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A New Cyanoacrylate Colloidal Polymer with Novel Antibacterial Mechanism and Its Application to Infection Control

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Abstract

The cyanoacrylate polymer particles, which do not contain antibiotics or antibacterial agents, bond with the cell wall of drug-resistant bacteria, and then induced an auto-destructive behavior that causes the bacteria to destroy itself. Because of this, the drug-resistant bacteria are unable to arise.

One noteworthy point of this nano-polymer is that it excels in safety given its induction of bacteria auto-destruction. The raw ingredients of this polymer consist only of biodegradable acrylic adhesives used in clinical practice and saccharides; thus, there have been no observed side effects in oral administration, tail vein administration, celiac administration, or intramuscular administration in mice. In this way, there are no other antibacterial agents that are superior to our nano-polymer in terms of safety given this antibacterial mechanism.

Keywords: Antibacterial agent; Bacterial auto-destruction; Biodegradable nano-polymer; Side effects

Introduction

Battle with pathogenic bacteria

Currently, measures taken for infections are roughly divided into treatments and infection controls (managements). The treatments mainly consist of antimicrobial medications and the infection controls consist of cleaning and improvement of environment with great role of disinfection. Characteristics of an antimicrobial drug used in treatment are specific to each bacterium. Based on mechanism of antimicrobial action the drugs are classified into drugs mainly acting extra-cellularly and those acting intra-cellularly (Table 1). [1] Cell wall synthesis inhibitors include penicillin, cephem, carbapenem, and beta-lactams represented by monobactam, as well as vancomycin, a glycopeptide to which sugar chain is bound. Furthermore, fosfomycin and a cyclic peptide derivative bacitracin are also effective for gram-positive bacteria. [2] Cell membrane dysfunction drugs include polypeptide drugs having a cyclic peptide structure that easily binds to the cell membrane components, and they bind to phospholipids in the cell membrane to impair membrane permeability or membrane enzymes. [3] Nucleic acid synthesis inhibitors are classified into quinolones that inhibit DNA replication and rifamycin drugs that inhibit RNA polymerase. [4] As protein synthesis inhibitors, macrolide drugs and chloramphenicol that act on the 50S subunit of bacterial ribosomes are known, and those acting on the 30S subunit include tetracycline and aminoglycosides. An aminoglycoside kanamycin acts on the bacterial ribosome 70S complex. [5] Folic acid synthesis inhibitors include sulfa drugs, which have a chemical structure similar to that of p-amino benzoic acid and therefore compete with folic acid metabolism by inhibiting conversion of p-amino benzoic acid to dihydrofolic acid. Since its antibacterial effect is weak, it is used as ST combination drug combined with trimethoprim in clinical settings.

Emergence of antibiotic-resistant bacteria and threat to daily life

Currently, we tend to feel as if the threat of infection has been eliminated from human life after the development of various antibiotics, but efficacies of the antibiotics continue to decrease year by year. Namely, resistant bacteria to which antibiotics are ineffective are gradually spreading. Bacteria create measures to disable cell-killing effects and antimicrobial activity of antimicrobial drugs to protect themselves and instruct the technical information to peer bacteria. The bacteria that survived infectious treatments-antibiotics resistant bacteria have proliferated and are now threatening human lives [1,2].

Antibiotic resistant bacteria, represented by penicillin-resistant bacteria, have been recognized since the 1970s, and today we are falling into an infection treatment spiral where a new antibiotic drug is developed and bacteria resistant to it immediately emerge. The particularly problematic resistant bacteria include penicillin resistant *Streptococcus pneumoniae* (PRSP), methicillin resistant *Staphylococcus aureus* (MRSA), and vancomycin resistant enterococci (VRE). They are referred to as ESKAPE based on the initials of the bacterial species having difficulty in infectious treatment (*Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter, Escherichia coli*) awareness for them has been raised [1].

Antimicrobial drugs acting extracellularly	i. Cell wall synthesis inhibitors Beta-lactams (penicillin, cephem, carbapenem, and monobactam) ii. Cell membrane dysfunction drugs Cyclic peptides (polymixin and bacitracin)
Antimicrobial drugs acting intracellularly	iii. Nucleic acid dysfunction drugs: Quinolones and rifamycin iv. Protein synthesis inhibitors:Macrolides, tetracycline, aminoglycosides and chloramphenicol v. Folic acid synthesis inhibitor: Sulfa drugs, trimethoprim and ST combination drug

Table 1: Classification of major antimicrobial drugs by mechanism of action.

