties of the resulting structure, as presented hereinbelow.

When compared to melt extrusion processing, solution cast processing has a profound effect on several mechanical and chemical properties of the afforded composite structures according to some embodiments of the present invention, primarily on their interaction with water and some of the mechanical properties derived therefrom. Studies that examined the relationships between structure/morphology and properties of series of composite structures prepared using these two processing techniques, namely solution casting and melt extrusion, showed that solution cast composite structures exhibit in general higher water uptake than melt extruded structures. Conductivity tests showed that at high levels of hydration, solution cast structures have in general higher conductivities than extruded samples. [Mokrini, A. *et al.*, *ECS Transactions*, 2010, 33(1), 855-865]. Studies using other polymeric materials investigated the effect of processing route on the structure and the thermal properties of the fabricated structures, wherein X-ray diffraction (XRD) testing indicated that a better dispersion of the incorporated additives can be achieved by solution cast intercalation, primarily due to the influence of the processing technique on the crystallization of the polymer [Marras, S.I. *et al.*, *J. Mat. Sci.*, 2010, 45(23), 6474-6480].

According to some embodiments of the present invention, the composition-of-matter presented herein is cast from an aqueous solution having a pH that ranges from 6 to 10. Alternatively, the pH is 7.2.

According to some embodiments of the present invention, the composition-of-matter presented herein is cast from an aqueous solution at a temperature that ranges from 25 °C to 70 °C. Alternatively, the temperature is 55 °C.

Additional discussion regarding the process of preparing a solution case composition-of-matter as disclosed herein is provided hereinafter.

Optimal mechanical properties:

Extensive studies and efforts are put into discovery and development of compositions exhibiting desired mechanical properties of substances. The mechanical properties of the composition-of-matter, or soy protein structures presented herein, determine to some extent the usability and applicability thereof.

As found by the present inventors, selection of ingredients, relative concentrations thereof as well as selection of chemical/thermal manipulation of the composition-of-matter or of the components comprising the same, afford an end-product soy protein structures with controllable, desired properties. Such selection of ingredients and their relative amounts as well as process parameters was found particularly beneficial for affording effective medical devices (e.g., wound dressing), as demonstrated hereinbelow.

Among several available criteria for describing the soy protein structures presented herein, tensile strength, Young's modulus and maximal strain have been selected as being widely accepted and standardized.

The phrase "tensile strength" as used herein describes the maximum amount of tensile stress that an object made of a given material can be subjected to before it breaks. As in the case of Young's modulus, tensile strength can be experimentally determined from a stress-strain curve, and is expressed in units of force per unit area (Newton per square meter (N/m²) or Pascals (Pa)).

Young's modulus (also known as the modulus of elasticity or elastic modulus) is a value which serves to determine the stiffness of a given substance. According to Hooke's law the strain of an object is proportional to the exerted stress applied thereto, and therefore the ratio of the two is a constant that is commonly used to indicate the

elasticity of the substance. Young's modulus is the elastic modulus for tension, or tensile stress, and is the force per unit cross section of the material divided by the fractional increase in length resulting from the stretching of an object. Young's modulus can be experimentally determined from the slope of a stress-strain curve
5 created during tensile tests conducted on a sample of the substance, and expressed in units of force per unit area (Newton per square meter (N/m²) or dynes per square centimeter), namely Pascals (Pa), megaPascals (MPa) or gigaPascals (GPa).

The term "maximal strain" is used herein as the breaking strain, namely the force exerted at the point of breaking an object.

10 According to some embodiments of the present invention, the obtained soy protein structures are characterized by at least one of:

a tensile strength that ranges from 2 to 30 MPa;

a Young Modulus that ranges from 50 to 600 MPa; and

a maximal strain before break that ranges from 50 % to 300 %.

15 According to some embodiments of the present invention, the tensile strength of a soy protein structure ranges from 5 to 30, or from 5 to 25 MPa. According to some embodiments of the present invention, the tensile strength ranges from 10 to 25 MPa, from 15 to 25 MPa or from 20 to 25 MPa, including any intermediate value.

According to some embodiments of the present invention, a soy protein structure
20 is characterized by a Young's modulus value that ranges from 50 to 600 MPa. According to some embodiments of the present invention, the Young's modulus value ranges from 50 to 300 MPa, or from 100 to 300 MPa. According to some embodiments of the present invention, the Young's modulus value ranges from 100 to 150 MPa, or from 150 to 200 MPa, or from 200 to 300 MPa, or from 150 to 300 MPa, including any
25 intermediate value.

According to some embodiments of the present invention, the maximal strain of a soy protein structure ranges from 5 % to 300 % or from 50 % to 300 %. According to some embodiments of the present invention, the maximal strain ranges from 50 % to 100 %, from 100 to 250 %, from 250 to 300 %, from 150 to 300 %, or from 200 % to
30 300 %.

Water vapor transmission rate:

One of the properties characterizing the composition-of-matter presented herein is its water vapor transmission rate, or WVTR. Water vapor transmission rate, also referred to as moisture vapor transmission rate (MVTR), is a measure of the passage of water vapor through a substance. When considering the provisions of the present invention in an exemplary embodiment of a primary wound dressing, the property of WVTR has an accentuated meaning. Exposed wound needs dressing as a protective means to form favorable environment for healing and revival, whereas healing of the wound is an important premise for convalescence.

Qualified primary wound dressing should have proper water vapor and gas transmission properties so as to form a favorable environment for speedy healing, which include relative humidity, acidity and oxygenation. Thus, water vapor transmission rate (WVTR) is a key index for manufacturers. As skin is the guard for internal stability and screening against external microorganism, many infections are related to the functional loss of skin, thus the main purpose of using primary wound dressing, severe wounds can be better healed. If water vapor transmission rate of primary wound dressing is too high, a humid environment could not be created. On the other hand, if the rate is too low, normal metabolism which promotes healing would be influenced adversely. Therefore, water vapor transmission rate should be regulated within a proper range. As humidity difference is formed between the two sides of the wound dressing at a certain temperature, water vapor permeates through the dressing and into the dry side.

According to some embodiments of the present invention, a soy protein structure is characterized by a water vapor transmission rate (WVTR) that ranges from 1000 to 4000 grams/m²/day. According to some embodiments of the present invention, the water vapor transmission rate (WVTR) ranges from 2000 to 3000 grams/m²/day, or from 2000 to 2500 grams/m²/day, or from 1000 to 2000 grams/m²/day, or from 2500 to 3000 grams/m²/day, or from 3000 to 3500 grams/m²/day, or from 3500 to 4000 grams/m²/day, including any intermediate value.

According to some embodiments of the present invention, the water vapor transmission rate (WVTR) ranges from 2000 to 2500 grams/m²/day.

Drug-eluting soy protein structures:

According to some embodiments of the present invention, the soy protein structures described herein further comprises one or more bioactive agent(s). In some embodiments, such a structure is designed to afford a drug-eluting structure. In other words, soy protein structures which contain a bioactive agent constitute a drug-eluting structure in which the bioactive agent is incorporated. In some embodiments, drug-eluting soy protein structures are formed such that the bioactive agent is released from therefrom upon contacting with a physiological medium. Thus, the soy protein structures, according to some embodiments of the present invention, can be used for various applications, as discussed herein, while at the same time serving as a reservoir and vehicle for delivering a bioactive agent.

It is noted herein that while the incorporation of a bioactive agent in the structure may affect its characteristics, the structure is designed to possess the desired properties presented hereinabove while adding the capacity of eluting bioactive agent(s) as discussed hereinbelow.

It is further noted herein that according to some embodiments of the present invention, the soy protein structure containing no bioactive agent, is meant for use primarily for its biocompatible mechanical properties. In such embodiments, other than the amount and rate of releasing a bioactive agent, all other characteristics and traits of a soy protein structure as described herein, as well as the main constituents and mode of preparation, apply for soy protein structures which do not include a bioactive agent therein.

The term "incorporated", as used in the context of a bioactive agent and the soy protein structure according to some embodiments of the present invention, is used synonymously with terms such as "sequestered", "loaded", "encapsulated" and the likes, all of which are used interchangeably to describe the presence of the bioactive agent, as defined hereinbelow, within the soy protein structure. An incorporated bioactive agent can elute or be released from the soy protein structure via, for example, diffusion, dissolution, elution, extraction, leaching, as a result of any or combination of wetting, swelling, dissolution, chemical breakdown, degradation, biodegradation, enzymatic decomposition and other processes that affect the soy protein structure. A bioactive

agent may also elute from the soy protein structure without any significant change to the structure, or with partial change.

As used herein, the phrase "bioactive agent" describes a molecule, compound, complex, adduct and/or composite that exerts one or more biological and/or pharmaceutical activities. In some embodiments, the term "bioactive agent" is used in place of the word "drug". The bioactive agent can thus be used, for example, to relieve pain, prevent inflammation, prevent and/or reduce and/or eradicate an infection, promote wound healing, promote tissue regeneration, effect tumor/metastasis eradication/suppression, effect local immune-system suppressed or increased response, and/or to prevent, ameliorate or treat various medical conditions.

"Bioactive agents", "pharmaceutically active agents", "pharmaceutically active materials", "pharmaceuticals", "therapeutically active agents", "biologically active agents", "therapeutic agents", "medicine", "medicament", "drugs" and other related terms may be used herein interchangeably, and all of which are meant to be encompassed by the term "bioactive agent".

The term "bioactive agent" in the context of the present invention also includes diagnostic agents, including, for example, chromogenic, fluorescent, luminescent, phosphorescent agents used for marking, tracing, imaging and identifying various biological elements such as small and macromolecules, cells, tissue and organs; as well as radioactive materials which can serve for both radiotherapy and tracing, for destroying harmful tissues such as tumors/metastases in the local area, or to accelerate or inhibit growth of healthy tissues; or as biomarkers for use in nuclear medicine and radioimaging.

Bioactive agents useful in accordance with the present invention may be used singly or in combination, namely more than one type of bioactive agents may be used together in one soy protein structure, and therefore be released simultaneously from the structure.

In some embodiments, the concentration of a bioactive agent in the soy protein structure ranges from 0.1 percents weight per volume to 10 percents, or from 1-3 percents by weight relative to the weight of dry soy protein used in the composition-of-matter, or more than 10 % w/w in some embodiments. Alternatively, the concentration of the bioactive agent is determined by the nature of the agent, the specific release

rate/profile and the desired effect. Higher and lower values of the content of the bioactive agent are also contemplated, depending on the nature of the bioactive agent used and the intended use of the soy protein structure.

When using the term "bioactive agent" in the context of releasing or eluting a bioactive agent, it is meant that the bioactive agent is substantially active upon its release.

As discussed hereinbelow, the bioactive agent may have an influence on the soy protein structure chemical and/or mechanical properties by virtue of its own reactivity with one or more of the soy protein and/or the crosslinking agent, or by virtue of its chemical and/or physical properties *per-se*. It is therefore noted that in general, the bioactive agent is selected suitable for being incorporated into the composition-of-matter which affords the soy protein structure such that it can elute from the structure in the intended effective amount and release rate, as discussed herein, and while allowing the soy protein structure to exhibit the desired properties, as discussed herein.

As discussed and exemplified in the Examples section that follows, some bioactive agents may exhibit one or more functional groups which may be susceptible to the crosslinking processes, chemical and/or thermal, and may therefore be affected and/or influence the characteristics of the resulting soy protein structure. For example, bioactive agents exhibiting a carboxylic group or a primary amine group may react with a crosslinking agent has reactivity towards such functional groups. In such cases, in order to maintain desirable characteristics of the resulting soy protein structure, some adjustments may be introduced to the composition in terms of the type of ingredients and their concentrations.

In general, the bioactive agent is selected or modified so as to be compatible with the entire preparation process (which may involve heat and/or the use of reactive chemicals). By "compatible" it is meant that the bioactive agent does not interfere with the processes involved in forming the composition-of-matter, and/or substantially retains its desired characteristics and function for which it was selected, and/or is capable of being released from the structure upon contacting the structure with physiological media or other aqueous media.

The bioactive agent may be selected to achieve either a local or a systemic response. The bioactive agent may be any prophylactic agent or therapeutic agent

suitable for various topical, enteral and parenteral types of administration routes including, but not limited to sub- or trans-cutaneous, intradermal transdermal, transmucosal, intramuscular administration and mucosal administration, as well as local administration to internal organs or tissues (e.g., during surgery).

5 A bioactive agent, according to some embodiments of the present invention, can be, for example, a macro-biomolecule or a small, organic molecule.

 The term “macro-biomolecules” as used herein, refers to a polymeric biochemical substance, or biopolymers, that occur naturally in living organisms. Polymeric macro-biomolecules are primarily organic compounds, namely they consist
10 primarily of carbon and hydrogen, along with nitrogen, oxygen, phosphorus and sulfur, while other elements can be incorporated therein but at a lower rate of occurrence. Amino and nucleic acids are some of the most important building blocks of polymeric macro-biomolecules, therefore macro-biomolecules are typically comprised of one or
15 more chains of polymerized amino acids, polymerized nucleic acids, polymerized saccharides, polymerized lipids and combinations thereof. Macromolecules may
 comprise a complex of several macromolecular subunits which may be covalently or non-covalently attached to one another. Hence, a ribosome, a cell organelle and even an
 intact virus can be regarded as a macro-biomolecule.

 A macro-biomolecule, as used herein, has a molecular weight higher than 1000
20 dalton (Da), and can be higher than 3000 Da, higher than 5000 Da, higher than 10 kDa and even higher than 50 KDa.

 Representative examples of macro-biomolecules, which can be beneficially incorporated in the soy protein structure described herein include, without limitation, peptides, polypeptides, proteins, enzymes, antibodies, oligonucleotides and labeled
25 oligonucleotides, nucleic acid constructs, DNA, RNA, antisense, polysaccharides, viruses and any combination thereof, as well as cells, including intact cells or other sub-cellular components and cell fragments.

 According to some embodiments of the present invention, the bioactive agent is a non-proteinous substance, namely a substance possessing no more than four amino
30 acid residues in its structure. According to some embodiments of the present invention, the bioactive agent is a non-peptide or non-protein substance.

According to some embodiments of the present invention, the bioactive agent is a non-carbohydrate substance, namely a substance possessing no more than four sugar (aminoglycoside inclusive) moieties in its structure.

According to some embodiments of the present invention, the bioactive agent is substantially devoid of one or more of the following functional groups: a carboxyl, a primary amine, a hydroxyl, a sulphydroxyl and an aldehyde.

As used herein, the phrase "small organic molecule" or "small organic compound" refers to small compounds which consist primarily of carbon and hydrogen, along with nitrogen, oxygen, phosphorus and sulfur and other elements at a lower rate of occurrence. Organic molecules constitute the entire living world and all synthetically made organic compounds, therefore they include all natural metabolites and man-made drugs. In the context of the present invention, the term "small" with respect to a compound, agent or molecule, refers to a molecular weight lower than about 1000 grams per mole. Hence, a small organic molecule has a molecular weight lower than 1000 Da, lower than 500 Da, lower than 300 Da, or lower than 100 Da.

Representative examples of small organic molecules, that can be beneficially incorporated in the drug-eluting soy protein structures described herein include, without limitation, antibiotic agents, analgesic agents, anesthetic agents, anti-inflammatory agents, angiogenesis-promoters, cytokines, chemokines, chemo-attractants, chemo-repellants, drugs, agonists, amino acids, antagonists, anti histamines, antigens, antidepressants, anti-hypertensive agents, antioxidants, anti-proliferative agents, immunosuppressive agents, clotting factors, osseointegration agents, anti-viral agents, chemotherapeutic agents, co-factors, fatty acids, growth factors, haptens, hormones, inhibitors, ligands, saccharides, radioisotopes, radiopharmaceuticals, steroids, toxins, vitamins, minerals and any combination thereof.

Representative examples of bioactive agents suitable for use in the context of the present embodiments include, without limitation, antimicrobial agents, antibiotic agents, antifungal agents, antiviral agents, anti-parasitic agents, analgesic agents, anesthetic agents, anti-inflammatory agents, clotting factors, antitumor and chemotherapy agents, agonists and antagonists agents, amino acids, angiogenesis-promoters, anorexics, antiallergics, antiarthritics, antiasthmatic agents, antibodies, anticholinergics, anticonvulsants, antidepressants, antidiabetic agents, antidiarrheals, antigens,

antihistamines, antihypertensive agents, antimigraine agents, antinauseants, antineoplastics, antioxidants, antiparkinsonism drugs, antiproliferative agents, antiprotozoans, antipruritics, antipsychotics, antipyretics, antisenses nucleic acid constructs, antispasmodics, antiviral agents, bile acids, calcium channel blockers, cardiovascular preparations, cells, central nervous system stimulants, chemo-attractants, chemokines, chemo-repellants, chemotherapeutic agents, cholesterol, co-factors, contraceptives, cytokines, decongestants, diuretics, DNA, Drugs and therapeutic agents, enzyme inhibitors, enzymes, fatty acids, glycolipids, growth factors, growth hormones, haptens, hormone inhibitors, hormones, hypnotics, immunoactive agents, immunosuppressive agents, inhibitors and ligands, labeled oligonucleotides, microbicides, muscle relaxants, nucleic acid constructs, oligonucleotides, parasympatholytics, peptides, peripheral and cerebral vasodilators, phospholipids, polysaccharides, proteins, psychostimulants, radioisotopes, radiopharmaceuticals, receptor agonists, RNA, saccharides, saponins, sedatives, small organic molecules, spermicides, steroids, sympathomimetics, toxins, tranquilizers, vaccines, vasodilating agents, viral components, viral vectors, virions, viruses, vitamins, and any combination thereof.

One class of bioactive agents which can be beneficially incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, is the class of antimicrobial, antibiotic, antifungal, antiviral and anti-parasitic agents.

Antibiotic/antimicrobial agents include, without limitation, lipopeptides, fluoroquinolones, ketolides, tetracyclines (glycylcyclines), aminoglycosides such as gentamicin, amikacin, kanamycin, neomycin, netilmicin, paromomycin, streptomycin and tobramycin; cephalosporins such as cefacetrile (cephacetrile), cefaclomezine, cefaclor, cefadroxil (cefadroxyl), cefalexin (cephalexin), cefaloglycin (cephaloglycin), cefalonium (cephalonium), cefaloram, cefaloridine (cephaloradine), cefalotin (cephalothin), cefamandole, cefaparole, cefapirin (cephapirin), cefatrizine, cefazaflur, cefazedone, cefazolin (cephazolin), cefcanel, cefcapene, cefclidine, cefdaloxime, cefdinir, cefditoren, cefedrolor, cefempidone, cefepime, cefetamet, cefetrizole, cefivitril, cefixime, cefluprenam, cefmatilen, cefmenoxime, cefmepidium, cefmetazole, cefodizime, cefonicid, cefoperazone, cefoselis, cefotaxime, cefotetan, cefovecin,

cefoxazole, cefoxitin, cefozopran, cefpimizole, cefpirome, cefpodoxime, cefprozil (cefproxil), cefquinome, cefradine (cephradine), cefrotil, cefroxadine, cefsumide, ceftaroline, ceftazidime, cefteram, ceftezole, ceftibuten, ceftiofur, ceftiolene, ceftioxide, ceftizoxime, ceftriaxone, cefuracetime, cefuroxime and cefuzonam; carbapenems such as imipenem, imipenem/cilastatin, doripenem, meropenem and ertapenem; quinolone antibiotics such as balofloxacin, ciprofloxacin, clinafloxacin, enoxacin, flumequine, gatifloxacin, gemifloxacin, grepafloxacin, levofloxacin, lomefloxacin, moxifloxacin, nadifloxacin, nalidixic acid, norfloxacin, ofloxacin, oxolinic acid, pazufloxacin, pefloxacin, pipemidic acid, piromidic acid, prulifloxacin, rosoxacin, rifloxacin, sitafloxacin, sparfloxacin, temafloxacin, tosufloxacin and trovafloxacin; macrolide antibiotics such as azithromycin, erythromycin, clarithromycin, dirithromycin, roxithromycin and telithromycin; penicillins such as amoxicillin, ampicillin, bacampicillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, nafcillin, oxacillin, penicillin g, penicillin v, piperacillin, pivampicillin, pivmecillinam and ticarcillin; sulfonamides such as sulfamethizole, sulfamethoxazole, sulfisoxazole and trimethoprim-sulfamethoxazole; tetracycline antibiotics such as demeclocycline, doxycycline, minocycline, oxytetracycline and tetracycline; oxazolidinones such as linezolid, and other antibiotics such as clindamycin, metronidazole, vancomycin, rifabutin, rifampin, nitrofurantoin and chloramphenicol.

Antibiotic agents which are typically used to treat infectious wounds include, without limitation, gentamicin, ampicillin, ampicillin-sulbactam, augmentin (amoxicillin trihydrate), cefazolin, cefotaxime, cefotetan, cefoxitin, ceftriaxone, cephalexin, ciprofloxacin, clavulanic acid, dicloxacillin, imipenem, metronidazole, piperacillin, tazobactam and ticarcillin.

Additional non-limiting examples of antibiotic agents include alpha and beta hydroxy acids, azelaic acid and its derivatives, benzoyl peroxide, bile salts, chlortetracycline, cholate, dalfopristin, deoxycholate, ethylacetate, flavinoid antibiotics, glycopeptide antibiotics, lincomycin and derivatives thereof, meclocycline, methacycline, nalidixic acid, octopirox, oxytetracycline, phenoxy ethanol, phenoxy propanol, quinolone antibiotics, quinupristin, rolitetracycline, scymnol sulfate and its derivatives, sebostat antibiotics, sulfabenzamide, sulfacetamide, sulfadiazine, sulfadoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole

streptogramin antibiotics, sulfur-based antibiotics, sulfonamide antibiotics, teicoplanin, tetracycline antibiotics, triclosan, zinc, and any combination thereof.

Non-limiting examples of antiparasitic agents include mebendazole, pyrantel pamoate, thiabendazole, diethylcarbamazine, ivermectin, niclosamide, praziquantel, albendazole, praziquantel, rifampin, amphotericin B, melarsoprol, eflornithine, metronidazole, tinidazole and miltefosine, as well as any anti-malarial agents or anti-leishmania agents.

One class of bioactive agents which can be encapsulated in the drug-eluting soy protein structures, according to some embodiments of the present invention, is the class of analgesic agents that alleviate pain. Representative examples of analgesic agents include, without limitation, non-steroidal anti-inflammatory drugs (NSAIDs); COX-2 inhibitors such as celecoxib and rofecoxib; opiates and morphinomimetics such as morphine and various other substances including codeine, oxycodone, hydrocodone, dihydromorphine, pethidine, buprenorphine, tramadol and venlafaxine; paracetamol, flupirtine and the likes.

In the context of the present embodiments, NSAIDs include, but are not limited to Ibuprofen (Motrin®, Advil®, Nuprin®), Acematacin, Alminoprofen, Aspirin (Ecotrin®, Bayer®, Anacin®), Azapropazone, Benorylate, Benoxaprofen, Carprofen, Celecoxib (Celebrex®), Choline magnesium trisalicylate (Trilisate®), Clindanac, Cp-14,304, Diclofenac (Cambia™, Cataflam®, Flector®, Pennsaid®, Solaraze®, Voltaren®, Zipsor™), Diflunisal (Dolobid®), Disalcid, Etodolac (Lodine®), Felbinac, Fenbufen, Fenclofenac, Fendosal, Fenoprofen (Nalfon®), Fentiazac, Feprazone, Flufenamic, Flurbiprofen (Ansaid®), Furofenac, Indomethacin (Indocin®), Indopropfen, Isoxepac, Isoxicam, Ketoprofen (Orudis®, Actron®, Oruvail®), Ketorolac (Sprix™, Toradol®), Meclofenamate (Meclomen®), Mefenamic Acid, Meloxicam (Mobic®), Miroprofen, Nabumetone (Relafen®), Naproxen (Naprosyn®, Aleve®, Anaprox®, Naprelan®), Niflumic Acid, Oxaprozin (Daypro®), Oxepinac, Oxyphenbutazone, Phenylbutazone, Piroxicam (Feldene®), Pirprofen, Pranoprofen, Safapryn, Salsalate (Salflex®, Disalcid®, Amigesic®), Solprin, Sudoxicam, Sulindac (Clinoril®), Suprofen, Tenoxicam, Tiaprofenic, Tiopinac, Tioxaprofen, Tolfenamic Acids, Tolmetin (Tolectin®), Trilisate, Trimethazone, Zidometacin and Zomepirac.

According to some embodiments of the present invention, the analgesic agent is a non-steroidal anti-inflammatory drug (NSAID) such as aspirin, ibuprofen, or naproxen, an amino amide such as bupivacaine, a COX-2 inhibitor such as celecoxib or rofecoxib, an opiate, a morphinomimetic, morphine, codeine, oxycodone, hydrocodone, dihydromorphine, pethidine, buprenorphine, tramadol, venlafaxine, paracetamol, flupirtine, including derivatives and any combinations thereof.

Another class of bioactive agents which can be incorporated in the drug-eluting soy protein structures, according to some embodiments of the present invention, is the class of anesthetic agents. Exemplary anesthetic agents include, without limitation, acetamidoeugenol, alfadolone acetate, alfaxalone, amucaine, amolanone, amylocalne, benoxinate, benzocaine, betoxycaine, biphenamine, bupivacaine, burethamine, butacaine, butaben, butanilicaine, buthalital, butoxycaine, carticaine, 2-chloroprocaine, cocaethylene, cocaine, cyclomethycaine, dibucaine, dimethisoquin, dimethocaine, diperadon, dyclonine, ecgonidine, ecgonine, ethyl aminobenzoate, ethyl chloride, etidocaine, etoxadrol, β -eucaine, euprocine, fenalcomine, fomocaine, hexobarbital, hexylcaine, hydroxydione, hydroxyprocaine, hydroxytetracaine, isobutyl p-aminobenzoate, ketamine, leucinocaine mesylate, levobupivacaine, levoxadrol, lidocaine, mepivacaine, meprylcaine, metabutoxycaine, methohexital, methyl chloride, midazolam, myrtecaine, naepaine, octacaine, orthocaine, oxethazaine, parethoxycaine, phenacaine, phencyclidine, phenol, piperocaine, piridocaine, polidocanol, pramoxine, prilocalne, procaine, propanidid, propanocaine, proparacaine, propipocaine, propofol, propoxycaine, pseudococaine, pyrrocaine, risocaine, salicyl alcohol, tetracaine, thialbarbital, thimylal, thiobutabarbital, thiopental, tolycaine, trimecaine, zolamine, phenol, and mixtures thereof.

