Tristel Rinse Assure:
A Potential Solution to the Issues Observed in Reverse Osmosis-based Water Purification Systems

Abstract

Reverse Osmosis (RO) is a widely used as water purification method. One of the applications of RO is the production of rinse water for medical devices. This White Paper outlines this process, presents its drawbacks and identifies a potential alternative that eliminates and/or mitigates the drawbacks.

Tristel Rinse Assure (RA) offers chemically-treated rinse water in conjunction with RO, utilising Tristel's proprietary chlorine dioxide chemistry. Tristel RA can be easily connected to an endoscope washer disinfector (EWD) (or automated endoscope reprocessor (AER)) to provide microorganism-free rinse water.

Introduction

RO removes ions and other molecules from water in which they are dissolved. RO reverses the process of osmosis, in which the solutes move from a high-solute concentration to a low-solute concentration. Solutes include salts, ions and microorganisms such as bacteria. In RO, the solutes concentrate in high-solute regions. RO uses a semi-permeable membrane to separate the solutes from water. The result of this process is purified water.

Reverse Osmosis

In the naturally occurring process of osmosis, the solutes move from a high-solute concentration (low water potential) to a low-solute concentration (high water potential) in order to maintain equilibrium (Haynie, 2001). The higher the concentration of the solutes, the higher the osmotic pressure. RO reverses this process by applying a greater pressure than that of osmosis which concentrates the solutes in one region. Only water is pushed through the semi-permeable membrane. This results in the separation of water molecules and solute molecules (Figure 1).

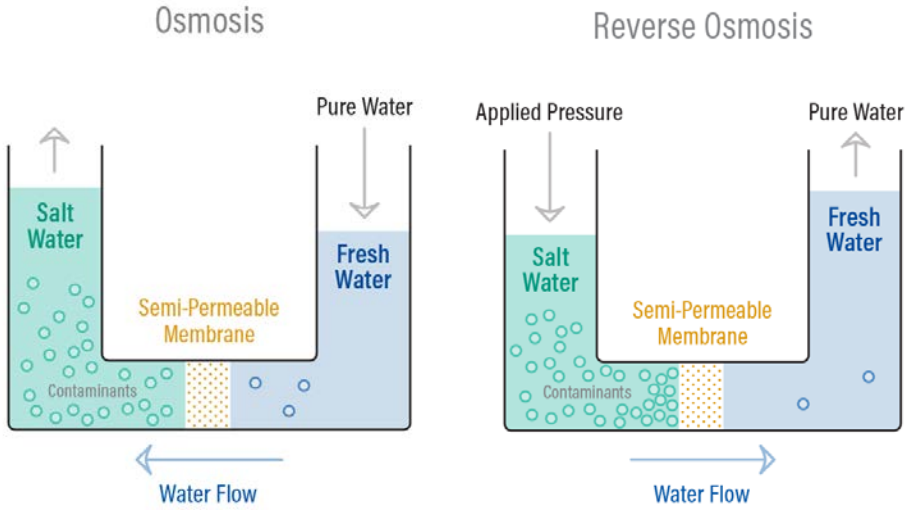

Figure 1: The processes of osmosis and reverse osmosis (https://puretecwater.com)

RO is most commonly used in water purification systems for obtaining drinking water from seawater (Shaffer et al., 2012). RO is also used to purify water for rinsing medical devices, such as endoscopes, after disinfection. The semi-permeable membranes used in such systems only allow passage of water particles thus resulting in separation of water and solute molecules.

A hydraulic pressure is applied to the high concentration side of the solution, forcing the water to filter through the membrane. A mechanical pump is used in RO to overcome the inherent osmotic pressure (Wimalawansa, 2013).

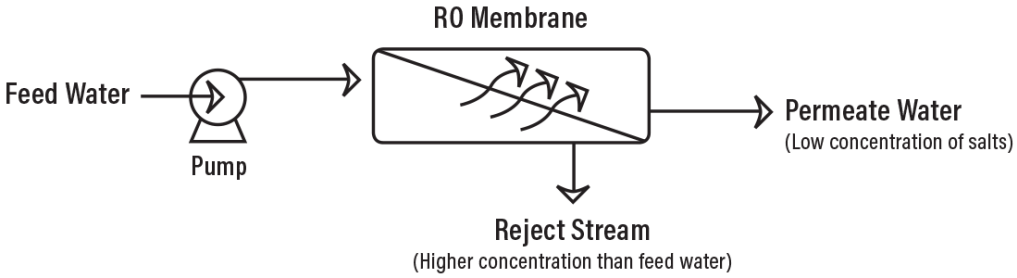

Figure 2: A simplified representation of the RO system (https://puretecwater.com)

The semi-permeable membranes used in RO systems are designed to only allow passage of water molecules. RO removes particles larger than 0.1 nanometres (nm). Membrane pore sizes can vary from 0.1 nm to 5,000 nm (0.0001 micrometre (µm) to 5 µm) depending on the filter type (Wimalawansa, 2013). The size of a water molecule is approximately 3 Angstorms (or 0.3 nm). Figure 3 portrays the particles removed through the RO system.