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MRSA stands for methicillin resistant *Staphylococcus aureus*, one of the major causative organisms of hospital infection. In the US, about 80,000 people are infected with MRSA each year, and about 18,000 of the infected patients die (FDA statistics). An antibiotic drug vancomycin is used as a specific for antibiotic resistant gram-positive bacteria such as MRSA. However, in recent years, enterococci resistant to the said vancomycin (VRE: vancomycin resistant enterococci) is spreading worldwide and gathering attention. (Fortunately, its detection rate is still low in Japan). The resistant bacteria were known as causative bacteria for hospital infection, and now the WHO (World Health Organization) has indicated the risk to cause resistant bacteria infection now, leading the issue of a warning to each country in the world to take precautions at a national level (August 20, 2010) [6].

In Japan, expensive antibiotics are broadly used under the universal healthcare system to improve treatment results, while, on the other hand, resistance rates to penicillin and macrolides are both as high as about 70%. On the contrary, the resistant rates are about 10% in Germany and the UK. The emergence of resistant bacteria is correlated with antibiotic use. One of the clinical problems associated with antibiotic resistant bacteria is cross-resistance, namely, bacteria that have acquired resistance to one antibiotic drug also show resistance to other antibiotics with similar structures.

Resistant bacteria are terrible because it may cause a lethal condition in immune compromised or elderly patients due to the reduced effect of antibiotic therapy when such patients are infected with the resistant bacteria, although they were originally indigenous bacteria that caused no disease in healthy people. Currently, problematic bacteria with high resistance includes penicillin-resistant *Streptococcus pneumoniae* (PRSP), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin- intermediate and vancomycin-resistant *Staphylococcus aureus* (VRSA), vancomycin resistant enterococci (VRE), metallo-beta-lactamase producing Serratia, beta-lactamase nonproducing ampicillin/penicillin resistant influenza bacillus and metallo-beta-lactamase producing multi-drug resistant *Pseudomonas aeruginosa* (MDRP), multi-resistant Acinetobacter, and New Delhi metallo-beta-lactamase 1 (NDM-1). Treatment of these infections is gradually becoming difficult [1,2]. With the popularization of long-term admission in nursing institutes or advanced medical care, many of the problematic bacteria proliferate in increasing number in compromised patients with lowered immunocompetence and are transmitted to the community and human to human via institution personnel or the patient's family. MRSA are major causative bacteria for rampant hospital infection from the 1980s to 1990s. Now MRSA are detected as frequently as in about 60% of outpatients. Vancomycin is used as a specific for antibiotic resistant gram-positive bacteria such as MRSA, but bacteria resistant to vancomycin have also emerged. Vancomycin resistant enterococci (VRE) encode a gene for strong resistance to glycopeptide antibiotics. Since the first isolation and identification of VRE in UK in 1986, the bacteria have spread from Europe to all over world. In US, the number of mortality from VRE infection is as high as that due to AIDS, pulmonary cancer, or traffic accident.

Mechanism of antibiotics disabling

The mechanism of resistance against antibiotics is specific to each drug, and multi-resistant bacteria survive combination antibiotic therapy utilizing their various systems (Table 2).

As a mechanism of resistance against cell wall synthesis inhibitor [3,4]: 3 mechanisms of resistance against beta-lactams are known. (a) Beta-lactamase production: the antibiotic drug is disabled by production of an enzyme that hydrolyzes beta-lactam cyclic amid bound by the drug. (b) Changes in PBP property and development: mecA resistance genes found in MRSA produce new PBP2' with low affinity for beta-lactam antibiotics or changes the existing PBP to reduce antimicrobial activities of antibiotics. (c) Decrease in antibiotic permeability of the outer membrane in gram negative bacteria: a mutation eliminates the porins in the outer membrane of gram negative bacteria to affect its permeability.

