BIOMATERIALS UTILISED IN MEDICAL TEXTILES: AN OVERVIEW

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ABSTRACT

An overview of the classification of textile fibres (both natural and man-made) is provided, along with information on the chemical structures, forms and properties of biomaterials (natural and modified carbohydrate polymers and proteins) utilised in medical textiles. The major carbohydrate polymers (and their derivatives) discussed include cellulose, dextran, chitin, chitosan, alginate, and hyaluronan. The major protein discussed is collagen (and gelatin). Examples of commercial products composed of such biomaterials are also presented. Finally, the future of the medical textiles industry with respect to wound healing, controlled release, smart technologies, etc, is discussed.

TEXTILE FIBRES AND THEIR CLASSIFICATION

'Textile' originally applied to only woven fabrics, but now includes fibres, filaments and yarns made from natural and synthetic materials (and products utilising such raw materials) [1]. Textile fibres are broadly classified as natural or man-made.

Natural Fibres

Natural fibres refer to fibres that occur naturally in nature, and are found in animals, vegetables and minerals, as detailed in Figure 1. Animal-derived proteinaceous fibres are divided into silk (protein filaments forming silkworm cocoons and wild silk fibres extruded by selected other insect larvae), wool (fibrous covering of sheep, *Ovis aries*), and hair (animal fibre other than silk or sheep's wool), e.g. from alpaca (*Lama pacos*), camel (e.g. *Camelus bactrianus*), cow (common ox, *Bos Taurus*), goat (genus *Capra*, including mohair - angora goat, *Capra hircus aegagrus*, and cashmere - originally from the Asiatic goat, *Capra hircus laniger*), horse (*Equus caballus*), mink (*Mustela (Lutreola) vison*), angora rabbit (*Oryctolagus cuniculus*), vicuna (*Lama vicugna*), etc [1].

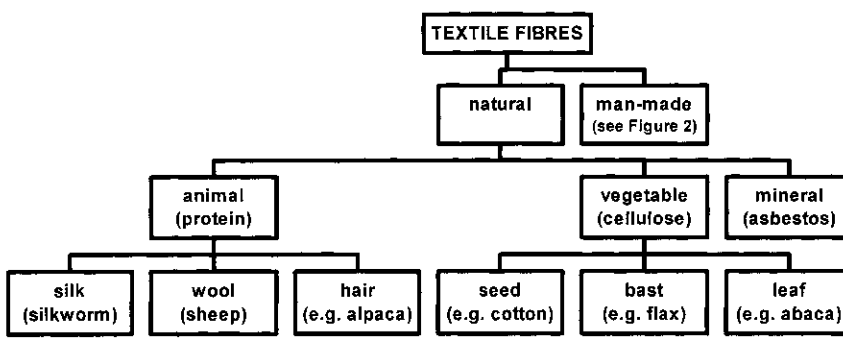

Figure 1. Classification of natural textile fibres [1].

Vegetable-derived fibres are subdivided into three classes, namely those isolated from seeds, bast, and leafs (Figure 1). Fibres can grow from the surfaces of some seeds and from the inner surfaces of some fruit cases or pods, and are formed by the marked elongation of epidermal cells. Cotton (from a wide variety of plants of the *Gossypium* family) is the most important natural seed fibre. Fibres from the bast layer of plants include flax (from *Linum usitatissimum*, used for making linen products), hemp (from *Cannabis sativa*), jute (from *Corchorus capsularis* and *Corchorus olitorius*), kenaf (from *Hibiscus cannabinus*), ramie (from the stems of *Boehmeria nivea*, which belongs to the *Urticaceae* or nettle family, and is in ribbon form and known as 'China Grass'), etc [1]. Fibres from plant leaves include abaca fibre (also known as manila fibre, from *Musa textilis*). Mineral-derived fibres include asbestos (a generic name used to describe a family of naturally occurring fibrous hydrated silicates.

Man-made Fibres

Man-made textile fibres are those that do not occur in nature, although they may be composed of naturally occurring materials. They are classified into three groups, namely synthetic polymers, natural polymers, and 'others' (Figure 2). Synthetic polymers covers fibres manufactured from chemically synthesised polymers such as acrylics (polyacrylonitrile), chlorofibres (e.g. PVC), fluorofibres (e.g. PTFE), nylons (polyamides), polyesters, polyolefins (e.g. polyethylene), polyurethanes, PVA, etc [1].

Natural polymers in man-made textile fibres are subdivided into alginates, rubber, regenerated protein (azlon), regenerated cellulose (rayon), and cellulose esters (Figure 2). Alginate fibres are composed of water-insoluble alginic acid and/or its salts, which are obtained from the cell walls of brown algae (*Phaeophyta*, such as the seaweeds *Laminaria* & *Ascophyllum* species), and are discussed in more detail in a subsequent section [2,3]. Rubber covers fibres in which the fibre-forming substance consists of natural or synthetic rubber, including amorphous polyolefins, diene-hydrocarbon copolymers, elastodiene, lastrile, polybutadiene, polychloroprene and its copolymers, etc [1,4].

A regenerated fibre is formed from a solution of a natural polymer or of a chemical derivative of a natural polymer and has the same chemical constitution as the natural polymer from which the solution or derivative was made. Azlon is a manufactured fibre in which the fibre-forming substance is composed of any regenerated naturally occurring protein [1]. Casein is the principal protein in milk, and has served as the raw material for some animal-derived regenerated protein fibres. Fibres can also be produced from regenerated vegetable-derived protein (such as zein) [5]. Rayon is the generic name for manufactured regenerated cellulose fibres (as well as those with no more than 15% of hydroxyl groups derivatised). Regenerated cellulose subdivisions include viscose, cupro, modal, lyocell and deacetylated acetate (Figure 2) [1].

Viscose fibre is produced by the viscose process, which involves the production of a cellulose xanthate solution (viscose) [6]. Cellulose xanthate is an alkali-soluble salt formed by the reaction of cellulose and carbon disulphide in the presence of strong alkali. Cupro (or cupra) fibre is obtained by the cuprammonium process [6]. Modal fibre is a regenerated cellulose fibre obtained by processes giving a high breaking strength and a high wet modulus. This includes polynosic fibres, characterised by a high initial wet modulus of elasticity and a relatively low degree of swelling in sodium hydroxide solution [1]. Lyocell fibre is a regenerated cellulose fibre obtained by extruding cellulose dissolved in organic solvent [1]. The viscose and cuprammonium processes, and cellulose solubilisation methodologies are discussed later.

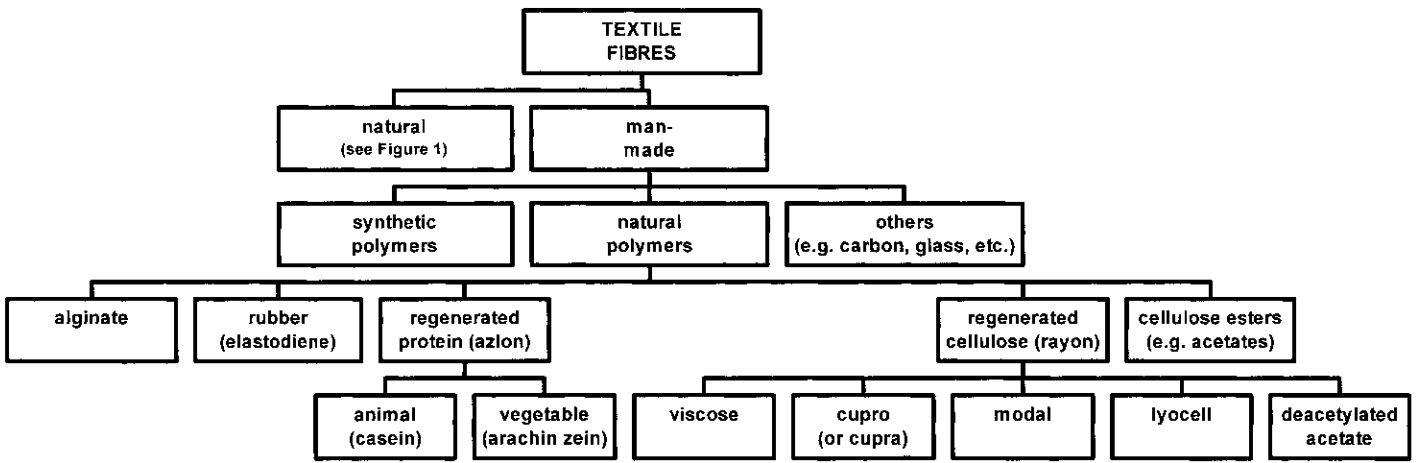

Figure 2. Classification of man-made textile fibres [1].

Deacetylated acetate fibre is a regenerated cellulose fibre obtained by almost complete deacetylation of cellulose acetate [1]. Cellulose esters, including acetates, are discussed in more detail in a subsequent section. The 'others' classification covers naturally occurring non-fibrous materials, such as carbon, glass, metal, ceramics, etc [1]. Carbon fibres are composed at least 90% carbon and are obtained by controlled pyrolysis of appropriate fibres [1]. Glass filaments or fibres are obtained by drawing molten glass, extrusion, or other spinning processes, and are described as inorganic products of fusion which have cooled to a rigid condition without crystallising [1]. Glass fibres used in textiles consist of vitreous silicates or borosilicates. Metal/metallic fibres covers fibres made from any metal, plastic-coated metal, metal-coated plastic, or a core completely covered by metal [1]. Ceramic fibres are refractory fibres composed of a metal oxide, metal carbide, metal nitride, or their mixtures. Alumina and silica are the most commonly used. In this context silicon and boron are regarded as metals. Ceramic fibres are used in electrical, thermal and sound insulation, in high temperature filtration, and as reinforcement in some composites [1].

NATURAL CARBOHYDRATE POLYMERS

All living organisms (e.g. plants, animals and microorganisms) contain and/or produce significant quantities of carbohydrate polymers (polysaccharides), which are naturally occurring biomolecules that perform a number of different functions, e.g. as structural components, energy reserves, lubricating agents, etc [7,8]. Polysaccharides are high molecular weight condensation polymers composed of monosaccharides which can be neutral, basic, acidic, or combinations thereof, making the polysaccharide neutral, basic, acidic, or possessing the ability to have mixed/variable charges according to its environment. Homo-polysaccharides are composed of a single type of monosaccharide, whilst hetero-polysaccharides contain two or more different monosaccharides. Polysaccharide structures can also be linear or branched. Linkage configuration and position plays an important part in determining three-dimensional structure [7,8].

D-Glucans (Cellulose, Dextran, etc)

The D-glucan homopolysaccharides are widely distributed amongst plants, animals and microorganisms. With four different glycosidic linkages ((1→2), (1→3), (1→4) and (1→6)) and two configurations (α and β), a wide variety of polysaccharide forms are possible, with both linear and branched structures, the latter being sequential or random in nature [7,8]. Such structural diversities, along with differences in molecular weight distribution and resultant molecular shape/size give rise to a range of differing physicochemical characteristics [7,8].

Cellulose is a linear structural polysaccharide composed of (1→4)-α-D-Glc*p* residues (Figure 3) and its major sources are cotton and wood pulp. It is rigid, highly crystalline, and is therefore difficult to solubilise [2,7,8]. Dextrans are essentially linear (1→6)-α-D-glucans (Figure 3), which differ only in chain length and degree of branching. Branching occurs via (1→3)- or (1→4)- α-D-Glc*p* linkages (and much less frequently by (1→3)-α-D-Glc*p* linkages) [2,7,8]. Many bacteria synthesise dextran from sucrose, *Leuconostoc mesenteroides* and *Leuconostoc dextranicum* are used commercially. Native dextrans have high molecular weights, whilst clinical dextrans are produced by synthesis or controlled degradation of native dextrans, thus having lower molecular weights [9,10]. Most of the physical and associated pharmacological properties of dextran fractions are dependent upon their molecular weight distribution.

(1→3)-β-D-glucans are present in most plants and microorganisms and occur as major structural or storage components [11]. A specific (1→3)-β-D-glucan that has been examined in some detail is lentinan, which also contains (1→6)-β-D-Glc*p* branches, and has antitumour activity, suppressing chemical and viral oncogenesis and reportedly preventing cancer recurrence or metastasis after surgical intervention [2,11]. There is also evidence that such (1→3)-β-D-glucans are also able to increase the host resistance to bacterial, viral and parasitic infections. A bio-artificial skin has been produced from gelatin and (1→3), (1→6)-β-D-glucan (from *Schizophyllum commune*) [12].

Chitin (and Chitosan)

Chitin is found in the outer shell of crustaceans. It is a (1→4)-β-D-glycan composed of 2-acetamido-2-deoxy-β-D-glucopyranose residues (N-acetyl-D-glucosamine residues, Figure 3) [2]. Chitosan is the name given to the partially deacetylated form of chitin and is therefore composed of 2-amino-2-deoxy-β-D-glucopyranose residues (D-glucosamine residues, Figure 3) [2,13]. Chitosan is biocompatible [14,15] (since its degradation products are natural metabolites) and can be produced in powder, film, bead, fibre and fabric forms via a range of different techniques [16-19]. Many of chitosans properties rely on its cationic nature, which allows it to interact with negatively charged biomolecules such as proteins, anionic polysaccharides and nucleic acids, many of which are located in skin. Chitin/chitosan is used in a broad range of orthopaedic, periodontal, tissue engineering, wound healing and drug delivery applications [20].

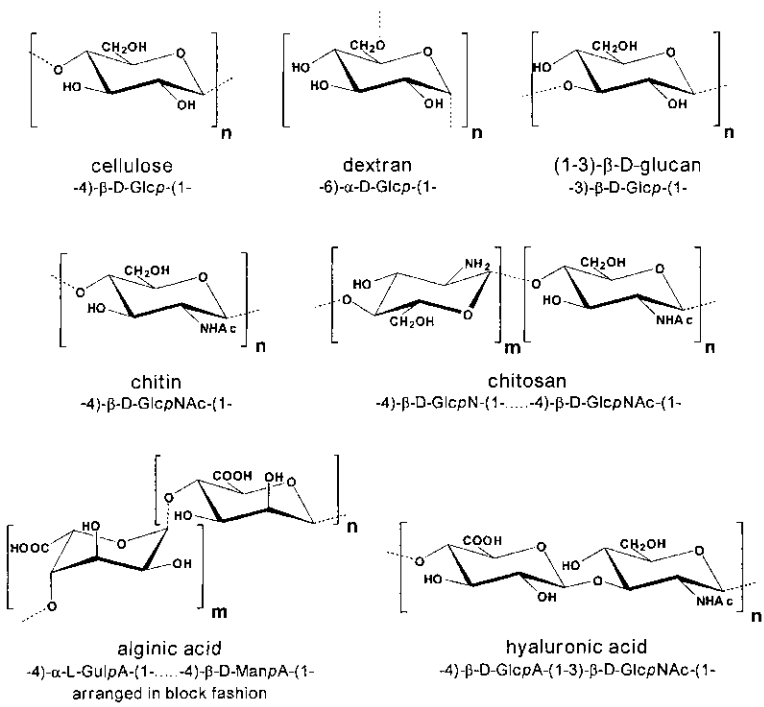

Figure 3. Neutral, basic and acidic polysaccharides used in medical textiles [2].

Alginate / Alginic Acid

The seaweed polysaccharide alginic acid is a linear block copolymer composed of two uronic acid residues, namely D-mannuronic and L-guluronic acid (Figure 3) [2,21]. The distribution of the uronic acids along the chain is non-random and involves relatively long sequences of each uronic acid. In the presence of divalent cations, such as calcium, alginate gels can be formed due to ionic cross-linking via calcium bridges between L-guluronic acid residues on adjacent chains [22].

Alginates have historically been known to have a haemostatic function and to be capable of absorbing specific solutes. Calcium alginate gels have a large pore size and high water absorbency making them potentially useful as hydrogel dressings. Hydrophilic sponges (xerogels) produced from calcium alginate are reported to have good absorptive properties for both blood and wound exudate [23].

Alginate fibres are generally prepared by injecting a solution of water-soluble alginate (usually sodium alginate) into a bath containing an acidic solution or calcium salt solution to produce the corresponding alginic acid or calcium alginate fibres, which can be used to produce yarns and fabrics for medical applications [17,21,22,24,25], and as drug carriers for wound healing [26].

Hyaluronan / Hyaluronate / Hyaluronic Acid

Hyaluronic acid is a naturally occurring polysaccharide, which is widely distributed in the connective tissue and vitreous and synovial fluid of mammals. It acts as a lubricant and shock-absorbing material in the fluid of joints [27,28]. It is a linear polysaccharide consisting of a disaccharide-repeating unit containing D-glucuronic acid and 2-acetamido-2-deoxy-D-glucose (linked as shown in Figure 3) [2,27,28].

Hyaluronan has a very high molecular weight, affording very viscous aqueous solutions, even at low concentrations. Commercial sources include Cock's combs, human umbilical cords and fermentation (*Streptococcus equi*). Biologically, it is far more than just a high viscosity space filler, since it is capable of interacting with a wide range of biomolecules, including tissue components, proteins, proteoglycans, growth factors, etc [27,28].

Sulphated Polysaccharides

The group of naturally occurring sulphated polysaccharides including heparin, chondroitin (sulphate), dermatan sulphate and keratan sulphate (Figure 4) [2,27] exhibit extensive biological activity. It is proposed that their biological activity is due to their anionic nature. They will undoubtedly find greater application in medical textiles in the not too distant future.

Complex Heteropolysaccharides

Research is now focusing on the suitability of more complex polysaccharides for use in wound management aids. An example of this is branan ferulate, a substituted arabinoxylan isolated from high fibre corn bran by alkaline extraction (Figure 5) [28,29]. Branan ferulate has also been incorporated into alginate fibres [25].

The ferulate ester groups in branan ferulate are enzymically cross-linked (using a peroxidase / hydrogen peroxide system) to form a commercial hydrogel product, Sterigel® (SSL International), which is used as a wound management aid [30].

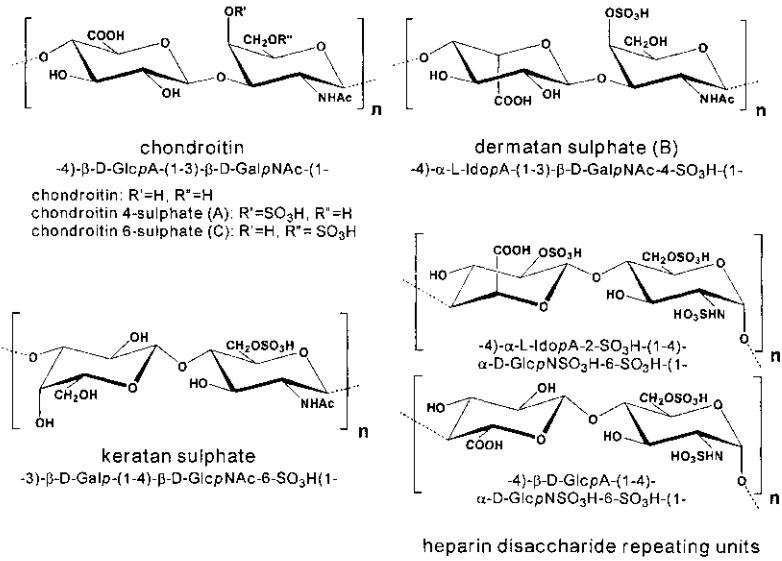

Figure 4. Sulphated polysaccharides with medical textiles potential [2].

