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(54) **BIODEGRADABLE FOAM**

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(57) **ABSTRACT**

The invention relates to a method for forming a dried absorbent foam having an open pore network and pores by preparing an aqueous dispersion comprising an enzymatically biodegradable biopolymer and a foaming agent and optionally one or more of gel-forming ions, a plasticizer, a crosslinking agent and a pH modifier, forming, preparing or mixing a foam from the aqueous dispersion and drying the foam to form a dried foam containing open pores. Gelled composites made from the foams are also provided. The invention is particularly useful in providing foams and composites for use in biomedical applications and as an anti-adhesive in tissue regeneration and wound management.

BIODEGRADABLE FOAM

[0001] This application claims the benefit of U.S. Provisional Application No. 60/777,869, filed Mar. 1, 2006; U.S. Provisional Application No. 60/872,844, filed Dec. 5, 2006; and U.S. Provisional Application No. 60/874,174, filed Dec. 11, 2006.

[0002] This invention is directed to a biodegradable foam comprising a biopolymer, a composite comprising a biopolymer a method of preparation of the foam, a composite and their uses. The foam and composite are particularly useful in biomedical, pharmaceutical, personal care, and industrial applications.

[0003] Foams made from biopolymers are known and known for use in many applications including wound management, tissue regeneration, tissue engineering and cell immobilization and the like. Chitosan foams are known for use in treating both topical and internal wounds. The polymer's antibacterial and bioadhesive properties also makes it a suitable polymer for wound treatment in addition to other applications where it may be desired that the foam should adhere to a tissue surface. Many of the products available today for use as a matrix for cell immobilization, or cell growth as an anti-adhesion scaffold, as a system for controlled release are made of mammalian products such as collagen. It is desirable to replace these with products made from non-mammalian materials and materials of higher purity.

[0004] Hyaluronic acid or hyaluronate is a natural component in mammalian organisms and is enzymatically biodegradable by hyaluronidases. Sodium hyaluronate is an abundant glycosaminoglycan found in the extracellular matrix of skin, joints, eyes and most organs and tissues of all higher animals. Non animal derived HA may be fermented from *Streptococcus zooepidemicus*

[0005] U.S. Pat. No. 5,840,777 (Eagles) discloses a method of forming a polysaccharide foam which comprises preparing an aqueous solution including a soluble polysaccharide and mechanically foaming the solution. The foam may be made by introducing a gas. The foam may be air dried after formation however this leads to the foam in an interior region collapsing.

[0006] We have now found that a foam produced in a particular manner and comprising an enzymatically biodegradable polymer provides an excellent combination of characteristics including structural integrity, strength, flexibility and biodegradability.

[0007] The invention provides q method for forming a dried absorbent foam having an open pore network and pores by:

[0008] a. forming a wet foam from an aqueous dispersion comprising a polysaccharide; a foaming agent; optionally a plasticizer; optionally a crosslinking agent; optionally gel-forming ions; optionally one or more additives, and water;

[0009] b. mixing a foam from the aqueous dispersion, optionally by mechanical agitation;

[0010] c. molding or shaping the wet foam and optionally forming a crosslinked foam; and

[0011] d. drying the foam to form a dried foam optionally by air drying and optionally further molding, shaping or compressing the dried foam.

[0012] The invention also provides a method for forming a dried absorbent foam comprising an open pore network and gel-forming ions by:

[0013] a. forming an aqueous dispersion comprising a polysaccharide; a foaming agent; gel-forming ions, optionally a plasticizer; optionally a pH modifier; optionally one or more additives and water;

[0014] b. preparing a foam from the aqueous dispersion; and

[0015] c. drying the foam to form a dried open pore foam containing pores and gel-forming ions;

[0016] in which the amount of gel-forming ions added will saturate less than 25% of the gelling sites of the polysaccharide.

[0017] The invention also provides a method for forming a dried absorbent foam comprising an open pore network and gel-forming ions suitable for gelling a subsequently added polysaccharide solution by:

[0018] a) forming an aqueous dispersion comprising a polysaccharide, a foaming agent, at least one gelling ion which does not gel the polysaccharide, optionally a plasticizer, optionally a pH modifier, optionally one or more additives and water;

[0019] b) forming a foam from the aqueous dispersion; and

[0020] c) drying the foam to form a dried foam containing open pores and gel-forming ions suitable for gelling a subsequently added polysaccharide solution.

[0021] In a preferred aspect the invention provides a method for forming a dried absorbent foam having an open pore network and pores by:

[0022] a) preparing an aqueous dispersion comprising an enzymatically biodegradable biopolymer or polysaccharide and a foaming agent, preferably a biologically-acceptable foaming agent and optionally one or more of a plasticizer, gel-forming ions, a crosslinking agent and a pH modifier;

[0023] b) forming, preparing or mixing a foam, preferably a wet foam, from the aqueous dispersion, optionally by mechanical agitation;

[0024] c) optionally molding or shaping the foam and optionally forming a crosslinked foam; and

[0025] d) drying the foam to form a dried foam containing open pores.

[0026] By "enzymatically biodegradable biopolymer" is meant, a biopolymer which is capable of degrading in vivo in a mammal as a consequence of enzymatic action.

[0027] Suitably the foam is dried by air drying. The dried foam may be molded, shaped or compressed as desired during or after drying.

[0028] Preferably the foam is formed by forming, preparing or mixing the aqueous dispersion comprising an enzymatically biodegradable, polysaccharide; gel-forming ions,

a foaming agent; and optionally one or more of a plasticizer, a crosslinking agent and a pH modifier and drying the foam to form a dried open pore foam containing pores.

[0029] Suitable gel-forming ions for use in the present invention include monovalent and polyvalent ions, preferably a divalent and/or a trivalent ions, or mixture of ions. It is a requirement of the invention that the gel-forming ions are capable of forming a gel with the enzymatically biodegradable biopolymer. Where the ions form a soluble salt with a particular biopolymer, these ions are generally not considered suitable for making a gel or foam with that biopolymer. Suitable gel-forming ions may be positively or negatively charged and be monovalent or polyvalent. Examples include, for example, calcium(2+), barium(2+), strontium(2+), iron(2+), zinc(2+), copper(2+), and aluminum(3+). Preferred cations are divalent metal cations, more preferably the calcium (2+) cation. A cation would not be considered as a suitable gel-forming ion for a particular biopolymer if it provided a soluble salt however it would be suitable with another biopolymer provided it did not form a soluble salt with that polymer. Where the enzymaticaly biodegradable biopolymer salt is positively charged, for example, chitosan, negatively charged gel-forming ions, for example phosphate may be employed.

[0030] A salt or combination of salts that provides the desired gel-forming ions or mixture of gel-forming ions may be used as the gel-forming ions. Gel-forming ions may be incorporated in the foam either during preparation or subsequently added to the foam, if applicable, prior to addition of the liquid with the polysaccharide. Typical washing solutions for the polysaccharide foam have about 30 mM to about 200 mM, more preferably from 50 to 100 mM, of a water-soluble gelling salt such as calcium chloride, barium chloride, or strontium chloride. Suitably, the rate of gelation may be controlled to delay gelling by using sparingly soluble salts under pH conditions which they are slowly solubilized, or by using soluble gel-forming ions in combination with sequestrants. Washing or soaking can be used to modify the properties of the composite where additional gel-forming ions may be added to strengthen or harden the composite and also to control cell proliferation, while other treatments such as sequestrants or non-gel-forming ions may be used to weaken or dissolve the composite.