1. Producing new enzymes that degrade or convert the antibiotics
2. Reducing affinity of target site for the antibiotics
3. Preventing penetration of the antibiotics
4. Enhancing extrusion of the antibiotics once taken-up into the cell

Table 2: Mechanism of resistance to major antibiotics.

The mechanism of resistance to vancomycin is unique. In this case, the bacterial cell structure itself has undergone complete mutation. Vancomycin resistant enterococci have D-alanyl-D-alanine in the amino acid sequence of the basic cell wall unit with the murein monomer changed to D-alanyl-D- lactate, which makes vancomycin unable to bind to a murein monomer. There are nine genes on the plasmid of the resistant enterococci. The most important genes are vanH and vanA. The vanH produces dehydrogenase to convert pyruvic acid to D-lactate, and vanA serves as ligase to synthesize D-alanyl-D-lactate that is incorporated in the cell wall. Furthermore, there are three genes of vanX, vanY, vanZ, which degrade, leaving D-alanyl -D-alanine that has not been replaced by the activity of vanA or vanH. Accordingly, cooperative functions of the genes completely eliminate the point of action for vancomycin.

As for fosfomycin resistance, the chromosome of an enzyme associated with intracellular transportation of the drug undergoes mutation to reduce its transfer ability, and the gene in the plasmid eliminates antimicrobial activity in association with fosfomycin modification.

Mechanism of resistance against intracellularly acting antibiotics [5,7-13]: The mechanism of resistance to quinolones involves decreasing intracellular uptake of the antimicrobial drug and reduced affinity due to mutations in DNA gyrase and topoisomerase IV. A major cause of resistance to rifamycin is mutation in the genes for the beta-subunit of RNA polymerase. The most frequent mechanism of resistance to macrolides is the acquisition of the erythromycin resistance methylase (erm) gene. Products of this gene change the conformation by methylating the specific adenine base of 23S ribosome RNA that constitutes the 50S ribosome subunit to inhibit binding of macrolides. Resistance by production of esterase that degrades cyclic lactone, production of phosphorylating enzymes, and enzymes extruding macrolide from the cell are also known. In tetracycline resistance, the tetracycline extruding protein is encoded by a plasmid or transposon. This gene actively extrudes the antibiotic drug and reduces the binding affinity of tetracycline for ribosomes by methylation of ribosomes. As for mechanisms of resistance to aminoglycosides, reduction in permeability into the cell, inactivation by aminoglycoside modifying enzyme and mutation in the action point on ribosome are known, and resistant bacteria having more than one of these mechanisms have also been found. Three modification enzymes are known, and genes producing these enzymes are distributed in plasmid and transposon of a number of bacterial species. Sulfa drug resistance includes resistance due to chromosomal mutation and resistance involving plasmid.

Efforts for the Global Issue

First, the major problem is multi-resistance where bacteria that

acquire a resistance to one antibiotic drug further acquire resistance to another different drug. As described above, many of the resistant bacteria are originally human or animal indigenous bacteria, and infection is easily provoked in compromised and elderly patients with lowered immunocompetence resulting in human-to-human spread. Bacteria have produced mechanisms of resistance in various ways to cope with various antibiotics, and humans have been repeating never-ending battles by novel antibiotics. As a result, the bacteria have been converted into multi-resistant bacteria that have acquired multiple antibiotics, resulting in the emergence of new types of multi-resistant bacteria with resistances to multiple antibiotics in parallel. The new bacteria originated from New Delhi, India, and Pakistan and have spread to Europe. These multi-resistant bacteria have generated a new resistance gene NDM-1, which degrades almost all antibiotics.

Improvement in the living environment is an important measure taken against community infection of multi-resistant bacteria. Multi-resistant *Pseudomonas aeruginosa or Acinetobacter* survive under low nutrition conditions with appropriate humidity. Their proliferation, particularly *Pseudomonas aeruginosa*, can be controlled by keeping the sink areas clean and dry because the bacteria are vulnerable to dryness. Gram negative bacteria are generally susceptible to dryness. Within the living environment, we are focusing attention on pathogenic bacteria of *Staphylococcus* and acid-fast bacteria, and controls for these bacteria are measures taken for issues in the living environment.