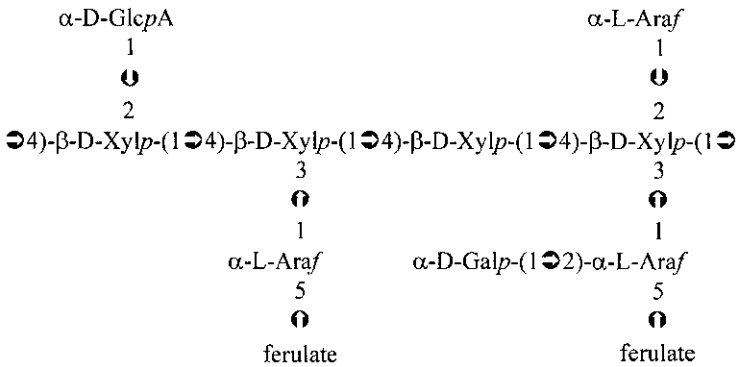

Figure 5. Structural features / monosaccharide configuration in branan ferulate [31].

MODIFIED CARBOHYDRATE POLYMERS

Polysaccharides contain a relatively broad range of functional groups (e.g. primary and secondary hydroxyl groups, free amino groups and carboxylic acid groups, etc.), which can be utilised as sites for chemical or enzymatic modification/derivatisation [32]. Therefore, naturally occurring polysaccharides can be modified in order to alter their physicochemical characteristics, which may result in a material with enhanced applicability for a specific application. The most common types of modification include esterification, etherification, oxidation, cross-linking (to increase molecular weight), and controlled hydrolysis (to reduce molecular weight) [32].

Cellulose Solubilisation

Cotton cellulose fibres are generally unsuitable for direct biomedical application in their native form (other than in simple woven dressing form) due to their inflexibility and/or lack of solubility in water (and most organic solvent systems). In order to overcome these problems methods of processing and/or chemical modification have been developed to produce flexible fibres and water-absorbing swellable derivatives [6,33-37]. Cellulose dissolution is an important aspect of both existing and potential applications of cellulose [38]. Solvents for cellulose include metal/amine solvents such as cuprammonium hydroxide (cuam) and cupriethylenediamine hydroxide (cuen) systems, ammonia/ammonium thiocyanate (NH_3/NH_4SCN), lithium chloride/dimethylacetamide (LiCl/DMAc), amine oxides (such as *N*-methylmorpholine-*N*-oxide), dinitrogen tetroxide/dimethylformamide (N_2O_4/DMF), and dimethylsulphoxide/paraformaldehyde (DMSO/PF), the latter two resulting in the formation of a cellulose derivatives, namely cellulose nitrite and methylol cellulose, respectively [38]. In the cuprammonium process cellulose is solubilised in basic copper sulphate ($CuSO_4.3Cu(OH)_2.1H_2O$ plus NaOH), or copper hydroxide, in ammonium hydroxide, giving cupritetramine hydroxide ($[Cu(NH_3)_4](OH)_2$) and spun into a bath containing warm (30-50°C), soft, air-free water. The filaments are then run through dilute acid and washed with water [6].

The Viscose Process

The production of a spinnable viscose solution involves the conversion of cellulose into alkali cellulose by steeping in sufficiently strong sodium hydroxide (mercerisation), the oxidative depolymerisation of the alkali cellulose (aging), conversion of the aged alkali cellulose into sodium cellulose xanthate with carbon disulphide (xanthation), dissolving the xanthate in dilute alkali to give viscose, filtration, de-aeration, and ripening of the viscose solution [6,49]. During ripening the DS decreases from ~ 0.5-0.7 as a function of time, and the xanthate groups undergo a redistribution to mainly the C6 position (trans-xanthation). When the required DS has been achieved the viscose solution is spun into regenerated cellulose fibres by coagulation/decomposition by injection into a bath containing sulphuric acid and sodium sulphate [6].

Cellulose Derivatisation

An enormous number of cellulose derivatives have been synthesised and characterised over the years [38,39]. The main classes of commercially produced cellulose derivatives are cellulose esters and cellulose ethers. Esterification is often performed using organic acids, anhydrides, or acid chlorides [37,40,41], a general reaction scheme is provided in Figure 6. Industrial modification processes normally take place in heterogeneous systems, i.e. both the cellulose substrate and the produced derivative are present as solids, either as dry matter or suspended in the reaction medium. Thorough mixing/stirring is often required to ensure uniform swelling, alkali distribution, and production of a homogeneous product (with respect to random distribution of added functionality). Non-random derivatisation can result in poor product solubility due to underivatised regions in the final product. Cellulose derivatives are among the oldest industrially exploited substances. Derivatives such as nitrocellulose (guncotton, collodium), cellulose ethers (e.g. carboxymethylcellulose, CMC), and cellulose esters (e.g. cellulose acetate and mixed organic cellulose esters) have been produced on an industrial scale for many years [37,42].

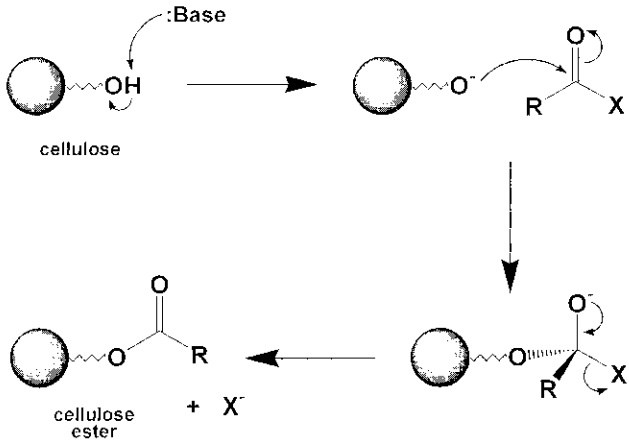

Figure 6. Cellulose esterification using acids, anhydrides, or acid chlorides [38].

A wide variety of cellulosic ethers have been synthesised and have broad ranging industrial applications [43-45]. Carboxymethylation of cellulose produces a range of cold-water soluble products, with high viscosity and polyelectrolyte behaviour (due to its anionic nature). CMC has a number of applications in the medical device and pharmaceutical area [17,22,25,27-29]. A variety of cellulose esters have been produced industrially. Cellulose acetate is recognised as the most important organic ester of cellulose due to its extensive applications in fibres, plastics and coatings. Cellulose acetate is produced by reacting high purity cellulose with acetic anhydride, using acetic acid as the solvent and sulphuric acid as catalyst. Applications of such derivatives rely on solubility in solvent systems, which is linked to degree of substitution (DS) [46].

Cellulose acetates of specific DS can be produced either by acetylation cellulose [47] or by deacetylation of a cellulose acetate with a higher DS (usually cellulose triacetate) [48]. The latter approach results in a more homogenous product, in terms of relative acetylation of the C2, C3 and C6 hydroxyl groups. In contrast, the former approach produces materials that are dependent upon the form/structure of the cellulose starting material since acetylation of the most accessible hydroxyl (C6) predominates. Materials of similar average DS produced by these different methods have different properties. Material with an acetate content of ~ 36-42 % (DS ~ 2.1-2.7) produced by the latter method is acetone soluble, but not if produced by the former method. Fibres are produced from both cellulose triacetate and acetone soluble cellulose acetate [6].

Cross-linked Dextran

Dextran appears to have beneficial activity in wound treatment but its high solubility is limiting. Reduction in solubility is achieved by making an emulsion polymerisation using epichlorohydrin as cross-linking agent to produce insoluble beads that swell in water. Such beads can be used in the treatment of skin lesions, to absorb wound exudate from secreting wounds with an associated reduction in wound healing time, and have been reported to assist the wound management process by stimulating macrophages [50].

Chitosan (and Chitin) Derivatives

It has been shown that in the area of wound healing, chitosan and chitosan derivatives can reduce scar tissue (fibroplasia) by inhibiting the formation of fibrin in wounds. Chitin, chitosan and chitosan derivatives affect macrophage activity, which will influence the wound healing process [51-53]. The biomedical applications of chitosan require some physicochemical properties that chitosan itself does not possess, e.g. dissolution in water, gel-forming ability, etc. Modification is therefore required to make materials that in contact with body fluids locally form gels and then dissolve, such as *N*-carboxybutyl chitosan [54,55]. Films and fibres can be produced from dibutyrylchitin, produced by esterification of chitin with butyric anhydride, which can also be used to produce regenerated chitin fibres [56,57]. Chemical modifications of the amino group and both primary and secondary hydroxyl groups are possible [13,55]. A commercial artificial skin material has been produced from chitin fibres [58].

Hyaluronan and Hylans

There are some limitations with respect to the direct use of hyaluronan in wound management due to its solubility, rapid resorption and short tissue residence time. Attempts have been made to overcome this by derivatisation, particularly esterification in the case of tissue engineering applications [59]. Hyaluronan derivatives of varying solubility (from water soluble to insoluble biodegradable derivatives) have been produced by use of different ester types and controlling the degree of esterification. Such derivatives can be manipulated by extrusion, lyophilisation and spray drying to produce different physical forms including membranes, fibres, sponges and microspheres.

The degree of esterification of benzyl and ethyl derivatives can be altered accordingly to produce materials that have a good absorbency capacity (> 100 % w/w) and that either dissolve rapidly or remain as a semi-solid hydrogel for long periods. Hyaluronic acid ethyl ester treated wounds end to accumulate collagen more slowly during the healing process, i.e. has a positive effect on scar formation [60].

Such materials have potential as tissue engineering supports where a biodegradable matrix is required to enable effective regeneration of skin in full-thickness burn injuries. Cross-linked hyaluronan derivatives are generally referred to as hylans. Water-insoluble soft hylan gels are suitable as viscosurgical implants to prevent postoperative adhesions and to control scar formation [61]. Hyaluronan and chondroitin sulphate have been chemically cross-linked and made into hydrogel films with potential wound healing application as bio-interactive dressings [62].

NATURAL AND MODIFIED PROTEINS

Proteins used in the formulation/production of medical textiles include collagen, gelatin, casein, zein, laminin and elastin. Collagen is a fibrous scleroprotein that is the major protein of connective tissue and the most abundant protein in higher animals (also being found inn skin, bones, teeth, tendons, cartilage, and blood vessels). Collagen has an unusual amino acid composition, with glycine (Gly) comprising approximately one-third, and proline (Pro) and 4-hydroxyproline (Hyp) comprising approximately one-third, of the total amino acid residues. The arrangement of the amino acids in the polypeptide chain gives collagen its strong secondary structure: a triple helix composed of three left-handed polypeptides helices twisted together to form tropocollagen [63,64].

Tropocollagen is the basic structural unit of collagen, with a molecular weight of ~ 300 kDa, and associates to form collagen fibrils, which associate to form larger fibres. Several types of collagen occur: Type I – the major adult form, widespread, lowest carbohydrate content, low hydroxylation of lysine; Type II – in cartilage, intermediate hydroxylation of lysine; Type III – in blood vessels and foetal skin, disulphide bonds, low hydroxylation of lysine; Type IV – in basement membranes, disulphide bonds, highest carbohydrate content, high content of hydroxylysine and hydroxyproline; Type V – in basement membranes, high content of carbohydrate and hydroxylysine [64].

The largest range of protein based medical textiles and wound dressings contain collagen [65]. Collagen in such dressings is in the form of extruded fibres, sponges and films [65]. Collagen peptides are thought to have a positive effect on collagen biosynthesis and collagen-based wound dressings can induce the spatial deposition of wound tissue [66]. A new product in the generation of biologic dressings is bilayered cellular matrix, (OrCel™), a collagen sponge supporting live human allogeneic skin cells. This bilayered composite of human cultured skin contains proliferating keratinocytes and fibroblasts and produces a variety of growth factors and cytokines [67].

Hybrid scaffolds with tissue repair potential have been produced from chitin and collagen [68]. Incorporation of hyaluronan into such collagen-containing dressings results in increased chemoattraction, replication and collagen deposition during wound healing [69]. Other carbohydrate polymer/collagen materials include oxidised regenerated cellulose/collagen sponges [70], and BGC matrix (Brennan Medical, Inc, St. Paul, MN) combines β-glucan with collagen in a meshed reinforced wound dressing [71].

Gelatin is produced by the thermal denaturation or acid/alkali degradation of collagen [64]. Carbohydrate polymer/gelatin materials of specific interest include Dextran dialdehyde cross-linked gelatin hydrogel films [72], and novel gelatin-alginate sponge artificial skin [73].

Elastin is a major scleroprotein of connective tissue, especially of the elastic tissue of tendons and arteries. It contains unusual covalent cross-links (demosine cross-link between four α-helical polypeptide chains), which form an elastic network that can stretch reversibly in any direction [64, 74].

Laminin is a large, complex, non-collagenous glycoprotein, synthesised by a variety of cell types, that is a major component of basement membranes, and is believed to be a critical adhesive protein of hepatocytes [64]. Hybrid peptides derived from laminin and elastin have been linked to alginate wound dressings [75].

Casein is a mixture of phosphoproteins (the most important being α-, β- and κ-casein) that are the principal proteins in milk [63,64].

Zein is the major storage protein of corn or maize (*Zea mays*), the only cereal crop indigenous to the Americas [5]. Commercial applications of zein include coatings for medical tablets [76], composite wound dressings [77], and controlled release [5]. Zein based textile fibres were sold in the 1950s under the name *Vicara* [5], and more recently cross-linked zein fibres have been produced by Du Pont [78-80].

COMMERCIAL APPLICATIONS AND PRODUCTS USING CARBOHYDRATE POLYMERS

Many commercial medical textile products (e.g. woven and nonwoven dressings) are made from natural polymers and their derivatives, the simplest being retention bandages, support and compression bandages, absorbents, gauzes, tulle dressings, and wound dressing pads produced from woven cellulose fibres (cotton & viscose) [27-29,81].

The primary cellulose derivatives produced for medical applications are biocompatible non-toxic esters and ethers (such as cellulose acetate and carboxymethyl cellulose) [82]. Commercial hydrogels containing cellulose derivatives include Intrasite® Gel (modified carboxymethyl cellulose, Smith & Nephew), Comfeel® (carboxymethyl cellulose, Coloplast, available in sheet, powder and pad form, Comfeel® Plus also contains Ca alginate) [83,84], Granugel® (pectin and Na carboxymethyl cellulose, ConvaTec), and Aquacel®, a hydrocolloid dressing containing carboxymethyl cellulose, ConvaTec) [81,85-87]. Surgicel® (Johnson & Johnson) is an oxidised regenerated cellulose wound dressing [88]. Debrisan® (Pharmacia & Upjohn) is the commercial product based on epichlorohydrin cross-linked dextran, and is available in bead and paste form [81].

There are a wide variety of commercial alginate products available. Dressings include Algisite® M (Ca alginate fibre nonwoven, Smith & Nephew), Algosteril® (Ca alginate, Beiersdorf) [84], Kaltogel® (Ca Na alginate gelling fibre, ConvaTec), Kaltostat® (Ca alginate fibres in nonwoven pads, ConvaTec) [84,88-90], Melgisorb® (Ca Na alginate gelling fibre, Molnlycke), Seasorb® (Ca Na alginate gelling fibre, Coloplast), Sorbalgon® (Ca alginate, Hartman), and Sorbsan® (Ca alginate fibres in nonwoven pads, Maersk) [84]. Hydrogels include Nu-Gel® (containing alginate, Johnson & Johnson), Fibracol® (Ca alginate with collagen matrix, Johnson & Johnson), Kaltocarb® (Ca alginate fibre, ConvaTec), and Purilon® Gel (Na alginate, Coloplast) [27,81].

MeroGel (Medtronic Xomed, Jacksonville, FL, USA) is an absorbable hyaluronic acid ester nasal dressing [91]. Hyalofill® (ConvaTec) is a commercial nonwoven hyaluronan embedded hydrofibre dressing material composed of Hyaff, a hyaluronan ester derivative [81,92].

THE FUTURE

The medical textiles industry continues to grow at an alarming rate, with constant expansion in areas of wound healing and controlled release, and development of new smart technologies [93]. One such novel area is 'textronics', the synergistic combination of textile science, electronics and computer science [94]. An example of novel uses for carbohydrates in textiles is modification of textiles with cyclodextrins [2,95], which can trap body odour compounds by inclusion complexation, or in the reverse scenario can be used to release perfumes, or deliver pharmaceuticals/cosmetics on skin contact [96,97].

This volume highlights advances in medical textiles and covers the use of biomaterials not only for their physicochemical characteristics, but also their potential beneficial biochemical activity. As more is understood regarding such biochemical activity, with respect to carbohydrate polymers and proteins (and their fragments), a greater number of materials containing some of the more unusual biopolymers (such as glycosaminoglycans and complex plant heteropolysaccharides) will be produced.

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NEW GENERATIONS OF RESORBABLE BIOMATERIALS WITH TEXTILE STRUCTURES

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ABSTRACT

The researches in the production field of the resobable biomaterials with textile structures have the following main objectives: the ensuring of the biological characteristics and especially of the biocompatibility of the products depending on the category they belong to (implant or medical article for external use); the using within the manufacturing process, of biomaterials that corresponds to the requirements imposed by the European legislation (90/385 EC) and American legislation in the field; the elaborating of the physico-chemical and toxicological testing methodologies depending on the intensity of interaction between the medical product and the human body, in conformity with the requirements of the international standards ISO 10993; the increasing of accuracy, precision, reliability, durability degree of the products.

Resorbable biomaterials, with textile structures can be used for any type of wound when by the destruction of the skin or of the live tissues, local haemorrhages or/and fluid secretion occur.

The employing fields of these biomaterials can be also applied to urgent interventions required by naval, road, air accidents, avalanches in areas difficult of access, natural calamities etc.

The researches regarding the obtaining of these products are part of bilateral collaborations with the Chemistry Centre of Polymers – Zabrze, Poland and the National University of Yokohama – Japan.

The paper presents the types of resorbable materials used in the making of these types of medical articles, the main results obtained within the experiments of transforming in fibres the biodegradable polymers made by the partner from Poland, the biomedical and biofunctional characteristics of implantable products imposed by the ongoing national and international norms, as well as the terms of manufacturing and marketing on European markets of these types of products.

GENERAL CONSIDERATIONS

Objectives at the international level in the production field of resorbable biomaterials with textile structures are:

- the ensuring of biological characteristics and especially of the products biocompatibility depending on the category they belong to (implant or medical article of external use);
- the use in the manufacturing processes, of the biomaterials that correspond to the requirements imposed by the European legislation (90/385/EC) and American regulation bodies in the field;
- the elaboration of the toxicological and physico-chemical methodology depending on the intensity of interaction between the medical product and human body, according to the demands of the international standards ISO 10993 and EN 30993;
- the increase of the accuracy, precision, reliability and durability degree of the products.