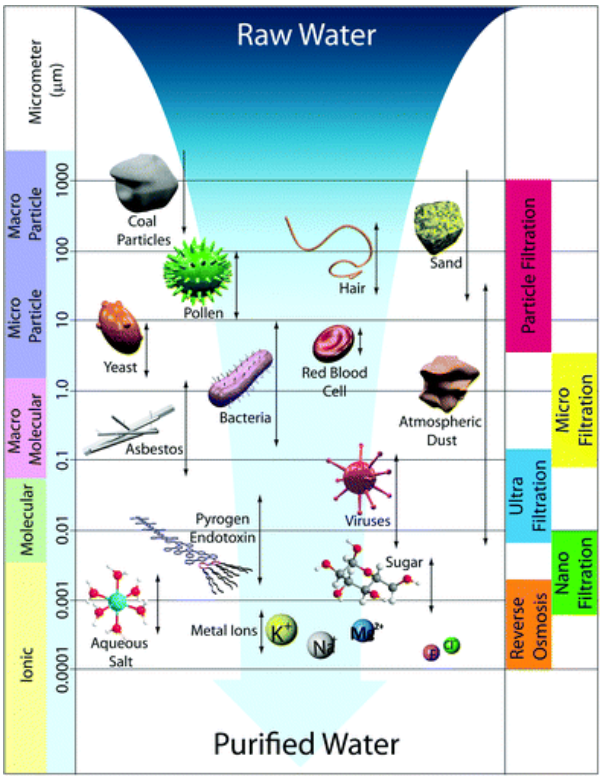

Figure 3: Membrane filtration spectrum (Lee et al., 2016)

The two most common RO membranes are spiral-wound and hollow-fibre. Figure 4 portrays the spiral-wound membrane used in RO systems. The feed water is filtered through the membranes, the solutes are unable to pass through. Purified water (permeate) is released.

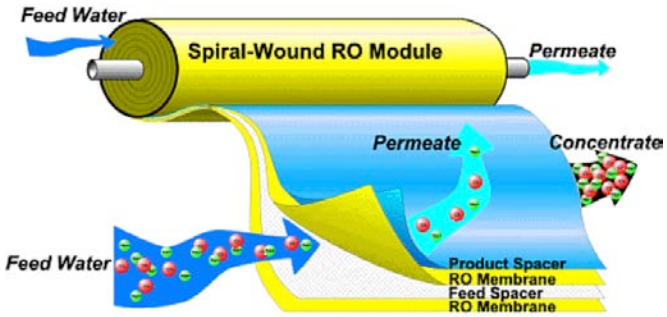

Figure 4: Spiral-wound RO membrane (http://www.sapphire-water.ca)

Figure 5 portrays the second type of membrane used in RO systems, the hollow-fibre membrane. The advantage of hollow fibre membranes is the increased surface area per unit volume as compared to flat sheet membranes, such as spiral-wound membranes (Sagle and Freeman, 2004)

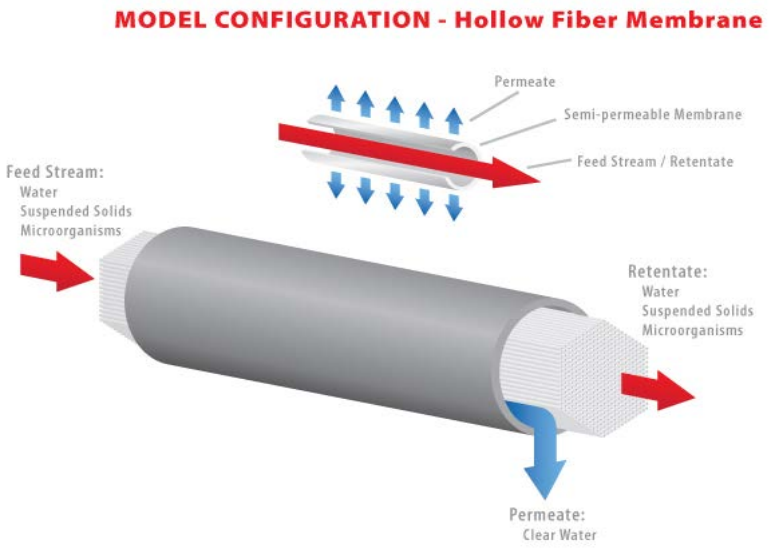

Figure 5: Hollow-fibre RO membrane (http://synderfiltration.com)

There are two types of RO systems. These are known as 'stage' and 'pass' systems. Each exists with two arrangements known as either 1 or 2 RO stage system, or 1 or 2 RO pass system (puretecwater.com).