One of the causes of transmission of pathogenic bacteria is imported animal husbandry and fishery products. A great amount of antibiotics are used in the animal husbandry, fishery and agricultural industries. WHO has recommended limiting antibiotic use in the animal husbandry industry, indicating the risks of transmission of resistant bacteria via the food chain, such as transmission to humans via food, spread of infection to livestock animals via grazing in pastures growing on soil contaminated with the excreta of livestock animals and diffusion from sludge in sewage treatment plants. However, withdrawal of antibiotic use is a difficult issue in reality because of rising infection risks. Efforts to improve hygienic environment and reduce the amounts of antibiotics used are made to take measures. Development of novel antimicrobial measures that overcome the resistant bacteria without causing resistance is an issue to be resolved urgently not only for animal husbandry and fishery industries but also for the assurance of a safe and secure life.

Roles of Disinfectants and Differentiation in their Use

Infection is the entry of pathogenic bacteria and their proliferation in the body. Infection control is to prevent the occurrence of infection in advance and control the infection so that the infection will not be spread anymore.

For this purpose, disinfection is performed to damage microorganisms harmful to animals and humans to reduce them to below the infectious level. Disinfection is totally different from a bactericide to kill microorganisms or sterilization to kill or eliminate all microorganisms, including spores. Namely, disinfection is not a method to kill all microorganisms but to reduce the number of pathogenic microorganisms. Decolonization is to reduce the number of microorganisms by cleaning, bactericide or filtration, bacteriostasis is a condition where no organism proliferates, and germfree is a condition where no microorganism exists.

Disinfection involves physical methods, such as heat, drying, and irradiation of UV, radiation or electron beams, and chemical methods using active oxygen like ozone or disinfectants. Physical disinfection has a characteristic that causes no resistant bacteria, including heat, drying, radiation of UV, radiofrequency waves or radiation, and filtration. Free-flowing steam sterilization allows materials to stand for 30 to 60 minutes in the steam at 100°C. Boiling sterilization keeps the material in boiling water for 15 minutes. Intermittent sterilization repeats to keep the material in hot water at 80°C to 100°C or steam for 30 to 60 minutes 3 to 6 times. Drying sterilization dries the material at 80°C for 10 minutes. Physical disinfection methods for living ware include automatic dishwashers, hot air sanitizers, hot water washing machines, and bedpan washers. Chemical disinfections are applicable to cases where heat method cannot be used, including disinfection of living organisms, environment and non-heat resistant instruments. Therefore, safety concentration for use of disinfectants, risks of emergences of resistant bacteria, their biological toxicity and adverse effects on global environment should be kept in mind. Chemical disinfections are divided into gas/vapor phase methods and liquid (disinfectant) methods. Vapor phase methods involve fumigation with ethylene oxide gas or formaldehyde gas, hydrogen peroxide (plasmanization) and ozone disinfection. They are unsuitable for disinfection of living organisms and living environment. Characteristics of major disinfectants often used today are summarized in Table 3. Note that emergences of resistance are also found for surfactant disinfectants that are relatively safe and frequently used in daily life.

Bactericide performance of a disinfectant is significantly affected by its concentration, temperature, duration of use, material and form of the object to be disinfected and organic substances that coexist. The

Substance	Characteristics					
	Environment	Instruments	Skin	Mucosa	General bacteria	MRSA
Ethanol	Δ	○	○	×	○	○
Sodium hypochlorite	Δ	Metals×	×	×	○	○
Povidone iodine	×	×	○	○	○	○
Benzethonium chloride	○	○	○	○	○	Resistant
Benzalkonium chloride	○	○	○	○	○	Resistant
Chlorhexidine	○	○	○	×	○	Resistant
Phenol	×	Δ	×	×	○	○
Cresol	×	Δ	Δ	Δ	○	○
Glutaral	×	○	×	×	○	○
Silver, copper, tungsten compounds	×	Limited	Δ	×	Limited to light area, insufficient effect	
(rare metals)	(unstable supply)					
Novel technology	○	○	○	○	○	○

Table 3: Characteristics of major disinfectant.