Another class of bioactive agents which can be incorporated in the drug-eluting soy protein structures, according to some embodiments of the present invention, is the class of therapeutic agents that promote angiogenesis. The successful regeneration of new tissue requires the establishment of a vascular network. The induction of angiogenesis is mediated by a variety of factors, any of which may be used in conjunction with the present invention (Folkman and Klagsbrun, 1987, and references cited therein, each incorporated herein in their entirety by reference). Non-limiting examples of angiogenesis-promoters include vascular endothelial growth factor (VEGF)

or vascular permeability factor (VPF); members of the fibroblast growth factor family, including acidic fibroblast growth factor (AFGF) and basic fibroblast growth factor (bFGF); interleukin-8 (IL-8); epidermal growth factor (EGF); platelet-derived growth factor (PDGF) or platelet-derived endothelial cell growth factor (PD-ECGF);
5 transforming growth factors alpha and beta (TGF- α , TGF- β); tumor necrosis factor alpha (TNF- β); hepatocyte growth factor (HGF); granulocyte-macrophage colony stimulating factor (GM-CSF); insulin growth factor-1 (IGF-1); angiogenin; angiotropin; and fibrin and nicotinamide.

Another class of bioactive agents which can be incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, especially in certain embodiments wherein tissue regeneration is desirable, and application involving implantable devices and tissue healing, are cytokines, chemokines and related factors. Control over these agents can translate into a successful medical procedure when the immune system plays a key role. Cytokines are any of several
10 small non-antibody regulatory protein molecules, such as the interleukins and lymphokines, which are released by cells of the immune system population on contact with a specific antigen and act as intercellular mediators in the generation of an immune response. Cytokines are the core of communication between immune system cells, and between these cells and cells belonging to other tissue types. There are many known
15 cytokines that have both stimulating and suppressing action on lymphocyte cells and immune response. They act by binding to their cell-specific receptors. These receptors are located in the cell membrane and each allows a distinct signal transduction cascade to start in the cell that eventually will lead to biochemical and phenotypical changes in the target cell. Typically, receptors for cytokines are also tyrosine kinases. Non-
20 limiting examples of cytokines and chemokines include angiogenin, calcitonin, ECGF, EGF, E-selectin, L-selectin, FGF, FGF basic, G-CSF, GM-CSF, GRO, Hirudin, ICAM-1, IFN, IFN- γ , IGF-I, IGF-II, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, M-CSF, MIF, MIP-1, MIP-1 α , MIP-1 β , NGF chain, NT-3, PDGF- α , PDGF- β , PECAM, RANTES, TGF- α , TGF- β , TNF- α , TNF- β , TNF- κ and VCAM-1
25

Non-limiting examples of immunosuppressive drugs or agents, commonly referred to herein as immunosuppressants, include glucocorticoids, cytostatics, antibodies, drugs acting on immunophilins and other immunosuppressants.
30

Glucocorticoids include steroids such as hydrocortisone (cortisol), cortisone acetate, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone, beclometasone, fludrocortisone, deoxycorticosterone and aldosterone; Cytostatic agents include alkylating agents, antimetabolites such as folic acid analogues, purine analogues, pyrimidine analogues and protein synthesis inhibitors, methotrexate, azathioprine and mercaptopurine and cytotoxic antibiotics; Antibodies include polyclonal and monoclonal antibodies, T-cell receptor directed antibodies, Muromonab-CD3 and IL-2 receptor directed antibodies; Drugs acting on immunophilins include Ciclosporin, Tacrolimus and Sirolimus; and other immunosuppressants include interferons, opioids, TNF binding proteins, mycophenolic acid, Fingolimod, Myriocin and the likes.

Additional bioactive agents which can be beneficially incorporated in the drug-eluting soy protein structures, according to some embodiments of the present invention, include cytotoxic factors or cell cycle inhibitors, including CD inhibitors, such as p53, thymidine kinase ("TK") and other agents useful for interfering with cell proliferation.

Additional bioactive agents which can be beneficially incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, include cell survival agents such as Akt, insulin-like growth factor 1, NF-kB decoys, I-kB, Madh6, Smad6 and Apo A-1.

Additional bioactive agents which can be beneficially incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, include genetic therapeutic agents and proteins, such as ribozymes, anti-sense polynucleotides and polynucleotides coding for a specific product (including recombinant nucleic acids) such as genomic DNA, cDNA, or RNA. The polynucleotide can be provided in "naked" form or in connection with vector systems that enhances uptake and expression of polynucleotides. These can include DNA compacting agents (such as histones), non-infectious vectors (such as plasmids, lipids, liposomes, cationic polymers and cationic lipids) and viral vectors such as viruses and virus-like particles (i.e., synthetic particles made to act like viruses). The vector may further have attached peptide targeting sequences, anti-sense nucleic acids (DNA and RNA), and DNA chimeras which include gene sequences encoding for ferry proteins such as membrane

translocating sequences ("MTS"), tRNA or rRNA to replace defective or deficient endogenous molecules and herpes simplex virus-1 ("VP22").

Additional bioactive agents which can be beneficially incorporated in the drug-eluting soy protein structures, according to some embodiments of the present invention, include gene delivery agents, which may be either endogenously or exogenously controlled. Examples of endogenous control include promoters that are sensitive to a physiological signal such as hypoxia or glucose elevation. Exogenous control systems involve gene expression controlled by administering a small molecule drug. Examples include tetracycline, doxycycline, ecdysone and its analogs, RU486, chemical dimerizers such as rapamycin and its analogs, etc.

Additional bioactive agents which can be beneficially incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, include the family of bone morphogenic proteins ("BMP's") such as BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 (Vgr-1), BMP-7 (OP-1), BMP-8, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-14, BMP-15, and BMP-16. Some of these dimeric proteins can be provided as homodimers, heterodimers, or combinations thereof, alone or together with other molecules. Alternatively or, in addition, molecules capable of inducing an upstream or downstream effect of a BMP can be provided. Such molecules include any of the "hedgehog" proteins, or the DNA's encoding them.

Additional bioactive agents which can be beneficially incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, include chemotherapeutic agents. Non-limiting examples of chemotherapeutic agents include amino containing chemotherapeutic agents such as daunorubicin, doxorubicin, N-(5,5-diacetoxypentyl)doxorubicin, anthracycline, mitomycin C, mitomycin A, 9-amino camptothecin, aminopertin, antinomycin, N⁸-acetyl spermidine, 1-(2-chloroethyl)-1,2-dimethanesulfonyl hydrazine, bleomycin, talysomucin, and derivatives thereof; hydroxy containing chemotherapeutic agents such as etoposide, camptothecin, irinotecan, topotecan, 9-amino camptothecin, paclitaxel, docetaxel, esperamycin, 1,8-dihydroxy-bicyclo[7.3.1]trideca-4-ene-2,6-diyne-13-one, anguidine, morpholino-doxorubicin, vincristine and vinblastine, and derivatives thereof, sulfhydryl containing chemotherapeutic agents and carboxyl containing chemotherapeutic agents.

In the context of embodiments of the present invention, antiviral agents include nucleoside phosphonates and other nucleoside analogs, AICAR (5-amino-4-imidazolecarboxamide ribonucleotide) analogs, glycolytic pathway inhibitors, anionic polymers, and the like. More specifically, antiherpes agents such as acyclovir, famciclovir, foscarnet, ganciclovir, idoxuridine, sorivudine, trifluridine, valacyclovir, and vidarabine; and other antiviral agents such as abacavir, adefovir, amantadine, amprenavir, cidofovir, delviridine, 2-deoxyglucose, dextran sulfate, didanosine, efavirenz, indinavir, interferon alpha, lamivudine, nelfinavir, nevirapine, ribavirin, rimantadine, ritonavir, saquinavir, squalamine, stavudine, tipranavir, valganciclovir, zalcitabine, zidovudine, zintevir, and mixtures thereof. Still other antiviral agents are glycerides, particularly monoglycerides that have antiviral activity such as monolaurin, the monoglyceride of lauric acid.

Additional bioactive agents which can be beneficially incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, include viral and non-viral vectors, such as adenoviruses, gutted adenoviruses, adeno-associated virus, retroviruses, alpha virus (Semliki Forest, Sindbis, etc.), lentiviruses, herpes simplex virus, ex vivo modified cells (i.e., stem cells, fibroblasts, myoblasts, satellite cells, pericytes, cardiomyocytes, skeletal myocytes, macrophage, etc.), replication competent viruses (ONYX-015, etc.), and hybrid vectors, artificial chromosomes and mini-chromosomes, plasmid DNA vectors (pCOR), cationic polymers (polyethyleneimine, polyethyleneimine (PEI) graft copolymers such as polyether-PEI and polyethylene oxide-PEI, neutral polymers PVP, SP1017 (SUPRATEK), lipids or lipoplexes, nanoparticles and microparticles with and without targeting sequences such as the protein transduction domain (PTD).

Antifungal agents include miconazole, terconazole, isoconazole, itraconazole, fenticonazole, fluconazole, ketoconazole, clotrimazole, butoconazole, econazole, metronidazole, 5-fluorouracil, amphotericin B, and mixtures thereof.

Other anti-infective agents which can be beneficially incorporated in the drug-eluting soy protein structures, according to some embodiments of the present invention, include miscellaneous antibacterial agents such as chloramphenicol, spectinomycin, polymyxin B (colistin), and bacitracin, anti-mycobacterials such as isoniazid, rifampin, rifabutin, ethambutol, pyrazinamide, ethionamide, aminosalicic acid, and

cycloserine, and antihelminthic agents such as albendazole, oxfendazole, thiabendazole, and mixtures thereof.

Additional bioactive agents which can be beneficially incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, include steroidal anti-inflammatory drugs. Non-limiting examples of steroidal anti-inflammatory drugs include, without limitation, corticosteroids such as cortisone, hydrocortisone, hydrocortisone-21-monoesters (e.g., hydrocortisone-21-acetate, hydrocortisone-21-butyrate, hydrocortisone-21-propionate, hydrocortisone-21-valerate, etc.), hydrocortisone-17,21-diester (e.g., hydrocortisone-17,21-diacetate, hydrocortisone-17-acetate-21-butyrate, hydrocortisone-17,21-dibutyrate etc.), alclometasone, alpha-methyl dexamethasone, amcinafel, amcinafide, beclomethasone dipropionate, betamethasone and esters thereof, betamethasone benzoate, betamethasone dipropionate, chlorprednisone, chlorprednisone acetate, clescinolone, clobetasol propionate, clobetasol valerate, clocortelone, cortodoxone, desonide, desoxycorticosterone acetate, desoxymethasone, dexamethasone, dexamethasone-phosphate, dichlorisone, diflorasone diacetate, diflucortolone valerate, difluorosone diacetate, difluorosone diacetate, diflurprednate, fluadrenolone, flucetonide, flucolorolone acetone, flucoloronide, flucortine butylesters, fludrocortisone, flumethasone pivalate, flunisolide, fluocinonide, fluocortolone, fluoromethalone, fluosinolone acetone, fluperolone, fluprednidene (fluprednylidene) acetate, fluprednisolone, fluradrenolone, fluradrenolone acetone, flurandrenolone, halcinonide, hydrocortamate, hydrocortisone acetate, hydrocortisone butyrate, hydrocortisone cyclopentylpropionate, hydrocortisone valerate, hydroxyltriamcinolone, medrysone, meprednisone, methylprednisolone, mometasone furoate, paramethasone, prednisolone, prednisone, triamcinolone, triamcinolone acetone, and mixtures thereof.

Additional bioactive agents which can be beneficially incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, include anti-oxidants. Non-limiting examples of anti-oxidants include ascorbic acid (vitamin C) and its salts, ascorbyl esters of fatty acids, ascorbic acid derivatives (for example, magnesium ascorbyl phosphate, sodium ascorbyl phosphate, ascorbyl sorbate), tocopherol (vitamin E), tocopherol sorbate, tocopherol acetate, other esters of tocopherol, butylated hydroxy benzoic acids and their salts, 6-hydroxy-2,5,7,8-

tetramethylchroman-2-carboxylic acid (commercially available under the trade name Trolox^R), gallic acid and its alkyl esters, especially propyl gallate, uric acid and its salts and alkyl esters, sorbic acid and its salts, lipoic acid, amines (for example, N,N-diethylhydroxylamine, amino-guanidine), sulfhydryl compounds (for example, glutathione), dihydroxy fumaric acid and its salts, lysine pidolate, arginine pilolate, nordihydroguaiaretic acid, bioflavonoids, curcumin, lysine, methionine, proline, superoxide dismutase, silymarin, tea extracts, grape skin/seed extracts, melanin, and rosemary extracts.

Additional bioactive agents which can be beneficially incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, include vitamins. Non-limiting examples of vitamins include vitamin A and its analogs and derivatives: retinol, retinal, retinyl palmitate, retinoic acid, tretinoin, isotretinoin (known collectively as retinoids), vitamin E (tocopherol and its derivatives), vitamin C (L-ascorbic acid and its esters and other derivatives), vitamin B₃ (niacinamide and its derivatives), alpha hydroxy acids (such as glycolic acid, lactic acid, tartaric acid, malic acid, citric acid, etc.) and beta hydroxy acids (such as salicylic acid and the like).

Additional bioactive agents which can be beneficially incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, include hormones. Non-limiting examples of hormones include androgenic compounds and progestin compounds such as methyltestosterone, androsterone, androsterone acetate, androsterone propionate, androsterone benzoate, androsteronediol, androsteronediol-3-acetate, androsteronediol-17-acetate, androsteronediol 3-17-diacetate, androsteronediol-17-benzoate, androsteronedione, androstenedione, androstenediol, dehydroepiandrosterone, sodium dehydroepiandrosterone sulfate, dromostanolone, dromostanolone propionate, ethylestrenol, fluoxymesterone, nandrolone phenpropionate, nandrolone decanoate, nandrolone furylpropionate, nandrolone cyclohexane-propionate, nandrolone benzoate, nandrolone cyclohexanecarboxylate, androsteronediol-3-acetate-1-7-benzoate, oxandrolone, oxymetholone, stanozolol, testosterone, testosterone decanoate, 4-dihydrotestosterone, 5 α -dihydrotestosterone, testolactone, 17 α -methyl-19-nortestosterone and pharmaceutically acceptable esters and salts thereof, and combinations of any of the foregoing, desogestrel, dydrogesterone, ethynodiol diacetate, medroxyprogesterone,

levonorgestrel, medroxyprogesterone acetate, hydroxyprogesterone caproate, norethindrone, norethindrone acetate, norethynodrel, allylestrenol, 19-nortestosterone, lynoestrenol, quingestanol acetate, medrogestone, norgestrienone, dimethisterone, ethisterone, cyproterone acetate, chlormadinone acetate, megestrol acetate, 5 norgestimate, norgestrel, desogestrel, trimegestone, gestodene, nomegestrol acetate, progesterone, 5 α -pregnan-3 β ,20 α -diol sulfate, 5 α -pregnan-3 β ,20 β -diol sulfate, 5 α -pregnan-3 β -ol-20-one, 16,5 α -pregnen-3 β -ol-20-one, 4-pregnen-20 β -ol-3-one-20-sulfate, acetoxypregnenolone, anagestone acetate, cyproterone, dihydrogesterone, flurogestone acetate, gestadene, hydroxyprogesterone acetate, 10 hydroxymethylprogesterone, hydroxymethyl progesterone acetate, 3-ketodesogestrel, megestrol, melengestrol acetate, norethisterone and mixtures thereof.

Additional bioactive agents which can be beneficially incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, include cells of human origin (autologous or allogeneic), including stem 15 cells, or from an animal source (xenogeneic), which can be genetically engineered if desired to deliver proteins of interest. Cell types include bone marrow stromal cells, endothelial progenitor cells, myogenic cells including cardiomyogenic cells such as procardiomyocytes, cardiomyocytes, myoblasts such as skeletomyoblasts, fibroblasts, stem cells (for example, mesenchymal, hematopoietic, neuronal and so forth), 20 pluripotent stem cells, macrophage, satellite cells and so forth. Cells appropriate for the practice of the present invention also include biopsy samples for direct use (for example, whole bone marrow) or fractions thereof (for example, bone marrow stroma, bone marrow fractionation for separation of leukocytes). Where appropriate, media can be formulated as needed and included in the preparation of the soy protein structures, 25 according to some embodiments of the present invention, so as to maintain cell function and viability.

Additional bioactive agents which can be beneficially incorporated into the drug-eluting soy protein structures, according to some embodiments of the present invention, also include both polymeric (macro-biomolecules, for example, proteins, 30 enzymes) and non-polymeric (small molecule therapeutics) agents and include Ca-channel blockers, serotonin pathway modulators, cyclic nucleotide pathway agents, catecholamine modulators, endothelin receptor antagonists, nitric oxide

donors/releasing molecules, anesthetic agents, ACE inhibitors, ATII-receptor antagonists, platelet adhesion inhibitors, platelet aggregation inhibitors, coagulation pathway modulators, cyclooxygenase pathway inhibitors, natural and synthetic corticosteroids, lipoxigenase pathway inhibitors, leukotriene receptor antagonists, antagonists of E- and P-selectins, inhibitors of VCAM-1 and ICAM-1 interactions, prostaglandins and analogs thereof, macrophage activation preventers, HMG-CoA reductase inhibitors, fish oils and omega-3-fatty acids, free-radical scavengers/antioxidants, agents affecting various growth factors (including FGF pathway agents, PDGF receptor antagonists, IGF pathway agents, TGF- β pathway agents, EGF pathway agents, TNF- α pathway agents, Thromboxane A2 [TXA2] pathway modulators, and protein tyrosine kinase inhibitors), MMP pathway inhibitors, cell motility inhibitors, anti-inflammatory agents, antiproliferative/antineoplastic agents, matrix deposition/organization pathway inhibitors, endothelialization facilitators, blood rheology modulators, as well as integrins, chemokines, cytokines and growth factors.

According to some embodiments of the present invention, the bioactive agent is an antibiotic (e.g., gentamicin or any other other aminoglycoside).

According to some embodiments of the present invention, the bioactive agent is an analgesic (e.g., bupivacaine or ibuprofen).

Drug release profile:

The rate of release of the bioactive agent, or the drug release profile from the soy protein structure, serving as its reservoir, can be controlled by various factors, such as the relative concentrations of the constituents in the soy protein structure described herein, as well as the processing parameters of its making.

A typical drug delivery mechanism, relevant in the context of the present embodiments, consists of a reservoir containing a predetermined and exhaustible amount of the drug, and an interface between the drug's reservoir and the physiological environment (media). Typically, the drug release commences at the initial time point when the reservoir comes in contact with the physiological environment/media, and follows typical diffusion-controlled kinetics with additional influences effected by water uptake (swelling), disintegration and biodegradation of the soy protein structure.

A "drug release profile" is a general expression which describes the temporal concentration of a drug (a bioactive agent) as measured in a particular bodily site of

interest as a function of time, while the slope of a concentration versus time represents the rate of release at any given time point. A drug release profile may be sectioned into rate dependent periods whereby the rate is rising or declining linearly or exponentially, or staying substantially constant. Some of the typically sought rates include the burst
5 release rate and the sustained release rate.

The phrase "burst release", as used herein, refers to the phase of the release profile which is consistent with a rapid release of a drug into the bodily site of interest, and is typically associated with an exponential increase of the drug's concentration, growing from zero to a high level at a relatively short time. Typically, the burst release
10 section of the drug release profile ends briefly and then gradually changes to a plateau, or a sustained release section in the release profile.

The phrase "sustained release", as used herein, refers to the section of the drug release profile which comes after the burst release part, and is typically characterized by constant rate and relative long duration.

The main differences between the burst and the sustain parts of a release profile are therefore the rate (slope characteristics) and duration, being exponential and short for the burst release, and linear and long for the sustained release; and both play a significant role in drug administration regimes. In most cases, the presence of both a burst release phase and a sustained release phase is unavoidable and stems from
20 chemical and thermodynamic properties of the drug delivery mechanism.

In the context of embodiments of the present invention, the phrase "high burst release" is an attribute of a drug-eluting soy protein structure, as described herein, which refers to the amount of drug that is being released from the soy protein structure during the initial stage of contacting the structure with the environment of its action
25 (e.g., physiological environment/media). According to some embodiments of the present invention, an amount that ranges from 30 % to 70 % of the total amount of the drug initially contained in the soy protein structure, are released during the first six hours from exposure to physiological environment/media.

In some embodiments of the present invention, "high burst release" describes an
30 attribute of a drug-eluting soy protein structure, as described herein, in which 20 %, 30 %, 40 %, 50 %, 60 % and even higher percentages of the bioactive agent (drug) are released during the first 6 hours of contacting the structure with a physiological

medium. Any value between 20 % and 100 % of the bioactive agent (drug) are contemplated.

Accordingly, the phrase "low burst release" refers to drug-eluting soy protein structures wherein less than 20 % of the contained drug is released within the first six hours of exposure. In some embodiments of the present invention, "low burst release" describes an attribute of a drug-eluting soy protein structure, as described herein, in which 15 %, 10 %, 5 % and even lower percentages of the bioactive agent (drug) are released during the first 6 hours of exposing the structure to a physiological medium. Any value between 20 % and 1 % of the bioactive agent (drug) are contemplated.

In general, and according to some embodiments of the present invention, at least 20 percents of the bioactive agent are released to the surrounding physiological medium within 6 hours of contacting the soy protein structure with physiological medium. According to some other embodiments of the present invention, no more than 20 percents of the bioactive agent are released to the surrounding physiological medium within 6 hours of contacting the soy protein structure with physiological medium.

According to some embodiments of the present invention, the remaining content of the bioactive agent which was not released during the burst release phase, is released at the sustained release phase, which may range from 6 hours to 1 month. Alternatively, the remaining of the bioactive agent is released at a sustained release profile for a time period of at least 1 day, 2 days, 3 days, at least one week, at least two week or at least 1 month.

According to some embodiments of the present invention, upon contacting a drug-eluting SPI structure, as presented herein, with a physiological medium, the bioactive agent incorporated therein is released (elute), at least to some extent, over a time period of at least 2 hours.

According to some embodiments of the present invention, 30-70 percents of the bioactive active agent are released during the first 6 hours of contacting the composition-of-matter with a physiological medium, and the remaining of the bioactive agent is released over at least 30 days of contact. Such a drug release profile is beneficial, for a non-limiting example, for treating an infectious wound by eluting an antimicrobial agent at a high concentration in the first few hours, and maintaining a moderate release over an extended period of time to prevent re-infection of the wound.

Thus, according to some embodiments of the present invention, there is provided a composition-of-matter as described herein, which further comprises an antimicrobial agent (e.g., an antibiotic such as gentamicin), which is capable of releasing the bioactive agent at a desired release profile, as described hereinabove.

5 According to some embodiments of the present invention, at least 90 % of the bioactive agent are released during two days from contacting the drug-eluting soy protein structure with a physiological medium. Such a drug release profile is beneficial, for a non-limiting example, for treating local pain by eluting an analgesic agent at a moderate release rate over an extended period of time.

10 Thus, according to some embodiments of the present invention, there is provided a composition-of-matter as described herein, which further comprises an analgesic agent (e.g., bupivacaine or ibuprofen), which is capable of releasing the bioactive agent at a desired release profile, as described hereinabove.

Shape of the soy protein structure:

15 The solution cast crosslinked soy protein structure presented herein, whether incorporating a bioactive agent or not, can take the shape of a film, a strip, a wound dressing, a bandage, a poultice, a compress, a fascia, a pack, a plaster, a pledget, a cataplasm or a patch.

 According to some embodiments of the present invention, the structure takes the
20 general form of a film. The term "film", as used herein, is a substantially two-dimensional body having a thickness which is 2, 4, 6, 8, 10 and 20 times or more smaller than any of its length or width dimensions, and typically having an overall shape of a thin sheet. According to some embodiments of the present invention, a film can be flexible and therefore can be shaped as desired when used. Alternatively, a film
25 can be used, if desired, to form tubes, bags and the likes, and can also be used to wrap other objects. For example, a medical device which comprises a film according to some embodiments of the present invention, can be shaped into a sleeve (tube) and be wrapped around an elongated bodily organ (finger or artery), or line the interior of a bodily organ (mouth and nasal cavity, or intestine).

30 The thickness of the film correlates to the drug-reservoir capacity, and can be tailored so as to suit any specific application for which the systems, according to some embodiments of the present invention, are used for. For example, for long-range

temporal drug delivery, a large reservoir of the drug is required, and hence relatively thick films are useful and desired in many applications. A relatively thick film is also required to encapsulate large bioactive agents, while the entrapment of relatively small drug molecules which are needed in small locally-distributed amounts may suffice with a relatively thin film. Therefore, the thickness of the film, according to the present embodiments, can range from about 10 μm to about 2000 microns and in certain cases can be even up to 1 cm.

The release profile of a bioactive agent from a drug-eluting soy protein film, according to some embodiments of the present invention, correlates at least in part, to diffusion controlled kinetics, and hence correlates to the surface area of the film (the interface between the film and the physiological environment).

Medical devices:

As discussed hereinabove, the soy protein structures, according to some embodiments of the present invention, is designed rationally to suite particular medicinal uses, such as medical devices, drug delivery systems in many medical applications and/or forming a part thereof.

Hence, according to a further aspect of the present invention there is provided a medical device which is based on the soy protein structures described herein.

In one basic embodiment, a soy protein structure is having a bioactive agent incorporated therein and is shaped as a film. The bioactive agent is typically released as the film contacts a wet environment such as a physiological media (mucus tissue, skin, exposed tissue, internal tissues and the likes), while the film is serving as a drug-delivery vehicle as well as a physical barrier *per-se*.

The term “delivering” or “delivery” as used in the context of the present embodiments refers to the act of enabling the transport of a substance to a specific location, and more specifically, to a desired bodily target, whereby the target can be, for example, an organ (e.g., skin), a tissue (e.g., mucous membrane), a cell, or a sub-cellular compartment such as the nucleus, the mitochondria, the cytoplasm, etc..

Exemplary medical devices include, without limitation, devices for topical applications and implantable devices.

Owing to its unique properties, before considering its capacity to deliver drugs, structures made from the composition-of-matter presented herein offer an ideal primary

wound dressing which can replace existing swab dressing which only functions as the covering for wounds. Such a wound dressing can meet modern clinical demands, protecting wounds, promoting organic revival, and accelerate healing; and due to its drug-eluting capacity, such a wound dressing also acts as germicide and a mean for relieving local pain.

According to some embodiments of the present invention, the medical device is adapted for transdermal and/or topical applications in a subject, or otherwise placed on an external part of the body. It is particularly important that such medical device would cause minimal tissue irritation when used to treat a given tissue.

Exemplary devices which can be used for topical application include, without limitation, a wound dressing, a bandage, an adhesive strip, an adhesive plaster, a skin patch, guided tissue matrices, tissue regeneration devices, tumor targeting and destruction devices, a drug delivery patch and occlusive burn bandage device.

Implantable medical devices based on or include a soy protein structure as described herein, are adapted for surgical applications in a subject, or otherwise placed on, in or near an internal bodily site, organ or tissue which is made accessibly during the surgical procedure, thereby eluding the bioactive agent at that internal bodily site at the desired high or low burst release.

Exemplary implantable devices which can be used in surgical applications include, without limitation, a sleeve, a tube, a strip, a sheet and a patch, a plate, dental implants, orthopedic implants, guided tissue matrices, tissue regeneration devices, tumor targeting and destruction devices and periodontal devices.