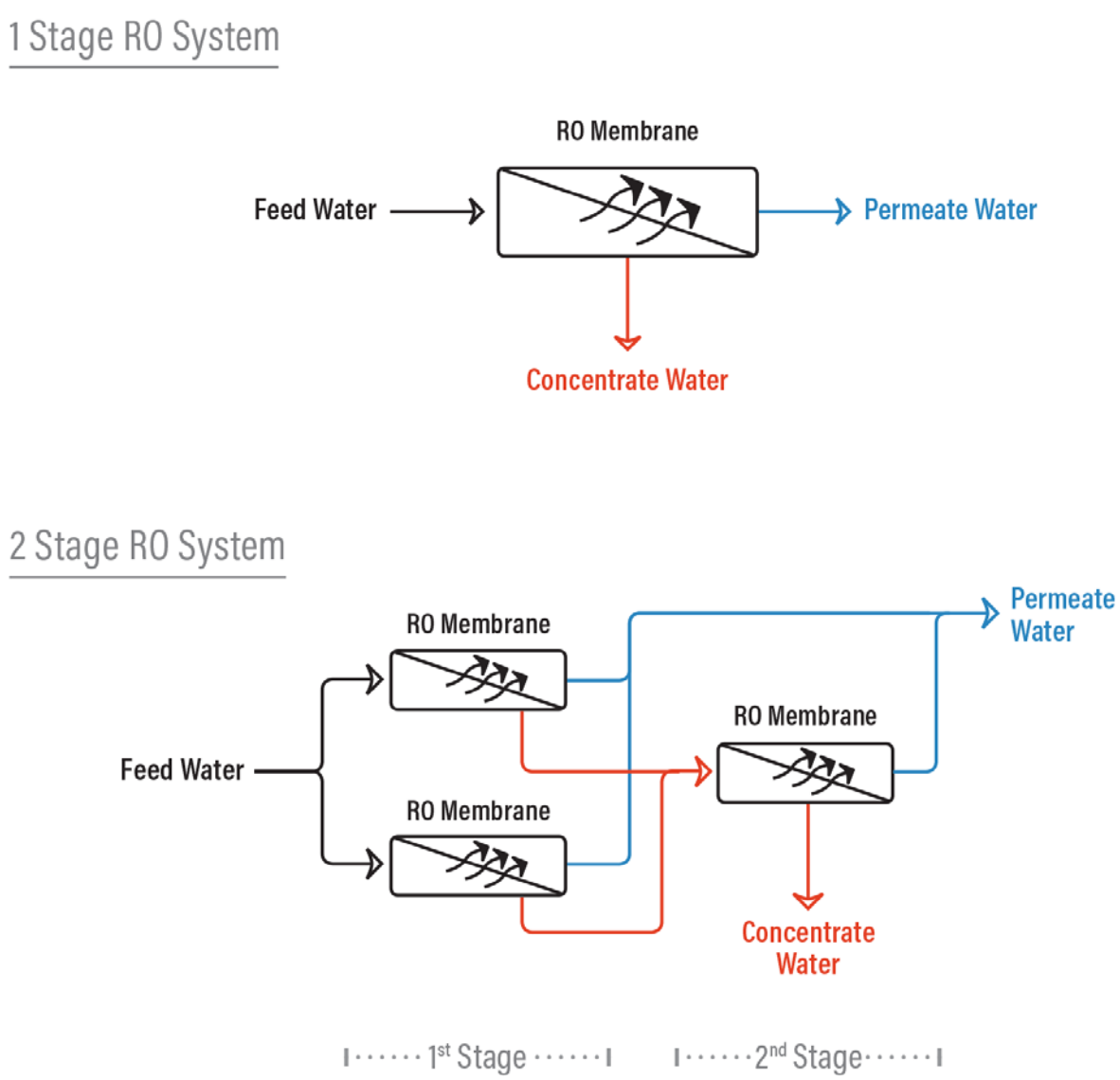

Figure 6: Visual representation of the 1 stage and 2 stage RO systems (https://puretecwater.com)

In 1-stage RO systems, the feed water enters the RO system as one stream and exits the RO as either concentrate (solute) or permeate water.

In 2-stage systems the concentrate from 1-stage becomes the feed water to the 2-stage. The permeate water collected from the 1-stage is combined with permeate water from the 2-stage. Additional stages increase the recovery from the system (Figure 6).

In addition to 1-stage and 2-stage RO, there are also 1-pass and 2-pass RO systems.

The difference between 1-pass and 2-pass RO is that in the 2-pass system, the permeate from 1-stage forms the feed water to the 2-stage. In 2-pass systems the water goes through two RO processes (Figure 7). This results in higher-quality water (puretecwater.com).