higher the disinfectant concentration is, the stronger its bactericide effect becomes, with rising biological toxicity. Therefore, there is an appropriate concentration of the disinfectant from view of safety. In general, chemical disinfectants themselves are altered by oxygen and UV, and consumption by chemical reaction with coexisting organic substances makes the sterilization performance weaker. Prewashing is important in chemical sterilizations, and attention should be paid to disposal of bacteria washed out during the prewashing. Concentrations of disinfectants are indicated as weight concentrations of the ingredients (w/v: weight per volume; 1% means 1 g of disinfectant is contained per 100 mL), volume concentrations (v/v: volume of disinfectant per volume; rubbing alcohol is indicated as 70% concentration, meaning that 70 mL of ethanol is contained per 100 mL) or ppm (1000 ppm = 0.1%) used in sodium hypochlorite and povidone iodine. Care should be taken to adjust the disinfectant to the appropriate concentration.

Bactericide effect of disinfectants mainly involves protein denaturation based on chemical reaction, and insufficient chemical reaction results in insufficient damage to bacteria. Chemical reaction in disinfection is weakened as temperature decreases. Disinfectant is usually used at 20°C or higher. Duration of disinfection depends on the disinfectant used. Since each disinfectant has indication of duration of use, sufficient time for disinfection should be ensured.

Disinfecting methods includes soaking, wiping, spraying, and perfusion. The general disinfection method for instruments is soaking. Instruments should be immersed completely without remaining bubbles. Prewashing and brushing are effective to improve the bactericide effect. For viruses, aldehyde or hypochlorite disinfectants are definitely effective. Attention should be paid to ventilation because toxic gas may be produced. Since these disinfectants themselves has strong toxicity, it is important, above all things, to prevent diffusion of the disinfectant gas and close the lid of the disinfectant tight assuming that toxic gas may be generated.

Wiping method uses quaternary ammonium salts, detergents, aqueous sodium hypochlorite at 500 to 5000 ppm or rubbing alcohol. Care should be taken because sodium hypochlorite tends to produce discoloration or decolorization. It is important that gauze, nonwoven fabric or a mop is sufficiently soaked in disinfectant solution to wipe the object in one direction before the disinfectant is dried. Floors are wiped from the back of the room to the entrance. During the wiping, gloves and special work wear should be worn, taking care not to inhale the disinfectant or toxic gas generated during the disinfection.

The spray method uses a spray instrument, allowing disinfection of niches or cracks that cannot be disinfected by wiping method. Attention should be paid to biological toxicity (Table 4), and disinfection operation should be conducted with perfect protection using gloves, a cap, and work wear covering the arms and legs, covering the face with a goggle or gas mask. Spray method often uses quaternary ammonium salts or detergent in view of safety. Rubbing alcohol is not used because of its risk of firing or combustion explosion.

Glutaral (aldehyde)	Local inflammation in the lungs or bronchi, pulmonary congestion, interstitial pneumonia, central nerve disorders like vertigo or ataxia, and skin irritation
Sodium hypochlorite	Contact dermatitis, respiratory irritation symptoms like cough, glottal edema or dyspnea.
Formaldehyde gas	Erythema at the site exposed to the gas, irritation in the throat and lungs, and asthmatic attack. Carcinogenicity at high concentration
Quaternary ammonium salts	Eruption, skin irritation and mucosal irritation
Detergents	Mucosal irritation

Table 4: Intoxication symptoms and biological toxicity of disinfectants.

Roles and Safety of Antiseptics/Preservatives

Antiseptics prevent putrefaction/fermentation in advance by suppressing invasion, growth and proliferation of microorganism and are used for bacteriostatic effects. They are totally different from disinfectants to kill microorganisms. Industrial antiseptics include coal tar for antisepsis of woods. As food antiseptics, benzoic acid is used as preservatives and food additives. As for antiseptics for drugs (quasi drugs), p-hydroxybenzoate (paraben) is added to cosmetics and eye drops.

Preservatives are used in a lot of processed food products and bread sold in convenience stores to ensure product quality within the expiration period. The idea that commercial food contains a great amount of preservatives because bread or foods prepared at home easily get moldy but commercial food is not is often unwise and wrong. Microorganisms exist everywhere on the Earth, and the number of microorganisms at home is definitely greater than that in the food factories under good hygienic management. Prefectural governors are responsible for control of manufacturing environment in such factories. It can be easily presumed that food prepared at home may have far more microorganisms colonized in the food than that prepared in food factories.