The device is shaped and sized according to the intended use thereof. For example, a wound dressing is typically a flat and thin rectangular or round film-like object which is laid upon the treated part of the skin such that the treated area is covered thereby. The wound dressing can be cut to any shape so as to cover any shape wound or skin area. For example, in the case of a wound dressing for treating an infected wound, the device would comprise a film-shaped soy protein structure designed for high burst release and the bioactive agent would be an antibiotic agent.

In another example, the implantable device is a thin sheet that is being shaped as an elongated sleeve or tube and then placed inside and against the inner walls of a nasal or oral cavity or parts of the gastric or intestinal tract.

In some embodiments wherein a drug-eluting soy protein structure is used as a wound dressing, the bioactive agent incorporated therein can be selected so as to exert a therapeutic effect which is beneficial for treating an infection associated with pathogenic microorganisms, namely an antimicrobial agent.

5 In some embodiments wherein a drug-eluting soy protein structure is used as, e.g., a wound dressing, bandage or skin patch, the bioactive agent incorporated therein can be selected so as to exert a pain relieving effect which is beneficial for treating local pain, namely an analgesic agent.

10 As demonstrated in the Examples section that follows below, the present inventors have designed and practiced wound dressings based on soy protein film structures. These dressings were prepared from aqueous solutions of SPI and were studied for the effects of the formulation and process parameters on the mechanical and physical properties and on the release profile of an exemplary antibiotic drug gentamicin therefrom.

15 The initial mechanical properties of these exemplary wound dressing structures were shown to be affected by the plasticizer and crosslinking agent as well as by thermal curing. Glycerol was chosen as an exemplary plasticizer for the soy protein structures and glyoxal was chosen as an exemplary crosslinking agent. The pH and temperature of the solution cast process also had an effect on the mechanical properties
20 of the soy protein structures. Soy protein structures that were formed as SPI films and crosslinked by a combination of crosslinking agent and thermal curing were found to be highly useful in combining relatively high resistance to tear (tensile strength of 17 MPa) and ductility (maximal strain of 160 %).

The physical properties of the wound dressing were also studied in terms of
25 water uptake and weight loss profile, which were also shown to be controlled by the crosslinking process. Film structures that were crosslinked by thermal curing or the addition of a crosslinking agent exhibited lower water uptake and weight loss rate than non-crosslinked films. A combination of both crosslinking methods resulted in higher trends of these results. The water vapor transmission rate of these exemplary soy
30 protein structures was in the desired range for wound dressings of about 2300 g/m²/day.

The drug (gentamicin) release profile exhibited by these exemplary soy protein structures showed a moderate burst effect followed by a decreasing release rate which

lasted for at least 4 weeks. The dominant release mechanism of gentamicin from crosslinked SPI films is diffusion. Crosslinking by a combination of glyoxal and thermal curing resulted in a lower burst release and lower total released drug, compared to crosslinking by glyoxal alone.

5 These exemplary SPI structures combine desirable mechanical properties with desired physical properties and controlled release of the antibiotic drug gentamicin, and can therefore qualify for use as wound, burn and ulcer dressings. Changing the process parameters enables adapting the desired properties to the wound characteristics, and can thus enhance wound healing.

10 Accordingly, there is provided a use of the composition-of-matter, or the soy protein structure presented herein, in the manufacturing of a product for treating a topical, internal or transdermal surgical incision or wound, a local/topical infection, local/topical pain, and the likes.

Methods of treatment:

15 The soy protein structures and/or devices containing same, as described herein, can be utilized in the treatment of various medical conditions in which release of the incorporated bioactive agent is desirable, whereby the composition-of-matter is designed so as to exhibit a release profile that suits the condition being treated. A soy protein structure and/or a medical device containing same which does not contain a
20 bioactive agent can also be used for its mechanical properties *per-se*.

 Thus, for example, in cases of treating an infection associated with a pathogenic microorganism, the structure incorporates an antibiotic agent and characterized by a high burst release of the antibiotic agent upon contact with the infected tissue. In some embodiments, a mediating layer may be used so as to facilitate the wetting of the soy
25 protein structure and improve the contact between the structure and the infected tissue. For such medical conditions, a device containing the soy protein structure can be designed as a wound dressing, an adhesive strip, a bandage, an adhesive plaster, a skin patch and an occlusive burn bandage device. The device is placed on the infected area so as to cover it and its immediate surroundings.

30 In cases of medical conditions in which prevention of infection is desired (prophylactic treatment against infections), such as in case of burns and other medical conditions that involve exposed tissues, the bioactive agent is an antibiotic agent and the

soy protein structure is characterized by a low burst release of the antibiotic agent upon contact with the infected tissue.

In cases of medical conditions which require a rapid or prolonged release of an analgesic agent, such as in case of treating local pain, the structure is characterized by a
5 respective high or low burst release of the analgesic agent upon contact with the afflicted tissue.

As demonstrated in the Examples section that follows below, the present inventors have designed and used exemplary soy protein film structures in a method of eradicating pathogenic microorganisms. In general, the strategy of drug release for
10 treating an infected wound depends on the condition of the wound. After the onset of an infection, it is crucial to immediately respond to the presence of large numbers of bacteria (more than 10^5 CFU/mL) which may already be present in the biofilm, and which may require antibiotic doses of up to 1000 times those needed in suspension. Following the initial release, sustained release at an effective level over a period of time
15 can prevent the occurrence of latent infection. Accordingly, it has been demonstrated that exemplary SPI film structures incorporating gentamicin as an antibiotic agent as described herein, comply with these requirements. All gentamicin release profiles from the SPI film structures exhibited a combination of a medium burst release followed by release in a decreasing rate over 4-5 weeks.

As presented hereinbelow, the time-dependent antimicrobial efficacy of exemplary SPI based structures used as wound dressing was tested *in vitro* by the disc diffusion test, which represents a typical clinical situation, where the treatment is effected by placing the drug-eluting wound dressing on the wound surface, allowing the drug to diffuse to the wound bed. The results from this method of treatment were found
20 to dependent on the rate of diffusion of the bioactive agent from the soy protein structure, set against the growth rate of the bacterial species growing on the bacterial lawn, and were found to dependent on the physicochemical environment.

Preparation:

In order to produce the soy protein structures described herein, and particularly
30 such structures which combine mechanical properties with the capacity to incorporate bioactive agents while retaining their activity and to controllably release these agents, there is provided a process of preparing the structures described herein.

The process is demonstrated and exemplified in details in the Examples section that follows below. Generally, the process of preparing the composition-of-matter presented herein is effected by providing an aqueous solution containing soy protein at a concentration that ranges from 3-7 percents weight per volume of water, a plasticizer at
5 a weight ratio compared to the amount of dry soy protein that ranges from 25 % w/w to 100 % w/w relative to the weight of dry soy protein used in the composition, as described herein, optionally a crosslinking agent at a concentration that ranges from 0.1 to 2 percents by weight relative to the weight of dry soy protein used in the composition, and the optional bioactive agent at a concentration that ranges from 0.1
10 percents to 10 percents relative to the weight of dry soy protein used in the composition.

As mentioned hereinabove, in some embodiments of the present invention, the soy protein structure may be prepared with additional additives, which can be added to the solution in order to confer color and finish to the resulting structure.

The solution's pH may be adjusted to a desired level, e.g., 7.2, and heated to 25-
15 70 °C or about 55 °C, then allowed to clarify and cool to room temperature before being poured (cast) on a surface and let dry at the ambient temperature and humidity for 2-4 days.

Once formed as a film or in any shape according to the casting mold, the structure may be treated with heat to effect thermal curing, as described herein, e.g., at a
20 temperature ranging from 40 °C to 100 °C for 12 to 48 hours.

The resulting soy protein structure can further be shaped, fashioned, and processed to take any desirable form, depending on its intended use.

Specific exemplary embodiments:

A soy protein film structure is prepared by solution cast. The solution is
25 prepared with 5 % weight per volume SPI, glycerol is added at a 50 % relative to the weight of dry soy protein used in the composition, glyoxal is added at a concentration of 1 % relative to the weight of dry soy protein used in the composition, and an optional bioactive agent is added at a concentration of 3 % relative to the weight of dry soy protein used in the composition. The pH of the solution is adjusted to 7.2 and the
30 solution is heated to 55 °C for 30 minutes and cooled at room temperature for another 30 minutes for bubble removal and degassing. The cooled solution is pours on a surface

and allowed to dry for 72 hours at ambient temperature and humidity. Thereafter the resulting film is further thermally cured at 80 °C for 24 hours.

General:

As used herein the term “about” refers to $\pm 10\%$.

5 The terms "comprises", "comprising", "includes", "including", “having” and their conjugates mean "including but not limited to".

The term “consisting of means “including and limited to”.

10 The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

15 The term “exemplary” is used herein to mean “serving as an example, instance or illustration”. Any embodiment described as “exemplary” is not necessarily to be construed as preferred or advantageous over other embodiments and/or to exclude the incorporation of features from other embodiments.

The term “optionally” is used herein to mean “is provided in some embodiments and not provided in other embodiments”. Any particular embodiment of the invention may include a plurality of “optional” features unless such features conflict.

20 As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

25 Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as
30 individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term “treating” includes abrogating, substantially inhibiting, substantially preventing, substantially slowing or substantially reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

It is expected that during the life of a patent maturing from this application many relevant soy-protein based structures will be developed and the scope of this term is intended to include all such new technologies *a priori*.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

5

EXAMPLE 1

Preparation of Soy Protein Structures

Materials and Methods:

Soy protein source: non-GMO soy protein isolate (SPI, Solpro 910™, minimum
10 90 % w/w protein, on dry basis) was produced by Solbar™ (Ashdod, Israel).

Plasticizers: glycerol (G-7893) and sorbitol (S-1876) were purchased from Sigma-Aldrich (Rehovot, Israel).

Crosslinking agents: glyoxal (50650) and L-cysteine (C-7352) were used as purchased from Sigma-Aldrich (Rehovot, Israel).

15 Bioactive agent: gentamicin sulfate (G-1264), an antibacterial drug (450-477 g/mol, $T_m = 218-237$ °C), was purchased from Sigma-Aldrich (Rehovot, Israel).

Protease: trypsin/EDTA Solution A (03-050-1): EDTA 0.02 %, trypsin 0.25 % with Phenol Red (Biological Industries, Beit Haemek, Israel).

Preparation of soy protein films:

20 Films of soy protein isolate (SPI) were prepared using the solution cast method as follows. SPI solutions were prepared by slowly dissolving the SPI in constantly stirred distilled water. When needed, the pH was adjusted with 1M sodium hydroxide or 1M HCl (using a pH meter, Mettler Toledo MP220). Thereafter plasticizer, crosslinking agent(s) and bioactive agent(s) were added to the mixture with constant
25 stirring. Thermal curing was optionally effected on the formed films by heat treatment.

While stirred, the solution was heated at a constant temperature for 30 minutes and cooled at room temperature for another 30 minutes for bubbles removal and degassing. Finally, the solution was cast into low-density polyethylene plates and dried at the ambient temperature and humidity for 72 hours.

30 The thickness of the file was controlled by casting the same amount of solution (50 ml) per plate and was determined to be approximately 0.5 mm.

Dried films were removed from the plates and specimens were cut for each test. In certain samples, a thermal curing by heat treatment was performed to formed films in an oven. The samples to be heated were placed in glass Petri dishes and were held down with covering plates to prevent curling and rippling during heating. Thereafter the samples were stored in desiccators (room temperature, 30 % relative humidity) until use.

Yellowish, transparent, homogenous, SPI films were created successfully using the solution-casting technique under various processing conditions and addition of various chemical agents. The films were approximately 0.5 mm thick and easily peeled off the plates. Low-density polyethylene plates prevented the film's adherence, contrary to polystyrene or glass Petri dishes. The potential of SPI films to serve as drug-eluting wound dressings was assessed by studying the effects of the process parameters and additives on the mechanical and physical properties of the films and on the drug release profile.

The preparation and processing parameters of exemplary SPI film structures, according to some embodiments of the present invention, are presented in Table 1.

Table 1

Parameter	Values used to prepare some exemplary film samples
Soy protein isolate	3 %, 5 % and 7 % w/v in aqueous solution
pH values of the aqueous solution	6, 7.2, 10
Temperature of the aqueous solution	25 °C, 40 °C, 55 °C, 70 °C
Plasticizer(s)	Sorbitol – 30 %, 40 %, 50 % and 80 % w/w relative to SPI
	Glycerol – 25 %, 30 %, 35 %, 40 %, 50 % and 80 % w/w relative to SPI
Crosslinking agent(s)	Cysteine – 1 % w/w relative to SPI

	Glyoxal – 0 %, 0.5 %, 1 % and 2 % w/w relative to SPI
Bioactive agent (drug)	Gentamicin sulfate – 1 % and 3 % w/w relative to SPI Ibuprofen and Bupivacaine – 3 % w/w relative to SPI
Heat treatment (thermal curing) temperature	60 °C, 80 °C and 100 °C
Heat treatment (thermal curing) duration	1, 2, 6 and 24 hours

EXAMPLE 2

Mechanical Properties of Soy Protein Structures

5 *Tensile mechanical properties:*

The SPI film's tensile mechanical properties were measured at room temperature, under unidirectional tension at a rate of 50 mm/min (according to a standard test method ASTM D 638-03), using a 5500 Instron™ machine. Each film sample was cut into a dog bone wound dressing shape (neck length 2 cm, width 5 mm).

10 The tensile strength was defined as the maximum strength in the stress-strain curve. The maximal strain was defined as the breaking strain.

Young's modulus was defined as the slope of the stress-strain curve in the elastic (linear) region.

At least three samples were tested for each type of specimen. The means and
15 standard deviations were calculated using the t-test.

Desired mechanical properties, i.e. combination of strength with ductility and flexibility, are beneficial for routine handling and mechanical stability during function. The effects of the plasticizer type, crosslinking method, and temperature and pH of the solution on the SPI's tensile properties were studied and the results are presented
20 hereinbelow.

Plasticizer effect:

While reducing the present invention to practice, the present inventors have realized that it may be beneficial to add a plasticizer to the composition for preparing the SPI films in order to augment the mechanical properties of the films, e.g., soften the films. Film samples were prepared using 5 % w/v SPI solution at pH 7.2 and 55 °C as presented hereinabove. 1 % w/w glyoxal was also added to the solution and the effect of glycerol and sorbitol as plasticizers on the SPI properties was studied. The effect of further crosslinking by means of thermal treatment after the film preparation step was also studied. The data which were obtained for some exemplary SPI film samples, and particularly the effect of the plasticizer on the mechanical properties of thermally treated (80 °C, 24 hours) and non-treated SPI films crosslinked with 1 % w/w glyoxal, are presented in Table 2.

Table 2

Sample		Ultimate Tensile Strength [Mpa]	Young's Modulus [Mpa]	Maximal strain [%]
Non-thermally treated	Glycerol 50 % w/w	9.3±0.2	128.5±12.4	158.8±20.5
	Sorbitol 50 % w/w	7.3±1	104.3±18.9	151.5±17.7
Thermally treated (80 °C, 24 hours)	Glycerol 50 % w/w	16±1.5	190.6±35.6	144.5±24.6
	Sorbitol/glycerol 50/50 (total of 80 % w/w plasticizer)	13.9±0.9	127.8±16.6	146.9±14.9
	Sorbitol 80 % w/w	18.9±1.6	273.2±28.2	9.0±3.7

As can be seen in Table 2, the results of non-treated films show a slight advantage to glycerol over sorbitol. However, when films were thermally treated (80

°C, 24 hours), 80 % w/w sorbitol samples enabled mechanical testing and were superior to 50 % w/w sorbitol samples. Therefore, thermally treated 50 % w/w glycerol films were compared to thermally treated 80 % w/w sorbitol and 80 % w/w glycerol/sorbitol (50/50) film samples.

5 As can further be seen in Table 2, the results show that both plasticizers showed an effect on the tested parameters with glycerol showing a more desired effect.

Bioactive agent effect:

Table 3 presents results showing the bioactive agent versus plasticizer effect on the Young's modulus (elasticity in MPa), the maximal strain before break (stretchability
10 in percents) and the maximal stress (strength in MPa) which were measured in SPI film samples prepared as discussed above using 5 % w/v SPI solution at pH 7.2 and 55 °C, 1 % w/w glyoxal and thermal curing at 80 °C for 24 hours. Some of the films were prepared with two exemplary analgesic agents, ibuprofen and bupivacaine, which can be used to manufacture exemplary medical devices based on SPI and useful in treating
15 local pain, according to some embodiments of the present invention.

Table 3

Percent glycerol and drug in SPI film sample	Young's Modulus [Mpa]	Max strain [%]	Max stress [Mpa]
50 % no drug	344.95 ±74.81	63.2 ±19.0	16.34 ±1.69
35 % no drug	342.57 ±95.94	73.1 ±35.7	17.20 ±2.82
30 % no drug	560.17 ±61.08	31.7 ±15.8	25.44 ±2.94
50 % and 3 % ibuprofen	205.29 ±66.53	108.5 ±34.7	14.15 ±1.77
50 % and 3 % bupivacain	185.98 ±26.01	112.5 ±27.7	13.38 ±2.86

Figures 1A-C present bar graphs showing tensile strength tests (Figure 1A),
20 maximal strain (Figure 1B) and Young's modulus tests (Figure 1C), measured for exemplary SPI film samples prepared with, 5 % w/v SPI content, 1 % glyoxal crosslinking agent, cast from high temperature solutions (55 °C, 24 hours) and thermally cured at 80 °C for 24 hours, showing the effect of various glycerol and bioactive agent contents on mechanical parameters.

25 As can be seen in Table 3 and Figures 1A-C, the glycerol content affects the elasticity and stretchability, namely an increase in the glycerol content lowered the

elasticity, and increased the stretchability. Glycerol was also shown to exhibit a strength-lowering effect.

As can further be seen in Table 3 and Figures 1A-C, glycerol content has a similar effect on elasticity and stretchability compared to that of the bioactive agent in the case of both ibuprofen and bupivacaine, namely an increase in the bioactive agent content lowered the elasticity, increased the stretchability, similar to an increase in glycerol content. In the case of strength, glycerol had a more notable strength-lowering effect than that of the bioactive agent.

It is noted that low glycerol content may confer higher biocompatibility in some applications and embodiments of the present invention, therefore an ability to lower the glycerol content may be desired. It is therefore shown herein that a glycerol content can be selected for controlling the capacity to incorporate a bioactive agent at a certain desired content and while maintaining desired mechanical properties.

Effect of thermal curing (heat treatment) duration:

Films were prepared from a solution cast of 5 % SPI, pH 7.2 at 55 °C using 50 % glycerol and 1 % glyoxal. All films underwent thermal treatment at 80 °C. The samples were subjected to different durations of thermal curing (crosslinking by heat treatment) of 1, 3, 6 and 24 hours, and the results are presented in Figures 1A-C.

Figures 2A-C present comparative bar-plots showing the effect of duration of heat treatment ("thermal" crosslinking or thermal curing) for 1, 2, 6 and 24 hours on SPI films, according to some embodiments of the present invention, prepared at 70 °C from 5 % w/v SPI solutions and further containing 1 % glyoxal as a chemical crosslinking agent and 50 % glycerol as a plasticizer, wherein Figure 2A shows the effect on tensile strength, maximal strain in Figure 2B and Young's modulus in Figure 2C.

As can be seen in Figures 2A-C, the tensile strength and Young's modulus of the SPI films showed a gradual increase as the thermal treatment duration was increased, while the maximal strain exhibited some decrease with the increase in the duration of the thermal treatment.

Effect of thermal curing temperature:

Films were prepared from a solution cast of 5 % SPI, pH 7.2 at 55 °C using 50 % glycerol and 1 % glyoxal, and subjected to different thermal curing temperatures of 60 °C, 80 °C and 100 °C, and the results are presented in Figures 3A-C.

Figures 3A-C present comparative bar-plots of tensile strength tests (Figure 3A), maximal strain (Figure 3B) and Young's modulus tests (Figure 3C), measured for exemplary SPI film samples prepared with 5 % w/v SPI content, 50 % w/w glycerol plasticizer and 1 % w/w glyoxal crosslinking agent, and cast from high temperature solutions (70 °C, 24 hours), and showing the effect of heat treatment (thermal curing) at 60 °C, 80 °C and 100 °C.

As can be seen in Figures 3A-C, the SPI films which were thermally cured at 100 °C showed higher tensile strength and Young's modulus and lower values of maximal strain compared to the films cured at 60 °C and 80 °C. There was no significant difference found between mechanical characteristics of the films cured at 60 °C and 80 °C.

Effect of soy protein content:

The effect of soy protein content was studied with SPI films which were prepared in 70 °C from SPI solutions containing 50 % w/w glycerol as a plasticizer and 1 % w/w glyoxal as crosslinking agent, and subjected to thermal curing treatment at 80 °C for 24 hours. The various soy protein contents were 3 %, 5 % and 7 % w/v.

SPI films prepared in 55 °C with 7 % w/v SPI were mechanically tested as well, however, the solutions containing 7 % w/v soy protein were difficult to stir and the films cast from it were non-homogeneous and slightly singed following the thermal treatment.

Figures 4A-C present comparative bar-plots of tensile strength tests (Figure 4A), maximal strain (Figure 4B) and Young's modulus tests (Figure 4C), measured for exemplary SPI film samples prepared with 3 %, 5 %, 7 % w/v SPI content, 50 % w/w glycerol plasticizer, and cast from high temperature solutions (70 °C and 55 °C, 24 hours), showing the effect of various SPI content and casting temperature on the tested parameters.

As can be seen in Figures 5A-C, the tensile strength and Young's modulus of the SPI films showed a gradual increase with the increase in the film's SPI content. The decrease in the maximal strain was less significant. The 7 % SPI films prepared in 55 °C showed higher tensile strength and Young's modulus compared to all the samples prepared in 70 °C.

Crosslinking effect:

Crosslinking was studied for its effect on the film's integrity and stabilization during swelling, degradation and drug release profile. Therefore, a proper crosslinking method for the SPI films was sought. Glyoxal (dialdehyde) crosslinked films were compared with L-cysteine (amino acid) crosslinked films. Glyoxal and L-cysteine are considered less toxic than formaldehyde-based crosslinking agents.

The effect of a crosslinking agent on the tensile properties of thermally treated (80 °C, 24 hours) SPI film samples prepared with 50 % w/w glycerol crosslinked with 1 % w/w crosslinking agent is presented in Table 4.

Table 4

Crosslinking agent used in the Sample	Ultimate Tensile Strength [Mpa]	Young's Modulus [Mpa]	Maximal strain [%]
Glyoxal 1 % w/w	16.5±1.7	244.8±8.6	128.2±12.3
L-cysteine 1 % w/w	10.5±3	142.8±57.3	110.5±14

As can be seen in Table 4, the results indicate that glyoxal may confer better and consistent mechanical properties compared to L-cysteine.

The effect of an exemplary combination of a crosslinking agent and a crosslinking method, e.g., use of glyoxal and effecting thermal curing by heat treatment, was studied and the combined effect was also investigated and compared to the non-crosslinked films, and the results are presented in Figure 5.

Figures 5A-C present comparative bar-plots of tensile strength tests (Figure 5A), Young's modulus tests (Figure 5B) and elongation at break tests (Figure 5C), measured for two types of exemplary SPI film samples prepared with 5 % w/v SPI content and 50 % w/w glycerol plasticizer, wherein the films cast from high temperature and high pH solutions (70 °C, pH=10) are marked by solid black and films cast from low temperature and neutral pH solutions (55 °C, pH=7.2) are marked by checked bars, and the crosslinking method is indicated below the bars, wherein "non-treated" refers to films prepared with no crosslinking agent and which did not undergo thermal curing.

As can be seen in Figures 5A-C, both crosslinking methods induced effects of increasing tensile strength and Young's modulus and decreasing maximal strain. However, solution conditions of 55 °C and pH=7.2 enabled improved results in terms of tensile strength and Young's modulus compared to films prepared in conditions of 70 °C and pH=10.

Without wishing to be bound by any particular theory, it is assumed that glyoxal creates "chemical" crosslinks mostly via free ϵ -amine groups, while thermal curing by heat treatment is believed to promote "thermal" crosslinks mostly by encouraging S-S associations and formation of new hydrogen and hydrophobic interaction. The combined effect of using both crosslinking methods is presumably achieved because each method acts on different types of protein-protein interactions. Furthermore, the crosslinking reaction with the protein chains is probably enhanced during heat treatment and more "chemical" crosslinks are created via amine groups.

It is noted that adding the contribution to the overall mechanical properties of the soy protein structure conferred by chemical crosslinking with glyoxal alone, to the contribution of thermal curing alone may amount to less than the overall mechanical properties of the soy protein structure conferred by a combination of both the chemical and thermal crosslinking.

Figure 6 presents comparative stress-strain curves of three exemplary 5 % w/v SPI film samples plasticized with 50 % w/w glycerol and cast as untreated films (no crosslinking agent and no thermal curing), crosslinked using 1 % glyoxal (no thermal curing), and crosslinked with 1 % glyoxal and thermally cured (heat treated) at 80 °C for 24 hours as indicated therein.

As can be seen in Figure 6, the film samples that were crosslinked by a combination of a crosslinking agent (glyoxal) and thermal treatment were found to be superior in combining relatively high resistance to tear (tensile strength of 17 MPa) and ductility (maximal strain of 160 %).

Effect of crosslinker content:

Films were prepared from a solution cast of 5 % SPI, pH 7.2 at 55 °C using 50 % glycerol and various glyoxal contents of 0 %, 0.5 %, 1 % and 2 % w/w, underwent thermal curing for 24 hours at 80 °C and tested for mechanical properties; the results are presented in Figures 7A-C.

Figures 7A-C present comparative bar-plots of tensile strength tests (Figure 7A), maximal strain (Figure 7B) and Young's modulus tests (Figure 7C), measured for exemplary SPI film samples prepared with 5 % w/v SPI content, 50 % w/w glycerol plasticizer, and cast from high temperature solutions (55 °C, 24 hours), and showing the effect of various glyoxal content at 0 %, 0.5 %, 1 % and 2 % w/w relative to SPI dry weight.

As can be seen in Figures 7A-C, there was no significant effect of the glyoxal content on the initial mechanical characteristics of the films. It is noted that these experiments were performed on SPI films which were cast using lower temperature of 55 °C found notable differences between 0 and 1 % w/w glyoxal.

The obtained results suggest that the thermal treatment has higher influence in determining the mechanical properties of the film compared to the chemical crosslinking.

Effect of pH and solution temperature:

A series of experiments using SPI films prepared at various pH values (6, 7.2 and 10) and casting temperatures (25 °C, 40 °C, 55 °C and 70 °C) were carried out in order to evaluate the effect of pH and temperature of the solution on the tensile properties of the crosslinked films. All film samples were prepared using 5 % w/v SPI solutions with glycerol (50 % w/w) as a plasticizer and glyoxal (1 % w/w) as a crosslinking agent, and underwent thermal treatment (80 °C, 24 hours), and the results are presented in Figures 8A-C.