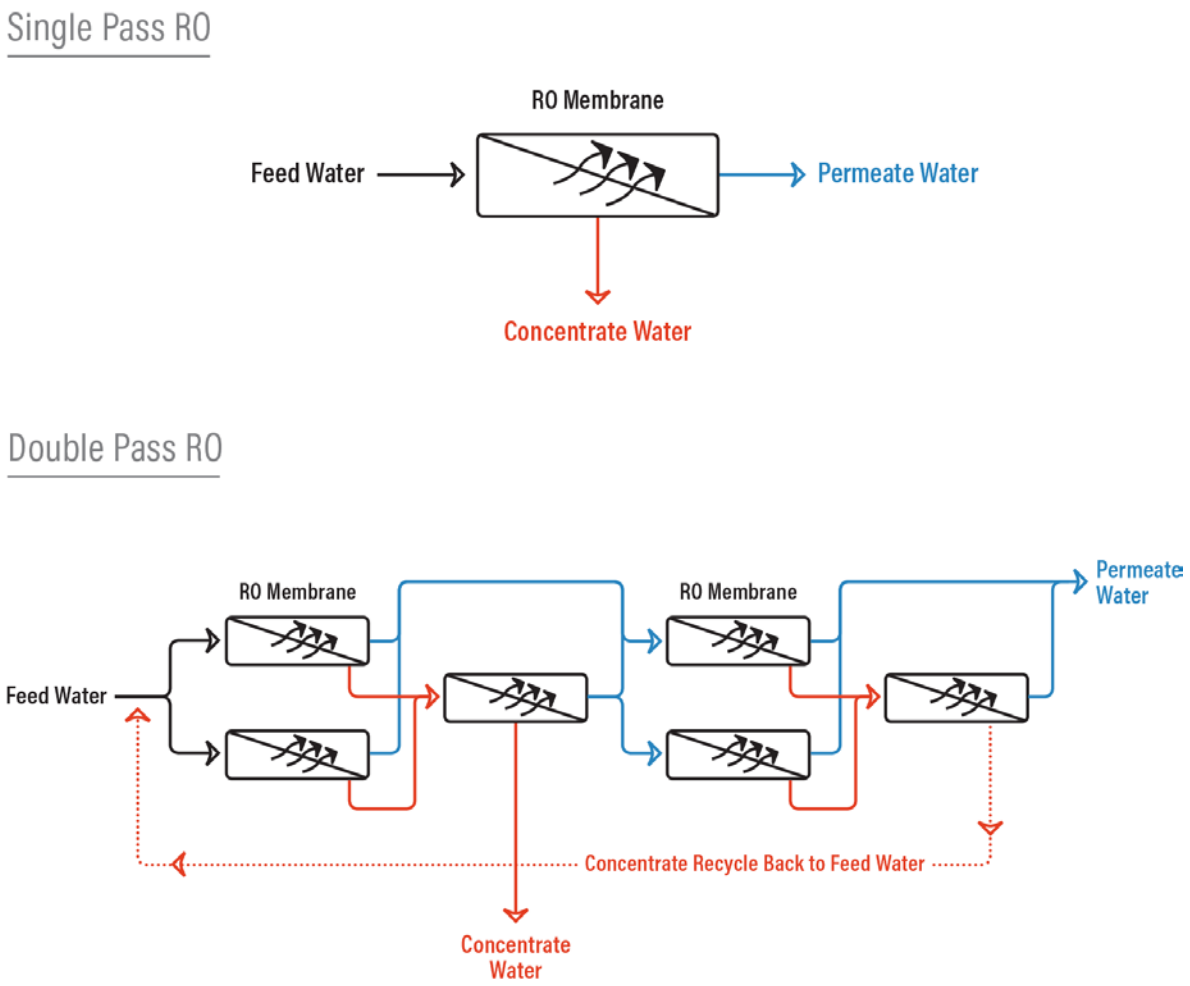

Figure 7: Visual representation of the 1 pass and 2 pass RO systems (https://puretecwater.com)

Concerns

Biofouling:

A major concern of RO systems is biofouling of the membranes. Biofouling is a term used for the growth and deposition of biofilms (Flemming, 2002). Biofilm is defined as a community of bacteria and extracellular material attached to a surface which cannot be easily removed (Donlan, 2002). Bacteria encapsulate themselves by releasing extracellular polymeric substances (EPSs) forming a protective layer. The EPS comprises mainly of polysaccharides and proteins (Flemming, 2002). This protective layer of EPS forms the biofilm (Herzberg and Elimelech, 2007) (Figure 8). Once released from the biofilm, the bacteria are viable and able to proliferate (Watnick and Kolter, 2000). Figure 9 shows a magnified image of *Staphylococcus* biofilm.

