Global Hot Topics

Biofilms

In

Food, Food Processing and Food Serving Environments

Part 1 to 7

By Globalhottopics. Published at Globalhottopics.com

Contents

BIOFILMS 1: In Food, Food Processing and Food Serving Environments - Detection

What are biofilms?

Biofilms are complex microbial ecosystems formed from one or several microbes bound in a complex extracellular matrix of their own secretions and formations. Each microbe in the ecosystem make contributions that strengthens the community, creating a strong common bond that translates genomically, chemically and structurally into collective effectiveness, efficacy and their ultimate communal survival.

The microbes include mainly bacteria or fungi or a mixture of different combinations and proportions, which depends on the type of food and environment particularly the micro-environmental factors such as humidity and temperature and the macro-environment like the type of surfaces. Generally, biofilms need water or moisture to continue to grow.

The microbial extracellular matrix is composed, in the main, of polysaccharides, such as cellulose, proteins or exogenous DNA. Biofilms attach to hard surfaces through specialised structures facilitated by the extracellular matrix.

Such hard surfaces in the food, food preparation and food serving areas include various kitchen equipment, tables, taps, hand wash basins, storage surfaces including plastic and glass bottles, jars, or containers, cans, dispensing materials, conveyor belts in food processing plants, transportation, soil and also on biological structures such as vegetables, meat, bones, fruits, etc. Even on supermarket food shelves are not spared.

It has been noted that the extracellular matrix has an important structural role in the determination of the impact of biofilms. Among others, such important roles include:

- provision of a strong persistence and protective biofilms around the microbes
- producing a complex gradient of nutrients and oxygen diffusion
- contains extracellular enzymes used for nutritional purposes
- allowing for the transfer of cell communication molecules, and
- protects the embedded cells against toxic compounds including antimicrobial disinfectants.

Biofilms and Food Public Health Concerns

Microbial biofilms can develop in most environment with the right conditions. They can develop in private and public bathrooms, toilets or restrooms and kitchens, restaurants, butchers' shops and groceries sections where they help to increase the rate of food spoilage.
Their growth is possible and enhanced when surfaces are not regularly cleaned and wiped dry after use. Biofilms form and grow in small puddles of water and areas that remain wet over a length of time particularly starting and spreading from hard to reach crevices.

A: Not regularly cleaned

B: not regularly cleaned. Corrosion by biofilm is visible

C: cleaned regularly after use

D: Wash basin in a supermarket – bottom end crevices of the waste water pipe showing accumulation of microbial biofilms. These are hard to reach areas with real risk of cross-contamination.

Three photographs A, B, C and D were taken from three different supermarkets selling almost every household item including cooked and uncooked food. Often the wash rooms are located very close to the restaurant part of the establishment for the convenience of the dinning customers and use these washrooms.

There are currently no studies to determine the relationship between the spread or incidence of food related health issues, the proximities of the toilets and washrooms, most common location for biofilms, and any existence of biofilms in the establishment.

Washrooms are often equipped with soap dispensers, which may be manual or automatic soap dispensers. The case housing the soap may be clean on the outside. However, the inside is often not easily accessible to the cleaning staff. The wet environment provides ideal opportunities for biofilms to thrive in the crevices of the dispensers:

Manual Soap Dispenser: The inside of a manual soap dispenser from a supermarket washroom. The brown spots inside the dispenser white surface are accumulating biofilms and around the metal rings. They have a real potential to contaminate the soap dispensed on to hand

Automatic Soap Dispenser: Bottom end of an automatic soap dispenser showing accumulation of biofilms around the pump, a potential source of cross contamination

Thorough cleaning regime is necessary to keep biofilms out. It is necessary to also including the cleaning of inside of dispenser at a determined regular interval. The biofilms can survive some challenging conditions. *Pseudomonas* spp and *Acinetobacter spp* have been found predominantly on food conveyor belts at simulated temperatures ranging from 3^{o}C – 76^{o}C.

Overall, biofilm formation offers advantages to the microbial cells in a food preparation and serving environment, such as physical resistance against drying or desiccation, mechanical resistance against liquid streams in pipelines and protection against antagonistic chemicals intended for their eradication.

Detection of Biofilms

Will Classical agar plating still do the job?

The complex composition and interaction in biofilms make detection and proper identification very challenging, hence their high potential to persist when contamination occurs. Classical detection methods consisted mainly of agar plating, isolation and identification by classical microbiology and / or biochemical analysis of isolates. Agar plating is obviously not suitable for modern big food industrial setting and related organisations.

The classical agar method is regarded as not a very effective and efficient method as some food borne microbes are capable of entering into what is referred to as "viable but non-cultural" VBNC states with low metabolic activity. VBNC cells are not detectable by culturing and may survive stress conditions such as low temperature regimes.