Comparing various food risks (frequency of health hazards after ingesting the food), the greatest risk is food intoxication. Microorganisms such as *Norovirus, Vibrio parahaemolyticus, Salmonella enterica,* O-157, and *Staphylococcus aureus* are colonized in food processed under unhygienic conditions and cause food intoxication. It can be found that cleaning of the food processing environment and suppression of microorganism growth are important for management of these risks. There is no food without any risks. Even oxygen in the air or sunlight may damage the genes, and vegetables contain great amounts of oxalic acid and metals.

Benzoic acid has antimicrobial effects and its water soluble sodium salt is added to soft drinks as a preservative. The Federal Institute for Risk Assessment (BfR) [14] in Germany reported that coexisting benzoic acid and ascorbic acid (Vitamin C) may produce benzene even in trace amounts. Benzene raises risks of various cancers and myelocytic leukemia. However, the concentration of benzene produced from soft drinks is 20 ppb or lower and its health hazard is assessed as being far smaller compared to the acceptable daily intake of benzene (no increasing risk has been found in exposure at 1 ppm as time-weighted concentration for 40 years).

Paraben has been used as a preservative since the early 19th century. It is added to cosmetic products (humectant, cleansing gel and emulsions), soaps, shampoo, deodorants, shaving cream and topical medicine. It is used to prevent alteration, strange odor, or mold growth in products due to contaminating microorganisms during the process from manufacturing to distribution to consumers, as well as between seal opening to using up of cosmetic products. The Pharmaceutical Affairs Law obligates the indication of 102 items that "may cause skin disorders such as an allergy," such as paraben on the product package when any of them is used. Some manufacturers considered that paraben is water soluble and does not penetrate into the deep skin, but Darbre PD et al. issued warnings in 2004 that "paraben was frequently detected in breast cancer tissue from breast cancer patients, and it may mimic estrogen that closely associated with breast cancer onset" [15] citing articles. This report caused seismic effects on the cosmetic industry

and became a hot topic that had great impact on consumers' awareness. No empirical research on whether paraben may trigger development of breast cancer or not has been published until now, and it cannot be concluded that paraben is safe.

Use of antiseptics or preservatives is effective to reduce risks and it may be inadvisable to reject antiseptic/preservative use unilaterally in view of risk management. Namely, switching to safer preservatives and development of additive-free processing technology and containers that suppress microorganism growth are in the mainstream. Safety should be prioritized in bacterial control, and therefore, development of safer antiseptics and preservatives is globally desired.

New Approach

Now plant-derived substances are gathering attention as new measures against bacterial infection. Focuses are placed on herbs or essential oils extracted from plant that have been used as folk medicine [16-18], and flavonoids, alkaloids and polyphenols are found to have antimicrobial activities. Tellimagrandin I in rose petals and corilagin in bearberries enhance drug susceptibility of beta-lactam antibiotics resistant MRSA. Photosensitizer of porphyrin [16] is also effective to MRSA and now being developed as an antimicrobial agent with new mechanism. Our laboratory reported interesting facts [19] that several substances in the Sasa family plant *Sasa albo-marginata* have activity against resistant bacteria and that they potentiate the effects of existing antimicrobial drugs.

Recently, Nottingham University (UK) reported that a protein contained in the brain tissue of cockroaches and locusts had antimicrobial activity against MRSA and pathologic *E. coli*. Furthermore, a caplazamycin derivative CPZEN-45 with new mechanism of action was found in *Actinomyces* culture medium. In an animal experiment where animals were infected with XDR-TB resistant to 10 anti-tuberculosis drugs, it reduced the amount of bacteria to 1/100. These approaches seem to be promising in the future, although proceeding step-by-step.

Finding Close Peers

We have developed a nano-antimicrobial technology as a novel approach, under a concept of "Finding close peers." We found a property of acryl polymer that it has nitrile groups serving as strong electron withdrawing groups with ηπ* electrons and carboxylic groups at alpha-position (Figure 1), presenting high affinity for glycoproteins. It is expected that application of this property to bacteria having the surface layer of sugar chain peptide may cause new changes in bacterial function through specific junction between the bacteria and nano-polymer. This is the point of origin for this invention.