The products are used for each type of wound, when by skin and living tissues deterioration, local hemorrhage and /or fluid secretions occur; in cases of urgent interventions imposed by naval, road, air accidents, avalanches in difficult accessible area, natural calamities etc.

The forms that are used in clinics are represented by: nonwoven materials, sponges, pads, dressings from knittings or fabrics.

The main producing companies are: Johnson & Johnson USA; ETHICON France; Japan Medical Supply Co. Japan; NIPPON KAYAKU Japan.

Currently, in the manufacturing of this category of products are used esters of glycolic acid; copolymers containing glycolic acid and lactic acid, or poly(N-acetyl-D-glucosamine); oxidised and regenerated cellulose are used.

The biomedical and biofunctional characteristics relate to the clinical applicability fields including: adequate conformity, flexibility, adaptability to wound topography (being able to migrate while the host tissue slips, the displaced fibrills reaching the wound are absorbed, being harmless); the maintainance of the resistance force for a minimum period of 7 days; biocompatibility; inertia from the chemical point of view.

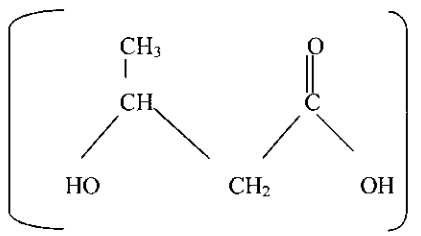
The achieving of this category of products is included in a bilateral collaboration with the Polish Science Academy – The Center of Polymers Chemistry Zabrze –Poland of which the main objectives point to:

- the establishing of the procedure of obtaining the filaments and specific technical parameters, including: temperature, solvent nature, concentration etc,
- the establishing of further finishing operations depending on the product destinations (medical articles, pharmaceutics, food industry),
- the establishing of studies regarding the setting, in time, of the biomedical characteristics of the polymeric characteristics,
- identification of variation limits of the physico-mechanical characteristics of the obtained filaments (fineness, resistance, number of filaments, protection torsion etc.),
- preliminary experimental "spinning" (obtaining of filaments from the variants of polymeric materials made by the partner from Poland),
- technological experiments of obtaining the forms clinnically applicable by weaving, knitting, technologies or nonwovens,
- complete characterisation of the textile supports obtained by physico-chemical, physico-mechanical and biological analyses,
- the making of preliminary clinical trials in clinics authorised by The Health Ministry in both countries,
- the technical-economical evaluation of the results obtained from the preclinic and clinic experiments of the technical verifications and the establishing of the optimal processing,
- the establishing of the technological parameters of obtaining the hemostatic dressings absorbed by the living tissue,
- the establishing of the sterilisation procedure depending on the nature of the raw material and the different parameters,
- the drawing up of the technical documentation of homologous medical products obtained in conformity with the European norms in progress, and
- the emphasizing of other potential fields of using the polymeric materials with biomedical characteristics in view of expanding their applicability fields (medicine, food industry pharmacy etc.).

EXPERIMENTAL

The preparation of the polymeric blend based on the natural polymer (3-hydroxybutyrate–co–3-hydroxyvalerate) is polymer synthetic and atactic.

Raw materials used:
Bacterial material (3-hydroxybutyrate–co–3-hydroxyvalerate) 10% molar concentration 3-hydroxyvalerate with the following characteristics:
$M_n = 36,000$
$M_w/M_n = 2,2$
Polyhydroxybutyrate characterised by:
$M_n = 38,000$
$M_w/M_n = 1,40$



monomer: α-butyrolactone
coordinative catalysis
temperature = 50-100°C

n = 2-12

Polyhydroxybutyrate

The blend is made by dilution in chloroform solution and evaporation at room temperature.

Obtaining of spinning solution and rheological study

In view of identification of the conditions for obtaining the spinning solution, the influence of the polymer concentration and of temperature on the rheology of concentrated solutions the polymer have been observed. In this respect, first the dissolution conditions have been identified. Because the solvent used was dimethylformamide, that at temperatures of 60-80°C starts to thermally degrade, the studies of dissolving were made at temperatures of 60-80°C, following the duration until any undissolved polymer is observed.

This way, by preliminary tests the following parameters have been identified as optimal:

temperature of dissolving	75 ± 5°C;
duration of dissolving	45 min

Under these conditions, four spinning solutions with concentrations of 15, 20.25 and 30% polymer were prepared; they were characterised rheologically at temperatures of : 50, 70 and 80°C. The viscometric determinations were made on a RHEOTEST device – with 2 coaxial cylinders. The results obtained underline that the tests present a dilatating character (the increasing of dynamical viscosity with shearing gradient).

This character is more emphasized in the field of small shearing gradients; in bigger shearing gradients, the curves reach a level where the cooling becomes Newtonian. This

information is important as in the die the shearing gradients are very big (about 10,000 s^{-1}), so the level of viscosity is almost equal to that of die orifices.

The second observation refers to the fact, as expected, that the dynamic viscosities of the samples decrease due to the rising of the temperature. There is also a reduction of the dilatating character of cooling in the field of small shearing gradients.

It was noticed that this dilatating cooling is characteristic to the majority of the fiable polymers in solution, the increase of viscosity with the shearing gradient having favourable effects on the stability of the solution jet extracted in the coagulation bath. From this point of view the polymer corresponds to the spinning in solution.

Also, the variation of dynamic viscosity of the solutions with the concentration of 25% PGBL in DMF has been analysed, depending on the shearing gradient. The samples were tested at 50, 70 and 80°C.

In this case also, the dilatating character of cooling in the field of the small shearing gradients and the reaching of a Newtonian cooling level was observed. Also, it was observed that the dynamic viscosity and the dilatating cooling character decrease by the raising of the solutions temperature. In comparison with the solutions of concentration 15% PGBL, the dynamic viscosity at 25% is bigger, and the dilalating character of cooling is more stressed.

Of the analysis of the shearing gradient of the solutions dynamic viscosity with the concentration of 30% PGBL in DMF, it has been observed that all the solutions present a dilatating character in small shearings and a Newtonian character in big shearings. The dynamic viscosity and the dilatating character again are decreased by the temperature solution rising. In this concentration, the dilatating cooling character is more emphasized than in solutions of small concentration. On the basis of this rheologic study the optimal spinning parameters of the polymer have been determined:

concentration of the spinning solutions:	30%
spinning temperature:	50°C.

Note that this concentration is not characteristic of the spinnings in solution in the wet way (where concentration of 25% is not exceeded) and it was imposed by the polymer characteristics. Because of the same reasons, there has been choice for the spinning temperature to be 50°C.

Production of the biodegradable yarns by spinning-etiration

Biodegradable yarns were obtained from 400 g polymer solution with a concentration of 30% and dynamic viscosity of 424 cP at 50°C, according to the following technological phases: polymeric solution filling out, debit adjusting, coagulation, plastifying, spinning, stretching. The fibre had the following characteristics:

- the polymer presents fiability, but due to reduced molecular mass, the coagulation occurs under harsh conditions;
- although very thin, the cable supports big stretching ratios (about 3);
- the fibre supports a dry stretching in relatively small ratios, but the total stretching ratio is almost the same as that used currently in the spinning installations in the wet way.

Characterisation of the obtained yarns

The yarns obtained were evaluated from the point of view of their physico-mechanical performances, the results obtained being presented in Table 1.

Table 1 –Physico-mechanical characteristics of the resorbable yarns

No. Crt.	Sample	Length Density dtex (din)	Resistance gf	Elongation %	Tenacity gf/dtex(gf/din)
0	1	2	3	4	5
1	1 NE	238,1 (214,3)	37	1,3	0,15 (0,17)
2	3 NE	228,3 (205,5)	43	1,6	0,19 (0,17)
3	3 E	235,8 (213,3)	93,2	2,2	0,39 (0,43)
4	4 E	206,2 (185,6)	105,9	3,4	0,51 (0,57)

Note: NE- nonstreched yarns; E-streched yarns

Observations:
- the nonstretched samples presented resistances to tear and much smaller tenacities, which underlines the importance of dry etiration;
- on comparing the nonstreched samples dry (sample 1 NE and sample 3 NE) it leads to the conclusion that, as expected, a more gentle coagulation (in bath with solvent) leads to superior mechanical properties. This conclusion is also strengthened by the results of the dry stretched samples (sample 3 E and sample 4 E);
-The 4E sample coagulated under the most gentle conditions (conc. by DMF in setting bath of 8% and temperature of 52°C) presented the best physico-mechanical properties.

Achieving of textile supports

Experimental models of biomaterials with textile structures were made by technologies of knitting on rectangular knitting machines. The number of the needles involved was Na = 45 x 2, and the position of the looping clip adjusted adequately to 12.5 divisions.

RESULTS

The physico-mechanical characteristics of watery extracts of the products obtained were evaluated according to the methodology of the norms in progress for the category of medical implants, the results obtained being presented in Table 2.

Evaluation of biocompatibility of the biodegradable copolymer was extensive. The biocompatibility studies had in view the tests of: toxical and hemolithical impurities, of acute toxicity in polar and nonpolar solvent, intradermal reactivity, sensitizing and cutaneous local tolerance.

To do these determinations two extracts were prepared such as: isotonic watery extract – obtained by the processing of 1250 cm^2 polymeric film /250 ml distilled water at 121 ± 1 °C, for 30 min, followed by isotonisation, and oily extract - obtained by incubating in the bath of 1250 cm^2 polymeric film /250 ml sunflower oil of pharmaceutic purity, at 70°C, for 16h.

The tests were been carried out by the subcutaneous and intravenous introduction of the two extracts in Swiss mice and New Zealand rabbits, the animals being kept under observation for 28 days.

Table 2. Physico-mechanical characteristics of watery extract

No. Crt.	Characteristics	Admission conditions	Results obtained
1	Appearance	Clear	Clear
2	Colour	Colourless	A little yellowish
3	Smell	None	None
5	Oxido-reducing substances, ml KMnO4; 0,01 N la 100 ml extract	Max 10	None
6	Content in NH_4^+, g/100 ml extract	Max 0.0002	None
7	Content in Cl^-, g/100 ml extract	Max 0.00025	None
8	Content in SO_4^{2-}, g/100 ml extract	Max 0.001	None
9	Content in heavy metals Pb^{2+}, g/100 ml extract	Max 0.0001	None
10	Content in Ca^{2+}, g/100 ml extract	Max 0.0003	None
11	Content in starch, dextrine	Max 0.3	None

The conclusions are: the lab animals had a good general state, the favourable weighing evolution, (they did not decrease in weigh); there weren't any toxical phenomena and mortality in any lot of treated animals; the polymeric film does not release in watery extract toxical and hemolithical impurities, it does not present any acute toxicity in polar or nonpolar solvents and intradermal reactivity, it does not have sensitive potential, and it is well tolerated and cutaned. Also, the copolymer had a good general tolerance, after oral administration.

In conclusion, the studies carried out emphasized the biocompatibility of the bioresorbable copolymer in view of its using in the hemostatical dressings manufacturing.

CONCLUSIONS

The copolymeric material made by the partner in Poland presents fiability and the fibre goes through a dry stretching in relatively small ratios, but the total stretching ratio comes near to that of the one used currently in the wet spinning installations.

The physico-mechanical determinations emphasized the stretched and coagulated samples under the most gentle conditions, (conc. by DMF in setting bath of 8% and temperature of 52°C) presented the best mechanical-physical properties.

The biodegradable yarns were transformed in clinically applicable forms by the mechanical–textile processing technologies.

The biological and physical-chemical characteristics were provided by complex finishing technologies and the microbiological characteristics, by the adequate correlation of the sterilisation parameters with the nature of the raw material and the technical characteristics of the textile supports.

The chemico-physical determinations emphasized the framing of all the parameters within the admission limits stipulated by the norms for this product category.

The biological studies carried out emphasized the biocompatibility of the copolymer PHBV/a-PHB, for its use in the manufacturing of the hemostatical dressings.

REFORMED COLLAGEN FIBRES

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ABSTRACT

Collagen is the primary structural component of connective tissue and because of its inherent biocompatibility is an ideal candidate for biomedical applications such as scaffolds for tissue regeneration. One route to create a collagenous biomaterial which can be produced in a variety of forms is to render the collagen soluble and then reconstitute it. The research reported here is concerned with reconstituted collagen fibres and specifically compares different solubilisation processes (acid soluble collagen, ASC vs pepsin soluble collagen, PSC), and the influence of polyethylene glycol (PEG) solution on fibre formation. The structure and mechanical properties of reformed fibres were examined. It was found that PEG was essential for the formation of fibres from PSC but its use caused a decrease in the strength of the fibres from ASC. The yield of fibres from PSC was much greater than that from ASC. Some dried reformed fibres produced from both acid soluble and pepsin soluble collagen had a tensile strength equal to that of dried native collagen fibres.

INTRODUCTION

The fibrillar protein, collagen, is a major component of the extra-cellular matrix and forms the biological 'scaffold' that holds cells in place[1] providing excellent support for cell adhesion and growth[2]. It is found in all animal phyla except arthropods and certain classes of coelenterata. Among skin proteins it is quantitatively the major component, accounting for 60-80% of its dry matter, and 20-30% of total animal body protein[3, 4]. Collagen performs many functions in the body, most of which require a tough, durable material. Collagen fibrils are the source of the mechanical strength of skin, tendon, cornea and blood vessels, as well as other tissues that are exposed to repeated tensile forces. The fibrils are very long and have a range of diameters, depending on the stage of development[5].

Collagen is one of the few fibrous proteins, which may be made available in a soluble native form from which it is possible to produce reconstituted structures (fibres, sponges, films). Such reformed collagen materials have great potential for use in the biomedical field (scaffolds for tissue culture, wound healing, implants) as well as finding application in the food industry (edible coatings)[6, 7].

Whilst collagen has been used for sutures for many years it has many desirable characteristics that make it a potentially widely acceptable choice as a biomaterial for a large number of biomedical applications. Commercial use of collagen in medical applications depends on its controlled biodegradation rate and biocompatibility, its availability in commercially useful quantities of high purity and its ease of formation into a variety of end-use forms. Reconstituted collagenous biomaterials have been proposed to augment tissue, control bleeding, support the growth of cells, act as a conduit for nerve regeneration, transmit tensile forces in joints, enhance healing of open dermal wounds and protect the cornea after ophthalmic surgery. Other valuable attributes of reformed collagen include its ability to promote wound healing by

attracting inflammatory and connective tissue cells, its high tensile strength and low extensibility, its amenability to various forms of controlled cross-linking, its low antigenicity, and its ability to promote cellular attachment and growth[7, 8, 9, 10].

Most research work has been based on acid soluble collagen (tropocollagen). However, collagen solubilized in acidic environment by enzymatic (pepsin) removal of non-helical polypeptides (atelocollagen), is expected to become more widely used in a clinical settings, since it is an inert, bio-absorbable natural material and has a very low antigenicity with superior biocompatibility[2, 7, 11]. Whilst there is some literature in the field there is relatively little understanding of what controls the biophysical characteristics of such materials produced either from tropocollagen or atelocollagen.

The aim of this study was to investigate the physical properties of reformed collagen fibres prepared from either acid soluble collagen (ASC) or pepsin soluble collagen (PSC) with or without the addition of polyethylene glycol (PEG).

MATERIALS AND METHODS

Collagen purification

The starting material used was bovine Achilles tendon since it is mainly composed of type I and III collagen[12]. Collagen was extracted as described previously[13] with slight modifications. Tendons were collected from a slaughterhouse, transferred in a thermo-protected container in ice and manually dissected out from the surrounding fascia. They were washed with distilled water and kept frozen until use. The frozen tendons were minced through a 1/4inch grinder plate with ice, in order to avoid heat denaturation and then kept frozen until use.

The minced frozen tendons were washed at 4^0C for an hour in three changes of 50vol (vol/wt) of 3.7mM disodium hydrogen orthophosphate, 0.35mM potassium dihydrogen orthophosphate, and 51mM sodium chloride, at pH 7.5.

Acid Extraction

The minced tendons were suspended in 0.5M ethanoic acid [50vol (vol/wt)] at 4^0C for 72 hours to extract soluble collagen. The suspension was filtered through a 5μm nylon screen to separate the solubilized collagen from the insoluble residue. The acid soluble collagen was purified by repeated precipitation in 0.9M sodium chloride, centrifugation at 12,000g, at 4^0C for 45min and re-dissolution in 1M ethanoic acid. The collagen solution was dialysed (8,000 MW cut off) against 0.01M ethanoic acid.

Pepsin Extraction

The insoluble material was re-suspended in 50vol (vol/initial wt) of 0.5M ethanoic acid and pepsin (1:100 of the initial wet weight) for 72h at 4^0C. The suspension was filtered through a 5μm nylon screen and the pepsin soluble collagen purified by repeated salt precipitation as described above.

The final collagen solutions (acid soluble and pepsin soluble) were either kept refrigerated or frozen until used. The collagen content of both ASC and PSC preparation was determined by hydroxyproline assay and the concentrations subsequently adjusted to 6mg/ml.

Fibre formation

A 5ml syringe containing either of the collagen solutions (6mg/ml) was loaded onto a syringe pump system and set to infuse at 0.5ml/min. One end of a silicone laboratory tube (30cm in length and 1.5mm internal diameter) was connected to the syringe with the other end placed at the bottom of a container, which was connected to a trough. Collagen was extruded into a 'Fibre Formation Buffer' (FFB) comprising of disodium hydrogen orthophosphate, sodium dihydrogen orthophosphate (with or without 20% PEG, MW 8,000) at pH 7.55 and 37^{0}C. The collagen instantaneously gelled on contact with the neutral pH solution and the fibre began to form. Since the free end of the silicone tubing was placed at the bottom of the container, the produced fibres could rise due to buoyancy. When they reached the surface, a stream of air at 0.5mm/sec was applied in order to direct them through the trough. Fibres were allowed to remain in this buffer for a maximum period of 10min and then immersed for a further 10min in a 'Fibre Incubation Buffer' (FIB) comprising of 5.5mM disodium hydrogen orthophosphate, 0.5mM potassium dihydrogen orthophosphate and 75mM sodium chloride at pH 7.10 and 37^{0}C. Finally, the fibres were partially dehydrated by immersing them into a 70% propan-2-ol solution for 10min and then air-dried under the tension of their own weight.

Mechanical tests

An Instron 1122 Universal machine was used for the mechanical tests at an extension rate of 10mm/min. The gauge length was fixed at 5cm for the PSC with PEG and the ASC without PEG. A 3cm gauge length was used for the ASC with PEG. Soft leather was used to cover the grips in order to avoid damaging the fibres at the contact points. Prior to testing the fibres had been conditioned in a 65% Relative Humidity room for at least 48h.

Microscopy

A Nikon Eclipse E600 optical microscope and a Hitachi Variable Pressure Scanning Electron Microscope (VPSEM) were respectively used to measure the diameter of the fibres and to carry out observations of the microstructure of the fibres. Each fibre cross sectional area was calculated assuming a circular cross section and by measuring the diameter at five places (every 1cm for the PSC with PEG and ASC without PEG and every 0.6cm for the ASC fibres with PEG) along the fibre using the optical microscope fitted with a calibrated eyepiece gratitude. When the cross section area was uneven, measurements were carried out in the uniform regions.

