The present invention provides a method for inhibiting the viability and/or growth of a microorganism, said method comprising contacting said microorganism with an alginate oligomer, wherein said alginate oligomer has a number average degree of polymerisation of 8-50 and wherein at least 90% of the monomer residues are guluronate. The invention is based on the surprising discovery that certain alginate oligomers are able directly to kill, or to inhibit the growth of microorganisms, and both medical and non-medical uses are provided to combat microbial infection or contamination.
Anti-microbial Alginate Oligomers

The present invention relates to the use of alginate oligomers as antimicrobial agents, that is agents capable of killing or destroying, or inhibiting the growth of microorganisms. More particularly, the invention provides alginate oligomers for use as microbicidal and/or microbistatic agents and is based on the surprising discovery that certain alginate oligomers are able directly to kill or destroy, or inhibit the growth of microorganisms. More particularly we have shown that alginate oligomers are cytotoxic and cytostatic to microorganisms such as bacteria.

Accordingly the invention provides a method for inhibiting the viability and/or growth of microorganisms, both in biotic and abiotic situations or locations.

Thus, both medical and non-medical uses and methods are provided to combat infection (put more particularly, microbial infection) or to combat microbial contamination (i.e. colonisation) (in other words, to decontaminate or inhibit microbial colonisation), at animate or inanimate sites or locations, e.g. for disinfection and cleaning purposes.

Many microorganisms, have little or no detrimental impact on human activities. Some are necessary for human well-being and others are even beneficial. However, many microorganisms have activities that are detrimental to human activities, for instance, by causing disease, by damaging crops and animal health, by spoiling food, or by negatively affecting the environment. The ability to control microbial populations, or microbial growth, is essential to mitigate the potentially detrimental effects of microbial activities. An effective mechanism for controlling the effects of microorganisms is to control the numbers of individuals in microorganism populations, or to control the activity of the individuals in microorganism populations. This can be achieved by treating target populations with agents that can destroy (e.g. kill or inactivate) the microorganisms or which can inhibit the growth of the microorganisms, i.e. antimicrobial agents. Agents with antimicrobial properties or functions include physical conditions such as UV, X-ray and gamma radiation, extremes of temperature, pH, osmotic pressure and atmospheric pressure, and also chemicals, e.g. disinfectants, antiseptics and...
antimicrobial chemotherapeutic compounds (antibiotics, antifungals, antivirals and antiprotozoals, for instance).

The benefits of controlling microbial populations are such that vast expenditures of time and resources are made each year globally to achieve this end. Alternative or improved strategies for control of microbial populations are always needed, especially in view of the emergence of microorganisms that have developed, or begun to develop, resistance to some of the control strategies currently available. Thus, there is a need for alternative methods to destroy or inhibit the growth of microorganisms.

As noted above, it has been found that a particular class of alginates, namely alginate oligomers, are effective in destroying, killing and/or inhibiting the growth of microorganisms. These compounds are therefore capable of addressing the various needs described above.

As such, according to the present invention there is proposed a new method or means to destroy, kill or inhibit the growth of microorganisms, including both prokaryotic and eukaryotic microorganisms, and, in particular, bacteria, and fungi.

Alginites are linear polymers of (1-4) linked β-D-mannuronic acid (M) and/or its C-5 epimer α-L-guluronic acid (G). The primary structure of alginates can vary greatly. The M and G residues can be organised as homopolymeric blocks of contiguous M or G residues, as blocks of alternating M and G residues and single M or G residues can be found interspacing these block structures. An alginate molecule can comprise some or all of these structures and such structures might not be uniformly distributed throughout the polymer. In the extreme, there exists a homopolymer of guluronic acid (polyguluronate) or a homopolymer of mannuronic acid (polymannuronate).

Alginites have been isolated from marine brown algae (e.g. certain species of Durvillea, Lessonia and Laminaha) and bacteria such as Pseudomonas aeruginosa and Azotobacter vinelandii. Other pseudomonads (e.g. Pseudomonas fluorescens, Pseudomonas putida, and Pseudomonas mendocina) retain the genetic capacity to produce alginates but in the wild they do not produce detectable levels of alginate. By mutation these non-producing pseudomonads can be induced to produce stably large quantities of alginate.

Alginate is synthesised as polymannuronate and G residues are formed by the action of epimerases (specifically C-5 epimerases) on the M residues in the polymer. In the case of alginates extracted from algae, the G residues are
predominantly organised as G blocks because the enzymes involved in alginate biosynthesis in algae preferentially introduce the G neighbouring another G, thus converting stretches of M residues into G-blocks. Elucidation of these biosynthetic systems has allowed the production of alginates with specific primary structures (WO 94/09124, Gimmestad, M et al, Journal of Bacteriology, 2003, Vol 185(12) 3515-3523 and WO 2004/01 1628).

Alginates are typically isolated from natural sources as large high molecular weight polymers (e.g. an average molecular weight in the range 300,000 to 500,000 Daltons). It is known, however, that such large alginate polymers may be degraded, or broken down, e.g. by chemical or enzymatic hydrolysis to produce alginate structures of lower molecular weight. Alginates that are used industrially typically have an average molecular weight in the range of 100,000 to 300,000 Daltons (such alginates are still considered to be large polymers) although alginates of an average molecular weight of approximately 35,000 Daltons have been used in pharmaceuticals.

It has now been found that alginate oligomers have a direct effect against the viability or growth of microorganisms, particularly bacteria, and on this basis an antimicrobial utility is proposed, not only against bacteria but also against other microorganisms. In this regard, it has been observed that alginate oligomers may exhibit cytotoxic activity against microorganisms, in particular bacteria.

Accordingly, in one aspect alginate oligomers are therefore proposed or believed to have a microbicidal effect. However, alginate oligomers may not necessarily, or only, have a microbicidareffect, and may also exhibit microbiostatic activity. Indeed, in other studies on the growth of bacteria in the presence of alginate oligomers we have shown a cytostatic effect. Thus in certain circumstances and/or conditions alginate oligomers may function also to inhibit the growth of microorganisms e.g. as cytostatic agents against microorganisms. The exact mechanism for these activities is not known, but the activities are believed to be a consequence of a direct effect on the microorganisms. Without wishing to be bound to any speculation, data provided in the Examples may suggest that the alginate oligomers of the invention can have a cytotoxic effect on microorganisms in or associated with a biofilm and a cytostatic effect on planktonic cells. It may also suggest that the alginate oligomers of the invention can be cytocidal for certain species and cytostatic for others, for instance alginate oligomers of the invention may be cytotoxic to Pseudomonas and cytostatic to Acinetobacter, Staphylococcus.
and Candida. What is clear however is that the alginate oligomers of the invention are capable of reducing microbial viability, that is killing or destroying a microorganism, or inhibiting the growth of microorganisms.

Accordingly, in a first aspect the invention provides a method for inhibiting the viability and/or growth of a microorganism, said method comprising contacting a microorganism with an alginate oligomer.

As noted above, alginates typically occur as polymers of an average molecular weight of at least 35,000 Daltons i.e. approximately 175 to 190 monomer residues, although typically much higher and an alginate oligomer according to the present invention may be defined as a material obtained by fractionation (i.e. size reduction) of an alginate polymer, commonly a naturally occurring alginate. An alginate oligomer can be considered to be an alginate of an average molecular weight of less than 35,000 Daltons (i.e. less than approximately 190 or less than 175 monomer residues), in particular an alginate of an average molecular weight of less than 30,000 Daltons (i.e. less than approximately 175 or less than 150 monomer residues) more particularly an average molecular weight of less than 25,000 or 20,000 Daltons (i.e. less than approximately 135 or 125 monomer residues or less than approximately 110 or 100 monomer residues).

Viewed alternatively, an oligomer generally comprises 2 or more units or residues and an alginate oligomer for use according to the invention will typically contain 2 to 100 monomer residues, preferably 2 to 75, preferably 2 to 50, more preferably 2 to 40, 2 to 35 or 2 to 30 residues. Thus an alginate oligomer for use according to the invention will typically have an average molecular weight of 350 to 20,000 Daltons, preferably 350 to 15,000 Daltons, preferably 350 to 10,000 Daltons and more preferably 350 to 8000 Daltons, 350 to 7000 Daltons, or 350 to 6,000 Daltons.

Alternatively put, the alginate oligomer may have a degree of polymerisation (DP), or a number average degree of polymerisation (DPn) of 2 to 100, preferably 2 to 75, preferably 2 to 50, more preferably 2 to 40, 2 to 35, 2 to 30, 2 to 28, 2 to 25, 2 to 22, 2 to 20, 2 to 18, 2 to 17, 2 to 15 or 2 to 12.

Other representative ranges (whether for the number of residues, DP or DPn) include any one of 3, 4, 5, 6, 7, 8, 9, 10 or 11 to any one of 50, 45, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13 or 12.
Other representative ranges (whether for the number of residues, DP or DPn) include any one of 8, 9, 10, 11, 12, 13, 14 or 15 to any one of 50, 45, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17 or 16.

Other representative ranges (whether for the number of residues, DP or DPn) include any one of 11, 12, 13, 14, 15, 16, 17 or 18 to any one of 50, 45, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20 or 19.

Other representative ranges (whether for the number of residues, DP or DPn) include any one of 14, 15, 16, 17, 18, 19, 20, or 21 to any one of 50, 45, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23 or 22.

In certain embodiments the alginate oligomer does not have a number of residues, DP or DPn of 9 or 13.

An alginate oligomer will, as noted above, contain (or comprise) guluronate or guluronic acid (G) and/or mannuronate or manuronic acid (M) residues or units. An alginate oligomer according to the invention will preferably be composed solely, or substantially solely (i.e. consist essentially of) uronate/uronic acid residues, more particularly solely or substantially solely of G and/or M residues. Alternatively expressed, in the alginate oligomer of use in the present invention, at least 80%, more particularly at least 85, 90, 95 or 99% of the monomer residues may be uronate/uronic acid residues, or, more particularly G and/or M residues. In other words, preferably the alginate oligomer will not comprise other residues or units (e.g. other saccharide residues, or more particularly other uronic acid/uronate residues).

The alginate oligomer is preferably a linear oligomer.

More particularly, in a preferred embodiment at least 30% of the monomer residues of the alginate oligomer are G residues (i.e. guluronate or guluronic acid). In other words the alginate oligomer will contain at least 30% guluronate (or guluronic acid) residues. Specific embodiments thus include alginate oligomers with (e.g. containing) 30 to 70% G (guluronate) residues or 70 to 100% G (guluronate) residues. Thus, a representative alginate oligomer for use according to the present invention may contain at least 70% G residues (i.e. at least 70% of the monomer residues of the alginate oligomer will be G residues).

Preferably at least 50% or 60%, more particularly at least 70% or 75%, even more particularly at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% of
the monomer residues are guluronate. In one embodiment the alginate oligomer may be an oligoguluronate (i.e. a homooligomer of G, or 100% G).

In a further preferred embodiment, the above described alginates of the invention have a primary structure wherein the majority of the G residues are in so-called G-blocks. Preferably at least 50%, more preferably at least 70 or 75%, and most preferably at least 80, 85, 90, 92 or 95% of the G residues are in G-blocks. A G block is a contiguous sequence of at least two G residues, preferably at least 3 contiguous G residues, more preferably at least 4 or 5 contiguous G residues, most preferably at least 7 contiguous G residues.

In particular at least 90% of the G residues are linked 1-4 to another G residue. More particularly at least 95%, more preferably at least 98%, and most preferably at least 99% of the G residues of the alginate are linked 1-4 to another G residue.

The alginate oligomer of use in the invention is preferably a 3- to 35-mer, more preferably a 3- to 28-mer, in particular a 4- to 25-mer, especially a 6- to 22-mer, in particular an 8- to 20-mer, especially a 10- to 15-mer, e.g. having a molecular weight in the range 350 to 6400 Daltons or 350 to 6000 Daltons, preferably 550 to 5500 Daltons, preferably 750 to 5000 Daltons, and especially 750 to 4500 Daltons or 2000 to 3000 Daltons.

It may be a single compound or it may be a mixture of compounds, e.g. of a range of degrees of polymerization. As noted above, the monomeric residues in the alginate oligomer, may be the same or different and not all need carry electrically charged groups although it is preferred that the majority (e.g. at least 60%, preferably at least 80% more preferably at least 90%) do. It is preferred that a substantial majority, e.g. at least 80%, more preferably at least 90% of the charged groups have the same polarity. In the alginate oligomer, the ratio of hydroxyl groups to charged groups is preferably at least 2:1, more especially at least 3:1.

The alginate oligomer of the invention may have a degree of polymerisation (DP), or a number average degree of polymerisation (DP\textsubscript{n}), of 3-28, 4-25, 6-22, 8-20 or 10-15, or 5 to 18 or 7 to 15 or 8 to 12, especially 10.

The alginate oligomer of the invention may have a degree of polymerisation (DP), or a number average degree of polymerisation (DP\textsubscript{n}), of 8-50, 8-40, 8-35, 8-30, 8-28, 8-25, 8-22, 8-20, 8-18, 8-16 or 8-14.
The alginate oligomer of the invention may have a degree of polymerisation (DP), or a number average degree of polymerisation (DPn), of 9-50, 9-40, 9-35, 9-30, 9-28, 9-25, 9-22, 9-20, 9-18, 9-16 or 9-14.

The alginate oligomer of the invention may have a degree of polymerisation (DP), or a number average degree of polymerisation (DPn), of 10-50, 10-40, 10-35, 10-30, 10-28, 10-25, 10-22, 10-20, 10-18, 10-16, 10-14, 10-13, 10-12, or 10-11.

The alginate oligomer of the invention may have a degree of polymerisation (DP), or a number average degree of polymerisation (DPn), of 11-50, 11-40, 11-35, 11-30, 11-28, 11-25, 11-22, 11-20, 11-18, 11-16, 11-14, 11-13, or 11-12.

The alginate oligomer of the invention may have a degree of polymerisation (DP), or a number average degree of polymerisation (DPn), of 12-50, 12-40, 12-35, 12-30, 12-28, 12-25, 12-22, 12-20, 12-18, 12-16 or 12-14.

The alginate oligomer of the invention may have a degree of polymerisation (DP), or a number average degree of polymerisation (DPn), of 13-50, 13-40, 13-35, 13-30, 13-28, 13-25, 13-22, 13-20, 13-18 or 13-16.

The alginate oligomer of the invention may have a degree of polymerisation (DP), or a number average degree of polymerisation (DPn), of 14-50, 14-40, 14-35, 14-30, 14-28, 14-25, 14-22, 14-20, 14-18 or 14-16.

The alginate oligomer of the invention may have a degree of polymerisation (DP), or a number average degree of polymerisation (DPn), of 15-50, 15-40, 15-35, 15-30, 15-28, 15-25, 15-22, 15-20, 15-18 or 15-16.

The alginate oligomer of the invention may have a degree of polymerisation (DP), or a number average degree of polymerisation (DPn), of 16-50, 16-40, 16-35, 16-30, 16-28, 16-25, 16-22 or 16-20.

Preferably the alginate oligomer of the invention is substantially free, preferably essentially free, of alginate oligomers having a degree of polymerisation outside of the ranges disclosed herein. This may be expressed in terms of the molecular weight distribution of the alginate oligomer of the invention, e.g. the percentage of each mole of the alginate oligomer being used in accordance with the invention which has a DP outside the relevant range.

The molecular weight distribution is preferably such that no more than 10%, preferably no more than 9, 8, 7, 6, 5, 4, 3, 2, or 1% mole has a DP of three, two or one higher than the relevant upper limit for DPn. Likewise it is preferred that no more than 10%, preferably no more than 9, 8, 7, 6, 5, 4, 3, 2, or 1% mole has a DP below a number three, two or one smaller than the relevant lower limit for DPn.

Representative suitable alginate oligomers have a DP$_n$ in the range 5 to 30, a guluronate/galacturonate fraction (F_G) of at least 0.80, a mannuronate fraction (F_M) of no more than 0.20, and at least 95 mole% of DP no more than 25.

Further suitable alginate oligomers have a number average degree of polymerization in the range 7 to 15 (preferably 8 to 12), a guluronate/galacturonate fraction (F_G) of at least 0.85 (preferably at least 0.90), a mannuronate fraction (F_M) of no more than 0.15 (preferably no more than 0.10), and having at least 95% mole with a degree of polymerization less than 17 (preferably less than 14).

Further suitable alginate oligomers have a number average degree of polymerization in the range 5 to 18 (especially 7 to 15), a guluronate/galacturonate fraction (F_G) of at least 0.80 (preferably at least 0.85, especially at least 0.92), a mannuronate fraction (F_M) of no more than 0.20 (preferably no more than 0.15, especially no more than 0.08), and having at least 95% mole with a degree of polymerization less than 20 (preferably less than 17).

Further suitable alginate oligomers have a number average degree of polymerization in the range 5 to 18, a guluronate/galacturonate fraction (F_G) of at least 0.92, a mannuronate fraction (F_M) of no more than 0.08, and having at least 95% mole with a degree of polymerization less than 20.

Further suitable alginate oligomers have a number average degree of polymerization in the range 5 to 18 (preferably 7 to 15, more preferably 8 to 12, especially about 10), a guluronate/galacturonate fraction (F_G) of at least 0.80 (preferably at least 0.85, more preferably at least 0.90, especially at least 0.92, most especially at least 0.95), a mannuronate fraction (F_M) of no more than 0.20 (preferably no more than 0.15, more preferably no more than 0.10, especially no more than 0.08, most especially no more than 0.05), and having at least 95% mole with a degree of polymerization less than 20 (preferably less than 17, more preferably less than 14).

