Advancing effective protein removal

David Jones outlines a research project in which ultrasonic energy is harnessed for the effective cleaning of surgical instruments. He explains how the research has led to the development of a patented solution that is capable of removing protein to an exceptionally low level.

Cleaning with high frequency sound is based on the effect of cavitation through the application of the sound into fluid. The principle of ultrasonic cleaning is to utilise transducers to apply flexure to the base and walls of a tank of fluid at high frequencies within the kilohertz range. This is distributed into the water via vacuum chambers or bubbles, the size of which are directly related to the frequency applied. On release, these bubbles rise up through the tank fluid until they hit an instrument, upon which they implode, drawing away contamination. Fluid based ultrasonic cleaning technology is classed as a "green" method of cleaning making it a more environmentally friendly and overall a cost-effective method.

Ultrasonics are used in many industries from anilox roll cleaning in the print industry, to cleaning steel hydraulic pipes for aerospace manufacturing, to name just a few. Most importantly, fluid based ultrasonics offers an excellent method for the removal of proteinaceous contaminants from surgical instruments – providing the correct parameters are used.

Tackling protein contamination

A literature search of protein contamination on surgical instruments found several studies looking at protein residue on "ready to use" instruments in rotation. The results indicated detection of high levels of protein (Some as high as 750µg and beyond).[1-8] In 2012, a further study[9] highlighted that incorrectly reprocessed surgical instruments can cause surgical site infections, and these can cost the NHS many millions of pounds a year.

Why are proteins difficult to remove from surgical instruments?

Due to the biomolecular structure, some proteins are hydrophobic or water repellent, and are more difficult to remove from surfaces.[10] Cleaning with alcohol has been noted to increase the difficulty of protein removal[11,12] and proteins can strongly adhere to materials if exposed to heat without adequate removal beforehand.

Most concerning of all is the prion protein. Found in higher concentrations in the brain, spinal tissue and tonsils, prions in particular have an affinity to stainless steel. Due to their molecular structure, prions exhibit a remarkable resistance to common chemical and physical decontamination procedures that effectively inactivate conventional pathogens.[13] There is potential for prion proteins to be passed from patient-to-patient through surgical instruments resulting in Iatrogenic Creutzfeldt-Jakob disease (iCJD) and, although very rare, there is no treatment for this fatal disease.

More recently, research has suggested that other proteins including Beta amyloid tau, the protein found in Alzheimer's patients, could be transmissible via surgical instruments.[14] However, further research is needed on this topic. Furthermore, proteins have the potential to become "baked on" in heat cycles if not adequately removed beforehand and mask other contaminants such as bacteria.

Due to the nature of ultrasonic cleaning and the vacuum method of contaminant removal, ultrasonics can remove hydrophobic proteins from surgical instruments. This can significantly reduce the potential of cross contamination of infectious proteins, such as prions, and also exposes any bioburdens that are potentially lurking underneath. Other benefits of Advanced Ultrasonics include a significant reduction of manual cleaning requirements, resulting in freeing up valuable time for technicians, and the removal of contamination on difficult to clean instruments – such as those with crevices, lumens and box joints.