RESULTS / DISCUSSION

The collagen yield from the PSC (37.5%) was significantly higher than that of the ASC (7.5%). Therefore, for industrial applications the PSC seems to be a better starting material. Furthermore, by digestion with pepsin, selective removal of the terminal nonhelical peptides takes place, and as a result antigenicity can be further reduced and a more competitive biomaterial can be produced[14].

Only the PSC without PEG failed to produce fibres suitable for testing. Fibres did form but they were extremely fragile and were impossible to transfer from the FFB to the FIB, even after extended incubation in the FFB (2 hours).

The length of the fibres produced deviated significantly between the batches (see Table 1). The PSC fibres appeared to be more consistent in their shape and diameter and their length was limited by the length of the FIB container. Therefore, all of them were approximately 50cm long. However, with other apparatus it was found that fibres longer than 120cm could be produced.

Table 1. Length of the fibres (± Standard Deviation).

Collagen Source	Mean Length (cm)
ASBAT (PEG)	7.0 ± 4.9
ASBAT (No PEG)	18.3 ± 4.4
PBAT (PEG)	» 50.3 ± 0.8*
PBAT (No PEG)	N/A

(*Limited by the length of the FFB container.)

Furthermore, the PSC fibres with PEG appeared to have most regular shape (see Figure 1c), in contrast to ASC fibres with or without PEG (see Figure 1a, 1b). It was observed that all the ASC fibres with and without PEG had an uneven surface texture throughout their length. As a result, accurate measurements of their diameter could not be made (see Table 2).

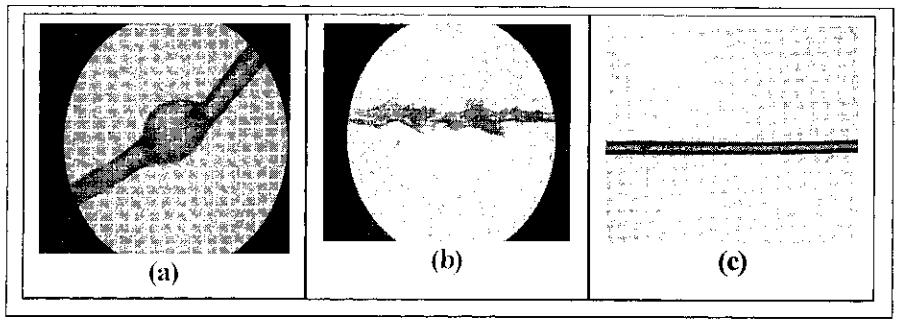

Figure 1. PSC and ASC fibres with and without PEG. (a) ASC with PEG; (b) ASC without PEG; (c) PSC with PEG

Table 2. Diameter of the produced fibres (± Standard Deviation).

Collagen Source	Mean Diameter (μm)	Diameter at Breaking Point (μm)
ASC (PEG)	66 ± 11	58 ± 13
ASC (No PEG)	36 ± 2	36 ± 2
PSC (PEG)	54 ± 9	54 ± 9
PSC (No PEG)	N/A	N/A

The in vitro fibrillogenesis of collagen is a process, which depends on the temperature, pH, collagen concentration and composition of the medium[15, 16]. Since all these parameters were kept constant for both preparations, we can speculate that either the ASC concentration was too low (6mg/ml) for the production of uniform fibres or the air stream utilised to forward the fibre within the FIB container could have been too strong and therefore able to tangle the thread beyond repair. These results though appear to deviate from previous work[13], where fibres were produced from a lower ASC concentration (5mg/ml) and it was reported that fibres could not be made from PSC due to its fragility.

A typical stress strain curve for a dry fibre is given in Figure 2. The shape of the graph shows that the relationship between the stress and strain is almost linear at higher strains but concave upwards in the lower strain region. The generally observed low strain concave down (toe) region found for dry native collagen fibres from rat tail tendon[17] and skin[18] is not found for these fibres, which suggests that they are comprised of a cohesive system of oriented fibrils.

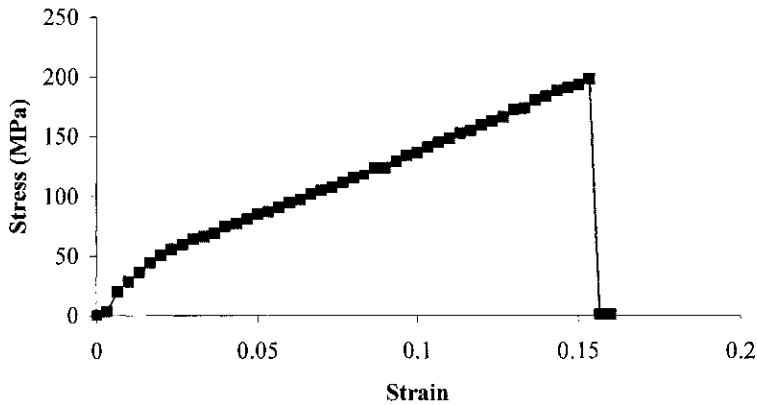

Figure 2. Typical stress-strain profile of reconstituted dry collagen conditioned at 65% RH.

Table 3. Mechanical properties of reconstituted collagen fibres (± Standard Deviation).

Collagen Source	Stress at break* (MPa)	Strain at break	Modulus at 2%strain (MPa)
ASC with PEG	40 ± 21	0.10 ± 0.06	1071 ± 535
ASC without PEG	191 ± 17	0.19 ± 0.03	2014 ± 320
PSC with PEG	139 ± 68	0.29 ± 0.05	1337 ± 615
PSC without PEG	N/A	N/A	N/A

(*Measurements carried out using the mean diameter.)

From Table 3 it can be seen that the mechanical properties of the fibres showed considerable scatter. This may be due the fact that some fibres were non-uniform (see Figures 1 and 3). The ASC without PEG produced fibres with the highest tensile strength approaching 200MPa, although their shape was the most complicated one (Figure 3). However, these may be artificial results due to the non-uniform cross section area of the produced fibres. It has been reported that dry collagen fibres from rat tail tendon shows a strain rate dependent stress-strain behaviour with the stress at break varying between 125 and 225MPa and a strain at break around 0.35[19]. We have therefore produced reformed collagen fibres whose tensile strength matches that of native collagen fibres. The role of PEG is interesting; with ASC strength is decreased with PEG addition whereas with PSC strength is improved. More work is required to understand this effect.

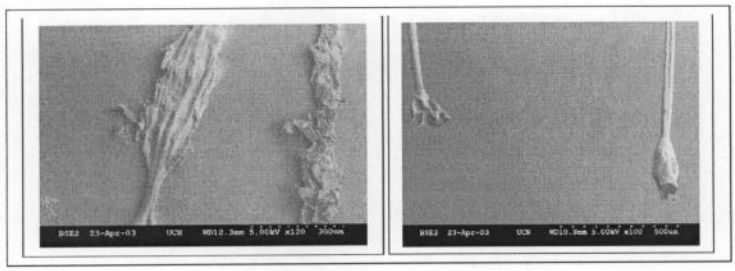

Figure 3. ASC fibres without PEG. Left: intact fibres; right: broken ends.

Strain at break values show that pepsin soluble collagen with PEG produced the most extendable fibres. Modulus values indicate the fibre stiffness. PSC with PEG and ASC without PEG showed higher modulus values. Finally, the break surfaces of all the fibres appeared similar, with the same apparent internal structure (see Figure 4). The stress at break was found to become lower as the fibre diameter was increased which seems to agree with previous observations[20].

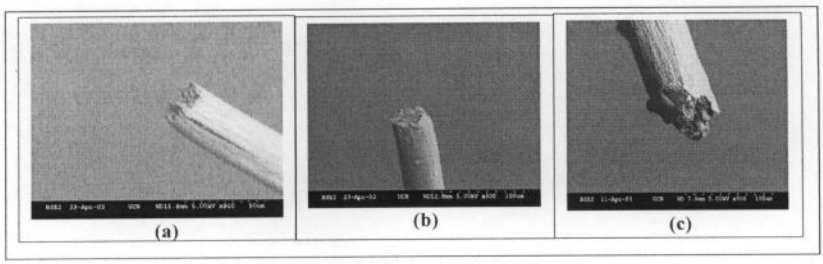

Figure 4. SEM pictures of breaking points of collagen fibres. (a) ASC fibre without PEG; (b): ASC fibre with PEG; (c): PSC fibre with PEG.

CONCLUSION

The differences between fibres produced from acid soluble and pepsin soluble collagen derived from bovine Achilles tendon and the effect of PEG on their self-assembly and

mechanical properties has been evaluated. The fibre diameter was found to be higher when PEG was utilised. It was found that the break surfaces of each type of fibres were similar and not dependent on the presence of PEG. The presence of PEG was vital for the formation of PSC fibres but its presence caused a decrease in the strength of the ASC fibres. Furthermore, PEG facilitated the production of fibres as long as 120cm when combined with the PSC, whilst its presence inhibited the production of long fibres from ASC. The yield of fibres from PSC was much greater than that from ASC. Reformed fibres produced from both acid soluble and pepsin soluble collagen can have a tensile strength equal to that of native collagen fibres.

ABBREVIATIONS

Abbreviation	Meaning
ASC	Acid soluble collagen
PSC	Pepsin soluble collagen
PEG	Polyethylene glycol
FFB	Fibre Formation Buffer
FIB	Fibre Incubation Buffer

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NOVEL CHITOSAN-ALGINATE FIBRES FOR ADVANCED WOUND DRESSINGS

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ABSTRACT

A range of commercial chitosans were sourced and their molecular size profiles and degrees of acetylation (DA) were determined by HPSEC and ^{1}H-NMR spectroscopy, respectively. Chitosans were subsequently utilised for modification of alginate fibres, and levels of chitosan incorporated into base fibres were estimated by elemental analysis. Elongation (%) and tenacity of resultant chitosan/ alginate fibres were determined in order to assess their potential application in wound dressings. A range of chitosan contents (~ 0-6 % w/w) resulted from a variety of alginate and chitosan starting materials. Fibres produced from sodium alginate (A1, 6%) using hydrochloric acid (0.2M) at a draw ratio of 1.18, and treated with chitosan (C1, 3.2%) were deemed to have the best properties. In general, use of a water washing stage resulted in the production of better chitosan/alginate fibres. Treatment with chitosans generally resulted in a reduction in tenacity (and a reduction in % elongation if a water washing stage was not used), i.e. no increase in fibre strength was observed, implying that the chitosan is more like a coating rather than penetrating/reinforcing the alginate fibre.

INTRODUCTION

Fibres have been extensively used in wound dressing applications because of their unique/advantageous properties, such as high surface area, softness, absorbency and ease of fabrication into many product forms. Fibres made from natural sources, especially polysaccharides, have been considered the most promising due to their excellent biocompatibility, non-toxicity, and potential bioactivity at the wound surface and beyond. Many commercial wound dressing products (woven and non-woven dressings, and hydrogels) are made from such natural polymers and their derivatives, the simplest being retention bandages, support and compression bandages, absorbents, gauzes, tulle dressings, and wound dressing pads produced from woven cellulose fibres (cotton and viscose) [1-4].

Among the various fibrous and hydrogel products, alginate-based products are currently the most popular ones used in wound management, since they offer many advantages over traditional cotton and viscose gauzes. They are biocompatible and form a gel on absorption of wound exudate. This eliminates fibre entrapment in the wound, which is a major cause of patient trauma/discomfort during dressing removal. Such gelation prevents the wound surface from drying out, which is beneficial since a moist wound environment promotes healing and leads to a better cosmetic repair of the wound [5]. Performance requirements for such gelled dressings (which often aim to replicate the inherent permeability/water content of natural skin) are obviously higher than mere absorbent coverings in order for the wound to remain moist during the contact period (which could be more than several days) [6]. Hence, it is also reported that

alginate-based dressings have haemostatic properties and can enhance the rate of healing of skin wounds [7,8].

Commercial alginate-based dressings include Algisite® M (non-woven calcium alginate fibre, Smith & Nephew), Algosteril® (calcium alginate, Beiersdorf), Kaltocarb® (calcium alginate fibre, ConvaTec), Kaltogel® (calcium/sodium alginate gelling fibre, ConvaTec), Kaltostat® (calcium alginate fibres in non-woven pads, ConvaTec), Melgisorb® (calcium/sodium alginate gelling fibre, Molnlycke), Seasorb® (calcium/sodium alginate gelling fibre, Coloplast), Sorbalgon® (calcium alginate, Hartman), and Sorbsan® (calcium alginate fibres in non-woven pads, Maersk) [2,3].

Another type of natural polysaccharide of interest with respect to wound management products is chitin, and its partially deacetylated derivative, chitosan. The presence of chitin/chitosan in a dressing is reported to promote fibroblast growth and affect macrophage activity, which accelerates the wound healing process [9-12]. Chitosans are biocompatible (since their biodegradation products are natural metabolites), and are used in a wide variety of commercial application areas, such as cosmetics, haemostatic agents, drug delivery vehicles, wound dressings, etc [13-15]. Although chitosan can be produced in powder, film, bead, fibre and fabric forms [16,17], products made from pure chitosan fibres have not been commercially viable due to the high processing costs involved (deproteination, demineralisation and deacetylation processes are required to produce chitosan materials of adequate purity) and the availability of such purified material is still insufficient for large industrial scale fibre production. Poor textile processing properties of resulting fibres has also been a major problem.

The chemical structures of sodium alginate/alginic acid and chitin/chitosan are displayed in Figure 1 [18]. Alginic acid is obtained from the cell walls of brown algae (Phaeophyta) such as the seaweeds *Laminaria* sp. and *Ascophyllum* sp. It is a linear block copolymer composed of uronic acid residues, namely β-D-mannuronic and α-L-guluronic acid, linked by (1→4)-linkages. The distribution of the uronic acids along the chain is non-random and involves relatively long sequences of each uronic acid. In the presence of divalent cations, such as calcium, alginate gels can be formed due to ionic cross-linking via calcium bridges between L-guluronic acid residues on adjacent chains.

Chitin is a naturally occurring polysaccharide found in the outer shell of crustaceans, and is composed of 2-acetamido-2-deoxy-β-D-glucopyranose residues (N-acetyl-D-glucosamine residues), linked by (1→4)-linkages. Chitosan is partially deacetylated chitin and is therefore composed of 2-amino-2-deoxy-β-D-glucopyranose (D-glucosamine) and N-acetyl-D-glucosamine residues.

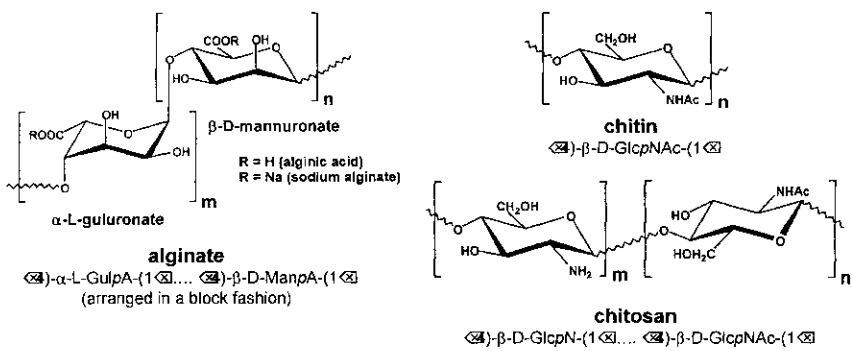

Figure 1. Chemical structures of alginic acid/sodium alginate and chitin/chitosan.

Alginate fibres are generally prepared by injecting a solution of water-soluble alginate (usually sodium alginate) into a bath containing an acidic solution or calcium salt solution to produce the corresponding alginic acid or calcium alginate fibres, which can be used to produce yarns and fabrics for medical applications [17,19,20]. Many of chitosans properties rely on its cationic nature, which allows it to interact with negatively charged biomolecules such as proteins, anionic polysaccharides and nucleic acids, many of which are located in skin. Therefore, under certain conditions, alginate and chitosan have opposite and therefore mutually attractive charges, making the use of mixed dope solutions of suitable concentrations unfeasible, with respect to chitosan/ alginate fibre production, since rapid coagulation/gelation of dope solution can occur.

Chitosan has been used to coat calcium alginate filaments (utilising the cationic interaction of the chitosan with the anionic nature of the alginate to produce a tight interaction) [21]. However, due to its high molecular weight the chitosan must be used in very low concentrations, as precipitation occurs in the presence of calcium ions, resulting in very low levels of chitosan incorporation (< 0.2% w/w) into the fibres. Problems in the direct production of chitosan/alginate fibres have been overcome using a variety of different approaches. Alginate and chitosan fibres have been separately produced and subsequently blended, and chitosan has been utilised as the insolubilising cation for production of an alginate fibre [22,23].

The approach adopted for the production of fibres presented in this paper was the use of an initial core fibre produced using one of the polysaccharide materials, and subsequently applying the other polysaccharide material by absorption into/coating onto this core fibre. The obvious route was to use an alginate core fibre (since the required methodologies for the production of fibres with suitable physicochemical characteristics are well known).

The aim of the research was to produce fibres that combine the biomedical properties of both alginate and chitosan, and that have good textile processing ability and relatively low production costs. Alginate would essentially manage excess liquid/exudate and chitosan would provide antibacterial, haemostatic and wound healing properties. The comparative analysis of a range of commercial chitosans are reported, along with the production and analysis of a range of chitosan/alginate fibres, produced by treating freshly extruded alginate fibres with chitosan solutions. The tensile properties of the produced fibres were also evaluated in order to evaluate their suitability for potential use in wound dressings applications.

MATERIALS AND METHODS

Materials

A number of different (in terms of viscosity, molecular weight profile, composition, etc) sodium alginate and chitosan starting materials were obtained for the production of chitosan/alginate fibres (as detailed in Table 1).

Non-carbohydrate materials utilised included hydrochloric acid (HCl, 37%, Riedel-de Haën®), acetic acid (AcOH, CH_3COOH, >99%, Fisher Chemicals), trifluoroacetic acid (TFA, CF_3COOH, 99%, Sigma-Aldrich), deuterium oxide (D_2O, 99.9%, Sigma-Aldrich), acetone (Fisher Chemicals), fused calcium chloride ($CaCl_2$, Fisher Chemicals), sodium acetate trihydrate ($NaOAc.3H_2O$, Acros Organics), sodium chloride (NaCl, Fisher Chemicals) and pullulan polysaccharide GPC calibration standard kit (Polymer Laboratories).

Table 1. Sodium alginate and chitosan materials obtained for fibre production.