Further suitable alginate oligomers have a number average degree of polymerization in the range 7 to 15 (preferably 8 to 12), a guluronate/galacturonate fraction (F_G) of at least 0.92 (preferably at least 0.95), a mannuronate fraction (F_M) of no more than 0.08 (preferably no more than 0.05), and having at least 95% mole with a degree of polymerization less than 17 (preferably less than 14).
Further suitable alginate oligomers have a number average degree of polymerization in the range 5 to 18, a guluronate/galacturonate fraction ($F_G$) of at least 0.80, a mannuronate fraction ($F_M$) of no more than 0.20, and having at least 95% mole with a degree of polymerization less than 20.

Further suitable alginate oligomers have a number average degree of polymerization in the range 7 to 15, a guluronate/galacturonate fraction ($F_G$) of at least 0.85, a mannuronate fraction ($F_M$) of no more than 0.15, and having at least 95% mole with a degree of polymerization less than 17.

Further suitable alginate oligomers have a number average degree of polymerization in the range 7 to 15, a guluronate/galacturonate fraction ($F_G$) of at least 0.92, a mannuronate fraction ($F_M$) of no more than 0.08, and having at least 95% mole with a degree of polymerization less than 17.

It will thus be seen that a particular class of alginate oligomers favoured according to the present invention is alginate oligomers defined as so-called "high G" or "G-block" oligomers i.e. having a high content of G residues or G-blocks (e.g. wherein at least 70% of the monomer residues are G, preferably arranged in G-blocks). However, other types of alginate oligomer may also be used, including in particular "high M" or "M-block" oligomers or MG-block oligomers, as described further below. Accordingly, it is alginate oligomers with high proportions of a single monomer type, and with said monomers of this type being present predominantly in contiguous sequences of that monomer type, that represent oligomers that are particularly preferred, e.g. oligomers wherein at least 70% of the monomer residues in the oligomer are G residues linked 1-4 to another G-residue, or more preferably at least 75%, and most preferably at least 80, 85, 90, 92, 93, 94, 95, 96, 97, 98, 99% of the monomers residues of the oligomer are G residues linked 1-4 to another G residue. This 1-4 linkage of two G residues can be alternatively expressed as a guluronic unit bound to an adjacent guluronic unit.

Thus, in a further embodiment more than 50% of the monomer residues of the alginate oligomer may be M residues (i.e. mannuronate or mannuronic acid). In other words the alginate oligomer will contain more than 50% mannuronate (or mannuronic acid) residues. Specific embodiments thus include alginate oligomers with (e.g. containing) 50 to 70% M (mannuronate) residues or e.g. 70 to 100% M (mannuronate) residues. Further specific embodiments also include oligomers containing 71 to 85% M residues or 85 to 100% M residues. Thus, a representative alginate oligomer for use according to this embodiment of the
The present invention will contain more than 70% M residues (i.e. more than 70% of the monomer residues of the alginate oligomer will be M residues).

In other embodiments at least 50% or 60%, more particularly at least 70% or 75%, even more particularly at least 80, 85, 90, 95 or 99% of the monomer residues are mannuronic. In one embodiment the alginate oligomer may be an oligomannuronate (i.e. a homooligomer of M, or 100% M).

In a further embodiment, the above described alginates of the invention have a primary structure wherein the majority of the M residues are in so called M-blocks. In this embodiment preferably at least 50%, more preferably at least 70 or 75%, and most preferably at least 80, 85, 90 or 95% of the M residues are in M-blocks. An M block is a contiguous sequence of at least two M residues, preferably at least 3 contiguous M residues, more preferably at least 4 or 5 contiguous M residues, most preferably at least 7 contiguous M residues.

In particular, at least 90% of the M residues are linked 1-4 to another M residue. More particularly at least 95%, more preferably at least 98%, and most preferably at least 99% of the M residues of the alginate are linked 1-4 to another M residue.

Other preferred oligomers are alginate oligomers wherein at least 70% of the monomer residues in the oligomer are M residues linked 1-4 to another M residue, or more preferably at least 75%, and most preferably at least 80, 85, 90, 92, 93, 94, 95, 96, 97, 98, 99% of the monomers residues of the oligomer are M residues linked 1-4 to another M residue. This 1-4 linkage of two M residues can be alternatively expressed as a mannuronic unit bound to an adjacent mannuronic unit.

In a still further embodiment, the alginate oligomers of the invention comprise a sequence of alternating M and G residues. A sequence of at least three, preferably at least four, alternating M and G residues represents an MG block. Preferably the alginate oligomers of the invention comprise an MG block. Expressed more specifically, an MG block is a sequence of at least three contiguous residues consisting of G and M residues and wherein each non-terminal (internal) G residue in the contiguous sequence is linked 1-4 and 4-1 to an M residue and each non-terminal (internal) M residue in the contiguous sequence is linked 1-4 and 4-1 to a G residue. Preferably the MG block is at least 5 or 6 contiguous residues, more preferably at least 7 or 8 contiguous residues.
In a further embodiment the minority uronate in the alginate oligomer (i.e. mannuronate or guluronate) is found predominantly in MG blocks. In this embodiment preferably at least 50%, more preferably at least 70 or 75% and most preferably at least 80, 85, 90 or 95% of the minority uronate monomers in the MG block alginate oligomer are present in MG blocks. In another embodiment the alginate oligomer is arranged such that at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, e.g. 100% of the G and M residues in the oligomer are arranged in MG blocks.

Although at its broadest, the invention extends to embodiments wherein at least 1% but less than 100% of the monomer residues of the oligomer are G residues (i.e. guluronate orguluronic acid), more particularly, and as defined further below, at least 30% of the monomer residues are G residues. Thus, at its broadest the MG block containing alginate oligomer may contain at least 1%, but less than 100%, guluronate (or guluronic acid) residues, but generally the MG block containing alginate oligomer will contain at least 30% (or at least 35, 40 or 45% or 50% G) but less than 100% G. Specific embodiments thus include MG block containing alginate oligomers with (e.g. containing) 1 to 30% G (guluronate) residues, 30 to 70% G (guluronate) residues or 70 to 99% G (guluronate) residues. Thus, a representative MG block containing alginate oligomer for use according to the present invention may contain more than 30%, but less than 70%, G residues (i.e. more than 30%, but less than 70%, of the monomer residues of the MG block alginate oligomer will be G residues).

Preferably more than 30%, more particularly more than 35% or 40%, even more particularly more than 45, 50, 55, 60 or 65%, but in each case less than 70%, of the monomer residues of the MG block containing alginate oligomer are guluronate. Alternatively, less than 70%, more preferably less than 65% or 60%, even more preferably less than 55, 50, 45, 40 or 35%, but in each case more than 30% of the monomer residues of the MG block containing alginate oligomer are guluronate. Any range formed by any combination of these values may be chosen. Therefore for instance the MG block containing alginate oligomer can have e.g. between 35% and 65%, 40% and 60% or 45% and 55% G residues.

In another embodiment the MG block containing alginate oligomer may have approximately equal amounts of G and M residues (e.g. ratios between 65% G/35% M and 35% G/65% M, for instance 60% G/40% M and 40% G/60% M; 55% G/45% M and 45% G/55% M; 53% G/47% M and 47% G/53% M; 51% G/49% M and 49%
G/51% M; e.g. about 50% G and about 50% M) and these residues are arranged predominantly, preferably entirely or as completely as possible, in an alternating MG pattern (e.g. at least 50% or at least 60, 70, 80, 85, 90 or 95% or 100% of the M and G residues are in an alternating MG sequence).

In certain embodiments the terminal uronic acid residues of the oligomers of the invention do not have a double bond, especially a double bond situated between the C₄ and C₅ atom. Such oligomers may be described as having saturated terminal uronic acid residues. The skilled man would be able to prepare oligomers with saturated terminal uronic acid residues without undue burden. This may be through the use of production techniques which yield such oligomers, or by converting (saturating) oligomers produced by processes that yield oligomers with unsaturated terminal uronic acid residues.

The alginate oligomer will typically carry a charge and so counter ions for the alginate oligomer may be any physiologically tolerable ion, especially those commonly used for charged drug substances, e.g. sodium, potassium, ammonium, chloride, mesylate, meglumine, etc. Ions which promote alginate gelation e.g. group 2 metal ions may also be used.

While the alginate oligomer may be a synthetic material generated from the polymerisation of appropriate numbers of guluronate and mannuronate residues, the alginate oligomers of use in the invention may conveniently be obtained, produced or derived from natural sources such as those mentioned above, namely natural alginate source materials.

Polysaccharide to oligosaccharide cleavage to produce the alginate oligomer useable according to the present invention may be performed using conventional polysaccharide lysis techniques such as enzymatic digestion and acid hydrolysis. In one favoured embodiment acid hydrolysis is used to prepare the alginate oligomers on the invention. In other embodiments enzymic digestion is used with an additional processing step(s) to saturate the terminal uronic acids in the oligomers.

Oligomers may then be separated from the polysaccharide breakdown products chromatographically using an ion exchange resin or by fractionated precipitation or solubilisation or filtration. US 6,121,441 and WO 2008/125828, which are explicitly incorporated by reference herein in their entirety, describe a process suitable for preparing the alginate oligomers of use in the invention.

Further information and discussion can be found in for example in "Handbooks of
Hydrocolloids", Ed. Phillips and Williams, CRC, Boca Raton, Florida, USA, 2000, which textbook is explicitly incorporated by reference herein in its entirety.

The alginate oligomers may also be chemically modified, including but not limited to modification to add charged groups (such as carboxylated or carboxymethylated glycans) and alginate oligomers modified to alter flexibility (e.g. by periodate oxidation).

Alginate oligomers (for example oligoguluronic acids) suitable for use according to the invention may conveniently be produced by acid hydrolysis of alginic acid from, but not limited to, *Laminaria hyperbora* and *Lessonia nigrescens*, dissolution at neutral pH, addition of mineral acid reduce the pH to 3.4 to precipitate the alginate oligomer (oligoguluronic acid), washing with weak acid, resuspension at neutral pH and freeze drying.

The alginates for production of alginate oligomers of the invention can also be obtained directly from suitable bacterial sources e.g. *Pseudomonas aeruginosa* or *Azotobacter vinelandii*.

In embodiments where alginate oligomers which have primary structures in which the majority of the G residues are arranged in G-blocks rather than as single residues are required, algal sources are expected to be most suitable on account of the fact that the alginates produced in these organisms tend to have these structures. The bacterial sources may more suitable for obtaining alginate oligomers of different structures.


The G content of alginates (for example an algal source material) can be increased by epimerisation, for example with mannuronan C-5 epimerases from *A.vinelandii* or other epimerase enzymes. Thus, for example in vitro epimerisation may be carried out with isolated epimerases from *Pseudomonas* or *Azotobacter*, e.g. AlgG from *Pseudomonas fluorescens* or *Azotobacter vinelandii* or the AlgE enzymes (AlgEI to AlgE7) from *Azotobacter vinelandii*. The use of epimerases from other organisms that have the capability of producing alginate, particularly
algae, is also specifically contemplated. The in vitro epimerisation of low G alginites with *Azotobacter vinelandii* AlgE epimerases is described in detail in Ertesvag *et al* (supra) and Strugala et al (Gums and Stabilisers for the Food Industry, 2004, 12, The Royal Society of Chemistry, 84 - 94).

To obtain G-block containing alginites or alginate oligomers, epimerisation with one or more *Azotobacter vinelandii* AlgE epimerases other than AlgE₄ is preferred as these enzymes are capable of producing G block structures. On the other hand AlgE₄ epimerase can be used to create alginites or alginate oligomers with alternating stretches of M/G sequence or primary structures containing single G residue as it has been found that this enzyme seems preferentially to epimerise individual M residues so as to produce single G residues linked to M residues rather than producing G blocks. Particular primary structures can be obtained by using different combinations of these enzymes.

Mutated versions of these enzymes or homologues from other organisms are also specifically contemplated as of use. WO 94/09124 describes recombinant or modified manuronan C-5 epimerase enzymes (AlgE enzymes) for example encoded by epimerase sequences in which the DNA sequences encoding the different domains or modules of the epimerases have been shuffled or deleted and recombined. Alternatively, mutants of naturally occurring epimerase enzymes, (AlgG or AlgE) may be used, obtained for example by site directed or random mutagenesis of the AlgG or AlgE genes.

A different approach is to create *Pseudomonas* and *Azotobacter* organisms that are mutated in some or all of their epimerase genes in such a way that those mutants produce alginites of the required structure for subsequent alginate oligomer production, or even alginate oligomers of the required structure and size (or molecular weight). The generation of a number of *Pseudomonas fluorescens* organisms with mutated AlgG genes is described in detail in WO 2004/01 1628 and Gimmestad, M., *et al*, 2003 (supra). The generation of a number of *Azotobacter vinelandii* organisms with mutated AlgE genes is disclosed in Gimmestad, M., *et al*, 2006 (supra). The skilled man would be able to use this teaching to produce new mutants that could be used to give rise to the alginate oligomers of the invention without undue burden.

A further approach is to delete or inactivate the endogenous epimerase genes from an *Azotobacter* or a *Pseudomonas* organism and then to introduce one or more exogenous epimerase genes, which may or may not be mutated (i.e. may
be wild-type or modified) and the expression of which may be controlled, for example by the use of inducible or other "controllable promoters". By selecting appropriate combinations of genes, alginates of predetermined primary structure can be produced.

A still further approach would be to introduce some or all of the alginate biosynthesis machinery of *Pseudomonas* and/or *Azotobacter* into a non-alginate producing organism (e.g. *E. coli*) and to induce the production of alginate from these genetically modified organisms.

When these culture-based systems are used, the primary structure of the alginate or alginate oligomer products can be influenced by the culture conditions. It is well within the capabilities of the skilled man to adjust culture parameters such as temperature, osmolarity, nutrient levels/sources and atmospheric parameters in order to manipulate the primary structure of the alginates produced by a particular organism.

References to "G residues/G" and "M residues/M" or to guluronic acid or mannuronic acid, or guluronate or mannuronate are to be read interchangeably as references to guluronic acid/guluronate and mannuronic acid/mannuronate (specifically α-L-guluronic acid/guluronate and β-D-mannuronic acid/mannuronate), and further include derivatives thereof in which one or more available side chains or groups have been modified without resulting in antimicrobial (e.g. microbiostatic or microbicidal) activity that is substantially lower than that of the unmodified oligomer. Common saccharide modifying groups would include acetyl, sulphate, amino, deoxy, alcohol, aldehyde, ketone, ester and anhydro groups. The alginate oligomers may also be chemically modified to add charged groups (such as carboxylated or carboxymethylated glycans), and to alter flexibility (e.g. by periodate oxidation). The skilled man would be aware of still further chemical modifications that can be made to the monosaccharide subunits of oligosaccharides and these can be applied to the alginate oligomers of the invention.

The term "microorganism" as used herein includes any microbial organism, that is any organism that is microscopic, namely too small to be seen by the naked eye. In particular as used herein the term includes the organisms typically thought of as microorganisms, particularly bacteria, fungi, archaea, algae and protists. The term thus particularly includes organisms that are typically unicellular, but which may have the capability of organising into simple cooperative colonies or structures such as filaments, hyphae or mycelia (but not true tissues) under certain conditions.
The microorganism may be prokaryotic or eukaryotic, and may be from any class, genus or species of microorganism. Examples of prokaryotic microorganisms include, but are not limited to, bacteria, including the mycoplasmas, (e.g. Gram-positive, Gram-negative bacteria or Gram test non-responsive bacteria) and archaeobacteria. Eukaryotic microorganisms include fungi, algae and others that are, or have been, classified in the taxonomic kingdom Protista or regarded as protists, and include, but are not limited to, for example, protozoa, diatoms, protophyta, and fungus-like molds. The microorganism may be aerobic or anaerobic. The microorganism may be pathogenic or non-pathogenic, or a be spoilage or an indicator microorganism. In particular preferred embodiments the microorganism is pathogenic.

Bacteria or fungi represent preferred classes of microorganism and accordingly the alginate oligomers may be preferably viewed as having antibacterial or anti-fungal activity (e.g. bacteriocidal or bacteriostatic or fungicidal or fungistatic).

Providencia alcalifaciens and Klebsiella oxytoca and Gram non-responsive bacteria such as Chlamydia trachomatis and Chlamydia psittaci.

Preferably the bacteria are selected from the following genera: Achromobacter, Acinetobacter, Actinobacillus, Aeromonas, Agrobacterium, Alcaligenes, Alteromonas, Bacteroides, Bartonella, Borrelia, Bordetella, Brucella, Burkholderia, Campylobacter, Cardiobacterium, Chlamydia, Chlamydophila, Chromobacterium, Chryseobacterium, Citrobacter, Clostridium, Comamonas, Corynebacterium, Coxiella, Cryptobacterium, Edwardsiella, Eikenella, Enterobacter, Enterococcus, Erwinia, Kingella, Klebsiella, Lactobacillus, Lactococcus, Legionella, Leptospira, Leptotrichia, Leuconostoc, Listeria, Listonella, Mobiluncus, Moraxella, Morganella, Mycobacterium, Mycoplasma, Neisseria, Nocardia, Nocardiosis, Pantoea, Parachlamydia, Pasteurella, Peptococcus, Peptostreptococcus, Prevotella, Propionibacterium, Proteus, Providencia, Pseudomonas, Ralstonia, Rickettsia, Salmonella, Shewenella, Shigella, Sphingobacterium, Sphingomonas, Staphylococcus, Stenotrophomonas, Streptobacillus, Streptococcus, Streptomyces, Treponem and Yersinia.