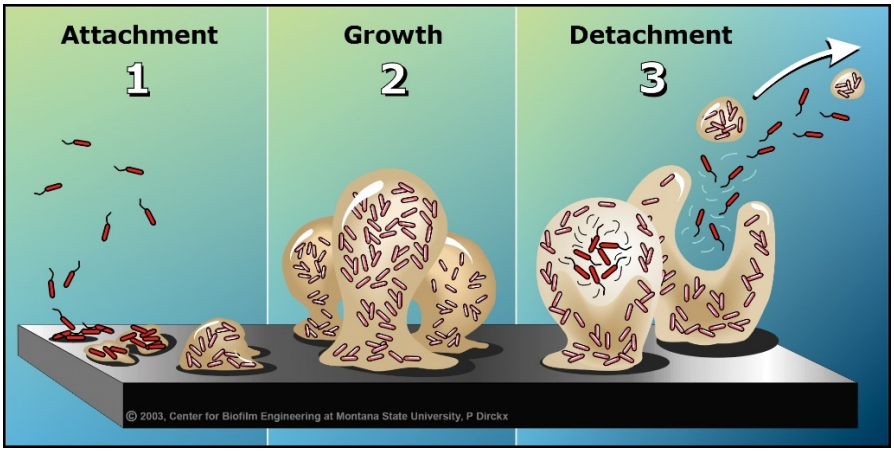

Figure 8: The life cycle of biofilm (http://www.biofilm.montana.edu)

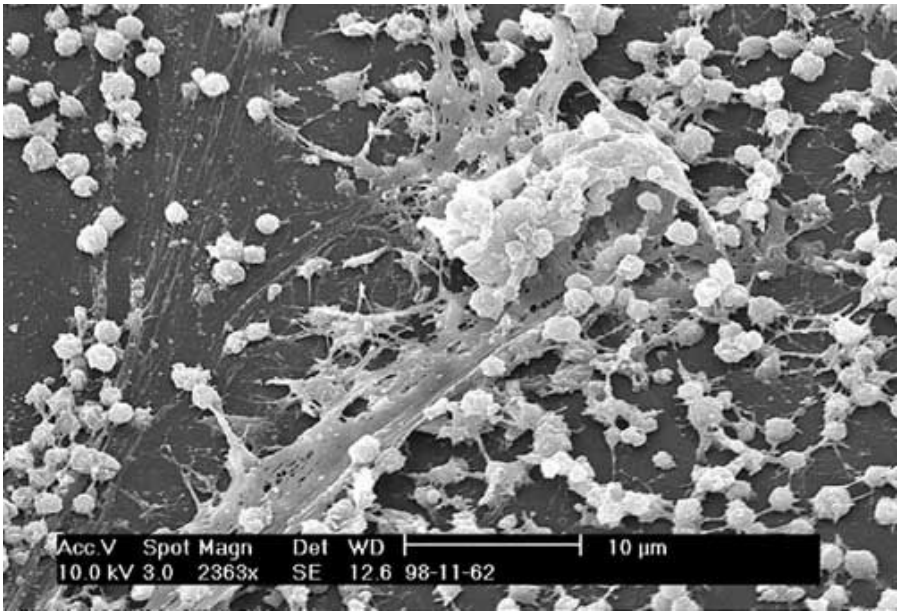

Figure 9: *Staphylococcus* biofilm on a needless connector (Donlan, 2001)

Biofouling results in a decline in permeate water flux due to lowered permeability and a decrease in solute passage (Herzberg and Elimelech, 2007). This requires an increased pressure to overcome the resistance caused by biofilm formation and results in greater energy requirements (Maddah and Chogle, 2017). Biofouling also increases maintenance costs. Chemical cleaning required to eliminate biofilm can account to up to 50% of total costs and can shorten membrane life (Maddah and Chogle, 2017).

Several different cleaning methods have been identified to tackle biofouling. This involves pre-treatment and membrane cleaning and disinfection (Maddah and Chogle, 2017). Pre-treatment of feed can include chlorination, ozonation, or ultraviolet (UV) radiation. Certain species of microorganisms produce colonies and spores which form large clusters (e.g. *Bacillus subtilis*) and chlorination of such clusters may destroy the microorganisms on the cluster surface but leave the organisms on the inside intact (Nguyen, Roddick and Fan, 2012). Ozone however, can form

mutagenic and carcinogenic agents such as bromate in treated water (Nguyen, Roddick and Fan, 2012).

UV radiation, although effective against bacteria and viruses, is expensive and difficult to control dosage (Nguyen, Roddick and Fan, 2012). Membrane cleaning can involve physical or chemical cleaning. High concentrations of surfactants, oxidising agents and enzymes are some of the chemical cleaning agents used for membrane cleaning. These however may weaken the membrane materials and cause hardening of foulant layers (Maddah and Chogle, 2017). Disinfection of feed water can also help prevent biofouling. Biocides can be applied; however, the dead biomass will remain in the feed which could be a source of nutrients for new bacterial cells (Flemming, 1997).