[0031] Alginate gels can be dissolved by addition of a recovery agent, for example an aqueous solution of citrate, EDTA or hexametaphosphate. Wash treatments for use with living cells must be isotonic. The properties of the composite may accordingly be tailored as desired.

[0032] The gel-forming ions may be able to form a gel with the polymer of the foam and/or the soluble polysaccharide. The gel-forming ions may form links between the foam and the soluble polysaccharide. Preferably, the "gel-forming ions" in the foam are donatable to the polysaccharide and are present in the foam at a level such that at least some of the gelling sites of the polysaccharide are occupied upon contacting the liquid component to the foam. Suitably, the gel-forming ions may be present in the foam at a sub-stoichiometric, stoichiometric or super-stoichiometric level with respect to sites in the foam for binding the gel-forming ions provided that sufficient gel-forming ions are present to occupy at least some of the gelling sites in the polysaccharide to be added.

[0033] In a preferred embodiment, gel-forming ions are present in the foam and may be incorporated into it during production of the foam or after it is formed but desirably before any addition of further polysaccharide to form a gelled foam composite. Preferably the gel-forming ions are suitable for forming a gel with a polysaccharide subsequently added to the foam. Suitably the amount of gel-forming ions added will saturate up to 200% of the gelling sites of the polysaccharide, for example less than 25% of the sites.

[0034] Foams of the present invention have open pore networks. Suitably, the pores in the open pore network are from 5 to 1000 microns, preferably from 25 to 500 microns. The foam is preferably capable of absorbing an added liquid component containing a polysaccharide into its pores. Suitably the foam has pores open on at least one surface and desirably have at least a portion of interconnected pores to enable transport within the foam of a liquid component added to the foam, for example a polysaccharide solution and/or effectively increase the volume of liquid which can be absorbed. The foam is suitably swellable and preferably may absorb up to 30 times its weight, more preferably from 1 to 20 times its weight of a liquid, for example an aqueous physiological solution or a polysaccharide solution. The foam can have a homogeneous or heterogenous distribution of pore sizes. Not all pores are required to absorb the liquid component.

[0035] The enzymatically biodegradable biopolymer is preferably selected from a polysaccharide, preferably chitosan and hyaluronic acid, By employing a biopolymer which is enzymatically biodegradable, a foam according to the invention and composites comprising the foam may be advantageously employed in wound management, as a bioadhesive and in other applications in the human or animal body. Enzymatic degradation allows the foam to be designed in such a manner that the product may perform its function and then be removed from the body through degradation. Biopolymers which are not enzymatically biodegradable either due to their intrinsic characteristics or due to the absence of suitable enzymes in the environment in which the foam is to be employed may take longer to degrade by other mechanisms for example hydrolysis, to a level where they may be excreted.

[0036] In an especially preferred embodiment, the biopolymer comprises chitosan. Chitosan is a linear polysaccharide comprising β-(1→4)-linked 2-acetamido-2-dexoy-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-D-glucopyranose (GlcN). Chitosan is N-deacetylated derivative of chitin, which consists nearly entirely of β-(1→4)-linked 2-acetamido-2-dexoy-D-glucopyranose (GlcNAc). Commercially chitosan is made by alkaline N-deacetylation of chitin. The heterogeneous deacetylation process combined with removal of insoluble compound results in a chitosan product which possesses a random distribution of GlcNAc and GlcN-units along the polymer chain. The amino group in chitosan has an apparent pK_a-value of about 6.5 and at a pH below this value, the free amino group will be protonized so the chitosan salt dissolved in solution will carry a positive charge. Accordingly, chitosan is able to react with negatively charged components it being a direct function of the positive charge density of chitosan.

[0037] Advantageously, the cationic nature of chitosan provides a bioadhesive property. In addition, chitosan may

precipitate red blood cells due to their negative charge providing benefits in forming blood clots and in reducing the level of fibrin during healing so reducing the formation of scar tissue. Chitosan may be degraded by lysozyme and other related enzymes occurring in a mammalian body, for example the human body. In use the chitosan in a foam of the present invention will suitably be degraded by lysozyme found in mammals in saliva, tears, blood serum and in interstitial fluid. A composite having a chitosan foam may advantageously be employed in wound management, as a bioadhesive and in other applications in the human or animal body.

[0038] As chitosan is also known to open the tight junctions between cells present, for example, in mucosal surfaces and skin as epithelial cells, the present invention is particularly useful for pharmaceutical and vaccines delivery applications. The chitosan foam may also be used as a matrix for cell immobilization, as an anti-adhesion scaffold and as a system for controlled release. Chitosan foams can provide matrices suitable for cell growth either in cell/tissue culture (in vitro) applications or as tissue

[0039] Enzymatic degradation allows the foam to be designed in such a manner that the product may perform its function and then be removed from the body through degradation. The degradation products of chitosan are glucosamine and N-acetylglucosamine which are non-toxic in mammals. The rate of biodegradation of implanted chitosan foams by the lysozyme can be modified by varying the degree of chitosan deacetylation since acetylation protects the polymer from enzymatic degradation. Chitosans with higher degrees of deacetylation are also more resistant to random depolymerization by acid hydrolysis due to a protective effect of the positive charge.

[0040] Chitosans suitable for use in the present invention may be in the form of the chitosan base, a water-soluble chitosan salt or a modified chitosan. Chitosan base may require dilute acid to dissolve for example 1 wt % acetic acid. Chitosan is soluble in aqueous media at acidic pH, where the polysaccharide will be highly positively charged. High molecular weight chitosans with a random distribution of monomer units and a degree of deacetylation (DA) between 40% and 60% are soluble at neutral pH. Chitosans shown increasing solubility at higher pH-values with decreasing DA. Also, by depolymerising chitosans with DA above 60%, their water solubility at neutral pH-values can be increased.

[0041] Generally, chitosan requires an acidic environment for dissolution. By dissolving chitosan in an appropriate acid, the chitosan salt is obtained upon drying. Suitable chitosan salts include chitosan chloride, chitosan glutamate, chitosan lactate, chitosan maleate, chitosan malate, chitosan malonate, chitosan succinate, chitosan formate, chitosan aspartate, chitosan acetate, chitosan propionate, chitosan nitrate, chitosan nicotinate, and chitosan adipate. For example, chitosan glutamate is chitosan converted into the glutamate salt form by dissolving chitosan in glutamic acid. Glutamic acid is present at a stoichiometric amount to the number of GlcN units. Chitosan chloride contains a stoichiometric amount of hydrochloride to the number of GlcN units.

[0042] Salts of chitosan are generally soluble water, and the pH of a 1% solution of chitosan salt is typically between 4 and 6. The functional properties of chitosan are influenced by the degree of deacetylation and molecular weight and molecular weight distribution. Suitably, the degree of acetylation ranges from 40% to 100%, preferably 50% to 100%. In some embodiments, the degree of acetylation is preferably 80 to 99%, more preferably 80 to 95%. Suitable molecular weights are in the range 10 kDa to 1000 kDa.