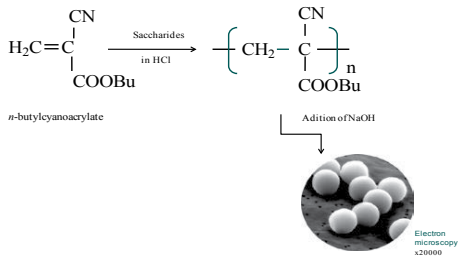


Figure 1: Synthesis of nano-polymer (PCT-JP2010/53426).

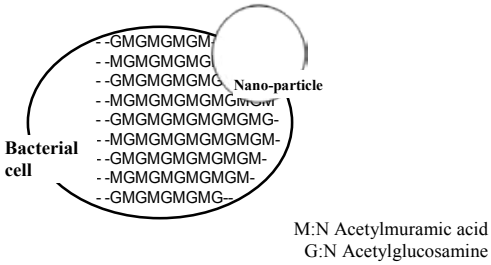


Figure 2: Schematic diagram of junction between nano-polymer and the cell wall of bacteria.

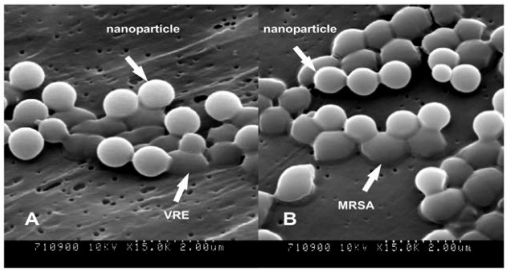
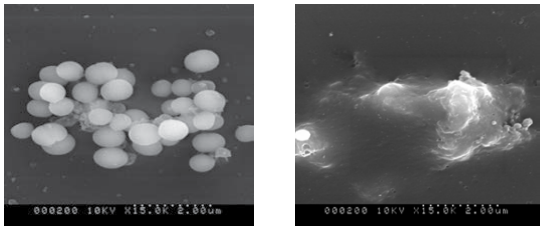


Figure 3: Scanning electron microscopic images taken 30 minutes after addition of the nano-polymer particles x15000 magnification. A: Junction with multi-resistant MRSA; B: Junction with multi-resistant VRE.



5C Untreated VSE

5D VSE bacteriolysis 6 hours after administration of nano-particle

Figure 4: Induction of bacteriolysis in VSE by the antimicrobial nano-polymer particle (Scanning electron microscopy x15000).

Bacteria must continue to produce the cell wall that keeps bacterial form and protects the cells from outer environment for survival. Cell wall synthesis basically consists of UDP-MurNAc-pentapeptide, which is next bound to fatty acid in the cell membrane and then forms lipid-MurNAc (GluNAc)-pentapeptide bound to GluNAc. The MurNAc is bound to GluNAc in the peptide glycan that is being synthesized to construct branching structure. A structure with affinity for branching structure consisting of sugar chain peptide can be created artificially so that specific junction with bacteria can suppress cell wall synthesis leading to bacteriolysis (Figure 2).

We found a phenomenon where our nano-polymer particles that contain no antimicrobial drug junction the cell wall of resistant bacteria (Figure 3), the resistant bacteria lysed with time (Figure 4), and autolysis phenomenon spreads to the whole bacterial colony (PCT/JP2008/073272). The changes in bacterial form by these nano-polymer particles are totally different from the changes in bacterial form by nano-capsules containing antimicrobial drugs. This bacteriolysis process has successfully been videotaped and proved in cooperation with Research Institute of Biomolecule Metrology Co., Ltd. Namely,

the polymer particles bound to the bacterial cell wall within a minute, and cell wall synthesis of the bacteria was locally inhibited due to the junction with the particle, producing partially weakened cell wall at the junction site, and the bacteria ruptured and lysed by themselves in 6 minutes and 30 seconds. The bactericide effect of these nano-polymer particle does not involve direct attack by antimicrobial toxicity that is found in nano-capsules containing antimicrobial drugs but induction of autolysis where bacteria lyses by themselves (compare between Figures 3 and 4). Accordingly, the novel antimicrobial technology using the present nano-polymer particles produces no resistant bacteria.