Modern microbiological technology

Great advances continue to take place in modern microbiology incorporating biotechnological methods of rapid screening and identification, including automation. Modern methods have been developed for rapid and mass detection of microbial biofilms. On-line tools monitoring and recording, including Polymerase Chain Reaction (PCR) amplification methods to show the number the different genotypes. Other methods include:

- metagenomics and metatranscriptomic for genotyping
- PCR amplification and gel electrophoresis to distinguish the different lengths

Application of new technology

For large food manufacturing settings, new strategies are being developed to detect biofilm formation, where biofilm development is monitored by the introduction of an external perturbation in the system. The external perturbation is then measured or calibrated using a suitable device. In some cases, the perturbation is amplified to make it measurable in values or changed into other forms such as heat and pressure transfers in the system.

For example, specially designed commercial on-line monitoring sensors are available and in use in the food and beverage industry. The sensors are based on thermal pulse analysis. They detect and measure the local thermal conductivity and heat variations resulting from the formation of biofilms. They are, however, able to detect deposits only a few micro-meters thick.

BIOFILMS 2: IN FOOD, FOOD PROCESSING AND FOOD SERVING ENVIRONMENTS – Control

Photographs taken from the washrooms three different supermarkets

A: Not regularly cleaned **B**: not regularly cleaned. **C**: cleaned regularly after use

How are biofilms controlled in the food sector?

Application of On-line technologies

On-line tools for monitoring the development, adhesion, growth and removal of biofilms from surfaces in big industrial environment is helping to reduce the cost of cleaning operations and minimizes production down times to enable regular maintenance operations so that cleaning operations can be planned, targeted and better managed.

In addition to the more recent technological approaches for monitoring and detecting biofilms and ultimately leading to their control, several new and classical specific, targeted methods could be used in individually or in a multimodal design and applications. Control operations should always be preceded by a proper identification of the microbes in the complex community. The control methods include the application of:

i. **Chemical control**

   A range of chemical families are used in disinfection and sanitation in the food and related industry. The challenge in the use of one chemical group is that a biofilm may compose of a complex group of pathogens, even of the same species, which may response to the chemical in different ways. While some may be killed, others may only be weakened or inhibited, only to emerge again when the chemical stress is reduced or removed.

   It is important to elucidate the complex microbial community of biofilms as far as possible using effective identification methods before applying chemical controls. Identification helps to make a target use of chemicals and obtain quick and cost-effective results. The main draw-back of chemical application is the development of different levels of resistance among the microbes in the biofilm community even when a specific species is dominant in that ecosystem. The biofilms provide microbes with the necessary apparatus for resistance against chemical through various mechanisms.

   Chlorine based disinfectants

   Chlorine based biocides or disinfectants are widely used in the food industry. Resistance to chlorine treatments has been known in some microbes. It is also considered that multiple applications of specific chemicals ultimately enhance the microbial selection mechanism and process to leaving some microbes behind to continue the pathogenic and /or spoilage fight.

In *Staphylococcus enterica*, chlorine resistance was correlated to the cellulose production phenotype, which in turn depended on the environmental stress conditions found in the food processing plants.

Among the chlorine product family, aqueous chlorine dioxide (ClO_2) is the most widely used disinfectant in the food industry. It has been shown to be more effective against *Bacillus cereus* endospores present in biofilms on food steel surfaces. In the case of E. coli O157:H7 biofilms, aqueous ClO2 was more effective than sodium hypochlorite (NaOCl) in particular if the application is designed so that the treated factory surface is allowed to dry after application.

Another negative side, chlorine-based treatments are usually applied at very low ppm concentrations, they often still leave chlorine odour on treated surfaces. Additional care may be required not to contaminate food materials particular dairy or fatty food. The possibility of residues on surfaces is also important.

Biofilms formed in raw milk on stainless steel and polypropylene surfaces by *Staphylococcus aureus* and *S. enterica were effectively eradicated by chemical treatment with* sodium hypochloride (NaOCl). Similar disinfection with NaOCl of *Cronobacter sakazakii* biofilms in the same environment was not effective, indicating the importance of environment factors such as surfaces in the application of a specific treatment regime and the inherent variability of results.

Quaternary ammonium compounds

Quaternary ammonium compounds, often referred to as 'quats' are commonly used in the food industry both for general disinfection and eradication of biofilms. They are often made of a combination of different types of quaternary ammonium moieties, which is claimed to enhance the efficacy of this group of products. Quats are soluble in water and commonly presented as water-based, non-alcohol disinfectants. The positive charge ions in the solution disrupt the bacterial cells to exact a killing effect. However, genes responsible for antimicrobial resistance (AMR) was found in certain places and microbial species and strains due to the use of quats. A multimodal application, combining various methods has been shown to be more effective and may well reduce the potential for the development of AMR. It was also found that a combination of NaOCl, H2O2, iodophor and benzalkonium chloride (quat) with steam heating eliminated ecosystems of biofilms formed by *E. coli* O157:H7, *S. enterica* and *L. monocytogenes* while achieving decreases in both the concentrations of disinfectants use and the duration of the application.