Figures 8A-C present comparative bar-plots of tensile strength tests (Figure 8A), Young's modulus tests (Figure 8B) and elongation at break tests (Figure 8C), measured for two exemplary SPI film samples prepared with 5 % w/v SPI content, 50 % w/w glycerol plasticizer and 1 % w/w glyoxal crosslinking agent, and cast from high temperature solutions (80 °C, 24 hours), wherein the results obtained for films cast from solutions at pH 6 are marked by solid white bars, films cast from solutions at pH 7.2 are marked by solid grey bars and films cast from solutions at pH 7.2 are marked by solid black bars, and the temperature of the cast solution is indicated below the bars.

As can be seen in Figures 8A-C, when the SPI films were cast from solutions at pH values of 6 and 7.2, the tensile strength and Young's modulus showed a gradual increase with temperature, while the maximal strain exhibited some decrease with the

increase in solution temperature. At pH 10, however, the maximal values of strength and elastic modulus were acquired at 55 °C. The effect of the solution's pH in the selected range was mostly minimal except for films prepared at 70 °C, where the tensile modulus was decreased and the maximal strain was increased with the increase in pH.

5 It is noted that films prepared from solutions at pH 6 exhibited a denser and more crumpled texture, while films prepared from solutions at pH 10 exhibited a less homogenous morphology.

These results suggest that solution parameters such as pH 7.2 and 55 °C enable to obtain high quality homogenous SPI-based films which combine relatively high
10 tensile strength and Young's modulus with good ductility.

Conclusive Remarks:

The mechanical properties of exemplary SPI films, according to some embodiments of the present invention, have been demonstrated to be suitable for wound protection and dressing performance. It has been demonstrated that the films exhibit the
15 capacity to withstand stress, strain, compression and other mechanical distortions while avoiding tear, thereby capable, for example, to protect topical dermal wounds or be used as an internal wound support, e.g. for surgical tissue defects. In the clinical setting, appropriate mechanical properties of dressing materials are needed to ensure routine handling, and the SPI films presented herein were shown to be capable of such
20 performance.

Table 5 presents comparative mechanical properties of an exemplary SPI film according to some embodiments of the present invention, and various biopolymer films known in the art.

Table 5

Sample	Tensile Strength [MPa]	Young's modulus [MPa]	Maximal strain [%]
Thermally treated and crosslinked solution-cast SPI films ¹	16.05±1.5	190±35	144±24
Thermally treated	14.7±0.4	-	6.1±0.7

solution-cast SPI films ^{2,3}			
Electrospun poly-(l-lactide-co-ε-caprolactone) (50:50) mat. ⁴	4.7±2.1	8.4±0.9	960±220
Electrospun gelatin mat. ⁴	1.6±0.6	490±52	17.0±4.4

¹ SPI film according to an embodiment of the present invention, containing SPI at 5 % w/v), 50 % w/w glycerol plasticizer, 1 % w/w glyoxal as crosslinking agent, cast from a solution at pH 7.2 and 55 °C, and thermally treated at 80 °C for 24 hours; ² 5 % SPI w/v, 50 % w/w glycerol, pH 10, 70 °C and thermally treated (90 °C, 24 hours); ³ Rhim J.W. *et al.*, *J. Agricultural and Food Chemistry*, 2000, 48(10), 4937-4941; and ⁴ Lee J. *et al.*, *Biomaterials*, 2008, 29(12), 1872-1879.

As can be seen in Table 5, initial mechanical properties of some polymers, such as collagen or gelatin, may provide some satisfactory attributes. However, considerable downfall in these properties is expected to occur quickly due to hydration and digestive enzymatic activity. Hence, the results presented hereinabove demonstrate that the solution-cast SPI films, according to embodiments of the present invention, exhibited superior tensile strength and maximal strain compared to other biopolymer membranes and previously studied soy protein films.

As described in the art, polyol-based plasticizers (compounds containing multiple hydroxyl groups) reduce stiffness and induce flexibility by penetrating between protein chains, associating hydrogen bonds and lowering Tg [Guilbert *et al.*, *Packaging Technology and Science*, 1995, 8(6), 339-346]. Sorbitol is known for its tendency to crystallize when films are stored under conditions of low humidity [Talja *et al.*, *Carbohydrate Polymers*, 2007, 67(3), 288-295]. Therefore, it could be expected that heat treatment of the cast plasticizer-containing film, which reduced the film's water content, would lead to sorbitol crystallization and thus to an increase in the film's brittleness.

Indeed, the addition of a plasticizer to the SPI films has been found to confer desirable characteristics. Hence, crosslinked films (5 % SPI w/v, 1 % glyoxal w/w, pH 7.2, 55 °C), containing sorbitol as plasticizer, exhibited mechanical properties similar to glycerol plasticized films. However, when the films were also thermally-treated, the glycerol-containing films exhibited higher maximal strain (see, Table 1).

As can be seen in the results presented hereinabove, using glycerol as plasticizer in the SPI films presented herein lead to films exhibiting superior mechanical properties.

The results also show that if one intends to reduce hydrophilic trait of the thermally-treated films by mixing glycerol and sorbitol, a 50/50 ratio and total quantity of 80 % w/w plasticizer in the film could be contemplated as an alternative to the use of glycerol alone.

The crosslinking agent glyoxal was found to be effective in imparting acceptable mechanical properties to the SPI films according to some embodiments of the present invention. The reaction of glyoxal with the free ϵ -amine groups (NH_2), sulfhydryl groups ($-\text{SH}$) and even side-chains of histidine and tyrosine, induces intra- and intermolecular crosslinks. Soy protein is susceptible to glyoxal crosslinking due to its high content of the basic amino acids lysine (about 6 %) and arginine (about 7 %); therefore effective crosslinking resulted in desired tensile properties. L-cysteine contains a thiol residue which is responsible for disulfide polymerization during heat treatment (thermal curing). Its activity is presumably limited primarily to disulfide linkages and primarily when heating of the protein is involved.

The study described herein shows that the combination of both crosslinking methods yielded a film with a higher mechanical strength than the film obtained by applying each method separately, with preservation of good ductility.

The pH and temperature of the SPI solution had a limited effect on the tensile properties of the films (see, Figures 8A-C). At relatively high pH values, such as 10, where more charged carboxylate groups are present, the dispersion of the chains probably causes higher rejection and less effective crosslinking activity, i.e. more intra- than inter- chain crosslinking which reduced the film's strength. This may be associated with the unexpected decrease in strength and modulus obtained for films processed at

70 °C and pH 10. It should be noted that the amount of carboxylic (COOH) groups in soy protein is approximately twice that of the amine groups (NH₂).

EXAMPLE 3

5 *Water Interaction of Soy Protein Structures*

Successful wound healing requires a moist environment, therefore, for an SPI film to be practical as a wound dressing platform, two parameters should be determined for desired levels: the water uptake ability of the film, and the water vapor transmission rate (WVTR) through the film. The weight loss profile in aqueous solutions affects the
10 drug release rate and the mechanical strength, and was therefore studied as well.

Some degree of water uptake is desired in order to provide adequate gaseous exchange and absorption of wound exudates. However, rapid water penetration should be avoided in order to prevent rapid release of the active agent from the wound dressing. Water uptake by the SPI films occurs as their hydrophilic polymer gradually
15 absorbs water, chain relaxation occurs and the matrix swells.

Water uptake ability:

The fluid absorbing capacity of a given used as a wound dressing is an important criterion for maintaining a moist environment over the wound bed. A swelling test was performed in order to determine the water sorption capacities of the various film
20 samples. Prior to testing, all films were conditioned for 10 days in a desiccator (room temperature, 30 % relative humidity), for equilibrium moisture content.

Round film samples (2 mm diameter) were pre-weighed and immersed in a PBS solution (pH=7) at 37 °C in an incubator. The weight of the samples was measured at several time points until 24 hours by removing the PBS and blotting them gently to
25 remove excess fluid.

The water uptake was calculated according to Equation 1:

$$\text{water uptake} = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{dry}}} \times 100[\%] \quad (\text{Equation 1})$$

Water vapor transmission rate:

The moisture permeability of the wound dressings was determined by measuring the water vapor transmission rate (WVTR) across the film. A Sheen Payne permeability cup with an exposure area of 10 cm^2 was filled with 5 ml PBS and covered with a circular wound dressing. The cup was placed in a straight position inside an oven containing 1 kg of freshly dried silica gel in order to maintain a relatively low humidity, at 37°C . The weight of the assembly was measured every hour and a graph of the evaporated water versus time was plotted. Measurements were taken until at least seven points were given on a straight line ($R^2 \geq 0.99$). The slopes of the curves (water loss rates) were calculated and the WVTR values were evaluated according to Equation 2:

$$WVTR = \frac{\text{slope} \times 24}{\text{area}} \left[\frac{\text{g}}{\text{m}^2 \cdot \text{day}} \right] \quad (\text{Equation 2})$$

In vitro weight loss profile:

The *in vitro* weight loss profile of the SPI films was studied in an aqueous medium. Round dry film samples (2 mm diameter) were pre-weighed (after 10 days in a desiccator, 30 % humidity at room temperature) and immersed in PBS solution (pH=7) at 37°C in an incubator for 28 days. Sodium azide (0.05 % w/v) was added as preservative. The films were taken out at certain time points (1, 7, 14, 21 and 28 days), dried in a vacuum oven at 60°C for 24 hours and weighed.

The percentage weight loss was calculated according to Equation 3:

$$\text{weight loss} = \frac{W_b - W_a}{W_b} \times 100[\%] \quad (\text{Equation 3})$$

whereas W_b is the sample weight before immersion and W_a is the weight after immersion and drying.

All films were cast from 5 % w/v SPI films plasticized with 50 % w/w glycerol.

The water uptake, water vapor transmission rate and weight loss profile of the following four samples were determined:

Non-crosslinked SPI films;

Crosslinked SPI films using 1 % w/w glyoxal;

5 Crosslinked films using thermal treatment at 80 °C for 24 hours; and

Crosslinked films using 1 % w/w glyoxal and thermal treatment at 80 °C for 24 hours.

Water uptake:

10 All samples were immersed in PBS (pH 7.0, 37 °C) to simulate the water absorption behavior in the presence of wound fluids. The water uptake values of the studied SPI films are presented in Figure 9.

Figure 9 presents a scatter-plot of the water reuptake of SPI film samples as a function of time, wherein the films are prepared with 5 % w/v SPI content, plasticized using 50 % w/w glycerol and cast from solution at pH 7.2 and 55 °C, whereas samples of un-crosslinked films are marked by solid rectangles, thermally crosslinked (80 °C for 15 24 hours) films are marked with X, films crosslinked using 1 % w/w glyoxal are marked with solid rhombs and filmed crosslinked using both thermal treatment and glyoxal are marked by solid triangles.

As can be seen in Figure 9, non-crosslinked films exhibited rapid water uptake, 20 reaching a value of 257 % after 6 hours. A slight decrease in water uptake was observed after 24 hours, probably due to some degradation. In general, crosslinked films exhibited reduced water uptake with similar water absorption patterns, consisting of the following stages:

A rapid initial water uptake within the first 30 minutes;

25 A slight decrease in water content during the following 30 minutes; and

A slight increase in water content during the following 23 hours.

Films which underwent both chemical and thermal crosslinking processes exhibited a water uptake peak of 67 % after 15 minutes and a constant water uptake of approximately 54 % after 1 hour.

30 ***Water vapor transmission rate (WVTR):***

The WVTR values of the four selected samples were measured as described hereinabove.

Figure 10 presents a bar-plot showing the water vapor transmission rate (WVTR) measured for SPI film samples prepared with 5 % w/v SPI content, plasticized using 50 % w/w glycerol and cast from solution at pH 7.2 and 55 °C, wherein the crosslinking process is indicated from left bar to right bar as thermally and chemically crosslinked samples (1 % w/w glyoxal and thermal treatment at 80 °C for 24 hours), chemically crosslinked samples, thermally crosslinked samples, un-crosslinked samples and an aqueous solution designated as "open-cup".

As can be seen in Figure 10, all samples exhibited WVTR values in the range of 2300-2700 grams/m²/day, with no statistical difference ($p > 0.05$), while the evaporative water loss through the various samples was found to depend linearly on time ($R^2 \geq 0.99$ in all cases), resulting in constant WVTR values. The WVTR of an exposed aqueous surface (open cup) was also determined experimentally in order to simulate a condition in which no dressing is applied to the wound surface. In this case, a WVTR of 6329 grams/m²/day was measured.

In vitro weight loss profile:

The weight loss profiles of the four samples of SPI films used in the previous study was determined and the results are presented in Figure 11.

Figure 11 presents a scatter-plot of the weight loss profile of SPI film samples as a function of time, wherein the films are prepared with 5 % w/v SPI content, plasticized using 50 % w/w glycerol and cast from solution at pH 7.2 and 55 °C, whereas samples of un-crosslinked films are marked by solid rectangles, thermally crosslinked (80 °C for 24 hours) films are marked with X, films crosslinked using 1 % w/w glyoxal are marked with solid rhombs and filmed crosslinked using both thermal treatment and glyoxal are marked by solid triangles.

In general, the weight loss rates (slope of the curve) of the un-crosslinked films were higher than those of the three types of crosslinked films. As can be seen in Figure 11, all samples lost from about 30 % to about 40 % of their initial weight after one day of immersion in an aqueous medium. This was followed by a slow weight loss for a period of 28 days. The films maintained their structural form during the entire experiment and did not disintegrate upon handling. Un-crosslinked film samples exhibited the highest weight loss rate and lost 50.5 % of their initial weight after one month. During the same period, glyoxal crosslinked or thermally crosslinked films lost

42.8 % and 41.2 %, respectively, and films that were crosslinked by both methods lost only 37.4 % of their initial weight.

Conclusive Remarks:

The aforementioned water interaction parameters and related properties of the SPI films, according to some embodiments of the present invention, were determined in order to study their function and practicability as a wound dressing.

Swelling of crosslinked film samples by water uptake was found to be significantly lower than that of un-crosslinked films (see, Figure 9). The crosslinked samples demonstrated a 3-stage water uptake pattern during the first 24 hours of immersion in the aqueous medium. In the first stage, a quick flux of water enters the film matrix. During the second stage, a small decrease in water content is exhibited, presumably due to "spring-like" contraction of the crosslinked polymer network and secretion of water and glycerol molecules. In the third stage, water uptake increases gradually as the chains undergo relaxation and the matrix swells again. While the crosslinked films undergo the second stage between 15 and 30 minutes of immersion, in the un-crosslinked films the second stage begins only after 6 hours of immersion.

It is noted that higher crosslinking density (as expressed by the mechanical properties) results in lower water uptake during each of the stages, and enables reaching equilibrium, i.e. constant water uptake, within a shorter period of time.

The WVTR of normal human skin is around 204 grams/m²/day and may reach up-to 5138 grams/m²/day in severe burn wounds. An effective wound dressing provides good WVTR management that retains a moist wound bed at the desired levels for the healing course. An excessive WVTR may lead to wound dehydration, whereas a low WVTR might lead to maceration and bacterial contamination. It has been asserted in the art that wound dressings should ideally possess a WVTR in the range of 2000-2500 grams/m²/day, however, commercial dressings do not always correspond to this range, and have been shown to cover a larger spectrum of WVTR, ranging from 90 (Dermiflex®, J & J) to 3350 grams/m²/day (Beschitin®, Unitika). Indeed, the WVTR value is related to the structural properties (thickness, porosity) of the dressing as well as to the hydrophilic nature of the material.

The results for all tested SPI film samples clearly demonstrate a WVTR in the desired range for a wound dressing, i.e. around 2300 grams/m²/day. This relatively high

value could be explained by the intrinsic hydrophilic nature of the soy protein and glycerol matrix. The results show that the WVTR practically does not change due to crosslinking. This observation probably relates to the relatively low density films that were prepared using the solution casting technique. In the case of denser melt processed (thermoplastic) SPI films, it was reported that crosslinking resulted in lower WVTR values [Silva *et al.*, *J. Materials Science: Materials in Medicine*, 2003, 14(12), 1055-1].

The primary weight-loss was obtained during the first day, as SPI film samples lost 30-40 % of their initial weight due to leaching of plasticizer and small uncrosslinked protein chains. This resulted from fast water uptake and was probably enhanced by the glycerol's hydrophilic nature. Thereafter a slow weight loss rate was observed for 28 days. It is assumed that protein chains are cleaved and diffuse from the matrix during that period. Indeed, a similar degradation profile was observed previously for chitosan/soy blended membranes. The crosslinked SPI films were found to have slower weight loss rates than the un-crosslinked films.

It is noted that the relatively high initial plasticizer content was used in these exemplary SPI film samples in order to obtain the desired ductility when handling the films as wound dressing. Water is known to act as a plasticizer for polymers, especially the hydrophilic ones. During usage, typical human topical wound's exudates will cause diffusion of the plasticizer, but this will occur in parallel to water uptake by the wound dressing.

It is further noted herein that while it has been reported that high glycerol content may lead to high water absorption and water-vapor permeability, which may be undesirable [Prudencio-Ferreira, S.H. *et al.*, *J. Food Science*, 1993, 58(2), 378-381], the data presented herein show that desired values of WVTR are obtained for films with a high glycerol content (e.g., 50 weight percents of the soy protein content).

EXAMPLE 4

Drug Release Profile of Soy Protein Structure

SPI films, according to some embodiments of the present invention, were cast from 5 % w/v solutions (55 °C, pH 7.2), plasticized with 50 % w/w glycerol and crosslinked using 1 % w/w glyoxal and used for the drug release studies. The

"chemically" crosslinked films were untreated with heat or further thermally cured (crosslinked) at 80 °C for 24 hours.

In vitro drug release study:

5 The SPI films (triplicate samples, 1 cm diameter each) were immersed in phosphate buffered saline (PBS, pH=7.0) at 37 °C for 56 days in order to determine the drug release kinetics from these structures, loaded with 1 % or 3 % w/w gentamicin.

The release studies were conducted in closed Eppendorf tubes containing 1.5 mL PBS. Sodium azide (0.05 % w/v) was added to the medium in order to prevent bacterial growth.

10 Periodically the medium was removed completely at each sampling time (2 hours, 6 hours, 12 hours, 1day, and 2, 3, 5, 7, 14, 21, 28, 35, 49 and 56 days), and fresh medium was introduced.

Gentamicin assay:

Determination of the medium's gentamicin content was carried out using an
15 Abbott Therapeutic Drug Monitoring System – TDX™ (Abbott Laboratories) according to the manufacturer's instructions. This drug content monitoring device enables the determination of the gentamicin concentration based on a polarization fluoroimmunoassay using fluorescein as a tracer. Briefly, the latter is excited by polarized light. Polarization of the emitted light is dependent on molecule size. Free
20 and labeled drug compete for binding sites. The drug concentration in the sample is proportional to the scatter of polarized light caused by free labeled drug. The measurable concentration range without dilution is 0 to 10.0 µg/mL. Higher drug concentrations were measured after carrying out manual dilution.

Residual drug recovery from the SPI films:

25 Residual drug recovery from the SPI films was measured as follows. Drug remains in the films were extracted by cleaving the film in trypsin A solution at 40 °C for 24 hours. Trypsin was used to cleave the protein chains and the gentamicin concentration was estimated using the above-described assay. The experiments were performed in triplicate.

30 Each film sample was loaded with either 1 % w/w or 3 % gentamicin and the drug release kinetics were studied for 2 months in triplicate samples. The cumulative release profiles from the four types of samples are presented in Figure 12 and the burst

release values and calculated release rates are presented in Table 6, presenting gentamicin release characteristics from SPI films.

Figures 12A-B present scatter-plots of cumulative gentamicin release from SPI film samples as a function of time from SPI (5 % w/v SPI, pH 7.2, 55 °C, 50 % w/w glycerol and 1 % w/w glyoxal), wherein measurements obtained from films not treated with heat are marked by solid rectangles, and film samples thermally cured at 80 °C for 24 hours are marked by solid triangles, while Figure 12A presents results obtained for films loaded with 1 % w/w gentamicin, and results obtained for films loaded with 3 % w/w gentamicin are presented in Figure 12B.

Table 6

Film type	Burst release (6 hours) [%]	Total release during 2 nd stage [%]	Sustained release during 2 nd stage [%/day]	Sustained release during 3 rd stage [µg/day]
Untreated (1 % drug)	66.7.3±6.1	94.3±7.4	0.094 (R ² =0.79)	0.21 (R ² =0.79)
Thermally treated (1 % drug)	46.2±4.1	65.7±6.7	0.150 (R ² =0.91)	0.34 (R ² =0.91)
Untreated (3 % drug)	63.2±6.5	94.7±8.2	0.119 (R ² =0.97)	0.80 (R ² =0.97)
Thermally treated (3 % drug)	47.8±7	70.6±3.6	0.314 (R ² =0.98)	2.12 (R ² =0.98)

As can be seen in Figures 12A-B and Table 6, the release profiles can be divided into three main stages:

- Burst release during the first 6 hours;
- Exponentially decreasing release rate during the first week; and
- Essentially sustained release rate during the following 2-8 weeks.

The thermal treatment had a significant effect on the gentamicin release profile for both samples, loaded with 1 % gentamicin and loaded with 3 % gentamicin. The first stage of the burst effect, and the total drug quantity released during the first week (second stage) of the untreated films were approximately 40 % higher than those of the thermally treated films. In contrast, the drug release rate from the thermally treated films during the third phase of release was higher than that measured from the untreated films.

After approximately two months, untreated films loaded with either 1 % or 3 % gentamicin released their total encapsulated drug, while the thermally treated films released only 73 % and 83 % of the total encapsulated drug, respectively. The effect of drug content on the release profile was clearly evident only during the third stage. Increasing the drug content from 1 % to 3 % had no significant effect on the first two stages of release, but resulted in a higher release rate during the third stage of release, especially in the thermally treated films.

Conclusive Remarks:

Gentamicin release profile obtained for exemplary SPI films, according to some embodiments of the present invention, showed a moderate burst effect of 46 to 66 % of the original drug content during the first 6 hours, accompanied by a stage of continuous decrease in release rate during the following week. This stage was followed by a third stage of zero-order release (sustained release rate) that lasted for the duration of the observation. It is noted that the SPI film samples maintained their integrity throughout the entire observation period. The burst effect and release profile during the first week were typical for diffusion-controlled systems. The third phase of sustained release rate presumably involved degradation of the soy protein matrix combined with diffusion of the remaining drug that was more firmly attached to the protein chains.

The observed release profile is highly suitable for applications such as, for example, antibiotic-eluting wound dressings. During the first hours of the wound treatment, it is essential to release a relatively high drug quantity into the wound bed in order to eliminate various infections that were not eliminated during wound cleansing and might create a resistant biofilm. Thereafter, the continued low release rate keeps the wound "infection-free" for more than two weeks, which is the time usually required for proper wound healing.

The burst release and overall amount of drug released during the first week from the thermally-treated films were significantly lower than those observed from the untreated films. This phenomenon is attributed to the limited swelling capacity of the former. The relatively low water uptake of densely crosslinked films limited drug diffusion during the first days. After initial degradation of the SPI matrix during the first week, more water could penetrate the matrix and the remaining drug leached out. Thus, after the second release phase, when higher drug amounts remained in the heat treated (thermally cured) film, it exhibited a higher diffusion gradient and faster release rate during the third phase of release compared to the untreated films.

The differences in the release profile can be harnessed for various applications including wound dressing. Thus, the thermally treated films can be used to treat burns which are not infected immediately after the trauma, but need a relatively long supporting period against infections, whereas the untreated films can be used for infected wounds.

It is noted that most of the gentamicin was released within the first week of the study (thermally treated 66 %, untreated 95 %), due to the hydrophilic nature of the antibiotic and the SPI matrix. The hydrophilic nature of soy protein enables relatively fast water intake, leading to full swelling of the matrix within a few hours of immersion (see, Figure 9). A faster and therefore unfavorable drug release rate has been reported in the literature for other antibiotic-eluting systems. Controlling the release of antibiotics from these systems is challenging due to the hydrophilic nature of both the drug and the host polymer. In most cases, the drug reservoir is depleted in less than two days, resulting in a very short antibacterial effect. Thus, the antibiotic-eluting SPI film described herein is advantageous over other systems. This advantage can be explained by the fact that various antibiotic drugs tend to bind proteins via various van der Waals or ionic interactions. The bonded portion may act as a reservoir and diffuse slower than the unbound form, and gentamicin binding to albumin is known to be 0-30 %. Furthermore, gentamicin is a highly charged polycation (+3.5, pH 7.4), whereas soy protein has many negatively charged carboxyl groups. It is thus probable that some ionic bonding took place. Such a binding mechanism was shown before between deprotonated carboxyl groups of succinylated collagen and positive anion groups of the gentamicin molecule.

EXAMPLE 5***Bacterial Growth Inhibition***

In this study, yellowish, transparent, homogenous, films were created using 5 % w/v SPI solution-casting technique as described hereinabove. The films were approximately 0.5 mm thick and easily peeled off the plates. Three exemplary SPI film samples were chosen for this study: films cast as untreated films, crosslinked SPI films using 1 % glyoxal, and SPI films crosslinked with 1 % glyoxal and thermally treated at 80 °C for 24 hours.

The microorganisms strains used in this study included *Pseudomonas aeruginosa* (*P. aeruginosa*), gram-negative, aerobic, rod-shaped bacterium; *Staphylococcus aureus* (*S. aureus*), facultative anaerobic, gram-positive coccus; *Staphylococcus albus* (*S. Albus*), gram-positive actinobacteria coccus; all bacteria were obtained from the microbiological laboratory, Rambam Medical Center, Haifa.

These bacterial strains were chosen for this study due to their frequent presence on human skin and their high pathogenicity in infections during wound occurrence and management. All three strains were clinically isolated and their minimal inhibitory concentration (MIC) values were evaluated, as presented in Table 7.

Table 7

Bacterial Strain	MIC [$\mu\text{g/ml}$]
Staphylococcus albus	3
Staphylococcus aureus	4.5
Pseudomonas aeruginosa	6.3

The strains were grown overnight on Müller-Hinton (Difco) agar plates at 37 °C prior to use. The bacterial cells were collected and re-suspended in saline, and adjusted to 1×10^7 CFU/mL (colony forming units) by visual comparison with a 0.5 McFarland standard. The corrected zone of inhibition test (CZOI) test was used to determine the time-dependence of the antimicrobial activity of the wound dressing.

The growth medium was Solid Mueller Hinton containing beef infusion (30 %), Bacto Casamino Acids (1.75 %), Starch (0.15 %) and Bacto-Agar (1.7 %) was purchased from Hy-Labs, Israel.

Host cells included primary human neonatal foreskin fibroblasts were obtained from Rambam Medical Center, Haifa.

Host cell culture medium was a modified Eagle's medium (MEM) with 10 % Fetal bovine Serum, 1 % L-glutamine and 0.1 % penicillin-streptomycin-nystatin all purchased from Biological industries, Beit Haemek, Israel.