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This bacteriolytic nanotechnology is unique, and the present bactericide effect has been confirmed in various countries since 2010. Namely, junction between nano-polymer and bacterial cell wall is the most important factor in the bacteriolytic effect, and no antimicrobial activity is found in gran negative bacilli that do not junction. The antimicrobial activity of the present nano-polymer is dose dependent and related to its structure.

Characteristics of the Novel Technology

We created the antimicrobial nano-polymer beyond the resistance mechanism of antibiotics by synthesizing 236 nano-strictures using biolytic drug n-butylcyanoacrylate used in the treatment of aneurysms or as surgical wounds and adhesives and screening them for antimicrobial activity. The antimicrobial effect of the present nano-polymer involves induction of bacterial autolysis caused by specific junction between the nano-particle and the bacterial cell wall (Figs 3 and 4). This is a globally unique technology (Application of international patent has been filed: PCT/JP2008/073272). The nano-polymer particle induces autolysis not only in bacteria resistant to multiple antibiotics such as MRSA and VRE but also in bacteria susceptible to antibiotics (Fig .4) without causing resistance. An in vivo study on antimicrobial activity of the present nano-particle was performed in mice with gastrointestinal infection in an institute specialized in infections (K Institute Medical Center Biolaboratory) to prove therapeutic and preventive effects on infection by oral administration.

The notable point of the present nano-polymer is high safety due to induction of bacterial autolysis. No unspecific toxicity has been found, such as mutagenicity. Formulation of raw materials of the nano-polymer are only clinically used biolytic acryl adhesive and sugars. No adverse reaction has been found after oral administration, intravenous administration in the caudal vein, intraperitoneal administration or intramuscular administration. It is not taken up into phagocytes, such as macrophages, and has anti-inflammatory effects without causing inflammation. Based on its mechanism of antimicrobial activity, its safety and antimicrobial activity are better than any other antimicrobial substances.

The present nano-polymer forms stable colloid in water solution and the colloid water solution is stable without mold growth after 3 years of storage at room temperature in the light laboratory condition. It can be used practically in the general living environment. Since no rare metal is used, no concern about contamination of the global environment is necessary. The characteristics of nano structure of acryl macromolecule enable oral administration, and gram-positive bacteria can be lead to autolysis only by mixing the nano-polymer in feed or drinking water. Therefore, it is favorable in application as antimicrobial drugs in animal husbandry, fishery and agriculture industries.

Examples in Use and Prospects

The expected examples in use include prevention of infection in advance and safe antimicrobial treatments. Antimicrobial drugs, safe disinfectant, veterinary drug or feed, pesticide, antiseptics/preservative and medical instruments are expected among others. In the public hygiene area, antimicrobial swab, sanitary articles, sport and health instruments, antimicrobial furniture, antimicrobial wallpaper, antimicrobial electric components, hand rail, strap, cosmetic products, antimicrobial bedding and antimicrobial garments are expected to prevent contact infection in advance. Application to sanitary transportation wares are also expected, such as antimicrobial containers or antimicrobial cartons.

Expected industries include trading company, non-life insurance, animal husbandry and fishery, agriculture, food, construction material, electric component, electrical equipment, sport instrument, sport facility, sanitary articles, transportation business, car industry, apparel, furniture, fermentation and bio-venture, cosmetics, medical and pharmaceutical industries.

Its market will be worldwide and huge demands are expected. Currently, basic investigations have almost been completed and the development has been advanced so that the polymer can be put into practical use soon. The development is now in the steps of commercialization suitable for practical use. It is desirable that companies conduct applied development and tests for practical use.

The present invention is totally novel antimicrobial technology that induces the mode of apoptosis where the bacteria are captured by nano-polymers and then lyse by themselves, with higher safety than any other products. Accordingly, global expansion is expected not only in medical industry but also in animal husbandry and fishery, agriculture, food, cosmetics, public hygiene, health business, and sanitary warehouse/logistics, and therefore, we would like to put it into practical use globally in cooperation with a company willing to develop this polymer.

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