Hydrogen peroxide (H_2O_2)

H_2O_2 is a potent oxidising disinfectant commonly used to eradicate pathogens in the food industry. It is non-toxic to users and does not leave residue on surfaces or on food. H_2O_2 generates free radicals when in contact with the microbial biofilm structures. The free radicals destroy the biofilms structures at very low to moderate concentrations (0.08% –5%) without leaving toxic side effects.

In combination with acetic acid, H_2O_2 generates peracetic acid in situ. The resulting solution is a strong oxidant with low acidic pH (pH2.8). This is used at low concentrations with high efficacy against biofilm community of *Listeria monocytogenes*, known to survive with or without oxygen, and *S. aureus* population in water pipes.

ii. Biosurfactants

Biosurfactants, like lichenysin are added to detergent formulations used in industry to control biofilms. Biosurfactants, biological surfactants or microbial surfactants non-synthetic chemicals generally of microbial origin that have similar characteristics as the synthetic surfactants with added value of being more readily biodegradable, less or even non-toxic to human and environment and in some cases more effective. The overall characteristics and performance depend on the class and source of the biosurfactant.

iii. Bacteriophages

The use of phages as biocides or sanitisers is regarded as an environmentally sustainable approach and a good alternative to antibiotic or chemical biocide applications as phages are innocuous to humans, animal and the environment. Commercial phages such as Listex P100 are successfully used to eliminate biofilms in processed meat factory work surfaces.

Bacteriophage application already authorised in the United States by the Department of Agriculture with the status of GRAS (Generally Recognized as Safe) biological agent. S. enterica or *E. coli* are among other bacteriophages targeted for commercial applications at industrial scales.

There may be limitations to the use of phases in biofilm control. They possess limited ability to access and target bacterial cell inside the specific biofilm community due to the collective protective mechanisms provided by the biofilm ecosystem.

The complex biofilm structure and the extracellular materials act as physical obstacle to the ability of phage to diffusion or penetrate into the bacterial cells. To counter this obstacle, some phages have developed specific enzymes, exopolysaccharide depolymerases to help take the battle to biofilms and win. These enzymes amply facilitate the diffusion by enhancing their capability to invade and disperse within the biofilm being treated.

iv. Bacteriocins

Bacteriocins offer a type of solution to the control of biofilms. They are antimicrobial agents of protein or peptide-based toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strains. Some of the identified advantages of using bacteriocins in the food industry include:

- prevention or preservation to stop the formation of biofilms in the first place
- extend the expiry or shelf life of food materials in storage including during refrigeration by stopping changes in the food
- reduce the use of chemical preservations both in terms of number and quantity applied

The World Health Organisation (WHO) approved a peptide, Lactococcus lactis, as an antimicrobial agent since 1969. The USA, Food and Drug Administration (FDA) approval followed in 1988 on grounds of being safe for consumption in animals and humans. Nisin is still the only FDA approved bacteriocin in the food industry. It is used as spray on surfaces used for food processing or manufacturing. It prevents adhesion and biofilm formation by *L. monocytogenes*.

Other bacteriocins, which are either in use or in ongoing research include: bacteriocins produced particularly by bacteria regarded as GRAS (Generally Recognized as Safe) such as lactic acid bacteria, novel bacteriocins produced by *Enterococcus spp* and active against *L. monocytogenes*, lactocins active against *Brochothrix thermosphacta* and produced by *Lactococcus spp*. and garvicin produced by *Lactococcus garvieae*, which is active against pathogenic strains of the same bacterium.

v. Quorum Sensing (QS) inhibition

This is based a complex biological systemic disruption mechanism. Bacterial biofilm formation and antimicrobial resistance development require different signalling pathways. These include the exchange of small organic molecules or proteins and the transmission of electrical signals. These activities will trigger the binding of inhibitors to the QS receptors, disrupting the enzymatic, biosynthetic and genomic systems.

Of the several identified signalling pathways, QS is one of the best characterised. It is a widely distributed intercellular signalling mechanism, used by bacteria to regulate gene expression in response to high environmental concentrations of small diffusible signalling molecules such as acyl homoserine lactones, peptides and the autoinducer-2. These QS regulated mechanisms include genes involved in biofilm formation, which can be inhibited.

vi. Essential oils

Plant-based essential oils have antibiofilm properties and are primarily a species-specific complex mixture of chemicals, which have been group based on type: monoterpenoids like borneol, camphor, carvacrol, eucalyptol, limonene, pinene, thujone; sesquiterpenoids like caryophyllene, humulene and flavonoids such as cinnamaldehyde and other phenolic acids. Some essential oils are commonly available and are widely regarded as from 'natural sources' with good for the environmental profile. It should be noted that some plant based antibiofilm agents are also classified as toxic or irritant to humans, animal and the environment.

vii. Enzyme disruption

Some detergents are being formulated with enzymatic components capable of disrupting the microbial cell structure. The enzymes used are mainly proteases or peptidases, glysosidases or DNase. They catalyse hydrolytic biological and genomic processes in the microbial cells and their extracellular matrices. The enzymes act on the bacterial cells and extracellular matrix of the biofilm.

viii. Steel coating

Steel surface maybe modified by coating with silver, copper or zinc nanoparticles, or by using the novel antibiofilm polymers with lysozyme or bacteriocins. Coated surface prevents the formation of biofilms. Antimicrobial surface coating is a growing area in the food and beverage industry and more evidence of its efficacy is demanded by consumers.