In certain embodiments the bacteria are selected from the genera, Acinetobacter, Klebsiella, Providencia, Pseudomonas and Burkholderia, e.g. the bacteria are from a species selected from Acinetobacter baumannii, Acinetobacter baylyi, Acinetobacter bouvetii, Acinetobacter calcoaceticus, Acinetobacter gerneri, Acinetobacter grimontii, Acinetobacter haemolyticus, Acinetobacter johnsonii, Acinetobacter junii, Acinetobacter Iwoffii, Acinetobacter parvus, Acinetobacter radioresistens, Acinetobacter schindleri, Acinetobacter tandoii, Acinetobacter tjernbergiae, Acinetobacter towneri, Acinetobacter ursingii, Klebsiella granulomatis, Klebsiella oxytoca, Klebsiella pneumoniae, Klebsiella singaporensis, Klebsiella variicola, Providencia stuartii, Providencia sneebia, Providencia rettgeri, Providencia rustigianii, Providencia heimbachae, Providencia burhodogranariae, Providencia alcalifaciens, Pseudomonas aeruginosa, Pseudomonas alcaligenes, Pseudomonas anguilliseptica, Pseudomonas argentinensis, Pseudomonas borbri, Pseudomonas citronellolis, Pseudomonas flavescens, Pseudomonas mendocina, Pseudomonas nitroreducens, Pseudomonas oleovorans, Pseudomonas pseudoalcaligenes, Pseudomonas resinovorans, Pseudomonas straminea, Pseudomonas cremoriclorata, Pseudomonas fulva, Pseudomonas monteilii, Pseudomonas mosselli, Pseudomonas ozyphabitans, Pseudomonas parafulva, Pseudomonas plecgoessica, Pseudomonas putida, Pseudomonas
balearica, Pseudomonas luteola, and Pseudomonas stutzeri, Burkholderia ambifaria, Burkholderia andropogonis, Burkholderia anthina, Burkholderia brasiliensis, Burkholderia caledonica, Burkholderia caribensis, Burkholderia caryophylli, Burkholderia cenocepa, Burkholderia cepacia, Burkholderia dolosa, Burkholderia fungorum, Burkholderia gladioli, Burkholderia glathei, Burkholderia glumae, Burkholderia graminis, Burkholderia hospita, Burkholderia kururiensis, Burkholderia mallei, Burkholderia multivorans, Burkholderia phenazine, Burkholderia phenolrurtrix, Burkholderia phymatum, Burkholderia phytofirmans, Burkholderia plantarii, Burkholderia pseudomallei, Burkholderia pyrrocinia, Burkholderia sacchari, Burkholderia singaporensis, Burkholderia sordidicola, Burkholderia stabilis, Burkholderia terricola, Burkholderia thailandensis, Burkholderia tropica, Burkholderia tuberum, Burkholderia ubonensis, Burkholderia unanae, Burkholderia vienniensis, and Burkholderia xenovorans. The Burkholderia species are of particular note, especially Burkholderia cepacia, Burkholderia multivorans, Burkholderia pseudomallei and Burkholderia mallei; e.g. Burkholderia cepacia.

Thus, the bacteria may be Gram positive or Gram negative bacteria, or indeed Gram-indeterminate bacteria. Gram-negative bacteria, for instance those particularised above, are of importance. Within the Gram-negative bacteria the Enterobacteriaceae and the Gram-negative bacteria non-fermenting bacteria are of particular note.

Enterobacteriaceae include, but are not limited to, bacteria from the genera Alishewanella, Alterococcus, Aquamonas, Aranicola, Azotivirga, Brenneria, Budvicia, Buttiauxella, Cedecia, Citrobacter, Cronobacter, Dickeya, Edwardsiella, Enterobacter, Erwinia, Escherichia, Ewingella, Grimontella, Hafnia, Klebsiella, Kluyvera, Leclercia, Leminorella, Moellerella, Morganella, Obesumbacterium, Pantoea, Pectobacterium, Phlomobacter, Photorhabdus, Plesiomonas, Pragia, Proteus, Providencia, Rahnella, Raoultella, Salmonella, Samsonia, Serratia, Shigella, Sodalis, Tatumella, Trabulsiella, Wigglesworthia, Xenorhabdus, Yersinia, Yokenella. Preferred genera of Enterobacteriaceae include Escherichia, Klebsiella, Salmonella, Shigella, and Yersinia and Providencia.

Non-fermenting Gram-negative bacteria include, but are not limited to, bacteria from the genera Pseudomonas, Acinetobacter, Stenotrophomonas and Burkholderia, Achromobacter, Algaligenes, Bordetella, Brevundimonas, Comamonas, Elizabethkingia (formerly Chryseobacterium), Methylobacterium,
Moraxella, Ochrobactrum, Oligella, Psychrobacter, Ralstonia, Roseomonas, Shewanella, Sphingobacterium, e.g. Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia, and Burkholderia spp..

Preferably the bacteria may be selected from the genera Pseudomonas, Acinetobacters, Burkholderia, Escherichia, Klebsiella, Streptococcus, Enterococcus, Providencia, Moraxalla, Staphylococcus, e.g. Pseudomonas aeruginosa, Acinetobacter baumannii, Burkholderia spp, E. coli, Klebsiella pneumoniae, Burkholderia cepacia, Burkholderia multivorans, Burkholderia mallei, Burkholderia pseudomallei, Acinetobacter Iwoffii, Providencia stuartii, Providencia rettgeri, Providencia alcalifaciens, Klebsiella oxytoxa, Pseudomonas anguilliseptica, Pseudomonas oryzyhabitans, Pseudomonas plecoglossicida, Pseudomonas luteola, Moraxalla catarrhalis, Enterococcus faecium, Streptococcus oralis and MRSA.

Thus, by way of representative example, the microorganism may be a bacteria of the genus Staphylococcus, Pseudomonas, Legionella, Mycobacterium, Proteus, Klebsiella, Fusobacterium or other enteric or coliform bacteria.

The microorganism may also be a, or from a, fungus, including for example fungi that may be, or may have been, classified as protista, e.g. fungi from the genera Candida, Aspergillus, Pneumocystis, Penicillium and Fusarium. Representative fungal species include, but are not limited to, Candida albicans, Candida dubliniensis, Cryptococcus neoformsans, Histoplasma capsulatum, Aspergillus fumigatus, Coccidiodes immitis, Paracoccidioides brasiliensis, Blastomyces dermitidis, Pneomocystis carnii, Penicillium marneffi, Alternaria alternate.

The microorganism may also be an, or from an, alga, including for example algae that may be, or may have been, classified as protista. Representative algal species include Chaetophora, Chlorella protothecoides, Coleochaete scutata, Coleochaeta soluta, Cyanidioschyzon merolae Aphanochaete, Gloëtaenium, Oedogonium, Oocystis, Oscillatoha, Paradoxia multisitia, Phormidium, Chroococcus, Aphanothece, Fragillaria, Cocconis, Navicula, Cymbella, Phaeodactylum as well as cyanobacteria (blue-green algae) and diatoms such as Nitzschia palea.

The microorganism may also be a protozoa, e.g. a member of the groups Amoebae, Sporozoa, Ciliates, and Flagellates. Representative protozoa include Toxoplasma species e.g. Toxoplasma gondii, Plasmodium species such as Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae. Trypanosoma
species e.g. *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania* species such as *Leishmania major*, and *Entamoeba* species such as *Entamoeba histolytica*.

Preferably the microorganism is selected from following genera: *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Serratia*, *Yersinia*, *Peptostreptococcus*, *Bacteriodes*, *Pseudomonas*, *Legionella*, *Staphylococcus*, *Enterococcus*, *Streptococcus*, *Klebsiella*, *Candida*, *Proteus*, *Burkholderia*, *Fusobacterium* and *Mycobacterium*, for instance, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Legionella pneumophila*, *Candida albicans*, *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Streptococcus Pyogenes*.

The term "microbicidal" means the ability negatively to impact the viability (i.e. to reduce or inhibit or ablate the viability) of a microorganism. In particular, "microbicidal" means the ability to kill or destroy a microorganism. The terms "kill" and "destroy" encompass the complete or partial destruction of the microorganism, e.g. the full or partial disintegration of the cellular structure of a microorganism. As used herein the term "microbicidal" is not necessarily specific to any particular field of use and therefore the use of the term, unless specifically stated otherwise, should not imply any particular technical context, e.g. environmental or clinical, medical or non-medical, etc.

"Microbiostatic" means the ability to inhibit the growth of a microorganism.

As described further below, the term "growth" is used broadly herein to refer to any aspect of growth of a microorganism, including both an increase in size or in the numbers of a microorganism. The term "growth" thus explicitly includes replication or reproduction of a microorganism. The term "inhibit" includes any degree of reduction of growth (as compared for example to growth which may be observed in the absence of the microbiostatic agent) as well prevention of growth. As used herein the term "microbiostatic" is not necessarily specific to any particular field of use and therefore the use of the term, unless specifically stated otherwise, should not imply any particular technical context, e.g. environmental or clinical, medical or non-medical, etc.

The term "microbicidal" thus includes a cytotoxic effect of an agent against a microorganism. Therefore, a microbicidal agent can be viewed as bactericidal, fungicidal, algicidal, protozoacidal and so on depending on the type of microorganism that the agent is cytotoxic against. Similarly, the term "microbiostatic" can be viewed as a reference to a cytostatic effect of an agent against a microorganism. Therefore, in relation to the term "microbe" a
microbiostatic agent can be categorised as bacteriostatic, fungistatic, algistatic,
protozoastatic and so on depending on the type of microbe that the agent is
cytostatic against.

The invention provides for the use of an alginate oligomer as a microbicidal
agent and/or a microbiostatic agent.

The term "viability of a microorganism" means the ability of a microbe to
survive under given conditions. Survival can be considered equivalent to remaining
alive. The alginate oligomers of the invention may reduce the viability of
microorganisms through their microbicidal effects. Determining the viability of a
microorganism can be done using the techniques detailed below for measuring
microorganism cell death (and viability).

Thus, "inhibiting the viability" of a microorganism can include any effect
which reduces the viability of a microorganism, or which renders it less likely to
survive, or non-viable. In particular this term covers killing or destroying a
microorganism.

The term "killing a microorganism" refers to the act of causing a
microorganism to cease to be alive, i.e. to become dead. A microorganism is
considered to be alive if it can be induced to replicate and/or grow, or at least
display morphological changes, when placed in a medium that would normally
support the growth of that microorganism and/or the microorganism is metabolising
nutrients to release energy to support cellular functions. Typically, a microorganism
can be considered to be dead if cell membrane integrity is lost.

Many routine assays are available to determine if a microorganism is alive
(viable) or dead. One option is to place the microorganism in conditions that would
normally support the growth of that microorganism and monitor the growth of the
microorganism by appropriate standard means, e.g. by monitoring the size of the
microorganism, the morphology of the microorganism, the number of
microorganisms in the colony over time, the consumption of nutrients in the culture
media, etc. Another option is to assess the microorganism for morphologies
characteristic of cell death, e.g. necrotic or apoptotic bodies, membrane blebs,
nuclear condensation and cleavage of DNA into regularly sized fragments, ruptured
cell walls or membranes and leakage of cell contents into the extracellular
environment. 

Other methods exploit the characteristic loss of cell membrane integrity in
dead microorganisms. Membrane impermeable dyes (e.g. trypan blue and
propidium iodide) are routinely used to assess membrane integrity. These dyes are excluded from intact microorganisms and so no staining occurs in such microorganisms. If cell membrane integrity is compromised, these dyes can access the microorganism and stain intracellular components. Alternatively, or in addition, dyes that only stain microorganisms with intact membranes are used to give an indication of the viability of the cell. The Live/Dead Assay of Invitrogen Ltd is an assay that uses two dyes, one to stain dead cells, the other to stain live cells. Another approach to assessing membrane integrity is to detect the release of cellular components into the culture media, e.g. lactate dehydrogenase.

A still further option is to measure the metabolism of the microorganism. This can be done routinely in a number of ways. For instance the levels of ATP can be measured. Only living cells with intact membranes can synthesise ATP and because ATP is not stored in cells, levels of ATP drop rapidly upon cell death. Monitoring ATP levels therefore gives an indication of the status of the microorganism. A yet further option is to measure the reducing potential of the cell. Viable microorganisms metabolising nutrients use reducing reactions, by applying a marker that gives different outputs whether in reduced or oxidised form (e.g. a fluorescent dye) to the microorganism, the microorganism's reducing potential can be assessed. Microorganisms that lack the ability to reduce the marker can be considered to be dead. The MTT and MTS assays are convenient examples of this type of assay.

By "growth of a microorganism" it is meant both an increase in the size of the microorganism or in the amount and/or volume of the constituents of a microorganism (e.g. the amount of nucleic acid, the amount of protein, the number of nuclei, the numbers or size of organelles, the volume of cytoplasm) and an increase in the numbers of a microorganism i.e. an increase in the replication of a microorganism.

Typically growth of a microorganism is accompanied by the enlargement of the microorganism. The growth of a microorganism can be measured with routine techniques. For instance, microscopic examination of microorganism morphology over time, or assays to measure changes in the quantities of protein or nucleic acid (e.g. DNA) in general, or the changes in the quantities of specific proteins or nucleic acids, can be used. The skilled man would easily be able to select suitable markers to follow. Conveniently, so called house keeping genes (e.g. β-actin, GAPDH, glyceraldehyde 3-phosphate dehydrogenase), SDHA (succinate dehydrogenase),
HPRT1 (hypoxanthine phosphoribosyl transferase 1), HBS1L (HBS1-like protein), AHSP (alphahaemoglobin stabilising protein), and β2M (beta-2-microglobulin), 16S RNA and virus genes, and their expression products can be monitored.

By "replication of a microorganism" it is meant the act by which a microorganism reproduces. Typically this is by binary fission where a microorganism divides into two, but can also be by budding mechanisms where multiple progeny can arise from a microorganism. To support the division of the microorganism into two, binary fission is normally preceded by enlargement of the dividing microorganism and an increase in the amount and/or volume of cellular constituents. Replication results in an increase in the number of cells/particles and so may be followed by any method of assessing microorganism numbers in a population. Another option is to follow the process in real time by visual examination with a microscope. The time it takes for a microorganism to replicate (i.e. produce another version of itself) is the generation time. Generation time will depend on the type of microorganism, its mode of replication and the conditions in which the microorganism is found. The rate of replication can be expressed in terms of the generation time.

By "inhibiting the growth of a microorganism" it is meant that measurable growth (e.g. replication) of a microorganism, or the rate thereof, is reduced.

Preferably measurable growth (e.g. replication) of a microorganism, or the rate thereof, is reduced by at least 50%, more preferably at least 60%, 70%, 80% or 90%, e.g. at least 95%. Preferably, measurable growth (e.g. replication) is ceased. Growth in terms of microbial size increase or expansion etc. may be inhibited independently of replication and vice versa.

The term "contacting" encompasses any means of delivering the alginate oligomer to the microorganism, whether directly or indirectly, and thus any means of applying the alginate oligomer to the microorganism or exposing the microorganism to the alginate oligomer e.g. applying the alginate oligomer directly to the microorganism, or administering the alginate oligomer to a subject within which or on which the microorganism is present, e.g. subjects with a microbial infection. It will be appreciated therefore that both in vitro and in vivo methods are included.

More particularly the microorganism will be contacted with an effective amount of the, alginate oligomer, more particularly an amount of the alginate oligomer effective directly to inhibit the viability of (e.g. to kill) the microorganism or to inhibit directly the growth of the microorganism.
By "directly" it is meant that it is the alginate oligomers do not recruit physiological systems or mechanisms (e.g. the immune system) to impart their microbicidal or microbiostatic (e.g. their cytotoxic or cytostatic) effects. Rather, the alginate oligomers act directly on the microorganism.

An "effective amount" of the alginate oligomer is that amount of alginate oligomer that results in the microbicidal or microbicostatic effects described above. The skilled man would easily be able to determine what an effective amount of alginate oligomer would be on the basis of routine dose response protocols and, conveniently, the routine techniques for assessing microbial death or growth inhibition etc., as discussed above. The direct effects of the alginate oligomers can be assessed by using routine in vitro systems familiar to the skilled man which are devoid of complete physiological systems or mechanisms that may interfere with the assessment of microbicidal or microbicostatic effects (e.g. simple cell culture systems, isolated cell/virus systems).

The site or location of the microorganism is not restricted. The microorganism may be present on a surface. The surface is not limited and includes any surface on which a microorganism may occur. The surface may be biotic or abiotic, and inanimate (or abiotic) surfaces include any such surface which may be exposed to microbial contact or contamination. Thus particularly included are surfaces on machinery, notably industrial machinery, or medical equipment or any surface exposed to an aquatic environment (e.g. marine equipment, or ships or boats or their parts or components), or any surface exposed to any part of the environment, e.g. pipes or on buildings. Such inanimate surfaces exposed to microbial contact or contamination include in particular any part of: food or drink processing, preparation, storage or dispensing machinery or equipment, air conditioning apparatus, industrial machinery, e.g. in chemical or biotechnological processing plants, storage tanks, medical or surgical equipment and cell and tissue culture equipment. Any apparatus or equipment for carrying or transporting or delivering materials is susceptible to microbial contamination. Such surfaces will include particularly pipes (which term is used broadly herein to include any conduit or line). Representative inanimate or abiotic surfaces include, but are not limited to food processing, storage, dispensing or preparation equipment or surfaces, tanks, conveyors, floors, drains, coolers, freezers, equipment surfaces, walls, valves, belts, pipes, air conditioning conduits, cooling apparatus, food or drink dispensing
lines, heat exchangers, boat hulls or any part of a boat's structure that is exposed to water, dental waterlines, oil drilling conduits, contact lenses and storage cases.