In vitro bacterial inhibition:

In a modified version of the Kirby-Bauer disc diffusion test, which is typically used to determine bacterial susceptibility to antibiotics, round pieces of SPI film samples ($D=10$ mm) were placed on a bacterial lawn (100 μ L of inoculum, about 10^7 CFU/mL), seeded on Müller-Hinton agar plates), incubated overnight at 37 °C, and then photographed.

The inhibition zone area around the SPI film samples containing 3 % gentamicin was measured from the images by means of digital image processing software (SigmaScan Pro) by placing a circular mark to cover the circumference of the round inhibition zone (ignoring unclear overlapping between adjacent samples, and the effective diameter (D) of the circle was calculated according to Equation 4.

$$D = \sqrt{\frac{4 \cdot A}{\pi}} \quad (\text{Equation 4})$$

The corrected zone of inhibition test (CZOI) was then calculated by subtracting the round film's diameter from the value of D . This procedure was repeated on SPI film samples which were incubated in PBS for 1, 3, 7 and 14 days prior to testing. The release medium was replaced at time points corresponding to these of the CZOI test. The test was performed in triplicate for each type of dressing and for each of the three microorganisms: *S. aureus*, *S. albus* and *P. aeruginosa*.

Figures 13A-B present photographs of Petri dishes in which a culture of *Streptomyces albus*, an actinobacteria species of the genus *Streptomyces* has been grown and round samples of SIP films, according to embodiments of the present invention, have been placed upon to demonstrate a modified Kirby–Bauer disc diffusion test, showing a growth inhibition circles around SIP film samples prepared with 3 %

gentamicin (Figure 13A) and no growth inhibition around samples of SPI films prepared with no antibiotic agent as a control experiment (Figure 13B).

Figures 14A-C present comparative histogram plots showing the effect of drug release on corrected zone of inhibition (CZOI) around the four types of exemplary gentamicin-eluting SPI films, as a function of pre-incubation time, as observed for three bacterial strains *S. albus* (Figure 14A), *S. aureus* (Figure 14B), and *P. aeruginosa* (Figure 14C), wherein solid black bars represents results obtained with SPI films crosslinked with 1 % glyoxal and thermally treated at 80 °C for 24 hours containing 3 % gentamicin, diagonally striped bars represent results obtained with SPI films crosslinked using 1 % glyoxal containing 3 % gentamicin, solid grey bars represent results obtained with SPI films crosslinked with 1 % glyoxal and thermally treated at 80 °C for 24 hours containing 1 % gentamicin, and dotted bars results obtained with SPI films crosslinked using 1 % glyoxal containing 1 % gentamicin.

As can be seen in Figures 14A-C, all three bacterial strains clearly show susceptibility towards gentamicin with *S. albus* being the most susceptible and *P. aeruginosa* the most resistant. The strains *S. albus* and *S. aureus* were effectively inhibited by all SPI film samples for a period of at least 14 days. Strain *P. aeruginosa* was inhibited effectively until the third day of immersion. This is compared to the control where vast bacterial growth was exhibited on the films.

The largest inhibition zones are evident for all samples at $t=0$ (without pre-immersion). As expected, inhibition zones created around films loaded with 3 % gentamicin were slightly larger than those loaded with 1 % gentamicin.

For *S. albus* and *S. aureus* the inhibition zones obtained by the thermally treated specimens were rather constant after burst effect, while the non-thermally treated films generated a decreasing CZOI values over time.

In general, gentamicin released from exemplary SPI film samples, according to embodiments of the present invention, showed great efficiency towards microorganisms, which are abundant on human skin and usually responsible for wound infections. *S. albus* and *S. aureus* exhibited relatively larger inhibition zones compared to *P. aeruginosa* (see, Figure 14) due to their higher sensitivity to gentamicin concentrations, as expected from the MIC values (see, Table 7). The CZOI values indicate that the films could effectively inhibit *S. aureus* and *S. albus* infections for at

least two weeks and *P. aeruginosa* for three days. However, it is noted that the method of evaluation used here simulated a "pessimistic" conditions that usually do not occur *in vivo*, i.e. the efficacy of the released drug that remained in the films after its immersion in an aqueous medium for certain periods of time was evaluated. Thus, it is assumed that the antibacterial effect would be much higher when used in *in vivo* applications.

Films loaded with 3 % gentamicin exhibited slightly higher inhibition zones compared to films loaded with 1 % gentamicin, due to the higher drug quantity released. The inhibition zones obtained during two weeks of immersion correlates with the *in vitro* release profile that was measured. i.e., during the first several days, the non-thermally treated films exhibited larger inhibition zones than the thermally treated films but after one week an opposite trend was observed and the thermally treated films exhibited larger inhibition zones. During the first several days of immersion in an aqueous medium the thermally treated films released smaller quantity of drug compared to non-thermally treated films. Thus the larger drug amount that remained in the thermally treated films enabled larger bacterial inhibition.

The results demonstrate that SPI films, according to some embodiments of the present invention, loaded with gentamicin as an exemplary bioactive agent, are effective against pathogenic bacterial strains and can therefore be used as biodegradable drug-eluting wound dressings. Drug quantities higher than the MIC values should be released in order to eradicate all bacteria within a few days and prevent infection. In fact, a release profile such as demonstrated by SPI films, according to some embodiments of the present invention, with a medium burst effect followed by decreasing release rate is highly desirable. Higher concentrations of gentamicin can be incorporated into wound dressings based on the SPI films presented herein in order to treat *P. aeruginosa* infections.

EXAMPLE 6

Drug Release Profile

Materials and Methods:

SPI film samples were prepared as described hereinabove. The SPI films used for the release study were cast from 5 % w/v solutions (55 °C, pH 7.2), crosslinked using 1 % w/w glyoxal and thermally treated at 80 °C for 24 hours. Four such films

were studied, plasticized with 25 %, 30 %, 40 % or 50 % w/w glycerol. Each film was loaded with 3 % w/w ibuprofen.

Ibuprofen sodium salt was purchased from Sigma-Aldrich Rehovot, Israel (Cat. No. I1892). Ibuprofen is commonly used for treating mild to moderate pain related to dysmenorrhea, headache, migraine, postoperative dental pain, management of spondylitis, osteoarthritis, rheumatoid arthritis and soft tissue disorder.

Ibuprofen release study:

SPI film samples were immersed in phosphate buffered saline (PBS, pH 7.0) at 37 °C and the drug release kinetics was studied for 42 days in quadruplicates. Sodium azide (0.02 % w/v) was added to the medium in order to prevent bacterial growth.

The medium was completely removed periodically, at each sampling time point, 1 hour, 6 hours, 12 hour, and 1 day, 2, 3, 5, 7, 14, 21, 28, 35 and 42 days, and fresh medium was introduced. The removed medium was filtered using a disposable filter unit (Whatman, 0.2 µm) and kept in HPLC glass vials in -20 °C until HPLC analysis.

Residual drug recovery from the SPI films was measured by extracting the remaining drug from the films using enzymatic degradation of the film in trypsin A solution at 40 °C for 24 hours. Once trypsin cleaved the protein chains completely, ibuprofen concentration was estimated using HPLC as described below.

The ibuprofen content of the medium samples was determined using Jasco High Performance Liquid Chromatography (HPLC) with a UV 2075 plus detector and a reverse phase column (ACE 5 C18, inner diameter d=4.6 mm, length=250 mm), with a guard cartridge (ACE 5 C18, inner diameter d=3.0 mm. length=10 mm), under a constant temperature of 40 °C. The mobile phase consisted of a mixture of PBS (pH 3.3) and acetonitrile (40/60 v/v) at a flow rate of 2 ml/min, with a quaternary gradient pump (PU 2089 plus) without gradient. 20-40 µl samples were injected with an auto sampler (AS 2057 Plus). The area of each eluted peak was integrated using EZstart software version 3.1.7, according to a pre-determined calibration curve.

Effect of plasticizer content on ibuprofen release profile from SPI films:

Figure 15 presents a plot showing the cumulative amount of ibuprofen released during the first week from SPI films, prepared with various plasticizer contents and loaded with 3 % w/w ibuprofen, wherein rhombs represent films prepared with 25 % glycerol, rectangles represent 30 % glycerol, triangles represent 40 % and X represent

films prepared with 50 % glycerol, and the insert shows a magnification of the cumulative release profile in the first day.

As can be seen in Figure 15, the release profiles show no significant effect of the film's glycerol content on the ibuprofen release profile. The first stage is a medium burst release, followed by a decreasing release rate with time, and the majority of the ibuprofen is released during the first day.

EXAMPLE 7

Biocompatibility of the Soy Protein Structures

Materials and Methods:

SPI film samples were prepared as described hereinabove. The SPI films used for the release study were cast from 5 % w/v solutions (55 °C, pH 7.2), plasticized with 50 % w/w glycerol and crosslinked using 1 % w/w glyoxal and thermally treated at 80 °C for 24 hours. For some studies, some films were plasticized with 25 %, 30 %, 40 % or 50 % w/w glycerol. Fibroblast cell cultures (3rd passage) were thawed and cultured in 75 mm² flasks with culture medium (37 °C, humidified, 5 % CO₂). When confluence of 70 % was reached, the cells were detached using trypsin solution and seeded into 12-well plates with concentrations of 5x10⁴ per well for the 24-hour extractions test and positive control test.

Alamar-Blue solution was purchased from Enco Scientific Services. Using Alamar-Blue assay and spectrophotometer analysis for cell growth and viability estimation in the presence of SPI film extracts with varying plasticizer percentages. This method is an alternative to the indirect extraction test based on cell counting which was performed for cytotoxicity evaluation in Example 5 hereinabove. Alamar-Blue is a dark blue non-toxic fluorogenic redox indicator that turns red as a result of reduction in living cells. While the oxidized blue form has only little intrinsic fluorescence, the red form is highly fluorescent. The Alamar-Blue reduction extent, which indicates the cell viability, can be quantified spectrophotometrically using wave lengths of 570 and 600 nm.

The procedure of using Alamar-Blue assay included replacing the original medium with fresh medium containing 10 % (v/v) Alamar-Blue and incubation the wells for 4 hours. Following the incubation, triplicates from each well were transferred

into 96-well plate for spectrophotometer analysis (Spectra max 340 PC384, Molecular Devices). The percentage reduction of the Alamar-Blue was calculated according to the manufacturer's protocol. The Alamar-Blue reductions in the presence of the SPI extractions were compared to the Alamar-Blue reduction of control cell's environment
5 in order to evaluate the SPI extracts cytotoxicity.

Cellular response in indirect contact:

In order to evaluate the effect of extractions released from the SPI films on cell viability, an indirect extraction test based on cell counting was performed. Fibroblast monolayers were cultured in the presence of SPI film extraction for up to 3 days. Each
10 day, the cultures were sacrificed in triplicates and cells were counted.

For the 24-to-48 hours extractions test, cells were seeded in concentration of 4×10^4 per well. One ml of culture medium was added and the plates were put in the incubator overnight to reach 80 % confluence.

Extractions from exemplary SPI films was conducted using pre-sterilized
15 (ethylene oxide) round SPI film samples (22 mm diameter) which were placed in 6-well plates with culture medium at a ratio of 1 cm²/ml. The plates were put in an incubator (37 °C, humidified, 5 % CO₂) for 24 hours to allow extraction.

Extracts medium from the first 24 hours was filtered (45µ disposable filters, Whatman, Germany) and culture medium in each well was replaced with 1 ml extracts
20 medium. The plates were then placed back in the incubator.

The described above process was repeated, and 24-to-48 hours extracts were placed in contact with cells monolayers without filtration (1 ml/well). The same process was implemented for culture medium that was used for control.

After 24, 48 and 72 hours of incubation, cell cultures were detached using
25 trypsin solution and cells were counted using Trypan blue exclusion. Cell suspensions (10 µL) were mixed with the same volume of Trypan blue solution. Cell numbers were determined in triplicates using hemocytometer.

Cell viability was determined after 72 hours of cell culture in the presence of films extracts, wherein staining was performed using Giemsa assay. Culture medium
30 was decanted and cells were fixated using methanol for 10 minutes following Giemsa staining for 5 minutes. The cultures were then photographed (Nikon coolpix 4500) and cytotoxic effect of the extracts was determined by a qualitative comparison to control.

For positive control, film components (except from gentamicin) were dissolved in culture medium in high concentrations compared to the approximated released extractions. Culture mediums with added glycerol, SPI and glyoxal were prepared in concentrations of 6.25 % (v/v), 2.5 % (w/v) and 0.25 % (v/v), respectively. The prepared components mediums were then filtered (45 μ disposable filters, Whatman, Germany) and the culture medium in the pre-seeded culture plates was replaced with 1 ml of components medium per well. The results were compared to this control.

Figures 16A-B presents comparative bar-plots showing the percentage of counted cells compared to the number of seeded cells in the presence of the 24-hours (Figure 16A) and the 24-to-48 hours (Figure 16B) SPI film extractions tests at different cultivation times, wherein dotted bars represent results obtained with untreated SPI film samples, diagonally stripped bars represent results from films crosslinked with 1 % glyoxal and thermally treated at 80 °C for 24 hours, solid red bars represent results from films crosslinked using 1 % glyoxal containing 3 % gentamicin, and solid blue bars represent results obtained from control experiments.

As can be seen in Figures 16A, after one and two days of cultivation, cell growth remained constant around 130 % with no statistical differences between the groups. After three days, cells cultured in the control as well as in the presence of the thermally crosslinked film extracts have grown up to around 300 % compared to the number of seeded cells. On the contrary, cells cultured in the presence of untreated SPI film extracts and extracts from thermally crosslinked films containing 3 % gentamicin remained around 150 %.

As can be seen in Figure 16B, after one day of cultivation, cells grown in the presence of extracts of untreated SPI films and thermally crosslinked SPI films exhibited high counts and reached around 170 % compared to low counts of about 100 % for extracts of the SPI films containing 3 % gentamicin and control. After 2 and 3 days of cultivation, all cell cultures reached around 200 % of growth with no statistical differences. The control has a higher growth of around 250 % after three days of cultivation.

Generally, cells cultured in the presence of 24-to-48 hours extracts, especially from untreated SPI films, showed higher proliferation after one and two days compared to cells cultured in the presence of 24-hours extracts.

Figures 17A-H present photographs of fibroblast cultures grown for 72 hours in the presence of 24 hours extracts of exemplary SPI film samples (Figures 17 A-D), and 24-to-48 hours extracts (Figures 17 E-H), wherein growth in the presence of extract of untreated SPI film samples are shown in Figures 17A and 16E, films crosslinked using 1 % glyoxal and containing 3 % gentamicin in Figures 17B and 16F, films crosslinked with 1 % glyoxal and thermally treated at 80 °C for 24 hours in Figures 17C and 16G, and controls in Figures 17D and 16H.

As can be seen in Figures 17A-H, cells grown for 72 hours in the presence of film extracts (24-hours and 24-to-48 hours) showed no visible differences in viability compared to control.

Figures 18A-B present the results obtained for the positive control experiment of fibroblast cultures growth for 72 hours, wherein Figure 18A shows a photograph of the cell culture, and Figure 18B is a bar-plot showing percentage of counted cells compared to the number of seeded cells in the presence of film components after 24 hours of cultivation, compared to the cytotoxic effects of glycerol and SPI on cell culture.

As can be seen in Figures 18A-B, cells cultured in the presence of dissolved SPI solution showed no difference compared to control in cell proliferation as 130 % more cells were counted compared to those seeded. As can be seen in Figure 18B, glycerol solution showed a cytotoxic effect and drastically reduced the number of cells seeded by 33 %. Cells seeded in the presence of glyoxal solution could not be counted due to cell fixation.

Cellular response in biocompatibility test:

The effect of the SPI films on living cells was evaluated by biocompatibility test as follows. In this test, evaluation of cell growth and viability in close proximity to the SPI films was performed. Fibroblast monolayers were cultured in the presence of SPI films for 5 days and were photographed for qualitative estimation of cells viability.

Reference SPI film samples (22 mm diameter) loaded with gentamicin were cut into halves and sterilized using 70 % ethanol solution for 90 minutes. The ethanol was decanted and films were washed with sterile PBS and left to dry in laminar flow. Thereafter, the films were suspended in 6-well plates with culture medium for 24 hours prior to cell seeding (37 °C humidified incubator, 5 % CO₂). Finally, medium was

replaced and round stainless steel weights were placed on top in order to prevent film movement.

Fibroblast cells (12th passage) were cultured in 75 mm² flasks with culture medium (37 °C, humidified, 5 % CO₂). When confluence of 70 % was reached, the cells were detached using trypsin solution and seeded into the 6-well plates with the SPI films that were prepared in advance with concentrations of 1.5×10^5 per well, 2.5 ml of culture medium was added, and the plates were put in an incubator.

After 5 days, the confluent cultures were observed under inverted-light microscope to determine cell viability. Cell cultures viability was assessed as described in the previous section. Cultures without the presence of SPI films served as controls.

Figures 19A-C present photographs of fibroblast cell cultures grown for 5 days in the presence SPI film samples, wherein cells grown in direct contact with films crosslinked using 1 % glyoxal and containing 3 % gentamicin are shown in Figure 19A, films crosslinked with 1 % glyoxal and thermally treated at 80 °C for 24 hours in Figure 19B, and control experiment in Figure 19C.

As can be seen in Figures 19A-C, cells detected near the films exhibited normal fibroblastic morphological features compared to the control. The neonatal fibroblasts reached full confluence after 5 days and revealed a normal fibroblastic growth pattern, consisting of elongated cells running as groups in different directions. Cells were observed in close proximity to the outer borders of the films. No differences in cell shape or number were observed between the cells grown close to the chemically crosslinked SPI film sample or the control. Cell culture near the thermally and chemically crosslinked SPI film samples was sparse compared to the control.

As can be recognized from the results presented herein, films extracts released during the first 24 hours from all studied film samples showed no significant effect on cell growth after one and two days of cultivation compared to the control. After three days of cultivation, cells grown in the presence of extracts released from non-crosslinked films and films crosslinked with glyoxal only (or loaded with gentamicin) showed a slight decrease in proliferation compared to the control.

Film extracts released during the 24-to-48 hours from all film samples showed no significant effect on cell growth after one, two and three days of cultivation

compared to the control. In addition, cell viability after 3 days of culture in the presence of all film extracts seemed unimpaired compared to the control.

Without being bound by any particular theory, it is assumed that the slight cytotoxic effect exhibited in the cell counting test can be explained by the presence of glycerol in the medium as shown by positive control experiments. During the first 24 hours of extraction most of the glycerol is leached out of the film due to its high hydrophilic nature. In films with lower crosslinking density more glycerol is leaching, affecting cell proliferation.

As shown previously, cells exposed to gentamicin concentrations as high as 1500 µg/mL remained confluent and with a gross appearance similar to the control, thus eliminating the possibility for its cytotoxicity in this test, where much lower concentrations are involved. Soy protein isolates were also found to be non toxic in high concentrations compared to the control.

Hence, film extractions were found to be generally non toxic and slightly impaired cell growth only in cases where large quantities of glycerol were released to the medium. The cells were viable and proliferated at a similar rate to the control. The results presented herein clearly demonstrate that SPI films, according to some embodiments of the present invention, are biocompatible with human fibroblasts. Morphology of cells grown next to the SPI films, i.e. elongation and dense nucleus, indicates high viability. No significant differences in cell population were observed between the control and the cells grown near the SPI films.

Effect of plasticizer content on cellular response to SPI films extracts:

The 24 hours and 24-to-48 hours films extractions and the indirect contact experiments were performed as detailed hereinabove while using the Alamar-Blue assay for cells viability estimation.

The samples chosen for this test were prepared in 55 °C from 5 % w/v SPI solutions with glycerol as plasticizer and 1 % w/w glyoxal as a crosslinking agent, and cured using thermal treatment for 24 hours at 80 °C. The samples varied in the plasticizer contents, namely glycerol at 25 %, 30 %, 40 % and 50 % w/w in content.

The cells viability was examined by measuring their Alamar-Blue reduction relative to the Alamar-Blue reduction of control cells (cells grown in medium without SPI film extracts).

Figures 20A-B present comparative bar-plots showing the effect exhibited by the 24 hours (Figure 20A) and the 24-to-48 hours (Figure 20B) SPI films extractions, wherein the white bars represent Alamar-Blue reduction after 1 day cell cultivation, grey bars after 2 days and black bars represent readings after 3 days of cell cultivation.

5 As can be seen in Figures 20A-B, all 24 hours SPI film extracts showed no toxic effect compared to the control. The cells viability in comparison to the control showed improvement during the cultivation periods. For 24-to-48 hours tests, all film extracts showed no toxic effect compared to the control. The 50 % glycerol samples showed a slightly toxic effect. The films with 25 % and 40 % glycerol content showed higher
10 cells viability in comparison to control with the increase in cultivation periods.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all
15 such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and
20 individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. A composition-of-matter comprising crosslinked soy protein isolate and a plasticizer, wherein an amount of said plasticizer ranges from 25 to 100 weight percents of the weight of said soy protein isolate.
2. The composition-of-matter of claim 1, wherein said plasticizer is selected from the group consisting of glycerol and sorbitol.
3. The composition-of-matter of claim 2, wherein an amount of said plasticizer ranges from 25 to 80 weight percents of a weight of said soy protein isolate.
4. The composition-of-matter of claim 1, wherein said plasticizer comprises glycerol.
5. The composition-of-matter of claim 4, wherein an amount of said glycerol ranges from 35 to 50 weight percents of a weight of said soy protein isolate.
6. The composition-of-matter of any of claims 1-4, wherein said soy protein isolate is chemically crosslinked.
7. The composition-of-matter of claim 6, wherein said chemically crosslinked soy protein isolate is obtained by contacting said soy protein isolate with a crosslinking agent.
8. The composition-of-matter of claim 7, wherein said crosslinking agent comprises glyoxal.
9. The composition-of-matter of claim 8, wherein an amount of said glyoxal is 1 weight percent of a weight of said soy protein isolate.
10. The composition-of-matter of any of claims 1-9, being thermally cured.

11. The composition-of-matter of claim 10, being thermally cured by heat treatment of said crosslinked soy protein isolate.

12. The composition-of-matter of any of claims 1-9, wherein said soy protein isolate is crosslinked by thermal curing.

13. The composition-of-matter of claim 12, wherein said thermal curing comprises heat treatment of said soy protein isolate and said plasticizer.

14. The composition-of-matter of any of claims 10-13, wherein said heat treatment which is effected at a temperature that ranges from 40 to 100 °C.

15. The composition-of-matter of claim 14, wherein said heat treatment is effected at a temperature that ranges from 60 to 80 °C.

16. The composition-of-matter of any of claims 10-15, wherein said heat treatment is effected for a time period that ranges from 12 to 48 hours.

17. The composition-of-matter of any of claims 1-16, being characterized by at least one of:

- a tensile strength that ranges from 5 to 30 MPa;
- a Young Modulus that ranges from 50-600 MPa;
- a maximal strain that ranges from 50 to 300 percents; and
- a water vapor transmission rate (WVTR) that ranges from 1000 to 4000 grams/m²/day.

18. The composition-of-matter of claim 17, wherein said water vapor transmission rate (WVTR) ranges from 2000 to 3000 grams/m²/day.

19. A composition-of-matter comprising a crosslinked soy protein isolate, the composition-of-matter being characterized by at least one of:

- a tensile strength that ranges from 5 to 30 MPa;

a Young Modulus that ranges from 50 to 600 MPa;
a maximal strain that ranges from 50 to 300 percents; and
a water vapor transmission rate (WVTR) that ranges from 1000 to 4000 grams/m²/day.

20. The composition-of-matter of claim 19, wherein said water vapor transmission rate (WVTR) that ranges from 2000 to 3000 grams/m²/day.

21. The composition-of-matter of any of claims 19 and 20, further comprising a plasticizer.

22. The composition-of-matter of claim 21, wherein an amount of said plasticizer ranges from 25 to 100 weight percents of a weight of said soy protein isolate.

23. The composition-of-matter of claim 22, wherein said plasticizer comprises glycerol.

24. The composition-of-matter of claim 23, wherein an amount of said glycerol ranges from 25 to 80 weight percents of a weight of said soy protein isolate.

25. The composition-of-matter of claim 24, wherein an amount of said glycerol ranges from 35 to 50 weight percents of a weight of said soy protein isolate.

26. The composition-of-matter of any of claims 19-25, wherein said soy protein isolate is chemically crosslinked.

27. The composition-of-matter of claim 26, wherein said chemically crosslinked soy protein isolate is obtained by contacting said soy protein isolate with a crosslinking agent.

28. The composition-of-matter of claim 27, wherein said crosslinking agent is glyoxal.

29. The composition-of-matter of claim 28, wherein an amount of said glyoxal is 1 weight percent of a weight of said soy protein isolate.

30. The composition-of-matter of any of claims 19-29, being thermally cured.

31. The composition-of-matter of claim 30, being thermally cured by heat treatment at a temperature that ranges from 40 to 100 °C.

32. The composition-of-matter of any of claims 19-25, wherein said soy protein isolate is crosslinked by thermal curing.

33. The composition-of-matter of claim 30, wherein said thermal curing is obtained by heat treatment at a temperature that ranges from 40 to 100 °C.

34. The composition-of-matter of any of claims 31 to 33, wherein said heat treatment is effected for a time period that ranges from 12 to 48 hours.

35. A composition-of-matter comprising crosslinked soy protein isolate and a plasticizer, wherein an amount and type of said crosslinking and said plasticizer are selected such that the composition is characterized by at least one of:

- a tensile strength that ranges from 5 to 30 MPa;
- a Young Modulus that ranges from 50 to 600 MPa;
- a maximal strain that ranges from 50 to 300 percents; and
- a water vapor transmission rate (WVTR) that ranges from 1000 to 4000 grams/m²/day.

36. The composition-of-matter of claim 35, wherein said water vapor transmission rate (WVTR) that ranges from 2000 to 3000 grams/m²/day.

37. The composition-of-matter of any of claims 35 and 36, wherein said crosslinking comprises chemical crosslinking by a crosslinking agent.

38. The composition-of-matter of any of claims 35-37, wherein said crosslinking comprises thermal curing.

39. The composition-of-matter of any of claims 1-36, further comprising a bioactive agent incorporated therein.

40. The composition-of-matter of claim 39, capable of releasing said bioactive agent upon contacting the composition with a physiological medium over a time period of at least 2 hours.

41. The composition-of-matter of claim 40, wherein from 30 to 70 percents of said bioactive active agent are released during 6 hours of said contacting and the remaining of said bioactive agent is released over at least 30 days of said contacting.

42. The composition-of-matter of claim 41, wherein said bioactive agent is an antimicrobial agent.

43. The composition-of-matter of claim 42, identified for use in treating an infection associated with a pathogenic microorganism.

44. The composition-of-matter of claim 40, wherein at least 90 percents of said bioactive agent are released during two days from said contacting.

45. The composition-of-matter of claim 44, wherein said bioactive agent is an analgesic agent.

46. The composition-of-matter of claim 45, identified for use in the treatment of local pain.

47. A composition-of-matter comprising crosslinked soy protein isolate and a plasticizer, and further comprising a bioactive agent incorporated therein, wherein an amount and type of said crosslinking and said plasticizer are selected such that the

composition is characterized as capable of releasing said bioactive agent upon contacting the composition-of-matter with a physiological medium over a time period of at least 2 hours.