Other type of surface-based controls are repelling surfaces with monolayers, hydrogels or modified topography, inhibiting bacteria or microbes from binding onto the surfaces.

ix. High hydrostatic pressure (HHP)

HHP treatment is better used as a key part of a multimodal approach by integrating other methods such as essential oil application, chemical application or Thermal treatment between 50°C – 100°C. HHP at 300–900MPa, for example, can destroy vegetative bacterial cells but leave the endospores unharmed. The endospores will go on to germinate. Thermal treatment ensures the endospores are destroyed.

By carrying out the operation in two steps, a better result is obtained. A low pressure (300 – 400 MPa) pre-treatment phase, which allows the endospores to germinate before application of the HHP to eradicate the vegetative bacterial cells.
An significant advantage of using HHP is that it has no effect on the organoleptic and nutritional qualities of the food such as taste, vitamin content, colour, etc.

x. Non-thermal plasma

Non-thermal plasma treatment consists of using partially ionised gas at low temperature to treat biofilms on surfaces. The ionised has antimicrobial properties. The gas is produced at atmospheric pressure by mixing UV light with oxygen, nitrogen, ozone, and water and helium, under an electrical discharge. The resulting ionised gas is able to relatively rapidly destroy bacteria in complex biofilms ecosystems made up of Gram-negative bacteria including *Pseudomonas spp*. and *S. enterica* or Gram-positive such as *Bacillus spp*. species in as little as 10 min.

This is a very expensive technology to deploy. Hence, though it has proven to be highly effective, it limited to certain applications only, mainly laboratory. A key advantage is that the ionised gas can be used effectively in difficult to reach surfaces, corners, compartments and crevices.

xi. Photocatalysis

Nanotechnological applications employing photocatalytic properties of specific types of metals have been developed or are in development. Photocatalytic properties of nanoparticles enable the absorption of a specific wavelength, which generates or accelerates a chemical reaction, including destruction of microbial cells, generally due to the generation reactive oxygen species (ROS) inactivating bacterial cells on the surfaces.

Biofilm contamination in the food industry can constitute significant challenge to any affected company both economically, socially and for the wider health of the affected human population. They are difficult and expensive to treat and eradication could be a prolonged strategic battle for any affected organisation. A business may suffer reputational damage due to public announcements of the recall of biofilm contaminated products. This the business may take time to recover

The biofilms can be recurrent and corrode machineries necessitating a considerable downtime or even total closure to allow for eradication. It may be necessary to replace costly parts after the treatment. Affected products should be recalled for either treatment where possible or disposal. Some bacterial species, such as *Pseudomonas spp*. and *Bacillus spp*., secrete many different proteolytic and lipolytic enzymes that convey unpleasant odours or rancidity or bitter taste. In this latter case, affected products must be withdrawn from the market.

Some type of products may be recovered by administering appropriate treatments which may include a multimodal approach ensuring both microbial spores and vegetative stages are eradicated.

The health impact to the community may also be significant. Biofilm formation in food factories is a critical public health issue for the communities where the pathogenic microbes can interfere in the wellbeing of the community. Biofilm ecosystem that is principally of *B. cereus* and *S. aureus* are known to cause food intoxication. Strains of *E. coli* (O157:H7) and *S. enterica* may cause gastroenteritis and other systemic diseases (*L. monocytogenes*).

The health impact of biofilm formation and the role in the development of antimicrobial resistance will be among our next topics of discuss.

BIOFILMS 3: Formation and growth

How do biofilms form?

Bacteria, fungi and other microbes have existed in nature for thousands of years. They generally ensure survival and growth by attaching to different types of surfaces, animate and inanimate surfaces including soil, hard surface such metals, plastics, living tissues and in aquatic environments.

The microbes generally require wet environment or moisture to thrive in.

What factors can impact on formation and growth of biofilms?

The nature of the surfaces is a critical factor for the growth of biofilms and growth will differ between water repelling (hydrophobic) such as plastic, latex, and silicone surfaces and highly charged, hydrophilic surfaces such glass and metals. Smooth materials like in the silicone and plastic materials or rough in texture such as some environmental surfaces and water pipes, also make a difference to the type and rate of development of biofilms.

Some surfaces may be pre-treated with an antimicrobial agent such as copper or silver and the alloys to inhibit attachment to the surfaces and formation of biofilms. In general, rougher and more hydrophobic surfaces tend to develop biofilms more rapidly than the opposite.