Code	Material	Product	Supplier	# Viscosity (mPa.s, 1% solution)
A1	sodium alginate	Protanal LF 10/60	Pronova	20-70
A2	sodium alginate	Manucol® DH	ISP Alginates	40-90
A3	sodium alginate	Manugel® GMB	ISP Alginates	110-270
C1	chitosan HCl	Seacure CL 310	Pronova	200-800
C2	chitosan	Seacure 443	Pronova	Unknown
C3	chitosan	Kate	Kate Int.	50-100 (in AcOH)
C4	chitosan	Type 222 (high viscosity)	France-Chitine	200-2000 (in AcOH)
C5	chitosan	Type 242 (high purity)	France-Chitine	20-100 (in AcOH)

(# information supplied by manufacturer)

Chitosan characterisation

Determination of molecular size profiles using HPSEC

Equipment: Knauer HPLC pump 64, Waters 712 WISP autoinjector, Knauer column oven & control unit (set to 30°C), Knauer differential refractometer (DRI) and Dionex UI 20 Universal interface (PC link).

Columns: Progel™-TSK PW_{XL} guard, Progel™-TSK G6000 PW_{XL}, Progel™-TSK G4000 PW_{XL} and Progel™-TSK G2500 PW_{XL}, linked in series.

Eluent: acetic acid (AcOH, 0.5M) containing sodium acetate (NaOAc, 0.2M), He degassed immediately prior to use.

Flow rate: 0.5 mL/minute.

Calibrants: Pullulan polysaccharides (M_P 853, 380, 186, 100, 48, 23.7, 12.2 & 5.8 kDa) dissolved overnight in eluent (1 mg/mL, 100 µL injections).

Samples: Chitosans dissolved overnight in eluent (1 mg/mL, 100 µL injections).

Data: Data collected by PC using Dionex Chromeleon software (v. 6.11) connected to the DRI detector via the UI20 interface. Collected data was analysed using Polymer Laboratories PL Caliber Reanalysis software (v. 7.04).

[All solutions were filtered (Titan nylon membrane filters, 0.20 µm) prior to injection]

Determination of degree of acetylation (DA) by ^{1}H-NMR spectroscopic analysis

Aliquots of dried hydrolysed chitosan were dissolved (20 mg/mL) in deuterium oxide (D_2O, 2 mL) containing trifluoroacetic acid (TFA, 5 % v/v) overnight at ambient temperature in glass screw cap vials (3 mL capacity). ^{1}H-NMR spectra of the resultant chitosan solutions were recorded at ambient temperature using a Brüker AMX 400 MHz NMR spectrometer equipped with an autosampler. Resultant spectra were manipulated and areas of interest integrated using Brüker 1D WINNMR software, in order to calculate the DA.

Production of alginate fibres

Alginate fibres were produced by a conventional wet spinning technique using a multi-functional laboratory extruder (designed in-house and built by Howden Engineering Services, UK, displayed in Figure 2).

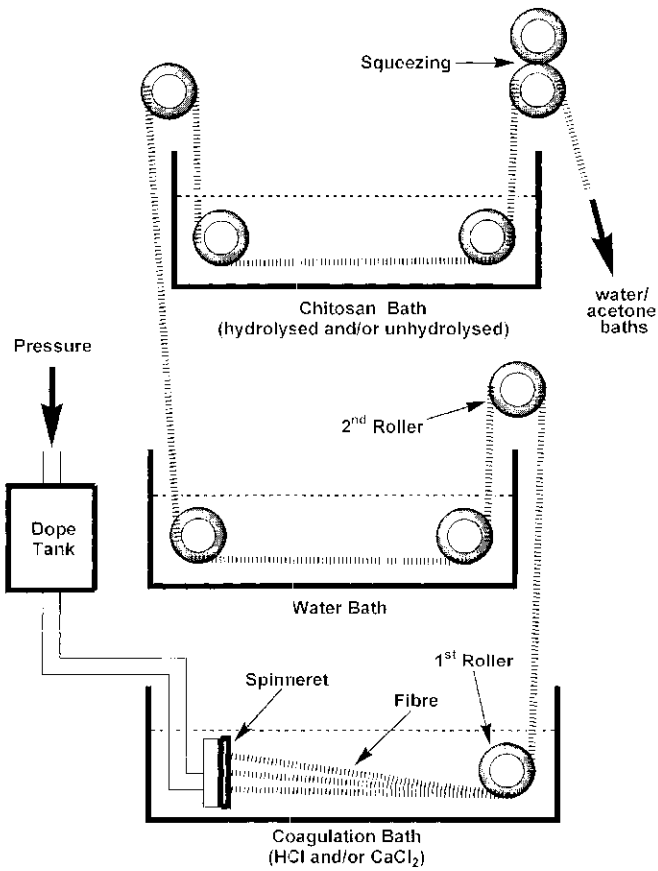

Figure 2. Production of chitosan/alginate fibres using a laboratory extruder.

Spinning dope solution (sodium alginate, 1-6% w/v, depending on type and viscosity) is extruded under pressure through a spinneret (200 holes, 76 μm diameter) into a coagulation bath containing either hydrochloric acid (0.2M) and/or calcium chloride (1-3% w/v) to afford the corresponding alginic acid and/or calcium alginate fibres. The resultant fibres/filaments are then drawn between a first and second set of rollers (the relative speeds of which determines the draw ratio) and are then passed through a water washing bath. The fibres are then squeezed (between rollers) to remove excess liquid, wound up, removed from the extruder and placed in a treatment bath containing chitosan (0-5% w/v in AcOH, 1% v/v) for 10 minutes, rinsed with deionised water and dried using acetone baths of increasing concentrations (50-100% v/v). Chitosan treatment, water washing and acetone drying can also be performed as continuous processes directly after production of initial fibres (as detailed in Figure 2). Finally, fibres are separated by hand and conditioned (24 hours at ambient temperature).

Fibre characterisation

Produced fibres were subjected to chemical and physical analysis. Moisture contents were determined by drying to constant mass using a Mettler Toledo HR73 Halogen Moisture Analyzer (80°C). Ash contents were determined using a standard ashing protocol (ashing samples to constant mass in a furnace, ~ 550°C). Sodium (Na) and calcium (Ca) contents were determined by flame photometry and atomic absorption techniques, respectively, and quantified from calibration curves prepared using suitable standard solutions. CHN contents were determined using an elemental analyser.

Elongation at break and tenacity measurements were performed using a Textechno Fafegraph M single fibre tensile tester. The fibre sample is held between a set of clamps and a constant rate of extension is applied via a load cell and load vs elongation is recorded until point of failure. Values for % elongation and tenacity are then generated automatically. Elongation describes the length by which a fibre extends when a load is applied. As the load is increased the elongation increases until the fibre breaks (under a specific load). The % elongation is determined from the ratio of the breaking length to the original length. Tenacity defines fibre strength with respect to linear density. Linear density is described in tex, which is fibre weight in g per 1000 m.

$$\% \text{ Elongation} = \left(\frac{l_b}{l_0}\right) \times 100$$

where:

l_b = breaking length

l_0 = original length

$$\text{Tenacity} = \left(\frac{L_b}{D_L}\right)$$

where:

L_b = breaking load (N)

D_L = linear density (tex)

RESULTS AND DISCUSSION

Chitosan molecular size profiles

Molecular size profiles from HPSEC analysis of the chitosans (detailed in Table 1) are presented in Figure 3. Retention times (R_T) for molecular weight calibration lines (10000, 5000, 1000, 500 & 100 kDa) were determined from linear regression of the pullulan calibration curve ($\log M_p = -0.1539\ R_T + 11.922$, $R = 0.9971$, M_p = peak MW).

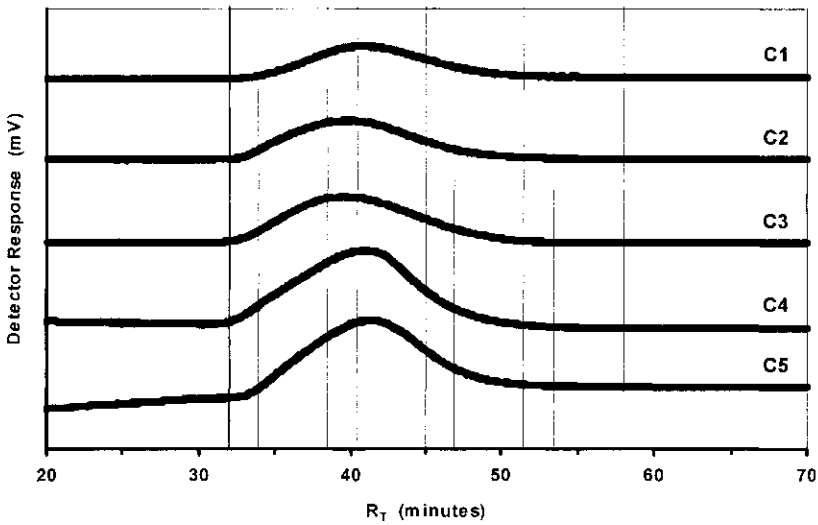

Figure 3. Chitosan molecular size profiles (from HPSEC analysis).

The results of mathematical analysis of the molecular size profiles (using Polymer Laboratories PL Caliber Reanalysis software v. 7.04) are presented in Table 2. Five molecular weight averages can be statistically calculated from molecular size profile data (M_n, M_w, M_z, M_{z+1} & M_v), and are defined by the equations detailed below. M_n is the number-average molecular weight and is simply the arithmetic mean, i.e. the sum of the molecular weights of all the molecules divided by the total number of molecules. M_w is the weight-averaged molecular weight, i.e. the weighted mean, and is the sum of the molecular weight squared of all the molecules divided by the total molecular weight of all the molecules. Increasing the weighting of the average gives rise to the *z* and z+1 averages. M_v is the viscosity average. w_i is the weight of *i* molecules with molecular weight M_i, N_i is the number of *i*th molecules with molecular weight M_i. *d* is the polydispersity and a *d* value close to unity indicates a narrow distribution.

$$M_n = \frac{\sum w_i}{\sum N_i} = \frac{\sum (N_i M_i)}{\sum N_i}$$

$$M_{z+1} = \frac{\sum (N_i M_i^4)}{\sum (N_i M_i^3)}$$

$$M_w = \frac{\sum (w_i M_i)}{\sum w_i} = \frac{\sum (N_i M_i^2)}{\sum (N_i M_i)}$$

$$M_v = \left[\frac{\sum (N_i M_i^{a+1})}{\sum (N_i M_i)} \right]^{1/a}$$

$$M_z = \frac{\sum (N_i M_i^3)}{\sum (N_i M_i^2)}$$

$$d = \frac{M_w}{M_n}$$

Table 2. Parameters obtained from analysis of chitosan molecular size profiles.

Chitosan	M_p (kDa)	M_n (kDa)	M_w (kDa)	M_z (kDa)	M_{z+1} (kDa)	M_v (kDa)	d (M_w/M_n)
C1	447.7	133.6	698.4	1920.6	3350.0	577.1	5.2
C2	888.1	209.8	1214.9	2937.9	4345.8	1009.3	5.8
C3	697.1	144.5	993.2	2666.7	4338.4	813.6	6.9
C4	402.5	204.4	1033.8	3021.2	5154.0	841.3	5.1
C5	368.4	152.0	748.4	1948.1	3080.7	619.9	4.9

Significant differences in the molecular size profiles of the analysed chitosan materials (C1-5) can be clearly seen from visual inspection of their HPSEC profiles (Figure 3) and scrutiny of their subsequent mathematical analysis data (Table 3).

Degree of acetylation (DA) of chitosans

Comprehension of the terminology detailed in Table 3 is required in order to understand degrees of acetylation (DA), substitution (DS) and deacetylation (DD).

Table 3. Definition of useful terms (as applied to the structure of chitin/chitosan).

Term	Definition
Acetylation	the presence or addition of N-acetyl groups (i.e. n, or conversion from m to n, in Figure 1)
Deacetylation	the absence or removal of N-acetyl groups (i.e. m, or conversion from n to m, in Figure 1)
degree of acetylation (DA) or degree of substitution (DS)	average number of acetyl groups per monosaccharide residue, i.e. DA of 0.1 means 1 out of every 10 GlcN residues acetylated (max. DA is 1, i.e. chitin).
degree of deacetylation (DD)	average number of amino groups per monosaccharide residue, i.e. a DD of 0.1 means 9 out of every 10 GlcN residues acetylated (max. DD is 1, fully deacetylated).
% DA (% acetylation)	number of acetyl groups present as a % of the total groups (assuming 0% acetylated, DA = 0, at start). Thus 20% acetylated is a DA of 0.2.
% DD (% deacetylation)	number of acetyl groups removed as a % of the total groups (assuming 100% acetylated, DA = 1, at start). Thus 20% deacetylated is a DA of 0.8.

(DA + DD = 1, likewise % DA + % DD = 100%)

The ^{1}H-NMR spectra of the analysed chitosans are composed of broad multiplets (rather than well resolved peaks) due to peak broadening as a result of high viscosity (Figure 4). The peak at ~ 1.8 ppm corresponds to N-acetyl methyl group hydrogen atoms, whilst the collection of peaks in the ~ 2.8-5.5 ppm region corresponds to chitosan backbone protons (i.e. single hydrogen atoms attached to the C_1-C_5 carbon atoms, & the two hydrogen atoms attached to the C_6 carbon atom). Trifluoroacetic acid (TFA) moves hydroxyl protons to a higher chemical shift (> 6 ppm, i.e. out of the area of interest).

DA is calculated from the ratio of the normalised acetyl group proton peak area (~ 1.8 ppm) to the chitosan backbone proton area (~ 2.8-5.5 ppm), determined by integration of respective peaks using Brüker 1D-WINNMR software and corrected to account for a single proton (Table 4). DA values are converted to % DA by multiplying by 100, and to % DD by subtracting the % DA value from 100.

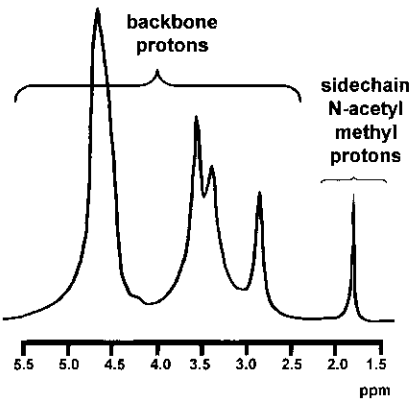

Table 4. DA of chitosans (from ^{1}H-NMR).

Chitosan	DA	% DA	% DD
C1	0.28	28	72
C2	0.33	33	67
C3	0.16	16	84
C4	0.14	14	86
C5	0.08	8	92

Figure 4. ^{1}H-NMR spectrum of chitosan (C3).

Analysis of chitosan/alginate fibres

Variables associated with production of alginate/chitosan fibres included sodium alginate type, method of alginic acid fibre production and chitosan type and concentration. The appropriate alginic acid (100%) fibres (i.e. not treated with chitosan) were also analysed as controls. Information for selected fibres (alginate fibre and chitosan solution production parameters, and fibre analysis/testing results for fibres produced using alginates A1-3 and chitosans C1 & 2) is provided in Table 5.

The chitosan contents of fibres were determined from their nitrogen (N) contents (based on the nitrogen contents of the pure alginate fibres and the chitosan used for their production). The Draw ratio (DR, or stretch ratio) is the ratio of the speeds of the first and second rollers (detailed in Figure 1) used for production of the initial alginate fibre, and provides an indication of the amount of molecular orientation the fibre has undergone.

$$DR = \left(\frac{S_2}{S_1}\right)$$

where:

DR = Draw Ratio
S_1 = speed of 1st roller
S_2 = speed of 2nd roller

Table 5. Composition and analysis of selected chitosan/alginate fibres.

Dope (% w/w)	Draw Ratio	1st Bath	2nd Bath	3rd Bath	Moisture (% w/w)	Ash (% w/w)	Chitosan (% w/w)	Elongation (%)	Tenacity (cN/dtex)
A1 (6%)	1.18	HCl (0.2M)	water	-	5.4	1.4	**0.0**	**20.4**	**2.2**
A2 (6%)	1.09	HCl (0.5M)	water	-	7.8	1.3	**0.0**	**5.8**	**0.8**
A3 (4%)	1.09	HCl (0.2M)	water	-	9.4	1.1	**0.0**	**13.1**	**1.2**
A1 (6%)	1.18	HCl (0.2M)	water	C1 (3.2%)	12.5	0.7	**2.1**	**23.4**	**2.0**
A2 (6%)	1.09	HCl (0.5M)	water	C1 (3.2%)	11.6	0.8	**5.5**	**tb**	**tb**
A3 (4%)	1.09	HCl (0.2M)	water	C1 (3.2%)	12.4	1.2	**2.8**	**tb**	**tb**
A1 (6%)	1.18	HCl (0.2M)	-	C1 (3.2%)	12.3	0.4	**1.1**	**15.0**	**1.0**
A2 (6%)	1.09	HCl (0.5M)	-	C1 (3.2%)	11.4	0.7	**0.9**	**tb**	**tb**
A3 (4%)	1.09	HCl (0.2M)	-	C1 (3.2%)	12.1	0.8	**1.1**	**tb**	**tb**
A1 (6%)	1.18	HCl (0.2M)	water	C2 (2%)	11.2	1.6	**0.7**	**29.1**	**1.4**
A2 (6%)	1.09	HCl (0.5M)	water	C2 (2%)	11.2	1.0	**1.7**	**4.8**	**0.6**
A3 (4%)	1.09	HCl (0.2M)	water	C2 (2%)	11.4	1.1	**2.3**	**10.5**	**0.7**
A1 (6%)	1.18	HCl (0.2M)	-	C2 (2%)	11.6	1.0	**1.1**	**12.5**	**1.0**
A2 (6%)	1.09	HCl (0.5M)	-	C2 (2%)	11.3	0.8	**2.3**	**10.9**	**1.1**
A3 (4%)	1.09	HCl (0.2M)	-	C2 (2%)	12.3	0.9	**0.1**	**tb**	**Tb**

(tb = too brittle to test)

Alginic acid/sodium alginate (A1-3) fibres were analysed separately (i.e. without chitosan treatment) as controls to provide a base for comparison of composition and

properties (as detailed in Table 5). Chitosan/alginate fibres produced from alginate A1 had significantly better physical properties than those fibres produced from both A2 and A3. Alginate A2 required a higher acid strength to produce suitable fibres (0.5M compared with 0.2M for A1 and A3), alginate A3 had to be used at a concentration of 4% (since 6% was too viscous), and both A2 and A3 fibres were produced at a draw ratio of 1.09 (compared with 1.18 for A1) due to the weakness of the fibres.

In general, use of a water washing stage resulted in the production of better chitosan/alginate fibres than without a water washing stage (in terms of both physical properties and chitosan contents). Treatment with chitosans generally results in a reduction in tenacity (and a reduction in % elongation if a water washing stage is not used). The best fibres (in terms of physicochemical properties) were produced using sodium alginate A1 (6%), a hydrochloric acid (0.2M) coagulation bath, a draw ratio of 1.18, and subsequent treatment of the produced fibres with chitosan C1 (3.2%). Chitosan C1 had the lowest number average (M_n) and weight average (M_w) molecular weights and may be better able to penetrate the base alginate fibres.