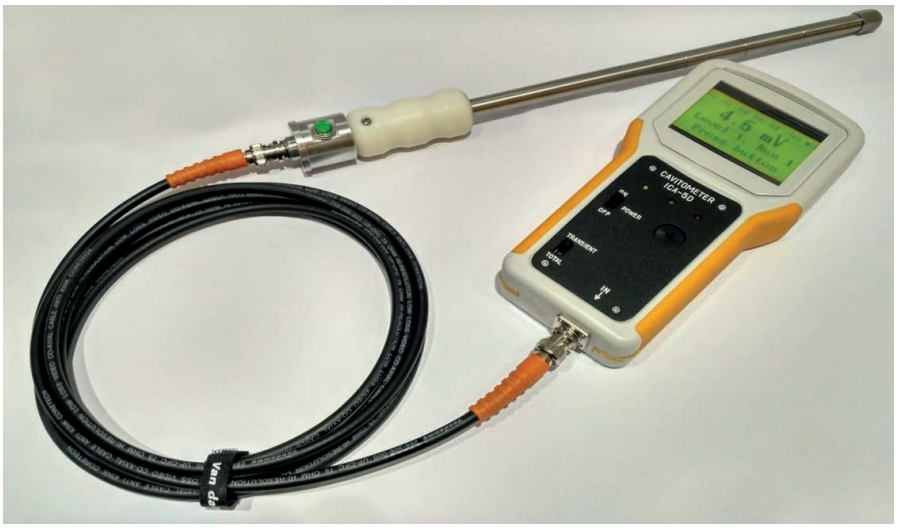

Fig 1 Cavitation validation device

Research and development

The initial work for Alphasonics' medical device project began with the procurement of a ProReveal protein measurement device, as advised by a local NHS Trust decontamination lead. This gave a means of measuring instruments contaminated with a variety of test soils (human blood, baked-on pig brain, Edinburgh soil, Browne's washer/disinfector test ▶

soil) combined with an array of variables – such as generator frequency, transducer location, tank shape, different chemistry pHs and cleaning time, to name a few.

All of the potential variables' performances were exhaustively assessed and the most successful combination of parameters were established to achieve the optimum in protein removal. This resulted in cleaning efficacy results repeatedly consistent to below ≤1µg of protein contamination per side, per instrument.

In 2014, this technology was presented to the Rapid Review Panel (RRP) and it was rated as R5: "Insufficient clarity and/or evidence presented to enable a full review of the product". The RRP panel suggested some additional work, which was taken on board. This included a comparison of competitors' technology that was available at that time. This gave clarity as to how Advanced Ultrasonics compared with competitors and the results strongly suggested that developing the technology further and moving it into the market would be a success.

Patented technologies

As a result of extensive research, to establish a reliable and consistent ultrasonic technology, there are several innovations that were developed and patented. Collectively, Advanced Ultrasonics includes: BetaSound, Basket Rotation Active Cavitation and a Cavitation Validation Device (a handheld device to monitor cavitation as a way of validating ultrasonic activity) BetaSound utilises multi-frequency technology. By modulating across several frequencies, a much more homogenous distribution of sound is achieved. Another technological advancement, developed through the process, is Active Cavitation.

As mentioned previously, cavitation is produced through the rapid flexure of the tank base or sides through the formation and collapse of millions of vacuum bubbles. These

Fig 3 Cavitation Validation Device measuring ultrasonic losses in a simulated lumen

Fig 2 Technician measuring cavitation with CVD

bubbles rise up through the tank fluid until they hit something or reach the surface, upon which they implode with great speed and high pressure.

This speed of collapse is so fast and the pressures so high that temperatures reach about 4727° C or 5000°K for a nano-second over a microscopic area. While this in itself is not enough to make the tank fluid boil, it is enough to rip open any O_2 molecules that may be present within the fluid. This is happening many thousands of times a second. This phenomenon is known as 'Sonolysis'. When the molecules are torn apart, what is left are free radicals (or an unpaired electrons) that are highly reactive with anything that is around them and will seek to pair with another molecule. In this case, it is the cleaning chemistry. Active Cavitation is achieved by introducing additional air into the fluid creating more oxygen molecules, thus building on the sonolysis principle. This increases the number of implosions of vacuum chambers in the fluid and results in a boost in cleaning, as well as aiding the consistency achieved. During efficacy trials, it was discovered that, if the basket gently oscillates during the cleaning cycle, this also increases the cleaning efficacy. This is known as 'Basket Rotation'.

In addition, to answer and improve the requirement for validation of the ultrasound and to monitor the performance of the ultrasonic transducers, a Cavitation Validation Device (CVD) (Fig 1) was developed that receives acoustic signals from the microscopic cavitation bubbles via a sensor in the probe and this, in turn, transfers these signals into electrical activity within the device. This electrical activity, measured in millivolts, is then displayed on the CVD screen. The device measures in triplicate and produces graphs to show the ultrasonic activity, as well as statistical analysis – such as co-efficient of variance. This device was developed, in an attempt to enable an improvement to the archaic method of foil testing. In 2016, Beta Sound, Active Cavitation and Basket Rotation were patented and, in 2017, ISO 13485 accreditation was obtained. Finally, in 2021, the Cavitation Validation Device was patented.

Further development

An important factor to consider is that ultrasonic energy becomes absorbed in materials and this is known as 'attenuation'. The development of the Cavitation Validation Device accelerated product R&D, as this technology allows for comparative measurements of ultrasonic activity from the tank to the target instruments (Fig 3).