The type of the microbial cell surface is as well important to the attachment and development of biofilms. Some microorganisms are thought to have specifically develop special appendages to enable and enhance their ability to attached onto surfaces, which is also linkage to their survival in or on their hosts. The possession of microbial flagella, pili, fimbriae, or glycocalyx have been shown to enhance attachment and ecosystem matrix, and linked to specific pathogenicity of the microbe.

Human have since learnt to live in communities and collaborate in order to be more efficient, increase productivity and their defences against enemies, hence the chances of survival. Microorganisms have similar ability to live in communities of different ecosystems for the same reasons. The formation of these ecosystems on different surfaces is particularly significant to human in the case of pathogenic microbes, diseases and public health.

Pathogenic bacteria and fungi in both healthcare settings and food production irreversibly attach to surfaces and grow. The microbial cells produce extracellular polymers to help hold the community together into a complex matrix. The new ecosystem enforces changes in the genetic makeup and behaviours (including pathogenicity and reproduction) of microorganism. New strains of a specific microbial type may develop in the biofilm with a different disease expression.

Importantly, biofilms render antimicrobial agents less effective against the microbial community, making medicinal antibiotics or surface disinfectants less effective. It has been indicated that these advantageous changes in the microbes may be natural or intrinsic due to the biofilm formation or acquired because of transfer of extra-genetic or chromosomal materials within individual cells the biofilm community.

Scanning Electron micrograph showing a developed biofilm (A), the substratum (B), and an attached cell (C). (Image by Rodney Donlan and Donald Gibbon, from the American Society for Microbiology Microbe Library. Taken from Donlan R.M. HEALTHCARE EPIDEMIOLOGY, CID 2001:33 (15 Oct), 1387

Understanding how biofilms are formed and growth will assist in devising good related public health policies, control measures and treatment regime in healthcare settings and in the food industry.

BIOFILMS 4: Important Healthcare Microbes in Food, Food Processing and Food Serving Environment.

Biofilm is described as a are complex microbial ecosystems formed from one or several microbes bound in a complex extracellular matrix of their own secretions and formations. In earlier discussions in this biofilm series, we have talked about general formation of the complex ecosystems, the detection, classical and modern control methods of biofilms in these ecosystems. In this part, some key microbes identified as clinically significant in food borne pathogens will be summarised.

Infection by the microorganisms or intoxication by the toxins they secret are two main routes of food borne diseases linked to biofilms. Bacteria and fungi secret toxins in food matrices or in human or animal body. Some of the toxins can be fatal if not identified early and treated appropriately. Disease outbreaks and food poisoning are not uncommon. The level of risk and attendant healthcare challenge will depend on the type of microorganisms in the biofilm ecosystem and the toxins they secret.

What are the key microbes identified in food, processing and serving environment and their impact?

Bacillus cereus

Bacillus cereus is an anaerobic or facultative anaerobic gram positive and spore-forming bacterium able to grow in different environments over a wide temperatures range of about 4◦C to 50◦C. It is known to be resistant to heat, chemical treatments and radiation with persistent vegetative forms in food processing surfaces. *B. cereus* can to survive industrial pasteurisation processes due the endospore, which is capable of a later growth after a non-sporicidal treatment.

The persistence of the spores complicates eradication of the biofilm with cleaning procedures. This is important in dairy factories where it can also reduce pasteurised milk and cream shelf-life leading to outbreaks of infections. Some strains produce toxins that cause diarrhoea and abdominal pain.

Automated milk pasteurisation process – a potential risk source

Enterohemorrhagic Escherichia coli

Human intestinal microbiota contains majority of *E. coli* strains without presenting any health problem. However, other noxious foodborne strains found in drinking water, fruits and vegetables including tomatoes, melons, parsley, cilantro, lettuce, spinach, etc., raw milk or fresh meat constitute adverse health conditions.

The carrier products could be contaminated from point of origin as part of the food manufacturing process. In the food industry, this contamination may take place during the pre-harvest period, due to the use of a contaminated water supply when cultivating the vegetables. Contamination may also occur in post-harvest environments, following washing and processing of the raw material such as carcasses, vegetables, etc. The storage temperatures encourage fast growth of certain pathogenic strains of *E. coli* in contaminated food and food materials.

Listeria monocytogenes

Though not resistant to pasteurisation treatment, *L. monocytogenes*, is a gram-positive, ubiquitous and dangerous foodborne pathogen. Examples of food products known to transmit this pathogen include seafood, dairy products, meat, ready-to-eat products, fruits, soft cheeses ice-cream, unpasteurised milk, can died apples, frozen vegetables, and poultry. Common contaminated foods are smoked fish, cold cuts and fresh cheese.

L. monocytogenes biofilms are mainly composed of teichoic acids able to grow on polypropylene, steel, rubber or glass surfaces throughout the industry and therefrom spreads to food batches, where it can replicate at refrigeration temperatures. Its ability to replicate at low temperatures enhances its hydrophilicity and induces biofilm status as a response to cold temperatures, increasing its attachment to surfaces and its resistance to cleaning procedures in many food factories. Resistance to treatments at up to 60◦C further complicates eradication strategies in food industries.