CONCLUSIONS

Achieved levels of chitosan incorporation were ~ 0-6% w/w, which were significantly higher than prior art. However, the fact that treatment of fibres with chitosan had no reinforcing effect, i.e. fibre strength did not increase, implied that the chitosan was behaving more like a coating and not penetrating the alginate fibre very well. It was therefore postulated that use of chitosan hydrolysates may result not only in significantly higher levels of chitosan incorporation into the alginate fibre, since fragments (i.e. molecules of lower molecular size than the parent unhydrolysed chitosan molecules) should be able to more easily penetrate the alginate fibre structure, but also an improvement in the physical properties of the resultant chitosan/alginate fibres by reinforcement of the structure through ionic interaction throughout.

A range of chitosan hydrolysates were prepared and analysed, and were used to produce the corresponding chitosan/alginate fibres. These fibres were subsequently analysed/tested accordingly (as detailed in the subsequent paper).

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MODIFICATION OF ALGINIC ACID FIBRES WITH HYDROLYSED CHITOSANS

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ABSTRACT

A range of commercial chitosans were subjected to controlled acid hydrolysis and their molecular size profiles and degrees of acetylation (DA) were determined (post-hydrolysis) by HPSEC and ^{1}H-NMR spectroscopy, respectively. Hydrolysed chitosans were subsequently utilised for modification of alginic acid/sodium alginate fibres, and levels of hydrolysed chitosan incorporated into base fibres were estimated by elemental analysis. Tensile properties (elongation % and tenacity) of resultant chitosan/alginate fibres were also determined. A broad range of hydrolysed chitosan contents (~ 7-25 % w/w) were obtained (significantly higher than using unhydrolysed chitosans). Fibres produced from either alginate A1 (6%), using HCl (0.2M), a draw ratio of 1.18, treated with hydrolysed chitosan C3 (3.9%), or from alginate A3 (4%), using HCl (0.5M), a draw ratio of 1.18, treated with hydrolysed chitosan C3 (3%), had the best properties.

INTRODUCTION

The aim of the research presented in this paper was to increase the levels of chitosan incorporation onto/into base alginic acid/sodium alginate fibres (compared with the 0-6% w/w chitosan incorporation levels achieved with a variety of different alginates and chitosans in the previous paper) [1]. This was to be facilitated by using a treatment bath containing hydrolysed chitosans (as opposed to the unhydrolysed chitosans used previously), since it was theorised that use of hydrolysed chitosans should result in higher levels of chitosan incorporation, since fragments (molecules of lower molecular size than the parent unhydrolysed molecules) should be able to more easily penetrate the base alginate fibre structure (as illustrated in Figure 1).

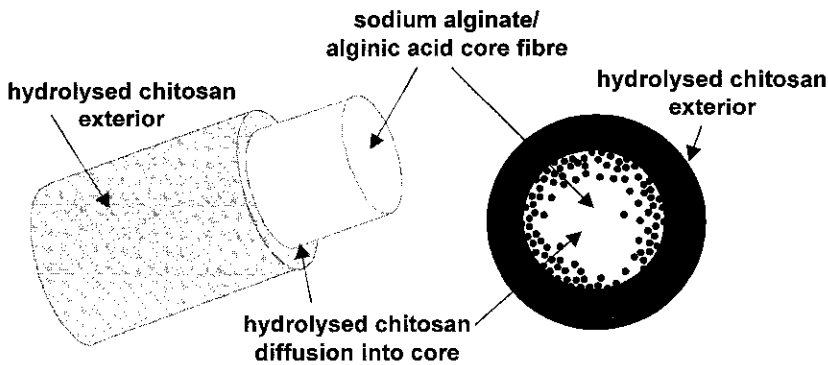


Figure 1. Representation of an alginate/hydrolysed chitosan fibre showing the absorption of hydrolysed chitosan onto/into the base alginate fibre.

It was also hoped that penetration of hydrolysed chitosan fragments into base alginate fibres would result in some reinforcement and thus increase/enhance tensile properties. Higher levels of chitosan incorporation are required in order to take greater advantage of antibacterial, haemostatic and wound healing properties [1].

MATERIALS AND METHODS

Materials

A number of different (in terms of viscosity, molecular weight profile, composition, etc) sodium alginate and chitosan starting materials were obtained for the production of chitosan/alginate fibres (as detailed in the previous paper) [1]. D-glucosamine hydrochloride (GlcN.HCl, Sigma-Aldrich) and N-acetyl-D-glucosamine (GlcNAc, Sigma-Aldrich) were used (as controls) to treat selected alginate fibres. Non-carbohydrate materials utilised as detailed in the previous paper [1].

Chitosan hydrolysis

Dried chitosan (30.5 g) was dissolved in deionised water (750 mL) plus acetic acid (AcOH, 7.5 mL). The solution was stirred until a viscous clear solution was obtained (~ 3-4 hours). Hydrochloric acid (HCl, 25 mL) was added and vigorous stirring was applied until a homogenous solution was obtained (~½-1 hour). The resulting solution (3.90% w/v, based on initial chitosan loading) was heated under reflux (for 8 hours), cooled, filtered to remove any insoluble material, and was ready for use in the treatment of alginate fibres. Hydrolysed chitosan solutions of different volume and/or chitosan concentration were prepared by scaling/altering the amounts used accordingly.

Aliquots of hydrolysed chitosan solutions (~ 50 mL) were placed in CelluSep® T1 regenerated cellulose tubular membrane dialysis bags (flat width 55 mm, nominal molecular weight cut off - MWCO 3.5 kDa, Membrane Filtration Products, Inc., USA) and were dialysed against running water (for 48 hours) to remove low molecular weight materials (especially salts). After dialysis, bag contents were quantitatively transferred into round bottom flasks with deionised water and were rotary evaporated to dryness (under reduced pressure at 50°C). Resultant hydrolysates were dried to constant mass using a Mettler-Toledo HR73 Halogen Moisture Analyzer (standard drying mode, 80°C) and stored over silica gel in a vacuum desiccator until required. Characterisation of the hydrolysed chitosans (molecular size profiling by HPSEC and DA determination by ^{1}H-NMR) was performed as detailed in the previous paper for unhydrolysed chitosans [1].

Fibre production

Spinning conditions for the production of base alginic acid/sodium alginate fibres, and subsequent chitosan treatment, water washing, and acetone drying conditions are detailed in the previous paper [1].

Fibre characterisation

Fibre characterisation methods (determination of moisture, ash, sodium, calcium, and CHN contents), and elongation to break and tenacity measurements are detailed in the previous paper [1]. Optical microscopy (and Image Pro software analysis) was used to compare and contrast the visual aspects and dimensions of selected produced fibres.

RESULTS

Molecular size profiles of hydrolysed chitosans

Molecular size profiles from HPSEC analysis of the hydrolysed chitosans are presented in Figure 2. Retention times (R_T) for molecular weight calibration lines (10000, 5000, 1000, 500 & 100 kDa) were determined from linear regression of the pullulan calibration curve (log M_p = -0.1539 R_T + 11.922, R = 0.9971, M_p = peak molecular weight). The results of mathematical analysis of the molecular size profiles (using Polymer Laboratories PL Caliber Reanalysis software v. 7.04) are presented in Table 1. Five molecular weight averages can be statistically calculated from molecular size profile data (M_n, M_w, M_z, M_{z+1} & M_v). Definitions of these averages and their equations are provided in the previous paper [1].

The molecular size profiles of the unhydrolysed chitosan starting materials were presented in the previous paper [1]. Chitosan C1 had the lowest initial M_n and M_w values and its hydrolysis has consequently produced the lowest molecular size averages. Chitosans C2 and C4 had similar initial profiles and have undergone similar degrees of hydrolysis. Chitosan C3 has undergone less hydrolysis than chitosans C2 and C4, since it has a higher proportion of higher molecular size material after hydrolysis, giving it the highest degree of polydispersity (*d*). Chitosan C5, although not having the highest initial molecular size averages, was the most resistant to hydrolysis, resulting in by far the highest molecular size averages after hydrolysis.

Acid hydrolysis causes depolymerisation, resulting in formation of fragments with a range of molecular sizes. The size distribution of the chitosan fragments will have an effect on their incorporation into alginate fibres. Large fragments may not be able to penetrate the fibre and will therefore only interact with the fibre surface, whereas smaller fragments could penetrate the fibre network and become entangled. Very small fragments could easily penetrate the fibre network without any entanglement.

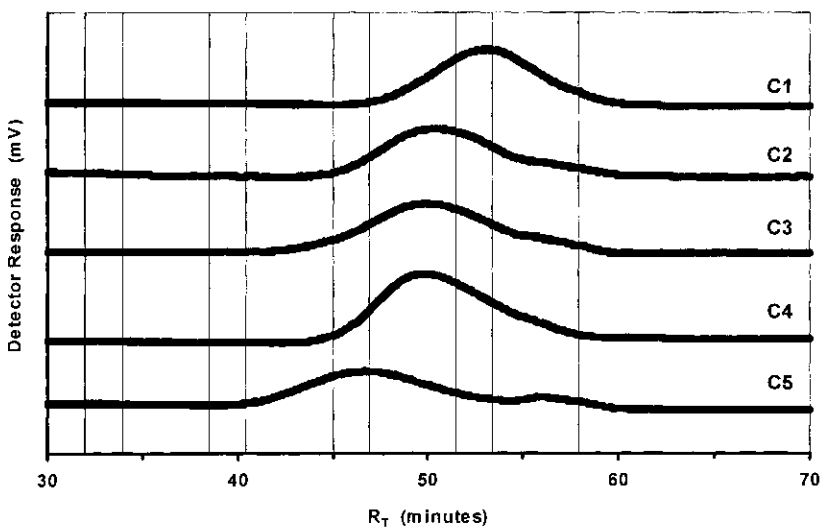


Figure 2. Molecular size profiles of hydrolysed chitosans.

Table 1. Parameters obtained from mathematical analysis of molecular size profiles of hydrolysed chitosans.

Hydrolysed chitosan	M_p (kDa)	M_n (kDa)	M_w (kDa)	M_z (kDa)	M_{z+1} (kDa)	M_v (kDa)	d (M_w/M_n)
C1	5.5	4.1	8.4	16.4	36.8	7.5	2.0
C2	14.8	6.6	18.1	36.9	58.1	16.0	2.7
C3	17.7	7.3	32.3	121.2	256.0	25.8	4.4
C4	18.6	7.0	20.0	42.0	68.1	17.5	2.8
C5	55.3	24.1	74.0	162.9	256.0	64.0	3.1

Degree of acetylation (DA) of hydrolysed chitosans

Definitions of associated terminology (acetylation, deacetylation, DA/DS, DD, % DA, % DD) are provided in the previous paper [1]. The ^{1}H-NMR spectra of hydrolysed chitosans are composed of broad multiplets (rather than well resolved peaks) due to peak broadening as a result of high viscosity (Figure 3). The peak at ~ 1.8 ppm corresponds to N-acetyl methyl group hydrogen atoms, whilst the collection of peaks in the ~ 2.8-5.5 ppm region corresponds to chitosan backbone protons (i.e. single hydrogen atoms attached to the C_1-C_5 carbon atoms, and the two hydrogen atoms attached to the C_6 carbon atom). Trifluoroacetic acid (TFA) moves hydroxyl protons to > 6 ppm, i.e. out of the area of interest. DA is calculated from the ratio of the normalised acetyl group proton peak area (~ 1.8 ppm) to the chitosan backbone proton area (~ 2.8-5.5 ppm), determined by peak integration using Brüker 1D-WINNMR software and corrected to account for a single proton (Table 2). DA values are converted to % DA by multiplying by 100, and to % DD by subtracting the % DA value from 100.

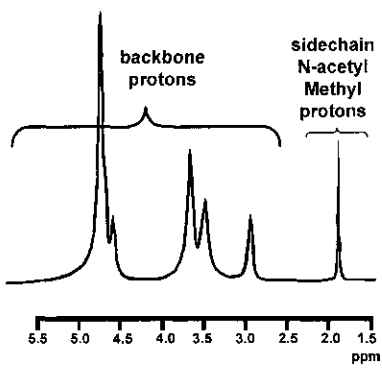


Figure 3. ^{1}H-NMR spectrum of chitosan hydrolysate (C3).

Table 2. DA of hydrolysed chitosans (from ^{1}H-NMR).

Chitosan	DA	% DA	% DD
C1	0.21	21	79
C2	0.26	26	74
C3	0.05	5	95
C4	0.07	7	93
C5	0.04	4	96

Analysis of alginate/hydrolysed chitosan fibres

Variables associated with production of alginate/hydrolysed chitosan fibres included sodium alginate type, method of alginic acid fibre production and hydrolysed chitosan type and concentration. A range of control fibres were also produced and analysed. These included alginic acid fibres, chitosan fibres, alginic acid fibres treated with glucosamine hydrochloride (GlcN.HCl, 3.9%), N-acetyl-D-glucosamine (GlcNAc, 3.9%), 0% chitosan bath (i.e. hydrolysis reagents) and dialysed hydrolysed chitosan (i.e. hydrolysis reagents removed). Information for selected fibres (alginate fibre and hydrolysed chitosan production parameters, and fibre analysis/testing results) is provided in Table 3.

Draw ratio (or stretch ratio) is the ratio of the speeds of the first and second rollers used for the production of the initial alginate fibre, and provides an indication of the amount of stretching the fibre has undergone during production. Draw Ratio = (S_2/S_1), where S_1 = speed of 1st roller and S_2 = speed of 2nd roller. The chitosan contents of fibres were determined from their nitrogen (N) contents (based on the nitrogen contents of the pure alginate fibres and the chitosan used for their production). The results in Table 3 for the fibres treated with glucosamine hydrochloride (GlcN.HCl) and N-acetyl-D-glucosamine (GlcNAc) show that very little incorporation into the A1 base alginate fibre occurred, showing that very small fragments are not retained and hence do not strengthen the A1 base alginate fibre, in fact the treatment process resulted in weakening of the A1 base alginate fibre.

Levels of hydrolysed chitosan incorporation using C1 and C2 chitosans were higher with A2 alginate base fibres, compared with A1 and A3 alginate base fibres, however those produced using A1 alginate base fibres had significantly better tensile properties. Use of hydrolysed C2 chitosan resulted in significantly higher levels of incorporation than use of hydrolysed C1 chitosan, which is interesting since hydrolysed C1 chitosan had the smallest molecular size profile (see Figure 2 and Table 1). Therefore fragments over a certain size may be required in order to produce internal ionic cross-linking/reinforcement effects (as presented in Figure 4). All of the base alginate fibres (A1-3) treated with hydrolysed C1 or C2 had significantly higher chitosan contents (~ 7-16% w/w) than fibres produced using unhydrolysed chitosans (~ 0-6% w/w) [1].

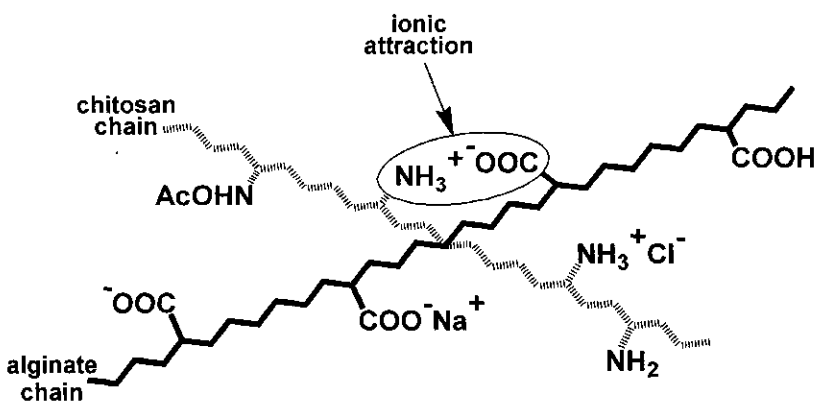


Figure 4. An alginic acid/alginate fibre treated with hydrolysed chitosan.

Table 3. Composition and analysis of selected alginate/hydrolysed chitosan fibres.

Dope (% w/w)	Draw Ratio	1st Bath	3rd Bath	Moisture (% w/w)	Ash (% w/w)	Chitosan (% w/w)	Elongation (%)	Tenacity (cN/dtex)
A1 (6%)	1.18	HCl (0.2M)	GlcN.HCl (3.9%)	9.8	0.3	**0.0**	**tw**	**tw**
A1 (6%)	1.18	HCl (0.2M)	GlcNAc (3.9%)	9.6	0.3	**1.8**	**tw**	**tw**
A1 (6%)	1.18	HCl (0.2M)	hydrol. C1 (3.2%)	11.9	0.3	**7.4**	**20.5**	**2.5**
A2 (6%)	1.18	HCl (0.5M)	hydrol. C1 (3.2%)	11.8	0.3	**9.6**	**5.6**	**1.2**
A3 (4%)	1.09	HCl (0.2M)	hydrol. C1 (3.2%)	11.8	0.4	**8.8**	**7.3**	**0.8**
A1 (6%)	1.18	HCl (0.2M)	hydrol. C2 (2%)	11.7	0.1	**11.9**	**29.3**	**2.7**
A2 (6%)	1.18	HCl (0.5M)	hydrol. C2 (2%)	11.7	0.3	**16.3**	**10.9**	**1.8**
A3 (4%)	1.09	HCl (0.2M)	hydrol. C2 (2%)	11.4	0.4	**10.1**	**6.0**	**0.7**
A1 (6%)	1.18	HCl (0.2M)	hydrol. C3 (2%)	9.9	0.7	**5.4**	**15.5**	**1.5**
A1 (6%)	1.18	HCl (0.2M)	hydrol. C3 (3%)	10.7	0.4	**14.7**	**19.2**	**1.5**
A1 (6%)	1.18	HCl (0.2M)	hydrol. C3 (3.9%)	10.4	0.9	**21.2**	**23.3**	**1.7**
A1 (6%)	1.18	HCl (0.2M)	hydrol. C3 (5.0%)	10.1	1.0	**25.2**	**tb**	**tb**
A3 (4%)	1.18	HCl (0.5M)	hydrol. C3 (2%)	11.4	0.6	**15.5**	**tw**	**tw**
A3 (4%)	1.18	HCl (0.5M)	hydrol. C3 (3%)	11.7	0.4	**23.3**	**18.5**	**2.4**
A3 (4%)	1.18	HCl (0.5M)	hydrol. C3 (3.9%)	11.9	0.1	**22.0**	**10.1**	**1.4**

(2nd bath contained water in all cases; tw = too weak to test; tb = too brittle to test)

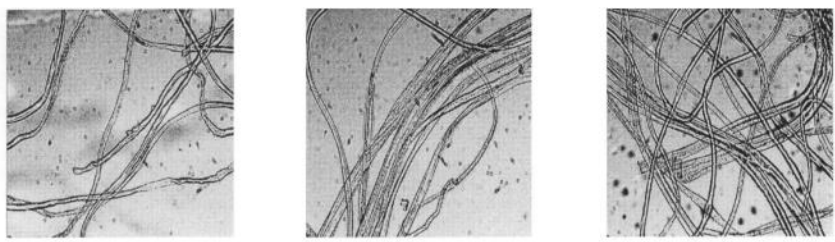
Treatment of A1 and A3 base alginate fibres with increasing concentrations of hydrolysed C3 chitosan showed some interesting trends. In the case of base A1 alginate fibres, treatment with increasing concentrations of hydrolysed C3 chitosan resulted in higher levels of chitosan incorporation into the base A1 alginate fibres. This was also paralleled by increases in fibre tensile properties (presumably due to fibre reinforcement by hydrolysed chitosan fragments penetrating the alginate structure). However, when the chitosan content was over 25 % w/w (from treatment using 5% w/w hydrolysed chitosan) the resultant fibres became too brittle.