[0043] Suitable modified chitosans contain moieties covalently linked to the chitosan for example peptide coupled chitosan. Modified chitosans can be tailored by selection of moieties and their concentration in the modified chitosan to add, modify or alter properties or functionalities of the chitosan such as crosslinking capability, solubility, rate of biodegradability of the ability to bind, for example, specific cells, pharmaceuticals or peptides.

[0044] A further preferred biopolymer comprises hyaluronic acid (HA), salts thereof and modified hyaluronate. Hyaluronic acid from a non-animal source is preferred for use in the present invention. Hyaluronic acid is a linear copolymer composed of (β-1,4)-linked D-glucuronate (D) and (β-1,3)-N-acetyl-D-glucosamine (N). The coiled structure of hyaluronate can trap approximately 1000 times its weight in water. These characteristics give the molecule advantageous physicochemical properties as well as distinct biological functions and is desirable for use as a building block for biocompatible and biointeractive materials in pharmaceutical delivery, tissue engineering and viscosupplementation.

[0045] Suitable modified hyaluronates include those containing moieties covalently linked to the hyaluronates and may include for example peptide coupled hyaluronates. A preferred modified hyaluronate suitably has a covalently modified carboxyl group and/or hydroxyl group on the D and N monomer units respectively. Modified hyaluronates can be tailored by selection of moieties and their concentration in the modified hyaluronates to add, modify or alter properties or functionalities of the hyaluronates such as crosslinking capability, solubility, rate of biodegradability of the ability to bind, for example, specific cells, pharmaceuticals or peptides.

[0046] Hyaluronic acid is thought to play an important role in the early stages of connective tissue healing and scarless fetal wound healing and regulate cell mobility, adhesion, and proliferation and is especially useful in tissue engineering and tissue regeneration applications.

[0047] The foaming agent is preferably biologically-acceptable so enabling use in relation to the human or animal body for example in wound management, as a bioadhesive and in other applications in the human or animal body. In a preferred embodiment, the foaming agent comprises a polymeric foaming agent which suitably produces a wet foam resistant to foam collapse. The foaming agent may be a single material or a mixture of materials that aid in foaming. Foaming agents which are not biologically-acceptable are not generally suitable for use in foams for use in medical applications. In an especially preferred embodiment, the foam is substantially free of non-polymeric surfactants and other non-biologically-acceptable foaming agents.

[0048] Polymeric foaming agents, such as hydrocolloids, are generally preferred for biological applications because they generally do not leach from the resulting foam than

surfactants. Examples of suitable hydrocolloids include methyl cellulose, hydroxy propyl methyl cellulose (HPMC), hydroxy propyl cellulose (HPC), hydroxy ethyl cellulose (HEC), albumin and glycol alginates, such as propylene glycol alginate. For some applications, it may be advantageous to add an additional polysaccharide, for example a cellulose derivative such as carboxymethyl cellulose, in addition to the foaming agent. The polymeric foaming agent is preferably soluble in water so that a homogeneous foam is produced. Especially preferred water soluble foaming agents include albumin and hydroxy propyl methyl cellulose as they produce small bubbles that result in fine pores in the foam.

[0049] When dried cross-linked foams containing high levels of calcium are soaked in water, the foam structure typically does not break down due to the high level of crosslinking of the foam. However, the soluble components in the foam, including water soluble foaming agents such as hydroxy propyl methyl cellulose, may diffuse out of the foam. This loss of foaming agent may be reduced or prevented in, for example a wound healing application, by use of a foaming agent that is not soluble under conditions of use. Some foaming agents form gels at body temperature, for example methyl cellulose forms gels above 35° C. When using a foam that comprises methyl cellulose as the foaming agent in an application in which the foam is at body temperature, the methyl cellulose will stay in the gelled state and remain in the foam and contribute to the wet strength of the foam.

[0050] When a polymeric foaming agent such as hydroxy propyl methyl cellulose is used, the concentration of the polymeric foaming agent in the aqueous dispersion is typically about 0.5 wt % to about 6 wt %, preferably about 1 wt % to about 4 wt %, more preferably about 1.5% to about 2 wt %. This produces a foam that comprises about 3 wt % to about 37 wt %, preferably about 6 wt % to about 25 wt %, more preferably about 6% to about 12.5 wt %, of the polymeric foaming agent, exclusive of water and any additive or additives that may be present in the foam.

[0051] The enzymatically biodegradable polymer foam can be prepared such that it will dissolve after hydration or the foam can be prepared to remain structurally in tact when hydrated by crosslinking. For example, a non-crosslinked chitosan foam can be prepared by dissolving a chitosan salt (such as PROTASAN UP CL) in water or in a salt solution. A wet foam can be prepared using a mixer, for example a kitchen aid mixer equipped with a wire whisk to aerate an aqueous solution of the enzymatically biodegradable polymer together with other ingredients such as plasticizers for example, glycerin and/or sorbitol or a foaming agent for example hydroxymethylcellulose and albumin. The pore structure of the foam is stabilized by the foaming agent and the structure frozen by drying the foam, at ambient or elevated temperature for example in an oven. The foam may also be prepared by freeze drying techniques.

[0052] The foam may be cross-linked. Cross-linking may be achieved by addition of an ionic component to the biopolymer or by using a covalent cross-linking agent. The biopolymer in the foam may be ionically or covalently cross-linkable but need not be cross-linkable. If the foam is to be used to make a composite by addition of a soluble polysaccharide to the foam, suitably the foam or a component of the foam, for example gel-forming ions, is cross-linkable with the soluble polysaccharide. Cross-linking may be achieved by addition of an ionic component to the biopolymer or by using a covalent cross-linking agent. An aqueous solution of an ion may be added to the biopolymer foam which is suitably aerated before casting. Addition of the ionic component suitably causes a gel to form and this may be cast. Alternatively the ionic component may be added to the wet foam suitably after the foam is cast in a mold for example by spraying, before drying.

[0053] Where a positively charged biopolymer is employed, for example chitosan, a negatively charged ionic component, for example tripolyphosphate or sodium citrate may be employed to effect cross-linking. In a preferred embodiment, β-Glycerophosphate can added to a positively charged biopolymer in the wet solution before the mixing step and gelling can be induced after molding by increasing the temperature. Other multivalent ions such as molybdates may be used to crosslink chitosan foams. Crosslinkers disclosed in Berger et al. (2004) "Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications", *European Journal of Pharmaceutics,* 57, 19-34 may also be useful in producing chitosan foams.

[0054] Cross-linking of the enzymatically biodegradable polymer may be achieved using a co-valent cross-linking agent known in the art for example an epoxide.

[0055] As desired a biopolymer film may also be cast onto the foam as a backing material.

[0056] Suitable plasticizers and other ingredients or additives include those described in WO2005023323 (Gaserod), the disclosure of which is herein incorporated by reference. Suitable pH modifiers are selected according to the nature of the foam, for example chitosan is positively charged and hyaluronate is negatively charged.

[0057] The present invention is particularly suited for use in biomedical applications. These applications may include pharmaceutical delivery or topical application or be for external use for example for tissue growth, tissue regeneration or wound applications or for internal use such as implantation or internal wound management. The biopolymer may be used in different levels of purity depending on the application. Ultra pure chitosan and alginate materials are biocompatible and have a purity sufficient to allow for implantation in a mammal, preferably in a human.