48. The composition-of-matter of claim 47, wherein said crosslinking comprises chemical crosslinking by a crosslinking agent.

49. The composition-of-matter of any of claims 47 and 48, wherein said crosslinking comprises thermal curing.

50. The composition-of-matter of claim 49, wherein said thermal curing is effected at a temperature that ranges from 40 to 100 °C.

51. The composition-of-matter of claim 50, wherein said thermal curing is effected at a temperature that ranges from 60 to 80 °C.

52. The composition-of-matter of any of claims 50 and 51, wherein said thermal curing is effected for a time period that ranges from 12 to 48 hours.

53. The composition-of-matter of any of claims 47-52, wherein from 30 to 70 percents of said bioactive active agent are released during 6 hours from said contacting and the remaining of said bioactive agent is released over at least 30 days of said contacting.

54. The composition-of-matter of claim 53, wherein said bioactive agent is an antimicrobial agent.

55. The composition-of-matter of claim 54, wherein at least 90 percents of said bioactive agent are released during two days from said contacting.

56. The composition-of-matter of claim 55, wherein said bioactive agent is an analgesic agent.

57. The composition-of-matter of any of claims 47-56, being characterized by at least one of:

- a tensile strength that ranges from 5 to 30 MPa;
- a Young Modulus that ranges from 50 to 600 MPa;
- a maximal strain that ranges from 50 to 300 percents; and
- water vapor transmission rate (WVTR) that ranges from 1000 to 4000 grams/m²/day.

58. The composition-of-matter of claim 57, wherein said water vapor transmission rate (WVTR) that ranges from 2000 to 3000 grams/m²/day.

59. The composition-of-matter of any of claims 1-58, being a solution cast composition.

60. The composition-of-matter of claim 59, obtained by casting an aqueous solution comprising said soy protein isolate, said plasticizer and a crosslinking agent, if present.

61. The composition-of-matter of claim 60, wherein a concentration of said soy protein isolate in said aqueous solution ranges from 3 to 7 percents weight per volume.

62. The composition-of-matter of any of claims 1-61, for use as wound dressing.

63. The composition-of-matter of claim 62, for use in the treatment of a wound.

64. A method of treating a wound, comprising contacting the wound with the composition-of-matter of any of claims 1-63.

65. Use of the composition-of-matter of any of claims 1-63, in the manufacture of a product for treating a wound.

66. A process of preparing the composition-of-matter of any of claims 1-63, the process comprising:

providing an aqueous solution containing soy protein isolate at a concentration that ranges from 3 to 7 percents weight per volume;

a plasticizer in an amount that ranges from 25 to 100 weight percents of a weight of said soy protein isolate;

an crosslinking agent, if present, in an amount that ranges from 0 to 2 weight percents of a weight of said soy protein isolate ; and

a bioactive agent, if present, in an amount that ranges from 0.1 to 10 weight percents of a weight of said soy protein isolate,

heating said solution to a temperature that ranges from 25 °C to 70 °C;

casting said solution onto a mold or a surface; and

drying said solution at room temperature and ambient humidity for a time period that ranges from to thereby obtain the composition-of-matter.

67. The process of claim 66, further comprising, following said drying, heating the composition-of-matter to a temperature that ranges from 40 °C to 100 °C.

68. The process of claim 67, wherein said temperature ranges from 60 to 80 °C.

69. The process of any of claims 67 and 68, wherein said heating is effected for a time period that ranges from 12 to 48 hours.

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FIG. 1A

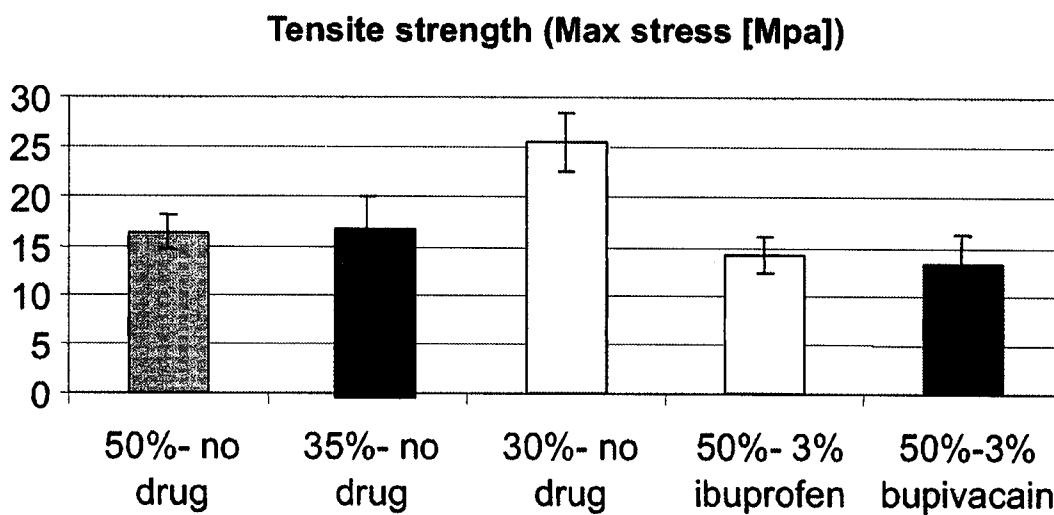
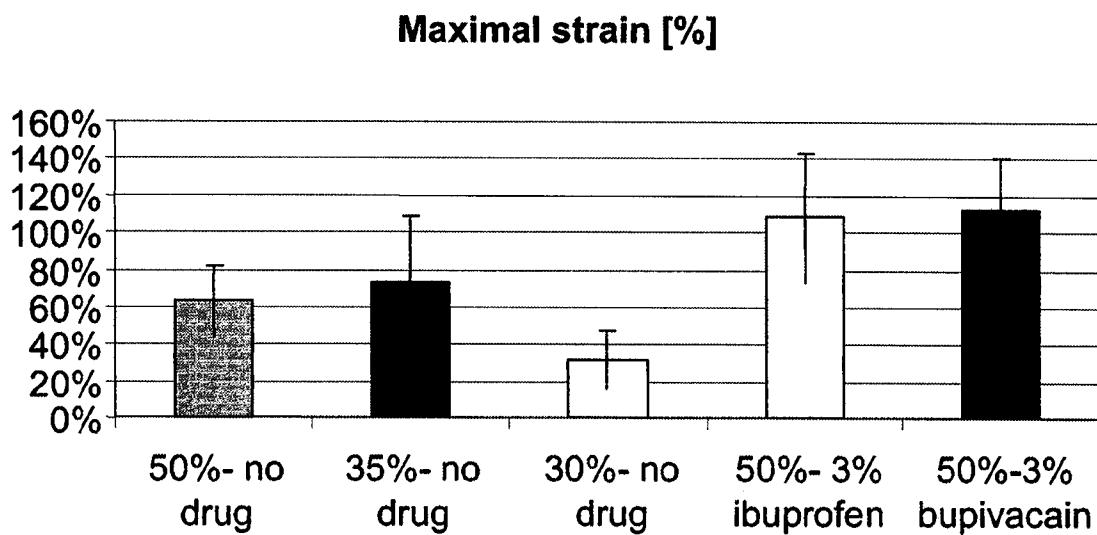


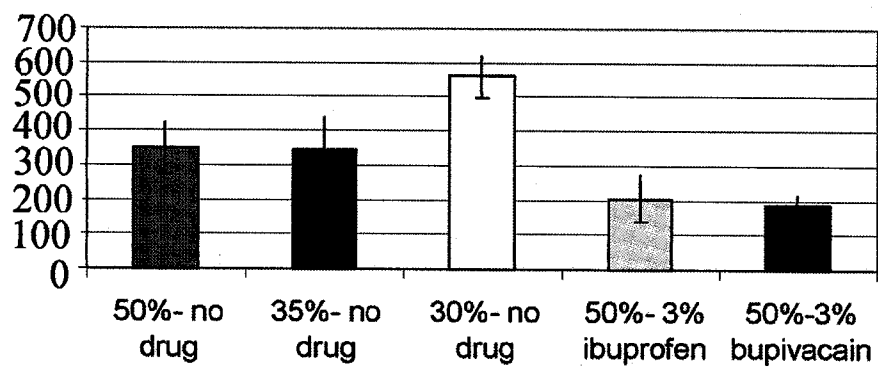
FIG. 1B



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FIG. 1C

Young's Modulus [Mpa]



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FIG. 2A

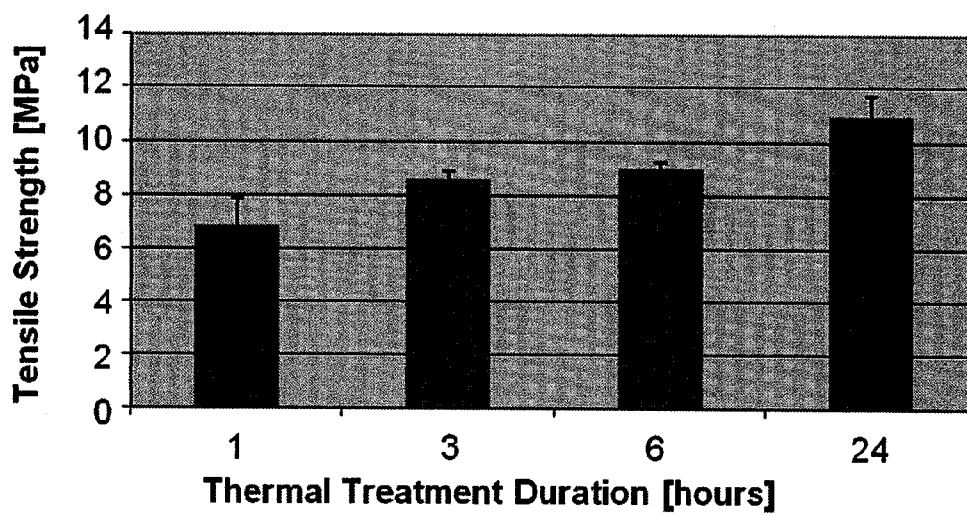
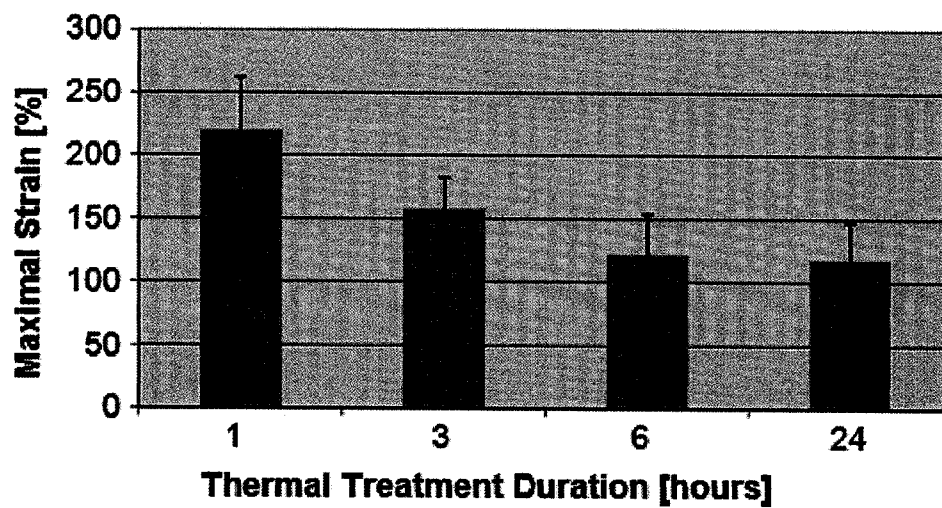
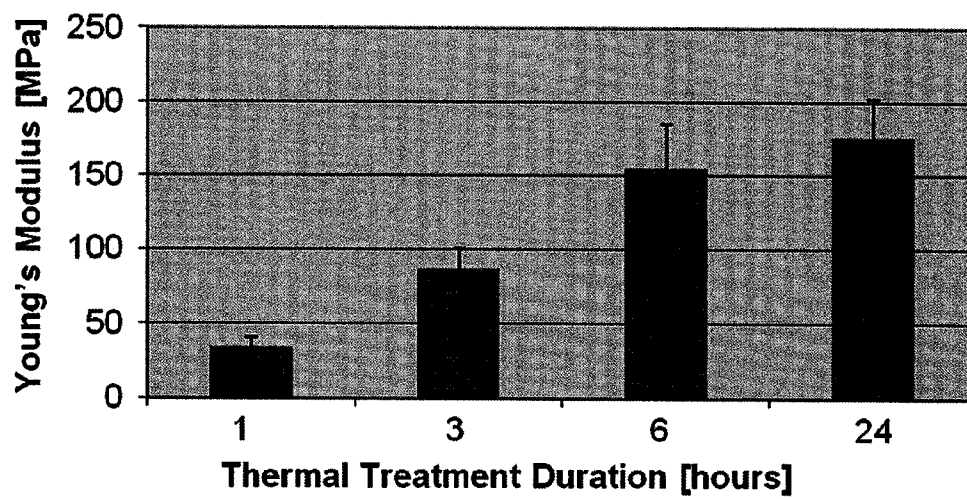


FIG. 2B



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FIG. 2C



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FIG. 3A

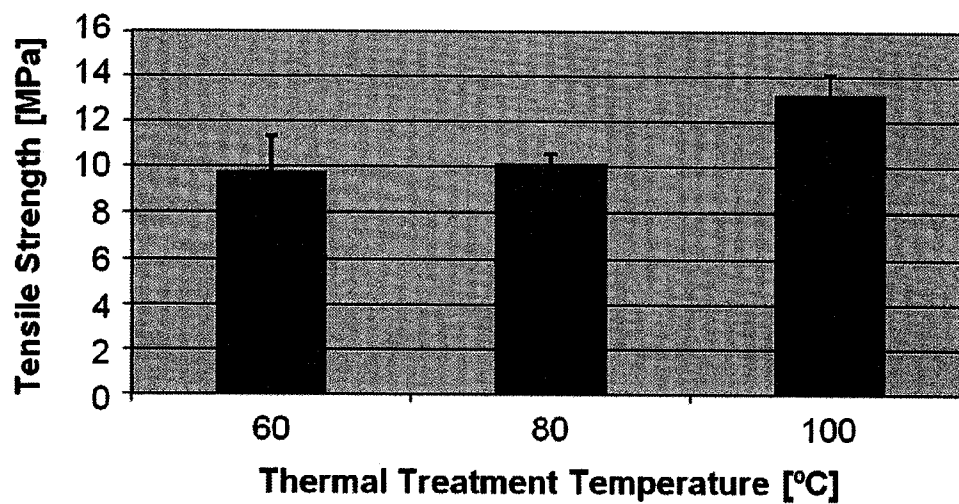
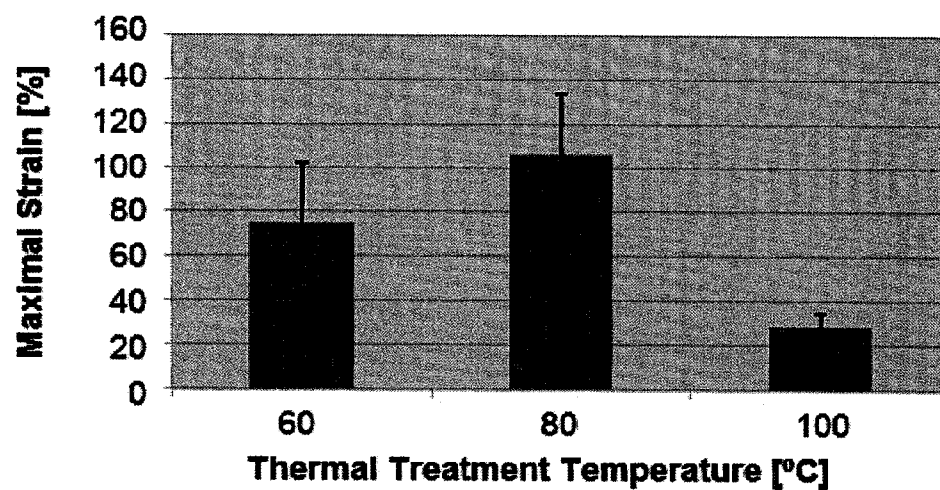
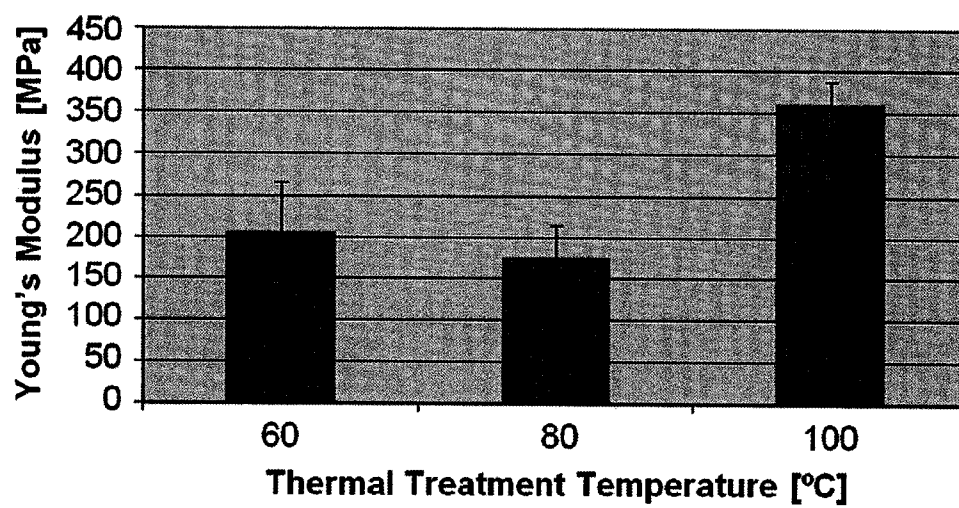


FIG. 3B



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FIG. 3C



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FIG. 4A

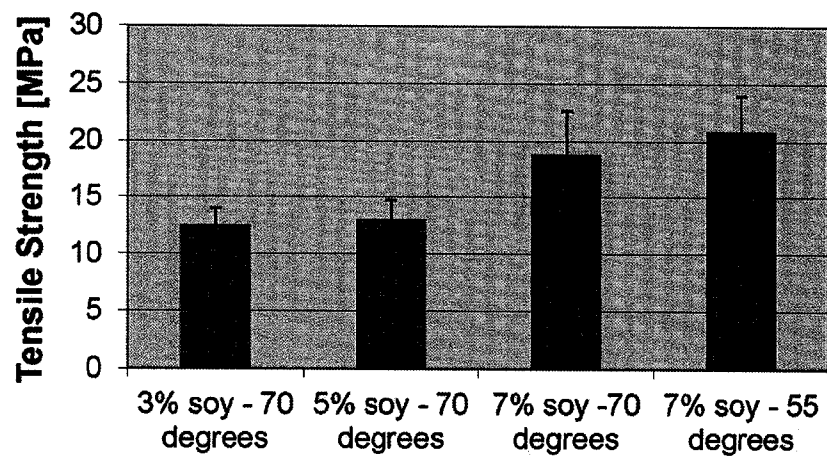
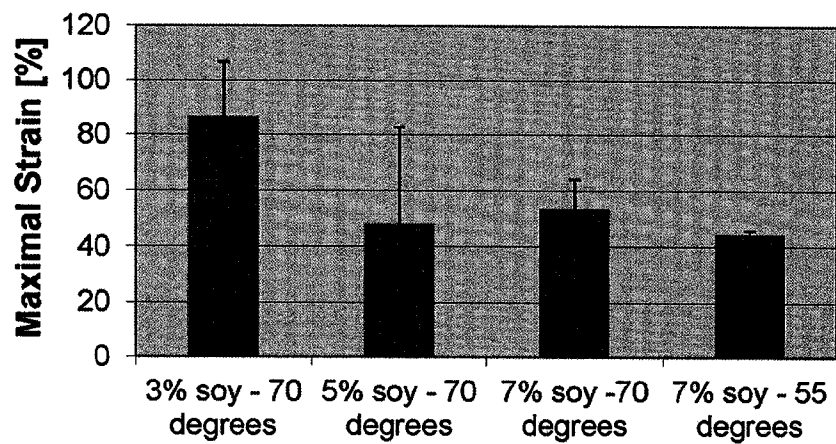
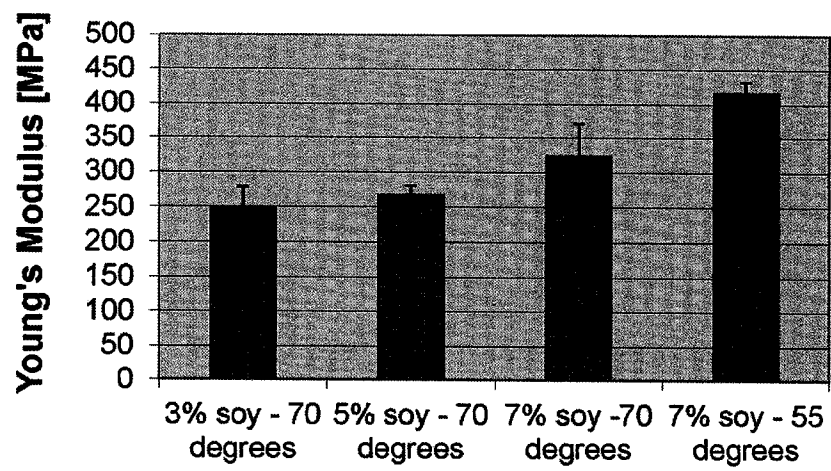


FIG. 4B



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FIG. 4C



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FIG. 5A

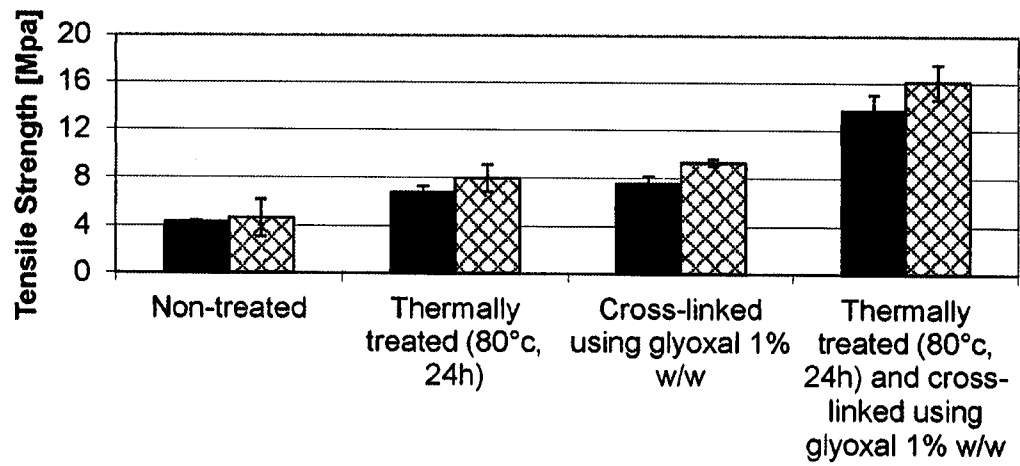
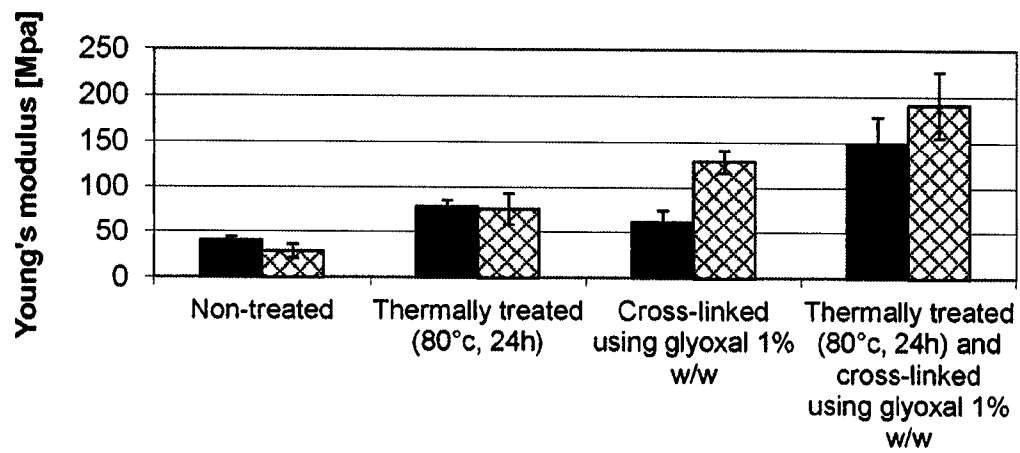
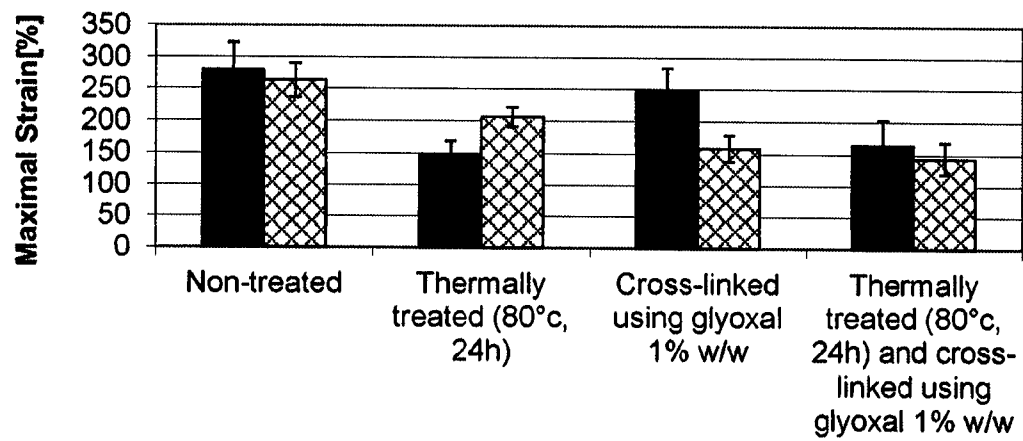