This pattern was also observed in the case of base A3 alginate fibres. Treatment of base A3 alginate fibres with 2% w/w hydrolysed C3 chitosan produced weak fibres, and treatment with 3% produced fibres with improved tensile properties, whilst treatment with 3.9% w/w hydrolysed C3 chitosan resulted in a reduction in the tensile properties.

The best two sets of fibres produced during the course of these investigations (in terms of maximising chitosan incorporation but retaining good physical properties) were from either sodium alginate A1 (6%), using a hydrochloric acid (0.2M) coagulation bath, a draw ratio of 1.18, and subsequent treatment of the produced alginate fibres with hydrolysed chitosan C3 (3.9%), or from sodium alginate A3 (4%), a hydrochloric acid (0.5M) coagulation bath, a draw ratio of 1.18, and subsequent treatment of the produced alginate fibres with hydrolysed chitosan C3 (3%).

Microscopic analysis

Selected fibres were examined by optical microscopy and the resultant images analysed using Image Pro software in order to compare and contrast their dimensions. Micrographs for a) untreated base A1 alginate fibres, and base A1 alginate fibres treated with b) 2%, and c) 5% w/w hydrolysed C3 chitosan, are displayed in Figure 5. Untreated base A1 alginate fibres (a) were ~ 8-15 μm wide (mean 10.0 μm), base A1 alginate fibres treated with 2% w/w hydrolysed C3 chitosan (b) were ~ 7-18 μm wide (mean 13.2 μm), and base A1 alginate fibres treated with 5% w/w hydrolysed C3 chitosan (c) were ~ 7-14 μm wide (mean 9.9 μm). Despite circular holes in the spinneret, extruded fibres appeared partially flat and ribbon like, were relatively uniform and displayed some striation along the fibre length.



a) alginic acid fibre

b) alginic acid fibre treated with hydrolysed C3 chitosan (2%)

c) alginic acid fibre treated with hydrolysed C3 chitosan (5%)

Figure 5. Longitudinal views of chitosan/alginate fibres (x 400).

CONCLUSIONS

The application of hydrolysed chitosans onto/into base alginic acid/alginate fibres results in significant increases in levels of chitosan incorporation (compared with use of unhydrolysed chitosans, and other literature methodologies). Lowering of the molecular weight of the chitosan clearly has a positive effect on its ability to penetrate the alginate fibres, rather than simply relying on a fibre surface interaction.

Penetration of hydrolysed chitosan fragments into the base fibre not only increases the chitosan content of the fibre, but can also reinforce the fibre structure and thus enhance its tensile properties. However, if the hydrolysed chitosan content of the fibre is too high then the physical properties of the fibre can be detrimentally affected (e.g. they become weak/brittle).

The aim is to maximise the chitosan content but retain desired physical properties (i.e. have good textile processing ability), since both are important factors with respect to their ultimate potential application in wound dressings. A broad range of chitosan contents (~ 7-25 % w/w) were produced from a variety of alginate and chitosan starting materials.

THE EFFECT OF DEGRADATION ON THE MECHANICAL PROPERTIES OF BIODEGRADABLE POLYLACTIDE YARNS AND TEXTILES

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ABSTRACT

A large research effort worldwide is directed at reproducing 3D tissue and one recent development in the production of tissue engineering scaffolds is the use of biodegradable materials. This research investigates the possibility of producing biodegradable knitted scaffolds from polylactic acid yarns. Degradation in the following biodegradable yarns is studied and the effect on their mechanical properties determined: a commercial polylactide (PLA) yarn Lactron (Kanebo Gohsen Ltd) and four yarns extruded in the Biomedical Textiles Research Centre (BTRC) from a medical grade Poly (L-lactide) (PLLA) polymer (Purac biochem). To determine the degradation effects the mechanical properties were evaluated on a Nene tensile testing rig and samples observed under the scanning electron microscope; before and after the yarns were immersed in phosphate buffer solution (pH=7.4) for various lengths of time at 37°C. The yarns extruded in-house showed a gradual decline in tenacity over the degradation period, unlike the commercial yarn where the tenacity was maintained. This work shows that the degradation process is affected by factors such as extrusion speed and twist. With further knowledge, it may be possible to produce polylactic acid yarns with known degradation and cell attachment characteristics for textile tissue engineering scaffolds. In conclusion, polylactic acid yarns have good potential and biodegradable textile tissue engineering scaffolds look promising for the future.

Keywords
polylactide yarns, biodegradable fibres, tissue engineering scaffolds, medical textiles.

INTRODUCTION

Tissue engineering has been described by Hutmacher *et al.*[1] as "A truly multidisciplinary field that applies the principles of engineering, life science and basic science to the development of viable substitutes, which restore, maintain or improve the function of human tissues." The concept is to grow cells on substrates artificially creating new organs. Current research areas include skin, cartilage, bone, nervous system tissues, muscles, liver and pancreatic cells[2].

For cells to be able to grow in three-dimensional structures there needs to be a scaffold to provide a basic framework for the cells to grow on[3]. There are a wide number of tissue scaffolds being researched today, constructed from numerous materials and each with different end uses. For example skin fibroblasts and epidermal cells form dermal tissue when grown on collagen lattices, while bone cells are cultured on 3-D poly(caprolactone) matrices[1]. The aim of this study is to investigate the feasibility of knitted biodegradable scaffolds, their characteristics and degradation properties.

BACKGROUND

Hutmacher *et al.*[1] describe what they consider to be essential characteristics for a scaffold:

- Highly porous with interconnected pore network for cell growth and flow transport of nutrients and metabolic waste
- Biocompatible and bioabsorbable with controllable degradation and re-sorption rate to match tissue replacement
- Suitable surface chemistry for cell attachment, proliferation, and differentiation
- Mechanical properties to match those of the tissue at the site of the implantation
- Be reproducible, processed into variety of shapes and sizes by solid free form fabrication.

Can a fabric be produced from a biodegradable polymer and fulfil these objectives creating a successful scaffold? In order to answer this question the potential of biodegradable yarns was studied.

Today there are many bio-absorbable polymers available for use in scaffold production. Hutmacher *et al.*[1] list nine of the major materials that can be used to produce scaffolds. The materials are split into the naturally occurring polymers: collagen, polysaccharides, alginate, agarose, chitin/chitosan and hyaluronan; and the synthetic polymers: poly(caprolactone), poly(dioxanone), poly(glycolic acid) and poly(lactic acid). Polylactic acid (PLA) has been used for implantable sutures for decades and researched as a promising candidate for tissue engineering scaffolds[1, 4].

Lactic acid exists in two optically active forms of isomers, known as L (Laevorotatory) and D(Dextrorotatory). After condensation the isomers form L-lactide, D-lactide or meso-lactide which undergo polymerisation to produce poly-L-lactic acid (PLLA), poly-D-lactic acid or poly-DL-lactic acid.

The biodegradation of the PURASORB® PLLA polymer occurs by random hydrolysis, which first decreases the molecular weight of the polymer, then affects the mechanical properties, with the final stage fragmentation and mass loss until the polymer is completely degraded[5]. In the body natural pathways, such as metabolism and excretion should eliminate the final degradation product, lactic acid. There are two models to explain the mode of degradation, bulk erosion (random degradation throughout the polymer) and surface erosion[6]. There appears to be a lot of uncertainty about the actual method of degradation in biodegradable polymers with considerable research directed in this area [7]. However, degradation can be affected by factors such as geometrical shape, size, polymer purity, polymer crystallinity, polymer processing, pH of degradation solution, temperature, implantation site, sterilisation, etc[5, 8].

When designing PLA tissue engineering scaffolds the length of time it takes the polymer to degrade and how the mechanical properties are affected during this period, must be determined. This study investigates the degradation of PLLA yarns over a ten week period; degradation is quantified by the change in material properties.

METHODS

Five PLLA yarns extruded (Table 1) by the Biomedical Textiles Research Centre (BTRC), Heriot-Watt University[9], from PURAC PURASORB® PLLA polymer (derived from corn, density 1.25-1.30 g/cm^3; melting temperature 170-200 °C; glass transition temperature 55-65 °C; degradation time > 24 months) were investigated. The yarns were compared with the commercial polylactide yarn Lactron®, Kanebo Gohsen

Ltd., referred to as PLA F (also produced from corn; tensile strength 0.4 N/Tex; Young's Modulus 400 – 600 kg/mm^2; and melting point 175 °C). The yarns were wound onto cotton reels and conditioned (65 % RH & 20 °C) 48 hours then weighed before the degradation tests.

All the yarns were immersed in phosphate buffered saline (PBS) solution (Sigma Aldrich, Poole, UK), pH 7.4, and kept in an oven at 37 °C, for degradation periods ranging from one to ten weeks. The pH was monitored during the degradation period and the PBS solution changed during week five. The following properties were measured on a Nene (M5, Nene Instruments Ltd.) tensile testing rig before and after degradation: Young's Modulus, load and displacement at yield point. After the degradation period the yarns were removed, rinsed three times with de-ionised water, then conditioned for 72 hours before testing.

Table 1 Production conditions for the five multifilament BTRC yarns

Yarn Name	Spinneret ratio 1:4 (no. holes)	Inherent Viscosity (dl g^{-1})	Extrusion Rate (rev min^{-1})	Extrusion Temperature (°C)	Twisted or Un-twisted
PLLA A	21	3.8	100	225	Un-twisted
PLLA B	21	3.8	300	225	Un-twisted
PLLA C	21	3.8	500	225	Un-twisted
PLLA D	37	6	100	200-235	Un-twisted
PLLA E	37	4	100	235	Twisted

Table 2 Initial properties of the yarns measured to breaking point

Yarn	Tex	Tenacity (N/Tex)	Elongation (%)
PLLA A	89.5	0.062 ± 0.001	3.6 ± 0.1
PLLA B	32	0.097 ± 0.011	79.1 ± 8.6
PLLA D	85	0.041 ± 0.004	2.9 ± 0.4
PLLA E	85	0.030 ± 0.007	1.7 ± 0.3
PLA F	9.2	0.203 ± 0.029	27.1 ± 4.0

The Nene was set up using a 100 N load cell, test speed of 250 mm/min and gauge length of 250 mm. The results were collected using the computer software Nenesoft Materials Test Program (Nene Instruments Ltd.) which calculated Young's Modulus and plotted the stress strain curve. It was found that after the yield point, in some yarns the fibres would break while other yarns were drawn, affecting the Tex. Therefore, in order to compare the yarns the load and displacement were obtained at the yield point. The initial tensile strength tests (n=5) on the yarns until breaking point are shown in Table 2. To minimise the amount of yarn on the cotton reels the Tex count was calculated by weighing one metre of yarn and multiplying the answer by 1000 (Table 3). The load and displacement (Table 3) were then used to calculate the tenacity [tenacity = load / Tex] and elongation [(displacement / original length) x 100]. The yarns, before and after ten weeks degradation, were sputter coated for 45 seconds then examined under the Scanning Electron Microscope (SEM) (Hitachi s-530).

Table 3 The effect of degradation in phosphate buffered saline solution on displacement and load at the yield point.

Yarn	Parameters	Week 0	1	2	4	6	8	10
PLLA A	Load (N)	5.56±0.15	4.66±0.61	4.02±0.55	3.05±0.94	1.45±0.06	1.66±0.30	1.3±0.40
	Displacement (mm)	9.90±0.22	6.30±0.84	6.20±0.91	4.48±1.37	2.90±0.64	2.12±0.39	2.38±0.77
	Tex (g/1000m)	85	85	87	85	88	88	91
PLLA B	Load (N)	2.48±0.07	2.27±0.38	2.53±0.10	2.47±0.06	2.46±0.20	1.60±0.12	1.69±0.36
	Displacement (mm)	7.80±0.27	8.10±0.74	7.30±0.67	8.40±0.22	7.60±0.54	4.80±0.76	5.20±1.30
	Tex (g/1000m)	30	32	30	31	30	30	30
PLLA C	Load (N)	1.95±0.09	1.91±0.07	1.836±0.06	1.78±0.11	1.676±0.08	1.57±0.15	1.37±0.16
	Displacement (mm)	9.40±0.22	8.00±0.35	8.50±0.87	7.90±1.14	8.10±0.22	7.60±0.42	5.80±0.84
	Tex (g/1000m)	20	23	21	22	22	21	22
PLLA D	Load (N)	2.95±0.38	2.89±0.13	2.77±0.84	2.20±0.92	1.88±0.52	1.73±0.58	1.69±0.57
	Displacement (mm)	5.80±1.04	5.08±0.73	4.76±1.30	4.42±2.05	3.30±0.38	2.96±1.04	3.12±1.05
	Tex (g/1000m)	82	87	85	78	82	84	85
PLLA E	Load (N)	2.52±0.65	--	1.47**	1.41±1.07*	1.73±0.13	1.45±0.24	0.78±0.20
	Displacement (mm)	4.05±0.95	--	3.80**	2.73±1.94*	3.91±0.30	3.00±0.51	2.00±0.60
	Tex (g/1000m)	85	--	--	85	81	89	81
PLA F	Load (N)	0.96±0.04	0.99±0.05	1.00±0.05	1.01±0.03	0.91±0.06	0.89±0.04	0.92±0.05
	Displacement (mm)	11.72±1.36	11.60±3.96	10.30±0.98	9.10±0.22	11.70±2.41	8.20±0.57	9.00±0.61
	Tex (g/1000m)	9	9	9	7	7	8	7

(n=5 except ** which represents n=1 and * which represents n=3)

The commercial yarn PLA F and two yarns made by the university, PLLA A and PLLA B, were knitted into fabrics on a six inch gauge weft knitting machine by Culzean Fabrics, Kilmarnock, UK. The fabric samples were weighed, then subjected to six weeks degradation under the same conditions, pH 7.4 and 37 °C, as the yarns. They were tested for strength before and after degradation. After degradation, the fabrics were washed three times with de-ionised water, then conditioned for 72 hours.

Fabric tests were carried out on an Instron (1122, Instron Ltd.) tensile testing rig (n=5). The crosshead and test speed were set at 100 mm/min and the tension compression load cell 200 N. The fabric was mounted onto a vice with a hole (91.6 mm) so that the probe pushed through into the fabric. The results are plotted as a graph, which shows the load at break (peak of graph), and distance the probe travels.

RESULTS

The pH of the buffer solution gradually increased throughout the degradation period, even after the buffer was changed in week five. A control experiment showed that the oven temperature affected the pH; probably caused by evaporation. Yarn PLLA E was impossible to measure in week one and only one tensile test result was obtained in week two, but no Tex value.

Yarn PLLA A had the highest Young's Modulus (Table 3) initially which fell from 0.67 ± 0.03 to 0.59 ± 0.07 in week ten. Over the weeks, yarns PLA F and PLLA C are relatively constant. Yarn PLLA E showed the greatest decrease in Young's Modulus falling by roughly 50 % from 0.63 ± 0.04 to 0.37 ± 0.02.

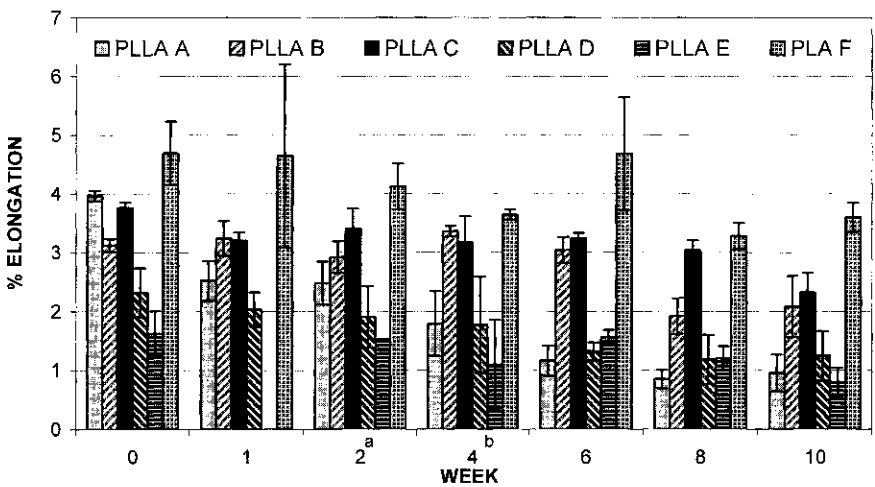

Fig. 1 The effect of degradation in phosphate buffered saline solution on the % elongation of the PLLA yarns.

(n=5 except a which represents n=1 and b which represents

Overall the % elongation, shown in Figure 1, was lowered after ten weeks degradation, with yarns PLLA A and PLLA D showing a progressive decrease. Yarn PLA F has the highest % elongation throughout the degradation period decreasing from 4.69 ± 0.54 to 3.60 ± 0.25 % in week ten.

Yarn PLLA A shows the greatest loss in % elongation, falling from 3.96 ± 0.09 to 0.95 ± 0.31 %, whereas yarn PLLA E has the lowest initial % elongation which decreased by approximately 50%.

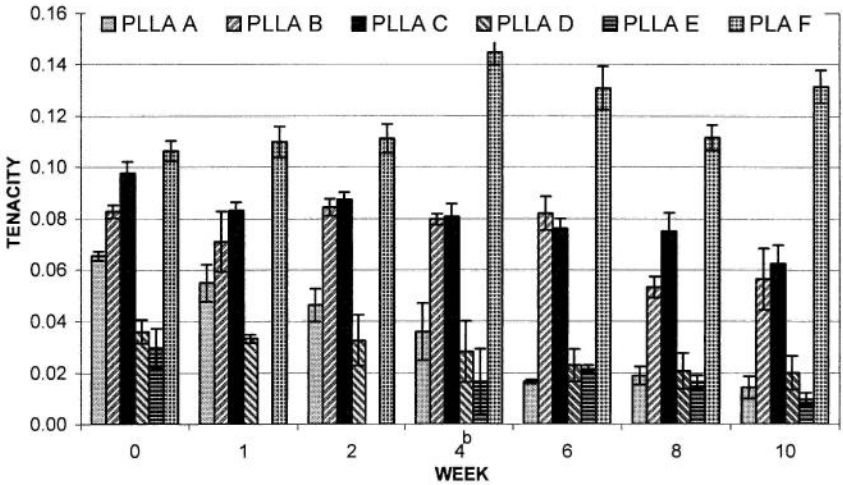

Fig. 2 The effect of degradation in phosphate buffered saline solution on the tenacity of the PLLA yarns.