[0058] The invention further includes combinations of foam compositions, for example, an alginate foam adfixed to the enzymatically biodegradable biopolymer foam for example by adding a dry or wet biopolymer foam to a wet gelled alginate foam and then drying. The alginate foam/biopolymer foam structure suitably provides improved properties such as mechanical strength. In a preferred embodiment, a composite comprising a chitosan foam and a gelled alginate foam may be employed to carry cells for use in biomedical applications.

[0059] Foams according to the invention are flexible, they can be cut in desired shape and be compressed. Foam properties can be varied by a number of parameters including the amount of air incorporated, biopolymer type, molecular weight and composition of the biopolymer, concentration, the amount of crosslinking and non-crosslinking,

and foam thickness. The biopolymer employed in the foam is biocompatible. By varying the amount of crosslinking and porosity, the foam integrity and foam degradation can be controlled, which makes the foam useful for many applications.

[0060] In a preferred embodiment, the foam contains added gel-forming ions suitable for gelling a subsequently added polysaccharide solution which is absorbed into the pores of the open pore structure of the foam. The gel-forming ions may comprise monovalent or polyvalent ions, typically a divalent and/or a trivalent ions, or mixture of ions capable of gelling the polysaccharide in the added polysaccharide solution. Gel-forming ions for specific polysaccharides are well known from the literature. For alginates, suitable polyvalent cations include, for example, calcium(2+), barium(2+), strontium(2+), iron(2+), zinc(2+), copper(2+), and aluminum(3+). Preferred cations are divalent metal cations, more preferably the calcium (2+) cation. The added gel-forming ions may be incorporated by using an appropriate salt solution during the manufacture of the foam or may be added during or subsequent to manufacture of the foam for example by spraying or soaking a salt solution.

[0061] In a further aspect, the invention provides a method to prepare sterile composites having for example, cells, drugs or particulates immobilized in gels within a sterile dried foam according to the invention. The composites are suitably formed by mixing a polysaccharide solution (such as a sterile alginate solution) comprising a soluble polysaccharide having gelling sites and a functional component such as cells, pharmaceuticals and particulates to form a liquid component, adding the liquid component to a foam according to the invention (such as a sterile, dried chitosan foam) having an open pore network and pores containing incorporated gel-forming ions, and reacting the gelling sites of the soluble polysaccharide with the gel-forming ions to form a gel which immobilizes the cells, drugs or particulates within the pores.

[0062] Suitably, the foam, the composite or a device containing the composite is sterilized, preferably by γ-irradiation, E-beam, ethylene oxide, autoclaving or contacting the foam with alcohol prior to addition of the liquid component or contacting with NOx gases, hydrogen gas plasma sterilization. Sterilisation should not be employed where it adversely affects the composite, or a functional component contained in the composite.

[0063] Applications for the sterile composite include cell immobilization and/or cell proliferation for in vitro or in vivo tissue culture applications, cell therapy and artificial organs, a delivery system used in vivo for controlled release, for wound management, or as an anti-adhesion layer in vivo. Ultrapure polysaccharides possessing a low content of endotoxins and documented safety profile are used, either for the foam or as the soluble polysaccharide, or both, as appropriate, depending upon what structure is intended for implantation into living animals and humans. By a low content of endotoxin it is meant that the endotoxin content must not exceed, for example, the U.S. Food and Drug Administration recommended endotoxin content for an implantable device. The current regulatory guidelines establish that the device may not release more than 350 endotoxin units (EU) to the patient. Ultrapure polysaccharides possessing a low content of endotoxins for example less than 350 EU/g, preferably less than 100 EU/g may be used, either for the foam or as the soluble polysaccharide, or both, as appropriate, depending upon what structure is intended for implantation into living animals and humans. For example, when alginates are used for implantation within the human body, the alginates suitably have an endotoxin content of less than 100 EU/g. In a preferred embodiment the composite has an endotoxin content of less than 10 EU/g

[0064] Cells immobilized in the composite may be implanted into animals wherein the gel acts as an immune barrier and prevents detection by the immune system thereby allowing the implantation of xenografts. Suitably strontium can be used as gel-forming ions when animal cells are desired for implantation (xenografts), since when using this type of artificial organ, it is important that the cells do not grow out of the implanted composite and become exposed to the immune system. The composite may also be used to establish cell, tumor and tissue xenografts in animals for, for example, cancer research. Immobilization of multicellular aggregates, such as islets Langerhans, in the composite allows said multicellular aggregates to be implanted into animals or humans without immune rejection and such implanted cell aggregates may then function as an artificial organ producing, for example, insulin.

[0065] Cell cultures can be used to manufacture many biological materials, for example enzymes, hormones, immunobiologicals (such as monoclonal antibodies, interleukins, lymphokines) and anticancer agents. Cells can be cultured in composites according to the invention to increase the total number of cells. For example, cells isolated from a patient can be cultured in a composite of the invention to increase the cell number, the cells can then be retrieved from the composite and used in tissue engineering applications. Cell cultures in a composite according to the invention can also be used to explore, characterize and specify cell differentiation and growth to produce tissue like structures. For example, cells are affected by the external stress and modifying the elasticity of the foam or composite (gel/foam) materials may influence gene expression.

[0066] Foams and composites according to the invention may be used in the treatment of the human or animal body to prevent adhesion between tissue. Surgical interventions may cause conglutination or growing together of tissues, e.g. between muscles, between muscles and tendons or nerves or other tissues. To prevent this undesired tissue growth, an anti-adhesion layer can be inserted between muscles, muscles and tendons or nerves to cover the wound and prevent postoperative adhesion formation during the healing process.

[0067] Foams and composites of the present invention can be formulated for use as an anti-adhesion layer by selection of materials for example a hyalouronate foam in the composite and gelling ions which retards or prevents cell growth and intrusion into the anti-adhesion layer thus avoiding adhesion between tissues during healing. The foam is suitably engineered from biodegradable materials which dissolve as the wound heals (by appropriately varying the amount of cross linking ions, type of polymer, polymer concentration) and are degraded or excreted from the body.

[0068] Depending upon the formulation properties, the foam or composite can be formulated to degrade over various periods of time and thereby release immobilized

materials such as therapeutic agents or tissue-regenerative agents. A preferred use of the invention is in tissue repair wherein organic or inorganic material can be immobilized within the composite and act as a scaffold for tissue regeneration. One such example would be the inclusion of hydroxyapatite in the gel within the foam and then implanted into or attached to a bone defect in order to induce bone regeneration into the foam or foam/gel composite. Another such example would be the inclusion of chemotactic or cell attractant substances within the composite followed by implantation of the composite in a tissue injury site in order to promote tissue regeneration.

[0069] The rigidity of the composite and the gel in which cells are immobilized are important factors for cell behavior since it appears that the mechanical properties of the gel regulates proliferation and differentiation has been observed based on cell type. The rigidity of the gel (as characterized, for example, by elastic modulus) in which the cell is immobilized determines the magnitude of the force generated from the exoskeleton and the extent of cell spreading that ensues. The gel properties are varied by polysaccharide concentration, saturation of gel-forming ions, and type of gel-forming ions. In addition, polysaccharides can be chemically modified by adding peptide sequences for cell adhesion, such as the cell adhesion peptide sequences, such as the RGD tripeptide.