FIG. 5B



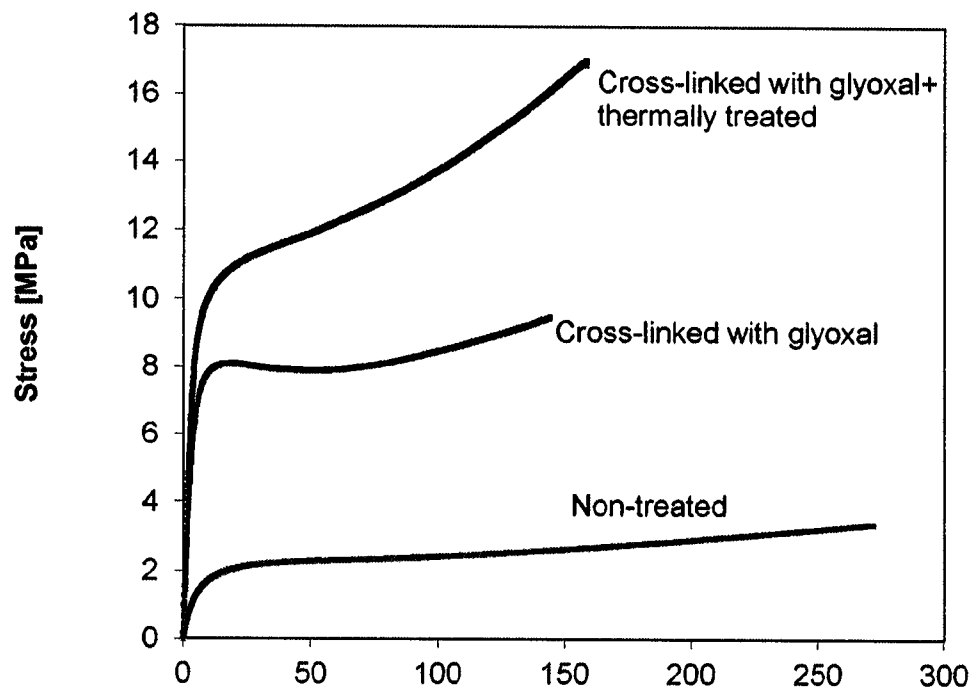
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FIG. 5C



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FIG. 6



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FIG. 7A

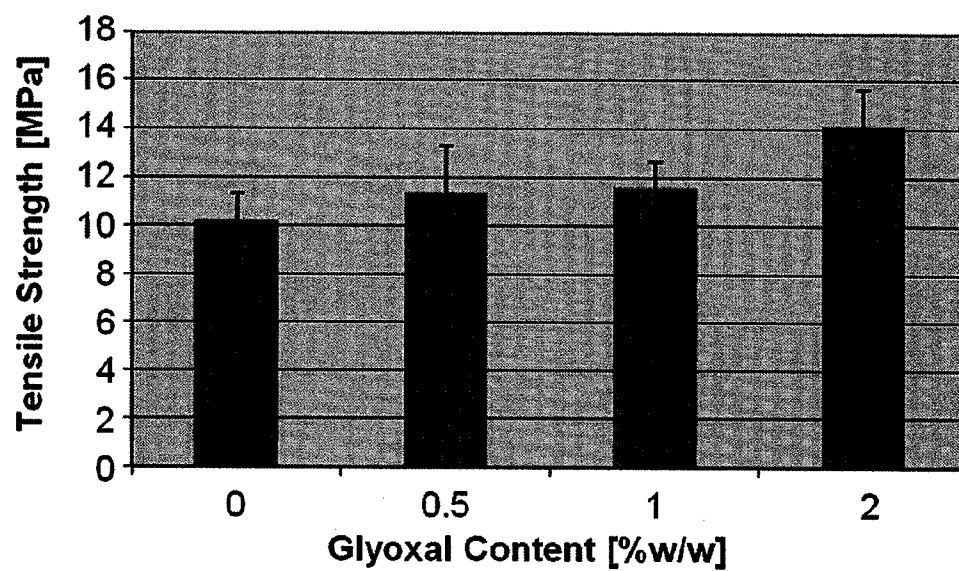
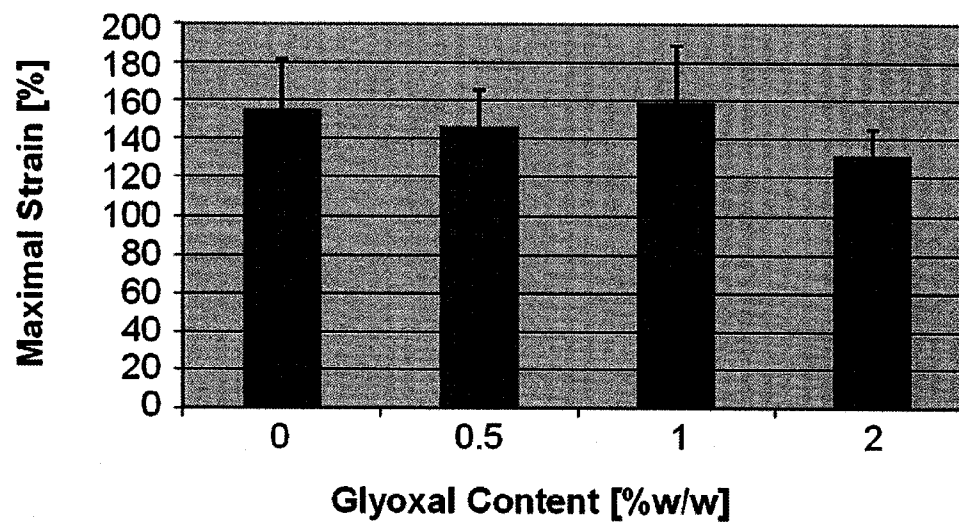
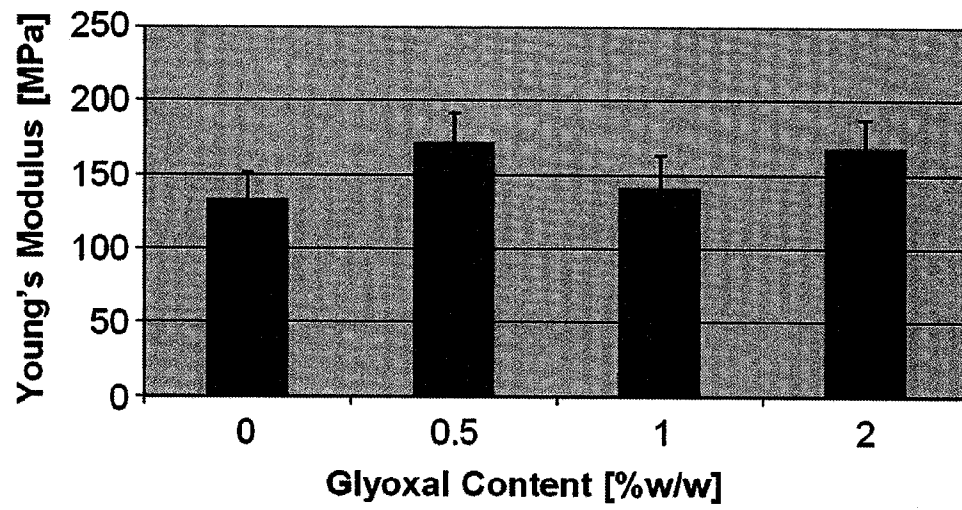


FIG. 7B



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FIG. 7C



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FIG. 8A

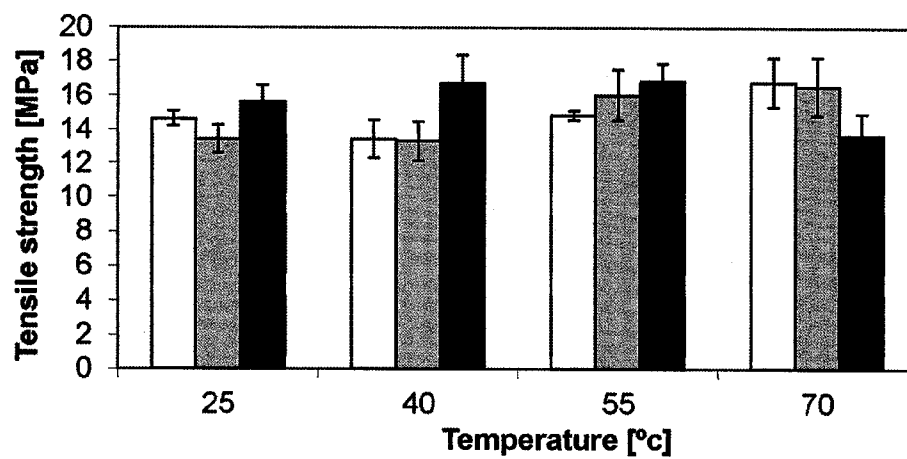
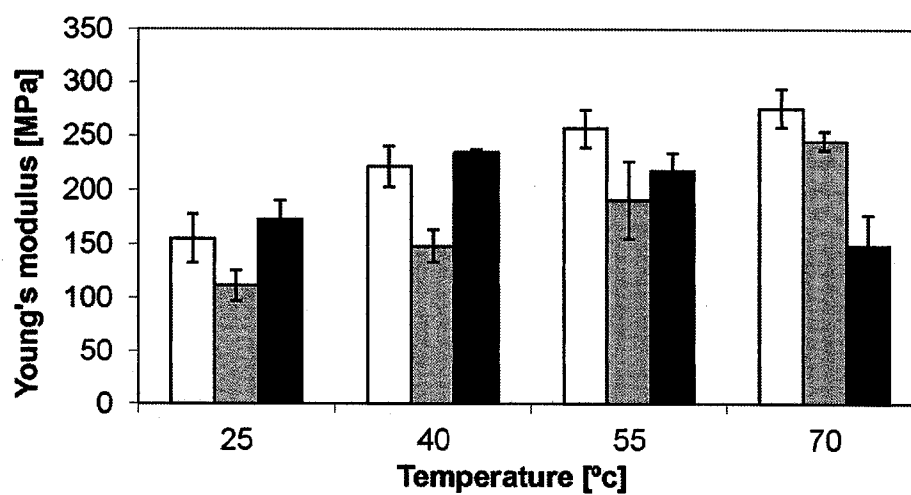
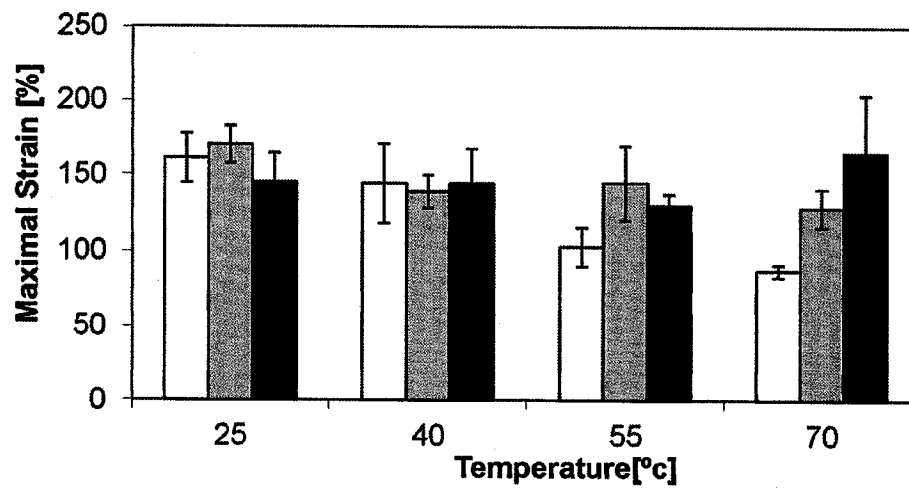


FIG. 8B



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FIG. 8C



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FIG. 9

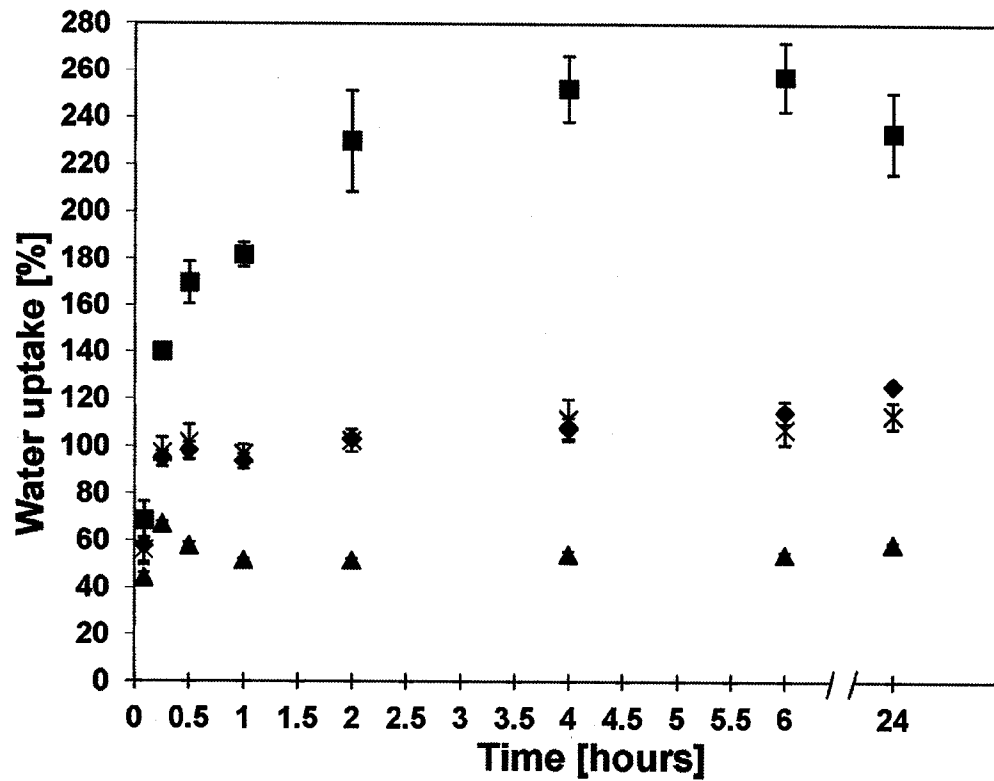
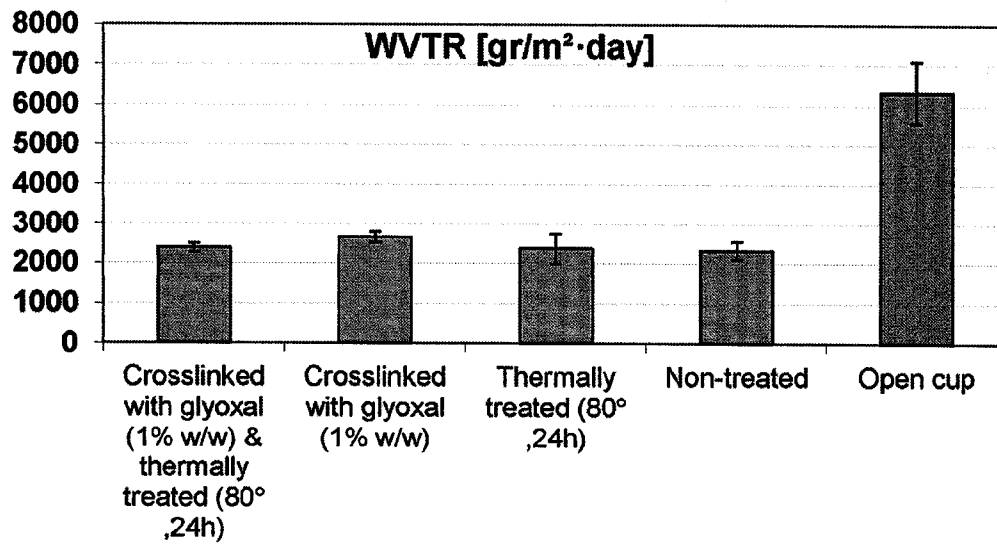
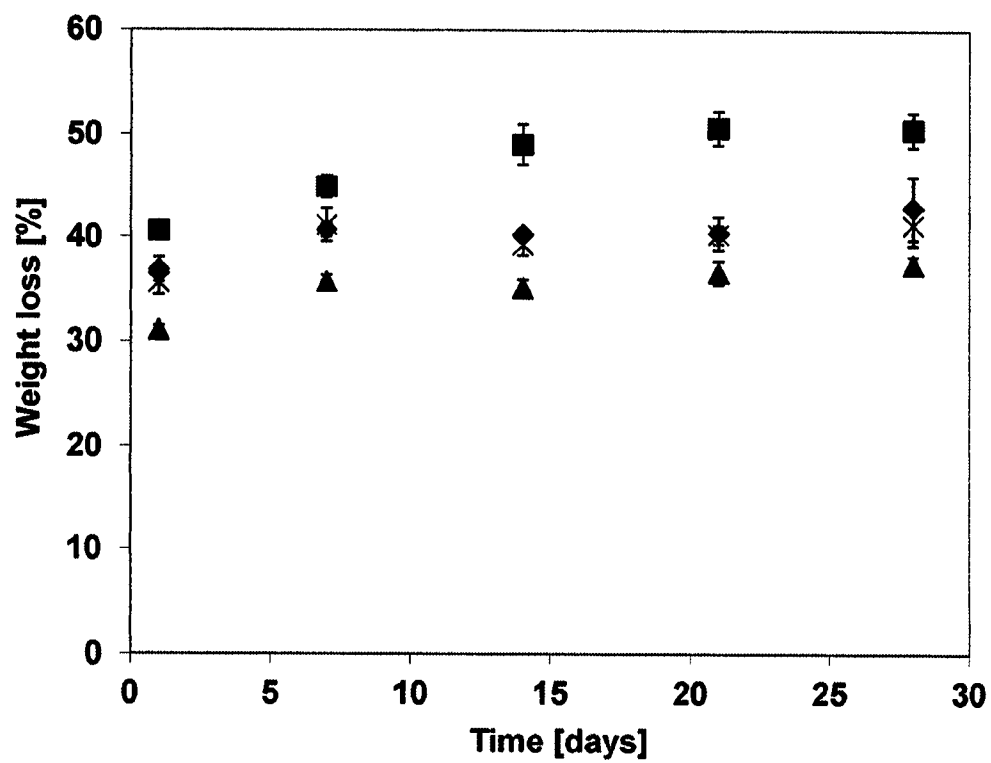


FIG. 10



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FIG. 11



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FIG. 12A

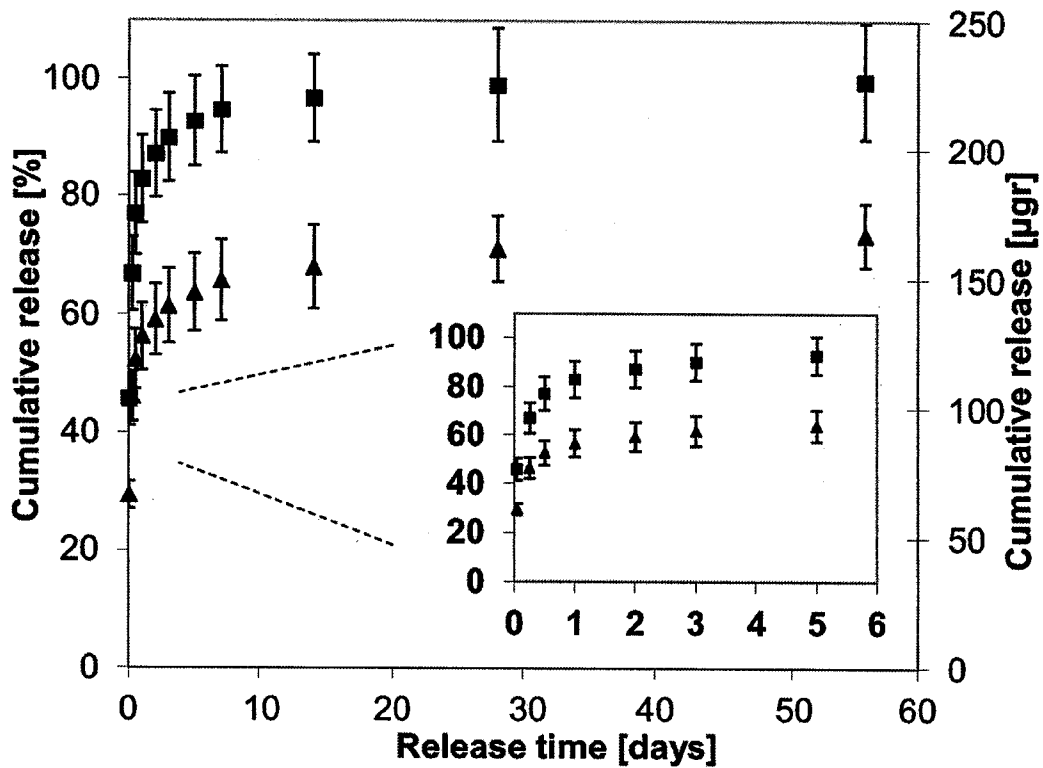
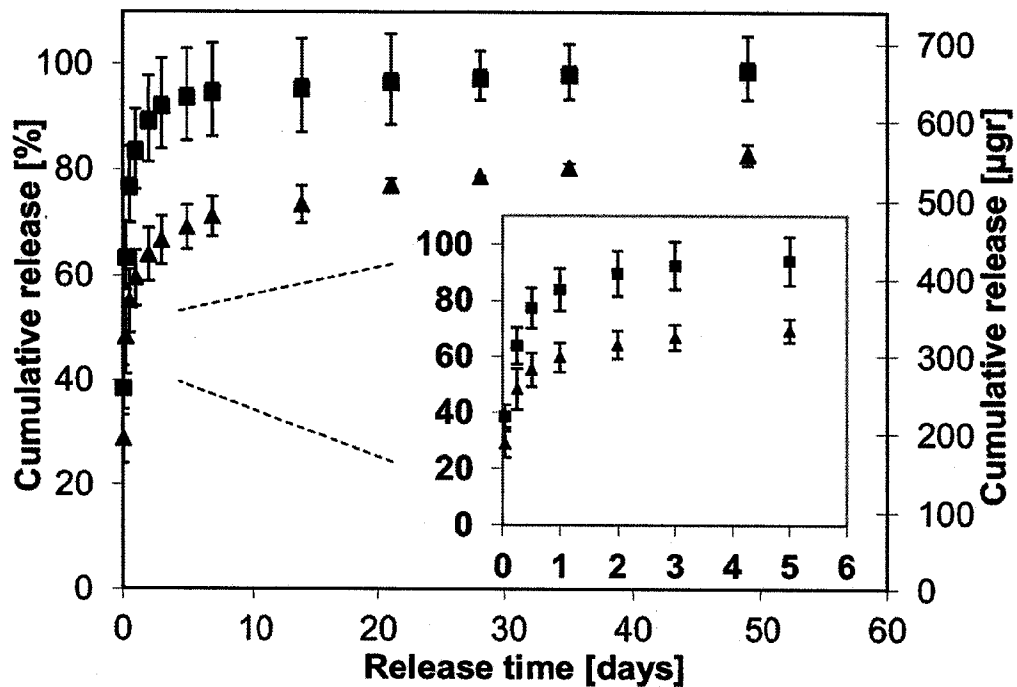


FIG. 12B



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FIG. 13A

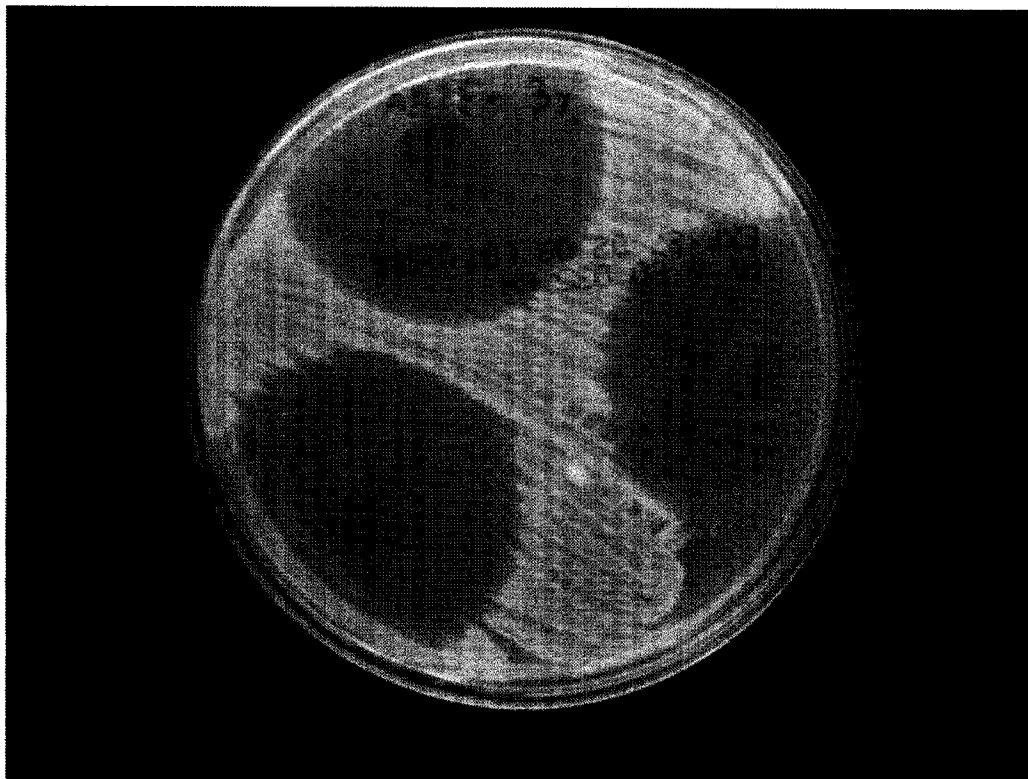
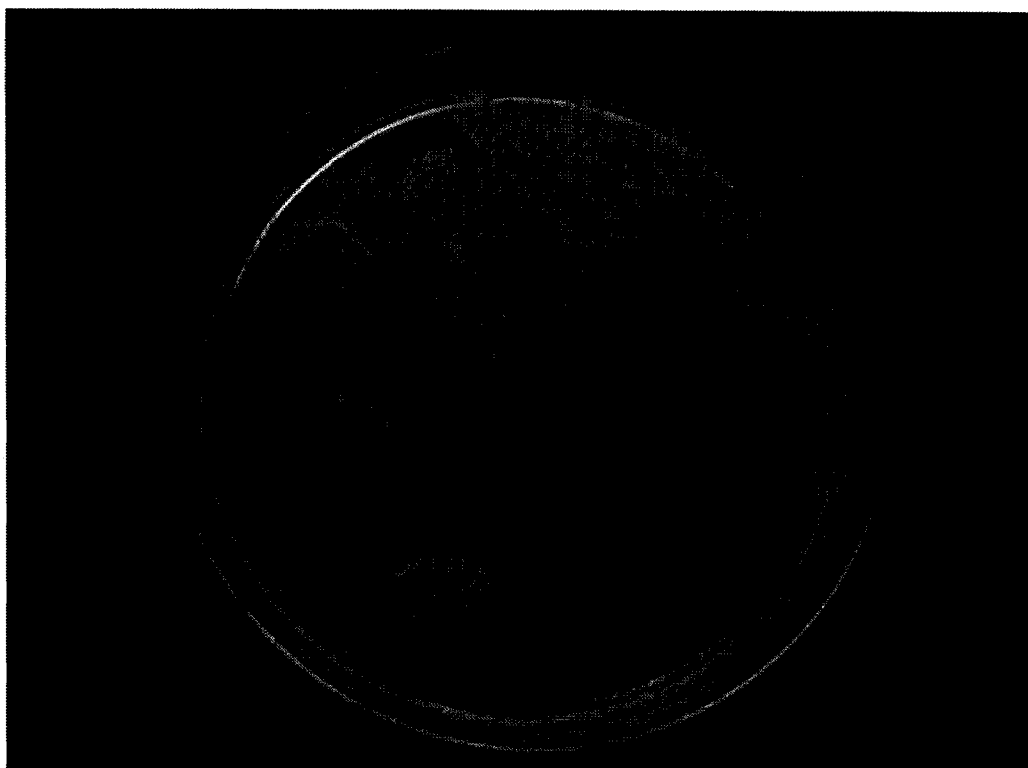