DIN baskets and external areas of hollowed instruments, such as lumened instruments or robotic shafts, significantly reduce ultrasonic energy and the ability to pass through to the difficult to reach contaminated part of an instrument. Studies showed a standard DIN basket with a perforated perimeter and a 6mm base can have ultrasonic losses of up to 28%. If ultrasonic energy is required to travel further, for example across a cannula or robotic instrument shaft, further losses incur and, when added together, this can equate to up to a 68% loss of ultrasonic energy to the target area.

Taking these losses into consideration, a second cleaning cycle was specifically designed for cannulated and robotic instruments. This research was conducted with several protein quantification methods, including Micro BCA Assays with SDS extraction and ProReveal, and an array of difficult to clean instruments.

The variables included time of

ultrasonification, pressure of irrigation and suitable proteolytic enzyme detergents to establish parameters that would thoroughly remove protein contaminants to a safe measure. Additionally, surrogate daVinci robotic instruments were designed (Fig 4). This was achieved by removing the shaft, replacing the distal working end with a valve that can increase or decrease the flow of water – mimicking the instrument back pressure flow method of cleaning.

The particular test instruments (Fenestrated Bipolar Forceps) when irrigated had a 4ml/per minute flow. The surrogates were attached to the irrigation system and the valve was manipulated to also allow 4ml/per minute of water through, so as to mimic the back pressure within the instrument. Soiling is achieved by delivering test blood via the flush ports in the housing of the instrument (Fig 5). This is then cleaned (using a low pH of chemical, as recommended in the reprocessing guidelines) and the shaft can be removed easily to assess visually.

With a ProReveal (with an extended drawer), the cleaning efficacy and protein contamination removal can be accurately measured. The protocol requires that a surrogate is placed within the batch being cleaned and, following the cleaning cycle, this can be quickly stripped-down to determine the cleaning efficacy. By mimicking the back pressure, the cleaning of the instrument will be the same as the surrogate. This process was designed as a less demanding method of spot checking that the instruments were being cleaned sufficiently in comparison to the ISO 15883-5:2021 (E) standard methods of protein extraction and quantification.

Furthermore, to tackle ultrasonic losses due to DIN baskets, these were redesigned for Advanced Ultrasonic systems – taking into consideration technician safety, but allowing for more ultrasonic energy to permeate the basket and clean the instrumentation thoroughly. Several baskets were designed and trialled using a Cavitation Validation Device to assess losses and, in addition, the cleaning efficacy of the instruments in the different prototypes.

Fig 4 daVinci surrogate instrument flow rate measurement

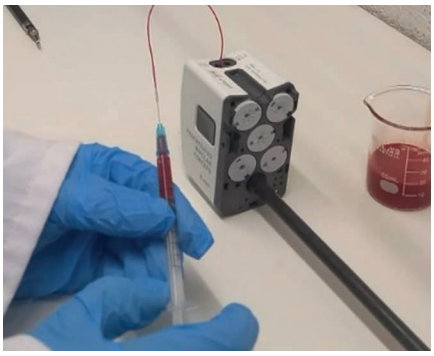

Fig 5 Soiling of daVinci surrogate instrument for cycle design

Cleaning efficacy decreases with ultrasonic energy losses. Instruments in baskets with small mesh will require a longer cleaning time than baskets with a larger mesh. Therefore, the basket choice was made (Figure 6) to have a skirt of smaller mesh to prevent instruments from sliding out of baskets but a larger base and perimeter to increase ultrasonic activity – thus achieving optimum cleaning at a desirable time.

External research

To corroborate the internal research and development, several external evaluations of the cleaning efficacy of this technology have been undertaken; firstly, assessing the removal of *E. faecium* and *S. aureus* – common pathogens in sepsis and other infections – with advanced ultrasonics and a proteolytic enzymatic in cold water. The results indicated a 5.5 log reduction on heavy soiled instruments and 6.6 log reduction on lightly soiled instruments.

The Infection Innovation Consortium (iiCON), led by the Liverpool School of Tropical Medicine, conducted scanning electron microscopy to determine that the results achieved through the use of the ProReveal device were accurate. Stainless steel coupons, soiled with porcine brain and cleaned with Advanced Ultrasonics, were examined.

The results suggested that there were very little remnants of nitrogen on the stainless-steel samples – thus denoting an absence of proteins. The same trial was conducted using ProReveal as a method of protein quantification. The results were almost identical, validating the ProReveal, in addition to advanced ultrasonics cleaning capabilities of protein removal.