As noted above, medical or surgical equipment or devices represent a particular class of surface on which microbial contamination may form. This may include any kind of line, including catheters (e.g. central venous and urinary catheters), prosthetic devices e.g., heart valves, artificial joints, false teeth, dental crowns, dental caps and soft tissue implants (e.g. breast, buttock and lip implants).

Any kind of implantable (or "in-dwelling") medical device is included (e.g. stents, intrauterine devices, pacemakers, intubation tubes (e.g. endotracheal or tracheostomy tubes), prostheses or prosthetic devices, lines or catheters). An "in-dwelling" medical device may include a device in which any part of it is contained within the body, i.e. the device may be wholly or partly in-dwelling.

The surface can be made of any material. For example it may be metal, e.g. aluminium, steel, stainless steel, chrome, titanium, iron, alloys thereof, and the like. The surface can also be plastic, for example, polyolefin (e.g., polyethylene, (Ultra-High Molecular Weight) polyethylene, polypropylene, polystyrene, poly(meth)acrylate, acrylonitrile, butadiene, ABS, acrylonitrile butadiene, etc.), polyester (e.g., polyethylene terephthalate, etc.), and polyamide (e.g., nylon), combinations thereof, and the like. Other examples include acetal copolymer, polyphenylsulfone, polysulfone, polyetheretherketone, polycarbonate, polyetheretherketone, polyvinylidene fluoride, poly(methyl methacrylate) and poly(tetrafluoroethylene).

The surface can also be brick, tile, ceramic, porcelain, wood, vinyl, linoleum, or carpet, combinations thereof, and the like. The surfaces can also be food, for example, beef, poultry, pork, vegetables, fruits, fish, shellfish, combinations thereof, and the like.

A biotic or animate surface may include any surface or interface in or on an animal, plant or fungal body. It may accordingly be viewed as a "physiological" or "biological" surface. It may be any internal or external body surface, including of any tissue or organ, which, in the case of an animal body, may include, haematological or haematopoietic tissue (e.g. blood). Dead or dying (e.g. necrotic) or damaged (e.g. inflamed or disrupted or broken) tissue is particularly susceptible to microbiological contamination, and such tissue is encompassed by the term "animate" or "biotic". The surface may be a mucosal or non-mucosal surface.

Representative biotic surfaces include, but are not limited to, any surface in the oral cavity (e.g. teeth, gingiva, gingival crevice, periodontal pocket) the
reproductive tract (e.g. cervix, uterus, fallopian tubes), the peritoneum, middle ear, prostate, urinary tract, vascular intima, eye, i.e. ocular tissue (e.g. the conjunctiva lachrymal duct, lachrymal gland, eyelid), corneal tissue, the respiratory tract, lung tissue (e.g. bronchial and alveolial), heart valves, gastrointestinal tract, skin, scalp, nails and the interior of wounds, particularly chronic wounds and surgical wounds, which may be topical or internal wounds. Other surfaces include the exterior of organs, particularly those undergoing transplantation, for example, heart, lungs, kidney, liver, heart valve, pancreas, intestine, corneal tissue, arterial and venous grafts and skin.

In one aspect the surface will not be mucosal, or more particularly will not have a hyperviscous mucus coating. The skilled person will be able to determine when the mucus at a given surface is hyperviscous. In one embodiment the surface will not be the surface of a mucus-secreting tissue. More particularly in such an embodiment the surface will not be the surface of a mucus-coated tissue.

The skilled person will know from his common general knowledge the tissues that secrete mucus and those that are mucus-coated.

The location may also be a location that is not a surface. In other words the microorganism can be found within an material as well as on its surface. The material can be chemically heterogeneous as well as chemically homogenous. The material can also be constructed or formed from or comprise different parts or components. The material can be a part of a larger material or entity. The material may or comprise the materials from which the above mentioned surfaces are formed. In some instances the material can be considered to be an object, which terms covers volumes of liquids wherever found. The material may comprise any of the above described surfaces. The material may be abiotic or biotic (inanimate or animate) as is discussed above in relation to surfaces. For instance, the material might be, completely or in part, a solid, a liquid, a semi solid, a gel or a gel-sol. Thus, for example, the microorganism might be present in body fluids (e.g. blood, plasma, serum, cerebrospinal fluid, GI tract contents, semen); tissues (e.g. adrenal, hepatic, renal, pancreatic, pituitary, thyroid, immune, ovarian, testicular, prostate, endometrial, ocular, mammary, adipose, epithelial, endothelial, neural, muscle, pulmonary, epidermis, osseous); cell and tissue culture media; cell and tissue cultures; clinical/scientific waste materials (which can comprise any of the preceding materials); pharmaceuticals (e.g. tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols,
sprays, compositions for use in nebulisers, ointments, soft and hard gelatine capsules, suppositories, sterile injectable solutions, sterile packaged powders; animal or human food stuffs (e.g. meat, fish, shellfish, vegetables, cereals, diary products, fruit juices, vegetable juices, sauces, stocks, soups, confectionary, alcoholic beverages, condiments); personal hygiene products (e.g. toothpaste, mouthwash, shampoo, soap, deodorant, shower gel); cosmetics (e.g. lip gloss, eye shadow, foundation); drinking water supplies; waste water supplies; agricultural feedstuffs and water supplies; insecticide, pesticide and herbicide formulations; industrial lubricants and so on. Liquids, semi solids, gels or gel-sols are of note.

The body fluids and tissues may be treated in vitro/ex vivo as well as it being possible to treat the same in vivo.

In one aspect the microorganism will not be in a biofilm. In another aspect the microorganism will be in a biofilm. Put differently, the microorganism will not be, or will be, in a biofilm mode of growth; or will be, or will not be, in a non-biofilm mode of growth.

By "biofilm" it is meant a community of microorganisms characterized by a predominance of sessile cells that are attached to a substratum or interface or to each other (some motile cells may also be present) and that are embedded in a matrix of extracellular polymers (more specifically extracellular polymers that they have produced) characterised in that the microorganisms of this colony exhibit an altered phenotype with respect to growth rate and gene transcription (for example as compared to their "non-biofilm" or free-floating or planktonic counterparts). By "in a biofilm" it is meant that the microorganism is within (completely or in part), on or associated with the polymer matrix of a biofilm. Viewed differently, microorganisms that are "not in a biofilm" are microorganisms that are either in isolation, e.g. planktonic, or if in an aggregation of a plurality of microorganisms, that aggregation is unorganised and/or is devoid of the matrix characteristic of a biofilm. In each case, the individual microorganisms do not exhibit an altered phenotype that is observed in their biofilm dwelling counterparts.

From the forgoing it is clear that the alginate oligomers of the invention, i.e. those described above, have medical and non-medical applications. In particular, the invention provides medical uses of the alginate oligomers as defined herein for the treatment of a microbial infection in a subject, for example infection with a pathogenic microorganism.
Thus, in one particular aspect the invention provides a method for inhibiting the viability and/or growth of a microorganism in a subject, said method comprising administering an effective amount of an alginate oligomer (which may be any alginate oligomer as herein defined) to a subject in need thereof.

Also provided is an alginate oligomer for use in inhibiting the viability and/or growth of a microorganism in a subject.

Alternatively put, this aspect of the invention provides the use of an alginate oligomer for the manufacture of a medicament for inhibiting the viability and/or growth of a microorganism in a subject.

Viewed differently the invention also provides an alginate oligomer for use as a therapeutic microbicidal agent and/or a therapeutic microbiostatic agent. In addition, the invention provides the use of an alginate oligomer for the manufacture of a microbicidal and/or a microbiostatic medicament.

These aspects of the invention can also be seen to provide an alginate oligomer for use in combating, and in particular in the treatment of, microbial infection in a subject, or the use of an alginate oligomer in the manufacture of a medicament for use in combating, and in particular in the treatment of, microbial infection in a subject. It will be seen in this aspect that the infection may be combated by inhibiting the growth and/or viability of a microorganism in a subject.

The subject may be any human or non-human animal subject, but more particularly may be a vertebrate, e.g. an animal selected from mammals, birds, amphibians, fish and reptiles. The animal may be a livestock or a domestic animal or an animal of commercial value, including laboratory animals or an animal in a zoo or game park. Representative animals therefore include dogs, cats, rabbits, mice, guinea pigs, hamsters, horses, pigs, sheep, goats, cows, chickens, turkeys, guinea fowl, ducks, geese, parrots, budgerigars, pigeons, salmon, trout, cod, haddock, sea bass and carp. Veterinary uses of the invention are thus covered. The subject may be viewed as a patient. Preferably the subject is a human.

The term "in a subject" is used broadly herein to include sites or locations inside a subject or on a subject, e.g. an external body surface, and may include in particular infection of a medical device e.g. an implanted or "in-dwelling" medical device.

In one embodiment the method of these aspects of the invention may comprise a step in which the subject will be diagnosed as a candidate that would benefit from an anti-microbial effect of the present invention, for example an effect...
in inhibiting the viability and/or growth of a microorganism, or in combating microbial
infection. In another embodiment the method of the invention may further comprise
a step in which the microorganism to be targeted by the treatment will be
determined as not being in a biofilm.

The microbicidal or microbiostatic (e.g. cytotoxic or cytostatic) effects of
alginate oligomers makes them suited to the combating of microbial populations,
including non-biofilm populations of microorganisms.

Thus in another aspect the invention provides a method to combat a
population of microorganisms, said method comprising contacting said
microorganisms with an alginate oligomer as defined herein.

More particularly the microorganisms will be contacted with an amount of
the alginate oligomer effective to kill or inhibit the growth of said microorganisms,
more particularly an amount of the alginate oligomer effective directly to kill or
inhibit the growth of said microorganisms.

In one embodiment of this aspect the microorganism or the population
thereof will not be in a biofilm or will not be in the process of forming a biofilm. For
instance, the microorganism or the population thereof will not be capable of biofilm
formation or the plurality of microorganisms in the population are not of sufficient
number or at a lifecycle stage that permits biofilm formation.

A population of microorganisms is considered to be at least 1000
microorganisms (e.g. cells), e.g. at least 10^5, 10^6, 10^7, 10^8 or 10^9 microorganisms.
The population of microorganisms may be homogenous (i.e. contain a single type
of microorganism) or may be heterogeneous (i.e. contain a plurality of types of
microorganism). For example, any or all of the various microorganism described
above may be found in the population. Preferably some or all of the
microorganisms in the population will be pathogenic. The population may be an
established population or be a partially established population. In other words the
location to be treated has previously been colonised by at least one microorganism
that has multiplied or recruited other microorganisms to establish the population.

By "combat a population of microorganisms" it is meant that the formation of
the population is prevented or the growth of the population is controlled.

By "control the growth of a population" it is meant that the rate of expansion
of the overall number of microorganisms in the population is reduced. Preferably
the rate of expansion is reduced by at least 50%, more preferably at least 75%,
85%, 95% or 99%. Most preferably the expansion is essentially stopped or
reversed, i.e. the overall number of microorganisms in the population is maintained or reduced. Preferably the overall number of viable microorganisms in the population is reduced by at least 50%, more preferably at least 75%, 85%, 95% or 99%. Most preferably the population is substantially or completely eradicated. By substantially eradicated it is meant that the population contains few, or virtually no viable microorganisms.

For populations containing microorganisms, control of the growth of the population can, in one embodiment, be achieved by controlling the rate of replication of the microorganisms in the population. In this regard, the rate of replication of the microorganisms in the population is preferably reduced by at least 50%, more preferably at least 75%, 85%, 95% or 99%. Viewed differently, preferably replication substantially ceases or virtually stops.

Alternatively, or in addition, population growth may be controlled by killing some or all of the microorganisms in the population.

By "prevent the formation of a population " it is meant that a small number (sub-population number) of microorganisms are prevented from expanding to reach population size, e.g. by preventing replication or killing the microorganisms already present or that are added to those already present.

The site or location of the population of microorganisms is not restricted and the various locations described above apply here also.

Thus, medical uses encompassed by the present invention may include the use of alginate oligomers to combat microbial populations within a subject. In this aspect the invention accordingly provides a method to combat a population of microorganisms in a subject, said method comprising administering a pharmaceutically effective amount of an alginate oligomer (which may be any alginate oligomer as herein defined) to a subject in need thereof.

Also provided is an alginate oligomer for use in combating a population of microorganisms in a subject.

Alternatively put, this aspect the invention provides the use of an alginate oligomer for the manufacture of a medicament for combating a population of microorganisms in a subject.

In one embodiment the method of this aspect of the invention may comprise a step in which the subject will be diagnosed as a candidate that would benefit from having a population of microorganisms within it combated. In another embodiment
the method of the invention may further comprise a step in which the population of microorganisms to be combated will be determined as not being, or in, a biofilm.

In a further aspect the invention provides a method for combating microbial contamination of a site, said method comprising contacting the site and/or the microorganism with an alginate oligomer.

More particularly the site (or location) and/or microorganism will be contacted with an effective amount of the alginate oligomer, more particularly an amount of the alginate oligomer sufficient directly to kill or inhibit the growth of the microorganism.

"Combating contamination" includes both preventative and reactionary measures or treatments and therefore covers the prevention as well as the reduction, limitation, or elimination of contamination.

By "contamination" it is meant the unwanted presence of a microorganism at a particular site or location. In abiotic locations this can be considered at its extreme to refer to the presence of any microorganism at the site. Contamination can be considered to cover colonisation of a location by the microorganism, i.e. the establishment of a microorganism at a location and the expansion of the numbers of that microorganism by replication or the recruitment of additional microorganisms, which may be of the same or of a different type. In one embodiment the colonisation process will not involve the formation of a biofilm.

The microorganism can be any microorganism described or listed above. In one embodiment of this aspect the microorganism is not in a biofilm. In another embodiment the microorganism is in a biofilm.

The site or location of the contamination or potential contamination is not restricted and can be any of the various sites or locations described or mentioned above, e.g. it can be in vitro or in vivo, but particularly in this aspect of the invention it will be an "in vitro" or "ex vivo" site or location (i.e. an inanimate or abiotic site or location). However, the site or location may be in a subject and in which case a therapeutically effective amount of the alginate oligomer is administered to the subject. In one embodiment the method of this aspect of the invention may comprise a step in which the subject will be diagnosed as a candidate that would benefit from having microbial contamination at a location in it combated. In another embodiment the method of this aspect of the invention may further comprise a step in which the contaminating microorganisms to be targeted by the treatment will be determined as not being, or in, a biofilm.
Alginate oligomers for use in such methods and the use of alginate oligomers in the manufacture of medicaments for use in such methods are also provided.

In one particular embodiment this aspect of the invention can be applied to the decontamination of clinical, scientific and industrial waste materials. In another particular embodiment this aspect of the invention can be used to decontaminant transplant tissue (e.g. heart, lungs, kidney, liver, heart valve, pancreas, intestine, corneal tissue, arterial and venous grafts and skin) prior to implantation. In another embodiment this aspect can be considered to cover the use of alginate oligomers as preservative agents in materials, especially solutions and liquids.

As noted above, the invention provides a method for combating an infection in a subject, and alginate oligomers for such uses.

"Combating an infection" can be viewed as the treatment or prevention of infection.

The location of the infection is not restricted and may be any of the sites or locations within a subject described above. Administering the alginate to the subject preferably results in the infected location being contacted with an alginate oligomer in an amount sufficient directly to kill or inhibit the growth of the microorganisms in the infection. The microorganism can be any of the microorganisms described above although bacteria are of note. In particular, the infection may a pathogen infection e.g. a bacterial or fungal pathogen. Representative examples of microorganisms that can cause infection are described above. For example the infection may be caused by bacteria from the following genera: *Achromobacter, Acinetobacter, Actinobacillus, Aeromonas, Agrobacterium, Alcaligenes, Alteromonas, Bacteroides, Bartonella, Borrelia, Bordetella, Brucella, Burkholderia, Campylobacter, Cardiobacterium, Chlamydia, Chlamydophila, Chromobacterium, Chryseobacterium, Chryseomonas, Citrobacter, Clostridium, Comamonas, Corynebacterium, Coxiella, Cryptobacterium, Edwardsiella, Eikenella, Enterobacter, Enterococcus, Erwinia, Kingella, Klebsiella, Lactobacillus, Lactococcus, Legionella, Leptospira, Leptotrichia, Leuconostoc, Listeria, Listonella, Mobiluncus, Moraxella, Morganella, Mycobacterium, Mycoplasma, Neisseria, Nocardia, Nocardiopsis, Pantoea, Parachlamydia, Pasteurella, Peptococcus, Peptostreptococcus, Prevotella, Propionibacterium, Proteus, Providencia, Pseudomonas, Raistonia, Rickettsia, Salmonella, Shewenella, Shigella,
Sphingobacterium, Sphingomonas, Staphylococcus, Stenotrophomonas, Streptobacillus, Streptococcus, Streptomyces, Treponem and Yersinia.