An alternative method of biofouling prevention is membrane surface modification. The semi-permeable membranes used in RO systems should be carefully designed to reduce bacterial adhesion. The surface should be smooth and hydrophilic, i.e. water loving (Maddah and Chogle, 2017).

The increased demand for cleaning and membrane replacement results in high treatment costs.

Some argue that the quality of RO-treated water is not high enough. According to the European Medicines Agency (EMEA) RO-treated water is not acceptable for production of water for injection (WFI) as 'there are no test methods currently available that would effectively identify all possible toxic contaminants'. EMEA also argues that 'biofilm cannot be destroyed' and that it 'can form on the permeate side of the membrane' resulting in possible contamination of the treated water (EMEA, 2008).

Endotoxins:

Endotoxins in rinse water are another great concern. Endotoxins are lipopolysaccharide (LPS) components of the cell membrane of Gram-negative bacteria which trigger the host's inflammatory response and can cause shock and death (Smith et al., 2011). This inflammatory response mediates clearance of the infection and bacterial toxins however, this response can also cause organ dysfunction and lead to death (Zivot and Hoffman, 1995). Bacteria shed endotoxins into their surroundings in small amounts when they are actively growing, and in large amounts when they die (Ryan, 2008). Figure 10 portrays the release of an endotoxin, which interacts with the cells of the immune system resulting in an inflammatory response.

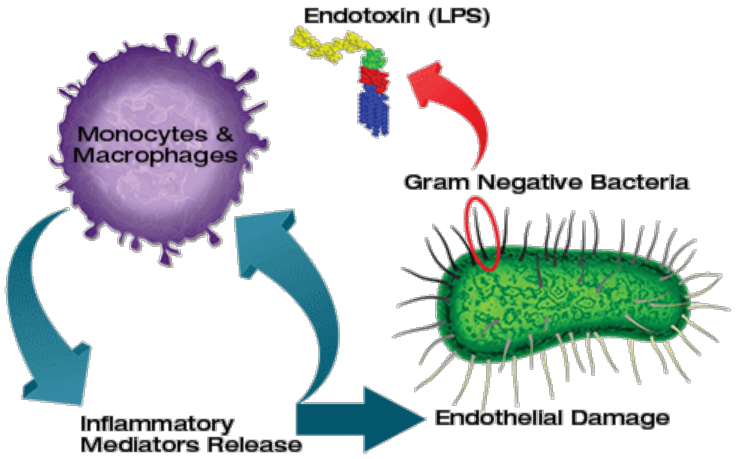

Figure 10: Gram-negative bacteria release endotoxins, triggering an immune response (https://www.clinicallabtests.com)

RO systems are usually effective in removing endotoxins. However, biofouling could inhibit this resulting in endotoxins remaining in the treated water (Mokhtar and Naoyuki, 2012). Residual endotoxins are a major concern for critical medical devices.

Solution

Tristel Rinse Assure (RA): A process that provides an alternative to RO and eliminates RO's shortcomings

Tristel RA is a water purification system, specifically designed to be connected to an endoscope washer disinfector (EWD). It doses low concentrations of Tristel's proprietary chlorine dioxide chemistry into the water used during an EWD's decontamination cycle. Tristel RA provides a constant supply of water chemically dosed with a low level of chlorine dioxide before a final 0.2µm filter. Dosage levels can be altered depending on the expected bioburden and in relation to the amount of water flowing through the system. It prevents bacterial proliferation and biofilm formation and protects the filter and rinse water from contamination.

Tristel RA is a Class I Medical Device which complies with the 93/42/EEC European Medical Device Directive. The chemically dosed water is compliant with HTM 01-06 (which supersedes CFPP 01-06) and EN ISO 15883 standards. HTM 01-06 is a Health Technical Memorandum issued by the UK Government offering guidance on the management and decontamination of flexible endoscopes. EN ISO 15883 specifies requirements for EWDs and the requirements for the "cleaning and disinfection" of EWDs and "accessories which may be required to achieve the necessary performance". Tristel RA also meets requirements set by the American AAMI (Association for the Advancement of Medical Instrumentation) Standards and the Australian/New Zealand Standard AS/NZS 4187:2014 regarding endotoxin levels in water.