L. monocytogenes may gastroenteritis in healthy individuals. Pregnant women, infants, the elderly and immunocompromised individuals, are known to be particularly susceptible to listeriosis, a critical disease which also involves septicaemia and meningitis. In pregnant women, listeriosis can lead to spontaneous abortion or damage to the foetus. Common contaminated foods are smoked fish, cold cuts and fresh cheese.

Salmonella enterica

Some strains of *S. enterica* cause gastroenteritis or septicaemia when contaminated food is consumed. *S. enterica* serovar Enteritidis is recorded as the most frequent strain causing nausea, vomiting, fever, diarrhoea and abdominal pain as the main symptoms. Poultry meat is an identified common reservoir for these bacteria in processed food.

The biofilms can form on multiple types of surfaces including cooked, pre-cooked food, raw food and cross contamination is possible between manufacturing plants and supermarkets as with other gram-positives. Cross country outbreaks occur due to the international nature of some of the products particularly poultry and meat. Inter-country control measures may be necessary to ensure eradication.

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive, non-spore forming, non-motile, facultative anaerobic bacterium. It is a human opportunistic pathogen, largely due to its characteristic production of enterotoxins at temperatures between 10◦C and 46◦C. Strains of S. aureus are able to multiply on the mucous membranes and skin of food handlers, a major issue for food factories because staphylococcal enterotoxins are heat-stable and are secreted during growth of this bacterium in a food matrix, eventually contaminated by the food handler or an animal. The bacteria grow well in food with a low water activity, such as those with high sugar or salt content.

Methicillin-resistant *S. aureus* (MRSA) has been noticed in farm animals and in farm animal-derived foods, which are a primary contamination sources for this resistant pathogen. *S. aureus* is able to form biofilms on many different kinds of animal surfaces, whether biotic or abiotic surfaces along the food production chain. This factor is of considerable economic importance since the removal measure is different and dependent on the inherent composition of the surface.



1. Fresh eggs
2. Fresh Turkey meat
3. Fresh spiced pork meat



4. Ready to use meat ball
5. Fresh spices chicken
6. Cut pork



7. Fresh milk
8. Ready to use chicken
9. Pre-prepared pizza

Potential microbial biofilm sources - improperly preserved processed, cooked, pre-cooked and raw meat and dairy products can be a breeding ground for biofilms and the origin of contamination and potential poisoning from secreted toxins.

BIOFILMS 5: In Food, Food Processing and Food Serving Environments – Location and Impact

Biofilm has been described as a are complex microbial ecosystems formed from one or several microbes bound in a complex extracellular matrix of their own secretions and formations. In earlier discussions in this biofilm series, we have talked about general formation of the complex ecosystems, the detection, classical and modern control methods of biofilms in these ecosystems and the key microbes that make up the ecosystems as well as the potential healthcare impact. In this part, we will discuss the areas where biofilms in food can original and pose the most public health threat.

Where can biofilms be found in food, food processing and serving environment?

The development of biofilms also depend on the type of factory, machineries, materials used in the various processes as well as the controls in place. Important material determinants may include the source and quality of water, added additives, other liquid pipelines, pasteuriser plates, reverse osmosis membranes, tables, contact surfaces, employee gloves, animal carcasses, storage silos for raw materials and additives, dispensing tubing, packing and packaging material, bottling equipment and even the cleaning and disinfecting agents made available from third party suppliers.

A small single unit food processing plant.
Biofilms may develop in any part of the food processing plant or machine part. Eradication may be easy due to less plant complexities.

A multi-unit food processing plant. Biofilms can develop on any part including the conveyor belts. The more complex the machine, the higher the risk of biofilm development and the control process and potential cost. Thorough, robust and regular cleaning regime is necessary.

With poor care strategies, multi-unit automated food processing machines can be attractive ground for biofilms. Biofilms in such environment can be even more challenging to eradicate. A multimodal control measure should be considered.

Food items whether solid matrix like meat or liquid such as milk kept in refrigerators particularly when overdue can be home to biofilms. When contaminated, these items may form films or gel-like materials on the food surfaces, separate into partitions, change in colour and produce repulsive odour due to the bacterial growth. Contaminated items should be discarded and the storage thoroughly cleaned with appropriate disinfectants.

Manual Soap Dispenser: The inside of a manual soap dispenser from a supermarket washroom. The brown spots inside the dispenser white surface are accumulating biofilms and around the metal rings. They have a real potential to contaminate the soap dispensed on to hand

Automatic Soap Dispenser: Bottom end of an automatic soap dispenser taken from a food serving supermarket showing accumulation of biofilms around the pump, a potential source of cross contamination

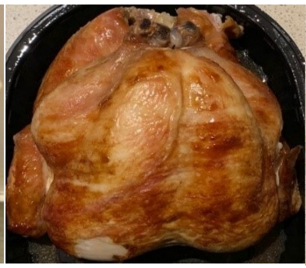
Biofilms contamination in food can be from farm or industrial processing sources - improperly cleaned and / or preserved, processed, pre-cooked, cooked and raw meat, dairy, fruits and vegetable products can be a breeding ground for biofilms leading to food poisoning from secreted toxins.