FIG. 13B



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FIG. 14A

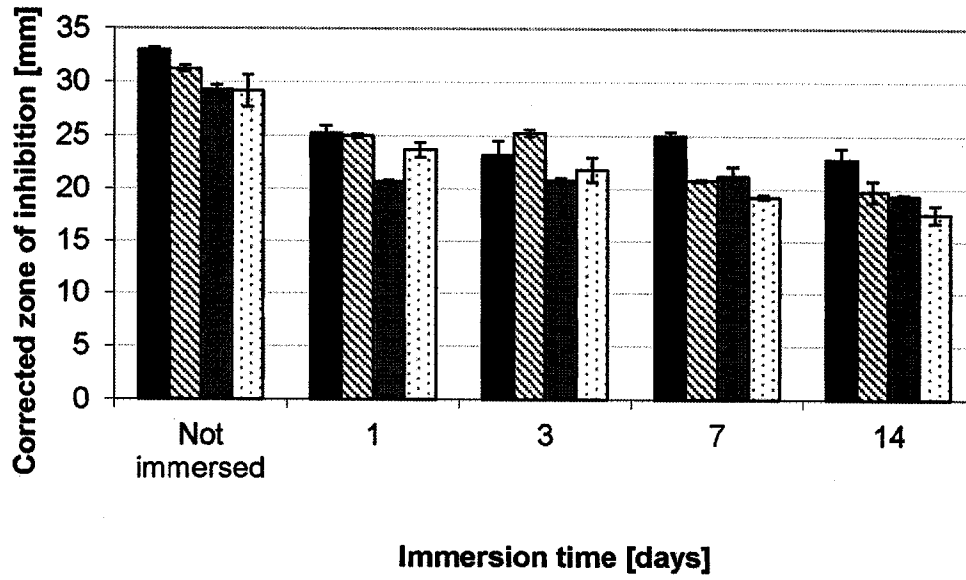
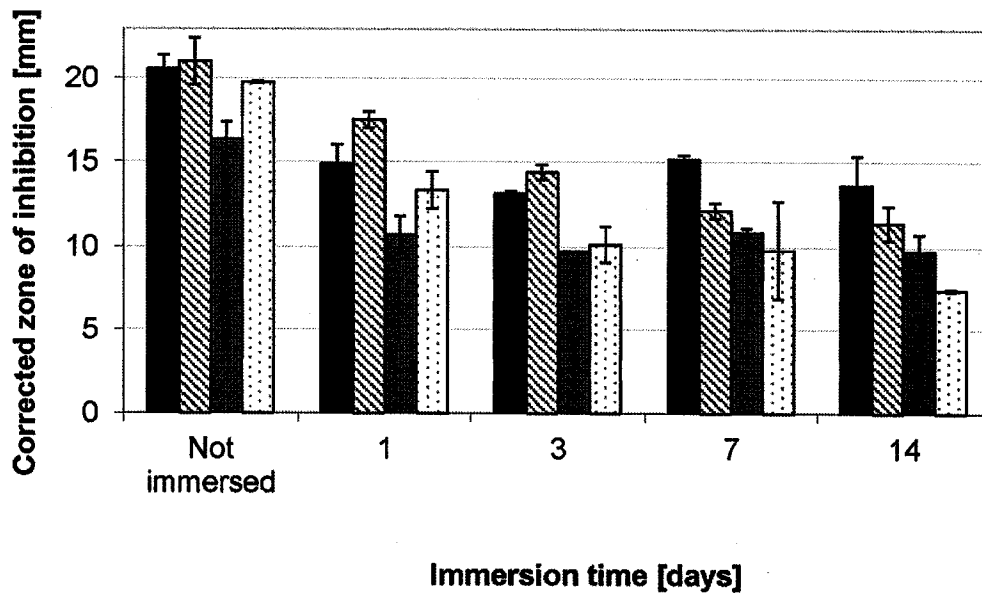
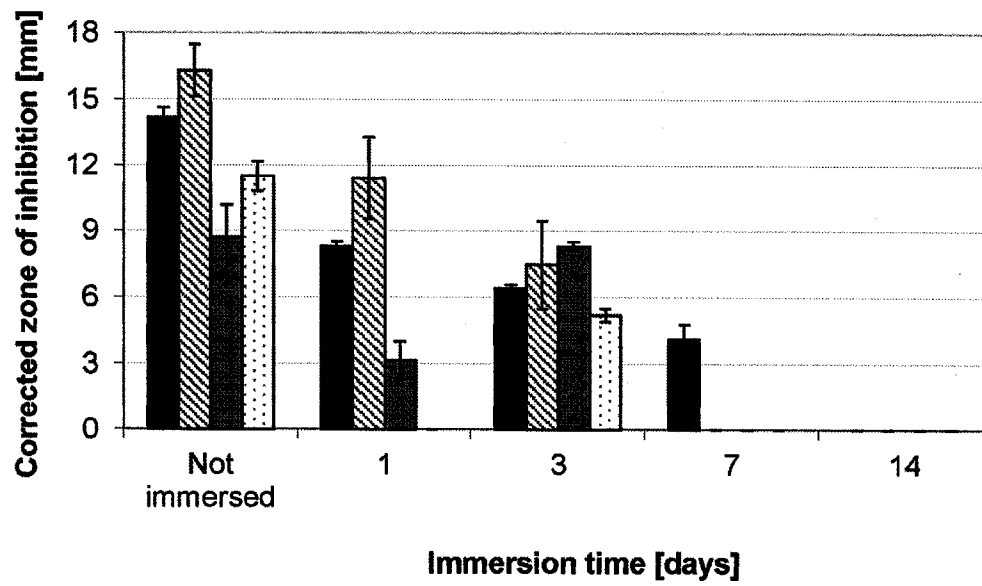


FIG. 14B



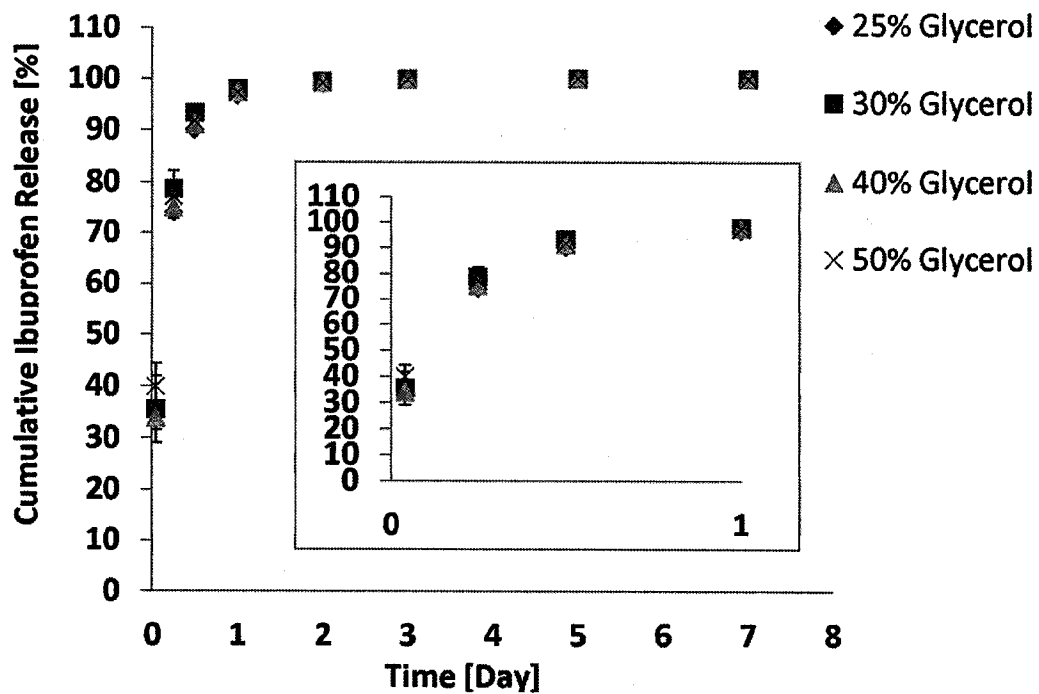
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FIG. 14C



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FIG. 15

Cumulative Released Ibuprofen (Normalized) for Different Plasticizer

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FIG. 16A

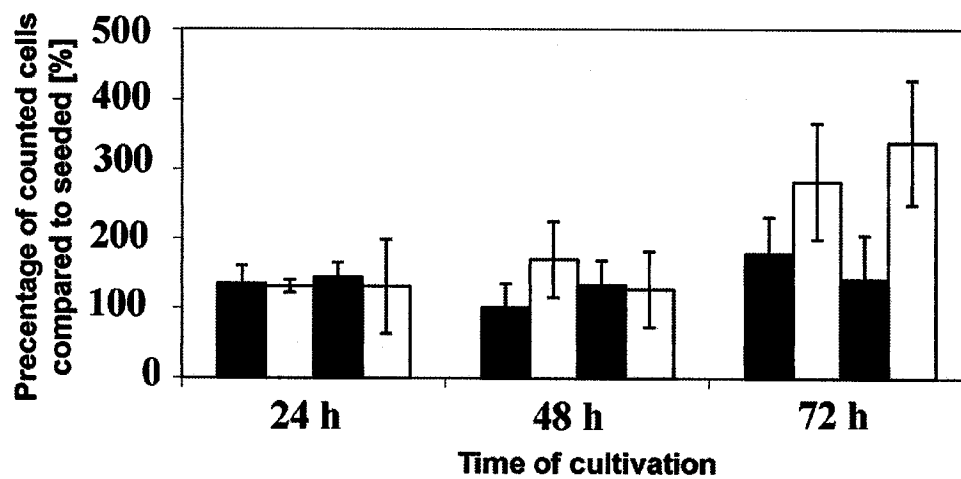


FIG. 16B

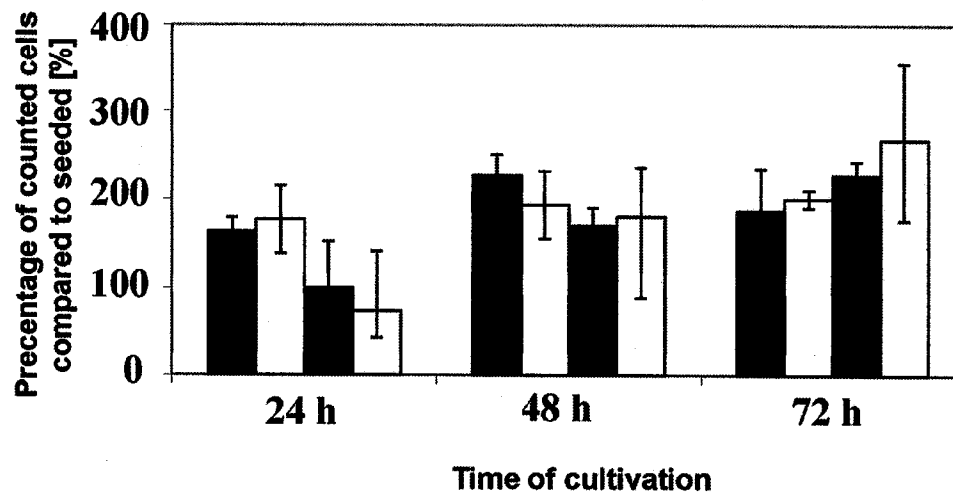


FIG. 17A

FIG. 17B

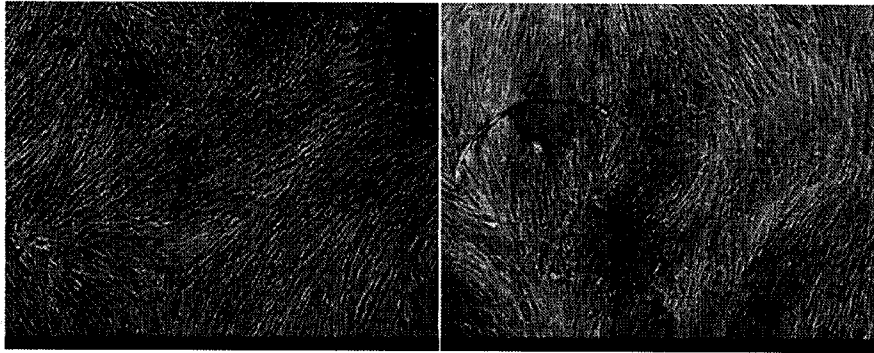


FIG. 17C

FIG. 17D

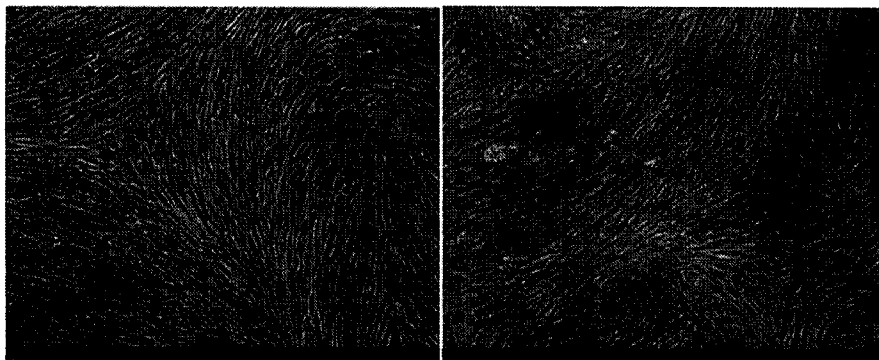


FIG. 17E

FIG. 17F

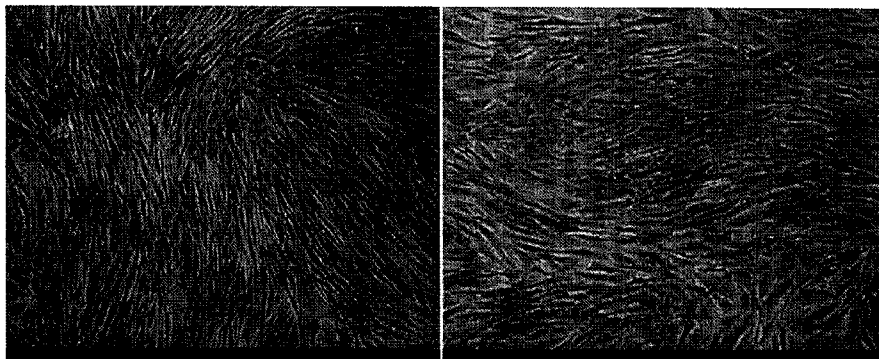


FIG. 17G

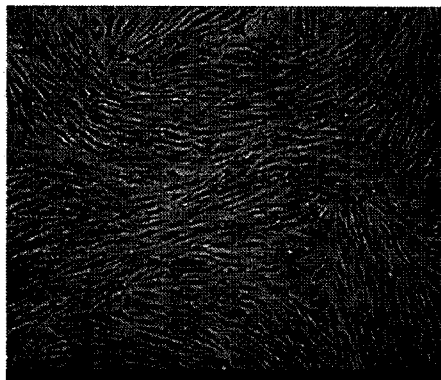
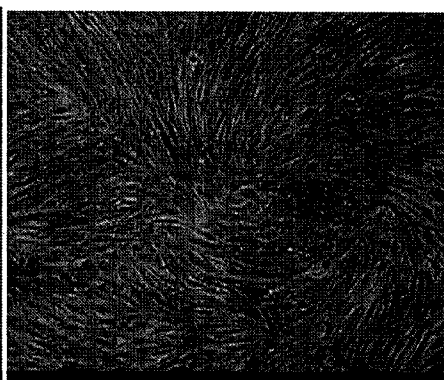


FIG. 17H



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FIG. 18A

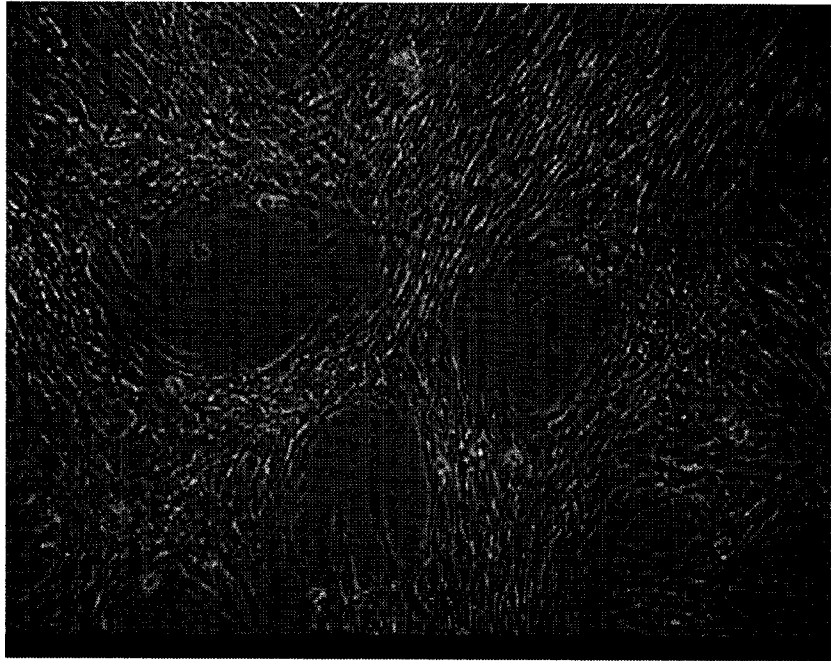
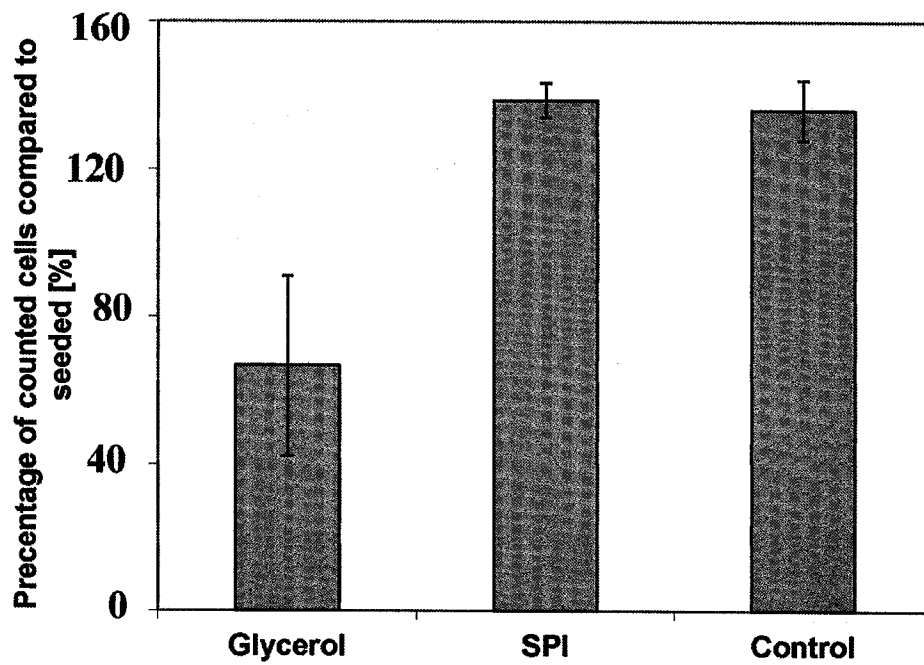


FIG. 18B



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FIG. 19A

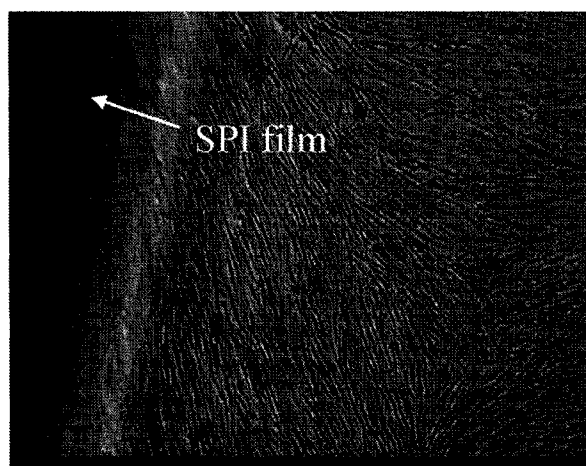


FIG. 19B

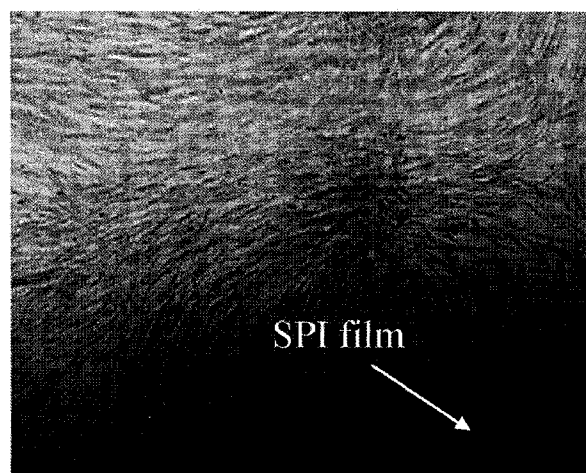


FIG. 19C



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FIG. 20A

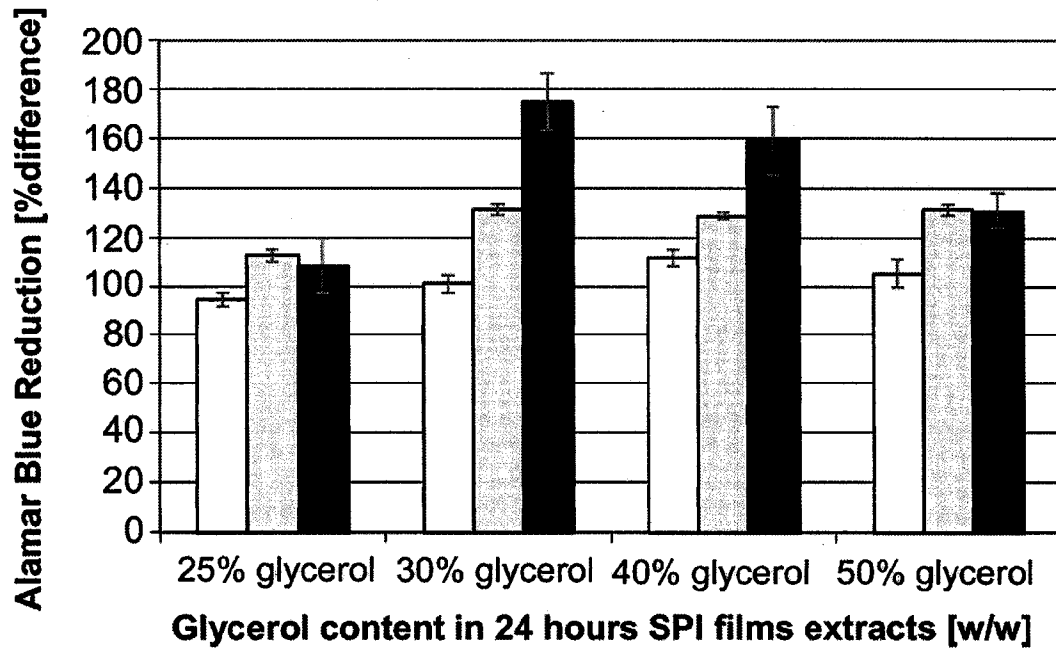
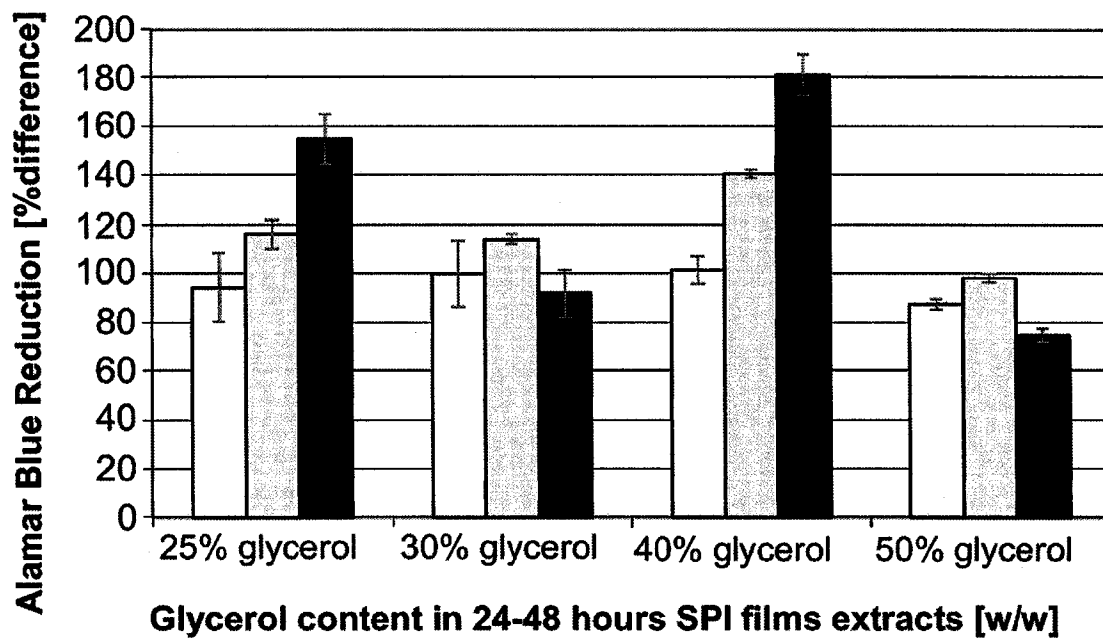


FIG. 20B



INTERNATIONAL SEARCH REPORT

International application No

PCT/IL2012/050079

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K9/70 A61K47/42 A61K38/16 A61L15/32
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61F A61L

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEN L ET AL: "Kinetics of the breakdown of cross-linked soy protein films for drug delivery", BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 29, no. 27, 1 September 2008 (2008-09-01), pages 3750-3756, XP022849905, ISSN: 0142-9612, DOI: 10.1016/J.BIOMATERIALS.2008.05.025 [retrieved on 2008-06-10]	1-23, 26-61
Y	pont 2.2; introduction ----- -/-	66-69



Further documents are listed in the continuation of Box C.



See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

22 August 2012

Date of mailing of the international search report

30/08/2012

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2012/050079

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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X	----- US 5 523 293 A (JANE JAY-LIN [US] ET AL) 4 June 1996 (1996-06-04) cited in the application column 7; table 2	1-38, 59-61
X	----- US 2011/027364 A1 (AMBROSIO LUIGI [IT] ET AL) 3 February 2011 (2011-02-03) paragraph [0026] - paragraph [0076]; example 7	19,62-65
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A	----- US 2004/081698 A1 (SANTIN MATTEO [GB] ET AL) 29 April 2004 (2004-04-29) cited in the application example 5	1-69

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International application No

PCT/IL2012/050079

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