Chlorine dioxide (ClO_2) is a gas, which remains in its gaseous state when it enters water. Tristel RA utilises this unique property. Tristel's patented chemistry is generated through the interaction of citric acid ($C_6H_8O_7$) (Base Solution) and sodium chlorite ($NaClO_2$) (Activator Solution). Common salt (NaCl), water (H_2O) and trisodium citrate ($Na_3C_6H_5O_7$) along with other citrate salts, are generated as by products. All of these compounds are non-toxic. Chlorine dioxide destroys microorganisms through oxidation resulting in the loss of electrons and ultimately cell death. Chlorine dioxide is

recognised by the World Health Organisation (WHO) for the treatment of drinking water (WHO, 2004).

The chlorine dioxide chemistry used in the Tristel RA system ensures the RO membrane stays clean and biofilm-free. It has been extensively tested in an accredited laboratory and proven efficacious.

The effectiveness of Tristel RA was evaluated by Nelson Laboratories under Good Laboratory Practice (GLP) test conditions. Nelson Laboratories is a leading United States laboratory used by medical device companies, including EWD companies, for disinfectant testing (Nelson Laboratories, 2018).

Tristel RA was challenged with a high bioburden (›10^6 CFU/mL) consisting of *Mycobacterium terrae* (mycobacterium) and *Pseudomonas aeruginosa* (bacterium) as outlined in the EN ISO 15883 standard. Organisms were injected directly into the tank of Tristel RA, bypassing the 5-micron filter and RO system situated prior to the tank, therefore representing a worst-case contamination scenario. The system achieved a ›6.62 log reduction for *M. terrae* and ~6.61 log reduction for *P. aeruginosa* (Pace, 2017).

Tristel RA protects against bacterial proliferation and biofilm formation. A tubing inoculated with *P. aeruginosa* biofilm (6.3 x 10^5 cfu/mL) was generated in accordance with BS EN 15883 and added into the internal RA water line. A self-disinfection cycle was run and water subsequently analysed. No bacteria were introduced into the tank following the self-disinfection cycle demonstrating total removal and kill from the tubing. This is supported by the HTM 01-06 Part B which in the Biofilm formation 2.82 section states: **"**The addition of a non-toxic biocide to the final rinse water may help prevent the formation of biofilm.**"** (HTM 01-06).

Tristel RA also lowers endotoxin levels in the incoming water supply. The final rinse water used in an EWD should not contain more than 30 endotoxin units (EU)/ml (HTM 01-06, 2016). The Water Quality Study performed at Nelson Laboratories demonstrated that there was a reduction in the level of endotoxins, from an average of 2.543 EU/mL to an average of 0.432 EU/mL (Pace, 2017). This achieved level of endotoxins meets the critical water requirement depicted by AAMI TIR34:2014 (‹10 EU/ml). This also meets the requirement depicted by AS/NZS 4187:2014 for 'Reprocessing of reusable medical devices in health service organizations', which declares 0.25 EU/mL as the maximum concentration level of endotoxins in the final rinse water for reusable medical devices.

In addition, Tristel RA removes organic matter, as measured by water conductivity levels. Samples were obtained from the pre- and post-treatment incoming water. Conductivity was measured in micro-Siemens per centimetre (µS/cm). Conductivity is a reliable and inexpensive method of measuring the ionic content of a solution (James, 2004). Filters within Tristel RA reduce incoming particles to 0.2µm in size. An approximate 80% reduction in ions was observed after Tristel RA treatment (Pace, 2017).

Tristel RA provides microorganism-free water, enables successful decontamination and prevents cross-contamination to medical devices.

Conclusion

Reverse osmosis appears to be an effective purification system for general use water. However, the rinse water for medical devices post-disinfection needs to be bacteria-free. Medical devices undergoing high-level disinfection need to be free of most viable microorganisms. It is crucial, therefore, for the water used for rinsing disinfected medical devices to be free of microorganisms, which could potentially contaminate the device. If the rinse water is not sufficiently pure, the medical device could become contaminated and pose a life-threatening risk to the patient.

The main concern of the RO system is the production and accumulation of biofilm, which results in contaminated permeate water.

Tristel offers a potential solution to this issue. Tristel RA utilises Tristel's proprietary chlorine dioxide chemistry to treat the water prior to its release. Chlorine dioxide eliminates bacteria and biofilm and significantly lowers endotoxin levels. This ensures microorganism-free rinse water.

References

AAMI TIR34:2014, 'Water for the reprocessing of medical devices.'

Clinical Lab Tests, Endotoxin release, image. Available at: https://www.clinicallabtests.com/endotoxin-production-and-function/. Accessed: 22/03/2018.

Dirckx (2003), Life Cycle of Biofilm, image. Center for Biofilm Engineering at Montana State University. Available at: http://www.biofilm.montana.edu/biofilm-basics/what_are_biofilms.html. Accessed: 22/03/2018.

Donlan (2001), *Staphylococcus* biofilm, image. 'Biofilms and Device-Associated Infections', *Emerg Infect Dis.*, **7**(2). Available at: https://wwwnc.cdc.gov/eid/article/7/2/70-0277-f1. Accessed: 22/03/2018.