[0070] When composites are to be used as controlled delivery applications, e.g. of drugs, growth factors, nutraceuticals, flavors or fragrances, the mechanical and chemical properties can be modified for proper release in the desired environment.

[0071] The dried foams of the present invention may generally contain a water content, on a weight percent basis, of less 40% by weight of all components in the dried foam, more specifically, less than 35%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, less than 3%, less than 2%, less than 1%, of all components in the dried foam.

[0072] The dried absorbent foams are capable of absorbing any liquid, for example, water, body fluids, etc.

[0073] Glossary

Albumin	Bovine albumin, Fraction V, approx. 99% (A-3059) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany)
$CaCl_2$	Calcium chloride dihydrate (1.02382.1000) (Merck KgaA, Darmstadt, Germany)
$CaCO_3$	HuberCAL 500 Elite, Calcium carbonate, particle size ~4.2 μm (Huber Engineered Materials, Hamina, Finland)
GDL	Glucono δ-lactone (Roquette, Alessandria, Italy)
Glycerine	Glycerin, Ph. Eur. (VWR Prolabo, Leuven, Belgium)
Hanks'	Hanks' balanced salt solution; (H8264) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany)
HPMC	Pharmacoat 603, Substitution type 2910, Hypromellose USP, (hydroxypropylmethylcellulose) (Shin-Etsu Chemical Co. Ltd., Japan)
Na_2HPO_4	Disodium hydrogen phosphate, art: 30427 (Riedel-de Häen, Seelze, Germany)
Na-triphosphate	Sodium triphosphate pentabasic (T5883-500G) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany)
PRONOVA UP MVG	Sodium alginate, batch: 701-256-11, viscosity (1 wt % aqueous solution at 20° C.) = 385 mPas (NovaMatrix, Oslo, Norway
PRONOVA UP LVG	Sodium alginate, batch: FP-502-04, viscosity (1 wt % aqueous solution at 20° C.) = 50 mPas (NovaMatrix, Oslo, Norway
PRONOVA SLG 20	Sterile sodium alginate, batch: Ch.-B.221105, viscosity (1 wt % aqueous solution at 20° C.) = 36 mPas (NovaMatrix, Oslo, Norway)
PROTASAN CL 210 (214)	Chitosan chloride, batch: 708-783-01, deacetylation: 94.5%, pH = 5.3, viscosity of 1% aqueous solution at 20° C. = 77 mPas (NovaMatrix, Oslo, Norway)
PROTASAN UP CL 213	Ultrpure chitosan chloride, batch: FP-104-02, viscosity (1 wt % aqueous solution at 20° C.) = 74 mPas, degree of deacetylation = 86% (NovaMatrix, Oslo, Norway)
Sodium Hyaluronate	Pharma grade 80, batch: 17053P, molecular weight: $1.08*10^6$ g/mole (NovaMatrix for Kibun Food Kemifa Co., Ltd., Kamogawa, Japan)
Sorbitol special	70% sorbitol solution (SPI Polyols, New Castle, DE, USA)
Sorbitol	D(−)sorbitol for biochemistry, dry, 100% (Merck, KGaA, Darmstadt Germany)

EXAMPLES

Example 1

[0074] This example presents a method for producing chitosan foams and their characteristics related to density and absorption.

[0075] An aqueous solution containing 4% chitosan salt was prepared using PROTASAN CL 210 (214). 77.0 g MQ-water and 14.0 g sorbitol (dry) were added a mixing bowl and the sorbitol were dissolved by gently swirling the bowl. 100 g of the chitosan solution, 6.0 g glycerin and 3.0 g HPMC were added to the same mixing bowl. The dispersion was blended with a Hobart kitchen aid mixer equipped with a wire whisk at medium speed for one minute to ensure homogeneity. The mixing continued at high speed for 2.5 minutes. The wet density was measured to be 0.23 g/ml (determined from the weight of wet foam required to fill a 100 ml container). The wet foam was cast in 2 mm and 4 mm high molds coated with Teflon and then placed in a drying oven at 80° C. for 30 minutes and 60 minutes, respectively.

[0076] Another foam was made by the procedure as above, but the wet foam was molded in a 8 mm deep mold. The foam was dried at 80° C. for 1 hour and then 3 hours at 40° C.

[0077] The resulting dry foams were flexible and soft with an open pore network. When water was added to the foam it was immediately absorbed and the foam expanded significantly. The hydrated foam retained its shape, but was relatively weak in that the wet foam could not be transferred in one piece by lifting it from one corner. Compressing the dry foam before hydration did not noticeably affect the foam's absorbency rate or absorption capacity.

[0078] To measure the absorption capacity foam pieces were cut at 3.5 cm by 3.5 cm with use of a scalpel. A foam piece was weighted and placed on a mesh (diameter 0.71 mm) and Hanks' Balanced Salt Solution, as a model physiological solution, was added using a pipette. Excess liquid was added and the foams turned transparent. When no dripping from the foam piece was observed, the weight of the wet foam was measured. The dry density and the

absorption capacity for the three different foams were measured, and the results are presented in table 1.

TABLE 1

Dry density and absorption capacity of a model physiological solution of chitosan foams of different thickness (n = 3, ± SD).

Thickness foam before drying, [mm]	Thickness dry foam, [mm]	Weight dry foam, 3.5 cm by 3.5 cm, [g]	Dry density, [g/cm^3]	Weight wet foam, [g]	Absorption, [g Hanks' absorbed/g foam]
2	1.95	0.101 ± 0.002	0.042 ± 0.001	2.02 ± 0.04	19.0 ± 0.1
4	3.20	0.164 ± 0.003	0.042 ± 0.001	3.20 ± 0.12	18.5 ± 0.8
8	5.50	0.390 ± 0.013	0.058 ± 0.002	6.76 ± 0.12	16.4 ± 0.3

Example 2

[0079] This example presents a two-layer foam material made comprising alginate foam as the first layer and chitosan foam as a second layer attached to the alginate foam. This type of composite may be used to modify integrity, strength, biodegradation and absorption capacity of the chitosan foam.

[0080] An alginate foam was made by first preparing an aqueous solution containing 4% alginate (PRONOVA UP MVG). 111.2 g of the alginate solution was transferred to a mixing bowl. To the same bowl 6.0 g glycerin, 18.0 g sorbitol special, 3.0 g HPMC, 0.85 g $CaCO_3$ (sufficient to saturate the guluronic residues in the alginate with 125%) and 33.3 g MQ-water were added. The dispersion was blended with a Hobart kitchen aid mixer equipped with a wire whisk at medium speed for 1 minute and 30 seconds to ensure homogeneity. The mixing continued at high speed for 7 minutes before a freshly mixed GDL solution of 2.69 g GDL and 25.0 g MQ-water was added. The mixing continued at high speed for 1 minute, which resulted in a foam with a wet density of 0.23 g/ml. The wet foam was cast in 4 mm and 2 mm high molds coated with Versi-Dry bench protector with the polyethylene side towards the foam (Nalgene Nunc International, NY, USA) and kept uncovered for 60 minutes at room temperature.