Infections caused by Citrobacter, Enterobacter, Escherichia, Hafnia, Serratia, Yersinia, Peptostreptococcus, Bacteriodes, Pseudomonas, Legionella, Staphylococcus, Enterococcus, Streptococcus, Klebsiella, Candida, Proteus, Burkholderia, Fusobacterium and Mycobacterium, for instance. Staphylococcus aureus, Staphylococcus epidermidis, Legionella pneumophila, Candida albicans, Pseudomonas aeruginosa, Burkholderia cepacia and Streptococcus Pyogenes are of note. Infections caused by and Pseudomonas, e.g. Pseudomonas aeruginosa, infections are of particular note.

Further infections of note are those caused by the genera Pseudomonas, Acinetobacter, Burkholderia, Escherichia, Klebsiella, Streptococcus, Enterococcus, Providencia, Moraxalla, Staphylococcus, e.g. Pseudomonas aeruginosa, Acinetobacter baumannii, Burkholderia spp, E. coli, Klebsiella pneumoniae, Burkholderia cepacia, Burkholderia multivorans, Burkholderia mallei, Burkholderia pseudomallei, Acinetobacter Iwoffii, Providencia stuartii, Providencia rettgeri, Providencia alcalifaciens, Klebsiella oxytoca, Pseudomonas anguilliseptica, Pseudomonas oryzihabitans, Pseudomonas plecoglossicida, Pseudomonas luteola, Moraxalla catarrhalis, Enterococcus faecium, Streptococcus oralis and MRSA.

The infection may be acute, or alternatively chronic, e.g. an infection that has persisted for at least 5 or at least 10 days, particularly at least 20 days, more particularly at least 30 days, most particularly at least 40 days.

In this aspect of the invention the infection may occur on a surface in or on the subject (i.e. a biotic surface as discussed above) and/or a surface of a medical device, particularly an implantable or "in-dwelling" medical device, representative examples of which are discussed above.

In one embodiment of this aspect the microorganism is not in a biofilm (the infection can therefore be considered to be a non-biofilm infection). In another embodiment the microorganism is in a biofilm. In one embodiment the method of this aspect of the invention may comprise a step in which the subject will be diagnosed as a candidate that is at risk of developing an infection or would benefit from having infection in it treated. In another embodiment, the method of this aspect of the invention may further comprise a step in which the infection to be targeted by the treatment will be determined as not being, or involving, a biofilm (i.e. a non-biofilm infection).
In this regard the infection may be an infection that is found at a location that is not a surface in a subject, e.g. an infection in a body fluid, including a blood infection and a cerebrospinal fluid infection, or an infection within a tissue. This aspect of the invention therefore provides a method for the treatment of septicaemia, septic shock, sepsis, meningitis, or poisoning by microbial toxins, e.g. cholera toxin and botulinum toxin.

In particular embodiments the invention may provide for the treatment of respiratory infections, e.g. cystic fibrosis, pneumonia, COPD, COAD, COAP, bacteraemia, septicaemia, septic shock, sepsis, meningitis, or poisoning by bacterially derived toxins.

An infection can occur in any subject but some subjects will be more susceptible to infection that others. Subjects who are susceptible to infection include, but are not limited to, subjects whose epithelial and/or endothelial barrier is weakened or compromised, subjects whose secretion-based defences to microbial infection have been abrogated, disrupted, weakened or undermined, and subjects who are immunocompromised, immunodeficient or immunosuppressed (i.e. a subject in whom any part of the immune system is not working normally, or is working sub-normally, in other words in whom any part of the immune response, or an immune activity is reduced or impaired, whether due to disease or clinical intervention or other treatment, or in any way).

Representative examples of subjects who are susceptible to infection include, but are not limited to, subjects with a pre-established infection (e.g. with bacteria, viruses, fungi or parasites such as protozoa), especially subjects with HIV, subjects with sepsis and subjects with septic shock; subjects with immunodeficiency, e.g. subjects preparing for, undergoing or recovering from chemotherapy and/or radiotherapy, organ (e.g. bone marrow, liver, lung, heart, heart valve, kidney, etc.) transplant subjects (including autograft, allograft and xenograft patients), subjects with AIDS; subjects resident in a healthcare institution, e.g. hospital, especially subjects in intensive care or critical care (i.e. those units concerned with the provision of life support or organ support systems to patients); subjects suffering from trauma; subjects with burns, subjects with acute and/or chronic wounds; neonatal subjects; elderly subjects; subjects with cancer (defined broadly herein to include any neoplastic condition; malignant or non-malignant), especially those with cancers of the immune system (e.g. leukaemias, lymphomas and other haematological cancers); subjects suffering from auto-immune conditions.
such as rheumatoid arthritis, diabetes mellitus type I, Crohn's disease, especially those undergoing immunosuppression treatment for those diseases; subjects with reduced or abrogated epithelial or endothelial secretion (e.g. mucous, tears, saliva) and/or secretion clearance (e.g. subjects with poorly functioning cilia on mucosal tissue and/or patients with hyperviscous mucous (e.g. smokers and subjects with COPD, COAD, COAP, bronchitis, cystic fibrosis, emphysema, lung cancer, asthma, pneumonia or sinusitis)) and subjects fitted with a medical device.

Thus, subjects in whom infections may particularly be combated according to the present invention include patients who are impaired, whether due to poor perfusion, repetitive trauma, poor nutrition, poor oxygenation or white cell dysfunction.

Of particular note are subjects that have undergone physical trauma. The trauma itself might cause a weakening in or compromisation of an epithelial and/or endothelial barrier of the subject or the subject may become immunocompromised in response to the trauma (a shock response). The term "trauma" refers broadly to cellular attack by foreign bodies and/or physical injury of cells. Included among foreign bodies are microorganisms, particulate matter, chemical agents, and the like. Included among physical injuries are mechanical injuries; thermal injuries, such as those resulting from excessive heat or cold; electrical injuries, such as those caused by contact with sources of electrical potential; and radiation damage caused, for example, by prolonged, extensive exposure to infrared, ultraviolet or ionizing radiations.

Also of particular note are subjects that have a burn. Any burn, in particular a severe burn, has a significant impact on the integrity of the epithelial and/or endothelial barrier of the subject and the subject will often become immunocompromised in response to the burn (a shock response).

Typical burn-causing agents are extremes of temperature (e.g. fire and liquids and gases at extreme temperature), electricity, corrosive chemicals, friction and radiation. The extent and duration of exposure, together with the intensity/strength of the agent, result in burns of varying severity. Scalding (i.e. trauma associated with high temperature liquids and/or gases) is considered to be a burn.

Epidermal burn severity is commonly classified in two ways. Most common is the classification by degree. First-degree burns are usually limited to erythema (redness) in the general area of the injury and a white plaque at the site of injury.
The cellular trauma of these burns extends only as deep as the epidermis. Second-degree burns also display erythema in the general area of the injury but with superficial blistering of the epidermis. The cellular trauma of second-degree burns involves the superficial (papillary) dermis and may also involve the deep (reticular) dermis layer. Third-degree burns are those in which the epidermis is lost with damage to the hypodermis. Damage is typically extreme including charring. Sometimes eschar, (dry, black necrotic tissue) will be present. Third-degree burns may require grafting. In fourth-degree burns catastrophic damage of the hypodermis occurs, e.g. the hypodermis is completed lost, with damage extending to the underlying muscle, tendon, and ligament tissue. Charring and eschar are observed. Grafting is required if the burn does not prove to be fatal.

Another common classification system is the classification by thickness. "Superficial thickness" burns correspond to first degree burns. The spectrum of second degree burns is covered by two classes of "partial thickness" burns. "Partial thickness-superficial" are burns that affect the epidermis only as far as the papillary dermis. "Partial thickness-deep" are burns that affect the dermis as far as the reticular dermis. "Full thickness" burns correspond to third and fourth degree burns.

Some physical injuries, e.g. some burns, and cellular attacks by foreign bodies result in the formation of a wound. More specifically a wound may be considered to be a breach in, or denudement of, a tissue. Wounds may also be caused by a spontaneously forming lesion such as a skin ulcer (e.g. a venous, diabetic or pressure ulcer), an anal fissure or a mouth ulcer.

Wounds are typically defined as either acute or chronic. Acute wounds are wounds that proceed orderly through the three recognised stages of the healing process (i.e. the inflammatory stage, the proliferative stage and the remodelling phase) without a protracted timecourse. Chronic wounds, however, are those wounds that do not complete the ordered sequence of biochemical events of the healing process because the wound has stalled in one of the healing stages. Commonly, chronic wounds are stalled in the inflammatory phase. In accordance with a particular aspect of the present invention, a chronic wound is a wound that has not healed within at least 40 days, particularly at least 50 days, more particularly at least 60 days, most particularly at least 70 days.

As discussed above, wounds are an ideal environment for infection, particularly chronic infection, due to their lack of an epithelial barrier and the availability of substrate and surface for microbial attachment and colonisation.
Problematically, infection of a wound often delays healing further and thus renders that wound more susceptible to established infection. The alginate oligomers of the invention are therefore effective in the treatment and prevention of infection of wounds and the treatment of wounds, especially chronic wounds, represents one preferred aspect of the present invention.

Therefore, in an embodiment of the invention there is provided a method for the treatment or prevention of infection, particularly chronic infection in the above-mentioned subjects, in particular in subjects with respiratory diseases or disorders e.g. cystic fibrosis, COPD, COAD, COAP, wounds, burns and/or traumas, said method comprising administering a pharmaceutically effective amount of an alginate oligomer as defined herein to the subject.

In an aspect of particular importance, the alginate oligomers may be used to treat or prevent infection in wounds, e.g. burns, for example in the treatment of infected wounds, e.g. burns.

Through the ability to treat and prevent infection of wounds the alginate oligomers defined herein can remove one of the obstacles to wound healing and therefore the alginate oligomers defined above are also effective in the promotion of healing of acute and chronic wounds.

By promotion of healing it is meant that the treatment accelerates the healing process of the wound in question (i.e. the progression of the wound through the three recognised stages of the healing process). The acceleration of the healing process may manifest as an increase in the rate of progression through one, two or all of the healing stages (i.e. the inflammatory stage, the proliferative stage and/or the remodelling phase). If the wound is a chronic wound that is stalled in one of the healing stages the acceleration might manifest as the restarting of the linear, sequential healing process after the stall. In other words, the treatment shifts the wound from a non-healing state to a state where the wound begins to progress through the healing stages. That progression after the restart may be at a normal rate or even a slower rate compared with the rate a normal acute wound would heal.

The alginate oligomers may be used to treat infections wherever they may occur in or on the body. Thus, in another embodiment, the infection may be an infection of a medical device, particularly an in-dwelling medical device.

The alginate oligomers may be used according to the present invention as oral healthcare agents, for example in the control of dental plaque, e.g. to reduce it
or to prevent, reduce or delay its development, by killing the microorganisms in the
plaque or inhibiting the replication or growth of said microorganisms. The alginate
oligomers may also be used in the treatment and prevention of infections or
infectious disease which may occur in the oral cavity, for example gingivitis and
periodontitis.

Conveniently, the alginate oligomers can be applied by any oral health/oral
hygiene delivery system. This may be through the use of toothpastes, dental gels,
dental foams and mouthwashes. Removable dentures and other removable dental
prostheses may be treated outside of the oral cavity with the same compositions or
other suitable pharmaceutically acceptable compositions. The alginate oligomers
can also be incorporated into compositions that are applied to the oral cavity (or
applied to removable dentures and other removable dental prostheses outside of
the oral cavity) to form a coating that persists on surfaces over time, or that
releases the alginate oligomers from the coated surfaces over time, and which
inhibit the viability and/or the growth bacteria in the oral cavity and on the surfaces
of removable dentures and other removable dental prostheses.

Whilst the treatment of infections of the lungs and respiratory tract and all
areas of the body is generally covered by the present invention, in one
embodiment, the medical uses of the invention are not directed to the treatment of
(i) infections in the respiratory tract of patients suffering from COPD's (chronic
obstructive pulmonary diseases), in particular the sinuses and the lungs, in
particular in the treatment of cystic fibrosis, chronic obstructive pulmonary disease,
emphysema, bronchitis and sinusitis; (ii) in the middle ear of patients suffering from
glue ear; or (iii) in the reproductive tract of female patients with impaired fertility; or
(iv) in the digestive tract of patients with digestive tract malfunction (e.g.
constipation).

In specific embodiments of the invention the alginate oligomers may be
used in the treatment of native valve endocarditis, acute otitis media, chronic
bacterial prostatitis, pneumonia (in particular ventilator associated pneumonia),
dental plaque, periodontitis, respiratory diseases associated with infection (which
may include COPD, COAD, COAP, pneumonia cystic fibrosis and asthma), and
device related infection associated with implantable or prosthetic medical devices
(e.g. prosthetic valve endocarditis or the infection of lines or catheters or artificial
joints or tissue replacements, or endotracheal or tracheotomy tubes).
In further embodiments the alginate oligomers of the invention are used to control infections in the eye, e.g. to reduce them, or prevent, reduce or delay their development. In particular, the alginate oligomers of the invention are used to treat or prevent bacterial conjunctivitis and the resultant keratoconjunctivitis sicca (also known as dry eye) that can result through the blockage of the lachrymal gland.

As mentioned previously, in one embodiment the above infections and associated conditions are not, or do not involve, biofilm, in other words they are non-biofilm infections. In another embodiment the above infections and associated conditions are, or do, involve biofilm.

A "pharmaceutically effective" amount of the alginate oligomer is the amount of alginate oligomer that provides a measurable microbicidal or microbiostatic (e.g. cytotoxic or cytostatic) effect on the targeted microorganism (as defined above) and/or a measurable effect on the condition being targeted. Preferably it is an amount sufficient directly to kill the microorganism or inhibit its growth. This amount can be determined with reference to standard practices for deciding dosage amounts and the skilled man will be able to detect evidence of successful treatment from his experience and with the aid of routine tests available to him.

Suitable doses of alginate oligomer will vary from subject to subject and can be determined by the physician or veterinary practitioner in accordance with the weight, age and sex of the subject, the severity of the condition, the mode of administration and also the particular alginate oligomer selected. Typically the alginate oligomers of the invention will be applied to the location undergoing treatment at a local concentration of at least 0.5%, preferably at least 2% or at least 4%, more preferably at least 6% and most preferably at least 10% weight by volume.

"Treatment" when used in relation to the treatment of a medical condition/infection in a subject in accordance with the invention is used broadly herein to include any therapeutic effect, i.e. any beneficial effect on the condition or in relation to the infection. Thus, not only included is eradication or elimination of the infection, or cure of the subject or infection, but also an improvement in the infection or condition of the subject. Thus included for example, is an improvement in any symptom or sign of the infection or condition, or in any clinically accepted indicator of the infection/condition (for example a decrease in wound size or an acceleration of healing time). Treatment thus includes both curative and palliative...
therapy, e.g. of a pre-existing or diagnosed infection/condition, i.e. a reactionary treatment.

"Prevention" as used herein refers to any prophylactic or preventative effect. It thus includes delaying, limiting, reducing or preventing the condition (which reference includes infection and contamination, as applicable, in the different aspects of the invention) or the onset of the condition, or one or more symptoms or indications thereof, for example relative to the condition or symptom or indication prior to the prophylactic treatment. Prophylaxis thus explicitly includes both absolute prevention of occurrence or development of the condition, or symptom or indication thereof, and any delay in the onset or development of the condition or symptom or indication, or reduction or limitation on the development or progression of the condition or symptom or indication.

Specifically, the alginate oligomers of the invention can be taken as a prophylactic treatment, for example to prevent, or at least minimise the risk, of infection or contamination (e.g. by a pathogen).

The aspect of the invention concerning the combating (treatment or prevention) of infection is of particular utility in the care of hospitalised patients as the risk of contracting a nosocomial infection (commonly known as hospital related/acquired infection or healthcare-associated infection), e.g. Staphylococcus aureus, Methicillin Resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia, Clostridium difficile, Mycobacterium tuberculosis and Vancomycin-Resistant Enterococcus, and Acinetobacter Iwoffii, Burkholderia cepacia, Burkholderia pseudomallei, Burkholderia mallei, Burkholderia multivorans, Providencia stuartii can be minimised with a prophylactic regime of the alginate oligomers defined herein. This aspect of the invention is also of particular utility in the care of subjects suffering from trauma, subjects with a burn and subjects with wounds, all of which, as discussed above, are more susceptible to pathogen infection than a subject that is not affected similarly.

Generally, subjects in need of treatment or prophylaxis according to the invention will be diagnosed as suffering or at risk from the target condition, e.g. identified as having or at risk of developing an infection.

Specifically, the alginate oligomers of the invention can be taken as a prophylactic treatment to prevent, or at least minimise the risk, of developing an infection, including for example the infection of wounds, native valve endocarditis,
acute otitis media, chronic bacterial prostatitis, periodontitis, infections of the respiratory tract and lungs (e.g. cystic fibrosis, COPD, COAD, COAP, pneumonia, or other respiratory diseases, dental plaque, pneumonia, or infection of a medical (e.g. in-dwelling) medical device.

In one advantageous embodiment of the invention the alginate oligomers may be used in the methods of the invention in conjunction or combination with a second or further anti-microbial agent (hereinafter “further anti-microbial agent”)

In the context of a medical use, such an anti-microbial agent may be any clinically-useful anti-microbial agent and particularly an antibiotic or an antiviral or antifungal agent. In the context of non-clinical uses, the anti-microbial agent may again be any anti-microbial agent used for such purposes, e.g. any disinfectant or antiseptic or cleaning or sterilising agent. The agents may be used separately, or together in the same composition, simultaneously or sequentially or separately, e.g. at any desired time interval.