Donlan (2002), 'Biofilms: microbial life on surfaces.' *Emerg Infect Dis.*, **8**(9): 881-90.

European Medicines Agency (2008), 'Reflection Paper on Water for Injection Prepared by Reverse Osmosis.' Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003403.pdf. Accessed: 26/02/2018.

Flemming (1997), 'Reverse osmosis membrane biofouling.' *Experimental Thermal and Fluid Science,* **14**(4): 382-391.

Flemming (2002), 'Biofouling in water systems--cases, causes and countermeasures.' *Appl Microbiol Biotechnol.*, **59**(6): 629-40.

Haynie (2001), 'Biological Thermodynamics.' *Cambridge University Press*, 130-136.

Health Technical Memorandum (HTM) 01-06 (2016), 'Decontamination of flexible endoscopes. Part B: Design and Installation'.

Herzberg and Elimelech (2007), 'Biofouling of reverse osmosis membranes: Role of biofilm-enhanced osmotic pressure.' *Journal of Membrane Science*, **295**(1-2): 11-20.

James (2004), 'Conductivity Analyzers and Their Application'. In Down and Lehr, *Environmental Instrumentation and Analysis Handbook*, pp 491-510.

Lee et al., (2016), 'Membrane materials for water purification: design, development, and application'. *Environ. Sci.: Water Res. Technol.,* **2**: 17-42.

Maddah and Chogle (2017), 'Biofouling in reverse osmosis: phenomena, monitoring, controlling and remediation.' *Applied Water Science*, **7**(6): 2637-2651.

Mokhtar and Naoyuki (2012), 'Microfiltration, Nano-filtration and Reverse Osmosis for the Removal of Toxins (LPS Endotoxins) from Wastewater'. *Journal of Membrane Science & Technology*, **2**(3).

Nelson Laboratories (2018), 'About Nelson Laboratories'. Available at: https://news.nelsonlabs.com/about. Accessed: 16/04/2018.

Nguyen, Roddick and Fan (2012), 'Biofouling of water treatment membranes: a review of the underlying causes, monitoring techniques and control measures.' *Membranes (Basel),* **2**(4): 804-40.

Pace (2017), 'Tristel Rinse Assure Disinfection Study GLP Report', Nelson Laboratories.

Pace (2017), 'Tristel Rinse Assure Water Quality Study GLP Report', Nelson Laboratories.

Puretec Industrial Water, 'What is Reverse Osmosis?'. Available at: https://puretecwater.com/reverse-osmosis/what-is-reverse-osmosis#pre-treatment-for-reverse-osmosis. Accessed: 20/03/2018.

Ryan (2008), 'Endotoxins and Cell Culture'. *Corning Technical Bulletin.* Available at: http://www.gongyingshi.com/item/doc/20141221/cc_endotoxins_tc_305_rev1.pdf. Accessed: 05/03/2018.

Sagle and Freeman (2004),'Fundamentals of Membranes for Water Treatment', University of Texas. Available at: http://www.twdb.texas.gov/publications/reports/numbered_reports/doc/r363/c6.pdf. Accessed: 27/03/2018.

Sapphire Water, 'Reverse Osmosis (RO) Filtration Process'. Available at: http://www.sapphire-water.ca/water-treatment-technology/reverse-osmosis-water-filter/. Accessed: 20/03/2018.

Shaffer et al., (2012), 'Seawater desalination for agriculture by integrated forward and reverse osmosis: Improved product water quality for potentially less energy.' *Journal of Membrane Science*, **415-416**: 1-8.

Smith et al., (2011), 'Removal of endotoxin from deionized water using micromachined silicon nanopore membranes' *Journal of Micromechanics and Microengineering*, **21**(5).

Synder Filtration, 'Hollow Fiber Membranes'. Available at: http://synderfiltration.com/learning-center/articles/module-configurations-process/hollow-fiber-membranes/. Accessed: 20/03/2018.

Tu et al. (2013), Boron as a surrogate for N-nitrosodimethylamine rejection by reverse osmosis membranes in potable water reuse applications. *Environ Sci Technol*, **47**(12): 6425-30.

Watnick and Kolter (2000), 'Biofilm, city of microbes.' *J Bacteriol.*, **182**(10): 2675-9.

Wimalawansa (2013), 'Purification of Contaminated Water with Reverse Osmosis: Effective Solution of Providing Clean Water for Human Needs in Developing Countries'. *International Journal of Emerging Technology and Advanced Engineering*, **3**(12): 75-89.

World Health Organization (WHO) (2004), 'Guidelines for Drinking-water Quality'. 3rd edition.

Zivot and Hoffman (1995), 'Pathogenic effects of endotoxin.*' New Horizons (Baltimore, Md.),* **3**(2): 267-75.