2. Fresh eggs | 2. Fresh Turkey meat | 3. Fresh spiced pork meat



4. Ready to use meat ball | 5. Fresh spices chicken | 6. Cut pork


7. Fresh milk | 8. Ready to use chicken | 9. Pre-prepared pizza



10. Cleaned ice berg lettuce | 11. Cleaned baby plum tomatoes | 12. Cleaned broccoli

BIOFILMS 6: Biofilms in Medical Devices and Impact

In earlier discussions in this series, biofilms and how they come about were introduced. Overview of the risk and impact in food, restauration, human wellbeing as well as control measures were also provided.

In this part, we provide information on biofilms and potential threat to an important aspect of healthcare delivery, the use of medical devices to treat disease conditions and / or improve patient wellbeing as in daily diagnosis, cardiac malfunction, kidney conditions, etc.

What is a medical device?

By European definition, the term medical device covers a very wide range of materials. This is demonstrated by the following regulatory definition**:**

- any instrument, apparatus, appliance, software, implant, reagent, material or other article intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the following specific medical purposes: — diagnosis, prevention, monitoring, prediction, prognosis, treatment or alleviation of disease,
- diagnosis, monitoring, treatment, alleviation of, or compensation for, an injury or disability,
- investigation, replacement or modification of the anatomy or of a physiological or pathological process or state,
- providing information by means of in vitro examination of specimens derived from the human body, including organ, blood and tissue donations, and which does not achieve its principal intended action by pharmacological, immunological or metabolic means, in or on the human body, but which may be assisted in its function by such means. The following products shall also be deemed to be medical devices
- devices for the control or support of conception,
- products specifically intended for the cleaning, disinfection or sterilisation of devices ……

In daily healthcare experience in hospitals, health clinics, healthcare centres, at the dental centres and eye clinics, the variety available may range from relatively uncomplicated to the very complex ones, which may be seen without particular notice.

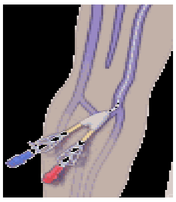
They include, among many, bandages for dressing wounds, stethoscopes, catheters, transfusion equipment, stethoscopes, injection needles, surgical aides and equipment, dentures, pacemakers, defibrillators, artificial body parts (limbs, bone parts, etc.) large and expensive healthcare equipment such as X-Ray machines and dialysis machines, etc. all of which could be contaminated by microbes and form potential growth sites for biofilms if adequate care is not in place.

Importantly, all medical devices directly or indirectly come in contact with patients to diagnose and treat a medical condition and improve wellbeing. It may contribute to the process and task of delivering better treatment and wellbeing such as an healthcare optimisation and delivery computer software.

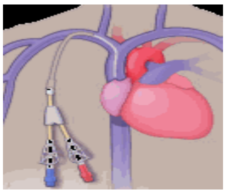
Medical Devices

Electronic medical diagnostic stethoscope

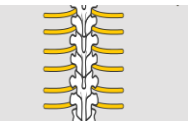
St John Ambulance Dual Headed Stethoscope



Catheters are tunnelled under the skin with the opening being external. They can last and function for a long time provided the they are very well cared for on the external parts and the skin around them to avoid microbial contamination, potential development of infection including biofilms, which can be complicated in certain cases depending on location on the body, type of microbes and other factors.



Hip and / or groin and spinal replacement surgery is an area of particular attention and care to prevent infection



So also, is spinal cord treatment with external bodies or objects including metals and their alloys

A Haemodialysis machine: the complexity of the machine means that a robust contamination prevention practice must be in place to stop microbes taking hold and causing infections.

Microbes Identified from Different Types of Medical Devices

Pathogens Associated with Cardiac Implantable Electronic Devices (CIED) Infection

(Saliba, E. *et al*. 2016).

The prevalence of in infection is very variable. *Staphylococcus aureus* and *S. epidermidis* infections were most prevalent particularly at the early stage. *Methicillin Resistance Staphylococcus aureus* (MRSA) infection occurs but rarely.