Thus, by way of representative example, the further anti-microbial agent may be used after the alginate oligomer, but a preceding or simultaneous use may be beneficial in some circumstances.

The choice of anti-microbial agent will of course need to be appropriate for the location undergoing treatment, but for instance anti-microbial agents, e.g. antibiotics, antifungals, antivirals, antiseptics may be used and/or sterilising conditions such as irradiation (e.g. UV, X-ray, gamma) extremes of temperature, and extremes of pH.

Representative antibiotics include, but are not limited to the aminoglycosides (e.g. amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin); the carbecephem (e.g. loracarbef); the 1st generation cephalosporins (e.g. cefadroxil, cefazolin, cephalaxin); 2nd generation cephalosporins (e.g. cefaclor, cefamandole, cephalaxin, cefoxitin, cefprozil, cefuroxime); 3rd generation cephalosporins (e.g. cefixime, cefdinir, cefditoren, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone); 4th generation cephalosporins (e.g. cefepime); the macrolides (e.g. azithromycin, clarithromycin, dirithromycin, erythromycin, troleandomycin); the monobactams (e.g. aztreonam); the penicillins (e.g. amoxicillin, ampicillin, carbenicillin, cloxacillin, dicloxacillin, nafcillin, oxacillin, penicillin G, penicillin V, piperacillin, ticarcillin); the polypeptide antibiotics (e.g. bacitracin, colistin, polymyxin B); the quinolones (e.g. ciprofloxacin, enoxacin, gatifloxacin, levofloxacin,
lomefloxacin, moxifloxacin, norfloxacin, ofloxacin, trovafloxacin); the sulfonamides (e.g. mafenide, sulfacetamide, sulfamethizole, sulfasalazine, sulfisoxazole, trimethoprim-sulfamethoxazole); the tetracyclines (e.g. demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline); the carbapenems (e.g. imipenem, meropenem, ertapenem, doripenem, panipenem/betamipron, biapenem, PZ-601); chloramphenicol; clindamycin, ethambutol; fosfomycin; isoniazid; linezolid; metronidazole; nitrofurantoin; pyrazinamide; quinupristin/dalfopristin; rifampin; spectinomycin; and vancomycin. The antibiotics vancomycin, tobramycin, meropenem, ciprofloxacin, pipercillin, colistin, aztreonam, ciprofloxacin and azithromycin are preferred. Azithromycin is particularly preferred.

For example, the antibiotic may be selected from amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, telithromycin, CarbomycinA, josamycin, kitasamycin, midecamicine, oleandomycin, spiramycin, tylosin, troleandomycin, aztreonam, imipenem, meropenem, ertapenem, doripenem, panipenem/betamipron, biapenem, PZ-601, cefixime, cefdinir, cefditoren, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, cefepime, demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, bacitracin, colistin, polymyxin B, ciprofloxacin, enoxacin, gatifloxacin, levofloxacin, lomefloxacin, moxifloxacin, norfloxacin, ofloxacin, and trovafloxacin. In particular, antibiotic may selected from ceftazidime, imipenem/cilastatin, meropenem, aztreonam, oxytetracycline, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, spiramycin and ciprofloxacin, and it is particularly preferred that the antibiotic is selected from ceftazidime, imipenem/cilastatin, meropenem, aztreonam, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, spiramycin and ciprofloxacin. More preferably the antibiotic is selected from aztreonam, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, spiramycin and ciprofloxacin.

Representative antiseptics include, but are not limited to chlorine bleach (sodium hypochlorite), quaternary ammonium compounds (e.g. benzalkonium chloride, cetyl trimethylammonium bromide, cetylpyridinium chloride), hydrogen peroxide, phenol compounds (e.g. TCP Triclosan), alcohols (e.g. ethanol), Virkon™, iodine compounds (e.g. povidone-iodine), silver compounds (e.g. elemental silver-nano/microparticles).
Antimicrobial surfactants are another class of antiseptics. These are compounds that disrupt microbial cell membranes and other structural components and therefore inhibit growth and/or viability of microorganisms. Antimicrobial surfactants and their use in antimicrobial compositions is well known in the art. Further guidance may be found in Kabara and Orth’s “Preservative-free and self-preserving cosmetics and drugs - Principles and practice”, Ed. Kabara and Orth, Marcel Dekker, NY, NY, 1997, is explicitly incorporated by reference in its entirety. Antimicrobial surfactants may be anionic, cationic, non-ionic or amphoteric. Examples of antimicrobial anionic surfactants include, but are not limited to, sodium dodecyl sulfate (sodium lauryl sulfate), sodium dodecyl aminopropionic acid, sodium ricinoleate, bile acids, alkylaryl sulfonates, Grillosan DS791, disodium undecylenic acid monoethanol amidosulfosuccinate. Examples of antimicrobial cationic surfactants include, but are not limited to, the quaternary ammonium compounds, the aminimides and chlorhexidine compounds. Examples of antimicrobial non-ionic surfactants include, but are not limited to, the monoesters of fatty acids, polyethylene glycomonoesters of alkyl/dihydroxybenzoic acids, glucosamine derivatives and diethanolamides of N-lauroyl dipeptides. Examples of antimicrobial amphoteric surfactants include, but are not limited to, the alkyl betaines, the alkylamidopropyrbetaaines, the alkyl aminopropionates, the alkyliminodipropionates and the alkylimidazolines.

Representative antifungals include, but are not limited to the polyenes (e.g. natamycin, rimocidin, filipin, nystatin, amphotericin B, candidin; the imidazoles (e.g. miconazole, ketoconazole, clotrimazole, econazole, bifonazole, butoconazole, fenticonazole, isoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole); the triazoles (e.g. fluconazole, itraconazole, isavuconazole, ravuconazole, posaconazole, voriconazole, terconazole); the allylamines (e.g. terbinafine, amorolfine, naftifine, butenafine); and the echinocandins (e.g. anidulafungin, caspofungin, micafungin).

Representative antivirals include, but are not limited to abacavir, acyclovir, adefovir, amantadine, amprenavir, arbidol, atazanavir, atripla, boceprevir, cidofovir, combivir, darunavir, delavirdine, didanosine, docosanol, edoxudine, efavirenz, emtricitabine, enfuvirtide, entecavir, famciclovir, famivirsen, fosamprenavir, foscarnet, fosfonet, ganciclovir, ibacitabine, imunovir, idoxuridine, imiquimod, indinavir, inosine, interferon type III, interferon type II, interferon type I, lamivudine, lopinavir, lovivride, maraviroc, moroxydine, nelfinavir, nevirapine, nexavir,
oseltamivir, penciclovir, peramivir, pleconaril, podophyllotoxin, raltegravir, ribavirin, rimantadine, ritonavir, saquinavir, stavudine, tenofovir, tenofovir disoproxil, tipranavir, trifluridine, trizivir, tromantadine, truvada, valaciclovir, valganciclovir, vicriviroc, vidarabine, viramidine, zalcitabine, zanamivir, and zidovudine

The further anti-microbial agent may conveniently be applied before, simultaneously with or following the alginate oligomer. Conveniently the further anti-microbial agent is applied at substantially the same time as the alginate oligomer or afterwards. For example, the further anti-microbial agent is applied at least 1 hour, preferably at least 3 hours, more preferably at least 5 and most preferably at least 6 hours after the alginate oligomer is administered. In other embodiments the further antimicrobial may conveniently be applied or administered before the alginate oligomer, e.g. at least 1 hour, at least 3 hours, at least 6 hours before the alginate oligomer. In these embodiments the alginate oligomer can be applied or administered with or without a further application of the further antimicrobial. To optimise the anti-microbial effect of the further anti-microbial agent it can be given (e.g. administered or delivered) repeatedly at time points appropriate for the agent used. The skilled person is able to devise a suitable dosage or usage regimen. In long term treatments the alginate oligomer can also be used repeatedly. This can be as frequently as the further anti-microbial agent, but will typically be less frequently. The frequency required will depend on the location of the microorganism, colony composition and the anti-microbial used and the skilled person is able to optimise the dosage or usage patterns to optimise results.

In an advantageous embodiment the alginate oligomer may be used or applied after physical removal or reduction (e.g. debridement) of the microbial colony/population causing the infection at the location undergoing treatment. The population may or may not be in a biofilm.

Following removal of, or an attempt to remove, the colony/population, the location may be contacted with the alginate oligomer for between 0 and 24 hours, particularly 2 and 12 hours, more particularly 4 and 8 hours, most particularly 5 and 7 hours, e.g. 6 hours. Following this, the further anti-microbial agent may if desired be applied. Such a scenario may be desirable or particularly applicable in a clinical setting. In the case of infected wounds the duration of incubation can be conveniently be designed to correspond to scheduled changes of the wound dressing.
Physical removal of the colony/population can be carried out with any suitable surgical, mechanical or chemical means. Conveniently this can be the use of a liquid, gel, gel-sol, semi-solid compositions or gas applied at pressure to the colony/population, sonication, laser, or by abrasive implement. A composition used in the removal itself or as a wash solution before, during or afterwards may conveniently contain the alginate oligomer.

Accordingly, in one specific embodiment there is provided a debridement or wash composition e.g. solution for wounds containing an alginate oligomer, particularly any alginate oligomer as herein defined for use, where appropriate, in the treatments and methods of the invention. Such a debridement composition will typically be a sterile solution, particularly an aqueous sterile solution or an oil-based sterile solution, and may additionally contain proteolysis enzymes (e.g. collagenase, trypsin, pepsin, elastase), an abrasive solid phase (e.g. colloidal silica, ground pumice, ground plant or animal shell).

Use of the alginate oligomers in combination or conjunction with immunostimulatory agents may also be beneficial in the application of the methods of the invention in a clinical situation. These immunostimulatory agents may conveniently be used at timepoints corresponding to those described above in relation to anti-microbial agents and may optionally be used in combination with an alginate oligomer and a further anti-microbial agent Suitable immunostimulatory agents include, but are not limited to cytokines e.g. TNF, IL-1, IL-6, IL-8 and immunostimulatory alginates, such as high M-content alginates as described for example in US 5,169,840, WO91/1205 and WO03/045402 which are explicitly incorporated by reference herein in their entirety, but including any alginate with immunostimulatory properties.

Use of the alginate oligomers in combination or conjunction with growth factors, e.g. PDGF, FGF, EGF, TGF, hGF and enzymes may also be beneficial in the medical uses of the invention. Representative examples of suitable enzymes include but are not limited to proteases, e.g. serine proteases, metalloproteases and cysteine proteases (examples of these types of proteases are listed in EP0590746, the entire contents of which are incorporated herein by reference); nucleases, e.g. DNase I and II, RNase A, H, I, II, III, P, PhyM, R; lipases and enzymes capable of degrading polysaccharides.

Use of the alginate oligomers in combination or conjunction with a physiologically tolerable mucosal viscosity reducing agent could also be beneficial,
e.g. a nucleic acid cleaving enzyme (e.g. a DNase such as DNase I), gelsolin, a thiol reducing agent, an acetylcysteine, sodium chloride, an uncharged low molecular weight polysaccharide (e.g. dextran), arginine (or other nitric oxide precursors or synthesis stimulators), or an anionic polyamino acid (e.g. poly ASP or poly GLU). Ambroxol, romhexine, carbocisteine, domiodol, eprazinone, erdosteine, letosteine, mesna, neltenexine, sobrerol, stepronin, tiopronin are specific mucolytics of note.

Use of the alginate oligomers in combination or conjunction with alpha blockers may also be beneficial in the medical uses of the invention, in the treatment of chronic bacterial prostatitis especially. Representative examples of suitable alpha blockers include but are not limited to the selective alpha-1 blockers (e.g. doxazosin, dilodosin, prazosin, tamsulosin, alfuzosin, terazosin), and the non-selective adrenergic blockers (e.g. phenoxybenzamine, phentolamine).

Use of the alginate oligomers in combination or conjunction with bronchodilators may also be beneficial in the medical uses of the invention, in the treatment of respiratory diseases associated with MDR bacteria especially (which may include COPD, COAD, COAP, pneumonia, cystic fibrosis, emphysema and asthma). Representative examples of suitable bronchodilators include but are not limited to the β2 agonists (e.g. pirbuterol, epinephrine, salbutamol, salmeterol, levosalbutamol, clenbuterol), the anticholinergics (e.g. ipratropium, oxitropium, tiotropium) and theophylline.

Use of the alginate oligomers in combination or conjunction with corticosteroids may also be beneficial in the medical uses of the invention, in the treatment of respiratory diseases associated with MDR bacteria especially (which may include COPD, COAD, COAP, pneumonia, cystic fibrosis, emphysema and asthma). Representative examples of suitable corticosteroids include but are not limited to prednisone, flunisolide, triamcinolone, fluticasone, budesonide, mometasone, beclomethasone, amcinonide, budesonide, desonide, flucinonide, fluocinolone, halcinonide, hydrocortisone, cortisone, tixocortol, prednisolone, methylprednisolone, prednisone, betamethasone, dexamethasone, fluocortolone, aclometasone, prednicarbate, clobetasone, clobetasol, and fluprednidene.

The alginate oligomers may be used optionally with any other therapeutically active agent it may be desired to use, e.g. anti-microbial agent, an anti-inflammatory agent, an immunostimulatory agent, a mucosal viscosity reducing agent, a growth inhibitor or an enzyme or an alpha blocker, a bronchodilator or a
corticosteroid. The combined use of an alginate oligomer with a further therapeutically active agent (e.g. an anti-microbial or anti-inflammatory agent, an immunostimulatory agent, a mucosal viscosity reducing agent, a growth inhibitor or an enzyme or an alpha blocker, a bronchodilator or a corticosteroid) may improve the clinical effects of the active agent and this may advantageously allow the dose (e.g. the usual or normal dose) of the further therapeutically active agent to be reduced e.g. it may be used at its normal or usual dose or at a lower dose, for example at up to 50% (or at 50%) of its normal dose.

The invention encompasses the use of a single alginate oligomer or a mixture (multiplicity/plurality) of different alginate oligomers. Thus, for example, a combination of different alginate oligomers (e.g. two or more) may be used.

In the case of medical use, the alginates of the invention may be administered to the subject in any convenient form or by any convenient means, e.g. by topical, oral, parenteral, enteral, parenteral routes or by inhalation. Preferably the alginate will be administered by topical, oral or parenteral routes or by inhalation.

The skilled man will be able to formulate the alginates of the invention into pharmaceutical compositions that are adapted for these routes of administration according to any of the conventional methods known in the art and widely described in the literature. Merely for guidance only, Examples 5 and 6 describe two possible compositions (a topical composition and a debridement liquid).

The present invention therefore also provides a pharmaceutical composition for use in any of the above-mentioned methods or uses comprising an alginate oligomer as defined herein together with at least one pharmaceutically acceptable carrier, diluent or excipient.

The active ingredient may be incorporated, optionally together with other active agents, with one or more conventional carriers, diluents and/or excipients, to produce conventional galenic preparations such as tablets, pills, powders (e.g. inhalable powders), lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), sprays (e.g. nasal sprays), compositions for use in nebulisers, ointments, soft and hard gelatine capsules, suppositories, sterile injectable solutions, sterile packaged powders, and the like. Sterile inhalable compositions are of particular note for use in the treatment of respiratory diseases associated with microorganisms (which may
include COPD, COAD, COAP, pneumonia, cystic fibrosis, emphysema and asthma).

Examples of suitable carriers, excipients, and diluents are lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, inert alginates, tragacanth, gelatine, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, water,-water/ethanol, water/ glycol, water/polyethylene, hypertonic salt water, glycol, propylene glycol, methyl cellulose, methylhydroxybenzoates, propyl hydroxybenzoates, talc, magnesium stearate, mineral oil or fatty substances such as hard fat or suitable mixtures thereof.

Excipients and diluents of note are mannitol and hypertonic salt water (saline).

The compositions may additionally include lubricating agents, wetting agents, emulsifying agents, suspending agents, preserving agents, sweetening agents, flavouring agents, and the like. As discussed above, the alginate oligomers proposed for use according to the invention may be used in combination with other therapeutic agents, for example to be administered together, in a single pharmaceutical formulation or composition, or separately (i.e. for separate, sequential or simultaneous administration). Thus, the alginate oligomers of the invention may be combined with a second (or further) therapeutically active agent, e.g. in a pharmaceutical kit or as a combined ("combination") product.

Thus a further aspect of the present invention provides a product containing an alginate oligomer as defined herein and a second active agent as a combined preparation for separate, simultaneous or sequential use (e.g. application to a microorganism and/or administration to a subject or location) for use in inhibiting the viability and/or growth of a microorganism; combating a population of microorganisms; combating contamination of a location with a microorganism; and/or combating an infection in a subject or any of the conditions defined herein.

Additional therapeutically active agents may be included in the pharmaceutical compositions, as discussed in relation to combination therapies above.

Thus the invention provides products (e.g. a pharmaceutical kit or a combined ("combination") product) or compositions (e.g. a pharmaceutical composition) including those described herein wherein the product or composition comprises an alginate oligomer as herein defined and a further active agent e.g. a therapeutically active agent, such as an anti-microbial agent e.g. an antibiotic, an immunostimulatory agent, a growth factor, a mucosal viscosity-reducing agent, an
agent effective in the treatment of respiratory diseases e.g. a bronchodilator, or an anti-inflammatory agent, e.g. an anti-inflammatory steroid.