[0081] Then wet chitosan foam was added on top of the gelled wet alginate foams as layers of 2 mm and 4 mm (by increasing the mold height) to the top of the 2 mm and 4 mm thick gelled alginate foams, respectively. The chitosan foam was made as described in Example 1 except 18.0 g sorbitol special was used in place of dry sorbitol and 73.0 g MQ-water was added for this foam. The mixing time at medium speed was 2 minutes and then 3 minutes of high speed mixing, which resulted in a foam with a wet density of 0.22 g/ml. The molds with the two-layered foams were then placed in a drying oven at 80° C. for 1.5 hours before it was transferred to an oven at 37° C. and the drying continued overnight.

[0082] The resulting dry foams were soft and flexible with an open pore network. The pores in the alginate foam part were smaller than in the foam made from chitosan. It was not possible to separate the two foam types after drying. Each foam layers absorbed water instantly (the absorption time of the first added drop was less than 1 second for the chitosan foam and about 3 seconds for the alginate foam) and they remained attached after hydration. The hydrated alginate part of the hydrated foam had a high tensile strength whereas the hydrated chitosan part was very weak. Pieces of the hydrated chitosan foam broke off when a finger was pushed against the chitosan foam side or when the chitosan foam was stretched by pushing against the reverse (alginate foam) side. The failure was not delamination.

Example 3

[0083] This example describes a method for cross-linking a chitosan foam for making it more stable related to biodegradation and providing higher wet integrity.

[0084] A chitosan foam was made as described in example 2 except that the mixing times were 1.5 minutes and 4.5 minutes at medium and high speed respectively. The resulting wet foam density was 0.20 g/ml. The wet foam was cast in 2 mm and 4 mm deep molds. Then a 100 mM solution of Na-triphosphate filled in a spray bottle with the nozzle adjusted to give fine droplets. The Na-triphosphate solution was sprayed onto the wet foams about 50 ml and 100 ml for the 2 mm and the 4 mm respectively. The wet foams absorbed some of the solution sprayed on, so the addition was performed several times with less than a minute between each addition. The wet foams were then dried in a drying oven at 80° C. for 1 hour and 2 hours for the foams cast in the 2 mm and 4 mm molds respectively.

[0085] The dry foams were soft, flexible and had an open pore network. The foams absorbed water instantly and they deformed less upon hydration and were stronger than the non-crosslinked chitosan foams in Example 1.

Example 4

[0086] This example describes the preparation of a chitosan foam that contains calcium ions. The calcium immobilized in the chitosan foam induced in situ gelling of an alginate solution when it was absorbed by the dry chitosan foam. Such structures may be useful in biomedical applications for cell immobilization or to provide controlled release of immobilized drugs, enzymes, hormones, etc.

[0087] A chitosan foam was made comprising the same amounts and ingredients as in example 2 except that 2.35 g of MQ-water were replaced with 2.35 g $CaCl_2*2H_2O$ (80 mM). A wet foam with a wet density of 0.20 g/ml were made by mixing at medium and high speed for 1.5 minutes and 6 minutes respectively. The wet foam was cast in 2 mm and 4 mm high molds as described earlier. Then they were placed in a drying oven at 80° C. for 1.5 hours. The dry foams were soft and flexible with an open pore network and a dry density

of 0.039±0.001 g/cm^3. The foam absorbed water instantly and had wet integrity similar to the foams of same thickness in Example 1. This foam expanded less when Hanks' solution was added this foam compared with the foams from Example 1. The absorption capacity of Hanks' solution for this foam was measured to be 16.8±1.9 g/g foam (average value of three samples±SD). The pores of this foam were somewhat larger than the 4 mm thick foam from Example 1, this may be described by more coalescence due to decreased viscosity of the chitosan because of the ionic strength of the solution.

[0088] Foam discs, from the foam molded in 4 mm high trays, were stamped out with use of a cork borer with a diameter of 2.1 cm. A dry foam disc was the placed on a Bohlin CVO 120 High Resolution Rheometer between serrated plates (PP25). Then 500 μl of a 1% alginate (PRONOVA UP LVG) solution was added with use of a pipette. The calcium content in the foam disc is enough to saturate gelling residues of the added alginate by 96%. After one minute the alginate solution is absorbed and the foam is close to be fully hydrated. The upper plate was lowered to 1.000 mm gap and measurements of the elastic modulus, G', were initiated. The frequency, strain and temperature were set to 1 Hz, 0.001 and 20° C. respectively. The results are presented in table 2.

TABLE 2

The elastic modulus, G', as a function of time for chitosan foams added alginate solution and water (n = 3).

Time, [min]	Elastic modulus, G' ± SD [Pa] (alginate)	Elastic modulus, G', ± SD [Pa] (water)
1	4987 ± 5	470 ± 16
2	5867 ± 40	501 ± 13
3	6346 ± 15	516 ± 15
4	6653 ± 72	515 ± 9
5	6850 ± 64	529 ± 17
7	7078 ± 65	531 ± 23
9	7191 ± 76	532 ± 23
11	7216 ± 122	536 ± 21
12	7260 ± 120	534 ± 21

[0089] The high value of G' for the foam discs added alginate solution and the increase in G' during the minutes just after addition, confirms donation of gel-forming ions to the added alginate solution from the chitosan foam.

Example 5

[0090] This example shows that a chitosan foam containing gelling ions will have the ability to induce gelling of an externally added chitosan solution in situ.

[0091] Foam disks (diameter=2.1 cm) were stamped out with use of a cork borer from the foam cast in the 4 mm high mold presented in Example 3. A foam disk was then placed on the serrated plate on the same rheometer as used in previous example. The disk was then added excess solution of either MQ-water or a 1.0% solution of chitosan (PROTASAN UP CL 213). The upper plate (PP25) was lowered to a gap of 500 μm and a stress sweep was performed with an applied shear stress from 0.5 Pa to 50 Pa. The oscillation measurements were initiated about three minutes after addition of solution. The frequency was set to 1 Hz. The sweep was performed two times for each foam patch. The elastic modulus, G', read in the linear viscoelastic region (G'_{lin}) and the phase angle are reported in Table 3.

TABLE 3

G'_{lin} and phase angle measured for cross linked chitosan foams added water and chitosan solution.

Solution added	G'_{lin} ± SD, [Pa]	Phase angle, [°]
MQ-water	502 ± 65	24.6 ± 0.3
1.0% chitosan solution	777 ± 29	17.6 ± 4.1

[0092] Based on both the elastic modulus and the phase angle indicate the results in the table a more gel like properties of the foam after addition of chitosan solution.

Example 6

[0093] This example shows how the mixing time and amount of air incorporated into the chitosan foams affects different foam properties.

[0094] Chitosan foams were prepared as described in Example 1 except different mixing times were used to obtain different foam densities. All foam ingredients for creating a wet foam was mixed at medium speed for 1.5 minutes. Then mixing at high speed was continued for 1 minute with a resulting wet density 0.45 g/ml. About half of the foam was cast in 4 mm and 2 mm high molds. Then the remaining foam was mixed at high speed for one additional minute. The resulting wet density was 0.29 g/ml and the rest of the foam was cast as above. A similar procedure as above was repeated except for the mixing times at high speed were first 45 seconds and the second 4 minutes and 45 seconds. The wet densities were 0.52 g/ml and 0.18 g/ml respectively. The two foams with highest wet densities got a thin film created at the surface against the mold. This is due to coalescence of the pores as the foam dries more slowly near the bottom. The dry foam density was determined by stamping out disks, from the foam cast in the 4 mm high mold, with a diameter of 1 cm with use of a cork borer and weighing them. The densities and thickness measured by a caliper of the different foams are presented in table 4. The foams were also characterized by its elastic modulus, G'_{lin}, with the same rheometer settings as described in Example 5 except the range of applied stress was 0.5 Pa to 18 Pa, and that three sweeps for each foam piece were performed. The results are included in table 4, presenting the average values of the two last sweeps for three different foams with a diameter of 1 cm. The foam pieces were kept in 2 ml Hanks' solution about five minutes before they were transferred to the rheometer. The tensile strength of the dried foams was measured with use of a SMS Texture Analyzer and A/TG tensile grips. The force required stretching the foam at 0.5 mm/s until breakage was read and maximum force and distance stretched when it ruptured are reported in table 4.