(n=5 except b which represents n=2)

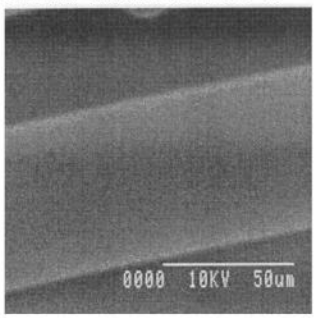

Fig. 3 SEM photomicrograph of PLLA A before degradation.

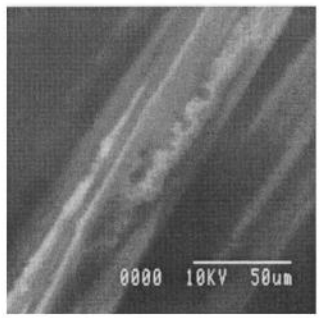

Fig. 4 SEM photomicrograph of PLLA A after degradation

Figure 2 depicts the tenacity of the yarns throughout the degradation period. Yarns PLLA A and PLLA D show a gradual decline in tenacity over the degradation period, unlike the commercial yarn PLA F; which had the highest tenacity throughout and increased by approximately 24 % from 0.106 ± 0.004 to 0.131 ± 0.006. Yarns PLLA D and PLLA E had the lowest tenacity values, which decreased by roughly 1/3 and 2/3 respectively.

All the fibres in the yarns were relatively smooth before the degradation period. After ten weeks in phosphate buffer solution, pH 7.4, the following yarns showed definite signs of degradation: PLLA A, PLLA D and PLLA E. The yarn PLLA A shows surface degradation (Figures 3 & 4), while the yarn PLLA E shows surface degradation and what appears to be hollowing of the fibres (Figures 5 & 6).

The fabric results (Table 4) show that before degradation PLLA A is the strongest fabric, load at break 110±9 N, while the fabric produced from the commercial yarn, PLA F, was the weakest. After six weeks degradation the fabrics produced from PLLA A and PLLA B had lost more than 80 % of their strength, while the fabric produced from the commercial yarn had retained its strength.

DISCUSSION

Each yarn had very different initial characteristics and no two yarns showed the same degradation changes.

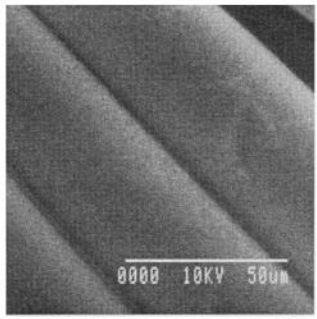

Fig. 5 SEM photomicrograph of PLLA E before degradation

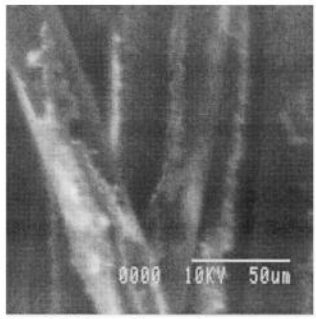

Fig. 6 SEM photomicrograph of PLLA E after degradation

Table 4 The effect of degradation on fabric load.

Yarn	Load (N)	
	Week 0	Week 6
PLLA A	110 ± 9	3 ± 1
PLLA B	77 ± 5	12 ± 2
PLA F	46 ± 10	51 ± 4

However, the ten-week degradation period had less effect on the commercial yarn, PLA F. This could be due to differences in the polylactide polymer but is more likely to be attributed to the production methods. In weeks one and two the PLLA E yarn fell apart making it difficult to test. During the extrusion process there may be small variations in the conditions resulting in an irregular yarn. Although the Tex counts for the degradation studies are only based on one sample the initial value compares favourably with the results in Table 2.

The pH was monitored to ascertain if the degradation product, lactic acid, could be detected. Reed and Gilding state that initially small amounts of lactic acid are released but this increases as the PLLA is broken down to low molecular weight oligomers [10]. In these experiments the pH rose throughout the degradation period mirroring the control PBS solution only. Either the amount of lactic acid released was very small and negated

by the buffer solution, or the changes in the buffer solution concealed the release of lactic acid. In future work an assay to detect lactic acid will be employed.

PLLA A had the highest Young's modulus values, which meant it was the stiffest yarn but the Young's modulus decreased by the end of week ten showing degradation. The initial elongation value for PLLA A was very high but after degradation fell dramatically. One explanation for this may be the relative thickness of the yarn fibres. This results in a greater surface area, per unit mass of fibre, available for degradation to occur on. The SEM photomicrographs show that the fibres have undergone what appears to be surface degradation rather than bulk degradation. Consequently the thinner fibres extending less and breaking earlier may explain the dramatic fall in elongation. When degradation occurs by surface erosion Gopferich[6] states that it should be easier to predict the degradation rate.

PLLA B had a higher tenacity value than PLLA A, a result consistent with extrusion at a faster rate. The molecules in the polymer should be more aligned and may have more crystalline regions holding the fibre together. As a result, less water should have been able to enter the structure, which would explain why the degradation period has a smaller effect on this yarn. PLLA C was produced under the same conditions as PLLA A and PLLA B but extruded at even faster speed. This sample should therefore have an even more aligned structure. When the tenacity values of the yarns are compared it would appear that this is indeed the case. SEM examination of the fibres shows little evidence of degradation. This backs up the theory that as the molecules in the polymer become more aligned, there are fewer amorphous regions present, so less water is able to enter the structure and cause hydrolysis.

The yarn most affected by degradation would appear to be PLLA E. The yarn started off with a high Young's Modulus. Thus it was one of the stiffer yarns, but over the weeks the Young's Modulus fell. The % elongation for PLLA E was initially quite low and remained the lowest after degradation. The tenacity values for this yarn also decreased over time. These results combined with the SEM observations suggest that the chemical bonds are broken during the degradation process and the polymer erodes. This process appears to occur relatively quickly in this yarn. The hollowing of the fibres shown by the PLLA E yarn was described by Gopferich[6] as bulk degradation. Moisture enters the yarn in the amorphous regions breaking some of the chemical bonds inside the fibre. Degradation occurs inside the fibre, until parts of it break off. One difference between PLLA E and the other BTRC yarns is the small twist applied to hold the yarns together. The act of twisting probably opens up the yarns, aiding the absorption of water, which controls the degradation process. It is also probable that the twist applied lowers the strength capacity of the yarn. If the yarn has been drawn in production, then the molecules that make up the polymer are aligned in the fibres. The more aligned the molecules are in one direction, the more strength the yarn should have in that direction. When the yarn is twisted, this strength characteristic is lost because the load is no longer applied in the same direction the molecules lie.

The PLA F yarn had a small amount of twist holding it together but was still a strong yarn relatively unaffected by degradation. As mentioned earlier this may be attributable to differences in the polymer and production methods. The yarn was stored in the conditioned room before the tests, which could account for the discrepancy between the tenacity value obtained and that quoted in the literature. The yarn was twist lively and produced a contorted fabric, making it difficult to apply a constant tension in the fabric test. Although the BTRC yarns had lower tenacity values initially, when knitted they produced stronger fabrics. Nevertheless, like the yarns their load bearing capacity was severely reduced after six weeks degradation.

CONCLUSION

This work shows that factors such as extrusion speed and twist have an effect on the degradation rate. For instance, it may be better to produce a weaker less drawn yarn that allows water molecules to enter the fibre, speeding up the erosion process. In conclusion, polylactic acid yarns have good potential for knitted tissue engineering scaffolds. With further knowledge, the scaffolds can hopefully be engineered to degrade slowly without impeding cell growth.

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CHITOSAN: CRAWLING FROM CRAB SHELLS TO WOUND DRESSINGS

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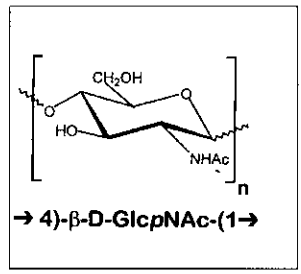
ABSTRACT

Chitin is a naturally occurring polymeric material with a structure similar to cellulose and was originally found to be present in fungi and later in the skeletons of insects and shellfish. Chitin is insoluble in water but when modified with boiling potassium hydroxide solution produces the derivative chitosan, which is soluble in weak acids. Chitosan is biocompatible, biodegradable and non-toxic with excellent antibacterial properties. These qualities make it suitable for use in many medical applications. It is a versatile material, able to be used as fibres, films, beads, gels and support matrices or blended with other materials. Fibres and films are suitable for use in wound care whereas beads or microcapsules are used as vehicles for drug delivery. Chitosan incorporated into a support matrix for tissue engineering or bone repair increases the rate of cell regeneration. The ability to form a hydrogel plus the biocompatibility and antibacterial properties have been used to good effect in the area of periodontal surgery. Chitosan has demonstrated hypocholesteric effects when used as a food supplement with an improvement in the HDL-cholesterol/total cholesterol ratio. Chitosan products have wide ranging uses throughout the pharmaceutical, dietary and water treatment industries. This poster illustrates the processing route from chitin to chitosan plus the varied application areas for chitosan products that can be found within the medical field.

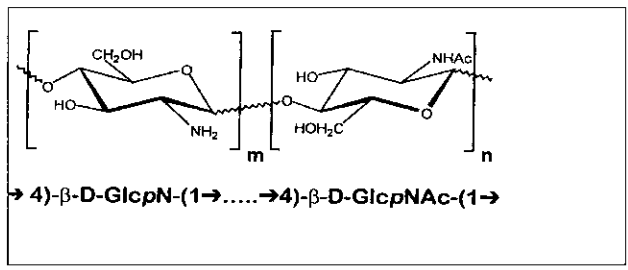
INTRODUCTION

Chitin, along with cellulose and starch, is one of the three most abundant carbohydrate polymers found naturally. It is found in the shells of crustaceans, in the skeletons of insects and was first discovered within fungi in 1811 by Bracannot, a natural historian, (Qin, 1994). It is estimated that the total global quantity produced annually is approximately 5.118×10^6 metric tonnes. The chitin in the waste shells is very slow to degrade therefore disposal of this waste has become a major problem for the seafood processing industry (Shahidi *et al*, 1999). Chitin and chitosan are produced in various countries including India, Japan, Norway and Australia (Kumar, 2000). This is a vast and a relatively unexploited source of biomaterial with some very useful properties that will be discussed later. Chitin, Figure $1^{(a)}$, is insoluble but chitosan, Figure $1^{(b)}$, the N-deacetylated derivative of chitin, is soluble in weak acid solutions. Although the waste shells are found in large quantities, chitosan is expensive to produce. Top quality chitosan for use in the pharmaceutical industry can cost as much as US$7.5/10g to purchase.

Chitosan is produced by crushing and grinding the crustacean shells, washing in alkali and acid to remove the proteins and minerals, leaving chitin which needs to be



(a) Chitin



(b) Chitosan

Fig 1 Structures of Chitin and Chitosan

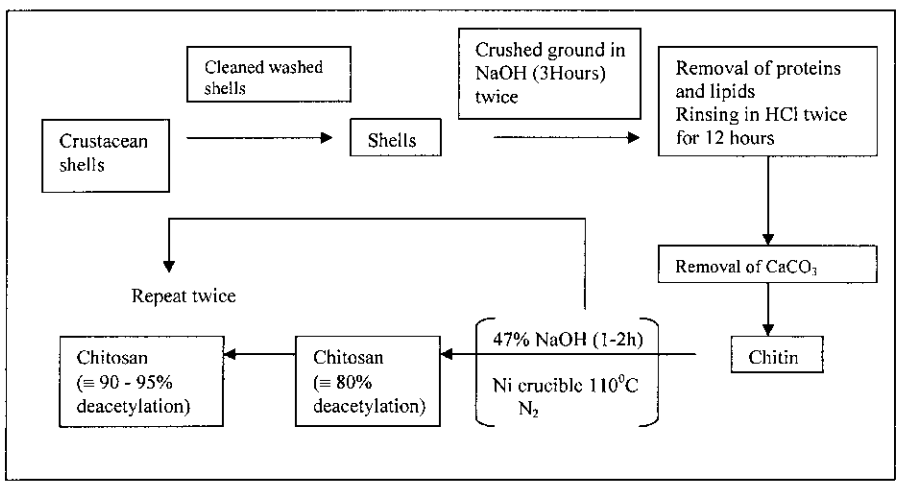


Fig 2 Manufacturing Route of Chitosan (Singla, 2001)

deacetylated. Deacetylation is achieved by treating the chitin in a strong solution of sodium hydroxide at raised temperature, see Figure 2 (Singla, 2001). As seen in Figure 2 the degree of deacetylation can be engineered during the process by increasing the number of sodium hydroxide treatments. An increase in the degree of deacetylation can increase the viscosity of chitosan in solution, along with an increase in chitosan concentration and a decrease in temperature. Chitosan has a high molecular weight and hydrolysation of chitosan solution decreases the viscosity considerably (Kennedy, 2003).

PROPERTIES

The properties that make chitosan an interesting material for medical applications are biocompatibility, biodegradability and bioactivity. Clinical tests show chitosan-based materials do not promote any inflammatory or allergic reaction following ingestion, implantation or injection into, or application onto the human body (Chatelet *et al*, 2000). Cytotoxicity tests to determine the biological response of cells to materials used in medical products show chitin and chitosan to be non-cytotoxic (Lam & Khor, 2001).

Chitosan tested for anti bacterial properties against three common bacteria, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, proved to be very effective in killing these bacteria. Bicomponent materials which included chitosan were shown to have superior antibacterial activity to a corresponding reference material (Li *et al*, 2002). MRSA (Methicillin Resistant Staphyloccocus Aureus) and MSSA (Methicillin Sensitive Staphyloccocus Aureus) infection is now a problem in many hospitals but research has shown that chitosan is effective in depressing growth of these organisms under both wet and dry conditions (Lee, 1999, Takai, 2002). Chitosan also stimulates the immune system against viral and bacterial infection (Peluso *et al*, 1994).

FIBRES AND FILMS

To produce chitosan fibres by the wet spinning process route requires a high quality chitosan of high purity, which is expensive to produce. The first chitosan fibres were produced in 1926 but the advent of synthetic fibres at around the same time made production of chitosan fibres a commercially unattractive proposition. The new synthetic fibres were much easier to produce and cheaper (Qin 1994). The prohibitive cost of producing 100% chitosan fibres has made researchers look into ways of blending chitosan with other materials or coating existing yarns or fabrics. Tamura *et al*, (2002) produced a chitosan coated fibre by extruding sodium alginate dope 1% into a coagulation bath containing very small amounts of chitosan. This procedure will only work with low concentrations of alginate in the dope and chitosan in the bath as chitosan and alginate normally gel when mixed together and extruded.

The viscose spinning route was chosen to produce fibres when chitosan was blended with collagen and tropocologen. Results show an improved blood compatibility for the chitosan/collagen blend for use in sutures and dressings (Hirano, 2000). Chitosan-coated cotton fibres were produced in Japan with good tensile properties and a biologically active fibre surface arising from the chitosan (Liu, 2001). Bicomponent fibres comprising alginic acid core with chitosan coating that displayed good tensile properties and soft handle were produced at Bolton Institute (Miraftab *et al*, 2003).

BIOMATERIALS AND WOUNDCARE

The wound healing process has three distinct stages (Muzzerelli *et al*, 1999):

- Stage 1: Inflammatory cells from the surrounding tissue move towards the site of the wound,
- Stage 2: Fibroblasts appear, producing collagen connective fibres which impart tensile strength to the regenerated tissue. Many capillaries form bringing oxygen and nutrients to the site. Epithelial cells at the edge of the wound start filling in the area under the scab,
- Stage 3: The wound is healed by the formation of the new epithelium

It is therefore very important that any material used in woundcare products actively promotes these three stages and additionally that the wound site has the correct environmental conditions for healing.

It is often assumed when discussing woundcare that it is applicable only to dressings that cover wounds to the outer surface of the body i.e. superficial skin wounds. Woundcare also applies to materials such as hydrogels for use in periodontal surgery or a matrix to be used for tissue engineering.

Surgical adhesions form stiff scars which can adhere to the abdominal wall after abdominal surgery. Chitosan when evaluated for the purpose of skin tissue repair was found to produce a less dense scar than other materials (Biagin *et al,* 1992). Skin grafts were treated with chitosan and found to heal a little quicker than those treated with a standard dressing but also the wound colour was improved after two months with less difference between grafted skin and original skin (Stone *et al*, 2000). Wounds covered with a bilayer chitosan dressing comprising of a dense skin layer supported by a sponge layer healed more quickly (Mi *et al*, 2001).

A hydrogel used in the treatment of infra-bone periodontal disease or extraction surgery plays a similar role to that of a blood clot in the natural healing process. An injectable material incorporating chitosan that gels on application will fill all the irregularities in the bone surface and provide an antibacterial presence that protects the site from infection (Gerentes *et al*, 2002).

The advances made in the repair of large deep wounds over recent years have needed new skills in tissue engineering. Scaffolds are already in use made from both natural and synthetic polymers. The polymer needs to be either tissue specific or have a broad application band that can be engineered to specific applications. The biocompatibility and non toxic properties of chitosan make it an ideal material for a support matrix. Scaffolds, with varying pore morphology, have been produced by freezing a chitosan solution followed by lyophilization to remove the water leaving a porous chitosan matrix (Suh, 2000). The pore morphology, Figure 3, is controlled by the rate of freezing, and pore orientation could be directed using the geometry of thermal gradients during the freezing process.

Chitosan scaffolds can be either molded to the correct size or cut to shape. As chitosan has a slow degradation rate, the structure will hold its shape while the tissue repair is underway.

Materials used for bone forming such as bone powder, ceramics and some synthetic polymers, while being effective, have all had some drawbacks, including low tissue compatabiltiy, inappropriate biodegradation and transmissions of disease but when chitosan has been incorporated with other materials improved performance has been reported. (Lee *et al*, 2002).

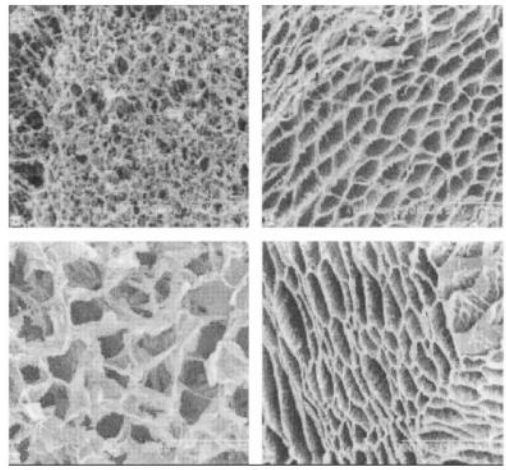


Fig 3 SEM Images Chitosan Scaffolds (Suh, 2000)

CONCLUSIONS

Chitosan is the biomaterial of the future with biocompatibility, antibacterial and biodegradable properties that can enhance the properties of many different materials for use within the medical textiles world. A bicomponent fibre can be produced for use in dressings, scaffolds for use in tissue engineering or bone repair, as well as hydrogels for use in periodontal surgery. Some of the properties can be engineered by varying the degree of deacetylation, solvents used or as in scaffolds, freezing times. Chitosan is a versatile material that can be incorporated into many medical end uses.

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