The invention also provides products (e.g. a pharmaceutical kit or a combined ("combination") product) or compositions (e.g. a pharmaceutical composition) including those described herein wherein the product or composition comprises an alginate oligomer as herein defined and an antibiotic selected from amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, telithromycin, CarbomycinA, josamycin, kitasamycin, midecamicine, oleandomycin, spiramycin, tylosin, troleandomycin, aztreonam, imipenem, meropenem, ertapenem, doripenem, panipenem/betamipron, biapenem, PZ-601, cefixime, cefdinir, cefditoren, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, cefitbuten, cefizoxime, ceftriaxone, cefepime, demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, bacitracin, colistin, polymyxin B, ciprofloxacin, enoxacin, gatifloxacin, levofloxacin, lomefloxacin, moxifloxacin, norfloxacin, ofloxacin, and trovafloxacin. In particular, antibiotic may selected from ceftazidime, imipenem/cilastatin, meropenem, aztreonam, oxytetracycline, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, spiramycin and ciprofloxacin, and it is particularly preferred that the antibiotic is selected from ceftazidime, imipenem/cilastatin, meropenem, aztreonam, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, spiramycin and ciprofloxacin. More preferably the antibiotic is selected from aztreonam, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, spiramycin and ciprofloxacin.

These products and compositions are specifically contemplated as for use in the methods of the invention. The products and compositions can be pharmaceutical or non-pharmaceutical. Therefore the products and compositions of this aspect of the invention can be used in any of the methods of the invention. The use of alginate oligomers as herein defined to manufacture such pharmaceutical products and pharmaceutical compositions for use in the medical methods of the invention is also contemplated.

The above and following discussion of additional active agents and excipients and the like is directly applicable in its entirety to these embodiments of the invention. The relative content of the alginate oligomer and the antibiotic, e.g. macrolide antibiotic can vary depending on the dosage required and the dosage
regime being followed and this will depend on the subject to be treated and the location and identity/constituents of the bacterium, contamination or population. Preferably the composition or product will comprise sufficient alginate oligomer that upon administration to a subject or application to a location, the local concentration of the oligomer will be at least 2%, preferably at least 4%, 6% or 8% and most preferably at least 10% (weight by volume). The antibiotic, e.g. macrolide antibiotic preferably will be present in an amount that is sufficient to provide a local concentration of at least 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 64, 128, 256, or 512, 1024, 2048 or 4096 µg/ml. The skilled man would know that the amounts of alginate oligomer and/or antibiotic, e.g. macrolide antibiotic can be reduced if a multiple dosing regime is followed or increased to minimise the number of administrations or applications. As mentioned above, the alginate oligomers of the invention may allow the dose of the antibiotic to be reduced. The compositions and products of this aspect will typically comprise between 1% and 99%, 5% and 95%, 10% and 90% or 25% and 75% alginate oligomer and 1% and 99%, 5% and 95%, 10% and 90% or 25% and 75% antibiotic, e.g. macrolide antibiotic, allowance being made for other ingredients.

Parenterally administrable forms, e.g., intravenous solutions, should be sterile and free from physiologically unacceptable agents, and should have low osmolarity to minimize irritation or other adverse effects upon administration and thus solutions should preferably be isotonic or slightly hypertonic, e.g. hypertonic salt water (saline). Suitable vehicles include aqueous vehicles customarily used for administering parenteral solutions such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection and other solutions such as are described in Remington's Pharmaceutical Sciences, 15th ed., Easton: Mack Publishing Co., pp. 1405-1412 and 1461-1487 (1975) and The National Formulary XIV, 14th ed. Washington: American Pharmaceutical Association (1975). The solutions can contain preservatives, antimicrobial agents, buffers and antioxidants conventionally used for parenteral solutions, excipients and other additives which are compatible with the biopolymers and which will not interfere with the manufacture, storage or use of products.

For topical administration the alginate oligomer can be incorporated into creams, ointments, gels, transdermal patches and the like. The alginate oligomers can also be incorporated into medical dressings, for example wound dressings e.g.
woven (e.g. fabric) dressings or non-woven dressings (e.g. gels or dressings with a gel component). The use of alginate polymers in dressings is known, and such dressings, or indeed any dressings, may further incorporate the alginate oligomers of the invention.

Accordingly, in a further specific embodiment, the invention further provides a wound dressing comprising an alginate oligomer (which may be any alginate oligomer as herein defined) for use, where appropriate, in the treatments and methods of the invention.

Further topical systems that are envisaged to be suitable are in situ drug delivery systems, for example gels where solid, semi-solid, amorphous or liquid crystalline gel matrices are formed in situ and which may comprise the alginate oligomer. Such matrices can conveniently be designed to control the release of the alginate oligomer from the matrix, e.g. release can be delayed and/or sustained over a chosen period of time. Such systems may form gels only upon contact with biological tissues or fluids. Typically the gels are bioadhesive. Delivery to any body site that can retain or be adapted to retain the pre-gel composition can be targeted by such a delivery technique. Such systems are described in WO 2005/023176.

For application to oral, buccal and dental surfaces, toothpastes, dental gels, dental foams and mouthwashes are mentioned specifically. Thus, in one particular aspect is included an oral health care, or oral hygiene, composition, comprising an alginate (which may be any alginate oligomer as defined herein), particularly a mouthwash, toothpaste, dental gel or dental foam for use, where appropriate, in the treatments and methods of the invention.

Inhalable compositions are also of note. The formulation of compositions suitable for inhalation is routine for the skilled man and has long been standard practice in the treatment of respiratory diseases. Inhalable compositions may, for instance, take the form of inhalable powders, solutions or suspensions. The skilled man would be able to select the most appropriate type of delivery system for his needs and be able to prepare a suitable formulation of the alginites of the invention for use in that system. Propellant-free nebulisable solutions and inhalable powder formulations are particularly preferred.

As noted above, a preferred composition of the invention is a debridement composition that is used in a debridement process to remove an infection colony or population, for example from a tissue. Typically such a composition will be liquid, but gels, gel-sols, or semi-solid compositions might be used. The composition
might be used to debride the colony/population (e.g. by application to the tissue under pressure) and/or may be used to bathe the tissue before, during and/or after debridement by other means such as by surgical, mechanical or chemical processes. The skilled person is readily able to formulate debridement compositions in accordance with the invention.

In some instances it may be beneficial to administer the alginate oligomers as defined herein to animals, e.g. to promote weight gain/growth. Administration can be achieved in the form of the pharmaceutical compositions described herein, but conveniently the alginate oligomers as defined herein may be used as a conventional feed additive, i.e. a compound that is added to animal feed in small, nutritionally inconsequential amounts. The use of feed additives in animal feeds is well established and it would be entirely routine for a skilled man to determine and use appropriate amounts of the alginites of the invention to achieve the desired effects, e.g. weight gain/growth.

In the case of microorganisms on an inanimate surface on an inanimate material, the alginate oligomer may be applied to the surface or material to be treated in any convenient composition or formulation, or by any convenient means. Thus the alginate oligomer may be in liquid, gel, gel-sol, semi-solid or solid form (e.g. solutions, suspensions, homogenates, emulsions, pastes, powders, aerosols, vapours). Typically the compositions for treating such inanimate surfaces or materials will be a non-pharmaceutically acceptable composition. The choice of composition form will be dictated by the identity of the microbe on the surface or in the material and location of the surface or material. For instance, if the location is a fluid line it might be convenient to apply a fluid composition. It might also be preferred to use a composition that persists on the surface or in the part of the fluid line to be treated but that will not leach into the fluid of normal use, e.g. an adhesive gel. The skilled person is readily able to prepare suitable compositions from his common general knowledge. For instance, the alginate oligomer may be added to a paint formulation and applied to the surface to be treated, e.g. a boat hull or other part of a boat's structure that is exposed to water, or to a building or any part thereof, a tank (e.g. a storage or processing tank) or indeed to any part of any industrial machinery. Such compositions may conveniently also comprise a further anti-microbial agent, as described above, e.g. an antibiotic, chlorine bleach, TCP, ethanol, Virkon™, povidone-iodine, silver compounds, antimicrobial surfactants, etc.

As the compositions need not be pharmaceutically acceptable, harsher
antimicrobials can be used subject to considerations of surface damage, environmental contamination, user safety and contamination of the treated surface and interaction with the other components of the composition.

The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the subject/surface by employing procedures well known in the art. Adhesive compositions are also preferred. Adhesive, sustained and/or delayed release formulations may be particularly convenient.

In a further aspect the invention provides products susceptible to microbial contamination/colonisation whose susceptible surfaces have been pretreated with an alginate oligomer as defined herein.

By "pretreated" it is meant that the susceptible surface is exposed to an alginate oligomer prior to an exposure to microorganism and that the alginate oligomer persists on the surface for a duration sufficient to prevent contamination/colonisation by a microorganism for an appreciable duration of time. Preferably the alginate oligomer will persist for substantially the useful life of the surface, e.g. the pretreatment results in a substantially permanent coating of an alginate oligomer. Thus a pre-treated surface/product is one to which the alginate oligomer is applied and on which it remains. Such a product/surface may be a coated product/surface.

Non-limiting examples of products and surfaces susceptible to microbial contamination/colonisation are described above. Particular mention may be made of medical and surgical devices and food or drink processing, storage or dispensing equipment. Pretreatment can be achieved by any convenient means, for example any form of applying the alginate oligomer to the surface, notably coating the surface, e.g. spray drying, polymer coating with a polymer incorporating the alginate oligomer, and painting, varnishing or lacquering with paint, varnish or lacquer formulations containing the alginate oligomer. Such a "coating" composition (e.g. a paint, varnish or lacquer) containing an alginate oligomer represents a further aspect of the present invention. Alternatively, the alginate oligomer can be incorporated into the material from which the object or its susceptible parts are manufactured. This approach is suited to objects, or constituent parts thereof, manufactured from polymers such as plastics and silicones, e.g. the medical and surgical devices described above.
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Products comprising an inanimate surface comprising an alginate oligomer coating or coating composition, or incorporating an alginate oligomer are therefore contemplated. Non-limiting examples of such products and surfaces are described above. Of particular note are medical and surgical devices. This may include any kind of line, including catheters (e.g. central venous and urinary catheters), prosthetic devices e.g., heart valves, artificial joints, false teeth, dental crowns, dental caps and soft tissue implants (e.g. breast, buttock and lip implants). Any kind of implantable (or "in-dwelling") medical device is included (e.g. stents, intrauterine devices, pacemakers, intubation tubes (e.g. endotracheal or tracheostomy tubes), prostheses or prosthetic devices, lines or catheters). Further products include food processing, storage, dispensing or preparation equipment or surfaces, tanks, conveyors, floors, drains, coolers, freezers, equipment surfaces, walls, valves, belts, pipes, air conditioning conduits, cooling apparatus, food or drink dispensing lines, heat exchangers, boat hulls or any part of a boat's structure that is exposed to water, dental waterlines, oil drilling conduits, contact lenses and storage cases.

In accordance with the invention the alginate oligomer may be incorporated into compositions to function as a preservative as well as, or instead of, functioning as the main active ingredient of the composition. The other components of the composition are not restricted and the above discussion of various compositions apply to this aspect of the invention. The composition will typically be a solid, liquid, semi-solid, gel or gel-sol. The amount of alginate used will depend on the susceptibility of the composition to microbial, contamination and the duration in which contamination must be prevented. Other considerations such as viscosity, turbidity, colour and taste of the composition may also affect the amount of alginate oligomer that can be used. Typical the alginate will be used in an amount of between 0.5 and 10%, preferably 1 to 8%, preferably 2 to 6% weight by volume.

The invention will be further described with reference to the following non-limiting Examples in which:

Figure 1 shows the effects of increasing concentrations of alginate oligomers on Pseudomonas cell structure as visualised by SEM. A: Mucin only; B: Mucin and 2% G-fragments; C: Mucin and 6% G-fragments; D: Mucin and 10% G-fragments

Figure 2 shows the effects of increasing concentrations of alginate oligomers on Pseudomonas cell structure as visualised by CSLM of FM® 1-43
stained Pseudomonas cells. A: Mucin only; B: Mucin and 2% G-fragments; C: Mucin and 6% G-fragments; D: Mucin and 10% G-fragments.

Figure 3 shows the effects of increasing concentrations of alginate oligomers on GFP-Pseudomonas aeruginosa biofilms, stained with BODIPY® 630/650-X SE, as visualised by CSLM. A: 0% G-fragments; B: 2% G-fragments; C: 6% G-fragments; D: 10% G-fragments.

Figure 4 shows the effects of increasing concentrations of alginate oligomers on Acinetobacter baumannii cell structure as visualised by SEM. A: 0% G-fragments; B: 2% G-fragments; C: 6% G-fragments; D: 10% G-fragments.

Figure 5 shows the effects of increasing concentrations of alginate oligomers on cell numbers in overnight cultures of Acinetobacter baumannii as visualised by CSLM of cultures stained with the Live/Dead® BacLight™ stain. A: 0% G-fragments; B: 2% G-fragments; C: 6% G-fragments; D: 10% G-fragments.

Figure 6 shows the effects of increasing concentrations of alginate oligomers on cell numbers in overnight cultures of MRSA as visualised by CSLM of cultures stained with the Live/Dead® BacLight™ stain. A: 0% G-fragments; B: 2% G-fragments; C: 6% G-fragments; D: 10% G-fragments.

Figure 7 shows the effects of increasing concentrations of alginate oligomers on cell numbers in overnight cultures of Candida albicans as visualised by CSLM of cultures stained with the Live/Dead® BacLight™ stain. A: 0% G-fragments; B: 2% G-fragments; C: 6% G-fragments; D: 10% G-fragments.

Figure 8 shows the effects of increasing concentrations of alginate oligomers on the growth of various Acinetobacter strains after 19 hours incubation in 0%, 2%, 6% or 10% alginate oligomers. Dotted bar: 0% G-fragments; light grey bar: 2% G-fragments; hatched bar 6% G-fragments; dark grey bar 10% G-fragments.

Figure 9 shows the effects of increasing concentrations of alginate oligomers on the growth of various Acinetobacter strains after 25 hours incubation in 0%, 2%, 6% or 10% alginate oligomers. Dotted bar: 0% G-fragments; light grey bar: 2% G-fragments; hatched bar 6% G-fragments; dark grey bar 10% G-fragments.

Figure 10 shows the effects of increasing concentrations of alginate oligomers on the growth of various Klebsiella strains and Moraxella catarrhalis after 19 hours incubation in 0%, 2%, 6% or 10% alginate oligomers. Dotted bar: 0% G-
fragments; light grey bar: 2% G-fragments; hatched bar 6% G-fragments; dark grey bar 10% G-fragments.

Figure 11 shows the effects of increasing concentrations of alginate oligomers on the growth of various Klebsiella strains and Moraxella catarrhalis after 25 hours incubation in 0%, 2%, 6% or 10% alginate oligomers. Dotted bar: 0% G-fragments; light grey bar: 2% G-fragments; hatched bar 6% G-fragments; dark grey bar 10% G-fragments.

Figure 12 shows the effects of increasing concentrations of alginate oligomers on the growth of various Pseudomonas aeruginosa strains and Providencia stuartii after 19 hours incubation in 0%, 2%, 6% or 10% alginate oligomers. Dotted bar: 0% G-fragments; light grey bar: 2% G-fragments; hatched bar 6% G-fragments; dark grey bar 10% G-fragments.

Figure 13 shows the effects of increasing concentrations of alginate oligomers on the growth of various Pseudomonas aeruginosa strains and Providencia stuartii after 25 hours incubation in 0%, 2%, 6% or 10% alginate oligomers. Dotted bar: 0% G-fragments; light grey bar: 2% G-fragments; hatched bar 6% G-fragments; dark grey bar 10% G-fragments.

Figure 14 shows the effects of increasing concentrations of alginate oligomers on the growth of various Burkholderia cepacia and Escherichia coli strains after 19 hours incubation in 0%, 2%, 6% or 10% alginate oligomers. Dotted bar: 0% G-fragments; light grey bar: 2% G-fragments; hatched bar 6% G-fragments; dark grey bar 10% G-fragments.

Figure 15 shows the effects of increasing concentrations of alginate oligomers on the growth of various Burkholderia cepacia and Escherichia coli strains after 25 hours incubation in 0%, 2%, 6% or 10% alginate oligomers. Dotted bar: 0% G-fragments; light grey bar: 2% G-fragments; hatched bar 6% G-fragments; dark grey bar 10% G-fragments.

Figure 16 shows the effects of increasing concentrations of alginate oligomers on the growth of various Staphylococcus aureus and Enterococcus faecium and Streptococcus oralis strains after 19 hours incubation in 0%, 2%, 6% or 10% alginate oligomers. Dotted bar: 0% G-fragments; light grey bar: 2% G-fragments; hatched bar 6% G-fragments; dark grey bar 10% G-fragments.

Figure 17 shows the effects of increasing concentrations of alginate oligomers on the growth of various Staphylococcus aureus and Enterococcus faecium and Streptococcus oralis strains after 25 hours incubation in 0%, 2%, 6%
or 10% alginate oligomers. Dotted bar: 0% G-fragments; light grey bar: 2% G-fragments; hatched bar 6% G-fragments; dark grey bar 10% G-fragments.