[0095] The foam pieces were bone-shaped cut with use of a scalpel with the dimensions; 3.15 cm long, 1.75 cm wide at the ends and 1.25 cm wide in the center, the narrowing start 1 cm from the ends. The foam was cut in this shape to ensure breakage in the middle of the foam and not where it was attached to the grips. Approximate 0.3 cm of each end of the foam piece was used to fasten it to the grips.

TABLE 4

Chitosan foams of different density and their properties. (n = 3, ± SEM)
(The foam with wet density of 0.23 g/ml is the foam from Example 1)

Foam wet density, [g/ml]	Foam dry density, [mg/cm^2]	Thickness, [mm]	Tensile strength, [g]	Distance before rupture, [mm]	G'_{lin}, [Pa]
0.52	24.8 ± 0.2	2.4	138 ± 10	20 ± 2	133 ± 23
0.45	22.4 ± 0.7	2.5	148 ± 8	14 ± 2	115 ± 6
0.29	17.4 ± 0.4	3.2	79 ± 1	5.1 ± 0.2	55 ± 3
0.23	15.4 ± 0.2	3.4	60 ± 1	6.6 ± 0.3	51 ± 1
0.18	12.5 ± 0.3	3.7	49 ± 1	6.7 ± 0.5	9 ± 1

[0096] The table shows that the foams with the highest wet densities collapsed most due to coalescence. It was also observed that the foams had increasing pore size by increasing wet density. The tensile strength and the elastic modulus decreased by increased amounts of air. Also the elasticity of the materials presented as the length the material could be stretched before it ruptured decreased by decreasing wet density. The three less dense materials had about the same elasticity.

Example 7

[0097] This example describes foam preparation with use of a foaming agent alternative to the one used in the previous examples.

[0098] A chitosan foam was made with the same ingredients as described in Example 1, except an aqueous albumin solution (0.25 g/ml) replaced HPMC as the foaming agent. The amounts of chitosan solution and plasticizers were the same as in Example 1, and to this blend 75.0 g MQ-water and 5.0 g albumin solution were added. The mixing started at medium speed for one minute and continued at high speed for six minutes. Then 2 ml albumin wad added and mixing continued at high speed for two minutes. The addition of 2 ml albumin solution and two minutes mixing was repeated another six times until 19 ml albumin solution was added. The resulting foam had a wet density of 0.34 g/ml. The foams dried in the 2 mm and 4 mm high mold were dried 1 hour and 2 hours respectively at 80° C.

[0099] The dry density of the foam cast in the 4 mm high mold was measured, as described in Example 6, to be 21.3 mg/cm^2±0.2 mg/cm^2. The tensile strength and elasticity described by distance stretched before rupture, measured as described in Example 6 were respectively 104 g±5 g and 33.4 mm±0.8 mm.

Example 8

[0100] This example describes the preparation of a hyaluronic acid (HA) foam with calcium ions incorporated. Also the foams ability to donate these ions to induce gelling of an externally added alginate solution is shown.

[0101] An aqueous solution containing 2.5 % HA was prepared and set aside. 49.65 g MQ-water, 2.35 g $CaCl_2$*$2H_2O$ and 10.5 g sorbitol (dry) were added a mixing bowl and the dry ingredients were dissolved by gently swirling the bowl. 130 g of the HA solution, 4.5 g glycerin and 3.0 g HPMC were added to the same mixing bowl. The dispersion was then blended with a Hobart kitchen aid mixer equipped with a wire whisk at medium speed for two minutes to ensure homogeneity. The mixing continued at high speed for 3 minutes and 50 seconds. The wet density was measured to be 0.21 g/ml (determined from the weight of wet foam required to fill a 100 ml container). The wet foam was cast in 2 mm and 4 mm high molds coated with Teflon and then placed in a drying oven at 80° C. for 50 minutes.

[0102] With use of a cork borer foam disks (diameter=2.1 cm) were stamped out from the foam cast in the 4 mm high mold. A 1.0% and 0.5% alginate solution was prepared from PRONOVA SLG 20 (batch: 221105) by addition of MQ-water. A dry foam disk was placed on a Bohlin CVO 120 High Resolution Rheometer between serrated plates (PP25). Then 350 μl of the alginate solution was added with use of a pipette. The calcium content in the foam disk is enough to saturate gelling residues of the added alginate by 124% and 248% for the 1.0% and 0.5% solution respectively. After one minute the alginate solution is absorbed and the foam is close to be fully hydrated. The upper plate was then lowered to 500 μm gap and measurements of the elastic modulus, G', was initiated. The frequency, strain and temperature were set to 1 Hz, 0.001 and 20° C. respectively. The results are presented in table 5.

TABLE 5

Elastic modulus, G', as a function of time after addition of water and alginate solutions to the HA foam with calcium ions incorporated.

	Elastic modulus, G', [Pa]		
Time, [min]	MQ-water	0.5% alginate	1.0% alginate
2	26	743	1665
4	31	660	2153
6	27	698	2544
8	25	750	3003
10	25	816	3322
15	—	1003	4167
20	—	1193	4732
25	—	1355	5608
30	—	1591	6292
35	—	1867	6602

[0103] The increase of G' during the minutes just after addition of the alginate solution, confirms donation of gelling ions and that a gelling reaction have been initiated. The difference in G' value between the three solutions confirms that a gel is being created and that he strongest gel is created from the most concentrated alginate solution.

Example 9

[0104] This example describes the preparation of a HA foam with phosphate ions incorporated. Also the foams ability to donate these ions to induce gelling of an externally added chitosan solution is shown.

[0105] The HA foam was made as described in Example 8, except that the calcium source was replaced with 2.27 g Na_2HPO_4 and the amount of water used was 49.7 g. The mixing time at high speed was 3 minutes with gave a wet density of 0.17 g/ml. The foams cast in 2 mm and 4 mm molds were kept in the drying oven at 80° C. for 45 min and 75 min respectively.

[0106] The same parameters for rheological measurements as described in Example A were used. Water and 1.0% chitosan solution was added in excess amount. The values describing the elastic modulus, G', and phase angle flattened off at the values presented in table 6.

TABLE 6

Elastic modulus, G', and phase angle of rehydrated HA foams with phosphate ions incorporated.

Solution added	Elastic modulus, G', [Pa]	Phase angle, [°]
1% chitosan solution	76	22
MQ-water	18	46

[0107] The results indicate that the foam added chitosan solution gets a more gel like behavior and is stiffer than the foam added MQ-water.