Study	N	Microbiology
Sandoe et al[1] (2015)	18 studies of at least 100 patients	Gram-positive (67.5%–92.5%) with CoNS being the most frequent one, followed by *Staphylococcus aureus* Gram-negative bacilli (1%–17%) Fungal infection (uncommon, no >2%) Polymicrobial (2%–24.5%) Negative culture (12%–49%)
Welch et al[2] (2014)	238	EARLY[a]: S. *aureus* (40.7%), CoNS (46.9%), and non-staphylococcal (15.4%) LATE[b]: S. *aureus* (25.9%), CoNS (52.9%), and non-staphylococcal (23.8%)
Durante-Mangoni et al[3] (2013)	82	Gram-positive cocci (84%) • *Staphylococcus epidermidis* (41%) • *S. aureus* (21%) • Streptococci/enterococci/peptococci (7.3%) • Other CoNS (15%) Gram-negatives (4.9%) Candida species (2.4%) *Propionibacterium acnes* (1.2%) Culture negative (7.2%)
Padeletti et al[4] (2011)	N/A	Staphylococcal (70%–90%): *S. aureus, S. epidermidis* Non-staphylococcal (10%–30%): *Enterococcus* spp., *Streptococcus* spp., *Proteus, Klebsiella, Escherichia coli, Pseudomonas aeruginosa, Salmonella, Mycobacteria*, Mycetes (rare) Polymicrobial (2%–7%) Culture negative (5%–20%) EARLY: *S. aureus* LATE: *S. epidermidis*, other Gram-positive bacteria, Gram-negative bacteria, or negative cultures
Sohail et al[5] (2011)	68	Methicillin-susceptible *S. aureus* (34%), methicillin-resistant *S. aureus* (13%) CoNS (25%) Polymicrobial (7%) *Serratia marcescens, Propionibacterium* spp., *Enterococcus* spp., *P. aeruginosa* (21%) Culture negative (7%)
Lekkerkerker et al[6] (2009)	75	CoNS (29%) *S. aureus* (25%) Polymicrobial (14%) Other (11%) Culture negative (15%) EARLY (1–12 months): *S. aureus*, CoNS LATE (>12 months): culture negative

BIOFILMS 7: The Bionic Man Experience - A true life story

Soon after the September 11, 2001, terrorist attacks in the USA (often referred to as 9/11 attacks), airports and other ports of entry worldwide increased the level of checks and controls that travellers have to undergo before boarding the plane, train or ship.

Entry into important buildings were similarly protected with increased technological surveillance. Different levels of conditions and equipment were introduced for liquids, sharp objects and other metallic objects, employing sophisticated metal detectors. These practices persist and are seen by travellers around the globe.

McVey, a frequent traveller, recently recounted his experience at one of the world's biggest and notable airports about six months after the attack. As travellers on the day took turn to pass through the security check machine, he did the same when it was his turn. The machine's detector blipped loudly with the lights blinking red.

He was respectfully asked to go back and pass again. This time the detector blipped even louder and the blinking light, turned into sharp flickering to and from either ends of the metal detector. McVey winked from the corners of both eyes in quick succession. With a small smile, he scanned around him to take a glance at the other travellers faces. The air of suspicion, curiosity, amazement and worry grew to almost palpable proportion.

McVey remained relaxed and calm. Nothing seemed alright or clear to the airport assistances and their supervisors. It was either him or the machine especially in those early days. These were days long before the present smart, hand-held metal detectors and rapid X-Ray machines.

It was seldomly done, but the guards asked McVey to go through the process for a third time and the same sound came on even louder. He explained that the more they made him go through the process, the louder the noise and the quicker and brighter the light.

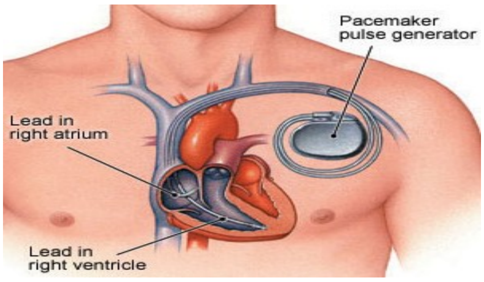
On the third occasion, he looked back at the guards again, this time with a broad, knowing and authoritative smile. Then, he proudly announced, "I am the bionic man". No body understood. All stood professionally and respectfully watching and speechlessly bewildered.

At 89 years old, McVey has had hip and groin replacement. Part of his shoulder has also seen partial replacement. These correction interventions happened at different times and circumstances in his life, partly due to natural wear and tear of the bone and / or accident. The replacement parts were made from mixture of materials including special metallic alloys. He was also carrying a pacemaker with metallic parts. He looked fit and went about his affairs on his own. On that occasion at the airport, McVey was travelling unaided. No stick, no wheel chair. However, to protect him from infection, he had a collection of medications including antibiotics. The medicines were appropriately packaged and sealed in a plastic bag, the type that has become fixtures in most the airports.

Hip and Groin – problems can be treated by replacement. Infection is a risk that must be avoided.

McVey's story illustrates the direction of travel of life particularly in an aging world population. It has been predicted that many more people worldwide will need support of different types of medical devices. Infection prevention post replacement should increase in tandem to prevent internal complications from the installed devices.

Developments in medical science and technology through medical devices will assist to prolonged human life span. This will come with different healthcare needs and costs.



Pacemaker: helps improve heartbeats. Different types exist for various heart conditions – the figure shows one to three flexible, insulated wires (leads) placed in a chamber of the heart. Care must be taken to avoid microbial infection.

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