**EXAMPLES**

**Example 1 - Materials and Standard Methods**

**Bacterial strains.**

*Pseudomonas aeruginosa* PAO1 (ATCC 15682, a wound isolate)

**Chemicals and bacterial media.**

Bacterial colonies were grown on blood agar base No2, (BA; Lab15, LabM, Bury, UK) supplemented with 5% sheeps' blood and were used to inoculate tryptone soya broth (TSB, CM0129, Oxoid, Basingstoke, UK) for overnight growth. Biofilms were generated in cation-adjusted Mueller-Hinton broth (CAMHB; Lab1 14, LabM). Pig gastric mucin glycoprotein (purified by Jeff Pearson, Newcastle University) and alginate oligomers CF-5/20 ("G-fragments"; 2600Da, %G 90-95) and G-block #0802 (6400 Da, %G 91) were provided by Algipharma AS, Sandvika, Norway.

**Growth of biofilms for imaging.**

After retrieval from -80°C storage, bacterial isolates were grown on BA and then grown overnight in TSB. After dilution of the bacterial cultures to 0.5 McFarland in CAMHB with mucin (2.5g/l), 1ml was transferred to the wells of a flat-bottom 12-well plate containing sterile glass coverslips for samples to be used in staining or sterile plastic Thermanox coverslips (Agar Scientific, Essex, UK) for samples to be used in scanning electron microscopy (SEM). Plates were then wrapped in parafilm to prevent dehydration and incubated at 37°C for 6 hr to allow biofilm formation. After biofilm formation, planktonic cells and supernatant were removed and each well was then washed with sterile phosphate buffered saline (PBS). After washing, cells were treated with G fragments and mucin (2.5g/l) in 100
µl CAMHB. Plates were then wrapped in parafilm and incubated at 37°C for 24hr with gentle tilting.

LIVE/DEAD staining of G-fragment treated biofilms.

The G-fragment treated biofilms were stained with the Live/Dead® BacLight™ stain (Bacterial Viability Kit, Invitrogen Ltd). The live/dead stain consists of two nucleic acid stains: The green fluorescent SYTO9 and the red-fluorescent propidium iodide which are used in combination to discriminate live and dead bacteria in a mixed population. Bacterial cells with a compromised membrane (those that are considered to be dead or dying) stain red, whereas cells with an intact membrane appear green. 2µl of each stain was added to 1 ml NaCl (0.85% w/v), mixed and 100 µl of this mixture was added to each test sample. Samples were incubated in the dark for 15 min and then analysed by confocal laser scanning microscopy (CLSM).

Scanning Electron Microscopy (SEM) of Pseudomonas biofilms.

Glutaraldehyde (final conc. 1.25%) was added to G-fragment treated biofilms and fixed at room temperature for 24 hours. The samples were dehydrated in a graded series of ethanol concentrations, dried in a critical point dryer (Balzers CPD 030, Germany), mounted on aluminium stubs, coated with gold in a sputter-coater (EMscope model AE 1231, UK), and then viewed on a scanning electron microscope (FEI-Philips XL-20, The Netherlands).

CLSM of undisturbed biofilms using FM® 1-43 stain

G-fragment treated biofilms were washed with sterile distilled water and stained with the FM® 1-43 stain (Invitrogen Ltd) which selectively stains Pseudomonas cell membranes. FM® 1-43 was added (100 µl at 1 µg ml⁻¹) to each biofilm sample. The preparation was incubated in the dark for 2h and then analysed by CLSM using a Leica TCS SP2 AOBS spectral confocal microscope (Leica, Wetzlar, Germany).
Example 2 - Effect of G fragments on Pseudomonas cell membrane integrity

The effect of G-fragments on cell membrane integrity (a measure of cell death) was investigated using LIVE/DEAD. Increased numbers of dead cells were evident with increasing G-fragment concentrations, demonstrating a direct cytotoxic activity with these alginate oligomers that is dose dependent (data not shown).

Example 3 - Analysis of Pseudomonas cell structure and effects of G-fragments with Electron Microscopy

The overall structure of Pseudomonas biofilms was followed using Scanning Electron Microscopy (SEM). Figure 1 shows the effect of increasing amounts of G fragments on Pseudomonas cell structure. The bacterial cells exposed to 0% and 2% G-fragments look healthy and normal. However, as the concentration of G-fragments increases from 2 to 10% the extracellular polysaccharide coating the cell surfaces appears to become increasingly disrupted with visible precipitates forming between the bacterial cells. This is evidence of the leakage of cellular contents and is an indication of cell death. This further demonstrates a direct cytotoxic activity with these alginate oligomers that is dose dependent.

Example 4 - Analysis of Pseudomonas cell structure and effects of G-fragments with Confocal Laser Scanning Microscopy

The effect of G fragments on the structure of the Pseudomonas cell membranes was investigated using confocal microscopy of undisturbed biofilms labelled with the fluorescent dye FM® 1-43. This dye selectively stains Pseudomonas cell membranes. Results are shown in Figure 2. The appearance of 'ghost' cells (hollow cells due to loss of cell contents) is seen with 2% G fragments. This is evidence of loss of cell integrity and therefore death. In addition to cells being hollow, cells appear shortened and fattened by treatment with 6% G-fragments compared with 'mucin only' control and 2% G-fragments. Effects are more pronounced with 10% G-fragments. These morphological changes are also characteristic of death. These effects are further evidence of a direct cytotoxic activity with these alginate oligomers.
Example 5 - Topical composition comprising alginate oligomer

An example of a topical composition (a moisturising skincare body lotion) comprising an alginate oligomer is prepared with the following ingredients.

Oil phase:
Mineral oil 3%
Cyclomethicone 4%
Isopropyl myristate 3%
Stearic acid 1.8%
Cetyl alcohol 1.0%
Glyceryl stearate 1.5%

Water phase:
Carbomer 984 0.10%
Glycerine 3%
Triethanolamine 0.90%
Alginate oligomer 0.1%
Water 81.60%

Example 6 - Debridement composition comprising alginate oligomer

An example of a liquid debridement composition comprising an alginate oligomer is prepared with the following ingredients.

Castor oil 77.81%
Balsam of Peru refined grade 10%
Collagenase 0.2%
ZnCl 0.5%
Water 5%
Polyoxyethylene (10) oleyl ether 4%
Colloidal silica 2%
Alginate oligomer 0.5%
Example 7 - Effects of G-fragment alginate oligomers on GFP-Pseudomonas biofilms

After retrieval from -80°C storage, GFP (green fluorescent protein) expressing PAO1 were grown on BA and then grown overnight in TSB. GFP-PAO1 was then subcultured in MH Broth including either 0, 2, 6 or 10% G-fragments. 200 µl of each was then transferred to Coverwell™ incubation chamber gasket (Invitrogen) with addition of BODIPY® 630/650-X SE stain (BODIPY® 630/650-X SE, Invitrogen Ltd) which selectively stains the matrix components (EPS) in Pseudomonas biofilms. A HistoBond™ (positively charged for adhesion) slide was pushed down onto the chamber gasket and incubated overnight at 37°C. Visualisation of biofilm was by CLSM. Results are shown in Figure 3. As can be seen increasing concentrations of G-fragments resulted in decreasing numbers of cells in the biofilm. This is consistent with the cytotoxic and biofilm disruptive effects observed in Examples 2, 3 and 4.

Example 8 - Analysis of Acinetobacter cell and biofilm structure and effects of G-fragments with Electron Microscopy

The overall structure of Acinetobacter baumannii biofilms was followed using Scanning Electron Microscopy (SEM). Acinetobacter baumannii biofilms were prepared for SEM as described in Example 1. Figure 4 shows the effect of increasing amounts of G-fragments on Acinetobacter baumannii cell structure. The bacterial cells exposed to 0% and 2% G-fragments look healthy and normal. However, as the concentration of G-fragments increases from 2 to 10% the overall morphology of the cells and biofilms changes significantly and it is difficult to resolve individual cells. This is suggestive of cell death. This further demonstrates a direct cytotoxic activity with these alginate oligomers that is dose dependent and that is seen in different microorganisms.

Example 9 - Analysis of live cell numbers in Acinetobacter, MRSA and Candida albican cultures exposed to G-fragments

Strains used
Planktonic cell cultures were generated overnight in MH broth with 0%, 2% G, 6% G or 10% G fragments. Aliquots of these cultures were removed and stained with the Live/Dead® BacLight™ stain (Bacterial Viability Kit, Invitrogen Ltd). Samples were incubated in the dark for 15 min and then analysed by confocal laser scanning microscopy (CLSM); Results are shown in Figures 5 to 7. As can be seen increasing concentrations of G-fragments result in reductions in cell numbers for each species tested. Of the cells present few cells stained with the propidium iodide, suggesting that although cell numbers have reduced, the cells that are present remain viable. This is possibility suggestive of a cytostatic effect.

The growth inhibitory effects of alginate oligomers can therefore seen in differing bacteria types (Gram negative/Gram positive) and in fungi. The effect can therefore be considered a general anti-microbial effect.

**Example 10 - Growth of various bacteria strains in the presence of increasing concentrations of G-fragments**

Growth was measured as the increase in OD600nm during the incubation. The following protocol was followed:

G-block alginites (OligoG CF-5/20, referred to above as G-fragments) were dissolved in Mueller-Hinton broth (Lab M limited, LAB1 14 Mueller-Hinton broth) to 1.25 times of the desired assay concentrations (2, 6 and 10%). OligoG CF-5/20 G-fragments were provided by Algipharma AS, Norway.

Frozen stock cultures were made from overnight cultures in TSB-broth for all strains by addition of glycerol to 15 % concentration prior to freezing at - 80°C. At the day of analysis, overnight TSB cultures (6 ml in 50 ml tube tilted to 45-degrees angle, 200 rpm, 2.5 cm amplitude, 37 0C) were diluted in TSB until the OD600 was 0.10, and further diluted 1:40 in Mueller-Hinton broth. Each well in the 384-well assay plates was inoculated with 7.5 µl of the diluted culture. The microplates were placed in plastic bags and incubated at 37 0C. The optical density at 600 nm in the microwells was measured after 19 hours of incubation. The microplates were
further incubated for 6 hours, and optical density in the cultures was measured again. Results are shown in Figures 8 to 17. As can been seen at the 19 hour timepoint virtually all strains displayed growth inhibition in 10% G fragments and as a whole this effect is dose responsive across with the amount of G-fragments used. At 25 hours, particularly in the Pseudomonas strains, the effects are less pronounced, but there is an overall trend of increasing G-fragments causing increasing inhibitions and 10% G-fragment inhibit growth in virtually every strain. The one exception to the effects of the G-fragments is *Klebsiella pneumoniae* (G-lyase strain). This strain excretes a lyase that degrades G-fragments and thus the lack of inhibition in this strain would seem to validate the growth inhibitory properties of the G-fragments.
Claims

1. A method for inhibiting the viability and/or growth of a microorganism, said method comprising contacting said microorganism with an alginate oligomer, wherein said alginate oligomer has a number average degree of polymerisation of 8-50 and wherein at least 90% of the monomer residues are guluronate.

2. The method of claim 1, wherein said microorganism is on an animate or inanimate surface or in an animate or inanimate material.

3. The method of any one of claim 1 or 2, wherein the microorganism is on a surface selected from surfaces of food or drink processing, preparation, storage or dispensing machinery or equipment, surfaces of air conditioning apparatus, surfaces of industrial machinery, surfaces of storage tanks, surfaces of medical or surgical equipment, surfaces of aquatic/marine equipment or the surfaces of buildings and other structures.

4. The method of claim 3 wherein the surface is selected from food processing, storage, dispensing or preparation equipment or surfaces, tanks, conveyors, floors, drains, coolers, freezers, equipment surfaces, walls, valves, belts, pipes, air conditioning conduits, cooling apparatus, food or drink dispensing lines, heat exchangers, boat hulls, dental waterlines, oil drilling conduits, contact lenses, contact lens storage cases, catheters, prosthetic devices or implantable medical devices.

5. The method of any one of claim 1 or 2, wherein the microorganism is in a material selected from clinical/scientific waste, animal or human food stuffs, personal hygiene products, cosmetics, drinking water supplies, waste water supplies, agricultural feedstuffs and water supplies, insecticide formulations, pesticide formulations, herbicide formulations, industrial lubricants, cell and tissue culture media, and cell and tissue cultures.

6. An alginate oligomer for use as a therapeutic microbicidal and/or microbiostatic agent, wherein said alginate oligomer has a number average degree
of polymerisation of 8-50 and wherein at least 90% of the monomer residues are guluronate.

7. An alginate oligomer for use in inhibiting the viability and/or growth of a microorganism in a subject, wherein said alginate oligomer has a number average degree of polymerisation of 8-50 and wherein at least 90% of the monomer residues are guluronate.

8. The method or alginate oligomer of any one of claims 1 to 7, wherein the microorganism is in a non-biofilm mode of growth.

9. An alginate oligomer for use in combating a microbial infection in a subject, wherein the microorganism of said infection is not growing in or on a biofilm, wherein said alginate oligomer has a number average degree of polymerisation of 8-50 and wherein at least 90% of the monomer residues are guluronate.

10. The method or alginate oligomer of any one of claims 1 to 9, wherein the microorganism is a prokaryotic or eukaryotic microorganism.

11. The method or alginate oligomer of claim 10 wherein the prokaryotic or eukaryotic microorganism is selected from bacteria, archaeobacteria, fungi or algae.

12. The method or alginate oligomer of any one of claims 1 to 11, wherein at least 95% of the monomer residues of the alginate oligomer are guluronate, preferably wherein at least 98% of the monomer residues of the alginate oligomer are guluronate.

13. The method or alginate oligomer of any one of claims 1 to 12, wherein at least 90% of the guluronate residues of the alginate oligomer are linked 1-4 to another guluronate residue.

14. The method or alginate oligomer of any one of claims 1 to 13, wherein the alginate oligomer has a number average degree of polymerisation of 10-50, preferably 14 to 50.
15. The method or alginate oligomer of any one of claims 1 to 14, wherein the alginate oligomer is substantially free of alginate oligomers having a degree of polymerisation outside of said average degree of polymerisation.

16. The method or alginate oligomer of any one of claims 1 to 15, wherein the terminal uronic acid residues of the alginate oligomer do not have a double bond.

17. The method or alginate oligomer of any one of claims 1 to 16, wherein the alginate oligomer is prepared by acid hydrolysis of an alginate polysaccharide.

18. The alginate oligomer of any one of claims 7 to 17, wherein the microorganism or the microbial infection is in or on an internal or external body surface, a body tissue or a body fluid.

19. The alginate oligomer of claim 18 wherein the internal or external body surface is selected from a surface in the oral cavity, the reproductive tract, the urinary tract, the respiratory tract, the gastrointestinal tract, the peritoneum, the middle ear, the prostate, vascular intima, the eye, including conjunctiva or corneal tissue, lung tissue, heart valves, skin, scalp, nails, the interior of wounds or the surface of adrenal, hepatic, renal, pancreatic, pituitary, thyroid, immune, ovarian, testicular, prostate, endometrial, ocular, mammary, adipose, epithelial, endothelial, neural, muscle, pulmonary, epidermis or osseous tissue.

20. The alginate oligomer of claim 18 wherein the body fluid is selected from blood, plasma, serum, cerebrospinal fluid, GI tract contents and semen; and the body tissue is selected from adrenal, hepatic, renal, pancreatic, pituitary, thyroid, immune, ovarian, testicular, prostate, endometrial, ocular, mammary, adipose, epithelial, endothelial, neural, muscle, pulmonary, epidermis or osseous tissue.

21. The alginate oligomer of any one of claims 7 to 20 wherein the subject is selected from a subject with a pre-established infection, an immunocompromised subject, a subject undergoing intensive or critical care, a subject suffering from trauma, a subject with a burn, a subject with an acute and/or chronic wound, a neonatal subject, an elderly subject, a subject with cancer, a subject suffering from
an auto-immune condition, a subject with reduced or abrogated epithelial or endothelial secretion and/or secretion clearance or a subject fitted with a medical device.

22. The alginate oligomer of claim 21 wherein the subject is selected from a subject with a condition selected from HIV, sepsis, septic shock, AIDS, a cancer of the immune system, rheumatoid arthritis, diabetes mellitus type I, Crohn's disease, COPD, bronchitis, cystic fibrosis, emphysema, lung cancer, asthma, pneumonia and sinusitis, a subject preparing for, undergoing, or recovering from chemotherapy and/or radiotherapy, an organ transplant subject, a subject resident in a healthcare institution or a smoker.

23. The method or alginate oligomer of any one of claims 1 to 22, wherein said alginate oligomer is used in combination with a further anti-microbial agent.

24. A product containing an alginate oligomer as defined in any one of claims 1, and 12 to 17 and a second active agent as a combined preparation for separate, simultaneous or sequential use in inhibiting the viability and/or growth of a microorganism or in combating non-biofilm infection in a subject.
Figure 9
Figure 10
Figure 14
Figure 15

Growth at 25h (OD600nm)

- *Burkholderia cepacia* (1322, V23)
- *Burkholderia cepacia* (LMG18941, ATCC-BAA-246)
- *Escherichia coli* (5702, Wales, V11)
- *Escherichia coli* (7273, V24)
- *Escherichia coli* (AIM-1, V5)

Strain
INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2010/001098

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/36
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C

See patent family annex

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Date of the actual completion of the international search
13 July 2010

Date of mailing of the international search report
22/07/2010

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Authorized officer
Cattel I, James
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