The most recent research involves testing the limits or robustness of Advanced Ultrasonics. This was achieved by assessing unfavourable conditions, such as absence of chemistry and/or very low water temperatures, and the effects this has on the protein cleaning efficacy have been evaluated.

However, these studies exclude bacteria removal or log reduction. Disinfection is not claimed, as these devices do not include a thermal disinfection cycle. However, a high log reduction is achieved through the unique application of Advanced Ultrasonics and is achieved as a 'side effect' of the primary aim of the technology (the removal of proteins). Advanced Ultrasonics does not include thermal energy and is preferably used with DI/RO water.

However, mains water is fully acceptable and does not affect the cleaning efficacy. Considering the potential of mains supply water being used by sterile service departments in colder months, cleaning efficacy trials were conducted with water temperatures of <5°C with detergent and the results were similar to those recorded within the standard parameters of a normal cleaning cycle (≤1µg).

Finally, cleaning efficacy testing was conducted in the absence of cleaning chemistry. Although this is not recommended for surgical instruments, the results for this trial are shown in tables 1-3. The importance of this trial is to ensure that, if for whatever reason, chemistry ▶

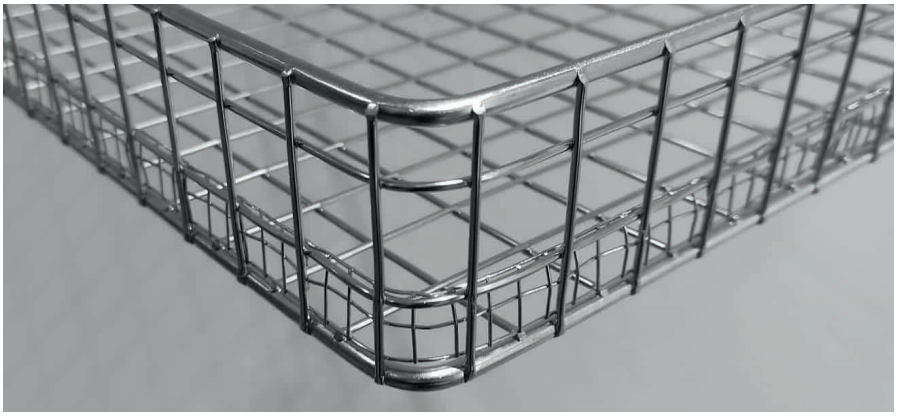

Fig 6. Alpha Basket B, designed to allow more ultrasonic energy to surgical instruments, with a 20mm base and perimeter and a protective 6mm skirt to prevent instruments from injuring technicians.

Attempt 1 Sample	Measurement ID	ProReveal Contamination Measurement (µg)
1	4023	0
2	4815	0
3	5633	0.82
4	0421	0
5	4844	0
6	5710	0.34
7	0759	0
8	2022	0.49

Attempt 2 Sample	Measurement ID	ProReveal Contamination Measurement (µg)
1	2635	1.59
2	3535	1.92
3	2731	0
4	3340	0.12
5	3723	0
6	4104	0.07
7	4457	0
8	5324	0

Attempt 3 Sample	Measurement ID	ProReveal Contamination Measurement (µg)
1	4452	0
2	5034	0
3	5434	0
4	5837	0
5	1704	0
6	2348	0.18
7	2741	0.43
8	4928	0.09

Tables 1-3 ProReveal results for assessing advanced ultrasonics in the absence of cleaning detergents

is not delivered during a cycle, that protein removal is still achieved to a safe level.

Almost a decade has passed since this project began and significant time has been spent developing, researching and obtaining accreditation. The transition from print to the medical device industry has not been a quick and straightforward process.

However, there are now Advanced Ultrasonic systems implemented in the NHS and more projected for 2024. It is safe to say the hard work has paid off. Reflecting on the process and the knowledge gained from the whole project, the Cavitation Validation Device sets the technology apart – giving the ability to monitor and validate ultrasonic systems. Additionally, removing protein to an exceptionally low level, without the need for a thermal cycle, helps to improve patient safety, while contributing to a reduction in CO_2 emissions. **CSJ**

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About the author

With a history of design engineering, David Jones started Alphasonics in 1993. Since then, he has steered the company's technological developments through solid engineering principles and practices. Medstar technology is David's brainchild, developed through a painstaking scientific approach. Several worldwide patents are being filed for the technology.