1. A method for forming a dried absorbent foam having an open pore network and pores by:

a. forming a wet foam from an aqueous dispersion comprising a polysaccharide; a foaming agent; optionally a plasticizer; optionally a crosslinking agent; optionally gel-forming ions; optionally one or more additives, and water;

b. mixing a foam from the aqueous dispersion, optionally by mechanical agitation;

c. molding or shaping the wet foam and optionally forming a crosslinked foam; and

d. drying the foam to form a dried foam optionally by air drying and optionally further molding, shaping or compressing the dried foam.

2. A method for forming a dried absorbent foam comprising an open pore network and gel-forming ions by:

e. forming an aqueous dispersion comprising a polysaccharide; a foaming agent; gel-forming ions, optionally a plasticizer; optionally a pH modifier; optionally one or more additives and water;

f. preparing a foam from the aqueous dispersion; and

g. drying the foam to form a dried open pore foam containing pores and gel-forming ions;

in which the amount of gel-forming ions added will saturate less than 25% of the gelling sites of the polysaccharide.

3. A method for forming a dried absorbent foam comprising an open pore network and gel-forming ions suitable for gelling a subsequently added polysaccharide solution by:

a) forming an aqueous dispersion comprising a polysaccharide, a foaming agent, at least one gelling ion which does not gel the polysaccharide, optionally a plasticizer, optionally a pH modifier, optionally one or more additives and water;

b) forming a foam from the aqueous dispersion; and

c) drying the foam to form a dried foam containing open pores and gel-forming ions suitable for gelling a subsequently added polysaccharide solution.

4. A method for forming a dried absorbent foam having an open pore network and pores by:

a) preparing an aqueous dispersion comprising an enzymatically biodegradable biopolymer or polysaccharide and a foaming agent and optionally one or more of gel-forming ions, a plasticizer, a crosslinking agent and a pH modifier;

b) forming, preparing or mixing a foam, preferably a wet foam, from the aqueous dispersion, optionally by mechanical agitation;

c) optionally molding or shaping the foam and optionally forming a crosslinked foam; and

d) drying the foam to form a dried foam containing open pores.

5. A method according to claim 4 in which the polysaccharide or biopolymer is selected from chitosan, modified chitosan, ultrapure chitosan, ultrapure modified chitosan, hyaluronate, ultrapure hyaluronate, modified hyaluronate, modified ultrapure hyaluronate or mixtures thereof.

6. A method according to claim 5 in which the polysaccharide or biopolymer is chitosan, hyalouronate or a mixture therof.

7. A method according to claim 5 in which the polysaccharide or biopolymer comprises chitosan having a degree of deacetylation from 40% to 100%.

8. A method according to any one of claim 5 in which the polysaccharide or biopolymer comprises chitosan having a molecular weight of at least 10 kDa.

9. A method according to claim 8 in which the chitosan has a molecular weight in the range of 10 kDa range 1000 kDa.

10. A method according to claim 4 in which the foaming agent comprises a polymeric foaming agent.

11. A method according to claim 4 in which the foaming agent comprises a biologically-acceptable foaming agent for use with the human or animal body.

12. A method according to claim 4 in which the foaming agent comprises a polymeric, biologically-acceptable foaming agent for use in the human or animal body which is substantially free of non-polymeric surfactant.

13. A method according to claim 4 in which the foaming agent is selected from hydroxypropyl methyl cellulose and albumin.

14. A method according to claim 4 comprising crosslinking the foam.

15. A method according to claim 14 wherein the crosslinking is ionic or covalent.

16. A method according to claim 4 in which gel-forming ions are added to the foam during or subsequent to its

production, said gel-forming ions being capable of forming a gel with a subsequently added polysaccharide solution.

17. A method according to claim 16 further comprising the step of adding a polysaccharide solution to the foam comprising the gel-forming ions to form a gel in the pores of the foam.

18. A absorbent dried foam having an open pore network comprising:

i) an enzymatically biodegradable biopolymer selected from chitosan, modified chitosan, ultrapure chitosan, ultrapure modified chitosan, hyaluroate, ultrapure hyaluronate, modified hyaluronate, modified ultrapure hyaluronate or mixtures thereof; and

ii) a biologically-acceptable, polymeric foaming agent.

19. A foam according to claim 18 comprising sufficient gel-forming ions to gel a subsequently added polysaccharide, preferably chitosan or alginate solution.

20. A foam according to claim 18 wherein the polymeric foaming agent is selected from hydroxyl propyl methyl cellulose and albumin and, optionally, one or more other polymeric foaming agents.

21. A foam according to claim 18 which is enzymatically biodegradable.

22. A foam according to claim 18 which has been sterilized by alcohol treatment, ethylene oxide, gamma irradiation, e-beam NO_x or autoclaving.

23. A foam according to claim 18 in which the dried absorbent foam structure is produced by freeze drying.

24. A foam according to claim 18 in which the absorbent structure is produced by spinning a biopolymer fiber and then knitting or weaving or layering said fibers to form a felt.

25. A foam according to claim 18 in which the absorbent structure comprises a woven or nonwoven fiber of the biopolymer.

26. A foam according to claim 18 comprising a plurality of layers, wherein at least one layer comprises an open pore network and comprises gel-forming ions suitable to induce gelling of an added polysaccharide solution.

27. A foam according to claim 18 comprising a layer comprising chitosan, modified chitosan, ultrapure chitosan, ultrapure modified chitosan, hyalouroate, modified hyalouronate or mixtures thereof and, adhered to the layer, a second layer comprising an alginate foam.

28. A composite comprising an absorbent foam according to claim 18 wherein the foam comprises gel-forming ions selected from calcium, barium and strontium ions or mixtures thereof and a polysaccharide gel.

29. A composite according to claim 28 in which the polysaccharide gel comprises an alginate, a pectin, a carrageenan, a modified alginate, a peptide coupled alginate, or a mixtures.

30. Use of a foam according to claim 18 or a composite according to claim 28 as a matrix for cell immobilization and/or proliferation for an in vitro tissue culture application or an in vivo tissue engineering application.

31. Use of a foam according to claim 18 or a composite according to claim 28 wherein the foam or composite further comprises an therapeutic agent to provide in vivo or topical controlled release of the active agent into a human or animal body.

32. Use of a foam according to claim 18 or a composite according to claim 28 in managing a wound to provide in vivo or topical wound management.

33. Use of a foam according to claim 18 or a composite according to claim 28 as an antibacterial barrier.

34. Use of a foam according to claim 18 or a composite according to claim 28 as a hemostatic agent.

35. Use of a foam according to claim 18 or a composite according to claim 28 as a matrix for cell immobilization and/or proliferation for in vitro tissue culture applications in vivo tissue engineering applications.

36. A method for inhibiting cell proliferation comprising forming a foam according to claim 18 or a composite according to claim 28 wherein the foam or the composite comprises strontium ions and cells.

37. A method for attachment of a composite according to claim 28 to tissue by fastening a foam according to claim 18 to said tissue, said foam having open pores and gel-forming ions and adding a liquid comprising a soluble polysaccharide and reacting the polysaccharide with the gel-forming ions.

38. A method for preventing adhesion of tissue to adjacent tissue comprising applying to the tissue a foam according to claim 18 or a composite according to claim 28 such that it provides a barrier between the tissue and the adjacent tissue.

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